



Special Issue

Abstracts of the International Symposium on Phytochemicals in Medicine and Food (ISPMF 2015, June 26-29 2015, Shanghai, China)

Edited by **Petra Högger** and **Jianbo Xiao**



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Preface

Introduction for International Symposium on Phytochemicals in Medicine and Food (ISPMF 2015)

The International Symposium on Phytochemicals in Medicine and Food (ISPMF2015), organized by the Phytochemical Society of Europe (PSE) and the Phytochemical Society of Asia (PSA), was held June 26-29, 2015, in Shanghai of China. This was the first time that a PSE meeting has been held in Asia and a PSE-PSA joint symposium provided an opportunity for communication between scientists from Europe and Asia and other continents. ISPMF2015 has been jointly sponsored by Fujian Agriculture and Forestry University, Guizhou Medical University, Shanghai Normal University, Yancheng Institute of Technology, Beijing Normal University, and Fudan University. More than 270 scientists from 48 countries attended this meeting and presented their research and opinions on phytochemistry, phytomedicine and phytoneering. The international organizing committee and scientific advisory board of ISPMF 2015 comprised of outstanding scientists from around the globe. Dr. Jianbo Xiao was the chairman of the International Organizing Committee of ISPMF2015 and moderated the open address on June 26.

The organizing committee of ISPMF2015 assembled an exciting and diverse program, featuring 16 sessions including 12 plenary lectures, 20 invited talks, 55 short oral presentations, and more than 130 posters, which were dedicated to creating a podium for exchanging the latest research results in the phytochemicals for food and human health.

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Programme of the International Symposium on Phytochemicals in Medicine and Food (ISPMF 2015)

	25 June, Thursday
14:00 - 18:00	Registration

	26 June, Friday	
From 07:00	Registration	
08:00 - 08:30	Opening Address <i>Jianbo Xiao, Simon Gibbons, Mei Han</i>	
08:30 - 10:15	Plenary Session 1 (Room 1) <i>Moderators: Simon Gibbons, Zhi-Hong Jiang</i>	
08:30 - 09:05	PL1: Phytotoxic saponins from Agavaceae family <i>Francisco A. Macías (Spain)</i>	
09:05 - 09:40	PL2: Antiaging - from discovery to clinical study <i>Chin-Kun Wang (Taiwan)</i>	
09:40 - 10:15	PL3: Phytochemicals: Potential sources of new antiinfectives and psychoactives <i>Simon Gibbons (United Kingdom)</i>	
10:15 - 10:45	Refreshment Break	
10:45 - 12:35	Session 1 (Room 1): Polyphenols and human Health <i>Moderators: Mingfu Wang, Anca Miron</i>	Session 2 (Room 3): Bioactivity of natural products (I) <i>Moderators: Marc Diederich, Amir Reza Jassbi</i>
10:45 - 11:10	IL1: Novel roles of phenolics in management of food quality and human health <i>Mingfu Wang (China)</i>	IL3: Prevention of phytochemicals on the toxicity of neo-formed compounds (NFM) during food processing <i>Fang Chen (China)</i>
11:10 - 11:35	IL2: Stilbene derivatives from <i>Pinus cembra</i> L. bark and their cytotoxic effects on HeLa cells <i>Anca Miron (Romania)</i>	IL4: Investigation of effect mechanisms and clinical study of polysaccharide of <i>Dendrobium officinale</i> in alleviating cigarette-induced COPD <i>Kalin Yanbo Zhang (Hong Kong)</i>
11:35 - 11:55	SL1: Modulating the properties of dietary flavonoids and isoflavonoids <i>Jean-Paul Vincken (Netherlands)</i>	SL4: Advances in phytochemicals, biological activities and toxicology of common edible flowers <i>Bai-Yi Lu (China)</i>

11:55 – 12:15	SL2: Anti-glycation and antioxidant properties of polyphenol-enriched fractions from Giant knotweeds (<i>Reynoutria</i> sp.) <i>Izabela Nawrot-Hadzik (Poland)</i>	SL5: Phytochemicals and power test inhibition spoon leaf extract (<i>Plantago major</i> L.) and fruit sugar-apple (<i>Annona squamosa</i> L.) on xanthine oxidase activity <i>Mohamad Mukhlis Saktiyawan (Indonesia)</i>
12:15 – 12:35	SL3: Phenolic-rich water fraction of edible medicinal fern <i>Stenochlaena palustris</i> is a potent antiglycosidase and antioxidant agent <i>Tsun-Thai Chai (Malaysia)</i>	SL6: Nutritional composition, physiological functions and processing of lotus (<i>Nelum bonucifera</i> Gaertn.) seeds: a review <i>Baodong Zheng (China)</i>
12:35 – 14:00	Lunch	
14:00 – 16:15	Session 3 (Room 1): Antimicrobial and antifungal activities of phytochemicals <i>Moderators: Franz Bucar, Zhi-Hong Jiang</i>	Session 4 (Room 3): Analytical techniques for phytochemicals <i>Moderators: Krystyna Skalicka-Woźniak, Francisco A. Macías</i>
14:00 – 14:35	IL5: Zingiberaceae - a source of antimicrobials and resistance modulators <i>Franz Bucar (Austria)</i>	IL6: Enzymatic bioautography on HPTLC: combined phytochemical and activity screening tool for medicinal plants quality assessment and <i>in vitro</i> cultivation bioprocess control <i>Evelyn Wolfram (Switzerland)</i>
14:35 – 14:55	SL7: Prenylation of phytochemicals through fungal elicitation of seeds to increase their antimicrobial potential <i>Carla Araya-Cloutier (Netherlands)</i>	SL10: New HPLC-MS methods for ginsenoside profiling and identification in roots and ginseng based products <i>Igor Rodin (Russia)</i>
14:55 – 15:15	SL8: Investigation of antibacterial mechanism and identification of bacterial protein targets mediated by three antibacterial plant extracts <i>Fai-Chu Wong (Malaysia)</i>	SL11: Structure and bioactivity of lactoferrin from milk <i>Ming Du (China)</i>
15:15 – 15:35	SL9: Antimicrobial activities and chemical compositions of <i>Pogostemoncablin</i> (Blanco) Benth. and <i>Perillafrutescens</i> (L.) Britt. in a traditional Chinese medicine formula <i>Wen-Wu Li (United Kingdom)</i>	SL12: Metabolomics combined with chemometrics reveals chemical features of <i>Pericarpium Citri Reticulatae</i> and <i>Pericarpium Citri Reticulatae Viride</i> <i>Lun-Zhao Yi (China)</i>
15:35 – 15:55	Technology report: Comprehensive two-dimensional liquid chromatography separations of TCM by DGLC coupled with Tandem Mass spectrometer <i>Jin Yan (Thermo Fisher, China)</i>	SL13: Rapid analysis of the key aroma compound, 2-acetyl-1-pyrroline, in rice plant at different growth stages using automate headspace-gas chromatography with nitrogen-phosphorus detector <i>Pittayaporn Boontakham (Thailand)</i>
15:55 – 16:55	Refreshment Break / Poster section 1 (No. 1-60) (Room 5)	

16:55 – 18:50	Session 5 (Room 1): Ethnomedicine and traditional food <i>Moderator: Elvira Gille, Fang Chen</i>	Session 6 (Room 3): Leads finding from Nature <i>Moderators: Simon Gibbons, Jun Lu</i>
16:55 – 17:20	IL7: Starting from Danube Delta folk medicine data to novel nutraceuticals <i>Elvira Gille (Romania)</i>	IL9: Potential biomedical use of New Zealand seaweed <i>Undaria pinnatifida</i> <i>Jun Lu (New Zealand)</i>
17:20 – 17:45	IL8: Pharmacological and chemical investigations of <i>Polygonum capitatum</i>-an ethnic Miao's herb used in the treatment of urinary tract infections <i>Shang-Gao Liao (China)</i>	IL10: Bioactive phytochemicals from shoots and roots of <i>Salvia</i> species <i>Amir Reza Jassbi (Iran)</i>
17:45 – 18:10	SL14: Studies on the proximate analysis, minerals, antinutritional factors and <i>in vitro</i> anthelmintic effects of <i>Moringa oleifera</i> leaves (FHI 109897) on bovine helminth eggs <i>Kehinde O. Soetan (Nigeria)</i>	IL11: Exploring for new secondary metabolites with pharmacological potential from the Mangroves of southern coast of China <i>Yuewei Guo (China)</i>
18:10– 18:30	SL15: Evaluation of Chinese rice wine on the antioxidant activities in D-galactose induced aging mice <i>Yue Huang (China)</i>	SL16: Biologically active compounds from hops and their prospects <i>Pavel Dostálek (Czech Republic)</i>
18:30– 18:50		SL17: Phytochemicals in fruits and vegetables <i>Shafika Abdelhamid Zaki (Egypt)</i>
19:00 – 21:00	Welcome Reception	

	27 June, Saturday
From 07:00	Registration
08:00 –09:45	Plenary Session 2 (Room 1) <i>Moderators: Satya Sarker, Franz Bucar</i>
08:00 – 08:35	PL4: Pharmacokinetics and cellular effects of a French maritime pine bark extract in humans <i>Petra Högger (Germany)</i>
08:35 – 09:10	PL5: Sphingolipidomic and cyclopeptidomic studies on <i>Cordyceps sinensis</i> <i>Zhi-Hong Jiang (Macau)</i>
09:10 – 09:45	PL6: Anthocyanins prevent atherosclerotic cardiovascular diseases – basic and applied studies <i>Wenhua Ling (China)</i>
09:45 – 10:15	Refreshment Break

10:15 – 12:25	Session 7 (Room 1): Isolation/Structure elucidation of natural products <i>Moderators: Milen I. Georgiev, Evelyn Wolfram</i>	Session 8 (Room 3): Bioseparations and downstream processing of natural products <i>Moderators: Francisco A. Macías, Jianbo Xiao</i>
10:15 – 10:40	IL12: Pharmaceutically important phenylethanoids: Isolation, bioactivities and biosynthesis <i>Milen I. Georgiev (Bulgaria)</i>	IL13: Suggestions on enhancing functional properties of phytochemicals <i>Inwook Choi (Republic of Korea)</i>
10:40 – 11:00	SL18: Counter-current chromatography as a modern and efficient tool for separation on biologically active compounds from plant matrix <i>Krystyna Skalicka-Woźniak (Poland)</i>	SL22: Extraction of α-humulene-enriched oils from clove by ultrasound-assisted supercritical carbon dioxide extraction and studies of its solubility <i>Ming Chi Wei (Taiwan)</i>
11:00 –11:20	SL19: Phytochemical products development and regulation for the global market including US, EU and Asia <i>Hua Deng (USA)</i>	SL23: The influence of a modifier on the recovery of bioactive aglycone from chamomile by superheated water <i>Aleksandra Cvetanović (Serbia)</i>
11:20 – 11:40	SL20: Quantitative TD-GC-ToF MS analysis of volatile compounds in a controlled-flow headspace extract from cured <i>Vanilla planifolia</i> pods <i>S. M. Gardiner (United Kingdom)</i>	SL24: Supercritical carbon dioxide extraction of ursolic acid from <i>Rabdosiarubescens</i> assisted by ultrasound and its solubility determination. <i>Yu-Chiao Yang (Taiwan)</i>
11:40 – 12:00	SL21: Bioactive guided fraction, isolation and characterization of antitumor phyto-constituents from <i>Aphanamixix polystachya</i> <i>Shailendra Gurav (India)</i>	SL25: Effects of packaging methods and storage time on anthocyanin contents in Thai Jao Hom Nin rice <i>Orranuch Norkaew (Thailand)</i>
12:00 – 13:30	Lunch	
13:30 – 15:40	Session 9 (Room 1): Biotechnology and bioprocess engineering for phytochemicals production <i>Moderator: Xiao-Hong Yu, Tatiana V. Matveeva</i>	Session 10 (Room 3): Natural antioxidants <i>Moderator: Chin-Kun Wang, Maurizio Battino</i>
13:30 – 13:55	IL14: Progress towards engineering high levels of unusual fatty acid nutraceuticals in plants <i>Xiao-Hong Yu (USA)</i>	IL15: The healthy effects of strawberry polyphenols: which strategy behind antioxidant activity? <i>Maurizio Battino (Italy)</i>
13:55 – 14:20	SL26: Influence of <i>Agrobacterium</i> oncogenes on secondary metabolism of plants: naturally transgenic <i>Linaria</i> plants as a new model for investigation <i>Tatiana V. Matveeva (Russia)</i>	SL31: Comprehensive evaluation of antioxidant activity of foods based on structure change of a target protein <i>Masaaki Terashima (Japan)</i>
14:20 – 14:40	SL27: Enhanced diterpenetanshinone accumulation and anti-cancer activity of transgenic <i>Salvia miltiorrhiza</i> hairy roots by pathway engineering <i>Guoyin Kai (China)</i>	SL32: Flavonoid profiles, antioxidant potential, and acetylcholinesterase inhibition activity of the extract from archeogoniophore of <i>Marchantia polymorpha</i> <i>Xin Wang (China)</i>

14:40 – 15:00	SL28: Production of ginsenoside F2 using Lactococcuslactis with improved expression of β-glucosidase from <i>Paenibacillus mucilaginosus</i> <i>Ling Li (Republic of Korea)</i>	SL33: Potential of <i>Brosimum alicastrum</i> as edible plant by its contents in phytochemicals and phytohormones and evaluation of antioxidant activities <i>Marcos Soto-Hernández (México)</i>
15:00 – 15:20	SL29: Manipulation of 3-hydroxy-3-methylglutaryl coenzyme A synthase for health-promoting isoprenoid production <i>Pan Liao (Hong Kong)</i>	SL34: Hypolipidemic effect of tetramethylpyrazine on HepG2 cells <i>Jicheng Chen (China)</i>
15:20 – 15:40	SL30: Production of phenolic acids from cereals using lactic acid bacteria <i>Wooha Joo (Republic of Korea)</i>	SL35: Stability of polyphenols under the cell culture conditions: Avoiding erroneous conclusions <i>Jianbo Xiao (China)</i>
15:40 – 16:40	Refreshment Break / Poster section 2(No. 61 -) (Room 5)	
16:40 – 18:10	Session 11 (Room 1): Carbohydrate Polymers <i>Moderator: Mingyong Xie, Satya Sarker</i>	Session 12 (Room 3): Pharmacokinetics of phytochemicals <i>Moderator: Petra Högger, Mei Han</i>
16:40 – 17:05	IL16: The structural characteristic and bioactivity of polysaccharide from <i>Dendrobium officinale</i> <i>Shao-ping Nie (China)</i>	IL17: Chemical composition, influence of digestion and biofunctional properties of green tea and GABA tea (<i>Camellia sinensis</i> L.) <i>Maria Daglia (Italy)</i>
17:05 – 17:30	SL36: Structural characteristics and physicochemical properties of lotus seed resistant starch prepared by different methods <i>Yi Zhang (China)</i>	SL39: Antithrombotic activity of oral administered low molecular weight fucoidan from <i>Laminaria Japonica</i> and possible mechanism <i>Xue Zhao (China)</i>
17:30 – 17:50	SL37: Isolation and structural characterization of pectic compounds from fruits of quince (<i>Cydonia oblonga</i>) and Chinese quince (<i>Pseudocydonia sinensis</i>) <i>Roman Bleha (Czech Republic)</i>	SL40: Snow lotus herb's investigation: pharmacokinetics study of <i>Saussurea laniceps</i> <i>Jia-yi Fan (Hong Kong)</i>
17:50 – 18:10	SL38: Structural and physicochemical properties of lotus seed starch treated with ultra-high pressure <i>Zebin Guo (China)</i>	SL41: The interactions between green tea and anticancer drug paclitaxel on pharmacodynamics <i>in vitro</i> and <i>in vivo</i> <i>Na Du (China)</i>
18:10 – 20:00	Dinner	

	28 June, Sunday	
From 07:00	Registration	
08:00 – 10:15	Plenary Session 3 (Room 1) <i>Moderator: Francisco A. Macías, Mei Han</i>	
08:00 – 08:35	PL7: Study on the structure and bioactivity of polysaccharides from food resources <i>Mingyong Xie (China)</i>	
08:35 – 09:10	PL8: Bioactive phytochemicals in spices and herbs in the development of pharmaceuticals and nutraceuticals <i>Young-Joon Surh (Republic of Korea)</i>	
09:10 – 09:45	PL9: A journey of a phytochemist - witnessing the changing face of phytochemical research <i>Satya Sarker (United Kingdom)</i>	
09:45 – 10:05	Refreshment Break	
10:05 – 11:35	Session 13 (Room 1): Bioactivity of natural products (II) <i>Moderator: Zhi-Hong Jiang, Maria Daglia</i>	Session 14 (Room 3): Cardioprotection with phytochemicals <i>Moderators: Yoshitaka Takahashi, Thomas Netticadan</i>
10:05 – 10:30	IL18: Piceatannol induces Nrf2-mediated antioxidant gene expression and inhibits NF-κB-mediated pro-inflammatory gene expression in human mammary epithelial cells <i>Hye-Kyung Na (Republic of Korea)</i>	IL19: Cardioprotection with polyphenols <i>Thomas Netticadan (Canada)</i>
10:30 – 10:55	SL42: Effect of whole coffee fruit extract phytochemicals on antiradical activity and BDNF in healthy subjects <i>Boris Nemzer (USA)</i>	SL45: Combinations of genistein, EGCG and/or resveratrol synergistically inhibit pre-adipocyte differentiation by suppressing PPAR-γ/C/EBP-α pathway <i>Hongwei Si (USA)</i>
10:55 – 11:15	SL43: Effect of polysaccharides from <i>Tremella fuciformis</i> on UV-induced photoaging <i>Lijun You (China)</i>	SL46: Novel anti-inflammatory daucane esters from <i>Laserpitium zernyi</i> Hayek (Apiaceae) <i>Višnja Popović (Belgium)</i>
11:15 – 11:35	SL44: ILG suppresses human T lymphocyte activation via modulation of cysteine 46 of IκBα kinase <i>Ting Li (Macau)</i>	SL47: Squalene improves lipid and glucose metabolism in high-fat-fed rats by activating peroxisome proliferator-activated receptor alpha <i>Minh Hien Hoang (Vietnam)</i>
11:35 – 12:20	Lunch	
12:20 – 20:00	Excursion	
20:00 – 23:00	Gala dinner	

29 June, Monday		
08:30 – 10:15	Plenary Session 4 (Room 1) <i>Moderator: Young-Joon Surh, Petra Högger</i>	
08:30 – 09:05	PL10: Inhibition of atherogenic 12-lipoxygenase activity by tea extracts <i>Yoshitaka Takahashi (Japan)</i>	
09:05 – 09:40	PL11: The role of flavonoids in the prevention of degenerative diseases <i>Didem Şöhretoğlu & Randolph Arroo (United Kingdom)</i>	
09:40 – 10:15	PL12: Biological function of acetic acid-Improvement of obesity and glucose tolerance by acetic acid in type 2 diabetic rats <i>Hiromi Yamashita (Japan)</i>	
10:15 – 10:35	Refreshment Break	
10:35 – 12:25	Session 15 (Room 1): Anti-cancer/Cancer prevention <i>Moderator: Young-Joon Surh, Marc Diederich</i>	Session 16 (Room 3): Functional phytochemicals in food <i>Moderator: Hiromi Yamashita, Randolph Arroo</i>
10:35 – 11:00	IL20: Crosstalk between cell death modalities as a pharmacological target <i>Marc Diederich (Republic of Korea)</i>	SL52: Polysaccharides with immunomodulating activity from <i>Codonopsis pilosula</i> roots <i>Yuan-Feng Zou (China & Norway)</i>
11:00 – 11:20	SL48: Phytochemical compounds from <i>Olea Europaea</i> leaf extracts have anti-pancreatic cancer activity <i>Chloe D. Goldsmith (Australia)</i>	SL53: Phyto-constituents investigation and <i>in-vivo</i> hair growth promotion of the leaf of <i>Hibiscus Rosa sinensis</i> plant <i>Olayinka Taiwo Asekun (Nigeria)</i>
11:20 – 11:40	SL49: Aquatic extract of Canadian manufactured phytoplankton significantly reduces clonogenic and metastatic activities in various cancer cells <i>Hongwei Cheng (Canada)</i>	SL54: Progress in natural products from tea and other food related materials <i>Guan-hu Bao (China)</i>
11:40 – 12:00	SL50: Development of inhalable curcumin nanoparticle for lung cancer treatment <i>Wing-Hin Lee (Australia)</i>	SL55: Colored mulberry fruit extract as a potential natural colorant <i>Shivraj Hariram Nile (Republic of Korea)</i>
12:00 – 12:20	SL51: Screening of Traditional Chinese Medicines for SIRT5 inhibitors as potential therapeutic agents in cancer <i>Bin He (China)</i>	
12:20 – 12:40	Awards and Grants Closing ceremony	

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PL: Plenary Lectures

PL2: Antiaging - from discovery to clinical trials

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ABSTRACT

Objective: Aging is a natural progress of life for human beings. Potential antiaging materials were well focused from the past till now. Green tea, tea polyphenols containing milk, roselle, chlorella, marigold flower and Si Wu Tang (traditional Chinese herb) were assayed for antiaging activity from animal model to human clinical, respectively.

Methods: SAM 8 mice were used to evaluate the function of the materials on longevity, memory, biochemical parameters and physical performance firstly. After transferring the dosage for human beings, a randomized, placebo-controlled, double-blind and crossover design was employed for each material. Elderly (older than 65 years) were involved in each clinical trial. The total study period was 13 months for each trial. In each trial, all subjects were assigned into two groups, one group was administered testing material in the first six months and placebo in the next six months with one month wash-out period in between; at the same time, the other group initially received placebo and then switched to the testing material.

Results: All enrolled materials showed a positive antiaging effects in SAM 8 mice, especially the life-span and some specific functions. All subsequent human clinical trials clearly indicated health benefits on the liver (green tea, chlorella), skin (Si Wu Tang, tea polyphenols containing milk and roselle), and eyesight (marigold flower). In addition, all antiaging materials significantly increased the antioxidative capacity within the human body and showed perfect antioxidant enzyme activity (SOD, GPx, GR and GST).

Conclusion: Antiaging effects from discovery to clinical trial clearly indicated that some natural materials have positive properties.

PL3: Phytochemicals: Potential sources of new antiinfectives and psychoactives

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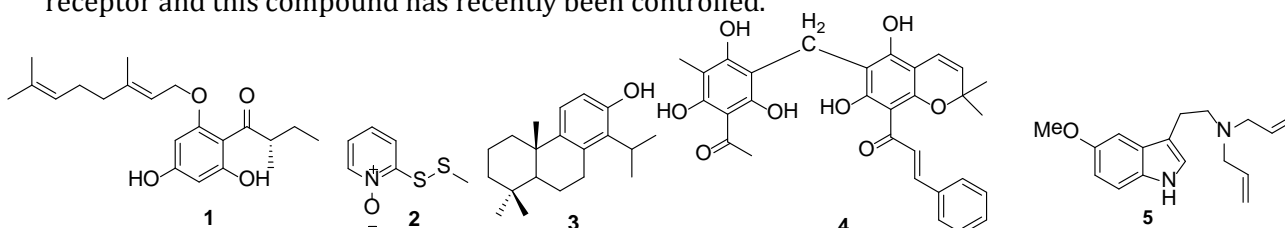
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ABSTRACT

Objectives: At present there is a pressing need for new classes of antibacterial to deal with multidrug-resistant (MDR) strains of clinically-relevant bacteria, which include methicillin-resistant *Staphylococcus aureus* (MRSA), extensively-drug-resistant *Mycobacterium tuberculosis* (XDR-TB) and the plethora of MDR Gram-negative bacteria including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Plants offer considerable opportunities in this area for new chemical scaffolds for antibacterials, resistance-modifying agents and plasmid-transfer inhibitors and we have screened a number of taxa for these properties. We are also evaluating Novel Psychoactive Substances (NPS), as some of these materials are modelled on existing and controlled natural product drugs of abuse and are becoming highly problematic globally.

Methods: Bioassay-guided fractionation of extracts from taxa such as *Hypericum* and *Allium*, followed by structure elucidation, has afforded a variety of antibacterial and resistance-modifying agents. Purchases from the internet of NPS have also provided access to chemistry which was evaluated using the Psychoactive Drug Screening Program (PDSP), run by Professor Bryan Roth at the University of North Carolina.

Results: Results included the anti-MRSA acylphloroglucinol (1) from *Hypericum olympicum*, 1 (minimum inhibitory concentration (MIC) = 2 mg/L) and anti-TB natural products such as the simple sulphur-containing alkaloid 2 from *Allium stipitatum* (MIC = 0.5 mg/L).² Work on efflux inhibitors has afforded 3,³ and an inhibitor of plasmid transfer between bacteria, has been characterised (rottlerin (4)). Evaluation of some synthetic tryptamine NPS included 5-methoxy-N,N-diallyl-tryptamine (5)⁴, which is a potent hallucinogen and an activator of the 5-HT_{2B} receptor and this compound has recently been controlled.



Conclusions: Phytochemicals demonstrate appreciable levels of activity against clinically-relevant strains of bacteria and in some cases the selectivity of these compounds warrants their further evaluation. NPS continue to be highly problematic and the chemists who produce these materials have opportunities to readily circumvent legislation. Consequently, new international control measures are needed to protect the general public from these harmful materials.

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PL4: Pharmacokinetics and cellular effects of a French maritime pine bark extract in humans

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ABSTRACT

The standardized extract of the French maritime pine *Pinus pinaster Ait.* (Pycnogenol®) is rich in procyanidins comprising of catechin and epicatechin subunits and also contains various polyphenol monomers, phenolic or cinnamic acids and their glycosides. It is monographed as a food supplement in the United States Pharmacopoeia (USP). In clinical studies, the intake of this pine bark extract has been shown to entail various beneficial effects, e.g. anti-inflammatory, anti-diabetic or cardiovascular effects.

Objective: To identify the background of the pharmacologic activity of Pycnogenol, it is necessary to determine which compounds are absorbed from the gastrointestinal tract after oral intake, how they distribute in the body and which cellular effects they exhibit.

Methods: Healthy volunteers and patients with severe osteoarthritis ingested daily doses of 200 or 300 mg of Pycnogenol. Blood samples and, in case of the patients, also synovial fluid samples were obtained and analyzed by liquid chromatography.

Results: In plasma samples of healthy humans highest concentrations of catechin, followed by taxifolin, caffeic and ferulic acid were detected. Additionally, a metabolite that is produced from catechin units by gut microbia, δ -(3,4-dihydroxy-phenyl) γ -valerolactone (M1), has been found in the plasma samples. M1 displayed a higher *in vitro* bioactivity than its metabolic precursor. The observation that M1 concentrations required to exert an effect *in vitro* were significantly higher than measured *in vivo* levels prompted further investigations. Blood and endothelial cells were identified as compartments in which M1 accumulated *in vitro*. Analysis of concentrations of M1 and other polyphenols in serum, blood cell and synovial fluid samples of patients with severe osteoarthritis confirmed an individual preferential distribution of polyphenols into these specimen. Moreover, the intake of Pycnogenol decreased various markers of cartilage destruction *in vivo*.

Conclusion: Constituents and metabolites of the maritime pine bark extract Pycnogenol are also distributed into compartments other than serum, e.g. the synovial fluid, which helps explaining its beneficial effects in osteoarthritis. Generally it can be concluded that analysis of serum samples might underestimate the true concentrations of polyphenols being present *in vivo*.

PL9: A journey of a phytochemist - witnessing the changing face of phytochemical research

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ABSTRACT

Since the introduction of the unique branch of chemistry, called 'phytochemistry', it has remarkably evolved through the advent of modern chromatographic, spectroscopic, bioassay and computational techniques.

Objective: The objective of this communication is to capture the changes in phytochemical research that the authors have witnessed and experienced since late 1980's.

Methods: Review of authors' more than 380 own publications, and also related literature available through Web of Science, Science Direct and Google Scholar.

Results: Medicinal properties of plants have been known to humans for thousands of years. Initially, a genuine curiosity for understanding why certain plants possess medicinal properties led to some of the earliest phytochemical studies, and the discovery of well-known drugs like, morphine, quinine, reserpine, atropine, and several others. Later, phytochemical work had been very much driven by the sheer excitement and quest of the chemists for identifying new chemical entities, irrespective of bioactivities, and understanding the chemistry of plants. This formed a new distinct branch of chemistry, called phytochemistry, which mainly deals with the chemistry and biochemistry of secondary metabolites of plant origins. Working with medicinal plants to identify medicinal compounds has never stopped as new drug-like molecules have continued to be discovered from medicinal plants. The advent of various separation and detection technologies further intensified the effective isolation and identification of various phytochemicals, and a new avenue in phytochemical research, called chemotaxonomy, emerged to classify plants on the basis of their chemistry. Whilst the popularity of chemotaxonomical work started to die down, the main focus of phytochemical research began to shift towards bioassay-guided isolation of active compounds from plants, and has become one of the main sources of new chemical entities for modern drug discovery process. During the evolution of 'combinatorial chemistry', a new approach for creating dereplicated natural products (phytochemical) libraries to feed into the high-throughput-screening (HTS) was incorporated. Most recently, a more holistic and multidisciplinary approach in phytochemical research, known as 'plant metabolomics', has been developed.

Conclusions: The face of phytochemistry has been ever changing to meet the demands of modern science and to cope with the advancements of technologies applicable to phytochemical research.

PL10: Inhibition of atherogenic 12-lipoxygenase activity by tea extracts

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ABSTRACT

Objectives: Leukocyte-type 12-lipoxygenase is the enzyme producing hydroperoxy acids, and oxygenates not only free fatty acids but also esterified fatty acids in the low-density lipoprotein (LDL) particle. Oxidative modification of LDL is the first key step for the development of atherosclerosis and the role of leukocyte-type 12-lipoxygenase in this process was established using leukocyte-type 12-lipoxygenase-knockout mice. In the present study we examined whether tea extracts which have been reported to show effects on inflammation and cardiovascular disease contained components inhibiting the leukocyte-type 12-lipoxygenase activity.

Methods: Guava leaves or Chinese teas were extracted with 50% ethanol in water. Inhibition of the catalytic activity of leukocyte-type 12-lipoxygenase was evaluated with the partially purified enzyme. The enzyme-expressing macrophage-like cells were incubated with LDL in the presence of extracts, and oxidized LDL in the medium that was quantified. We fractionated the extracts by reverse-phase HPLC. The structure of the isolated components showing potent inhibitory activity of leukocyte-type 12-lipoxygenase was analyzed by electrospray ionization mass spectrometry, ¹H-NMR and ¹³C-NMR.

Results: Guava leaf extracts inhibited not only leukocyte-type 12-lipoxygenase activity but also LDL oxidation. Oral administration of guava leaf extracts to apoE-knockout mice significantly reduced the area of atherogenic lesions. The major components inhibiting leukocyte-type 12-lipoxygenase were identified as ethyl gallate and quercetin (1). Among 12 extracts from Chinese teas that were tested, the extracts from Qing Shan Lu Shui showed potent inhibitory activity of leukocyte-type 12-lipoxygenase and contained the lowest amount of catechins known to inhibit lipoxygenases. The active components contained in the extracts were identified as ligulosides A and B, novel monoterpenoid glycosides (2).

Conclusions: The antiatherogenic effect of tea extracts might be attributed to the inhibition of leukocyte-type 12-lipoxygenase activity.

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PL12: Biological function of acetic acid-Improvement of obesity and glucose tolerance by acetic acid in type 2 diabetic rats

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ABSTRACT

Objective: Previously we found out that exogenous acetic acid incorporated into cultured hepatocytes has been found to have an inhibitory effect on the activity of the carbohydrate-responsive element-binding protein (ChREBP) that regulates several genes required for the conversion of glucose to fatty acids in the liver. The aim of this study was to investigate whether an oral administration of acetic acid would contribute to reducing lipogenic genes and protecting against obesity.

Methods: Five-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats were randomly assigned to two groups: water-injected and acetic acid-injected. The water-injected group was given distilled water at 5 ml/kg of body weight, and the acetic acid-injected group was given 52 mg/kg BW (1 % (v/v) acetic acid of 5 ml/kg of body weight) daily 5 days a week for 6 months. At 31 weeks of age, an oral glucose tolerance test was performed, and at 32 weeks of age, the rats were anesthetized by an intraperitoneal injection of Nembutal. The liver was immediately isolated, weighed, frozen in liquid nitrogen and stored at -80°C for the subsequent isolation of RNA, and then analyzed by Northern blotting. A part of the liver of each rat was subjected to a histochemical analysis, western blotting, and nucleotides analyses. Each data value is presented as the mean \pm SE. The water-administered OLETF rats and LETO rats, as well as the water-administered OLETF rats and acetic acid-administered OLETF rats were respectively compared by an unpaired Student's test.

Results: Orally administered acetic acid was absorbed by tissues and activated AMP-activated protein kinase (AMPK) by increasing the AMP/ATP ratio. Treatment with acetic acid showed a marked reduction in lipid accumulation in the adipose tissue, protection against accumulation of fat in the liver, and improved glucose tolerance in obesity-linked type 2 diabetic OLETF rats. It decreased the transcripts of the lipogenic genes in the liver. Furthermore, acetic acid treatment showed a higher rate of oxygen consumption and a smaller size of lipid droplets in white and brown adipose tissues.

Conclusion: On the basis of those results, exogenous acetic acid is a potential compound to improving obesity and obesity-linked type 2 diabetes.

IL: Invited Lectures

IL2: Stilbene derivatives from *Pinus cembra* L. bark and their cytotoxic effects on HeLa cells

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ABSTRACT

Pinus cembra L. (cembran pine) is widespread in the Alps and Carpathian Mountains. The species is considered to be a glacial relict and it is resistant to high altitude stress factors (low temperatures, high levels of ozone and UV radiation). The species has been poorly investigated despite its stress resistance properties.

Objective: This study aimed to isolate compounds from the bark of *Pinus cembra* L. with potential antiproliferative effects on HeLa cells.

Methods: The hydromethanolic extract of cembran pine bark was subjected to solvent fractionation to obtain diethyl ether, ethyl acetate, *n*-butanol and aqueous fractions. The ethyl acetate fraction was purified by CC over polyamide 6 and preparative RP-HPLC to yield compounds 1 and 2. The structures of the isolated compounds were elucidated by NMR (¹H-NMR, ¹³C-NMR, 2D-COSY) and MS analysis (FTMS). Their effects on HeLa cells were evaluated by spectrophotometric and flow cytometric methods. In this respect, protein quantification, proliferation, viability, apoptosis and cell cycle assays were performed.

Results: Spectral analysis showed the presence of a stilbene type structural moiety in the isolated compounds: compound 1: *trans*-3,5,4'-trihydroxystilbene-4'-O-β-D-glucopyranoside, compound 2: *trans*-3-methoxy-5-hydroxystilbene-4'-O-β-D-glucopyranoside. The compounds dose-dependently reduced protein synthesis, proliferation and viability of HeLa cells. At 100 μg/mL, both compounds almost completely inhibited protein synthesis: 97.46±0.18% (*p*< 0.001) inhibition for compound 1 and 97.95±0.10% (*p*< 0.001) inhibition for compound 2. At the same concentration, compounds 1 and 2 increased the percentage of dead HeLa cells to 61.28±7.39% (*p*< 0.01) and 59.16±2.93% (*p*< 0.001), respectively and the proportion of sub-G1 phase cells to 89.36±0.56% (*p*< 0.001) and 89.71±0.59% (*p*< 0.001), respectively. Elucidation of the mechanisms responsible for the cytotoxic activity is in progress.

Conclusion: This is the first report on stilbene derivatives occurring in *Pinus cembra* L. bark that induce cytotoxic effects on HeLa cells.

IL3: Prevention of phytochemicals on the toxicity of neo-formed compounds (NFM) during food processing: a review

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ABSTRACT

In recent years, neo-formed compounds (NFCs) during food processing have attracted the attention of the scientific community since many of them exhibit diverse toxicity such as neurotoxicity, genotoxicity, potentially carcinogenic and reproductive toxicity. Acrylamide, benzo(a)pyrene and heterocyclic aromatic amines are the main types of NFCs during heating foods enriched with carbohydrate, fats and proteins. In recent years, a number of papers have been published that focused on the evaluation of various phytochemicals in alleviation of their related toxicities both in vitro and vivo.

Objective: This review aimed at providing evidence on the prevention of phytochemicals against the diverse toxicity induced by three NFCs.

Methods: The proposed mechanisms of the prevention of phytochemicals on the toxicity of three NFCs during food processing were summarized.

Results: Three potential aspects involving excellent antioxidant activity, DNA prevention function and enzyme induction contribute to the successful protection mechanism. For the excellent antioxidant capacity, phytochemicals can improve antioxidant defense by directly scavenging ROS, increasing the oxygen-radical absorbing capacity of cells, reducing the formation of oxidative damage in cells as well as altering the apoptosis/proliferation-related signaling pathways. Meanwhile, they achieve DNA protection mainly through blockage of metabolic activation and DNA binding of carcinogens, repair of DNA damage, reduction of oxidative DNA damage, stimulation of detoxification and lowering the formation of DNA adducts. In addition, phytochemicals reduce phase I enzymes to avoid carcinogenesis activation, and then induce phase II enzymes which may inactivate or accelerate excretion of NFCs activated by phase I enzymes, therefore inhibiting possible further damage by the NFCs. Finally, the limitations from existing knowledge have been illustrated and the possible perspective for further studies has also been considered.

Conclusion: The information from this review provides an easier and better way to improve human health when considering the possibility of using foods enriched with phytochemicals for prevention of the toxicity of exogenous pollutants.

IL4: Investigation of effect mechanisms and clinical study of polysaccharide of *Dendrobium officinale* in alleviating cigarette-induced COPD

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ABSTRACT

Objective: Cigarette smoke (CS) is a potent pro-inflammatory stimulus and a major risk factor of chronic obstructive pulmonary disease (COPD), which is characterized by chronic inflammation with a progressive and irreversible airflow limitation. *Dendrobium officinale* polysaccharides (DOP) exhibited anti-inflammatory and body fluid promotion activities. Therefore, the effects of DOP on patients with CS induced COPD were investigated.

Methods: 40 patients with CS induced COPD were treated with placebo or DOP for 30 days. Assessments included spirometry, serum and bronchoalveolar lavage fluid pro-inflammatory mediators, and immunohistochemistry analysis.

Results: Lung functions index including forced expiratory volume in one second (FEV1)/ forced vital capacity (FVC) (%), FEV1% predicted data, V50% predicted data, and V25% predicted data were significantly increased after DOP intervention. Pro-inflammatory mediators including interleukin-6, interleukin-8, C reactive protein and tumor necrosis factor- α were also remarkably reduced both in the serum and bronchoalveolar lavage fluid of the patients. Interestingly, DOP could markedly increase aquaporin-5 protein expression and decreased mucin-5AC protein expression in the airway submucosal glands. *In vitro* studies confirmed the up-regulation of aquaporin-5 protein and gene expressions and down-regulation of mucin-5AC protein and gene expressions by DOP.

Conclusion: Altogether, our results for the first time showed that DOP could improve lung functions and alleviate lung inflammation, while up-regulating aquaporin-5 and down-regulating mucin-5AC expressions in patients with CS induced COPD. Therefore, DOP is a potential therapeutic for patients with CS induced COPD.

IL6: Enzymatic bioautography on HPTLC: combined phytochemical and activity screening tool for medicinal plants quality assessment and *in vitro* cultivation bioprocess control

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ABSTRACT

High Performance Thin Layer Chromatography (HPTLC) is an analytical tool of long term tradition in quality control of medicinal plant extracts and other natural multi compound mixtures. It is a rapid and standardizable technique, which delivers an informative picture of separated compounds as a result, which are fixed on the solid silica phase of the plate – like a compound library. By direct performance of visualizable enzyme reactions on the plate, this compound library can also be used for activity screening, called enzymatic bioautography. Similarities and differences of phytochemical and activity fingerprints can be compared for quality control purposes as well as for bioprocess control for *in vitro* medicinal plant cultivation.

Objective: The application of direct coupling of enzymatic assays and phytochemical fingerprint analysis by HPTLC shall be demonstrated. Assays procedures are optimized and applied for screening of medicinal plants extracts and fractions from conventional and *in vitro* cultivation.

Methods: HPTLC standardized fingerprint analysis by automated equipment (CAMAG, Muttenz) and HPTLC plates (Merck, Darmstadt). Derivatization by Neu's and PEG reagent. Detection at different wavelength. Optimization of published methods: Xanthine Oxidase (XOD) assay by Ramallo 2011; Lipase assay by Hassan 2011. Acetylcholinesterase (AchE) assay Marston et al 2002.

Results: When attempts to reproduce bioautographic enzyme assays from literature failed, extended optimization experiments resulted in validated, robust procedures. Screening of less studied medicinal plant species from the Balkan region from conventionally grown and *in vitro* cultivated origin showed the following results:

	XOD	Lipase	AchE
<i>Hypericum</i> sp.	+ (1-2)	-	(+)
<i>Pulsatilla</i> sp.	+ (2-4)	+ (1)	(+)
<i>Inula britannica</i>	+ (1-2)	(+)	-
<i>Sideritis scardica</i> .	-	+ (1)	+ (2-4)
<i>Artemisia alba</i>	+ (1)	-	-
<i>Clinopodium vulgare</i>	+ (2-3)	++ (1)	+(1)

Legend: + inhibition, (+) faint inhibition, – no visual inhibition, (number) number of inhibition zones

Conclusion: Bioautography offers a rapid and simple tool for screening of secondary metabolite profiles of medicinal plant biomass and derived products by HPTLC combined with screening of potential health beneficial activities. Direct coupling with MS detection (HPTLC-MS Interface or HPTLC-MALDI-TOF) could yield rapid additional mass information of the active molecule.

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IL7: Starting from Danube Delta folk medicine data to novel nutraceuticals

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ABSTRACT

The study includes folk medicine data from locals of the Danube Delta area in Romania, who use many medicinal plant species as remedies for various ailments. We have studied 50 plant species for which folk medicine data were collected after ethnomedicinal surveys conducted in 15 villages in the area. Three species were selected and phytochemically and biologically investigated.

Objective: The research focused on three species (*Typha angustifolia*, *Scirpoides holoschoenus* and *Helichrysum arenarium*), which were commonly used by locals for digestive and liver diseases. Simultaneously, we have processed data from traditional medicine on some species and analysed them in order to detect the active principles which justify the traditional uses.

Methods: Qualitative and quantitative methods were used to investigate the MAPs. We have obtained three extracts using proper solvents. The amounts of total phenolics, flavonoids, essential oils and triterpens and/or phytosterols were measured (spectrophotometry, TLC, HPLC, GC-MS). The antioxidant activity was measured by ABTS (1) and DPPH methods (2).

Results: The ear of the cattail (*Typha angustifolia*) contains 1.261 g eq. caffeic acid per 100 g and the flower of immortelle (*Helichrysum arenarium*) contain 0.677 g eq. rutoside per 100 g, fact that brings antioxidant properties to the extracts obtained from them. For example, in one of the villages from the Danube Delta – Jurilovca, the locals (Russian Lippovans) are most often using the plant species *Scirpoides holoschoenus* ("dicop" or "root of life") in the fight against alcoholism and liver disease for local anglers.

The highest antioxidant activities were measured of *Typha angustifolia* by ABTS and DPPH (281.50±5.03 and 153.81±3,44 µM Trolox g DW) and *Scirpoides holoschoenus* ABTS and DPPH (278.32±4.62 and 146.91±0.18 µM Trolox g DW).

Conclusion: After phytochemical analyses and the determination of the plant extract antioxidant capacity, it can be concluded that some of the studied species can be used in the composition of new nutraceuticals.

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IL8: Pharmacological and chemical investigations of *Polygonum capitatum* - an ethnic Miao's herb used in the treatment of urinary tract infections

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ABSTRACT

Polygonum capitatum is a medicinal plant widely used by the Miao people for the treatment of various urologic disorders including urinary tract infections (UTIs) with considerable therapeutic efficacy. Statistic data shows that *P. capitatum*-based Relinqing Granule is among the best-selling TCM preparations for UTIs. The antibacterial and anti-inflammatory activities and chemical constituents of the plant have been reported but no correlations between chemical components and pharmacological effects were reported. Besides, initial chemical analysis showed that quite a number of compounds have not yet been identified.

Objective: To evaluate the antibacterial and anti-inflammatory activities of the structure-based fractions of *P. capitatum* and to clarify the compounds responsible for its clinical use.

Methods: Water and 70% aqueous ethanol extracts were separated, respectively, into a fraction enriched in polysaccharides and proteins (PP) and four fractions enriched in gallic acid and its analogues (GAA), flavonoids (FV), tannins (TN), and triterpenoids and steroids (TS). The antibacterial activities were assessed *in vitro* by determining the MICs and MBCs. The anti-inflammatory activity was evaluated by employing the xylene-induced mouse ear edema model. A tannin-free phenolic fraction (TFPF) containing GAA and FV was subjected to UPLC-PDA-ESI-MS/MS for chemical analysis.

Results: FV and TN are the most potent anti-bacterial fractions (MICs of 0.255-10.2 and 0.375-18.75 mg/kg, respectively), and FV and TS the most potent anti-inflammatory fractions (inhibition rates of 86.15 % and 73.71 %, respectively, at 0.6 g/kg; $P < 0.01$). Fragmentation patterns of the polyphenolic glycosides present in TFPF have been established. 40 phenolics including flavonoid and polyphenolic glycosides were identified or structurally characterized in TFPF. Among the compounds, four were new and 19 were described in the plant for the first time.

Conclusion: The study suggested that the traditional use of *P. capitatum* for the treatment of UTIs was attributed to the presence of biologically active compounds, and that flavonoid and polyphenolic glycosides, which were the most typical chemical markers, might be the major principles responsible for the therapeutic effects.

IL9: Anticancer potential and antioxidant property of extracts from New Zealand seaweed *Undaria pinnatifida* – potential use in cancer treatment and cardiovascular improvement

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ABSTRACT

The brown algae *Undaria pinnatifida* is a source of biologically active phytochemicals, including a wide range of components such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, tocopherol, and phycocyanins. The major components in *U. pinnatifida* such as fucoxanthin and fucoidan have been reported to have various health benefits.

Objective: This study aimed to screen components from New Zealand grown *U. pinnatifida* for anticancer and antioxidant activities.

Methods: *U. pinnatifida* samples grown in Marlborough Sounds, New Zealand were collected. Fucoidan and fucoxanthin fractions were extracted from the samples. Antioxidant tests were carried out using DPPH and CUPRIC assays. Cytotoxicity of fractions in a number of cancer cell lines was determined by MTT assay. Then, fractions were combined with gemcitabine, and the effectiveness of combination treatment was determined.

Results: Fucoxanthin and fucoidan fractions showed strong antioxidant activities in both DPPH and CUPRIC assays. The algal fucoidans specifically suppressed the proliferation of three cancer cell lines (lung, colon and breast) with less cytotoxicity against the non-cancer cells. The extract containing fucoxanthin suppressed the proliferation of melanoma and cervix cancer cell lines with less cytotoxicity against the non-cancer cells. Fucoxanthin combined with gemcitabine showed synergy in cell killing in pancreatic cancer cell lines with no significantly increased cytotoxicity to non-cancer cell lines. Fucoidan combined with gemcitabine showed synergy in cell killing in melanoma cancer cell lines with no significantly increased cytotoxicity to non-cancer cell lines.

Conclusion: New Zealand grown *U. pinnatifida* is a promising source of phytochemicals with highly potent antioxidant activities, which may be developed into cardiovascular health products. It also contains rich phytochemicals with anticancer property, which may be developed into supplement of a combination chemotherapy to treat some types of cancer.

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IL10: Bioactive phytochemicals from shoots and roots of *Salvia* species

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ABSTRACT

Objective: The plants of the genus *Salvia* are well-known as source of biologically active natural products (NPs) isolated from their shoots (1) and roots (2) such as antimicrobials, cytotoxic and antioxidant secondary metabolites. These NPs have important ecological roles like allelopathic and antifeedant activity. In this paper we will describe the biologically active and ecologically important NPs, including terpenoids, phenolics and volatile organic compounds from different Iranian *Salvias*.

Methods: In a cytotoxic and antibacterial bioassay-guided purification and identification of natural products from different Iranian sages including: *Salvia rhytidea*, *S. hydrangea*, *S. chloroleuca*, *S. reuterana*, and *S. persepolitana*, several abietane- and labdane-type diterpenoids which were isolated by different chromatographic methods including column chromatography, thin layer chromatography and HPLC and identified by various spectroscopy techniques such as mass spectrometry and 1 and 2 D NMR spectroscopy.

Results: Sahandinone (2), 6,7-dehydroroyaleanone, 7 α -actoxyroyaleanone, and tanshinone like diterpenoids were purified from root's extracts of *S. rhytidea* and *S. hydrangea*. Furthermore, several antibacterial labdane diterpenoids such as sclareol, epoxy sclareol and other labdane diols along with β -sitosterol and its glycosides were isolated from the aerial parts of *S. chloroleuca*, *S. persepolitana* and *S. reuterana*. Two diterpenoids from the roots of *S. hydrangea* and those which were isolated from the roots of *S. rhytidea* showed high anti-cancer properties against three human cancer cell lines.

Conclusions: In this paper we compare the cytotoxic potential of the isolated compounds with each other and with standard anticancer agents. We also correlate the anti-cancer potential of the isolated compounds with their phytotoxic potential on seed germination of some selected test plants such as tobacco as a way of choosing anticancer agents.

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IL13: Suggestions on enhancing functional properties of phytochemicals

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ABSTRACT

Although, consumption of phytochemicals is known to improve health of the human body, low bioavailability and low effectiveness of their metabolites on the target tissues can limit their utilization as potent nutraceuticals.

Objective: In this study, we developed some bio-enhancers to modify absorption or hepatic metabolism of phytochemicals for the purpose of increasing their bioavailability as well as improving functional properties on target tissues.

Methods: Phytochemicals and bio-enhancers were co-treated in human hepatoma HepG2 cells, human liver microsomes, cytosols and human recombinant UGT, SULT isoforms. The production of pro-inflammatory markers such as nitric oxide (NO), prostaglandin (PG)E₂, interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α were examined in LPS-stimulated a murine macrophage RAW264.7 cells. In addition, the anti-inflammatory activity of metabolites of phytochemicals was investigated against carrageenan-induced paw edema in mice. The paw volume was measured at 1 and 3 hrs following carrageenan-induced paw edema in mice.

Results: Bio-enhancers inhibited hepatic metabolism of phytochemicals by phase II conjugating enzymes including UGT, SULT. Co-treatment of phytochemicals and bio-enhancers reduced the production of proinflammatory mediators in LPS-stimulated RAW 264.7 cells. Administration of phytochemicals and bio-enhancer also markedly attenuated paw edema formation after carrageenan injection. These results suggest that bio-enhancer plays synergistic roles in maintaining anti-inflammatory effect of phytochemicals after hepatic metabolism.

Conclusion: Our data provide a new insight on possible improvement in functional properties of phytochemicals on target cells by modifying their metabolic pathway in hepatocytes.

IL14: Progress towards engineering high levels of unusual fatty acid nutraceuticals in plants

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ABSTRACT

Objective: The low production cost of oils from crop plants make them desirable platforms for the production of unusual fatty acids with desirable health promoting, or “nutraceutical” properties.

Method: The examples include: the omega-7 fatty acids, palmitoleic acid (16:1 Δ 9c), *cis*-vaccenic acid (18:1 Δ 11c) and eicosenoic acid (20:1 Δ 13c); a family of conjugated linolenic acids (CLNAs) comprising conjugated isomers of linolenic acid (LNA); and the very long chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA, 20:5 Δ 5,8,11,14,17) and docosahexaenoic acid (DHA, 22:6 Δ 4,7,10,13,16,19). The general strategy for creating plants that accumulate significant levels of these unusual fatty acids is to introduce their biosynthetic genes into a host plant engineered to produce high levels of substrate for the heterologously expressed pathway.

Results: In this review we will describe their natural occurrence, their beneficial properties, the isolation of genes responsible for their syntheses and progress made towards achieving commercial levels of the desired fatty acids in crop plants. Detailed understanding of the form of the fatty acids i.e., as a lipid-, coenzyme A (CoA)- or acyl carrier protein (ACP)- esters and of the compartmentalization of the biosynthetic route is necessary for successful heterologous recapitulation of the pathway in a crop plant. Each of the three pathways described herein contain multiple separate steps. To identify optimal combinations of biosynthetic genes, often from disparate organisms, demonstration of feasibility was performed in model plants such as *Arabidopsis* and tobacco before final pathway assembly in a crop plant.

Conclusion: Progress to date along with new biotechnological advances will surely lead to new affordable sources of health promoting nutraceuticals.

IL16: The structural characteristic and bioactivity of polysaccharide from *Dendrobium officinale*

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ABSTRACT

It has long been demonstrated that *Dendrobium officinale* has various bioactivities. Polysaccharide, as one of the main component, plays an important role in exhibiting these activities. Therefore, it is necessary to understand the fine structure of *D. officinale* polysaccharide and discuss the relationship between structural characteristic and bioactivity.

Objective: This study aimed to reveal the structural characteristic and bioactivity of polysaccharide from *D. officinale*.

Methods: A purified polysaccharide, named Dendronan[®], was extracted and isolated from *D. officinale* herbs. Its chemical composition, molecular weight (MW) and monosaccharide composition were detected. Fourier transform infrared (FTIR) spectroscopy, methylation analysis, nuclear magnetic resonance (NMR) spectroscopy, as well as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-tof-MS) were employed to investigate the detailed structure of Dendronan[®]. Its immunomodulatory activity was studied both *in vitro* and *in vivo*, using macrophage line RAW 264.7 and immunosuppressed mice model respectively.

Results: Dendronan[®] had an MW of 312 kDa, consisted dominantly of mannose and glucose in a molar ratio of 6.9:1. Dendronan[®] was proved to be a glucomannan, consisting of 1,4- β -d-mannopyranosyl and 1,4- β -d-glucopyranosyl residues, with O-acetyl groups attached at the O-2 or O-3 position of certain mannose residues.

The bioactivity studies indicated that Dendronan[®] could enhance the cell proliferation and phagocytic ability, as well as upregulating the expression of NO, TNF- α , IL -6, IL-12 cytokine of RAW 264.7 *in vitro*. The *in vivo* study showed that Dendronan[®] could stimulate the function of spleen lymphocytes, promote the secretion of serum cytokines, immunoglobulin and serum hemolysin, improve the phagocytotic function of peritoneal macrophage, as well as protect the organ from oxidative stress in immunosuppressed mice.

Conclusion: Dendronan[®] has a favourable immunomodulatory activity. Its bioactivity has a close relationship with the structural characteristics, especially the existence of O-acetyl group.

IL17: Chemical composition, influence of digestion and biofunctional properties of green tea and GABA tea (*Camellia sinensis* L.)

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ABSTRACT

Increasing evidence suggests that consumption of tea is inversely associated with the risk of developing many diseases, especially chronic pathologies induced by oxidative stress.

Objective: The aim of this study was to evaluate the metabolic profiling, the influence of *in vitro* simulated digestion on healthy components, the *in vivo* protective activity against oxidative stress and the antidepressant-like activity on post-stroke depression of green tea and GABA green tea.

Methods: The metabolic profiling of teas was determined by RP-HPLC-UV-DAD-ESI-MSn and NMR spectroscopy. Then green tea extract was submitted to *in vitro* simulated gastroduodenal digestion and flavan-3-ols content was determined. Finally, tea extracts were submitted to *in vivo* tests to evaluate their effect on post-stroke depression.

Results: The results showed that the metabolic profiling of GABA tea is quite different in comparison with that of green tea: in GABA tea, a larger number of tannins and flavonols was detected, whereas less flavan-3-ols were identified. Metabolite analysis of digested samples showed that green tea polyphenols are stable under gastric conditions, whereas were degraded under gastroduodenal digestion. Regarding pharmacological tests, both tea extracts were effective against oxidative stress. The effect of tea extracts on mood state showed that GABA tea was more effective than green tea in the modulation of antidepressant-like symptoms.

Conclusion: This is the first report on the phytochemical characterization of GABA tea and the first attempt to demonstrate the positive effect of tea on post-stroke depression.

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IL18: Piceatannol induces Nrf2-mediated antioxidant gene expression and inhibits NF- κ B-mediated pro-inflammatory gene expression in human mammary epithelial cells

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Objective: Piceatannol (3,4,3',5'-tetrahydroxy-*trans*-stilbene, PIC), derived from the seeds of *Euphorbia lagascae*, has been reported to have chemopreventive properties. However, the underlying molecular mechanisms remain largely unresolved. As oxidative stress and inflammation are two major culprits implicated in pathogenesis of carcinogenesis, we intended to determine the antioxidant and anti-inflammatory effects of PIC.

Methods: Effects of PIC on expression of Nrf2, Keap1, heme oxygenase-1 (HO-1), cyclooxygenase-2 (COX-2) and IKK were measured by Western blot analysis in the human mammary epithelial (MCF-10A) cells with or without stimulation with 12-O-tetradecanoylphorbol-13-acetate (TPA). Antioxidant response element (ARE)-driven Nrf2 transcriptional activity in the MCF-10A cells was determined by the luciferase reporter gene assay. Nuclear localization of Nrf2 and P-p65 was measured by immunocytochemistry. To determine whether PIC induces antioxidant gene expression and/or inhibits anti-inflammatory gene expression, we transfected MCF-10A cells with Nrf2 siRNA or mutant IKK construct.

Results: PIC significantly upregulates the expression of the antioxidant enzyme HO-1 in MCF10A cells through activation of Nrf2. In addition, PIC treatment induced degradation of Keap1, which led to nuclear accumulation of Nrf2. PIC induced ARE-driven transcriptional activity and DNA binding activity of Nrf2 in MCF-10A cells. Furthermore, a chromatin immunoprecipitation assay revealed that up-regulation of HO-1 expression by PIC is mediated by direct interaction of Nrf2 to the ARE site present in the promoter region of many antioxidant genes. PIC-induced expression of HO-1 was attenuated in the MCF-10A cells harboring DN Nrf2 or siRNA-Nrf2. Likewise, DNA binding and transcriptional activities induced by PIC were blunted by dominant negative mutation or silencing of Nrf2 gene. In another experiment, PIC markedly inhibited phosphorylation and subsequent degradation of I κ B α and nuclear translocation of the phosphorylated form of p65 in the MCF-10A cells treated with the tumor promoter TPA. In addition, TPA-induced phosphorylation and catalytic activity of IKK were attenuated by PIC. This led to suppression of NF- κ B DNA binding and expression of its target protein COX-2. The inhibitory effects of PIC on NF- κ B activation and COX-2 induction were blunted in cells harboring mutant IKK β (C179A) in which cysteine 179 was replaced by alanine.

Conclusion: PIC induces Nrf-2 mediated HO-1 expression and suppresses the TPA-induced COX-2 expression by blocking IKK-NF- κ B signaling. This may protect MCF-10A cells against oxidative stress- and inflammation-associated carcinogenesis.

IL19: Cardioprotection with polyphenols

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ABSTRACT

Despite the success of existing therapies, cardiovascular disease (CVD) remains to be one of the major causes of death worldwide. Exploring new strategies to improve survival of patients with CVD is therefore of great importance; examining the potential of phytochemicals may be one such avenue. Polyphenols are a class of compounds that have received increased attention for their potential health benefits. These compounds are synthesized by plants to serve a wide variety of functions, including defense.

Methods: Over the past decade, we have examined *in vivo* the ability of the polyphenol, resveratrol, to prevent or reverse the development of abnormalities in cardiac structure and function in animal models of CVD. We also examined *in vitro* the effects of resveratrol in protecting diseased adult rat cardio myocytes. The molecular mechanisms underlying the effects of resveratrol was studied in both diseased adult rat cardio myocytes and heart tissues from the animal models of CVD.

Results: Our results showed that administration of 2.5 mg/kg/day of resveratrol was able to prevent/reverse abnormalities in cardiac structure and function in animal models of hypertension (the spontaneously hypertensive rat) and obesity/type II diabetes (high fat fed rat), as well as the myocardial infarction (coronary artery ligated rat). We also demonstrated that 30 micromolar resveratrol protected cardio myocytes against exposure to norepinephrine (NE), a potent hypertrophic and cell-death trigger.

The strong cardio protective effects observed with resveratrol led us to examine the potential of a polyphenol rich source – blueberry. We examined *in vitro* the effects of a total phenolics enriched blueberry fraction in protecting diseased adult rat cardio myocytes. Our results showed that the total phenolics enriched blueberry fraction protected cardio myocytes against exposure to NE, similar to that observed with resveratrol.

Conclusion: On the basis of the results from our studies, we conclude that polyphenols have strong cardio protective properties and may therefore have a potential in the prevention and treatment of CVD.

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SL: Short Lectures

SL1: Modulating the properties of dietary flavonoids and isoflavonoids

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ABSTRACT

Dietary isoflavonoids receive considerable attention worldwide because of their presumed health-promoting activities, including (anti)estrogenic activities.¹ Nevertheless, dietary (iso)flavonoids can also have a bitter off-taste, which should be considered when enriching foods in such substances.²

Objective: To alter the structure of dietary (iso)flavonoids and understand their structure-activity relationships with respect to estrogenic properties and bitter taste.

Methods: Various isoflavonoids were obtained by micromalting legume seeds under biotic stress.^{3,4} Extracts of seeds and (elicited) seedlings were analyzed by RP-UHPLC-PDA-MSⁿ. Various prenylated isoflavonoids were purified by LC.³ Besides, (iso)flavonoids were purified from licorice roots and purchased from various companies. The molecules obtained were tested for estrogenicity and bitterness with engineered yeast or mammalian cells.^{2,1}

Results: Over 20 (iso)flavonoids differing in skeleton, substitution with OH and OCH₃, and kind of prenylation were tested for estrogenic (EC₅₀) and anti-estrogenic effects (IC₅₀). They can induce a range of estrogenic responses, in which the configuration of the prenyl substituent plays a crucial role, as evidenced by molecular docking studies. The potency of almost 100 (iso)flavonoids (no prenyl substituents) were tested against bitter receptors hT2R14 and hT2R39.² Furthermore, a potential bitter blocker was identified.⁵ Molecular docking suggested that bitter tastants and blockers bind differently to hT2R39.

Conclusion: Emerging structure-activity relationships of (iso)flavonoids with respect to estrogenicity and bitterness show that relatively small structural modifications can have a large impact on the molecule's potency and mode of action.

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SL2: Anti-glycation and antioxidant properties of polyphenol-enriched fractions from Giant knotweeds (*Reynoutria* sp.)

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ABSTRACT:

Reynoutria japonica Houtt. is an important traditional medicinal herb in Eastern Asia (*hu zhang*) and a noxious invasive plant in Europe. Many studies have demonstrated antioxidant activity of *R. japonica* rhizome. However, little data is available to compare the antioxidant activity between *R. japonica* and similar but less common species like the *R. sachalinensis* and their inter specific hybrid *R. × bohemica*.

Objective: The aim of the study was to research antioxidant activities of rhizome and leaves of three species of *R. Reynoutria* and evaluate them for the inhibitory activity on AGEs formation *in vitro*.

Methods: Dried leaves and rhizomes were extracted and fractionated. DPPH scavenging activity and phosphomolybdenum assay of the fractions were determined. All the fractions were evaluated for the inhibitory activity on AGEs formation *in vitro*. Advanced Glycation End Products (AGEs) (Pentosidine, Vesperlysine A and B) were measured.

Results: Extracts and fractions from the rhizomes possess the strongest antioxidant activities in EtOAc extracts. EC₅₀ values for the DPPH scavenging activity of EtOAc fractions of *R. sachalinensis*, *R. japonica*, *R. ×bohemica* were 4,60 µg/mL, 6,57 µg/mL, 6,21 µg/mL, respectively. EC₅₀ for ascorbic acid was 8,60 µg/mL. Phosphomolybdenum assay revealed similar results. Leaf fractions had lower antioxidant activity than rhizomes. EC₅₀ values for the DPPH scavenging activity of diethyl ether, EtOAc, BuOH fractions were 8,30 µg/mL, 9,20 µg/mL, 9,53 µg/mL for *R. ×bohemica*, respectively and 9,16 µg/mL, 9,64 µg/mL, 10,17 µg/mL for *R. japonica*. Leaves of *R. sachalinensis* showed significantly lower activity. The strongest reduction in formation of AGEs revealed the EtOAc fraction of *R. ×bohemica* rhizome (91,27% reduction) and the EtOAc fraction of *R. japonica* rhizome (74,79% reduction), measured for Vesperlysine A i B. The strongest antioxidant EtOAc fraction of *R. sachalinensis* rhizome showed weaker reduction (63,42%). When pentosidine derivatives were measured, the strongest reduction was noticed in the EtOAc fraction of *R. japonica* leaves (79,25%) and the EtOAc fraction of *R. x bohemica* leaves 76,02%.

Conclusion: The EtOAc fraction of rhizomes showed the strongest antioxidant activity which may contribute to the strong reduction in formation of AGEs. However, the bioactivity of leaf constituents need further studies.

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SL3: Phenolic-rich water fraction of edible medicinal fern *Stenochlaena palustris* is a potent antilglucosidase and antioxidant agent

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ABSTRACT

Alpha-glucosidase is a therapeutic target in the management of type 2 diabetes. Commonly used antilglucosidase drugs have side effects. Thus there is considerable interest in searching for alternative antilglucosidase agents from food and natural sources.

Objective: We aimed at isolating a potent solvent fraction by exhibiting concurrent antilglucosidase and antioxidant activities from *Stenochlaena palustris*.

Methods: Antilglucosidase and DPPH scavenging activities as well as ferric reducing antioxidant power (FRAP) of hexane, chloroform, ethyl acetate, methanol (ME), and water extracts of *S. palustris* were evaluated. ME was partitioned into chloroform, ethyl acetate, butanol and water fractions (WF). Antilglucosidase and antioxidant activities of fractions were determined. Enzyme kinetic study was performed to pinpoint the mode of glucosidase inhibition exerted by WF. WF was also tested for anti-amylase, superoxide and hydrogen peroxide scavenging, and copper chelating activities. Total phenolic, flavonoid, hydroxycinnamic acid and proanthocyanidin contents were determined.

Results: ME had the strongest antilglucosidase and antioxidant activities. Among ME solvent fractions, WF had the highest antilglucosidase and antioxidant activities. EC₅₀ values for antilglucosidase activity of WF and quercetin (positive control) were 2.9 and 114.1 µg/mL. EC₅₀ values for DPPH scavenging activity of WF and quercetin were 7.7 and 6.3 µg/mL. FRAP value of WF was 9749 µmol Fe²⁺/g. Lineweaver-Burk plot analysis revealed that WF inhibited alpha-glucosidase competitively. WF had weak anti-amylase (EC₅₀ 2386 µg/mL) and moderate copper chelating (EC₅₀ 436 µg/mL) activities. Notably, WF (EC₅₀ 220 µg/mL) was equally as strong as quercetin (EC₅₀ 199 µg/mL) as superoxide scavenger. WF (EC₅₀ 364 µg/mL) was stronger than gallic acid (EC₅₀ 838 µg/mL) as hydrogen peroxide scavenger. Phytochemical analysis found WF to have the highest total phenolic content (62% by weight) and hydroxycinnamic acid content (15% by weight) among all extracts and fractions. Total phenolic contents correlated positively with DPPH scavenging activity ($R^2 = 0.76$, $p < 0.05$) and FRAP ($R^2 = 0.96$, $p < 0.05$). Total hydroxycinnamic acid contents correlated positively with antilglucosidase activity ($R^2 = 0.86$, $p < 0.05$).

Conclusion: Phenolic-rich WF of the edible fern *S. palustris* is a promising source of phytochemicals with highly potent antilglucosidase and antioxidant activities.

SL5: Phytochemicals and power test inhibition spoon leaf extract (*Plantago major* L.) and fruit sugar-apple (*Annona squamosa* L.) on xanthine oxidase activity

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ABSTRACT

Xanthine oxidase enzyme (EC.1.17.3.2) plays an important role in the formation of uric acid in the body. High levels of uric acid can cause uric acid. During this leaf spoon and sugar-apple fruit has been traditionally used to treat uric acid.

Objective: This study aims to determine the type of secondary metabolites contained in extracts of leaves and fruit spoon sugar-apple and it is power inhibition against xanthine oxidase activity relative to uric acid medication allopurinol.

Objective: This research was carried out in phases: 1) extraction of leaf spoon (ethanol 70% and water) and sugar apple fruit flesh (boiling and without boiling) by maceration method, 2) phytochemical test includes testing tannins, polyphenols, flavonoids, saponins and alkaloids, 3) isolation of the enzyme xanthine oxidase from fresh cow's milk and 0-40% ammonium sulfate fractionation, 4) inhibition of leaf extract power test spoon and sugar-apple fruit on xanthine oxidase activity relative to Allopurinol.

Results: The results showed that 1) the secondary metabolites contained in extracts of leaf spoon are a class of polyphenols, flavonoids, and alkaloids, whereas the sugar-apple fruits are flavonoids and alkaloids, 2) the yield of the water extract of leaf spoon, spoon leaf ethanol extract, water extract of sugar-apple fruit boiled and boiled meat without sugar-apple fruit are respectively 12.04%, 12.42%, 10.64%, and 11.88%, 3) power inhibition of water extract of leaf spoon, spoon leaf ethanol extract, extract broth Sugar-apple fruit flesh boiled and boiled sugar apple without row is 62.5%, 75%, 50%, and 87.5%, and 4) the mass needed to produce extracts that have a similar inhibition with 1 tablet Allopurinol (100 mg) was 465.72 grams (fresh spoon leaves with solvent water), 376.24 grams (fresh spoon leaves with ethanol 70%), 42.39 grams (sugar-apple fruit flesh boiled with water solvent) and 21.63 grams (Sugar-apple fruit flesh without boiled with water solvent).

Conclusion: Phytochemical test results showed that the sugar apple fruit and spoon leaf are secondary metabolites that are effectively used to treat uric acid such as allopurinol.

SL7: Prenylation of phytochemicals through fungal elicitation of seeds to increase their antimicrobial potential

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ABSTRACT

Prenylation of phytochemicals has shown to modulate their antimicrobial activity (1, 2) and it is a common defence response in plants from the Fabaceae family. With their lipophilic tails, prenylated molecules might exert their action by increasing membrane permeability (2). Production of prenylated phytochemicals is of great interest, because these molecules can potentially be used as therapeutic agents or as natural antimicrobials in foods.

Objective: To study the effect of fungal elicitation of legume seedlings on the content, composition and antimicrobial properties of the phytochemicals were induced.

Methods: Legumes seeds (mung bean, soybean, kidney bean, peanut, white, yellow and blue lupine) were simultaneously germinated and stressed by *Rhizopus* sp. Elicited seedlings were extracted and analyzed for their composition of phytochemicals by RP-UHPLC-ESI-MS. The extracts were tested for antibacterial activity against *L. monocytogenes* and antibiotic-resistant *S. aureus*. Minimum inhibitory and bactericidal concentrations were determined, as well as time-dependent killing kinetics.

Results: The content of prenylated compounds in the elicited seedling extracts increased 1 to 50-fold, compared to the non-elicited seedling extracts. The extracts of elicited peanut, yellow and blue lupine, contained the highest concentration of prenylated phytochemicals (22±1 mg/g for yellow lupine, 25±3 mg/g for blue lupine and 51±6mg/g for peanut) and showed antibacterial activity against *L. monocytogenes* and MRSA strains (MIC 0.5-1.0 mg/mL). The non-elicited seedling extracts did not have antimicrobial activity at 1.0 mg/mL (max. concentration tested). Chain-prenylation was predominant in isoflavones and stilbenes from the active extracts, while pyran ring-prenylation was found in pterocarpanes from soy bean (inactive). Taken together, the type of prenylation, as well as the skeleton of the phytochemical seem to have an effect on the antibacterial properties of these compounds.

Conclusions: Prenylation is a promising strategy to increase the antimicrobial properties of phytochemicals and fungal elicitation of seedlings is a simple and natural process to obtain these antimicrobial compounds.

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SL8: Investigation of antibacterial mechanism and identification of bacterial protein targets mediated by three antibacterial plant extracts

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ABSTRACT

Objective: Investigating the antibacterial mechanism and identifying bacterial protein targets, mediated by three antibacterial medicinal plants: *Melastoma candidum*, *Callicarpa formosana* and *Scutellaria barbata*.

Methods: The three selected plants were extracted with methanol and accessed for their effects on bacterial protein expression profiles. Upon treatment with the aforementioned plant extracts, bacterial proteins were extracted and resolved using denaturing gel electrophoresis. Differentially-expressed bacterial proteins were excised from the gels and subjected to protein sequence analysis using MALDI TOF-TOF mass spectrometry. Additionally, following treatment with antibacterial plant extracts, a scanning electron microscope was also applied to observe for morphological changes induced on the bacterial membrane surfaces.

Results: In this study, seven differentially expressed bacterial proteins (triacylglycerol lipase, N-acetylmuramoyl-L-alanine amidase, flagellin, outer membrane protein A, stringent starvation protein A, 30S ribosomal protein S1 and 60 kDa chaperonine) were identified via proteomic approach through treatments with the antibacterial plant extracts. Moreover, the scanning electron microscope study also indicated damages induced on bacteria cell surfaces, following treatment with an antibacterial *M. candidum* extract. To the best of our knowledge, this represents the first time these differentially expressed bacterial proteins were being reported, following exposure to antibacterial medicinal plant extracts.

Conclusion: Potential antibacterial protein targets were identified. Further studies in this direction could lead to the detailed understanding of their inhibition mechanisms and discovery of target-specific antibacterial agents.

SL9: Antimicrobial activities and chemical compositions of *Pogostemon cablin* (Blanco) Benth. and *Perilla frutescens* (L.) Britt. in a traditional Chinese medicine formula

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ABSTRACT

Huo-Xiang-Zhen-Qi-Wan is a well-known traditional Chinese Medicine formula and is widely used to "induce diaphoresis and clear away summer-heat, to resolve damp and regulate the function of the spleen and stomach". *Pogostemoncablin* (Blanco) Benth. (Huo Xiang, 藿香) and *Perilla frutescens* (L.) Britton (Zi Su, 紫苏) are the two major aromatic components in this formula.

Objective: The aim of this study was to evaluate their antimicrobial activities and to identify the bioactive compounds.

Methods: Both the hexane and ethanol extracts of these two plants were obtained by maceration. The disk diffusion method and microplate Alamar blue assay were used to evaluate their antimicrobial activities against a set of five Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Bacillus subtilis*, and *Enterococcus faecalis*), four Gram-negative bacteria (*Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*) and a fungus, *Saccharomyces cerevisiae*. The potential bioactive compounds were characterized by gas chromatography-mass spectrometry.

Results: The hexane extracts of *P. cablin* (PCH) consisted of two major compounds (patchouli alcohol and pogostone) among other minor components (α -pinene, β -pinene, β -patchoulene, β -caryophyllene, α -guaiene, seychellene, α -patchoulene). The hexane extracts of *P. Frutescens* (PFH) consisted of the major compounds (hexadecanoic acid ethyl ester, linoleic acid ethyl ester, linolenic acid ethyl ester, and octadecanoic acid ethyl ester) together with other minor compounds including β -caryophyllene, linalool, perilla ketone, myristicin, elemicin, and asarone. PCH showed the strongest antimicrobial effect against both *S. aureus* and *A. faecalis*, and also showed moderate antibacterial activity against *B. subtilis*, *E. coli*, and *E. cloacae*. PFH showed antibacterial activity against *B. subtilis*, *E. cloacae*, *S. aureus*, and *A. faecalis* with a MIC value of 1024 μ g/ml. MICs values of patchouli alcohol against *S. aureus* (11832), *S. epidermidis* (2008) and *E. coli* (8277) were determined to be 512, 256 and 64 μ g/ml, respectively.

Conclusion: The hexane extracts of both *P. cablin* and *P. Frutescens* showed antibacterial activities against a range of Gram-positive and negative strains.

SL10: New HPLC-MS methods for ginsenoside profiling and identification in roots and ginseng based products

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ABSTRACT

The pharmacological properties of old Chinese medicine (Ginseng) are generally attributed to its triterpene glycosides, which are called ginsenosides. More than 600 ginsenosides have been isolated from *Panax* species. One of the main goals of the ginseng researches was the differentiation of the ginsenosides patterns between the different traditional processing of *P. ginseng* roots such as white and red ginseng and different *Panax* species.

Objective: This study was aimed to propose new methods for fast ginsenoside profiling and identification in challenging matrices such as roots, extracts and food products.

Methods: Ultra-sound assistant extraction was performed in order to achieve good recovery of the ginsenosides. Analysis of extracts was carried out using a reversed-phase chromatography with SB-C18 sorbent. For compounds identification, electrospray ionization and quadrupole/linear ion trap mass-spectrometer (ESI-LITMS) in different modes were used.

Results: Several binary combinations of methanol, acetonitrile, ethanol and water were evaluated for use as the extraction mixture in order to achieve maximal efficiency. Methanol:water (1:4) mixture was chosen. The absence of ginsenosides Rg3 and Rh2 decomposition products of added ginsenosides Rb1 and Rc was shown. Observed recovery was between 79 and 107%. The study of ginsenoside fragmentation in the linear ion trap was made. Several samples of fresh and dry *Panax ginseng* roots and processed products were undergone the extraction process followed by HPLC/ESI-LITMS analysis in order to investigate the number and elucidate structure of ginsenosides present. The chromatographic run took 45 min. Saponin profiles of several root and processed products extracts were compared. The inter-day precision was performed on three different days. Intra-day RSD values (N=3) were about 3% while inter-day RSD were on the level of 3-4%. Good linearity was observed in a range 0.01—5 µg/mL.

Conclusion: The result of this study is that a HPLC/ESI-LITMS method was developed. This method can be easily applicable for quality control purposes of marketed products and allows the rapid and direct identification of ginsenosides in crude plant extracts.

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SL12: Metabolomics combined with chemometrics reveals chemical features of *Pericarpium Citri Reticulatae* and *Pericarpium Citri Reticulatae Viride*

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ABSTRACT

The herbal materials of *Pericarpium Citri Reticulatae* (PCR) and *Pericarpium Citri Reticulatae Viride* (PCR_V) are tangerine peels, while their harvest time is different. The recently developed metabolomics approach is effective to detect a changing trend of metabolites during ripening and provides an opportunity to reveal the different chemical features of the closely related traditional Chinese medicines (TCMs).

Objective: This study aimed at demonstrating the metabolic footprints of tangerine peels during ripening and revealing chemical features of PCR and PCR_V.

Methods: High-performance liquid chromatography-diode array detection (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS) were employed to fingerprint the tangerine peels during growth, from July to December. Volatile and non-volatile components were identified and quantified with the help of UV-visible spectra, mass, heuristic evolving latent projection (HELP) method, and temperature-programmed retention indices (PTRIs). After that, principal component analysis (PCA) was applied to investigate the metabolic footprints of tangerine peels and screen the characteristic components. Finally, a heat map and Pearson correlation analysis were employed to screen the characteristic compounds representing their different ripening stages.

Results: Footprints of volatile and non-volatile metabolites were obtained, which suggested that July might be the best harvest time for PCR_V, November and December were better for PCR. Overlapped components in these HPLC/GC fingerprints were resolved by the HELP method for further qualitative and quantitative analysis. 45 non-volatile and 82 volatile components were identified. Furthermore, hesperidin, nobiletin, tangeretin, D-limonene and linalool were screened as chemical markers by loadings of PCA. In addition, we suggested that compounds such as 4-carene, 3-carene, β -pinene and γ -terpinene were as major components for the pungent smell of PCR_V. Geranyl acetate, farnesyl acetate and three alcohols (6-hepten-1-ol, 3-methyl-1-hexanol, 1-octanol) may lead to the pleasant odor of PCR.

Conclusion: We propose that metabolomics focusing on ripening process will be an effective strategy for quality control of closely related herbal medicines. It has shown its potential in optimization of harvest time and chemical markers' screening, which will have wide perspective in analysis of closely related TCMs.

Acknowledgements: This work was supported financially by National Nature Foundation Committee of P.R. China (No.21465016, No.21105129).

SL13: Rapid analysis of the key aroma compound, 2-acetyl-1-pyrroline, in rice plant at different growth stages using automate headspace-gas chromatography with nitrogen-phosphorus detector

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ABSTRACT

2-Acetyl-1-pyrroline (2AP) is the key aroma compound of aromatic rice that its content has been widely used for assessment of rice aroma quality. A headspace-gas chromatography (HS-GC) with nitrogen-phosphorus detector (NPD) method has been developed in this study for rapid quantitative analysis of 2AP in rice leaves and grains.

Objective: This study aimed to develop a method employing automated HS-GC/NPD for determination of 2AP in rice leaves and grain at different growth stages.

Methods: Fresh leaves of the Thai aromatic rice, Khao Dawk Mali 105 (KDML105), at different growth stages and their grains were collected and ground to small pieces prior to extraction of rice volatiles by HS and further analysis of 2AP by GC/NPD. All HS and GC parameters were optimized. Authentic 2AP was synthesized and used to construct a standard calibration curve for quantitative analysis using an internal standardization method.

Results: A rapid HS-GC method for quantitative analysis of 2AP in the rice leaf and grain samples was successfully developed with total analysis time of only 10 min per sample. The amount of 2AP in rice leaves following growth stages was found to continuously increase after seedling (10 days) until booting stage, in which 2AP reached its highest amount of 35.77 $\mu\text{g/g}$. During booting stage to the end of grain, the amount of 2AP decreased down to 12.51 $\mu\text{g/g}$. This was probably due to the lower rate of secondary metabolite production during these lately stages of rice growth. In addition, it was found that the amounts of 2AP in rice leaves were approximately three times higher than those found in rice grains. This newly developed method for analysis of 2AP in rice leaves offers an alternative way to assess aroma quality of the rice yield as its content in grains can readily be predicted.

Conclusion: Determination of 2AP in rice leaves was successfully accomplished using a newly developed HS-GC/NPD method, which is a green, rapid, and non-laborious method that requires low amounts of a sample. The amount of 2AP in grain can be correlated to its amount in leaves, thus, this method reduces sample preparation time in terms of planting which has made it more attractive and convenient.

SL14: Studies on the proximate analysis, minerals, antinutritional factors and *in vitro* anthelmintic effects of *Moringa oleifera* leaves (FHI 109897) on bovine helminth eggs

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ABSTRACT

Moringa oleifera is a plant used for food and medicinal purposes.

Objective: The need to control helminths resistance to the conventional anthelmintics necessitated the evaluation of phytochemicals in *M. oleifera* leaves for their possible anthelmintic effects. *M. oleifera* leaves (FHI 109897) were analysed for nutritional components, antinutritional factors and *in vitro* anthelmintic effects on bovine helminth eggs.

Methods: The nutritional components and the antinutritional factors were analysed according to standard methods. The quantitative faecal egg counts (FEC) were determined using the modified McMaster technique. The anthelmintic efficacy of *M. oleifera* leaves FHI 109897 was evaluated using different extracts prepared in different solvents: hexane, ethyl acetate and methanol. The different extracts from *M. oleifera* leaves were tested on the viability of the faecal eggs of two gastrointestinal nematodes of cattle: *Oesophagostomum spp.* and *Syngamus laryngeus*, using *in vitro* assays. The different volumes of the extract used were 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

Results: The results obtained for proximate analysis were crude protein (15.04±0.18%), crude fat (3.91±0.04%), crude fibre (17.27±0.02%), ash (9.85±0.02%), moisture (7.10±0.02%) and gross energy (3.52±0.01%). Elemental analysis revealed that potassium was 0.93±0.002% while calcium was 0.34±0.03%. Sodium was 0.19±0.003%, magnesium was 0.58±0.001% and phosphorus was 0.38±0.014%. Copper was 4.93±0.03 mg/kg, zinc was 52.3±0.01 mg/kg, iron was 191.3±0.02 mg/kg and selenium was 0.01±0.001 mg/kg. The antinutritional factors analysed were phytate (1.58±0.02%), oxalate (0.87±0.02%), saponin (0.45±0.01%), tannin (0.05±0.01%), alkaloids (1.08±0.02%), flavonoids (0.004±0.001%), cyanogenic glycoside (0.19±0.001%), phenol (0.12±0.001%), trypsin inhibitor (2.77±0.03 mg/g), haemagglutinin (15.81±0.04 HU/mg) and chymotrypsin (6.32±0.04 CU/mg). Phytochemical screening revealed the presence of alkaloids, cyanogenic glycosides, flavonoids, saponins, steroids, tannins and triterpenoids in *Moringa oleifera* leaves. The results of the FEC and anthelmintic assay revealed that the hatching of the *Oesophagostomum spp.* and *Syngamus laryngeus* eggs were totally inhibited by only the methanolic extract of *M. oleifera* leaves at 1.0 ml, 1.5 ml and 2.0 ml.

Conclusion: The study concluded that *M. oleifera* is rich in nutritional component and antinutritional factors. The methanolic extract of *M. oleifera* leaves has a better potential as an anthelmintic than the other extracts.

SL15: Evaluation of Chinese rice wine on the antioxidant activities in D-galactose induced aging mice

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ABSTRACT

Chinese rice wine (CRW) is a fermented alcohol beverage made from grain (rice, glutinous rice or millet), which is widely consumed by the general public in China. Recent studies have demonstrated that several kinds of commercial CRW owned effective antioxidant activity in vitro, while in vivo experiments have not been reported.

Objective: The purpose of this study was to evaluate the effect of CRW on antioxidants activities of D-gal induced aging mice, and to determine the phenolics content in CRW.

Methods: CRW was concentrated to 1/3 of initial volume, and the content of total and 13 individual phenolic compounds were determined. Sixty ICR mice were randomized into 5 groups, the D-gal were injected subcutaneously at doses of 150 mg/(kg·day) to the mice in the aging model group and the CRW groups, and simultaneously the mice in the CRW groups were oral administrated concentrated CRW at three different doses of 1.5, 3.0, 9.0 mg/(kg·day) respectively, the effect of CRW on the content of malondialdehyde (MDA), protein carbonyl group (PCO), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in serum, brain and liver were observed after 6 weeks.

Results: In serum, brain and liver of the mice in the aging model group, the content of MDA and PCO were higher and the activities of SOD and GSH-Px were lower than those in the normal control group ($P < 0.01$). Compared to the index in serum, brain and liver of the mice in aging model group, the content of MDA and PCO decreased and the activities of SOD and GSH-Px increased significantly in the Middle and High dose CRW groups. The total phenolic compound and the total of the 13 phenolic compounds in the concentrated CRW used in the experiment were 721.87 $\mu\text{g}/\text{mL}$ and 204.95 $\mu\text{g}/\text{mL}$ respectively, which retained more than 95 % phenolic active ingredients of the original CRW.

Conclusion: CRW could reverse the oxidative damage occurred in D-gal induced aging mice and phenolic compounds existed in CRW may be helpful in the antioxidant effect in a certain degree.

SL16: Biologically active compounds from hops and their prospects

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ABSTRACT

Hops are used in the manufacturing of beer. Nowadays there is an increasing interest in possible uses of hops for non-brewing purposes, especially in the pharmaceutical industry. Hops contains flavonoids, hop resins and essential oils which contain many compounds with very interesting medicinal effect.

Objective: This study reviews biologically active compounds from hops and their possible prospects in future.

Methods: Hop compounds were studied by using of microbial tissue cultures and animal model organisms. Only several hop compounds and hop components was tested in clinical studies.

Results: Among pharmaceutically important compounds from hops are flavonoids (1), having proven anticarcinogenic, antioxidant, antimicrobial, anti-inflammatory and estrogenic effects. One of these substances, with apparent 'miracle effects' is the prenylated chalcone, xanthohumol. Nowadays, considerable attention is being paid to 8-prenylnaringenin due to its reported strong phytoestrogenic activity, stimulating estrogen receptors in the body. Phytoestrogens can therefore substitute for human steroidal hormones and suppress menopausal symptoms in women, or reduce the risk of cancers associated with changes in the hormonal system.

Hop bitter acids are other potential sources of treatment or prevention of many diseases, including cancer, diabetes, osteoporosis, cardiovascular diseases, inflammatory and metabolic disorders. In addition, the antimicrobial effects of beta acids against different microorganisms are significant and can be utilized for human treatment.

The Committee on Herbal Medicinal Products (HMPC) of the European Medicine Agency (EMA) issued a statement in 2007 on the traditional use of hops (*Humulus lupulus* L.) for the treatment of mild symptoms of stress and insomnia. For these purposes, a hop extract is combined with an extract of valerian (*Valeriana officinalis*, *Valerianaceae*). Until recently, this preparation represented the most frequently administered form of plant-based sleeping agents and sedatives. Hop was identified as a medicinal plant for use in the treatment of mental state disorders such as anxiety, restlessness, and sleep disturbances.

Conclusion: Hop is a medicinal plant with a promising future. Among pharmaceutically important compounds from hops are flavonoids, namely prenylated, hop resins and their transformation products and hop essential oils.

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SL19: Phytochemical products development and regulation for the global market including US, EU and Asia

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ABSTRACT

The presentation focuses on development of phytochemical products and regulations in global markets. The first part outlines the process of developing phytochemical ingredients into dietary supplements, food and personal care products. The trend of phytochemical products in global markets is also discussed. The second part is an overview of international regulations on phytochemical products development especially in the US, EU and Japan. The third part provides some examples of phytochemical products in the US, EU and Japanese markets.

Objective: The purpose of this presentation is promoting understanding of global regulations for phytochemical product development and providing a link between academic research and industry.

Methods: Global regulatory requirements are outlined using examples of phytochemical products in the global market place, and the global trend of phytochemical products is discussed.

Conclusion: Phytochemical products have a strong potential for global markets. The knowledge and understanding of country-specific regulations is essential to develop phytochemical products for international market needs.

SL20: Quantitative TD-GC-ToF MS analysis of volatile compounds in a controlled-flow headspace extract from cured *Vanilla planifolia* pods

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ABSTRACT

The distinctive flavour and aroma of vanilla develops during senescence and post-harvest curing of the vanilla pod. The degradation of internal tissues and processing of important precursor metabolites enables the production of important compounds such as phenolics and aliphatic acids. It is believed that genetic variations, geographical location and post-harvest methodology can influence the final phytochemical profile.

Objective: This study describes a new reproducible, qualitative and quantitative method for profiling constituent compounds in vanilla extracts derived from controlled-flow headspace in conjunction with thermal-desorption gas chromatograph time-of-flight mass spectrometry (TD-GC-ToF).

Methods: Cryo-ground cured vanilla pods were weighed (0.5-5 g), placed in a Micro-Chamber™ (Markes International) and maintained at 20°C or 40°C whilst 100 ml/min air (carbon filtered) purged each chamber in parallel. Volatiles were collected in thermal-desorption tubes containing TENAX/Carbotrap adsorbent. Adsorbent exposure times of 1-5 min were used, and secondary tubes were attached to check for breakthrough constituent compounds emerging from the primary tube. Thermal-desorption temperatures (220-300°C) were tested for the possibility of the thermal decomposition of larger compounds. To prevent saturation of the detector, the ratio of gas flows through the GC injector was also varied to determine a suitable proportion of extract to be desorbed. Over 40 compounds previously identified in *V. planifolia* extracts were used to create a 'master-mix' standards series (0-1000 ng/μl).

Results: The optimised extraction method using the Micro-Chamber™ was at 40°C for 3 minutes. Thermal-desorption temperature was to remain at 300°C for 8 minutes to ensure complete desorption. For each vanilla sample, 2 tubes were taken for each replicate and run at different split-ratios; 1:90 to obtain a signal within range of detection for vanillin, and 1:30 for the remaining compounds. Using the optimised method, 40 compounds including key groups of phenolics, aliphatic acids and alcohols were identified and subsequently quantified. The optimisation process took into account previously unconsidered factors, producing quality separation and robust accuracy of quantitation.

Conclusion: Using this method, vanilla ecotypes and species can be compared and thus biomarkers could be attributed as a recognisable fingerprint of volatile compounds specific to each vanilla source.

SL21: Bioactive guided fraction, isolation and characterization of antitumor phyto-constituents from *Aphanamixix polystachya*

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ABSTRACT:

Aphanamixix polystachya (Wall) R.N. (Meliaceae), commonly known as 'Amoora rohitaka' is a traditional medicinal plant used in the Indian system of medicines.

Objective: The present research work dealt with the bioactive guided fractionation, isolation of ten phytoconstituents from an ethanolic fraction and their cytotoxic evaluation by *in-vitro* methods.

Methods: Dried roots of *A. polystachya* were subjected to size reduction and the powder was macerated with ethanol to obtain an ethanolic extract. The ethanolic fraction was subjected to flash chromatography and column chromatography to get different phytoconstituents. The ethanolic fraction and isolated compounds were evaluated for their antitumor activity on HepG2, Vero, MCF7 and HCT cells using *in-vitro* methods.

Results: Preliminary phytochemical screening of the ethanolic extract revealed presence of alkaloids, triterpenoids, steroids, flavonoids and tannins. Column chromatography along with flash chromatography of the ethanolic fraction resulted in isolation of ten phytoconstituents, viz. 7-keto-octadec-cis-11-enoate, Rohituka 3, Aphanamixin, Rohituka 15, Aphanamgrandin E, Aphanamixod A, Amoorinin-3-O- α -rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside, 8-C-methyl-5,7,3',4',-tetrahydroxyflavone-3-O- α -L-arabinopyranoside, Stigmasta-5,24(28)-dien-3 β -O- β -D-glucopyranosyl-O- α -L-rhamnopyranosyl (1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside with promising anticancer activity on different cell lines *in-vitro*.

Conclusion: *A. polystachya* is promising medicinal candidate with antitumor potential.

SL23: Subcritical water extraction - a novel extraction approach for hydrolysis and recovery of bio-potent molecules

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ABSTRACT

For efficient isolation and recovery of valuable compounds from natural sources, extraction process is a crucial step. In last few decades, special attention of scientific community has been directed towards the development of techniques that could respond to several demands simultaneously: to provide high yields of target components; to be environmentally-friendly; and finally to be economically viable. Green extraction approaches tend to respond well to set requirements. Among different solvents water in its subcritical state is particularly attractive and extraction with subcritical water has gained remarkable popularity in different applications. Moreover, high hydrolytical potential of superheated water can be used for simultaneous extraction and derivatization of myriad of natural organic compounds.

Objective: In the present study the influence of a modifier (hydrochloric acid) on the extraction and hydrolysis of apigenin-7-O- β -glycoside from chamomile flowers by using superheated water was investigated. Obtained results were compared with those obtained without the addition of a modifier.

Methods: Four different concentrations of hydrochloric acid (0.001; 0.005; 0.01; 0.05 mol/L) were used in order to examine the influence of this modifier on the hydrolytical potential of superheated water in the recovery of apigenin from its bound forms. Determination of apigenin and apigenin-7-O- β -glycoside was performed by HPTLC/UV-VIS. Total phenols and flavonoids were quantified by spectrometry.

Results: Different yields of phenols and flavonoids were noticed for different concentrations of acid modifier. Total phenols content was in the range from 3.97 (for the concentration of 0.05 mol/L) to 5.74 mg/mL (for the concentration of 0.001 mol/L). As in the case of phenols, the greatest contents of flavonoids were observed for acid concentration of 0.001 mol/L (1.12 mg/mL). Hydrolysis (or more precisely acetolysis) of apigenin-7-O-glucoside to free apigenin was seen even in weak acidic conditions confirming high hydrolytical potential of superheated water. Even lowest investigated acid concentration of 0.001 mol/L was sufficient for the hydrolysis of O-glycoside.

Conclusion: Obtained results clearly demonstrate high hydrolytical potential of subcritical water. Furthermore, high concentrations of total phenols and flavonoids in obtained extracts demonstrate good solvation properties of subcritical water for the isolation of bioactive compounds from natural sources.

SL25: Effects of packaging methods and storage time on anthocyanin contents in Thai Jao Hom Nin rice

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ABSTRACT

Thai Jao Hom Nin rice (*Oryza sativa* L.) is one of the Thai black rice varieties that have widely been consumed due to their functional pigmented components. In order to ensure the therapeutic quality of the rice, an appropriate method of packaging for preserving these black rice pigments during storage is required.

Objective: This study aimed at determining the anthocyanin pigments in Thai Jao Hom Nin rice after being subjected to four different packaging methods and being stored for six months.

Methods: Two weeks after harvesting and drying, Jao Hom Nin rice was sealed in four different types of packaging; 1) Nylon added with laminate bag 2) Nylon added with laminate bag and filled with N₂ 3) Nylon added with laminate bag and kept under vacuum at 0.8 bar, and 4) Aluminium bag and kept under vacuum at 0.8 bar. Identification of anthocyanins in the rice extracts was performed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Over to six months, the anthocyanin contents in the rice samples were determined at the end of each month using liquid chromatography-mass spectrometry (LC-MS) until 6 months.

Results: Two peaks of anthocyanins were detected in all rice extracts by LC-MS/MS at 9.80 and 10.53 min, which provided major ions at m/z 449 and 463, respectively. The loss of one glucose moiety caused the fragmented ions in these two mass spectra at m/z 287 and 301, leading to the identification of cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside, respectively. The identification was also confirmed by the MS/MS spectra of the two fragmented ions, which showed many related dissociation pathways. Overall, the contents of both anthocyanins in four rice samples from different packages decreased steadily during storage. After the first month of storage, rice samples sealed in aluminium bag and kept under vacuum at 0.8 bar retained higher contents of both anthocyanins. However, at the end of the six month storage, the rice packed by sealing in Nylon added with laminate bag and filled with N₂ preserved higher contents of cyanidin-3-*O*-glucoside (29%) and peonidin-3-*O*-glucoside (24%) than other packaging methods.

Conclusion: Nylon added with laminate bag and filled with N₂ is recommended as an appropriate method of packaging for maintaining the anthocyanin contents of black rice during storage.

SL26: Influence of *Agrobacterium* oncogenes on secondary metabolism of plants: naturally transgenic *Linaria* plants as a new model for investigation

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ABSTRACT

Transgenic hairy root cultures have revolutionized the role of tissue culture of plants in the production of secondary metabolites. It was shown that hairy roots often exhibit about the same or higher biosynthetic capacity for secondary metabolite production comparing to their normal roots. Using this methodology, a big number of chemical compounds has been synthesized. The role of single *rol* genes in secondary metabolite production was studied. Stimulatory effect on the production of secondary metabolites has been shown for genes *rolA*, *rolB*, *rolC*. These genes are present in naturally transgenic *Linaria* plants that are discussed as a new model for study of possible evolutionary function of *rol* genes in the control of secondary metabolites. Thus, increasing the amount of secondary metabolites is a characteristic of tissues where *rol* genes are expressed. This property can be useful for plants, because secondary metabolites may contribute to the resistance of plants to pests and disease.

Objective: This study aimed to characterize a big number of *Linaria* species from different sections of genus for presence of *rol* genes and perform a comparative analysis of sensitivity to fungal pathogens of *Linaria* plants containing *rol*-gene-like sequences and *Linaria* species without *rol* genes.

Methods: Screening of species, containing *rol*-gene-like sequences was done by digenerate Real time PCR, followed by sequencing of PCR positive samples. Sequences were analyzed for possibility to code full size proteins *in silico*. The leaves of *Linaria vulgaris* and *L. maroccana* were inoculated by infection mycelium suspension (40 mg/ml). Disease assessment was carried out three days after inoculation on the leaf necrosis.

Results: While analyzing *Linaria* species for presence of T-DNA it was shown, that all of the nine species analyzed in section *Linaria* and all of the five species from section *Speciosae* contained *rol* genes. None of the three species analyzed from sec. *Diffusae*, one species from sec. *Macrocentrum*, one species from sec. *Pelisserianae*, six species from sec. *Supinae* and two species from sec. *Versicolores* contained *rol*-genes. It means that naturally transgenic *Linaria* plants form a monophyletic group. In general, species composition of micromycetes on *L. vulgaris* and *L. genistifolia* is rather poor as compared to mycobioties of other plants. During all survey, no dead or highly impaired by fungus infection *Linaria* plants were found. The evaluation of virulence against *L. vulgaris* and *L. maroccana* for 14 strains of seven pathogenic micromycetes indicated that *L. vulgaris* plants were less amenable to pathogenic micromycetes than *L. maroccana*. We assume that regulation of plant disease resistance may happen at the level of regulation concentrations of Antirrhinoside and its derivatives.

SL27: Enhancement of cardio-cerebrovascular disease treatment drug tanshinone production in *Salvia miltiorrhiza* hairy roots by pathway engineering

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ABSTRACT

Salvia miltiorrhiza Bunge (Dan-shen in Chinese) is a well-known traditional Chinese medicine for the treatment of many cardiovascular diseases in China and other Asia countries with a long history. Tanshinones, a group of diterpenes mainly from *S. miltiorrhiza* with various biological activities such as anti-oxidative, anti-inflammatory, anti-proliferative and anti-tumor properties, have also roused extensive attention with increased market demand.

Objective: Due to huge consumption, low content and serious quality degradation, it is very significant to improve the content of tanshinones by modern biotechnology methods.

Methods: In our laboratory, based on the optimization of *S. miltiorrhiza* hairy root cultures and molecular cloning of genes involved in the tanshinones biosynthetic pathway as well as screening of key regulatory target, overexpression of single gene or different gene combinations lead to increasing tanshinones production in genetically engineered hairy root as well as related biological activity.

Results: Overexpression of *SmGGPPS* and/or *SmHMGR* as well as *SmDXS* in transgenic hairy root lines can significantly enhance the production of tanshinone, and co-introduction of *SmHMGR* and *SmGGPPS* resulted in highest production of tanshinone in line HG9, which was about 4.74-fold higher than that of the control. Furthermore, methyl jasmonate (MJ) and salicylic acid (SA) were used to investigate their effects on tanshinone accumulation and biosynthetic genes expression in the hairy roots of *SmGGPPS*-overexpressing line (G50) in *S. miltiorrhiza*, and total tanshinone content in G50 was obviously increased by 3.10-fold with MJ at 36 H and 1.63 times after SA treatment for 36 H in comparison with control. In addition, co-regulation of MVA and MEP pathways (by *SmHMGR* and *SmDXR*) were carried out to enhance the yield of tanshinone in *S. miltiorrhiza* hairy root, and transgenic hairy root harboring *HMGR* and *DXR*(HD42) exhibited higher tanshinone content after elicitation by yeast extract and/or Ag⁺ than before.

Conclusion: In a word, our results provide a useful strategy to improve tanshinone content as well as other natural active products by combination of genetic engineering with elicitors.

SL28: Production of ginsenoside F2 using *Lactococcus lactis* with improved expression of β -glucosidase from *Paenibacillus mucilaginosus*

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ABSTRACT

The major active compounds of ginseng are ginsenosides which have various pharmacological activities such as anti-cancer, anti-inflammatory, anti-aging and neuro-protective activities (1). Among them, the minor ginsenosides which have significant pharmaceutical functions were difficult to extract from raw ginseng due to the low contents.

Objective: The aim of this study was to produce minor ginsenoside F2 from major ginsenosides Rb1 and Rd by using recombinant *Lactococcus lactis* expressing heterologous β -glucosidase gene.

Methods: For this, the nucleotide sequence for β -glucosidase gene (BglPm) from *Paenibacillus mucilaginosus* was synthesized after codon-optimization and the two genes (unoptimized and optimized) were overexpressed in *L. lactis* NZ9000 by using pNZ8008 plasmid (pNZBgl-uno and pNZBgl-opt, respectively).

Results: After optimization, the percentage of unfavorable codons decreased by half. The enzymes were successfully expressed in *L. lactis* and purified by Ni-NTA column chromatography; the total activities were 0.001 unit/mL (unoptimized) and 0.017 unit/mL (optimized). This result showed that the expression level (total activity) of β -glucosidase significantly increased after codon optimization. The β -glucosidase hydrolyzed outer glucose of C3 and C20 position of major ginsenoside (Rb1 and Rd) and produced the minor ginsenoside F2. When the cell lysates and permeabilized cells of recombinant *L. lactis* harboring pNZBgl-opt were reacted with PPD type ginsenoside mixture (PPDGM), the substrate (Rb1) was completely consumed within 24 hours. The bioconversion yields of whole cells, cell lysates, and permeabilized cells were 38 %, 62% and 63%, respectively. In addition, when the transformant was used as a starter culture for yogurt fermentation, the population of *L. lactis* reached a maximum level (109 CFU/mL) after 24 hours, but the bioconversion yield in yogurt was lower (14 %) due to the decrease of pH during the fermentation.

Conclusion: This is the first report of F2 production using recombinant lactic acid bacterium, which showed high specific activity of ginsenoside transforming activity.

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SL29: Manipulation of 3-hydroxy-3-methylglutaryl coenzyme A synthase for health-promoting isoprenoid production

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ABSTRACT

Isoprenoids, a group of functional diverse natural products with economical and pharmaceutical value, are biosynthesized from the mevalonate (MVA) and the methylerythritol phosphate (MEP) pathways. 3-Hydroxy-3-methylglutaryl-coenzyme A synthase (HMGS) is the second enzyme in the MVA pathway. Extensive studies have reported the functional identification and manipulation of the MVA and the MEP pathways for health-promoting isoprenoid production. One strategy consists of manipulating 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) activity, while little attention has been paid to HMGS.

Objective: This study summarizes the progress on HMGS for health promoting isoprenoids production.

Methods: *Agrobacterium*-mediated plant transformation was used to obtain transgenic plants overexpressing wild-type (OE-wtBjHMGS1) and mutant HMGS (S359A) (OE-S359A). Gas chromatography-mass spectrometry was utilized to analyze phytosterol content in transgenic lines. Quantitative reverse transcription-PCR was performed to check the effects of HMGS overexpression on isoprenoid biosynthesis genes.

Results: Transgenic plants overexpressing wt and mutant HMGS (S359A) resulted in a higher production of total sterol than the vector-transformed control (1,2). OE-S359A showed higher sterol increase than the OE-wtBjHMGS1. The overexpression of HMGS resulted in the up-regulation of several genes in the isoprenoid pathway. Interestingly, HMGS was found to play an important role in regulating plant growth, pollen development and seed production (2,3). The specific inhibitor of HMGS (F244) was shown effective on *Brassica* seedlings and tobacco BY-2 cells in retarding growth (3).

Conclusion: HMGS (S359A) can promote phytosterol production, plant growth and seed yield.

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SL30: Production of phenolic acids from cereals using lactic acid bacteria

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ABSTRACT

Cereals contain not only carbohydrates, dietary fiber, proteins and minerals but also phenolic acids. Caffeic, p-coumaric and ferulic acids are the representative phenolic acids found in plant tissues in free or bound form. Phenolic acids give various beneficial effects when consumed; ferulic acid has antioxidant, cholesterol-lowering, antimicrobial, anti-inflammatory, anti-thrombosis and anti-atherosclerosis activities. The phenolic acids on bran of the cereals mainly present as bound form through ester bonds to arabinoxylan chains. However, these bound phenolic acids are not hydrolyzed by human digestive enzymes and are mostly excreted in the feces.

Objective: The aim of this study was to produce free phenolic acids from various cereals during lactic acid bacteria (LAB) fermentation to be easily absorbed into the human body.

Methods: Four LAB were selected based on *in silico* and *in vitro* analysis results of ferulyl esterase (FE) activities, and four cereals were chosen based on the bound-phenolic acid contents among nine cereals. LAB used were as follows: *Lactobacillus casei* KCTC 3109 as a negative control, *Lb. acidophilus* KCTC 3164, *Lb. reuteri* KCTC 3594 and *Lb. gasseri* KACC 12424 as positive strains having high FE activities. Rice bran, barley bran, corn bran and sugar beet pulp were used for cereal fermentation. Selected LAB were cultured in the MRS medium with cereals for 24 hr at 37 °C and production of free phenolic acids from cereal fermentation were analyzed by high performance liquid chromatography.

Results: In order to obtain information about enzymes, *in silico* and *in vitro* analysis was performed. Corn bran contained higher concentration of phenolic acids including caffeic acid, p-coumaric acid and ferulic acid. When cereals were fermented with four LAB, *Lb. acidophilus* KCTC 3164 resulted in the highest production of free phenolic acids from four cereals; among cereals, corn bran released the highest concentration of free phenolic acids and ferulic acid was produced as dominant product. Meanwhile, *Lb. casei*, the negative control having no FE activities, produced little amount of phenolic acids from four cereals. Additionally, in this study, the optimal fermentation condition was established for maximum production of phenolic acids from corn bran by using *Lb. acidophilus*.

Conclusion: This study demonstrates that fermentation of cereals by LAB having high FE activities can significantly increase the free phenolic acid content in fermented foods. Furthermore, it proposes that the potential to develop a cereal-based synbiotic products with improved bioavailability of dietary phenolic acids.

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SL31: Comprehensive evaluation of antioxidant activity of foods based on structure change of a target protein

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ABSTRACT

We have developed an assay method (myoglobin method) to evaluate antioxidant activity against four ROS (hypochlorite ion, peroxy radical, hydroxyl radical, and peroxy nitrite) (1). This method, based on the structural change of myoglobin, expresses antioxidant activities as myoglobin protection ratio (0–100%). Further, antioxidant activities of a specimen were comprehensively evaluated by plotting myoglobin protection ratio against four ROS and DPPH scavenging activity in a 5-axe cobweb chart. This protocol is applied to evaluate antioxidant activities of various standard antioxidants, vegetables and beans, and Japanese traditional seasoning miso.

Objective: This study aimed to evaluate the antioxidant activities of antioxidants and foods against four reactive oxygen species by the myoglobin method.

Methods: Myoglobin protection ratio was determined from the differences of absorbance at 409 nm in the presence of a reactive oxygen species (hypochlorite ion, peroxy radical, hydroxyl radical, or peroxy nitrite) with or without antioxidant specimen. The conventional DPPH scavenging activity was also determined. The antioxidant activities were comprehensively evaluated by plotting these results on 5-axe cobweb charts.

Results: Different antioxidants (ascorbic acid, ferulic acid, trolox, polyphenols, glutathione, capsaicin, carnosine, and beta-carotene) showed different patterns on the cobweb charts. However, four flavonoids (rutin, quercetin, luteolin, and kampferol) showed a similar pattern on the cobweb charts (2). The antioxidant activities of water extracts of vegetables (daikon sprouts, spinach, broccoli floret, onion, qing-egng-cai, Chinese cabbage, and cabbage) and beans (red bean, soy bean, common bean, and cowpea) were successfully evaluated by the proposed protocol (3). Further, 10 brands of miso, a traditional Japanese seasoning, were classified into three groups based on the activities against peroxy nitrite (4).

Conclusion: The myoglobin method is a convenient method to evaluate the antioxidant activities against hypochlorite ion, peroxy radical, hydroxyl radical, and peroxy nitrite. The 5-axe cobweb chart plotting these results and the DPPH scavenging activities is a useful tool to characterize the antioxidant activities of foods.

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SL32: Flavonoid profiles, antioxidant potential, and acetylcholinesterase inhibition activity of the extract from archeogoniophore of *Marchantia polymorpha*

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ABSTRACT

Marchantia polymorpha belongs to the family of Marchantiaceae and genus *Marchantia*, and is used as a traditional Chinese medicinal herb for scald and pneumonia. The phytochemicals found in *M. polymorpha* are terpenes and flavonoids, which show significant biological benefits for human health.

Objective: The flavonoids profiles, antioxidant and acetylcholinesterase (AChE) inhibition activities of the extracts from archeogoniophore and gametophyte of *M. polymorpha* were compared.

Methods: Radical scavenging assay (DPPH, ABTS, O²⁻), reducing power assay, inhibiting AChE assay and LC-MS analysis.

Results: The total flavonoids content in the archeogoniophore is determined as 47.4 mg/g, which is about 10-time higher than that of gametophyte (4.6 mg/g). The archeogoniophore and gametophyte of *M. polymorpha* possess different flavonoids profiles based LC-MS analysis. The main flavonoids in the archeogoniophore of *M. polymorpha* are flavone glycosides.

The antioxidant potential of these extracts from the archeogoniophore is much higher than that from the gametophyte by means of radical scavenging assay (DPPH, ABTS, O²⁻) and reducing power assay. The extract from the archeogoniophore showed significant inhibition against AChE. However, the extract from the gametophyte hardly inhibited AChE.

Conclusion: The archeogoniophore of *M. polymorpha* contains higher flavonoids content and shows stronger bioactivities than that of the gametophyte.

SL33: Potential of *Brosimum alicastrum* as edible plant by its contents in phytochemicals and phytohormones and evaluation of antioxidant activities

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ABSTRACT

Brosimum alicastrum (Mayan nut) has been described as an underexploited species, native from the Mesoamerican region and the Caribbean with significant potential as food or feed because it contains good values of energy, dietetic fibre, fats, proteins, carbohydrates minerals and vitamins. Worldwide there is a considerable interest to search for alternative foods from plants.

Objective: This study aimed to analyze the phytochemical content, phytohormones and antioxidant activities from edible plant *Brosimum alicastrum*.

Methods: DPPH scavenging activities of hexane, dichloromethane, methanol (ME), and water extracts from seeds, fruits and leaves of *B. alicastrum* were evaluated. ME was further analyzed by the phytochemicals content through colorimetric assays and thin layer chromatography. Phytohormones as indolbutiric, indolacetic acids, gibberelic and abscisic acids were determined by liquid chromatography (HPLC). Total phenolic, flavonoids and proanthocyanidin contents of all samples were also determined.

Results: Assays performed on the extracts found the strongest antioxidant activities in ME. Analysis of the extracts revealed that the presence of terpenoids, flavonoids and phenolic compounds evident in the ME had the highest antioxidant activity. EC₅₀ values for the DPPH scavenging activity of ME and quercetin (positive control) were 3.29 mg/mL and 5.9 µg/mL, respectively. The analyses of phytohormones showed a strong presence of indolbutiric acid with 66.95 ng g⁻¹ dw in the leaves and 32.15 ng.g⁻¹ dw in the fruits. Phytochemical analysis found ME to have the highest total phenolic content (0.7% by weight) and Flavonoids content (0.5% by weight) among all extracts. Total phenolic contents correlated positively with DPPH scavenging activity ($R^2 = 0.85$, $p < 0.05$) mainly in the seeds with coat with values of 2.61 mg g⁻¹. Total flavonoid contents in the leaves were 1.82 mg g⁻¹ dw correlated positively with antioxidant activity.

Conclusion: Phenolic-rich ME of edible plant *B. alicastrum* is a promising source of phytochemicals with highly potent antioxidant activity.

SL34: Hypolipidemic effect of tetramethylpyrazine on HepG2 cells

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ABSTRACT

Tetramethylpyrazine (TMP) is a bioactive compound isolated from Chinese black vinegar and it has protective effects for cardiovascular disease (CVD), but its mechanism of action is not clear. The objectives of this work were to determine TMP content of selected vinegar powder commonly consumed in China and to investigate the potential activity of TMP in modulating cellular cholesterol.

Objective: The objectives of this work were 1) to determine the TMP content of vinegar, 2) to investigate the potential activity of TMP in modulating cholesterol efflux and 3) to determine the effects of TMP on PPAR and LXR expression in regulating lipid homeostasis.

Methods: TMP was extracted and purified from Chinese black vinegar powder and determined. The intracellular cholesterol efflux, the level of reactive oxygen species (ROS), superoxide dismutase (SOD) and catalase (CAT) in HepG2 cells were determined after TMP treatment. The expression of the peroxisome proliferator-activated receptors (PPAR) and liver X receptor (LXR) were investigated.

Results: The amount of TMP in vinegar powder closely relate to fermentation processing and strain. Furthermore, affected by stocking and ageing of vinegars were attributable to different sources of vinegar. The results demonstrated that TMP could induce intracellular cholesterol efflux in a dose and time-dependent fashion ($P<0.05$) and the maximum decrease of cellular cholesterol was reaching 37.3% at 50 $\mu\text{g}/\text{mL}$ after treated for 12 hours. TMP may also improve endothelial function through their antioxidant effects. The level of ROS in HepG2 cells decreased dramatically by 10-15% ($P<0.05$) after TMP treatment. The activities of antioxidant enzymes, including SOD and CAT increased significantly ($P<0.05$) in a dose-dependent manner, maximal increase by 156.0 % and 237.2 %, respectively. TMP doubled LXR expression ($P<0.05$) in HepG2 cells, whereas it increased the PPAR gene expression up to 125-275% ($P<0.05$).

Conclusion: TMP could induce intracellular cholesterol efflux, inhibit reactive oxygen species, and enhance activities of SOD and CAT. The possible hypolipidemic mechanism of TMP is to up-regulate the expression of PPAR and LXR to induce cholesterol efflux. TMP might be a potential protective agent for dyslipidemia and atherosclerosis.

SL35: Stability of polyphenols under the cell culture conditions: Avoiding erroneous conclusions

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ABSTRACT

Most data of bioactivity from dietary polyphenols have been derived from in vitro cell culture experiments. In a typical cell experiment, the polyphenols or the extracts, rich in polyphenols, are incubated with various cells lines from 10 min to 72 h. In this context, little attention is paid to potential artifacts due to chemical instability of these natural antioxidants.

Objective: This study aimed to investigate the structure-stability relationship of polyphenols incubated with DMEM under cell culture conditions up to 180 minutes.

Methods: An early degradation time (${}^cT_{10}$) and half-degradation time (${}^cT_{50}$) were defined to characterize the stability of natural antioxidants incubated in Dulbecco's modified Eagle's medium (DMEM) at 37 °C.

Results: The degree of hydroxylation of flavones and flavonols significantly influenced the stability in order resorcinol-type > catechol-type > pyrogallol-type, with the pyrogallol-type being highly unstable. In contrast, any glycosylation of polyphenols obviously enhanced their stability. However, the glycosylation was less important compared to the substitution pattern of the nucleus rings. Methoxylation of flavonoids with more than three hydroxyl groups typically improved their stability as did the hydrogenation of the C2=C3 double bond of flavonoids to corresponding flavanoids. There was no significant correlation between the antioxidant potential of polyphenols and their stability. Notably, the polyphenols were clearly more stable in human plasma than in DMEM, which may be caused by polyphenol-protein interactions.

Conclusion: It is strongly suggested to carry out stability tests in parallel with cell culture experiments for dietary antioxidants with catechol or pyrogallol structures and pyrogallol-type glycosides in order to avoid artifacts.

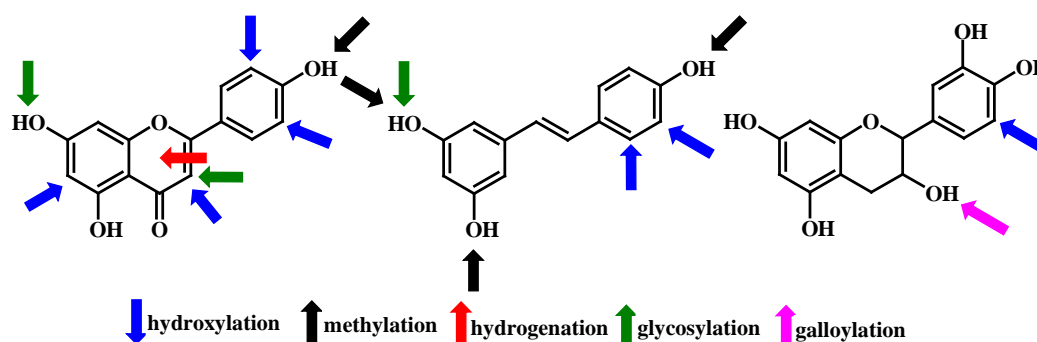


Figure 1. The potential sites of polyphenols affecting the stability are schematically illustrated. The up arrows represent increasing the stability, the down arrows represent decreasing the stability.

SL36: Structural characteristics and physicochemical properties of lotus seed resistant starch prepared by different methods

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ABSTRACT

Lotus seed resistant starch (LRS) is commonly known as resistant starch type 3 (LRS3). Different preparation methods played a very important role in the structural and physicochemical properties of RS3 which further affected its biological activity.

Objective: The objective of this study was investigating the effect of different preparation methods on the structural characteristics and physicochemical properties of LRS3.

Methods: LRS3 was prepared by use of the autoclaving method (GP-LRS3), microwave-moisture method (MP-LRS3) and ultrasonic-autoclaving method (UP-LRS3), respectively. Their structure was characterized by size exclusion chromatography connected with multi-angle laser light scattering and refractive index (SEC-MALLS-RI) system, environmental scanning electron microscopy (ESEM), X-ray diffraction (XRD), fourier transform infrared (FT-IR), and solid state ¹³C nuclear magnetic resonance (NMR) spectroscopy. Furthermore, the solubility, swelling power, iodine absorption curve and thermal properties were studied and compared.

Results: The molar mass of LRS3 prepared by autoclaving method (GP-LRS3) and ultrasonic-autoclaving method (UP-LRS3) was mainly distributed in the range of $1.0 \times 10^4 \sim 2 \times 10^4$ g/mol while a decrease of LRS3 prepared by microwave-moisture method (MP-LRS3) was observed. The particle of MP-LRS3 was smaller and relatively smoother while UP-LRS3 was bigger and rougher compared to GP-LRS3. Among these samples, GP-LRS3 exhibited the highest degree of ordered structure and crystallinity, the amorphous region of MP-LRS3 was the biggest and UP-LRS3 displayed the highest degree of double helical structure. The structural differences were attributed to the different preparation methods. Additionally, MP-LRS3 displayed the strongest solubility and swelling power while UP-LRS3 exhibited the strongest iodine absorption ability and thermostability, which were affected by their structural characteristics.

Conclusion: The different physicochemical properties of GP-LRS3, MP-LRS3 and UP-LRS3 were attributed to their different structures. Interestingly, the property was a result of a combination of multiple factors.

SL38: Structural and physicochemical properties of lotus seed starch treated with ultra-high pressure

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ABSTRACT

Ultra-high pressure (UHP) treatment is a non-thermal method for starch modification. UHP-treated starch has unique gelatinization and retrogradation properties compared to heat-gelatinized starch.

The mechanism of pressure-induced starch gelatinization and the effects of UHP treatment on the structural and physicochemical properties of starch attracted widespread interest.

Objective: The objective of this study was evaluating the effects of ultra-high pressure (UHP) treatment on the structural and physicochemical properties of lotus seed starch.

Methods: Aqueous lotus seed starch suspensions (15%, w/w) were subjected to ultra-high pressure treatment (UHP, 100–600 MPa) for 30 min. The structure of lotus seed starch samples was characterized by scanning electron microscopy (SEM), laser diffraction particle size analyzer, X-ray diffraction (XRD), and high performance size exclusion chromatography connected with multi-angle laser light scattering and the refractive index (HPSEC-MALLS-RI) system. Furthermore, the swelling power, solubility, pasting, and thermal properties were studied and compared.

Results: The SEM and laser diffraction particle size analysis revealed that UHP treatment affected the shape and size distribution of starch granules. The morphological structure of starch was completely destroyed at 600 MPa, indicating complete gelatinization. Analysis of HPSEC-MALLS-RI suggested that the dispersity index of UHP-treated starch were decreased from 1.28 to 1.11. According to XRD analyses, UHP treatment converted native starch (C-type) to a B-type pattern. The swelling power and solubility presented a significant decrease at 85 °C and 95 °C, but opposite trends were found at 55 °C–75 °C. The DSC results indicated a reduction in gelatinization temperatures and enthalpy with increasing pressure treatment. The RVA viscosgrams revealed that UHP-treated starch showed a decreased breakdown and setback viscosity, reflecting lower retrogradation tendency compared to native starch.

Conclusion: The results provide a basic and fundamental information of ultra-high pressure processing for starch modification.

SL39: Antithrombotic activity of oral administered low molecular weight fucoidan from *Laminaria japonica* and possible mechanism

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ABSTRACT

Fucoidans extracted from brown algae represent an intriguing group of natural fucose-enriched sulfated polysaccharides, which have excellent antithrombotic activity. However, it is unknown if the fucoidans also have antithrombotic activity when administered orally, a highly desirable feature of antithrombotic agents.

Objective: To establish the relationship among the molecular weight, absorption, and antithrombotic activity of the orally administered fucoidans and the underlying molecular mechanisms.

Methods: The absorption and bioavailability of fucoidan administered by oral route was evaluated by reversed-phase HPLC analysis. The antithrombotic and endotheliocytes regulation effects of fucoidans and action mechanism were evaluated in rats using an electrical induced arterial thrombosis model, adrenaline-induced endotheliocytes injury rat and BaF cell-based models of fibroblast growth factor (FGF) /fibroblast growth factor receptor (FGFR).

Results: Fucoidan fractions from *Laminaria japonica* with different molecular weight (LMW 7600 Da and MMW 35000 Da, respectively) and sulfate ester content were prepared. After single dose of oral administration, the fucose content both in plasma and urea of the LMW fucoidan-treated rats increased up to 2-fold and peaked at 15 h, which was much higher than the MMW fucoidan. Oral administration of the LMW fucoidan for 30 days inhibited thrombosis formation effectively induced by electrical shock in rats, accompanied by moderate anticoagulant activity, significant antiplatelet activity and effective fibrinolysis. However, MMW fucoidan exhibited lower antithrombotic activity for poor absorption. LMW fucoidan also showed more specific protection effect than MMW fucoidan on adrenaline-induced endothelial cells denudation in rat, accompanied with down regulation of von willebrand factor in rat plasma. Meanwhile, the highly sulfated MMW fucoidan and LMW fucoidans could induce FGFR1c-expressing BaF cell proliferation in the presence of FGF-1, -2, -7, -8, -9 and -10, indicating the excellent protection and stimulation on endotheliocytes of LMW fucoidan.

Conclusion: The antithrombotic activity of low anticoagulant LMW fucoidans might be related with the high absorption, significant antiplatelet activity, effective fibrinolysis, stimulation of endotheliocytes and up regulation of FGF/FGFR cell signaling pathways, which is quite different from the MMW fucoidans.

SL40: “Snow lotus” herb’s investigation: pharmacokinetics study of *Saussurea laniceps*

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ABSTRACT

Objective: As a source of Tibetan “Snow lotus”, *Saussurea laniceps* Hand.-Mazz (SL) has the reputation of treating rheumatoid arthritis, stomachache and dysmentorrhea. The current study aimed to find the metabolism pathways and patterns of its key components, umbelliferone and scopoletin.

Methods: Eight components are in the potential metabolism pathways: skimmin, scopolin, umbelliferone, scopoletin, umbelliferone glucuronide, umbelliferone sulfate, scopoletin glucuronide and scopoletin sulfate. A selective and sensitive UPLC-MS/MS method was developed and validated for the determination and pharmacokinetic study of them in rat plasma and urine after oral administration of SL extract.

Results: Umbelliferone and scopoletin were rapidly absorbed into bloodstream after oral administration of the SL extracts to rats. Most of these two compounds started to be biotransformed at around 0.5 hour. We have elucidated that the main metabolic pathway of umbelliferone and scopoletin is their hydroxyl groups be glucuronidated by the UDP-glucuronosyltransferase (UGT), and become umbelliferone glucuronide and scopoletin glucuronide. Meanwhile, some of the hydroxyl groups in umbelliferone and scopoletin were sulfated by the tyrosylprotein sulfotransferases (TPST) into umbelliferone sulfate and scopoletin sulfate.

Conclusion: The described UPLC-MS/MS method was proved to be sensitive and accurate in pharmacokinetic study. The potential metabolic pathways have been concluded based on the results.

SL41: The interactions between green tea and anticancer drug paclitaxel on pharmacodynamics *in vitro* and *in vivo*

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ABSTRACT

Tea drinking, especially green tea, is widely practiced in the world and has recently increased among cancer patients. The study of interactions between green tea and the anti-cancer drug paclitaxel provides an insight for preclinical research.

Objective: This study aimed to preliminarily investigate the interactions between green tea and paclitaxel on pharmacodynamics *in vitro* and *in vivo*.

Methods: For *in vitro* study, cytotoxicity analysis was performed by ATP assay. For *in vivo* study, pre-treatment Balb/c mice group was administered with GTE for 18 days to get into a tea drinking habit. 4T-1 breast cancer cells were inoculated into the mice to establish a transplantation model.

Results: For the *in vitro* study, a low concentration of green tea extract (GTE, 3 µg/ml) significantly increased the inhibition rates of paclitaxel from 40.31 % to 70.23 % in human breast cancer cell lines MCF-7 and from 31.99 % to 41.24 % in mouse breast cancer cell lines 4T-1. Most interestingly, only a high concentration of EGCG (25 µg/ml) could significantly increase the inhibition rates of paclitaxel from 49.69 % to 70.59 % in MCF-7 and from 31.99 % to 57.06 % in 4T-1. This data demonstrates that green tea as a whole may be a more reasonable mixture for paclitaxel chemotherapy than EGCG alone. For the *in vivo* study, the combination significantly inhibited tumour growth at end, whereas the single-agent activity was poor. Moreover, the combination had no significant effect on body weight, compared with paclitaxel alone.

Conclusion: Green tea has the potential to enhance efficacy of paclitaxel on pharmacodynamics for breast cancer.

SL42: Effect of whole coffee fruit extract phytochemicals on antiradical activity and BDNF in healthy subjects

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ABSTRACT

The possible interaction between brain-derived neurotrophic factor (BDNF) and oxidative stress markers (OSM) has not been investigated enough. Whole coffee fruit extracts with high polyphenols content can be used for antiradical activity and BDNF study in healthy humans.

Objective: This study aimed to provide phytochemical composition, antiradical activity and perform to assess the effects of a whole coffee fruits extract (WCFE) on blood levels of BDNF in healthy humans.

Methods: 19 phytochemicals were characterised by LC-MSⁿ and quantified by HPLC-DAD. The antioxidant activity was performed by total free radicals absorbance capacity (ORAC 5.0) assay. *Ex-vivo* formation of reactive oxygen species (ROS) in whole blood was measured using electron spin resonance (ESR) spectroscopy and spin probe CMH. Analysis was performed before and after 60, 120 and 180 minutes after supplementation of a single dose (100 mg) of WCFE or a placebo by five healthy fasted subjects. BDNF was measured using ELISA immunoassay. 25 healthy subjects were randomly divided into groups of five to receive one of the five treatments: WCFE, green coffee caffeine powder (GCCP), grape seed extract (GSE), green coffee bean extract (GCBE) or a placebo (silica dioxide). Plasma samples were collected at time zero and at 30 min intervals afterwards, up to 120 min after consumption of a single 100 mg dose of each material.

Results: A single dose of WCFE significantly decreased a cellular and mitochondrial ROS formation, inhibited generation of extracellular NADPH oxidase-dependent superoxide (O₂⁻) and peroxidase-dependent hydrogen peroxide (H₂O₂). WCFE increased the BDNF plasma level in patients by an average of 137% with respect to baseline (range 65-222%; *P*=0.001 v. placebo). GCCP showed an increase of 42%, but was not statistically significant (*P*=0.49). GCBE did not cause a significant increase in BDNF. GSE increased BDNF levels in plasma by 30%, though not significant (*P*=0.65). Treatment with placebo resulted in 34% reduction in BDNF blood levels (*P*=0.09).

Conclusion: This study results indicate that WCFE could be used to inhibit ROS formation and other oxidative stress markers and for modulation of BDNF-depend health conditions to support an optimal health in humans.

SL43: Effect of polysaccharides from *Tremella fuciformis* on UV-induced photoaging

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ABSTRACT

Overexposure to UV radiation is the main cause of skin photo damage, which is characterized by wrinkling, scaling, dryness, irregular pigmentation, poor elasticity and glossiness and skin cancer. It is worth noting that skin photo aging is currently perceived as a major problem for consumers based on their expenditures for skin care products. Thus, the development of effective preventive agents and strategies on the reduction or control of UV-induced skin damage is desired by consumers.

Objective: The purpose of the present work was to study the effect of oral treatment with polysaccharides from *Tremella fuciformis* Berk (TP) on UV-induced photo aging and begin to determine the possible mechanism(s) for any positive effect of the oral treatment.

Methods: The structural characteristic of in TP were determined using GC-MS, HP-GPC and FT-IR spectrophotometer. The anti-photo aging effects of TP were evaluated using a 30-day UV-irradiated animal assay. The skin moisture, collagen content and total glycosaminoglycan content were measured, as well as the histological analysis in terms of the thickness of the epidermis and dermis and immunohistochemistry for type I and III collagen. Additionally, the activities of SOD, GSH-Px, CAT and the contents of MDA in the serum were also determined.

Results: In the present work, *T. fuciformis* polysaccharides (TP) were extracted with hot water and it was found that TP mainly consist of mannose, followed by xylose, fructose, glucose, and galactose, as well as 10.77% (w/w) uronic acid. TP could efficiently reduce the loss of skin moisture and collagen content and inhibit the increase of glycosaminoglycans. Moreover, a histopathological study indicated that after oral treatment of TP, UV-induced skin structural alterations were alleviated as well as repairing endogenous collagen breakdown and maintaining the ratio of type I/III collagen. The activities of SOD, GSH-Px and CAT were increased. The content of malondialdehyde (MDA) was decreased compared to the irradiated control group without treatment.

Conclusion: TP is a potential therapeutic agent to prevent skin photo aging and can be utilized as a potential functional food supplement for skin function protection.

SL44: ILG suppresses human T lymphocyte activation via modulation of cysteine 46 of I κ B α kinase

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ABSTRACT

Owing to the genomic variations, personalized medicine has drawn more and more attention. It is necessary to clearly elucidate the underlying mechanisms and targets of the drugs or agents which is the premise to fully implement the personalized medicine.

Objective: The inhibitory effect, underlying mechanism and molecular target of ILG, which is derived from a traditional Chinese Medicinal herb, on human T cells activation *in vitro* and *in vivo*, was explored in our current study.

Methods: The proliferation of human T lymphocytes induced by ionomycin plus PMA was measured with the BrdU method. IL-2 and IFN- γ secretion were determined with ELISA methods. The expression of CD25, CD69 and CD71, the T cell activation markers, were determined by flow cytometry. The cell signaling pathways were examined by western blotting. IKK β assay was employed to investigate the effect of ILG on the activity of IKK β . Competition assay was used to elucidate the molecular target of ILG, and transgenic mouse was established to validate the target of the compound.

Results: Our results showed that ILG dose dependently suppressed human T-cell activation resulted from suppression on phosphorylation and degradation of I κ B, and nuclear translocation of NF- κ B. As molecular docking results predicted that Cys-46 probably is the binding site of ILG on IKK β , the competition assay and kinase assay was performed to verify the prediction of molecular docking. Moreover, the IKK β C46A knock-in mice were generated, and we found ILG has less potent immune-suppressive effect in homozygous IKK β C46A mice than IKK-wt mice.

Conclusion: In the current study, we not only explored for the first time the suppressive effect of ILG on the T cells *in vitro* and *in vivo*, but also deeply investigated the underlying molecular mechanisms, suggesting that ILG mediated immune-suppressive effect resulted from direct interaction with IKK β C46A *in vivo* and *in vitro*. ILG might serve as a model compound to develop more potential immunosuppressive agents with clear molecular mechanism for applications in inflammatory and autoimmune diseases in future.

SL45: Combinations of genistein, EGCG and/or resveratrol synergistically inhibit pre-adipocyte differentiation by suppressing PPAR- γ /C/EBP- α pathway

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ABSTRACT

Natural bioactive compounds for preventing and treating obesity are considered as an excellent alternative strategy for developing effective, safe and cost-effective anti-obesity agents. However, the anti-obesity effect of natural bioactive compounds is still controversial in human studies because the high dosages working in cells and animals cannot be reached in humans by consuming foods or supplements.

Objective: The goal of this study is to test whether the combinations of bioactive compounds soy bean genistein (G), green tea epigallocatechin-3-gallate (EGCG or E), and/or grape resveratrol (R) synergistically or additively inhibit pre-adipocyte differentiation and further define its molecular mechanisms in cells.

Methods: Individual or combinations of G (30 μ M), E (30 μ M) and/or R (30 μ M) were treated in differentiated 3T3-L1 cells for 10 days, and G (15 μ M), E (15 μ M) and/or R (15 μ M) were treated in differentiated human primary pre-adipocytes (HPAs) for 15 days. Cell differentiation was evaluated by intracellular lipid accumulation. Total proteins extracted from the cells were subjected to Western blot to measure specific proteins expressions.

Results: Combinations of G, R or E significantly inhibited pre-adipocyte differentiation both in both in 3T3-L1 cells (E+G, 73.0% of control, $P < .05$); R+G, 89.0% of control, $P < .01$; G+E, 62.0.0% of control, $P > .05$; G+E+R, 39.3% of control, $p < 0.01$) and HPAs (E+G, 70.0% of control, $P < .05$; R+G, 77% of control, $P < .01$; G+E, 85% of control, $P > .05$; G+E+R, 60% of control, $p < 0.01$). We also observed a similar pattern that combinations of G, E and/or R synergistically reduced protein expressions of CCAAT-binding proteins alpha (C/EBP α) and peroxisome proliferator activated receptor gamma (PPAR- γ), the two key pre-adipocyte differentiation regulators, both in differentiated 3T3-L1 cells and in HPAs. This suggests that the synergistic anti-adipogenic effect of G, E and/or R may be mediated by the PPAR- γ and C/EBP- α signaling pathways. Moreover, combined G, E and/or R synergistically reduced protein expression of fatty acid binding protein 4 (FABP4), a key molecule in fat drop accumulation in a very similar pattern of these compounds in inhibiting differentiation in 3T3-L1 cells.

Conclusion: This synergistic anti-adipogenic effect of combinations of G, E and/or R may be mediated by suppressing PPAR- γ and C/EBP- α signaling pathways, suggesting that obesity may be prevented simply by consuming several foods containing high level of bioactive compounds.

SL46: Novel anti-inflammatory daucane esters from *Laserpitium zernyi* Hayek (Apiaceae)

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ABSTRACT

Pro-inflammatory transcription factors nuclear factor κ B (NF- κ B) and activator protein 1 (AP-1) play a key role in inflammatory and immune responses. Glucocorticoids repress NF- κ B- and AP-1-mediated inflammatory processes via activating the glucocorticoid receptor (GR), but their prolonged use is associated with detrimental side-effects (1).

Species of the genus *Laserpitium* L. (Apiaceae) are plants used in European traditional medicine to treat inflammatory disorders. Previous investigation showed that some *Laserpitium* species are rich in daucane esters (2).

Objective: From the herb extract of *L. zernyi*, we isolated eight daucane esters, that were tested in bio-assays targeting NF- κ B and AP-1 pro-inflammatory pathways and for their ability to affect GR-driven GRE (Glucocorticoid Response Element)-dependent reporter.

Methods: Phytochemical separation was performed by a flash chromatography and preparative HPLC. Entities were characterized by NMR and HR-MS. Anti-inflammatory activity was tested in a TNF-induced stably integrated recombinant NF- κ B-dependent reporter gene and in the PMA-induced stably integrated recombinant AP-1-dependent reporter gene in A 549 cells.

Results: We isolated one known and six novel jaeschkeanadiol derivatives as well as laserpitin. The highest activity was exerted by a novel compound 10 α -acetoxy-6-angeloyloxy jaeschkeanadiol, that at a concentration of 30 μ M expressed a repression degree of 35.12 \pm 11.44% (TNF at 100%) on NF- κ B and 50.66 \pm 13.51% of repression (PMA at 100%) on AP-1.

Conclusion: Our results suggest that daucane esters isolated from *L. zernyi* herb may be good candidates for treatment of disorders connected with an uncontrolled regulation of NF- κ B and AP-1 signalling pathways, as well as other related inflammatory processes.

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SL48: Phytochemical compounds from *Olea Europaea* leaf extracts have anti-pancreatic cancer activity

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ABSTRACT

Pancreatic cancer is the fifth leading cause of cancer related death in western countries. Resistance to conventional treatment options and toxicity of current chemotherapy agents (e.g. gemcitabine) makes pancreatic cancer a target for the development of novel therapeutic agents. Oleuropein is the most abundant phytochemical compound found in olive leaf products, which has purported anti-cancer, anti-atherogenic and anti-inflammatory properties. Most of the literature regarding olive products has been focused on this compound; however, there are as yet no investigations reporting on olive leaf phytochemicals and pancreatic cancer.

Aims: To analyse olive leaf extracts for their phytochemical properties (including oleuropein content) and assess their anti-pancreatic cancer activity.

Methods: Extracts were prepared with previously optimised methods using water, ethanol or methanol. Phenolic compounds in the extracts were measured with the Folin Ciocalteu's total phenolic compound (TPC) assay and HPLC while the antioxidant capacity was assessed using the FRAP, DPPH and CUPRAC assays. The CCK-8 viability assay was used to assess the cell viability of pancreas cells after treatment with olive leaf extracts compared to controls.

Results: The methanol and ethanol extracts contained higher levels of oleuropein than the water extracts ($p < 0.05$). However, there was no difference in the levels of TPC or antioxidant activity in the organic solvent extracts compared to the water extracts ($p > 0.05$). At 100 and 200 $\mu\text{g/mL}$, all leaf extracts significantly decreased ($p < 0.05$) cell viability of the pancreatic cancer cells compared to control. However, at 50 $\mu\text{g/mL}$, the water extract exhibited the highest anti-proliferative activity ($p > 0.05$) but the effect could not be explained by the phytochemical properties.

Conclusion: Water represents a potentially viable alternative to organic solvents for the extraction of biologically active phenolic compounds from olive leaves. Furthermore, olive leaf compounds possess anti-pancreatic cancer activity and additional investigations are needed to identify these biologically active compounds.

SL49: Aquatic extract of Canadian manufactured phytoplankton significantly reduces clonogenic and metastatic activities in various cancer cells

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ABSTRACT

There has been an increased interest to study marine algae for their multiple bioactivities related to human health. Although the anticancer effects of many edible marine phytoplankton products have been continuously promoted in the natural health product market, comprehensive research studies in this field are still not well implemented.

Objective: The current study aimed to evaluate the anticancer effects of the aquatic extract from a Canadian manufactured phytoplankton product, the Cellton/Celllight phytoplankton, in multiple cancer cell lines.

Methods: Cell growth inhibition and anti-clonogenicity assays were performed in 8 common human cancer cell lines: PC3 and DU145 prostate cancer, MCF7 breast cancer, A549 and H460 lung adenocarcinoma, HCT116 colorectal cancer, BxPC3 pancreatic cancer and MNNG osteosarcoma. The anti-metastasis effect of the phytoplankton product was assessed in mouse renal subcapsule xenograft model with PC3 cells, in comparison with the vehicle control arm. Student's t-test statistic analysis was used to measure the significance of the experimental results.

Results: Cell line-base *in vitro* study demonstrates that the aquatic extract of the Canadian phytoplankton product significantly decreases the clonogenic capacities in all the 8 tested cancer cell lines ($p < 0.01$). *In vivo* xenograft experimental study revealed that the metastatic activity of the tested PC3 cells was greatly diminished in the phytoplankton treated mice, compared with that in controls ($P = 0.034$).

Conclusion: This study clearly revealed the anti-cancer effect of the phytoplankton water extract, although further research is still required to identify the functional active ingredients that are responsible for its anticancer activities.

SL50: Development of inhalable curcumin nanoparticle for lung cancer treatment

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ABSTRACT

Current cancer treatments are not adequate to cure cancer disease as most chemotherapeutic drugs do not differentiate between cancerous and non-cancerous cells; which lead to systemic toxicity and adverse effects. Curcumin is a natural yellow-colored spice derived from *Curcuma longa* with the ability to suppress and inhibit survival of cancer cells but not towards healthy cells.

Objective: This study aimed to fabricate curcumin nanoparticles (Cur-NPs) with uniform and tuneable size (30 to 200 nm) distributions for lung cancer therapy via pulmonary administration.

Methods: Cur-NPs were formulated using solvent and anti-solvent precipitation method. To engineer Cur-NPs with various sizes, different concentrations of pluronic F-127 and incubation time were used. The cytotoxicity of Cur-NPs in different sizes was evaluated on different lung cancer cell lines such as A549 and Calu-3. Different masses of Cur-NPs powders were dispersed in PBS while maintaining the curcumin content at 20 mg/ml. The experiment was carried out in a polycarbonate chamber with controlled temperature ($20 \pm 1^\circ\text{C}$) and relative humidity (above 95%).

Results: In this study, by manipulating of fabrication conditions (i.e. concentration of pluronic F127, temperature) resulted in nanoparticles with tunable size distributions. In general, the cytotoxicity effect followed a size-dependent relationship where NP size was inversely proportional to cytotoxic effect (Cur-NP30 > Cur-NP100 > Cur-NP200 > raw curcumin). The IC₅₀ values of Cur-NP30, Cur-NP100 and Cur-NP200 for A549 cells were 17.3 μM , 24.3 μM , and 26.7 μM ($p < 0.05$), respectively. Meanwhile, the cytotoxicity effect of Cur-NP30, Cur-NP100, and Cur-NP200 on Calu-3 cell line ranged between 14.9 to 25.5 μM . The IC₅₀ value of raw curcumin on A549 was 30.6 μM , which significantly ($p < 0.05$) lower than IC₅₀ value of raw curcumin on Calu-3. The impactor study revealed that, 21.9 ± 3.2 to 26.4 ± 4.6 of total curcumin (from Cur-NPs) was successfully deposited into the lung region.

Conclusion: Raw curcumin is the least cytotoxic towards cancer cells compared to Cur-NP, whereby nanoparticles with the smallest mean size were the most effective in killing cancer cells. Cur-NPs could be delivered via pulmonary administration.

SL51: Screening of Traditional Chinese Medicines for SIRT5 inhibitors as potential therapeutic agents in cancer

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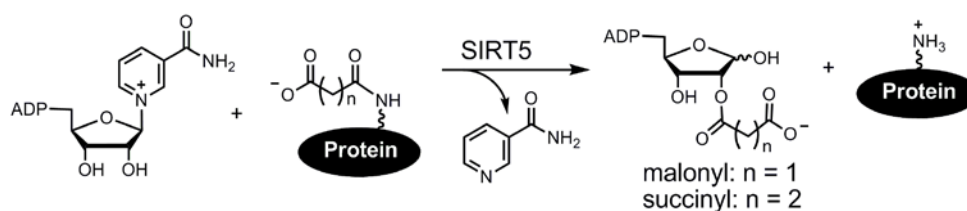
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ABSTRACT

Sirtuins are a class of evolutionarily conserved enzymes with nicotinamide adenine dinucleotide (NAD)-dependent deacylase activity which play important roles in a variety of biological processes including transcription, metabolism, aging (1). Humans have seven sirtuins, SIRT1 to SIRT7. SIRT5, as one of these seven sirtuins, has been demonstrating the potential for treating several human diseases including cancer. Based on our recent discovery that SIRT5 is a demalonylase and desuccinylase (Scheme 1) (2), a novel enzymatic assay for SIRT5, has been developed (3), which becomes a powerful tool to screen SIRT5 inhibitors from Traditional Chinese Medicine (TCM).



Scheme 1. Demalonylation and desuccinylation of SIRT5

Objective: To screen SIRT5 inhibitors from TCM and then to validate their inhibitory potency at both enzymatic and cellular levels.

Methods: An in-house collection of TCM with hundreds of compounds and High-performance Liquid Chromatography (HPLC)-based assay has been used in this screen and IC₅₀ measurements (4). HPLC can exploit the difference in retention time between the acylated substrate (H3K9Su) and deacylated product (H3K9) which can further correlate with sirtuin activity. We also checked the global succinylation level in PC-12 cells to confirm their cellular inhibition.

Results: ~20 compounds from the collection have shown more than 50 % SIRT5 inhibitory effect at 300 μM. Particularly, echinocystic acid (EA) demonstrated a very good inhibitory potency in both enzymatic and cellular levels with an IC₅₀ of 12.5 μM.

Conclusion: Based on the lead compound of EA, this will open up possibilities for the development of more potent and more cell-permeable inhibitors for Sirt5 to explore the therapeutic potential of Sirt5 inhibition in cancer.

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SL52: Polysaccharides with immunomodulating activity from *Codonopsis pilosula* roots

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ABSTRACT

Codonopsis pilosula polysaccharides have been shown to have lots of biological activities, such as immunomodulating, antitumor and radical scavenging activity. Only a few of these compounds have been characterized, and most of them are neutral polysaccharides. It was of interest to characterize the pectic polysaccharides with immunomodulating activity from *C. pilosula*.

Objective: This study aimed to isolate pectic polysaccharides and their enzyme degradation products, and determine their structure and complement fixing activity.

Methods: The roots of *C. pilosula* were sequentially extracted with ethanol, 50% ethanol, 50 and 100°C water using an accelerated solvent extractor. Ion exchange chromatography and gel filtration were applied to purify the polysaccharides from water extracts. The most abundant polysaccharides were further degraded by pectinase, and the main degradation products were obtained by gel filtration. The structures of these fractions were characterized by GC, GC-MS and NMR. Complement fixation assay was applied to guide the purification progress, and investigate the activity of all purified fractions.

Results: Two pectic polysaccharides, 50WCP-II-I and 100WCP-II-I, and their pectinase degradation sub-fractions 50WCP-II-Ia and 100WCP-II-Ia were obtained by ion exchange chromatography and gel filtration. These four fractions showed strong complement fixation activity, with ICH₅₀ value of 31.7 µg/ml (50WCP-II-I), 6.5 µg/ml (50WCP-II-Ia), and 32 µg/ml (100WCP-II-I), 12.2 µg/ml (100WCP-II-Ia). The study of the sub-fractions obtained after pectinase degradation showed that the complement fixation activities of these pectins are expressed mainly by their ramified regions. The structure studies of native and sub-fractions showed the 50WCP-II-I is a pectic polysaccharide, with long homogalacturonan regions (some of the galacturonic acid units were methyl esterified), interrupted by one short rhamnogalacturonan I (RG-I) region. The side chains of the RG-I region are arabinogalactan type I (AG-I) and type II (AG-II) attached on position 4 of rhamnose. The 100WCP-II-I has two main ramified regions, one is galacturonan region with AG-I side chain on position 2 of GalA, and the other one is RG-I region with AG-II side chain on position 4 of Rha.

Conclusion: The polysaccharides from *C. pilosula* showed high activity in complement fixation assay, and may therefore have effect on the human immune system. This might be seen as a rationale for the traditional use of *C. pilosula* to boost immunity.

SL53: Investigating phyto-constituents and *in-vivo* hair growth promotion of the leaf of *Hibiscus rosa sinensis* plant

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ABSTRACT

Hair growth promoting agents are in high demand because of psychosocial effects of alopecia on humans, especially cancer patients undergoing chemotherapy. Plants have been employed since ancient times in traditional medicine for hair growth promotion.

Objective: In this study, the leaves of *Hibiscus rosa-sinensis*, a common herb in Nigeria which is acclaimed traditionally for hair growth-promoting potential, was investigated.

Methods: The ethanolic extract of the leaves of *H. rosa-sinensis* was subjected to phytochemical screening using standard methods already adopted (1). The proximate compositions were determined by the official methods of analysis (2) and the hair growth investigation of the ethanolic extract was carried using established protocols at concentrations of 2.5 mg/ml, 5 mg/ml and 10 mg/ml (3). Four groups of albino rats were used.

Results: The results of the phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenols, steroids and terpenoids. The proximate analysis showed that the leaves have a high protein (48.57%) content. Carbohydrates, ash, water, fiber and lipids were also present in descending order. The micro- and macronutrients were determined to be magnesium (91.52 mg/100g), sodium (20.40 mg/100g), iron (12.31 mg/100g), potassium (9.70 mg/100g), manganese (8.90 mg/100g), calcium (7.57 mg/100g), zinc (4.80 mg/100g), and copper (0.23 mg/100g). Complete hair regrowth was observed after 21 days of treatment with the 5 mg/ml concentration of the leaf ethanolic extract, petroleum jelly was the control.

Conclusion: The result of this study suggests that the leaves of *Hibiscus rosa-sinensis* have hair growth and probably hair loss prevention potentials.

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SL55: Colored mulberry fruit extract as a potential natural colorant

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ABSTRACT

There is an increasing consumer demand for scientific interest in new natural colorants. Mulberry is known to be rich in phenolics and anthocyanins. These compounds give berries their distinctive colours and, more importantly, have several health benefits including the prevention of heart disease, cancer and inflammatory diseases.

Objective: Extracts from berries are examples of food-derived natural colorants. This study aimed to extract potent solvent fraction exhibiting good colorant with stable pH and temperature property and excellent biological activities

Methods: Mulberry fruits (*Morus alba* L.) in water develops a black red color ($\lambda_{\text{max, visible}} = 480 \text{ nm}$) in a time-dependent manner. Heat treatment of the fruit extract prevented colour development, whereas the addition of exogenous polyphenol oxidase (PPO) but not peroxidase restored colour development. Colour development was also inhibited by the addition of tropolone, an inhibitor of PPO. Colour formation resulted in a decrease in the concentration of polyphenols indicating utilization for colour formation. Antioxidant activities of fractions were determined by using DPPH and FRAP reagent.

Results: The black red colour intensified as the pH was adjusted from 3.0 to 12.0, and these changes were only partially reversible when pH was adjusted from 7 to 11 in the presence of oxygen, but completely reversible when the pH was changed in the absence of oxygen. The colour was found to be stable in solution at $-20 \text{ }^{\circ}\text{C}$ for 2 mo. All mulberry extracts showed potent antioxidant activity towards DPPH and FRAP reagents as compared to quercetin as a standard.

Conclusion: There is a growing public and scientific interest in the development of natural alternatives to synthetic colorants in foods. Our results suggested that the mulberry fruit extract represents a potential source of new natural colorants for functional food development.

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P: Posters

P1: HPLC isolation, purification and analysis of saponins from ginseng oolong

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ABSTRACT

Ginseng based medicines help to build up general vitality and increase resistance to physical, chemical and biological stress. As a result, ginseng based products are widely used as biologically active dietary supplements. Ginsenosides are considered as the main active principle of the traditional medicine “ginseng” but the chemical composition of different ginseng based formulations, food products and supplements are not fully investigated.

Objective: The goal of this study was to obtain significant amounts of the bioactive compounds from ginseng oolong tea for their further structural analysis using NMR and HPLC-MS/MS.

Methods: Ultra-sound assistant extraction with methanol and water was performed. Dried extract was dissolved in water. Ether was used for liquid-liquid extraction of impurities. In the next step, the compounds of interest were extracted with n-butanol and the extract was dried and dissolved in methanol. HPLC isolation was carried out using a reversed-phase chromatography with SB-C18 sorbent. For compounds identification, electrospray ionization and quadrupole/linear ion trap mass-spectrometer (ESI-LITMS), ^{13}C and ^1H NMR were used.

Results: Ginseng oolong extract HPLC-LITMS analysis in positive and negative detection modes have showed the group of peaks which mass-spectra were close to those of ginsenosides from the roots of *Panax ginseng*. Obtained mass-spectra have shown new fragmentation pattern of the unknown saponin. The composition of the sugar chains was also determined using MS/MS data. The comparison of the obtained mass-spectra and mass-spectra of standard protopanaxadiol, protopanaxatriol, ocotillol-type ginsenosides was made. HPLC-MS based separation and collection of the saponin fraction containing one of the compounds in the selected ion monitoring mode was done. Dried extract was dissolved in pyridine- d_5 : D_2O mixture (7:1). Different NMR experiments including ^1H - ^1H COSY и ^1H - ^{13}C HMBC were conducted. Obtained NMR data was analysed to confirm proposed new saponin structure.

Conclusion: The structures of several compounds present in ginseng oolong were proposed based on HPLC-MS/MS data and the NMR analysis data of the isolated saponin fraction have confirmed the saponin structure of these compounds.

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P2: Anti-inflammatory and antioxidant potencies of ethyl acetate fractions of *Pandanus tectorius* fruits from keys part on LPS-stimulated RAW264.7

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ABSTRACT

Inflammation is a complex process involving a multifactorial network of chemical signals to mediate the action. Infections by microorganisms will trigger inflammation as a part of host defensive responds. Some of the antioxidant or phenolic compounds are capable to evade the immune response by inhibiting or reducing parts of inflammatory pathways.

Objective: This study aimed to investigate the anti-inflammatory and antioxidant potencies of phenolic-rich ethyl acetate fraction from keys part of *Pandanus tectorius* fruits.

Methods: Ethyl acetate extract was obtained by sequential extraction based on solvent polarity and the fractions were yielded using silica gel column chromatography. The cytotoxicity of the ethyl acetate fractions against RAW264.7 cell lines was conducted via MTT assay. The anti-inflammatory activity was observed by iNOS inhibition pathway using Griess reagent. Antioxidant activity of the ethyl acetate fractions of *P. tectorius* fruits were determined by DPPH free radical scavenging. Total phenolic and TLC profiling were also determined.

Results: Result showed that some ethyl acetate fractions of *P. tectorius* fruits revealed strong antioxidant property (>70%) and high phenolic contents (>65%). Furthermore, all of ethyl acetate fractions were considered non-toxic against RAW 264.7 cell lines, with the IC₅₀ more than 30µg/mL. The higher anti-inflammatory potency was shown for fraction F3x and F3y with the percentage of NO reduction at 46% and 35%, respectively. This study proved that ethyl acetate extract and its fractions from *P. tectorius* fruits in Batu rakit, Kuala Terengganu, Malaysia have antioxidant and anti-inflammatory potencies.

Conclusion: Ethyl acetate fractions of *P. tectorius* fruits from keys part are a source of phenolic compounds with anti-inflammatory and antioxidant activities.

P3: Morphological, physicochemical and antioxidant profile of non-commercial banana cultivars

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ABSTRACT

Though not sold on commercial basis, mostly due to their flavour, shape, size and number of fingers per bunch, indigenous banana cultivars are said to contain functional and health beneficial properties which can be utilized on profiling of these cultivars.

Objective: Banana cultivars - Luvhele (*Musa* ABB), Mabonde (*Musa* AAA) and Muomva-red (*Musa balbisiana*) were characterized for morphological, physicochemical and antioxidant properties.

Methods: Morphological properties of fruit length (outer and inner curve of fruit) and girth (distal end, widest midpoint and proximal end of fruit) as well as physicochemical properties of pH, titratable acidity, total soluble solids and ash content were profiled for all three banana cultivars. Non-commercial banana cultivars were pre-treated with ascorbic, citric and lactic acid, milled to flour and viewed under a scanning electron microscope. Antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method while total polyphenols of cultivars was determined using the Folin-Ciocalteu colorimetric method.

Results: All three cultivars varied significantly ($p < 0.05$) in their morphology, pH, titratable acidity and total soluble solids with no significant difference in their ash content. Individual cultivars showed variations in flour starch granule when observed under the scanning electron microscope. Characterization of cultivars for total polyphenols (TPs) and antioxidant activity upon pre-treatment with ascorbic, citric and lactic acid shows that the DPPH radical scavenging assay results varied significantly with the Muomva-red cultivar (1.02 ± 0.01 mg GA/g) expressing the highest DPPH activity at a lactic acid concentration of 20 g/l. Total polyphenol content was also highest for the Muomva-red [1091.76 ± 122.81 mg GAE/100 g (d.w.)] non-commercial banana cultivar.

Conclusion: The high amount of TPs present in these cultivars make them a suitable source of bio-nutrients with medicinal and health functions.

P4: The qualitative analysis of phytochemicals present in some medicinal plants of Nepal

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ABSTRACT

Objective: Plants containing active biocompounds which are used for curing different human diseases are termed as Medicinal Plants. The main objective of our research work was to check the presence or absence of the phytochemical constituents in the selected medicinal plants.

Method: This present study involves phytochemical analysis from aqueous leaf extract of four different medicinal plants of different taxonomical identification viz: Rubus Ellipticus, Moringa Oleifera, Cajanus Cajun, and chenopodium Album, which were collected from Biratnagar and Dhankuta region of Nepal for the phytochemical screening. The aqueous leaf extract (3ml) of all the plants was taken and preliminary phytochemical screening was done for the presence of alkaloid, tannin, saponin, flavonoid, terpenoid, reducing sugar, and phlobatannin.

Results: Among these plants Rubus ellipticus contain all the phytochemical where as Moringa oliefera and Chenopodium album contain all the phytochemical except phlobatanin and reducing sugar and Cajanus cajan contain all the phytochemical except reducing sugar and saponin. The presence of all these phytochemical in the plant indicates that they could be use in the treatment of burns and wounds and the presence of alkaloid , flavonoid and terpenoid content of plant suggest theirs antioxidant potential and a source of therapeutic agent.

Conclusions: It is expected that the important phytochemical properties recognized in our study by the indigenous medicinal plants of Nepal will be very helpful in the curing of various diseases of this region and led a base to study the respective plants for others regions, too and also develop scope for the pharmaceutical industries for the development of new bio drugs.

P5: Antitussive and bronchodilatory properties of glycoconjugates from medicinal plants

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ABSTRACT

The curative effects of medicinal plants used in traditional medicine were evaluated only on the basis of the empirical practice. Searching for active compounds with antitussive and bronchodilatory properties, several plants were investigated in term of chemical structure and pharmacodynamics properties of herb glycoconjugates.

Objective: The study focused on extractable polymeric compounds from flowering parts of medicinal plants with interesting pharmacodynamics properties.

Methods: Flowering parts of herbs were minced and extracted by alkaline solutions. Supernatants were neutralized and extracted with organic solvents, dialyzed and freeze-dried to give crude glycoconjugates. Carbohydrate, phenolic and protein contents were estimated by the phenol-sulfuric acid, Folin-Ciocalteu and Lowry methods, respectively. Uronic acids were determined by m-hydroxybiphenyl reagent. Neutral sugars were estimated by g.l.c. and molecular mass by HPLC. The method of citric acid for assessing the cough reflex (1) and the plethysmograph technique for evaluation of bronchoactive effect were used (2).

Results: Alkaline extractions of herbs afforded the dark-brown glycoconjugates differing in molecular mass, carbohydrate, phenolic and protein contents. Carbohydrate parts of conjugates were rich mainly in rhamnogalacturonans/galacturonans and arabinogalactans. Antitussive activity tests showed in some conjugates the reduction of the number of cough efforts in the dose-dependent manner, while in some conjugates this dose dependence was not observed. The tests evaluating the influence of different doses of conjugates on airway smooth muscle reactivity revealed more significant effect of some plant conjugates in comparison with a commercial bronchodilator used. Comparative tests showed that antitussive activity of the most effective conjugate was lower than that of codeine, the strongest antitussive drug used in the clinical practice.

Conclusion: Glycoconjugates (polysaccharide-phenolic complexes) from *E. canadensis*, *L. salicaria*, *A. montana* and *E. purpurea* were shown to be promising candidates for the application in the herbal medicine as antitussive and bronchodilatory agents.

Acknowledgements: Study was supported by the VEGA Grant 2/0018/15 and the APVV-0305-12, and 0125-11.

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P6: Production of Angiotensin-I-converting enzyme inhibitory peptides from tilapia protein using cyclic batch enzymatic membrane reactor

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ABSTRACT

The use of enzymatic membrane reactor to integrate a reaction vessel with a membrane separation unit is emerging as a beneficial method for producing bioactive peptides.

Objective: In this study, the cyclic batch enzymatic membrane reactor (CBEMR) was employed to produce Angiotensin-I-converting enzyme (ACE) inhibitory peptides from tilapia protein.

Methods: A hollow fiber membrane (MWCO 1 kDa) was equipped with stirred reactor tank. The enzymatic hydrolysis and separation of peptides conditions were performed at constant temperature (50 °C) and pH (8). The investigation was focused on the effect of process parameters on ACE inhibitory peptides conversion and productivity during CBEMR. Furthermore, the influence of gas sparging, with gas injection factor of 0, 0.25, 0.35 and 0.50 on permeate flux and ACE inhibitory peptides capacity were studied. This work used Completely Randomized Design for experiments.

Results: It was found that proteins have to be pre-hydrolyzed for 90 min with Alcalase 2.4L before introduce to CBEMR to reduce flux decline due to fouling. Operating at transmembrane pressure (TMP) of 1.3 bar and cross flow velocity (CFV) of 1.5 m/s, gave the highest ACE inhibitory conversion and productivity. The use of gas-liquid two-phase flow could reduce membrane fouling and increase the permeate flux, depending on gas injection factor. Moreover, the gas sparging at gas injection of 0.5 led to a significant increase of ACE inhibitory peptides capacity. The ACE inhibitory activity conversion and productivity were 202 % and 0.9 mg ACE inhibitory peptides/unit of enzyme, respectively.

Conclusion: This result indicates that CBEMR was successfully employed to produce bioactive peptides.

P7: Using LC-MS based metabolomics to discriminate cold pressed rice bran oils producing from two different cultivars of *Oryza sativa* L. ssp. *indica* in Thailand

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ABSTRACT

Objective: A newly developed liquid chromatography mass spectrometry (LC-MS) method for analysis of cold pressed rice bran oil (RBO) was established and used to discriminate RBO produced from two different cultivars of major Thai fragrant rice species.

Method: Two different cultivars of fragrant rice, Khaw-Hom-Mali and Khaw-Hom-Pathum, were collected and the cold pressed RBO was prepared using the screw compression method. The samples were subjected into LC-MS with a quadrupole-ion trap to analyze and quantify the active pharmaceutical compounds. The LC-MS data was preprocessed with MZmine 2.10 program before analyzing with principal component analysis using SIMCA 13 software (1, 2).

Results: The LC-MS method was capable of detecting and quantifying several kinds of valuable constituents such as, fatty acids, vitamin E, and γ -oryzanol. The chromatographic condition was feasible. Short time for analysis and simple method were achieved. Ten metabolites were identified by comparing the retention times and mass spectrum of the RBO sample with reference standards. From PCA score plot and loading plot, two rice cultivars samples can be clearly separated and it was revealed that Khaw-Hom-Pathum was more suitable than Khaw-Hom-Mali in cold pressed RBO production since it contained a high amount of total γ -oryzanol and small amount of saturated free fatty acid.

Conclusion: Ten metabolites were selected as the markers in LC-MS analysis of RBO. To the best of our knowledge, this is the first report which can be used to analyze fatty acids, vitamin E, and γ -oryzanol in cold pressed RBO from Thai fragrant rice species in one single run. Moreover, the LC-MS based metabolomics platform in this study can be used in a quality control process of RBO dietary supplements.

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P8: Variation of phytochemical profiles, antioxidant and antiproliferative activities in different parts of *Citrus reticulata* Blanco cv. Chachiensis during fruit ripening and maturity

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ABSTRACT

Citrus is a rich source of nutritive and bioactive compounds. Dried peel of *Citrus reticulata* Blanco cv. Chachiensis (Chachi) is the major source of the Pericarpium Citri Reticulatae (Chenpi), which has been widely used as dietary condiment and traditional medicine to remedy indigestion and respiratory diseases.

Objective: This present study aimed to evaluate the impact of fruit tissues and ripening stage on the phytochemicals in *C. reticulata* Blanco cv. Chachiensis (Chachi) and to analyze the relation between antioxidant property, antiproliferative activity and the measured variables.

Methods: The variation in content of vitamin C, total phenolics, flavonoids and phenolic composition as well as antioxidant and antiproliferative activity among peel, flesh and seed of immature, semi-mature and mature Chachiensis was investigated.

Results: Significant variations were observed in vitamin C, total phenolics and flavonoids contents, phenolic compounds as well as antioxidant and antiproliferative activities among peel, flesh and seed of immature, semi mature and mature Chachiensis. Elevated levels of total phenolics, phenolic compounds such as hesperidin and naringin, and strong antioxidant capacities were found in peel at maturity, whereas the strongest antiproliferative activity against MCF-7 and highest amount of nobiletin were deliberated in immature peel. In flesh, the vitamin C content increased during ripening, while total phenolic content, ORAC value and antiproliferative activity decreased. The flavonoid content and antioxidant capacity in seed were relatively constant during ripening and comparable with flesh, which suggested seeds as a valuable source of flavonoid extraction for further utilization.

Conclusion: Hopefully, these results will promote the comprehensive utilization of phytochemicals in different parts of this medicinal and edible citrus fruit.

P9: Green tea polysaccharide ameliorate high-fat diet-induced metabolic syndrome (MetS) in type 2 diabetic mellitus (T2DM) mice

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ABSTRACT

Metabolic syndrome (MetS), a cluster of medical disorders that poses a risk of diabetes and cardiovascular disease, has become a significant public health problem worldwide. Promising substances from natural resources have become a new direction for researchers on treatment of MetS.

Objective: The aim of the study was to figure out the efficacy of green tea polysaccharide (GTPS) on high-fat diet-induced MetS in type 2 diabetic mellitus (T2DM) mice.

Methods: Mice were fed with high fat diet for four weeks to induce obesity and insulin resistance and injected intraperitoneally with streptozotocin (STZ) to develop type 2 diabetes model. Model mice were gavaged once daily with either distilled water, GTPS or rosiglitazone for four weeks.

Results: The results showed that the lipid profile was ameliorated nearly to the normal level. Oral glucose tolerance test (OGTT) and level of serum insulin indicated that GTPS reduced the insulin resistance. The high blood pressure of the mice was also relieved by the administration of GTPS. Enzyme activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in liver and kidney of were notably upregulated. GTPS also exert cytoprotective action on tissues in the experiment. The biochemistry index in metabolic syndrome (MetS) in type 2 diabetic mellitus (T2DM) mice was determined.

Conclusions: Green tea polysaccharide prevented high-fat diet-induced metabolic syndrome (MetS) in type 2 diabetic mellitus (T2DM) mice and it might be a good candidate for healthy food industry.

P10: Total tanshinones induce apoptosis and autophagy via reactive oxygen species generation in lung cancer 95D cells

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ABSTRACT

Objective: Tanshinones are main bioactive constituents isolated from the root of *Salvia miltiorrhiza* Bunge (Danshen). The study mainly focused on the anti-cancer effect and mechanisms of total tanshinones in 95D lung cancer cells.

Methods: The constituents of total tanshinones (TDT) were analyzed with HPLC. The quantitative determination of apoptosis using Annexin V/7-AAD double staining and the generation of reactive oxygen species (ROS) were performed on flow cytometry. The morphological assay such as MDC staining was utilized to determine vacuoles. The effect of TDT on mitochondrial membrane potential (MMP) was investigated qualitatively and quantitatively. The proteins' expressions were measured by western blot.

Results: The contents of dihydrotanshinone I, cryptotanshinone, tanshinone I, and tanshinone IIA in TDT were 5.56 %, 0.10 %, 0.73 %, and 55.13 %, respectively. The dose-dependent manner and time-dependent manner inhibitory effect of TDT on 95D cells were observed with calculated IC₅₀ of 4.4 µg/mL after 24 h treatment. TDT induced apoptosis in 95D cells as evidenced by the decrease in MMP and the expression of apoptotic proteins. TDT also induced autophagic vacuole accumulation in MDC staining and autophagic proteins expression. Furthermore, TDT induced ROS generation and inhibition of ROS partly reverse apoptosis and autophagy. In addition, autophagy inhibitors 3-MA and bafilomycin A1 facilitated that TDT induced cell death.

Conclusion: Our study demonstrated that TDT, a total tanshinone extract, inhibited 95D cancer cells proliferation in vitro. TDT induced apoptosis and pro-survival autophagy by increasing intracellular ROS formation.

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P11: Analysis of the transcriptase of *Dryopteris erythrosora* and predict genes for the biosynthesis of flavonoids

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ABSTRACT

Dryopteris erythrosora is a fern specie and used as a medicinal plant in China. The main active ingredients in this plant are flavonoids. However, the biosynthetic pathway of flavonoids in most ferns is still unclear. Furthermore, there is no reference genome sequence of this plant.

Objective: This study aimed to analyse the information of *D. erythrosora* transcriptome and find key genes related to the biosynthesis of flavonoids.

Methods: A cDNA library from *D. erythrosora* was constructed and sequenced by Illumina Hiseq 2000. De novo was assembly carried out with short reads into length of unigenes. Then, the unigenes were annotated to the NR, NT, Swiss-Prot, KEGG, COG, GO databases.

Result: Using the RNA-Seq technology, 100,542,050 raw sequencing reads were generated and assembled into 143,604 unigenes, of which about 48.7% (69,917 unigenes) were annotated. The species distribution showed that the transcripts are more similar to cryptogams, *Physcomitrella patens* (18.09%), *Selaginella moellendorffii* (17.94%), which reflect their close evolutionary relationship. Biological pathway analysis revealed 212 unigenes in flavonoid biosynthesis. The unigenes encoding chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol reductase (DFR), leucoantho-cyanidin dioxygenase (LDOX) and other six genes. Six chalcone superfamily genes, which might encode chalcone isomerase transforming naringenin chalcone into naringenin, were discovered.

Conclusion: To our knowledge, this is the first report of a transcriptome sequence from *D. erythrosora*. Candidate genes involved in flavonoid biosynthesis were identified. This study provides the basis for further genomics research in ferns.

P12: Mass spectrometry-based metabolomics approach for the metabolite profiling and biomarkers discovery of *Clinacanthus nutans* and its tissue cultures

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ABSTRACT

Plant metabolomics has become a new frontier in phytochemical analysis to identify and quantify the metabolites contained in plant cells which correlate with its biochemical phenotype. *Clinacanthus nutans* (Acanthaceae) is known as “Daun Belalai Gajah” or “Sabah Snake Grass” in Malaysia, which is gaining research interest since the plant has been used by local people to treat various ailments and also has been reported to possess various bioactivities.

Objective: The present study aimed to profile and identify the biomarker compounds of *C. nutans* plants and its tissue cultures using mass spectrometry-based metabolomics approach.

Methods: In the study, the chemical profiles of the purchased *C. nutans* plants, self-cultivated plants (6 month-old), regenerated plants via micropropagation (6 month-old), callus (3 month-old) and adventitious roots (1 year-old) were analysed using gas chromatography-mass (GC-MS) spectrometry approach. The generated raw data files were processed with Agilent’s MSD Chemstation Data Analysis and Mass Professional Profiler software for analysis. Then, key biomarker compounds of the plants and its tissue cultures were studied and identified.

Results: Using this high-throughput instrument coupled with advanced bioinformatics software, a large number of bioactive compounds were found in *C. nutans* and its tissue cultures. The plants were found to be rich in four categories of compounds which were (a) terpenes such as phytol and squalene; (b) pentacyclic triterpenoids such as such lupeol, betulin, beta-amyrin; (c) phytosterols such as stigmaterol, beta-sitosterol, campesterol; and (d) others such as alpha-tocopherol, Didecan-2-yl phthalate, beta-tocopherol. Via micropropagation of the node parts, the 6 month-old regenerated plants were found to accumulate higher abundance of the identified compounds (at least 3-fold and above) as compared to other samples. Besides, the 3 month-old callus and 1 year-old adventitious roots were also found to accumulate squalene and lupeol and the abundance was higher than the plants.

Conclusion: As the metabolome of *C. nutans* unfolds, it is believed that such information will play critical role in the standardization and development of herbal drugs derived from the plant in the future.

P13: A mixture of grape pomace and omija fruit alleviates hyperglycemia and oxidative stress in diet-induced obese mice

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ABSTRACT

Objective: A high-fat diet (HFD) is thought to be one of the main environmental factors for obesity in which state oxidative stress is an important pathogenic mechanism of obesity-associated metabolic syndrome including diabetes. Polyphenols-rich food plants are known to improve obesity-related diseases. The aim of this study was to investigate the effects of grape pomace and omija fruit (GPOF) on hyperglycemia and oxidative stress in diet-induced obese (DIO) mice.

Methods: Male C57BL/6J mice were fed a HFD (20% fat, 1% cholesterol, w/w) with grape pomace (GP, 0.5%, w/w) or GP plus omija fruit (GPOF, 0.5% GP plus 0.05% omija fruit, w/w) for 12 weeks. A 1 g GP diet contains 0.26 mg of total flavonoid and 0.475 mg of total polyphenol and a 1 g GPOF diet contains 0.2635 mg of total flavonoid and 0.491 mg of total polyphenol.

Results: GP and GPOF significantly lowered fasting blood glucose level and insulin/glucagon ratio compared to the control, whereas increased plasma glucagon level. Supplementation with GP or GPOF improved glucose tolerance and expressions of pancreatic insulin and glucagon with preservation of α - and β -cells. GPOF particularly seems to improve insulin sensitivity by reducing plasma insulin and homeostasis model assessment of insulin resistance levels. Erythrocyte glutathione peroxidase and glutathione reductase activities were significantly lowered in the GPOF group than in the control group, along with decreases in erythrocyte hydrogen peroxide and thiobarbituric acid-reactive substance levels.

Conclusions: These results suggest that GPOF rich in flavonoids may be beneficial in preventing increased risk factors of diabetes in obesity including fasting hyperglycemia, glucose intolerance and oxidative stress.

P14: Long-term supplementation of a high functional sugar alters inflammatory cytokine levels via body weight reduction in C57BL/6J mice given iso-caloric high-fat diet

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ABSTRACT

Recently there has been a global shift in diet towards an increased intake of energy-dense foods that are high in fat and sugars. Sugar substitutes are used to reduce calories while maintaining a sweet taste in the diet. D-psicose, a C-3 epimer of D-fructose, has been reported to playing a role as an anti-obese and anti-diabetic agent, however, its anti-inflammatory activity has not yet been completely understood.

Objective: We hypothesized that D-psicose may exert direct or indirect effects on the regulation of inflammatory cytokines in mice.

Methods: Sixty male C57BL/6J mice which are prone to diet induced obesity were divided into six dietary groups. They were fed a normal diet, a high-fat diet (HFD) (20%fat, 1 % cholesterol, wt/wt), HFD substituted with Erythritol (ERY), D-glucose (GLU), D-fructose (FRU) and D-psicose substituted sugar for 16 weeks. We analysed both biochemical changes to compare the biological effect of sugar substitutes.

Results: Long-term consumption of D-psicose reduced body weight as well as adipose tissue weight. Also, D-psicose supplementation in HFD-fed mice led to a remarkable decrease in peroxide levels, towards normal levels. To test whether D-psicose plays a role as a direct anti-oxidant, we determined activities of anti-oxidant enzymes including SOD, CAT, GSH-Px, GR, H₂O₂ and paraoxonase. However, those markers were not significantly affected by D-psicose supplementation in HFD fed-mice. Plasma inflammatory adipokines including TNF- α , IL-1 β , IL-6, MCP-1, IFN- γ and PAI-1 were elevated in the HFD group compared to the ND group. However, D-psicose supplement led to a significant decrease in plasma inflammatory adipokine levels with a simultaneous decrease in WAT mass towards a similar level in ND fed mice.

Conclusion: D-psicose supplement with an iso-caloric high-fat diet decreased inflammatory cytokine concentration to normal levels with concomitant reduction in body fat mass. It is plausible that anti-inflammatory property of D-psicose is exerted via body weight reduction in high-fat fed mice.

P15: Inhibitory effects of geranic acid derivatives on melanin biosynthesis

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ABSTRACT

Citronellol (3,7-dimethyl-6-octen-1-ol), citronellic acid (3,7-dimethyl-6-octenoic acid), geraniol (3,7-dimethylocta-2,6-dien-1-ol) and geranic acid (3,7-dimethyl-2,6-octadienoic acid) are flavor compounds that are used as perfume in certain cosmetics. These compounds are widely distributed aroma components that occur naturally in plants, and are detected principally in *Cymbopogon citrates*, *Rosa spp.* and *Vitis vinifera L.*

Objective: The principal objectives of this study were to evaluate the inhibitory effects of these compounds on cell viability and melanin production in melanocytes, and also to assess their effects on the expression of melanin biosynthesis-associated enzymes, including tyrosinase.

Methods: To evaluate the depigmenting ability of the geranic acid derivatives, Melan-a cells were employed in this study. Melan-a cells are highly pigmented melanocytes and provide an excellent parallel non-tumorigenic cell line derived from C57BL/6 mice. In addition, we assessed the regulatory effects of geranic acid on the expression of melanin generation-related protein in Melan-a cells via Western immunoblotting analysis. Tyrosinase activity was measured by its DOPA-oxidase activity.

Results: Treatment with geranic acid from 5 to 500 μ M dose dependently reduced cellular melanin contents with low toxicity. Geranic acid suppressed only 8.3% of viable cells, but reduced the melanin production by 43.9% at 500 μ M. In contrast, geraniol and citronellol evidenced high cell toxicity above 50 μ M, and citronellic acid did not inhibit melanin production at any concentration. Moreover, geranic acid also inhibited tyrosinase activity and intracellular tyrosinase expression in a dose-dependent manner. This is the first report of depigmenting properties of geranic acid in melanocytes. Overall, these results indicated that geranic acid may prove useful not only as a perfuming agent but also as a skin depigmentation agent.

Conclusion: These results showed that geranic acid may function as a skin depigmenting agent via the inhibition of tyrosinase activity and expression within melanocytes.

P16: The influence of a modifier on the recovery of bioactive aglycone from chamomile by superheated water

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ABSTRACT

For efficient isolation and recovery of valuable compounds from natural sources, the extraction process is a crucial step. In the last few decades, special attention of scientific community has been directed towards the development of techniques that could respond to several demands simultaneously: providing high yields of target components being environmentally friendly; and finally being economically viable. Green extraction approaches tend to respond well to set requirements. Among different solvents, water in its subcritical state is particularly attractive and extraction with subcritical water has gained remarkable popularity in different applications. Moreover, the high hydrolytical potential of superheated water can be used for simultaneous extraction and derivatization of myriad of natural organic compounds.

Objective: In the present study the influence of a modifier (hydrochloric acid) on the extraction and hydrolysis of apigenin-7-O- β -glycoside from chamomile flowers by using superheated water was investigated. The obtained results were compared to those obtained without the addition of a modifier.

Methods: Four different concentrations of hydrochloric acid (0.001; 0.005; 0.01; 0.05 mol/L) were used in order to examine the influence of this modifier on the hydrolytical potential of superheated water in the recovery of apigenin from its bound forms. The determination of apigenin and apigenin-7-O- β -glycoside was performed by HPTLC/UV-VIS. Total phenols and flavonoids were quantified by spectrometry.

Results: Different yields of phenols and flavonoids were noticed for different concentrations of acid modifier. The total phenols content was in the range from 3.97 (for the concentration of 0.05 mol/L) to 5.74 mg/mL (for the concentration of 0.001 mol/L). As in the case of phenols, the greatest contents of flavonoids were observed for acid concentration of 0.001 mol/L (1.12 mg/mL). Hydrolysis (or more precisely acetolysis) of apigenin-7-O-glucoside to free apigenin was seen even in weak acidic conditions, confirming high hydrolytical potential of superheated water. Even the lowest investigated acid concentration of 0.001 mol/L was sufficient for the hydrolysis of O-glycoside.

Conclusion: Obtained results clearly demonstrate high hydrolytical potential of subcritical water. Furthermore, high concentrations of total phenols and flavonoids in obtained extracts demonstrate good solvation properties of subcritical water for the isolation of bioactive compounds from natural sources.

P17: Optimization, identification and antioxidant activities of polyphenol compounds from kiwi fruit (*Actinidia chinensis* Planch.) seeds

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ABSTRACT

Kiwi fruit (*Actinidia chinensis* Planch.) has been widely used in food and pharmacy industry. But the kiwi fruit seeds are regarded as solid wastes and remain underutilized yet.

Objective: The objective of this study was optimizing the extraction conditions of the bioactive compounds and analyzing the main phytochemicals from kiwi fruit seeds.

Methods: The optimum extraction conditions of kiwi fruit seed polyphenols (KSP) were evaluated using response surface methodology according to highest recovery of total phenolic content (TPC) and DPPH scavenging capacities (DPPHsc). The main phytochemicals contents of the samples were also determined.

Results: The most efficient extraction conditions were an extraction time of 79.65 min, 59.45 % acetone, 38.35 °C and 1:11.52 (w/v) solid/liquid ratio for TPC, and 79.85 min, 59.95 %, 38.20 °C and 1:9.30 (w/v) for DPPHsc, respectively. There was no significant difference between the two conditions. DPPHsc and ferric reducing antioxidant power (FRAP) results showed that antioxidant activities of the samples decreased in order of grape seed polyphenols > KSP > BHT. Results of identification of KSP indicated that the main compounds were p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid, caffeic acid and ferulic acid, accounting for 204.81 mg/g KSP.

Conclusion: All the results indicated that kiwi fruit seeds, as solid wastes resulting from the food industries, were rich in bioactive phytochemicals and may be a potential source of natural antioxidants.

P18: Beneficial effects of mixture of persimmon leaf and *Citrus junos Seib* on dysregulation of lipid metabolism and oxidative stress in diet- induced obese mice

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ABSTRACT

Objective: Persimmon leaf and *Citrus junos Seib* contain various phytochemicals which have positive health benefits including anti-inflammatory, antioxidant and lipid-lowering properties. The aim of this study was investigating the effects of persimmon leaf and *Citrus junos Seib* mixture (MPJ) on the lipid profiles and antioxidant capacity in diet-induced obese C57BL/6J mice.

Methods: The mice were randomly divided into 3 groups and either fed normal diet (ND, 5% fat), a high -fat diet (HFD, 20% fat + 1% cholesterol + 0.25% cholate) and HFD supplemented with MPJ(0.5% ethanol extracts of persimmon leaf + 0.3% ethanol extracts of *Citrus junos Seib*) for 10 weeks. Body weight, food intake, food efficiency ratio (FER), plasma and hepatic lipid profiles, hepatic lipid-regulating enzymes activities and erythrocyte antioxidant enzymes activities were measured.

Results: The initial body weights of three groups were not significantly different. However, final body weights of the MPJ and ND groups were significantly lower than that of the HFD group. Food intake was significantly lower in HFD group than in the ND group, whereas FER was significantly higher in the HFD group compared with the ND group. MPJ supplementation also significantly lowered the FER compared to HFD group. The levels of plasma triglyceride, total cholesterol (TC), non-HDL-cholesterol, apolipoprotein B and atherogenic index were significantly higher in the HFD group than in the ND group. Among them, plasma TC and non-HDL-cholesterol levels were significantly lowered by MPJ. The supplementation of MPJ also significantly lowered the increase in hepatic cholesterol contents induced by HFD. In addition, hepatic fatty acid synthase (FAS) activity was significantly higher in the HFD group than in the ND group, while supplementation of MPJ led to significant inhibition of hepatic FAS activity compared to the HFD group. Hepatic β -oxidation activities were also significantly higher in the MPJ and ND groups than the HFD. Moreover, levels of thiobabaturic acid reactive substance level, marker of lipid peroxide, and H₂O₂ was significantly lower in the erythrocyte of MPJ group compared to the HFD group.

Conclusion: Our findings suggest that a mixture of persimmon leaf and *Citrus junos Seib* can ameliorate dysregulation of lipid metabolism and oxidative stress induced by HFD.

P19: Isolation, purification, partial structure characterization and anti-inflammatory properties of a neutral exopolysaccharide from submerged mycelial fermentation of *Schizophyllum commune*

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ABSTRACT

In the last few years there has been an upsurge of interest in mushroom polysaccharides which have been evaluated to be dietary fibers with medicinal effect. *Schizophyllum commune* is a filamentously growing fungus that produces exopolysaccharides.

Objective: This study aimed to characterize the *S. commune* exopolysaccharide and evaluate the anti-inflammatory effects of this exopolysaccharide.

Methods: An exopolysaccharide from mycelial culture of *S. commune* was obtained by isolation and purification using DEAE-52 cellulose and Sephadex G-150 chromatography. The molecular weight (MW), monosaccharide compositions, chemical compositions and physical properties were investigated by fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), atomic force microscope (AFM) and nuclear magnetic resonance (NMR) spectroscopy. The anti-inflammatory activity of exopolysaccharide was assessed by inhibiting the production of nitric oxide (NO), inducible nitric oxide synthase (iNOS) and 5-lipoxygenase (5-LOX) from macrophages.

Results: A homogeneous protein-bound heteropolysaccharide with MW of 1.7×10^5 Da and contained a β -type glycosidic linkage. This exopolysaccharide contained high amount of glucose. This exopolysaccharide significantly ($p < 0.05$) inhibited lipopolysaccharides (LPS)-induced iNOS expression levels in the cells in a dose-dependent manner. It also inhibited the production of NO and 5-LOX in cells, but not in dose dependently.

Conclusion: It indicated significant anti-inflammatory effects, which showed that exopolysaccharide might be exploited as an effective anti-inflammatory agent for application in NO-related disorders such as inflammation and cancer.

P20: *Rosmarinus officinalis* and its chemical constituents as possible antimicrobial agents

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ABSTRACT

Rosmarinus officinalis L. (Rosemary) (RO), belonging to the Lamiaceae family, is well known for its antioxidant and antimicrobial actions. It is used for flavouring food, beverages and applied in several pharmaceutical preparations. The main chemical constituents include essential oils, phenolic acids (rosmarinic acid, caffeic acid, gallic acid), phenolic diterpenes (carnosol, carnosic acid), triterpenes (ursolic, oleanolic, betulinic acid) and flavonoids (apigenin, hesperidin, luteolin). Antimicrobial action of RO extract and its chemical constituents have been reported.

Objective: This review aimed to discuss the antimicrobial potency and efficacy of RO extracts and their chemical constituents.

Methods: The spectrum of action, potency, synergism with antibiotics and mechanism of action of RO extracts and its major chemical constituents are explicated. The influence of the chemical composition on the antimicrobial action is also discussed.

Results: Antimicrobial action of the essential oils and the non-volatile fraction phenolics of RO have been reported. Overall effectiveness was observed against gram positive bacteria rather than gram negative bacteria. The polyphenol composition was found to influence the antimicrobial action. Among the detected phytochemicals, abietane diterpenes like carnosol and carnosic acid were found to have potent antimicrobial action. Furthermore, carnosic acid was found to be an efflux pump modulator. RO extracts were reported to have synergistic action with cefuroxime and antioxidants.

Conclusion: Though the RO extracts and essential oils contribute to the antimicrobial action, phytochemical lead molecules like carnosic acid, carnosol, rosmarinic acid are reported to have potent action when compared to the extract. Thus these compounds can be focused on in future to develop them as adjuvant to existing antibiotics.

P21: L-amino acid carbamate prodrugs of natural cardiovascular protective agent scutellarin with improved physicochemical property, Caco-2 cell permeability and *in vitro* anti-oxidative activity

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ABSTRACT

Objective: This study aimed to design and synthesis of a series of 4'-L-amino acid carbamate derivatives of scutellarin methylester, which is a primary active ingredient in breviscapine, which is extracted from the Chinese herb *Erigeron breviscapus* (1).

Methods: Scutellarin methyl ester was coupled with L-amino acid ester isocyanate in anhydrous DMF to obtain scutellarin methyl ester-4'-L-amino acid carbamate *tetra*-butyl ester conjugates, which then by deprotecting with TFA to obtain the target compounds (**4a-h**). Compounds **4a-h** was evaluated for their physicochemical properties, Caco-2 cell permeability and *in vitro* anti-oxidative activity by using accepted methods (2-4).

Results: Physicochemical evaluation results showed that the designed target compounds have higher chemical and enzymatic stability, and aqueous solubility. The permeability ($P_{appAP\ to\ BL}$) of **4c,4f** and **4g** in Caco-2 cell were 8, 7 and 13 times higher than that of scutellarin respectively, especially **4g** had highest $P_{app\ AP\ to\ BL}$ value ($1.85 \pm 0.29 \times 10^{-6}$ cm/s) and lowest ER ($P_{appBL\ to\ AP}/P_{appAP\ to\ BL}$) value 0.56. *In vitro* anti-oxidative evaluation results revealed that **4g** can protect against H₂O₂-induced PC12 cells oxidative damage by attenuating the MMP loss and decreasing H₂O₂-induced ROS production.

Conclusion: Scutellarin methyl ester-4'-L-amino acid carbamate conjugates is a promising kind of cutellarin prodrug with improved physicochemical properties and enhanced bioactivities.

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P22: Antibacterial effect of gallic acid against *Aeromonas Hydrophila* and *Aeromonas Sobria* through damaging membrane integrity

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ABSTRACT

Objective: In the study, we investigated the antibacterial activity and mechanism of gallic acid on *Aeromonas hydrophila* and *A. sobria*.

Methods: The antimicrobial activity of Gallic acid was determined by Agar well-diffusion assay, minimum inhibitory concentration (MIC) and time-kill assay. Furthermore, the antibacterial mechanism of gallic acid (0, 0.5, 1, 2 mg/mL) was performed by membrane integrity assay, scanning electron microscopy (SEM) assay and SDS-PAGE of bacterial proteins.

Results: Gallic acid showed strong antimicrobial activity against the two bacteria. Furthermore, gallic acid notably increased the released material absorption value at 260, 280 nm and electric conductivity in a dose-dependence manner; SEM assay showed that gallic acid induced severe shrink of bacterial intima, irregular morphology in a dose-dependence manner. The SDS-PAGE profiles further confirmed that gallic acid could damage bacterial cells.

Conclusion: These results indicated gallic acid exhibited an antibacterial effect by destroying membrane integrity of *A. hydrophila* and *A. sobria*. Hence, gallic acid has great potential as a new natural food preservative in food fresh-keeping and storage.

P23: A randomized, double-blind, placebo-controlled crossover trial of bayberry juice in young adults with non-alcoholic fatty liver disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is currently the most prevalent chronic liver disease. Oxidative stress and inflammation are involved in the pathogenesis of NAFLD. It has been found that bayberries contain high levels of anthocyanins and phenolic acids that possess anti-oxidative and anti-inflammatory properties in rodents.

Objective: This pilot study was designed to investigate if bayberry supplementation, in the form of pasteurized juice, will beneficially improve plasma biomarkers of oxidative stress, apoptosis and inflammation in young individuals with features of NAFLD.

Methods: A total of 44 subjects between 18–25 years of age were randomised in a double-blind crossover study to placebo versus 250 mL of bayberry juice (contains 675 mg polyphenols and 207 mg anthocyanins) twice a day. The bayberry-flavored placebo beverage matched the color, caloric content, acidity, sugar content, and ascorbic acid profile of the 100% juice but did not contain polyphenols. Treatment duration was 4 weeks, with a 2-week washout. Anthropometric characteristics were measured and fasting blood samples were drawn before and after each intervention period. Plasma glucose, insulin, lipids, and some NAFLD-related biomarkers were determined.

Results: No significant effects on anthropometric parameters and homeostasis model assessment for insulin resistance were observed. Compared with the placebo, the bayberry juice significantly decreased plasma levels of protein carbonyl groups ($P = 0.04$), tumor necrosis factor alpha ($P < 0.01$) and interleukin-8 ($P = 0.02$). The apoptosis markers analysis revealed significant differences between the treatment and placebo in levels of tissue polypeptide-specific antigen ($P < 0.01$) and cytokeratin-18 fragment M30 ($P < 0.01$).

Conclusion: The present study demonstrated that treatment with bayberry juice for four weeks was more effective in lowering plasma markers of oxidative stress, inflammation and apoptosis in young individuals with features of NAFLD than with a placebo. Our findings provide novel insights into the potential use of berries to prevent and/or treat liver-related complications in early-stage NAFLD patients.

P24: Optimization of microwave assisted-extraction of rice bran protein

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ABSTRACT

Worldwide there has been an extensive effort to valorize wastes or by-products of the food industry to increase profitability and to overcome environmental problems. Rice bran is a by-product of rice milling industry, which is not efficiently valorized and contains valuable components.

Objective: This study aimed to increase the value of rice bran through the application of microwave assisted-extraction to recover the protein at highest yield.

Methods: For this purpose, after establishing a design of experiment by using response surface methodology, three parameters (solid/liquid ratio, power and time) of microwave treatment were investigated on defatted rice bran. The optimized process parameters for protein extraction were determined.

Results: The highest protein yield was obtained from rice bran samples, which were microwave treated at 1/2 solid: liquid ratio, 175 W and extraction time of 175 sec. The response surface model showed that the total protein yield was significantly ($p>0.05$) affected by solid/liquid ratio and microwave power while microwave application time has no effect on the protein yield.

Conclusion: Optimum process parameters to obtain protein from a by-product, rice bran, at maximum yield were determined for microwave assisted extraction.

P25: Design and synthesis of bio-orthogonal probes for identifying scutellarin therapeutic targets

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ABSTRACT

Scutellarin (or scutellarein-7-O-glucuronide) is a natural drug found among the total flavonoids of *Erigeron breviscapus* (Vant.) *Hand-Mazz.* (Compositae). It has been widely used for the treatment of cerebral infarction, coronary heart disease, angina pectoris and other cardio-cerebrovascular diseases in China. After oral administration, the major form in body is scutellarein which is derived from the hydrolysis of scutellarin. Recently, scutellarin has been demonstrating the potential for treating several malignant tumors. However, molecular mechanisms of its antitumor activity still leave a blank. In this study, bio-orthogonal probes of scutellarin have been designed, synthesized and evaluated for the application of identification on scutellarin therapeutic targets.

Objective: To design and synthesize the biotin labelled probes of scutellarin and then to evaluate their antitumor activity as bioorthogonal probes.

Methods: The probes **A** and **B** (Figure 1) were obtained by introducing biotin into the 4' position of scutellarin via esterification, amide formation, hydrolysis, substitution and other reactions using scutellarein and biotin as starting materials. And then by using MCF-7 and MCF-10A cells, the antitumor activity of the probes **A** and **B** has been investigated.

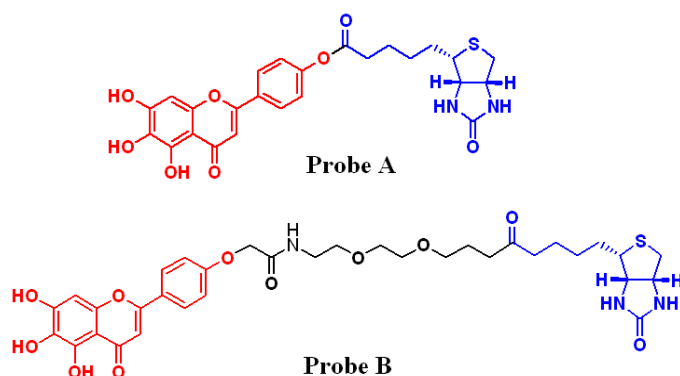


Figure 1: Structures of the bio-orthological probes **A** and **B**.

Results: All probe compounds and their intermediates have been characterized and verified by $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and ESI-MS. Cellular studies showed that the probes **A** and **B** demonstrated good and selectively antitumor activity.

Conclusion: The probes **A** and **B** could be the bio-orthogonal probes for scutellarin since the antitumor activity of probes **A** and **B** is comparable with that of scutellarin. They will further facilitate the discovery of scutellarin targets for treating malignant tumors.

P26: Immunomodulating pectins from elderberries

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ABSTRACT

Sambucus nigra L., also called European elder, is a tree-like shrub, and belongs to the Caprifoliaceae family. The fruit of *S. nigra* (elderberries) is a source for juices and wines and is used for flavoring or dyeing. Furthermore, it is globally utilized as a medicine or a source of dietary supplements. Elderberries have traditionally been used and are still used to treat respiratory illnesses such as cold and flu in Europe, Asia and America.

Objective: The aims of this study were to investigate the relationship between the chemical characteristics of the polysaccharides from the 50% ethanol–water extract, the 50 °C and 100 °C water extracts of elderberries and their immunomodulating properties.

Methods: Structural elucidation was performed by methylation and GC-MS. In order to elucidate the immunomodulating activities, the complement fixing and macrophage stimulating activities were determined. For further structural analysis of the pectic polymers, enzymatic treatment with endo- α -D-(1 \rightarrow 4)-polygalacturonase and weak acid hydrolysis were performed.

Results: All the purified fractions obtained from the 50% ethanol, 50 °C water and 100 °C water extracts showed potent dose-dependent complement fixating activity and macrophage stimulating activity. The tested fractions showed statistical significant macrophage stimulating effects on NO release at 100 μ g/mL. The isolated fractions consisted of long homogalacturonan regions, in addition to arabinogalactan-I and arabinogalactan-II, probably linked to a rhamnogalacturonan backbone. After the reduction of arabinose residues and 1 \rightarrow 3,6 Gal by weak acid hydrolysis, a reduced bioactivity was observed. Rhamnogalacturonan regions were isolated after enzymatic degradation and showed higher activity compared to the native polymer. The results from the linkage analysis and bioactivity tests led to the assumption that the branched moieties of the arabinogalactans, linked to rhamnogalacturonan region are important for the immunomodulating activity seen in elderberries.

Conclusion: Immunomodulating polysaccharides might be responsible for the claimed effect of berry extracts on cold and influenza, but the ability of these polysaccharides to contribute to immunomodulating activity in humans remains to be fully investigated.

P27: Squalene improves lipid and glucose metabolism in high-fat-fed rats by activating peroxisome proliferator-activated receptor alpha

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ABSTRACT

Hypertriglyceridemia is associated with type II diabetes, obesity, and metabolic syndrome. Squalene, an intermediate of cholesterol biosynthesis which can be obtained from algal oil, has been shown to reduce serum cholesterol, triglyceride and glucose in both of humans and animals, but their underlying mechanisms of action and protein targets are unknown.

Objective: This study aimed to investigate the molecular mechanism of action of squalene isolated from *Schizochytrium mangrovei* in hyperlipidemia by measuring peroxisome proliferator-activated receptors (PPAR) activity.

Methods and results: We demonstrated that squalene is a novel natural PPAR α agonist, identified from quantitative PCR analysis. PPAR- α transcription factor analysis shows that the squalene enhances the binding of PPAR- α to the peroxisome proliferator response element in PPAR- α -responsive genes. The differential effects of squalene on PPAR α were confirmed by measuring the expression of unique responsive genes for each PPAR subtype in vivo. High-fat diet (HFD)-fed rats were orally administered squalene (500 or 1000 mg/kg body weight) for eight wk. Squalene reduced adipocyte size, and plasma leptin concentrations, plasma triglyceride, total cholesterol and low-density-lipoprotein cholesterol concentrations, while it elevated the high-density-lipoprotein cholesterol concentration significantly compared with controls. Squalene induced the expression of PPAR α and its responsive genes involved in fatty acid uptake and β -oxidation in the livers, whereas genes involved in lipogenesis were down regulated. Squalene administration improved glucose tolerance and insulin sensitivity significantly compared with the HFD-fed control livers.

Conclusion: Our findings suggest that squalene significantly ameliorates dyslipidemia and diabetes in HFD fed rats by the activation of PPAR α and its target genes.

P28: Role of polyphenols in vascular health

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ABSTRACT

Diabetes mellitus and its related cardiovascular complications are among the leading causes for death worldwide. Hyperglycemia-induced vascular endothelial dysfunction (ED) is considered as a major factor in the pathogenesis of micro- and macroangiopathy (1). Endothelial nitrous oxide synthase (eNOS) takes a pivotal place in maintaining the cardiovascular homeostasis (2) and therefore this enzyme is object of a complex regulation, involving multiple mechanisms, such as cellular trafficking, protein-protein interactions and posttranslational modifications. Among them the protein phosphorylation has been shown to be a key regulator of eNOS activity (3).

Objective: Our study aimed to investigate the effects of polyphenols from diverse structural subclasses (flavones, multihydroxyflavonols, flavonols, flavanones, isoflavones, catechins, and stilbenoids) on eNOS activity *in vitro*.

Methods: We investigated the effects of the compounds on eNOS activity and related signaling pathways, using EA.hy926 cells, cultivated in medium with low glucose (LG – 100 mg/dl) and high glucose (HG – 450 mg/dl) concentrations. The cells were treated with 10 μ M of the respective substance. Mixture of 10 μ M histamine and 1 μ M insulin served as a positive control. The effects of the compounds on eNOS and related signaling pathways were investigated by analyzing the phosphorylation level at different enzyme sites, using immunoblotting techniques. Phosphospecific anti-bodies were used to detect the respective targets: peNOS (Ser1177), peNOS (Thr495), pAkt (Ser473), pAkt (Thr308), pERK1/2 (Thr202/Tyr204, Thr185/Tyr187), pp38 MAPK (Thr180/Tyr182), pAMPK- α (Thr172), pPKA (Thr197).

Results: Our data suggest that the exposure of the endothelial cells to hyperglycemic conditions might influence the Akt/PKB signaling and alter the activity of eNOS. Resveratrol was used as a model compound and its effect was tested on HG- and LG-cultivated cells. The results indicate that resveratrol showed capability to increase the phosphorylation at Ser1177 residue of eNOS, both in HG- and LG-conditions.

Conclusions: Further studies are required to elucidate the potential of polyphenols from different structural classes to activate eNOS, as well as their mechanism of action and the signaling pathways, that might be involved in the process.

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P29: Effects of ultrasonic, infrared and conventional drying methods on qualitative and quantitative of the essential oil of Bakhtiari savory (*Saturejabachtiarica Bunge*)

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ABSTRACT

Bakhtiari savory (*Saturejabachtiarica Bunge*.) of the mint family (Lamiaceae) is an endemic species plant widely distributed in the south-west of Iran. The aerial parts of Bakhtiari savory were dried using different drying methods.

Objective: The aim of the present research was studying different new methods such as infrared (40, 50 and 60 °C), ultrasonic (40, 50 and 60 °C), sun and shade drying to dry Bakhtiari savory for determining the best method.

Methods: The essential oils of fresh and dried samples were obtained through hydro-distillation, and analyzed using gas chromatography–mass spectrometry (GC–MS). The data was statistically analyzed using one-way ANOVA with the program SPSS version 17.0.

Results: The effects of drying methods and drying temperature were significant on essential oil yield at the 1% level. The highest essential oil yields were obtained with ultrasonic at 40 °C (2.2%), followed by ultrasonic at 50 °C (2.1%), and infrared at 40 °C (2%). It should be noted that the drying rate with the ultrasonic dryer was higher than when using other investigating methods, however the mentioned method yielded essential oil at 1.7%. Twenty-seven components were determined in essential oils of *S. bachtiarica*, which were mostly oxygenated monoterpenes and hydrocarbons monoterpenes. The main components in essential oils of fresh and dried Bakhtiari savory aerial parts were carvacrol (33–41%), γ -terpinene (10.2–17.6%), thymol (12.3–18.1%).

Conclusion: When both factors of drying rate and essential oil yields are examined, ultrasonic at 50°C was the most suitable drying method.

P30: Biflavones and furanone glucoside from *Zabelia tyaihyonii*

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ABSTRACT

Zabelia tyaihyonii (T. H. Chung ex Nakai) Hisauti & H. Hara (formerly, known as a synonym of *Abelia tyaihyonii* Nakai), an endemic Korea species, is a deciduous shrub belonging to the family of the Linnaeaceae. It occurs mainly in the central and northern parts of the Korean peninsula. Previous phytochemical studies on the genus *Zabelia* have resulted in the isolation of bis-iridoid and secoiridoid glucosides.

Objective: To date, the chemical constituents of *Z. tyaihyonii* have never been reported. This study was undertaken to investigate the chemical constituents of *Z. tyaihyonii*.

Methods: The MeOH extract of the leaves of *Z. tyaihyonii* was partitioned with *n*-hexane, CH₂Cl₂, EtOAc, and water, successively. The EtOAc-soluble fraction was separated using several column chromatography including MPLC and preparative HPLC. The structures of the isolated compounds were elucidated by interpretation of 1D- and 2D-NMR, HR-ESI-MS, and circular dichroism (CD).

Results: Repeated column chromatography of the EtOAc fraction *Z. tyaihyonii* led to the isolation of two new biflavones, (a*R*)-3'-methoxycupressuflavone and (a*R*)-3',3'''-dimethoxycupressuflavone, and two new furanone glucosides, zabeliosides A and B. The two known biflavones were identified as cupressuflavone and amentoflavone by comparing their physicochemical and spectroscopic data to those of published values.

Conclusion: In the present study, two new biflavones and two new furanone glucosides along with two known biflavones were isolated from the MeOH extract of the leaves of *Z. tyaihyonii*. Their structures were elucidated on the basis of spectroscopic methods and CD analysis. This is the first report on the chemical constituent of *Z. tyaihyonii*. We report herein the isolation, and structure determination of these isolated compounds.

P31: Latifolanone A, a new dimeric sesquiterpene with an unprecedented carbon skeleton from *Echinops latifolius*

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ABSTRACT

Echinops latifolius Tausch. (Asteraceae) is mainly distributed in Korea, China, and Japan. It has long been used in traditional medicine for the treatments of mammitis, arthralgia, and mumps. Previous phytochemical studies of *E. latifolius* have reported the isolation of thiophenes, alkaloids, and sesquiterpenes. The pharmacological studies have shown that it possesses anti-inflammatory, antitumor, and antineoplastic effects.

Objective: In order to find bioactive compounds, a detailed phytochemical investigation of this plant has been conducted.

Methods: The roots of *E. latifolius* were extracted with MeOH and partitioned with *n*-hexane, CH₂Cl₂, and H₂O, successively. The CH₂Cl₂ extract was chromatographed on silica gel and the resultant fractions were purified using a combination of Sephadex LH-20 and MPLC as well as preparative RP-HPLC. The structures of the compounds were determined by spectroscopic analysis, including 1D and 2D NMR techniques, and mass spectrometry.

Results: Repeated chromatography of the MeOH extract from the roots of *E. latifolius* yielded five pure compounds; a new dimeric sesquiterpene (**1**), together with four known compounds atractylenolide-III (**2**), 5-(1, 2-dihydroxyethyl)-2-(*Z*)-hept-5-ene-1,3-diynylthiophene (**3**), 5-(1,2-dihydroxyethyl)-2-(*E*)-hept-5-ene-1,3-diynylthiophene (**4**), and arctinol-b (**5**).

Conclusion: In this study we report the isolation and structure determination of the new compound **1** along with four known compounds. The structures of the compounds were established as ¹H-, ¹³C-NMR, 2D-NMR (HSQC-DEPT, HMBC, COSY, and ROESY) spectroscopic data analysis, and HRESIMS data. The compound **1** was determined as latifolanone A, a dimeric sesquiterpene with an unprecedented carbon skeleton.

P32: Radiomodulatory role of *Psidium guajava* leaf extracts against X-ray induced genotoxicity, oxidative stress and apoptosis in albinowistar rat model

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ABSTRACT

Natural herbs are found to contain high levels of antioxidants that can offer protection against radiation induced cytotoxicity. Although a number of plants have been screened for their radioprotective effects, none have been successfully employed in the clinical scenario. Therefore it is the need of hour, to identify new plants that may prove to be potential radioprotectors.

Objective: This study aims at determining the antioxidant activities, anti apoptotic activity and antimutagenic activity of leaf extracts of the tropical shrub, *P. guajava*, in animals exposed to 4gy of X-rays.

Methods: Optimum dose for P.G extract administration to the study groups was first determined by survival analysis of the animals. Albinowistar rats were then categorized into 4 groups. Sham control (Group 1) was administered with distilled water (0.01 ml/kgbodywt). Group 2 was exposed to 4 gy of x-rays. Group 3 was administered with an oral dose of 200 mg/kgbodywt of P.G. Group 4 was pretreated with the same dose of P.G, 5 days prior to exposure to x-rays. Animals in each group were sacrificed after 24 hrs of radiation treatment. Liver tissue homogenate was prepared and analyzed for antioxidants like SOD, reduced glutathione and catalase and oxidative parameters like lipid peroxides, NO and protein carbonyls. Hepatocytes were also used to study apoptosis and DNA damage through comet assay. All the four groups were compared by ANOVA. When results were found to be significant ($p < 0.05$), further comparison between individual groups was done by post hoc tukey tests using IBM SPSS software version 20 and the results were indicated as significant at $p < 0.05$.

Results: Optimum dose of P.G to be administered to the study groups was 200mg/kg body wt as determined by the survival curve. It was observed that there was a significant increase in NO, lipid peroxides and protein carbonyls and a significant decrease in antioxidants like SOD, catalase and reduced GSH in the irradiated group compared to others. Pretreatment with P.G prior to radiation exposure, has resulted in a significant decrease in protein carbonyls, lipid peroxides and NO and a significant increase in reduced GSH and SOD and also an increase in catalase which was non significant. Apoptotic index and DNA damage also decreased in the group that received P.G before irradiation but the difference between groups was not significant.

Conclusion: *P. guajava* has undoubtedly a predominant role in improving the oxidative stress induced by X-rays. To a certain extent it can also prevent DNA damage and inhibit apoptosis in the irradiated animals.

P33: Effect of 5,7-dimethoxyflavone on exercise capacity by increasing mitochondrial biogenesis

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ABSTRACT

Physical inactivity causes an estimated 3.2 million deaths annually in the world. Mitochondrial biogenesis, the increased number of mitochondria, enhances exercise endurance, which is essential for physical performance and health.

Objective: In the present study, the effect of DMF on exercise capacity through enhancing mitochondrial biogenesis was investigated in L6 cells and C57BL/6J mice.

Methods: DMF, isolated from *Kaempferia parviflora* Wall. Ex Baker (*K. parviflora*), was used to investigate mitochondrial contents in L6 cells by MitoTracker green. The effect of DMF (5 mg/kg/day for 6 weeks) on exercise capacity was evaluated in high-fat diet (HFD)-induced mice by using treadmill. At the molecular level, the regulation of mitochondrial biogenesis by DMF was determined with Western Blot, reverse transcriptase polymer reaction chain (RT-PCR) and luciferase reporter gene assay.

Results: In L6 skeletal muscle cells, DMF increased mitochondrial contents without reactive oxygen species (ROS) production change. DMF increased the expressions of AMP-activated protein kinase (AMPK), which induces the expression of PGC-1 α . Also, DMF positively regulated the expressions of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α), nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factor A (TFAM), estrogen-related receptor alpha (ERR α), and peroxisome proliferator-activated receptor delta (PPAR δ), which are mitochondrial biogenesis related factors. *In vivo* experiment shows that DMF increased running distance and time. Specifically, in the muscle tissue, the expression of mitochondrial DNA (mtDNA), PGC-1 α , NRF-1, ERR α , and TFAM were increased by DMF.

Conclusion: DMF might be a potential natural agent for enhancing exercise capacity.

P34: The effects of Schizandrin A on dyslipidemia and insulin resistance in high fat diet-induced obese C57BL/6J mice

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ABSTRACT

Obesity is one of the world's health problems in contemporary society. It can cause metabolic diseases, such as dyslipidemia, insulin resistance and type 2 diabetes. Schizandrin A is a major component of Omija, a fruit herb which is used as a traditional Korean drink. It has been reported to have antioxidant and anti-inflammatory effects. However, little is known about its role in obesity-related metabolic disturbances.

Objective: The aims of this study were to investigate the effects of schizandrin A on dyslipidemia and insulin resistance in high fat diet-induced obese C57BL/6J mice.

Methods: Male C57BL/6J mice were divided in 2 groups: High fat diet (20% fat, w/w, HFD) and HFD supplemented with schizandrin A (0.006%, w/w, SCH). The animals were provided with free access to food and water during the entire experiment period of 16 weeks.

Results: There was no significant difference in body weight between the two groups. SCH also did not significantly alter plasma levels of fasting blood glucose and plasma insulin as well as insulin resistance markers such as HOMA-IR and glucose tolerance. However, SCH supplement significantly improved plasma lipid profiles, including triglyceride (TG) and free fatty acid (FFA), and increased fecal lipids excretion.

Conclusion: Schizandrin A may have beneficial effect on dyslipidemia by increasing fecal lipids excretion, even if it did not reduce body weight and improve insulin resistance.

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P35: Binding of green tea catechins to beta-amyloid peptide studied by electrospray ionization time-of-flight mass spectrometry

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ABSTRACT

Green tea catechins, such as (-)-epigallocatechin gallate (EGCG), showed considerable potential as a drug candidate for the treatment of neurodegenerative diseases due to the inhibitory effect on the fibrillogenesis of beta-amyloid peptide ($A\beta$). The noncovalent interaction of four major catechins (EGCG, ECG, EGC and EC) of green tea with the $A\beta_{(1-40)}$ peptide was studied by ESI-TOF-MS technique.

Objective: This study aimed to investigate the binding stoichiometry and affinity of four major catechins of green tea with $A\beta_{(1-40)}$.

Methods: ESI-TOF-MS technique was utilized to observe the direct interaction of four catechins with $A\beta_{(1-40)}$ peptide. Stock solutions of $A\beta_{(1-40)}$ peptide monomer and catechins were prepared in 0.02% ammonium solution and methanol, respectively. Test solution with a final concentration of 30 μ M and 90 μ M for the peptide monomer and each catechin, respectively, was prepared in 1 mM ammonium acetate (pH 7.4) containing 20% methanol, and injected directly into a positive mode TOF mass spectrometer. Binding affinity of each catechin with $A\beta_{(1-40)}$ peptide was calculated according to the literature method (1-2).

Results: Multiple binding stoichiometries from 1:1 to 4:1 of each catechin with $A\beta_{(1-40)}$ peptide were observed. The binding affinity of four catechins with $A\beta_{(1-40)}$ peptide follows the trend of ECG > EGCG > EC > EGC (Figure 1), indicating that the galloyl group of ECG and EGCG plays an important role in binding with $A\beta_{(1-40)}$ peptide. This study provided some molecular insights into inhibitory effects of these catechins on $A\beta$ peptide aggregation.

Conclusion: Four major green tea catechins bind to $A\beta_{(1-40)}$ peptide following the trend of ECG > EGCG > EC > EGC with multiple stoichiometries from 1:1 to 4:1.

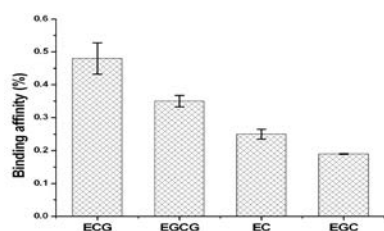


Figure 1. Binding affinity of four catechins with $A\beta_{(1-40)}$ peptide by ESI-TOF-MS analysis

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P36: Expression of deoxyxylulose phosphate reductoisomerase and copalyl diphosphate synthase increases tanshinones accumulation and anti-bacterial activity in *Salvia miltiorrhiza* hairy roots

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ABSTRACT

Tanshinones, a group of health-promoting diterpenes, are mainly produced in *Salvia miltiorrhiza* Bunge (Dan-shen in Chinese) and widely used in treatment of cardiovascular diseases. Because of shortage of natural *Salvia* plants, huge consumption and serious quality degradation of cultivated Dan-shen, it is very important to increase the content of tanshinones by plant biotechnology.

Objective: This study aimed to increase the content of tanshinones in *S. miltiorrhiza* hairy roots by overexpression of selected deoxyxylulose phosphate reductoisomerase (DXR) and copalyl diphosphate synthase (CPS).

Methods: The complete DXR and CPS cDNAs were cloned from *S. miltiorrhiza* according to the sequences reported in NCBI and constructed into plant expression vector *pCAMBIA2300*. Different plasmids constructed above were used to obtain genetically modified hairy roots using routine genetic transformation followed by molecular analysis. The expression profiles of introduced genes were analyzed by qRT-PCR. The content of tanshinones in *S. Miltiorrhiza* hairy roots were determined by HPLC. Antioxidant activities by DPPH and ABTS scavenging activities as well as anti-bacterial activity of tanshinones extracts of transgenic *S. Miltiorrhiza* hairy roots were evaluated.

Results: In this study, overexpression of *DXR* and/or *CPS* in transgenic *S. Miltiorrhiza* hairy root lines can significantly enhanced the production of tanshinones with different levels of control ($P < 0.05$). Tanshinones yield was higher in *CPS*-overexpressing lines (C lines) in comparison to *DXR*-overexpressing lines (D lines), implying that downstream specific *CPS* showed much more powerful pushing effect than upstream *DXR* for tanshinones accumulation in *S. miltiorrhiza*. Furthermore, co-introduction of *DXR* and *CPS* genes (DC Lines) caused a highest yield of tanshinones, showed that simultaneous up-regulation of *DXR* and *CPS* can produce a cooperative effect on tanshinones biosynthesis in *S. miltiorrhiza*. All the tested transgenic hairy root lines showed higher antioxidant activity than control. The antibacterial experiments confirmed that tanshinones extract from the transgenic hairy roots owned good antibacterial effects, and the best tanshinones-producing root line (DC15) exhibited the strongest effect.

Conclusion: Overexpression of *DXR* and/or *CPS* increased diterpenes tanshinones production and anti-bacterial activity. *CPS* showed a more pronounced enhancing effect, implying that *CPS* is a new regulatory target for improving metabolic flux in tanshinones biosynthetic pathway.

P37: Increased accumulation of anti-cancer drug camptothecin in *Ophiorrhiza pumila* hairy roots by metabolic engineering

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ABSTRACT

Camptothecin (CPT), originally isolated from the bark of the Chinese happy tree *Camptotheca acuminata*, is a modified terpenoidindole alkaloid (TIA) and exhibits excellent anti-tumor activity with increasing clinical need. So, it is important to develop sustainable and alternative production sources of CPT in order to resolve the worldwide scarcity of natural sources of CPT.

Objective: This study aimed at optimizing hairy root induction procedure and enhancing the production of the anti-cancer drug CPT in *O. pumila* hairy roots.

Methods: Different factors such as explants, *Agrobacterium rhizogenes* strains and culture media, which normally affect hairy root induction efficiency, were optimized for plant *O. pumila*. The complete *G10H* and *STR* cDNAs were cloned from the sterile seedlings of *Catharanthus roseus* according to the sequences reported in NCBI and Constructed into plant expression vector *pCAMBIA1304+*. Different plasmids constructed above were used for plant genetic transformation to obtain genetically modified hairy roots screened by molecular analysis. HPLC was used to determine camptothecin content in *O. pumila* roots. Anti-tumor activity of transgenic hairy roots was evaluated by MTT analysis.

Results: In this study, an efficient sterile plant culture system was established and optimized to induce hairy root formation in *O. pumila*. CPT production was significantly improved in *G10H*-overexpressing lines (G lines) compared to non-transgenic hairy root cultures (NC line) and *STR*-overexpressing lines (S lines), implying that *G10H* showed much more powerful pushing effect than *STR* for biosynthesis of CPT in *O. pumila*. Furthermore, co-introduction of *G10H* and *STR* genes (SG Lines) caused a highest yield of CPT, showing that simultaneous overexpression of *G10H* and *STR* can produce a synergistic effect on CPT biosynthesis in *O. pumila*. The MTT assay results indicated that CPT extracted from different root lines showed similar anti-tumor activity, suggesting that transgenic *O. pumila* hairy root lines could be an alternate way to obtain CPT instead of natural plant resources.

Conclusion: Anti-cancer drug CPT production can be enhanced with *O. pumila* hairy roots by metabolic engineering, which provides a promising strategy to obtain CPT by large-scale culture of transgenic hairy roots in the near future.

P38: Quality characteristics of fermented ginseng seed oil treated by different extraction methods

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ABSTRACT

The Ginseng seed fermentation was assumed to produce useful physiologically active substances as in the case of ginseng root. The ginseng seed was fermented using *Bacillus*, *Pediococcus*, and *Lactobacillus* strains for oil extraction. The analyzed quality characteristics were the extraction yield, color and amounts of phenolic compounds, fatty acids, and phytosterol.

Object: This study was to investigate the effect of different extractions on the quality and characteristics of fermented ginseng seed oil.

Methods: For ginseng seed fermentation, 1% of each strain was inoculated on sterilized ginseng seeds and then incubated at 30°C for 24 hours in an incubator. Fermented ginseng seed oil extraction was performed using compression extraction, solvent extraction, and supercritical fluid extraction.

Results: The extraction yield differed depending on the extraction method, and the highest yield was observed in the solvent extraction method. The color of fermented ginseng seed oil did not vary significantly according to the fermentation treatment and extraction method. Phenolic compounds detected in fermented ginseng seed oil were maltol, ρ -coumaric acid, vanillic acid, caffeic acid, ferulic acid, and trans-cinnamic acid. The fermentation condition with the highest phenolic compound content was supercritical fluid extraction combined with fermentation using the *Bacillus subtilis* KFRI 1127 strain. The fatty acid composition of fermented ginseng seed oil did not differ significantly with respect to fermentation or extraction method. Unsaturated fatty acids constituted over 90% of the total fatty acids and had the highest oleic acid content. Phytosterol contents varied significantly according to the fermentation strain and extraction method particularly in campesterol, stigmasterol, β -sitosterol, and sitostanol. The extraction method with the highest total phytosterol content was supercritical fluid extraction, followed by solvent extraction and compression extraction. In particular, the phytosterol content of ginseng seed oil fermented with *Bacillus subtilis* KFRI 1127 and extracted by the supercritical fluid method was the highest at 983.58 mg/100g.

Conclusion: The fermented ginseng seed oil treated by different extraction methods showed a high content of bioactive ingredients such as phenolics, fatty acids, phytosterols as compared to non-fermented ginseng seed oil.

P39: Secondary metabolites of mosses: a valuable source of biologically active compounds

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ABSTRACT

Mosses are one of the groups in the second largest division of plant kingdom-bryophytes. Due to their small size and complicated identification, biologically active substance extraction is difficult, thus they are rarely used as study object.

Objective: This study aimed to isolate secondary metabolites of mosses characteristic for Northern Europe and to evaluate their biological activity.

Methods: 15 moss species were chosen and extraction optimization was done using various methods (supercritical CO₂, microwave, ultrasound and conventional extraction) as well as different solvents (ethanol, methanol, DMSO, water and others). In the extracts total polyphenols, flavonoid and carbohydrate content as well as radical scavenging activity were evaluated using two methods (DPPH and ABTS). After extraction optimization two extraction types (microwave and conventional extraction) with 60% of ethanol was chosen and extracts were tested for their antimicrobial and anti-proliferative activity. Antimicrobial activity was tested on several bacteria (*Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus mirabilis*) and fungi (*Candida albicans* and *Aspergillus niger*). To test anti-proliferative activity, six cancer cell lines were chosen (rat glioma (C6), human breast adenocarcinoma (MCF-7), human epidermoid carcinoma (A431), human lung carcinoma (A549) and mouse melanoma cell lines (B16-F10)).

Results: Optimization of extraction showed that the most effective method for secondary metabolite extraction was microwave extraction at 150°C and ethanol concentration either 60% or 80% aqueous ethanol solution. Optimization was guided by total polyphenol content and antiradical (DPPH) activity. The difference between conventional extraction and microwave extraction was up to 10 times judging by their total polyphenol content. After optimal extraction conditions were established, antimicrobial and anti-proliferative activities were tested. No antimicrobial activity was found on two fungi used. Highest antimicrobial activity was observed against *Bacillus cereus* and *Pseudomonas aeruginosa*. Some anti-proliferative activity was observed on all used cancer cell lines, but the best activity was found using rat glioma cell line and human epidermoid carcinoma, results showed that some extract IC₅₀ was 0.9 and 13 µl/ml respectively.

Conclusion: Moss secondary metabolite extracts do show biological activity both as antimicrobial and anti-proliferative agents. Extraction optimization does significantly increase both secondary metabolite quantity and biological activity of extracts.

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P40: Allantoin inhibits LPS or palmitic acid induced inflammatory responses by regulating production of nitric oxide and cytokines in Raw 264.7 cells

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ABSTRACT

Allantoin, 5-ureide-hydantion, can be isolated from many plant species such as sugar beet and leguminous plants. It is non-toxic and used in various fields, such as wound healing, anti-irritating and skin protection. Although allantoin is commonly used, research on this compound is insufficient. Especially the potential pharmacological mechanisms of allantoin in anti-inflammatory regulation are still poorly understood.

Objective: The purpose of this study was to assess the anti-inflammatory effects of allantoin in murine macrophage Raw 264.7 cells with or without LPS and palmitic acid treatment.

Methods: 75 mM palmitic acid (PA) was dissolved in heated ethanol, filter sterilized and diluted 1:100 in DMEM containing 2% BSA to yield a final PA concentration of 0.75 mM. For the analysis of cell viability, cells were treated with the indicated concentrations of allantoin and viability was determined by XTT. Nitrite release into culture media was determined using the Griess reagent. Levels of IL-6 in the culture media were quantified using ELISA. cDNA was produced from total RNA using a RT-PCR. Amplification of the target genes was performed by using specific oligonucleotide primers in a normal PCR system.

Results: The XTT assay showed that allantoin was not cytotoxic up to 500 μ M. Allantoin decreased the LPS or PA induced NO production and led to inhibition of LPS or PA induced mRNA expression of iNOS and COX2 in Raw 264.7 cells. In addition, allantoin suppressed LPS or PA induced inflammatory cytokine release such as IL-6. Consistent with this result, IL-6 mRNA expression was also reduced. Our RT-PCR analysis showed that the mRNA expression levels of other inflammatory cytokine were also reduced by treatment with allantoin.

Conclusion: Allantoin has anti-inflammatory effects in LPS or PA stimulated Raw 264.7 cells. Thus, allantoin can potentially be a useful therapeutic strategy for prevention and treatment of inflammatory disorders caused by external harmful stimuli or obesity.

P41: Anti-adipogenic effects of the *Ecklonia cava* extract Seapolynol™ in adipocytes and high-fat diet-fed zebrafish

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ABSTRACT

Ecklonia cava, a brown alga native to the coastal of Korea and Japan, contains several bioactive polyphenols. The effects of Seapolynol™ (SN), the extract of *Ecklonia cava*, on adipogenesis has not been fully studied in adipocytes and *in vivo*.

Objective: The aim of the present study was to determine whether SN inhibits lipid accumulation in the adipocyte cell line 3T3-L1, as well as in high-fat diet-fed zebrafish.

Methods: SN was provided from BotaMedi (Jeju, South Korea). Differentiated 3T3-L1 cells, which were washed with PBS, were fixed with 4% formaldehyde at 4 °C for 1 h and stained with Oil red O solution. Total RNA was extracted from differentiated (day 6 or 8) or undifferentiated cells using the TRIZOL reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Protein (25 or 50 µg) extracted from differentiated cells was subjected to SDS-PAGE and immunoblot analysis with indicated antibodies.

Results: SN influenced the early stage of adipogenesis during mitotic clonal expansion (MCE). SN inhibited mRNA expression of early adipogenic genes, such as Krueppel-like factor 4 (KLF4), KLF5, CCAAT-enhancer-binding protein-β (C/EBP β), C/EBP, and ETS2. In contrast, SN up-regulated the mRNA expression of the anti-adipogenic factor KLF2. The results indicated down-regulation of late adipogenic factors, such as peroxisome proliferator-activated receptor-γ (PPAR γ), C/EBPβ, fatty acid bind protein (FABP4) and fatty acid synthase (FAS), resulting in lowered triacylglycerols. Also, the triglyceride synthetic enzymes DGAT1, Lipin1 and GPAT3 were decreased. Furthermore, SN reduced lipid accumulation in high-fat diet-fed zebrafish using Nile Red staining and a triacylglycerol kit assay.

Conclusion: In this study, we investigated the inhibitory effect of SN on lipid accumulation in 3T3-L1 cells, focusing on early adipogenesis in the cell cycle. The anti-adipogenic effect of SN was further studied in a zebrafish model.

P42: Ginsenoside Rg1 inhibits lipid accumulation and ROS production in 3T3-L1 cells during adipocyte differentiation

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ABSTRACT

Objectives: Ginsenoside Rg1 is one of the major components in panaxa ginseng, as a class of steroid glycosides, and triterpene saponins. The effect of ginsenoside Rg1 on adipogenesis lipid accumulation, as well as the mechanisms by which ginsenoside Rg1 affect adipogenic factors and ROS production during adipocyte differentiation have not been fully understood. In this study, we determined anti-adipogenic and anti-oxidant effect of ginsenoside Rg1 during adipogenesis.

Methods: The suppressive effect of ginsenoside Rg1 on lipid accumulation was investigated using 3T3-L1 cells. Cells were performed Oil Red O and NBT staining for measuring lipid accumulation and ROS production on 8day. In addition, cells were harvested and performed western blot and RT-PCR to investigate expression of adipogenic and lipogenic factors. The mechanism of action of ginsenoside Rg1 with respect to adipogenesis was elucidated.

Results: Ginsenoside Rg1 inhibited protein and mRNA expression of adipogenic genes, such as CCAAT-enhancer-binding protein- α (C/EBP α), peroxisome proliferator-activated receptor- γ (PPAR γ) and adipocyte protein 2 (aP2), as reduced lipid accumulation by Oil Red O staining. These results were reflected in down-regulation of late adipogenic factors, such as C/EBP α , PPAR γ and aP2, resulting in a decrease in triacylglycerol content and down-regulation of triglyceride synthetic enzymes. Furthermore, ROS production was reduced in the NBT assay. In conclusion, our data suggest that ginsenoside Rg1 inhibits lipid accumulation and ROS generation during adipocyte differentiation.

Conclusion: Our data demonstrate that ginsenoside Rg1 inhibited lipid accumulation in the 3T3-L1 cell line, as well as in animal models. Our study suggests that ginsenoside Rg1 represents a novel treatment for metabolic disorders, owing to its regulation of lipid accumulation and oxidative stress.

P43: Seapolynol™ reduces insulin resistance by inhibiting lipid accumulation in 3T3-L1 cells and db/db mice

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ABSTRACT

Seapolynol™ (SN) is a polyphenol extract of the marine brown algae *Ecklonia cava* with reported effects on hyperglycemia, hypercholesterolemia, hair growth, and body weight loss. Obesity is a worldwide problem which induces metabolic diseases. So research to prevent or treat obesity is urgently needed. We therefore evaluated whether SN improved glucose homeostasis in obesity and obesity-induced insulin resistance condition.

Objective: The objective of the current study was to determine the anti-obesity and anti-diabetic effects of SN.

Methods: Fully confluent preadipocytes were induced by hormonal cocktail with SN (25, 50, 100 μ M) for 8 days. Growth medium was changed every 2 days with SN (25, 50, 100 μ M) and insulin. On day 8, differentiated cells were harvested and measured using Western blot analysis with specific antibodies. Data were expressed as means and standard deviations of three replicates. Results were evaluated by ANOVA and Duncan's test ($p < 0.05$). (CON; control, fully differentiated cells, ND; non-differentiation, preadipocyte, hormonal cocktail, MDI; differentiation cocktail, IBMX (isobutylmethylxanthine) + dexamethasone + insulin). Male db/db mice were divided into four groups receiving SN (60, 150 mg/kgBW/day), rosiglitazone (10 mg/kgBW/day), or vehicle with chow diet for 6 weeks.

Results: In 3T3-L1 cells, the expression of insulin signaling such as PI3K and Glut4 was increased in a dose-dependent manner. Rosiglitazone served as a positive control. In db/db mice, the expression of adipogenic factors was decreased dose dependently. The protein levels of insulin signaling markers were increased in the SN supplement group. The results showed that the anti-adipogenic properties of SN affected the obesity-related insulin resistance.

Conclusion: Our study provides a potential agent to treatment of type 2 diabetes.

P44: Silibinin inhibits the inflammatory responses induced by lipopolysaccharide or palmitic acid in murine macrophages

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ABSTRACT

Silibinin is the major active component of silymarin, which is a flavonolignan mixture extracted from milk thistle. It is known as a treatment of hepatitis and for its anti-inflammatory effects. Recently, studies suggested that Silibinin may support blood glucose control in type II diabetes patients. It is known that silibinin inhibits LPS-induced inflammations but it is not clear yet whether it also inhibits palmitic acid-induced inflammation in murine macrophages.

Objective: Our study aimed to determine whether silibinin suppressed the LPS or palmitic acid (PA) induced inflammatory responses in RAW264.7 cells.

Methods: 75 mM palmitic acid (PA) was dissolved in ethanol and heated to 38 °C. It was diluted 1:100 in DMEM containing 2% BSA to yield a final PA concentration of 0.75mM and filter sterilized. The cell viability was measured using the XTT assay. Cells were pretreated with the suitable concentrations of silibinin before a treatment with LPS (1 µg/ml) and PA. Nitrite release into the culture media was measured using the Griess reagent. An ELISA was used to quantify levels of IL-6 in the culture media. Total RNA was isolated to obtain cDNA employing RT-PCR. Amplification of the target genes was performed by using specific oligonucleotide primers in a normal PCR system.

Results: The XTT assay indicated that Silibinin was not cytotoxic up to 100 µM. Silibinin decreased the NO production induced by LPS or PA. Moreover, it inhibited the LPS or PA stimulated mRNA expression of iNOS and COX-2 in Raw 264.7 cells. In addition, silibinin regulated LPS or PA induced inflammatory cytokine secretion such as IL-6. Furthermore, IL-6 mRNA expression was reduced. RT-PCR data revealed that the mRNA expression levels of other inflammatory cytokines were also decreased by treatment with silibinin.

Conclusion: Silibinin is a natural phytochemical for therapy of inflammatory disorders caused by external and internal harmful stimuli such as LPS by pathogens and palmitic acid in the obesity patient.

P45: Pterocarpan-rich fraction from soybean leaf has beneficial effects on glucose metabolism and inflammation in obese subjects with mild metabolic syndrome

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ABSTRACT

Metabolic syndrome is a worldwide disease in modern times. Many researchers have attempted to investigate the effects of a variety of herbal medicine on metabolic syndrome. Pterocarpan, a type of natural phenol, is the main component of ethyl acetate extracts of soy leaves and is known to prevent atherosclerosis and to improve glucose metabolism in metabolic disease animal models. However, effects of pterocarpan-rich soy leaf on metabolic disease in human subjects have not been reported yet.

Objective: In the present study, we investigated the functions of pterocarpan-rich fraction isolated from the soybean leaf (PT) in glucose metabolism and inflammation in obese subjects with mild metabolic syndrome.

Methods: Subjects were randomly assigned to two groups and administered six capsules containing starch (3 g/day, Placebo) and PT (2 g/day) for 12 weeks. Blood samples were collected with heparin from a vein on the flexor side of arm after 12 weeks to investigate the changes of plasma markers of glucose metabolism and inflammation.

Results: Body weight and body mass index were not significantly decreased by the PT supplement. However, PT supplement significantly lowered the baseline-adjusted fasting blood glucose and HOMA-IR levels compared to the Placebo group, although the plasma insulin level was not significantly lowered. Moreover, the baseline-adjusted plasma resistin and glucagon levels were significantly decreased by PT supplement. In addition, the baseline-adjusted plasma PAI-1, MCP-1 and TNF- α , the markers of inflammation, were significantly lowered by PT supplement. The plasma GOP and GPT levels were not significantly different between the PT supplement group and placebo group.

Conclusions: Taken together, PT has beneficial effects on glucose metabolism and inflammation in obese subjects with mild metabolic syndrome. Therefore PT is expected to be a useful phytomedicine to improve mild metabolic syndrome.

P46: Analysis of loganin content in *Cornus officinalis* fruits depending on ripening stages and extraction conditions

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ABSTRACT

The fruits of *Cornus officinalis*, which belong to the Cornaceae family, are used for anti-diuretic, antihypertensive and immune improvement in Oriental medicines. Loganin, a major iridoid constituent, has been reported as an active constituent.

Objective: The content of constituents in natural products is affected by many factors. Therefore, this study was performed to analyze the loganin content depending on ripening stages and extraction conditions.

Methods: The fruits of *C. officinalis* were divided into three groups, such as Green, Green-Red, and Red, depending on ripening stages. The content of loganin in *C. officinalis* extract was quantitated by HPLC analysis. The effect of extraction conditions such as extraction solvent, extraction time and extraction temperature was investigated by quantitation of loganin content in each extraction condition. Optimized extraction condition for maximum loganin content was also suggested by response surface methodology with three-level-three-factor Box-Behnken design.

Results: The loganin content in *C. Officinalis* was the highest in Green fruits (58.8 μ g/mg extract) and followed by Green-Red (38.0 μ g/mg extract) and Red fruits (30.0 μ g/mg extract). The loganin content in *C. officinalis* extract was also greatly affected by extraction solvent, whereas extraction time and extraction temperature exerted relatively little effect on loganin content. Further investigation on optimized extraction condition for maximum loganin content from *C. officinalis* fruits using response surface methodology suggested as ethanol concentration, 100%; temperature 55°C and extraction time, 82.2 min. The loganin content under optimal conditions was found to be 38.2 μ g/mg extract, which was well matched with the predicted value of 39.7 μ g/mg extract.

Conclusion: The content of loganin in *C. officinalis* fruits was greatly affected by ripening stages and extraction condition. Therefore, our present study will be beneficial to developing loganin as a functional product.

P47: Characterization of melanogenesis inhibitory constituents of *Morus alba* leaves and optimization of extraction condition using response surface methodology

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ABSTRACT

Melanin is a natural pigment that plays an important role in the protection of skin, however, hyperpigmentation is associated with several problems. Therefore, melanogenesis inhibitory natural products have been developed for skin medications in the cosmetics industry.

Objective: This study was aimed to characterize the melanogenesis inhibitory constituents of *Morus alba* leaves and optimize the extraction conditions for maximum efficacy.

Methods: The CH₂Cl₂ and EtOAc-soluble fractions of *M. alba* leaves was subjected to various column chromatography to isolate active constituents. The structures of the isolated compounds were determined on the basis of spectroscopic analysis. The anti-melanogenesis activity of isolated compounds was evaluated by measuring tyrosinase activity and melanin content in B6F10 melanoma cells. Optimization of extraction conditions with maximum tyrosinase inhibitory activity and maximum phenolic content was determined using response surface methodology with three-level-three-factor Box-Behnken design (BBD).

Results: Fractionation of CH₂Cl₂ and EtOAc-soluble fractions resulted in the isolation of 22 compounds including 8 benzofurans, 10 flavonoids, 2 coumarins, 1 silbenoid and 1 chalcone. These phenolic constituents showed significantly inhibited tyrosinase activity and melanin content in B6F10 melanoma cells. Optimization of extraction conditions of *M. alba* leaves for maximum tyrosinase inhibition and the phenolic contents yielded extraction conditions as a methanol concentration of 85.2 %; an extraction temperature, 53.2 °C; an extraction time 2 h. The tyrosinase inhibitory activity and total phenolic content under optimal conditions were found to be 74.8 % inhibition and 24.8 µg GAE/mg extract, which were well-matched with the predicted values of 75.0 % inhibition and 23.8 µg GAE/mg extract.

Conclusion: Our present study suggested an anti-melanogenesis inhibitory activity of *M. alba* leaves and characterized their active constituents as phenolic constituents. Optimized extraction condition for maximum efficacy was also suggested. Therefore, these results will provide useful information about *M. alba* leaves for the development as cosmetic therapeutics to reduce skin hyperpigmentation.

P48: Flavonoids from the roots of *Cudrania tricuspidata* and pancreatic lipase inhibitory activity

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ABSTRACT

Obesity is a serious health problem due to its association with diverse pathological disorders. Pancreatic lipase which plays a key role in fat absorption is one of the targets for therapeutics for obesity.

Objective: The *Cudrania tricuspidata* (Moraceae) is a deciduous tree which is widely distributed in Asia including Korea. In a continuation of our search for natural anti-obesity products, the roots of *C. tricuspidata* significantly inhibited pancreatic lipase. Therefore, we tried to elucidate the active constituents of roots of *C. tricuspidata* on pancreatic lipase.

Methods: The total extract was partitioned with *n*-hexane, CH₂Cl₂, EtOAc, *n*-BuOH and H₂O. The CH₂Cl₂ and EtOAc fractions were purified by silica column chromatography, MPLC, Sephadex LH-20 and HPLC. The structures and relative configuration of isolated compounds were determined on the basis of spectroscopic analysis including 1D, 2D NMR and ESI-MS. The absolute configurations were defined by CD spectrum. The isolated compounds were tested for their inhibition on pancreatic lipase activity.

Results : Fifteen flavonoids, namely genistein (1), biochanin A (2), 3'-*O*-methylroborol (3), wighteone (4), kaempferol (5), morin (6), naringenin (7), 5,7,3',5'-tetrahydroxyflavanone (8), aromadendrine (9), (2R,3R)-2,3-dihydro-3,5,6,7-tetrahydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (10), 8-prenylnaringenin (11), cudraflavanone D (12), cudraflavanone A (13), 2',5,7-trihydroxy-4,5'-(2,2-dimethylchromeno)-8-(3-hydroxy-3-methylbutyl)flavanone (14) and cudracuspiflavanone A (15), as well as a coumarin, umbelliferone (16) and two chromones, 5,7-dihydroxychromone (17) and 5-hydroxy-2,2-dimethyl-2H,6H-benzodipyran-6-one (18) were isolated from the roots of *C. tricuspidata*. This is the first time that Cudracuspiflavanone A (15) has been reported. Compounds **12** and **13** showed significant inhibitory effect on pancreatic lipase activity.

Conclusion: Our present study reports 18 compounds, including a new compound, from roots of *C. tricuspidata*. The isolated compounds showed inhibitory effect on pancreatic lipase. Therefore, the roots of *C. tricuspidata* can be valuable source for the treatment of obesity.

P49: Optimization of extraction condition for osthol, a melanogenesis inhibitor from *Cnidium monnieri* fruits

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ABSTRACT

Melanin is a natural pigment that plays an important role for protection of skin. However, hyperpigmentation is associated with several problems such as freckles and age spots. Coumarin is a phenylpropanoid derivative with a benzo- α -pyrone moiety and is reported to inhibit melanin synthesis.

Objective: Osthol is a major coumarin of *Cnidium monnieri* fruits and reduced melanin content. Extraction conditions greatly affect the yield of natural products and optimization of extraction conditions is essential for maximum yield. Therefore, extraction condition for osthol from *C. monnieri* fruits was optimized for maximum yield.

Methods: Extraction factors such as extraction solvent, extraction time and sample/solvent ratio were optimized for maximum yield of osthol from *C. monnieri* fruits using response surface methodology with Box-Behnken design (BBD). Anti-melanogenesis effect of osthol was measured using B16F10 melanoma cells and mushroom tyrosinase.

Results: The optimal condition was obtained as sample/solvent ratio, 1500 mg/10 ml; extraction time 30.3 min; methanol concentration, 97.7% by response surface methodology and predicted the value of 14.9 mg osthol/g dried samples. Regression analysis showed a good fit of the experimental data and the osthol yield under optimal conditions was found to be 15.0 mg/g dried samples, which were well matched with the predicted. Osthol inhibited melanin content in B16F10 melanoma cells not by inhibition of tyrosinase activity but by inhibition of the expression of melanogenesis genes, such as tyrosinase, TRP-1 and TRP-2.

Conclusion: These results will provide useful information about optimized extraction condition for the development of osthol as cosmetic therapeutics to reduce skin hyperpigmentation.

P50: Optimization of extraction conditions of torilin from *Torilis japonica* fruits using response surface methodology

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ABSTRACT

Abnormal accumulation of melanin is responsible for hyperpigmentation disorders, which can be improved by tyrosinase inhibitors. The fruits of *Torilis japonica* (Umbelliferae) have been reported to inhibited melanin production in melanoma cells. Torilin, a major sesquiterpene of *T. japonica* fruits, reduced the melanin synthesis by downregulation of tyrosinase levels.

Objective: This study evaluated the effects of extraction conditions on torilin yield and optimize extraction conditions of *T. japonica* fruits for maximum yield of torilin.

Methods: For the quantitation, torilin was isolated from *T. japonica* fruits and identified on the basis of spectroscopic analysis and reported data. The effect of extraction conditions such as extraction solvent, extraction temperature and sample/solvent ratio was investigated by quantitation of torilin content using HPLC analysis in each extraction condition. Optimized extraction condition for maximum torilin yield was also suggested by response surface methodology with three-level-three-factor Box-Behnken design. The statistical significance of the coefficients in the regression equation was checked by analysis of variance (ANOVA). The fitness of the polynomial model equation to the responses was evaluated with the coefficients of R^2 and the lack of fit was evaluated using F -test.

Results: Column chromatographic separation of the *n*-hexane soluble fraction of *T. japonica* fruits resulted in the isolation of torilin, which was determined using spectroscopic data, including ^1H and ^{13}C NMR data. Optimization of extraction conditions yield using response surface methodology suggested the importance of extraction solvent for maximum torilin yield. Regression analysis showed F -value of 12.48 and p -value of 0.006, which supported the reliability of model system. The optimal extraction condition obtained in this study was as follows; extraction solvent, EtOAc-MeOH (31.8%-68.2%); extraction temperature, 30°C; sample/solvent ratio, 1000 mg/2ml. The torilin yield under optimal conditions was found to be 93.0% which were well matched with the prediction.

Conclusion: Our present results suggested the optimized extraction condition for torilin, which provide useful information for the development of torilin as whitening agents.

P51: Synthesis and biological evaluation of resveratrol derivatives as melanogenesis inhibitors

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ABSTRACT

Resveratrol (**1**) is a naturally occurring stilbene compound. It has been suggested to a potential whitening agent due to its strong inhibition on melanin synthesis. However, the use of resveratrol in cosmetics has been limited due to its instability and acetylated derivatives have been reported to improve both efficacy and stability.

Objective: For the development of more stable and effective compounds, resveratrol derivatives were synthesized and their effects on melanin synthesis were assessed. Therefore, in attempt to improve anti-melanogenic activity and stability synthetic resveratrol derivatives were prepared.

Methods: Nine resveratrol derivatives including five alkyl ether derivatives (**2a-2e**) and four ester derivatives (**3a-3d**) were synthesized and the structures were confirmed by spectroscopic analysis including NMR and MS analysis. All the derivatives were determined the effect on tyrosinase activity in vitro with mushroom tyrosinase. Melanin synthesis and cell viability were examined on B16F10 melanoma cells and expression of tyrosinase, TRP-1 and TRP-2 were measured by Western Blot Analysis.

Results: Nine resveratrol derivatives including five alkyl ether derivatives with C₂H₅, C₄H₉, C₅H₁₁, C₆H₁₃, and C₈H₁₇ (**2a-2e**) and four ester derivatives with CH₃, CH=C(CH₃)₂, CH(C₂H₅)C₄H₉, C₇H₁₅ (**3a-3d**) as side chains were synthesized. All the derivatives as well as resveratrol efficiently reduced the melanin content in α -MSH stimulated B16F10 melanoma cells. Further investigation showed that inhibition of melanin synthesis was achieved not by the inhibition of tyrosinase activity but by the inhibition of melanogenic enzyme expressions such as tyrosinase, tyrosinase-related protein (TRP)-1 and 2.

Conclusion: Resveratrol derivatives exerted anti-melanogenic activity with improved stability and will provide wide application for the development for cosmetics.

P52: *In vitro* fermentation of gallic acid by the human intestinal microbiota

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ABSTRACT

In recent years, there has been growing interest by food scientist in the physiological relevance of antioxidant material reaching the lower gut and their relationship with human intestinal bacteria. There are few studies investigating the influence of polyphenols on the composition and activity of the nonpathogenic gut microbial community.

Objective: The aim of this study was to investigate the prebiotics properties of gallic acid on intestinal microbiota by using *in vitro* fermentation.

Methods: Fermentation by intestinal bacteria was conducted in anaerobic and pH-controlled faecal batch vessels with gallic acid (2 mg/ml) for 24 h. Fructooligosaccharide (FOS) (1 mg/ml) which is a known prebiotic was used as a control. Samples were collected at 0, 12, and 24 h of fermentation for bacterial enumeration by spreading on the selective agar medium according to species dependency. Short chain fatty acid (SCFA) was analyzed by a high-performance liquid chromatography (HPLC).

Results: Gallic acid induced the growth of total bacteria, achieving a statistical increase at 12 and 24 h of incubation compared with the control vessel. Particularly, gallic acid caused a significant increase in the growth of bacteria associated with beneficial effects such as *Bifidobacterium* sp. (Table 1). Furthermore, amount of SCFA and lactate increased in incubation with the gallic acid until 24 h. The major SCFAs produced by gallic acid and FOS was butyric acid which play critical role for healthy condition of gut epithelium.

Conclusion: This study demonstrates that the gallic acid in daily meals or beverages may reach the large intestine and give prebiotic effect modulating the composition of intestinal microbiota.

Table 1. Bacterial population changes in pH-controlled, stirred faecal batch cultures at 0, 12, and 24 h using gallic acid (GA) and fructooligosaccharides (FOS)

Carbon Source	Time (h)	Total Bacteria	<i>Bifidobacterium</i> sp.	<i>Bacteroides</i> sp.	<i>Clostridium</i> sp.
Control	0	5.38±0.06	6.38±0.06	5.20±0.05	5.43±0.03
	12	7.17±0.10	5.74±0.00	7.06±0.00	7.04±0.02
	24	8.09±0.04	5.18±0.06	7.95±0.03	9.00±0.00
GA	12	8.74±0.04	8.82±0.00	8.60±0.04	8.47±0.04
	24	9.06±0.03	8.30±0.07	8.44±0.03	8.09±0.60
FOS	12	9.08±0.00	9.01±0.10	9.08±0.09	9.04±0.07
	24	8.19±0.02	8.96±0.08	8.09±0.04	8.73±0.01

Data were expressed as Log CFU/mL±SD.

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P53: Chemical composition of essential oil from *Plectranthus zuluensis* (T. Cooke)

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ABSTRACT

Objectives: This study was carried out to evaluate the chemical composition of essential oil from the leaves of *Plectranthus zuluensis* (T. Cooke).

Methods: Essential oil was extracted from the leaves of *Plectranthus zuluensis* by hydrodistillation. The chemical composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS).

Results: The yield of essential oil of *Plectranthus zuluensis* was 0.38%. In total, 10 compounds were identified, representing 99.8% in the oil. The main compounds were Benzene,1,2,4-trimethoxy-5-(1-propenyl)-,(Z)-(70.95%), 2,4,6-Trimethoxystyrene(13.49%), asarone (12.41%) and Benzene, 1-ethyl-2,3,4,5,6-pentafluoro- (0.99%) and minor compounds is ethanone, 1-(2-hydroxy-4,6-dimethoxyphenyl)- (0.19%), Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]- (0.13%), p-Xylene (0.12%) and Carbamic acid, phenyl-, 1-methylethyl ester (0.08%).

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P54: Phytochemical profile of an essential oil from fruits of Mexican tejocote (*Crataegus* spp.)

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ABSTRACT

The name of the species of *Crataegus* in México is “tejocote”, to which are attributed medicinal properties since ancient times. In traditional medicine is used for treatment of several respiratory diseases. There is not information about the profile of essential oils or volatiles in the fruits of tejocote located in México. The structural diversity in the composition of the oil of the fruit of tejocote justify the biological activity presented by the members of the genus.

Objective: This study aimed to identify the components of the essential oil in eleven genotypes in fruits of tejocote.

Methods: The oils were extracted from the fruits of tejocote by hydrodistillation, with a microdistillation equipment type Clevenger. For this analysis was used a Hewlett Packard gas chromatograph coupled to a mass selective detector HP 5973 (EM). The quantification of the components was done by normalization method for each peak. The identification of the components was by the comparison of the spectra with those of the NIST library, using the own algorithms of the spectrometer. Also was obtained the retention index or Kovats index (KI) each compound.

Results: We identified 52 components, outstanding esters (21 %), terpenes (15 %) and organic acids (13 %). Hexanoic, heptanoic, octanoic and nonanoic acids are the most abundant metabolites in eight of the eleven studied genotypes. From the 52 compounds identified only linalool oxide and R- α -terpineol were related in the North American species (*C. robesoniana*, *C. flabellata* and *C. jackii*) and European (*C. azarolus*) (1).

Conclusion: We could not find any relation between the component profile identified and the origin of the samples analysed, nor with the color or the size of the fruit.

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P55: Cytotoxic Reactive Carbonyl Species Sequestering Action of Black Rice with Giant Embryo

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ABSTRACT

The rapid increase in prevalence of metabolic disorders, which is associated with a state of elevated systemic oxidative stress, is projected to cause future increases in the prevalence of chronic diseases such as diabetes and cardiovascular diseases. Oxidation of polyunsaturated fatty acids and sugars produces reactive carbonyl species, which, due to their electrophilic nature, react with nucleophilic sites of proteins and DNA and lead to cellular dysfunction. Therefore, an effective reactive carbonyl species sequestering agent is expected to prevent such cellular dysfunction.

Objective: This study aimed to determine cytotoxic reactive carbonyl species sequestering action of various rice varieties including black rice with giant embryo using a recently developed high resolution mass spectrometry.

Methods: A model protein, ubiquitin, was incubated *in vitro* with various reactive carbonyl species such as 4-hydroxy-*trans*-2-nonenal (HNE) in the presence of different concentrations of various rice varieties. After 24 hours of incubation, the reaction was stopped and the incubation products were analyzed by a high resolution mass spectrometry. Using this new state-of-the-art technique, the extent of ubiquitin modification by reactive carbonyl species and its alteration in the presence of various rice extracts were identified and characterized. Bioactive components acting as HNE sequestering agents were then identified by LC-ESI-MS/MS using an isotopic signature approach based on deuterated and non-deuterated HNE mixture in a 1:1 molar ratio.

Results: The different rice extracts prevented protein modifications induced by reactive carbonyl species in different degrees. In particular, the black rice with giant embryo strongly protected protein modification by the HNE ($IC_{50} \sim 29.1$). The isotopic signature approach allowed us to identify several reactive carbonyl species sequestering components in black rice with giant embryo such as histidine and histidine containing peptides.

Conclusion: The current study indicates that certain bioactive components in black rice with giant embryo effectively sequester cytotoxic reactive carbonyl species. Thus the black rice with giant embryo can be valuable for dietary strategy to prevent oxidative stress associated chronic diseases and to achieve optimal health.

Acknowledgements: This work has been supported by the Rural Development Administration (PJ010059), Republic of Korea.

P56: Effects of water-soluble oligosaccharides extracted from lotus (*Nelumbo nucifera* Gaertn.) seeds on growth ability of *Bifidobacterium adolescentis*

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ABSTRACT

Many microorganisms including some probiotic species in human gastrointestinal tract are sensitive to gastric acid secretions. The use of simulated gastrointestinal environments is a suitable approach to study the effects of a single factor on the stability of probiotics in the gastrointestinal tract (GIT).

Objective: In this study, the growth effects of lotus seed oligosaccharides and purified lotus seed oligosaccharides on *Bifidobacterium adolescentis* were assessed in a simulated gastrointestinal tract environment.

Methods: Frozen lotus seeds, whose cores had been removed, were mixed with distilled water (3/10, w/v) for 1 h. The suspensions were homogenized and heated at 90 °C for 2 h, mixed with five volumes of 95 % ethanol and allowed to precipitate overnight. The supernatant was collected to obtain the crude lotus seed oligosaccharides (LOS). LOS purified with a macroporous resin (P-LOS) was obtained by using a macroporous resin. The constitute of P-LOS was analysed by high-performance liquid chromatography (HPLC). Growth rate of *B. adolescentis* in presence of different carbon sources was tested *in vitro*. Tolerance tests were assessed by the acid tolerance test, the bile acid tolerance test and the gastrointestinal solution tolerance test.

Results: Compared to glucose (Glc), xylo-oligosaccharides (XOS), and fructooligosaccharides (FOS), lotus seed oligosaccharides (LOS), and purified lotus seed oligosaccharides (P-LOS) are more effective on promoting the growth of *B. adolescentis*. The final bacterial mass was higher in LOS and P-LOS than in Glc. The viability of *B. adolescentis* incubated at pH 1.5, 2.0, or 3.0 was improved by LOS and P-LOS. Additionally, LOS and P-LOS increased the viability of *B. adolescentis* in bile salts up to 10 g/L and in digestive juices.

Conclusion: The results revealed that LOS is an effective growth-accelerating factor of *B. adolescentis*, which improves the viability of *B. adolescentis* in gastrointestinal conditions.

P62: The therapeutic potency of *Echinacea* glycoconjugate

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ABSTRACT

Increased interest is observed in new active natural drugs without undesired side effects which are observed at conventional medications. *Echinacea purpurea* has a long history in traditional medicine. Recent trends are focused on its immune-modulatory effects, particularly prevention and treatment of airways diseases.

Objective: Verification of therapeutic potency of *E. glycoconjugate* (EC) in induced animal asthma model and comparison of its effect with those of classic bronchodilators, anti-inflammatory and anti-asthmatic drugs.

Methods: Alkaline extract of *Echinacea* flowers was neutralized, subsequently extracted with organic solvents, dialyzed and freeze-dried to give the EC. Effect of EC on airways smooth muscle reactivity was studied by *in vivo* and *in vitro* methods (1, 2) with complementary ciliary beating frequency measurement. Anti-inflammatory effect of EC was determined by cytokines levels assessment in plasma and BALF and exhaled NO levels.

Results: *Echinacea* conjugate, composed of phenolics, carbohydrates and protein, has molecular mass 10 000. It has a *strong bronchodilator effect* on airways reactivity after mediated contraction of airways, especially at low concentration of histamine (*in vitro*). This effect was confirmed also by changes in basal specific airway resistance measurement (*in vivo*). Measurements of ciliary beat frequency did not show negative impact of EC on mucociliary clearance. EC has reduced exhaled NO levels as well as cytokines levels in plasma and BALF. These facts confirmed its *anti-inflammatory activity*.

Conclusion: Our results are important in the context of *Asthma inflammation* because *Echinacea* conjugate has shown both, bronchodilator and anti-inflammatory effects (very efficient in local pulmonary inflammation).

Acknowledgements: Study was supported by the VEGA Grants 2/0018/15 and 1/0165/14, and APVV-0305-12.

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P64: Expression of fucosyltransferase gene from *Helicobacter pylori* in *Lactococcus lactis* and synthesis of 2-fucosyllactose by the enzyme reaction

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ABSTRACT

2-Fucosyllactose(2-FL) is a functional oligosaccharide present in human milk which plays important roles in stimulating growth of Bifidobacteria and preventing adhesion of pathogens to epithelial surface. 2-FL can be synthesized through the enzymatic fucosylation of lactose by α -1,2fucosyltransferase (FucT2) which catalyzes the fucosyl transfer from guanosine-diphosphate(GDP) to the acceptor molecules to form an α -glycosidic linkage (1).

Objective: The aim of this study was to synthesize 2-FL from GDP-L-fucose and lactose by using recombinant *Lactococcus lactis* expressing heterologous fucosyltransferase gene.

Methods: For this, the nucleotide sequence for FucT2 from *Helicobacter pylori* 26695 was synthesized after codon-optimization and the two genes (unoptimized and optimized) were expressed in *L. lactis* NZ9000 by using pNZ8008 plasmid. Protein expression and enzyme activity were analyzed by HPLC and TLC.

Results: After optimization, the number of unfavourable codon was significantly reduced. The FucT2 gene was subcloned into pNZ8008 vector to construct pNZ-HPwt and pNZ-HPopti plasmids, and transformed into *L. lactis* NZ9000. Wild type FucT2(HPwt) and codon-optimized FucT2(HPopti) were successfully expressed with approximately 33kDa of molecular weight. When the transformants were cultivated at low temperature (15°C), HPwt and HPopti were expressed as active enzymes to generate 2-FL. When they were reacted with GDP-L-fucose and lactose, 2-FL was synthesized. When analyzed by HPLC, a product peak with retention time of 10.6 min was shown and it corresponded to 2-FL. These results clearly demonstrate that the recombinant fucosyltransferases (HPwt and HPopti) expressed in *L. lactis* NZ9000 have a functional activity to produce 2-FL.

Conclusion: This is the first report about expression of FucT2 genes in recombinant *L.lactis* and production of 2-FL by the enzyme.

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P65: Discovery of two new cardioprotective compounds from the roots of *Pseudostellaria heterophylla*

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ABSTRACT

Except for as a tonic agent, the roots of *Pseudostellaria heterophylla* were also used for the treatment of cardiopalmus in traditional Chinese and ethnic medicines. Previous investigations showed that its extracts possessed cardioprotective effects either in rat myocardial infarction or in norepinephrine-induced cardiac myocyte injury. In the previous report, two active fractions (i.e., fractions PHP enriched in polysaccharides and PHS enriched in glycosides) responsible for the cardioprotective effect of *P. heterophylla* were obtained. Investigations on the chemical constituents of the active fractions and their cardioprotective effects are of significance for the discovery of cardioprotective agents.

Objective: This study aims to isolate, identify, and biologically evaluate the cardioprotective compounds from the roots of *P. heterophylla*.

Methods: The cardioprotective fraction PHS was firstly extracted by H9c2 cells and analysed by UHPLC-Q-TOF-MS, and then subjected to semi-preparative high performance liquid chromatography for the isolation of the target compounds extracted by H9c2 cells. The compounds were structurally elucidated on the basis of UV, IR, NMR, and CD spectroscopic data as well as calculated ECD. The cardioprotective effect was evaluated for their protective effect against H₂O₂-induced injury in H9c2 cells.

Results: Two new compounds, pseudoheterins A and B (**1** and **2**), that were extracted by H9c2 cells and possess unreported molecular structures were isolated from the fraction PHS. Compounds **1** and **2** significantly prevented H9c2 cells from oxidative damage induced by H₂O₂ in a dose dependent manner, restoring cell survival from 24% to 60.1% and 62.2%, respectively, at 50 μmol/L (Figure 1).

Conclusion: Pseudoheterins A and B are two promising cardioprotective candidates deserving further research and development.

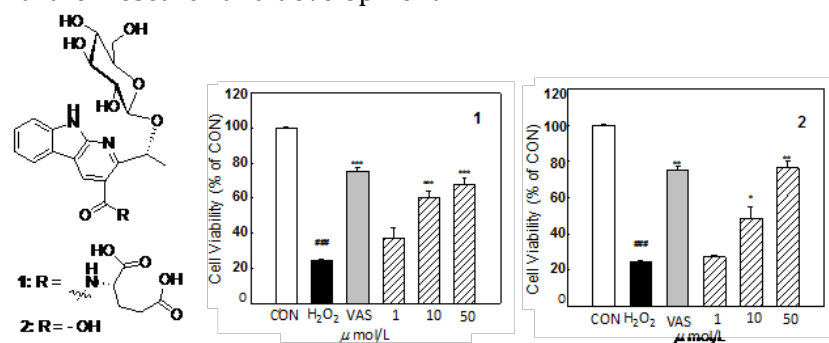


Figure 1. Cardioprotective effects of pseudoheterins A (**1**) and B (**2**) from *P. heterophylla* against H₂O₂-induced oxidative damage in H9c2 cells. ###P < 0.001 vs. the control group; ***P < 0.001 vs. H₂O₂ group; **P < 0.01 vs. H₂O₂ group; *P < 0.05 vs. H₂O₂ group. VAS: Vasorel (20 μmol/L)

P67: L-amino acid carbamate prodrugs of natural cardiovascular protective agent scutellarin with improved physiochemical property, Caco-2 cell permeability and *in vitro* anti-oxidative activity

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ABSTRACT

Objective: This study aimed to design and synthesis of a series of 4'-L-amino acid carbamate derivatives of scutellarin methyl ester, which is a primary active ingredient in breviscapine, which is extracted from the Chinese herb, *Erigeron breviscapus* (1).

Methods: Scutellarin methyl ester was coupled with L-amino acid ester isocyanate in anhydrous DMF to obtain scutellarin methyl ester-4'-L-amino acid carbamate *tetra*-butyl ester conjugates, which then by deprotecting with TFA to obtain the target compounds (4a-h). Compounds 4a-h was evaluated for their physiochemical properties, Caco-2 cell permeability and *in vitro* anti-oxidative activity by using accepted methods (2-4).

Results: Physiochemical evaluation results showed that designed target compounds have higher chemical and enzymatic stability, and aqueous solubility. The permeability ($P_{appAP\ to\ BL}$) of 4c, 4f and 4g in Caco-2 cell were 8, 7 and 13 times higher than that of scutellarin respectively, especially 4g had highest $P_{app\ AP\ to\ BL}$ value ($1.85 \pm 0.29 \times 10^{-6}$ cm/s) and lowest ER ($P_{appBL\ to\ AP}/P_{appAP\ to\ BL}$) value 0.56. *In vitro* anti-oxidative evaluation results revealed that 4g can protect against H₂O₂-induced PC12 cells oxidative damage by attenuating the MMP loss and decreasing H₂O₂-induced ROS production.

Conclusion: Scutellarin methyl ester-4'-L-amino acid carbamate conjugates is a promising kind of cutellarin prodrug with improved physiochemical properties, enhanced bioactivities.

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P68: Comparative metabolomic studies on alkaloids from four different origins

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ABSTRACT

Objective: *Lycopodium japonicum* Thunb. Has attracted great interest due to its rich alkaloids with significant anti-cancer activities. Because significant chemical differences often existed in a plant species from different origins, and thus affected its quality and bioactivities, the objective of this study is to develop comparative metabolomic strategies for the differential analysis of *Lycopodium* alkaloids from four different origins, and to further reveal their chemical and pharmaceutical distinctions at the molecular level for the first time which may provide a good molecular foundation for better practical pharmaceutical applications in the future, and pave the way for the discovery and development of new remedies from *Lycopodium* alkaloids.

Methods: Dried plant materials of *L. japonicum* Thunb from four different origins were crushed and ultrasonically extracted with 90% ethanol for 3 times in parallel. The extracts were further dispersed with 1% hydrochloric acid, and total alkaloids were obtained after further extraction and separation. The total alkaloids were determined by acid dye method, and then subjected to the comparative metabolomic analysis using HPLC-UV-ESI-MS/MS. At last, the comparative activity studies were conducted using CCK-8 cell based assays.

Results: Compared with lycopodium alkaloids from Kenya, Guangxi province and Zhejiang province with anti-HepG2 inhibitory rate at 26.72%, 20.26% and 33.62%, respectively, while lycopodium alkaloids from Hubei exhibited higher inhibitory rate *in vitro* at 65.95% at 10 µg/mL. To better understand the chemical bases underlying the anti-cancer activity, the total contents of alkaloids of Hubei were determined at 7.8‰ (w/w) of the plant material by acid dye colorimetry. By comparing the fingerprints of lycopodium alkaloids from four origins, we found the similarity of Hubei origin was 26.3% with the standard chromatography by Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A). Especially, the apparent phytochemical differences were exposed by the fingerprints ranged from 5 to 10 min and 20 to 40 min. Altogether, 48 peaks corresponding to lycopodium alkaloids were detected, 28 of which were tentatively identified. Peak 4 identified as 14-hydroxy-magellanineone was the most abundant lycopodium alkaloid and only found in the extracts from Hubei origin.

Conclusion: A comparative metabolomics strategy combined with corresponding bio-activity assay was developed to reveal their chemical and activity distinctions at the molecular level, and resulted in the successful identification of a series of alkaloid markers of four different origins, which provided valuable information for their quality control and further activity studies.

P70: Oxymatrine as a therapeutic agent for TGF- β -induced cardiac fibroblast proliferation and differentiation by targeting TGF- β /Smad signalling *in vitro*

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ABSTRACT

Myocardial fibrosis is a key pathological basis for cardiac dysfunction and adverse cardiovascular events. Transforming growth factor- β (TGF- β) has been shown to be a powerful cytokine in the pathogenesis of cardiac remodeling and fibrosis, which are mediated via a heteromeric serine/threonine receptor complex signalling through the Smad signaling pathway. We previously had confirmed that oxymatrine (OMT) possessed inhibitory activity on myocardial fibrosis induced by acute myocardial infarction in rats.

Objective: In the present study concentrates on the inhibitory effects of OMT on TGF- β -induced cardiac fibroblast proliferation and differentiation, and its mechanism on TGF- β -Smads signal pathway.

Methods: The cardiac fibroblasts (CFs) were separated by trypsin methods and differential adhesion from 1-3 d sucking rats. The cardiac fibroblast was identified by immunocytochemical methods as following: actin, vimentin, and fibronectin, respectively. At present study, CFs were used the 3-5 generation. Cellular morphological changes were observed by inverted phase contrast microscope and Giemsa staining. Cell viability was analyzed by MTT assay. Types I and III collagens were quantified by using the enzyme linked immunosorbent assay (Elisa) kit. The hydroxyproline contents were measured by commercial kits. The expressions of the Smad 2 and Smad 3 were investigated by western blotting. Moreover, the mRNA levels of t of the Smad 2 were measured by real-time PCR.

Results: The immunocytochemical results confirmed that all of three authenticated protein was classically positive characteristics including actin, vimentin, and fibronectin. After exposure to 20ng/mL TGF- β , the cardiac fibroblasts were emerged fibroblasts abnormal pathological characters, including morphology alterations, increased cell proliferation, and abnormal collagen expression. OMT ameliorated TGF- β -induced morphology changes and it significantly inhibited TGF- β -induced cardiac fibroblasts proliferation. Moreover, OMT alleviated the I and III collagen secretion after TGF- β exposure by Elisa and hydroxyproline commercial kits. OMT could down-regulated Smad 2 and Smad 3 proteins, and Smad 2 mRNA levels compared with TGF- β group.

Conclusion: OMT could ameliorate cardiac fibroblasts abnormal induced by TGF- β 1, and the mechanism involve down-regulating Smad 2 and Smad 3 protein signal of the down-stream of TGF- β 1-Smads.

P71: Determination of apigenin in *Scutellaria barbata* D. Don using power ultrasound extraction and chromatography

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ABSTRACT

Apigenin has been recently considered of interest in oncology because it exhibits an antineoplastic activity on several kinds of cancers.

Objective: In this study, apigenin was extracted from *Scutellaria barbata* D. Don using conventional heat-reflux extraction (HRE) and a hyphenated process (HUAE) of HRE and ultrasonic assisted extraction (UAE) with different extraction schemes and parameters and was analyzed by the high-performance liquid chromatography (HPLC).

Methods: HUAE was performed six times on each sample and was conducted using an ultrasonic cleaning bath with a working frequency of 40 kHz and 185 W of power. Each extraction was conducted using an intermittent pulse mode that provided 90 s of pulse followed by 30 s of no pulse (75% on/off time, performed manually). The effects of various parameters on the extraction efficiency for apigenin were evaluated.

Results: Comparable extraction results were obtained with the best HUAE and HRE conditions. The analytical performance of the HPLC method was also validated for its linearity, limits of detection (LOD) and quantification (LOQ), precision and accuracy. The relative standard deviations (RSDs) of both the intra-day and inter-day precision for apigenin were below 5.83%. The accuracy at different concentrations was within the range of -5.24 to 3.19%. The data have shown that HUAE had higher yields of bioactive apigenin as well as a shorter extraction time at less harsh operating conditions compared to HRE.

Conclusion: The study suggested that the combined HUAE and HPLC method proved to be sensitive, rapid, accurate and suitable for the determination of apigenin in *S. barbata* D. Don. HUAE is considered to be a good alternative process for obtaining high concentration of apigenin from *S. barbata* D. Don. The results may provide a theoretical basis for the medical utilization of *S. barbata* D. Don in the future.

P72: An analysis of the influence of different stages of growth and water stress on phytochemical and biochemical compounds compositions of *Aloe vera* L.

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ABSTRACT

Aloevera is one of the most economically important medicinal plants in many countries.

Objective: In order to study the effects of water stress and date harvest on phytochemical and biochemical compositions of *Aloe vera*.

Methods: An experiment was conducted in research greenhouse of Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran in 2013 and 2014. The experimental design was a randomized complete block design arranged in split-plot treatments included water stress (20, 40, 60 and 80% of the field capacity (FC) and date harvest (90, 180 and 270 days after imposing the treatments). Growth changes, phytochemical and biochemical compounds were evaluated during growing period.

Results: Generally, the results indicated that highest aloin percentage was obtained 90 days after imposing the treatments when the plants were irrigated after depleting 80% of the FC. On the contrary, the lowest aloin percentage was obtained 180 days after imposing the treatments when the plants were irrigated after depleting 40% of the FC. The highest fructose and glucose content was observed when the plants were irrigated after depleting 80% of the FC. Proline accumulation increased with increasing water stress intensity compared with non stressed plants so that the highest proline accumulation was registered 90 days after imposing the treatments when the plants were irrigated after depleting 40% of the FC.

Conclusion: According to the results, severe water stresses a significant increase in phytochemical and biochemical compounds. Increased phytochemical and biochemical compounds, as a water stress resistance mechanism, was observed in water stressed plants which led to adjusted osmotic.

P73: Identification of Allium volatiles using headspace combined with surface-enhanced Raman scattering

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ABSTRACT

Microwave heating method was utilized to obtain Ag colloids. The SERS spectra of volatile of fresh garlic, garlic chives and scallion were investigated and calculations were performed. The calculated result from gaussian 03 was compared with the experimental ones.

Objective: This study aimed to direct analysis of volatile organic compounds of fresh plants without sample preparation.

Methods: The SERS of gas state reference standards each was obtained as follows: 1 mL reference standard was placed in the headspace vial (volume: 250 mL) and sealed; after 5 minutes 30 mL of gas were drawn from the vial with a syringe and were injected very slowly into 1.5ml of Ag colloids for this experiment. Garlic, Chinese chives and scallion were obtained from a local market and chopped into pieces 2-3 mm long. Then 13.00 gram of each was placed in the headspace vial (volume: 250 mL) and sealed; they are then well shaken to ensure their volatility. No further sample preparation was used. After 5 minutes, 30ml of gas was drawn from the vial with a syringe and were injected very slowly into 1.5ml of Ag colloids for test.

Results: For garlic: high intensity bands are present in 1622, 1397, 1287, 1182, 711, 569, 461 cm^{-1} and low intensity bands are present in 1017, 979, 918, 307 cm^{-1} . For Chinese chives: the high intensity band is present in 672 cm^{-1} and low intensity bands are present in 1618, 1396, 1289, 1185, 575, 412, 274 cm^{-1} . For scallion high intensity bands are present in 699, 1023 cm^{-1} . and low intensity bands are present in 369, 887, 1084, 1314 cm^{-1} . The main volatiles of fresh garlic, Chinese chive and scallion are diallyl disulfide, allyl methyl sulfide and 1-propanethiol respectively. As the bulk concentration changes, different conformers are adsorbed on silver.

Conclusion: The fresh garlic, Chinese chive and scallion volatile in the headspace are diallyl disulfide, allyl methyl sulfide and 1-propanethiol respectively, which provide the highest affinity to bind to silver. The presented results illustrate that combining headspace with SERS is a powerful tool for identifying Allium volatiles. The volatile can be detected in fresh Allium directly and quickly without extraction.

P74: Ameliorated effects of the essential oil from *Fructus Alpiniaezarumbet* on thoracic aorta in spontaneously hypertensive rat (SHR)

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ABSTRACT

Endothelial dysfunction (ED) is the key pathological process in cardiovascular diseases (CVD), inflammatory and lipiddeposition is involving in the process.

Objective: The present study concentrates on the ameliorated effects of EOFAZ against SHR vascular endothelial injury.

Methods: The EDof SHR was reproduced by treated with high cholesterol diet for 12 weeks. The SHRs were randomly divided into 6 groups as following: the control group, model group, captopril 25 mg/kg group, EOFAZ 520 mg/kg group, EOFAZ 260 mg/kg group, EOFAZ 130mg/kg group. The SHR was administrated with the different drug for 12 weeks from the exposed with high fat diet 20g/(Rat-day).The blood pressure(BP) was determined after 1 h of administration. The TNF- α , CRP, and ET-1 in serum, and the blood lipid contents were determined by the Elisa methods. The NO contents were assayed by the nitrate reductase method. The pathological morphology was stained with Victoria blue (VB). The protein and mRNA expressions of NF- κ B were detected by RT-PCR and western blot, respectively.

Results: The Captoril and EOFAZ groups could alleviate the BP of SHR in various degrees compared to the control group. EOFAZ significantly attenuated the TNF- α and CRP levels ($p < 0.01$) and the ratio of NO/ET-1 and LDL-C/HDL-C in serum compared with the model group.TC and TG contents of 520 mg/kg and 260 mg/kg EOFAZ were significantly lower than the model group. The VB staining results indicated that elastic fibers decreased or disappeared, however the collagen fibers significantly increased in the model group. Administration with EOFAZ could ameliorate the deteriorated pathologic morphology. The mRNA expressions of NF- κ B in 520 mg/kg and 260 mg/kgEOFAZ were decreased in the thoracic aorta tissue. The western blot confirmed that NF- κ B (pp65/p65) was inhibited after treatment with 520 mg/kg EOFAZ.

Conclusion: EOFAZ could protect against the ED of SHR induced by high fat diet which may involve reducing vascular endothelial inflammatory injury, ameliorating lipid metabolism, and inhibiting NF- κ B activation.

P76: Flavonoid profiles, antioxidant potential, and acetylcholinesterase inhibition activity of the extracts from archegoniophore and gametophyte of *Marchantia polymorpha*

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ABSTRACT

Marchantia polymorpha belongs to family Marchantiaceae and genus *Marchantia* and is used as a traditional Chinese medicinal herb for scald and pneumonia. The phytochemicals found in *M. polymorpha* are terpenes and flavonoids, which show significant biological benefits for human health.

Objective: The flavonoids profiles, antioxidant and acetylcholinesterase (AChE) inhibition activities of the extracts from archegoniophore and gametophyte of *M. polymorpha* were compared.

Methods: Radical scavenging assay (DPPH, ABTS, O²⁻), reducing power assay, inhibiting AChE assay and LC-MS analysis.

Results: The total flavonoids content in the archegoniophore is determined as 47.4 mg/g, which is about 10-time higher than that of gametophyte (4.6 mg/g). The archegoniophore and gametophyte of *M. polymorpha* appear different flavonoids profiles based LC-MS analysis. The main flavonoids in the archegoniophore of *M. polymorpha* are flavone glycosides.

The antioxidant potential of these extracts from the archegoniophore is much higher than that from the gametophyte by means of radical scavenging assay (DPPH, ABTS, O²⁻) and reducing power assay. The extract from the archegoniophore showed significant inhibition against AChE. However, the extract from the gametophyte hardly inhibited AChE.

Conclusion: The archegoniophore of *M. polymorpha* contains higher flavonoids content and shows stronger bioactivities than that of the gametophyte.

P78: The ameliorated effects of Ginkgo biloba extract (GBE) on primary culture rat cardiac myocytes apoptosis induced by the AngII *in vitro*

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ABSTRACT

Cardiac myocytes apoptosis is the key pathological process in the cardiac remodeling, finally results in chronic heart failure and sudden death. GBE (Ginkgo Biloba extract), flavonoids and terpene lactones and other compounds, is bioactive ingredients extracted from ginkgo leaves for anti-cardiovascular and cerebrovascular diseases. However, there is unclear that GBE protect the apoptosis of cardiac myocytes.

Objective: In the present study concentrated on the GBE alleviated apoptosis of primary culture rat cardiac myocytes induced by AngII, and then explore the mechanism for the clinical application GBE ameliorating cardiac remodeling.

Methods: The primary culture rat cardiac myocytes (PCRCMs) were separated by trypsin methods and differential adhesion from 1-3 d sucking rats. The PCRCMs were randomly allotted into 5 groups as following: negative control group, AngII group, 6 µg/mL SB431542, 2 µg/ml GBE, 20 µg/ml GBE group. Preincubated with the different agents, and then the PCRCMs were exposed to 10⁻⁵ mol/L AngII. The histopathological morphology was determined by HE staining. The flow cytometry and Tunel staining were used to measure the apoptotic ratio. The Bcl-2 and Bax protein expression were detected by immunofluorescence methods with confocal laser scanning microscope.

Results: After exposed PCRCMs to 10⁻⁵ AngII, there were classic apoptotic characteristics by HE staining as following: the cell membrane shrinkage, horsehoe-shaped nuclei, etc. The apoptotic ratio was significantly increased by flow cytometry and Tunel staining after incubated with the 10⁻⁵ AngII 48 h. The immunofluorescence methods results confirmed that the Bcl-2 protein expression was significantly inhibited and Bax protein expression was significantly increased, finally attenuated Bcl-2/Bax. Pre-treated with GBE or SB431542 could ameliorated the deteriorated characteristics of PCRCMs.

Conclusion: GBE could ameliorate the primary culture rat cardiac myocytes apoptosis induced by AngII, the mechanism maybe involve regulating the relevant apoptosis protein expression such as increasing the Bcl-2 protein apoptosis, alleviating Bax protein expression, and enhancing the Bcl-2/Bax ratio.

P79: Evaluation to the antioxidant, cytotoxic and apoptotic activity of crude extract from different fractions of *Stenoloma chusanum* (L.) Ching

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ABSTRACT

Stenoloma chusanum (L.) Ching is known as popular herb in China. Because of its wide use in private, there is a considerable concern worldwide over its components and bio-activities recently.

Objective: The purpose of this work is to evaluate multiple biological potentials of *S. chusanum* (L.) Ching and identify its different soluble fractions to find the one with the highest specific biological activity.

Methods: All the fractions were extracted from its crude ethanol extract, which were respectively petroleum ether (PF), chloroform (CF), ethyl acetate (EF), n-butanol (NF) and water fractions (WF). They were tested for total flavonoid content, antioxidant (DPPH• and ABTS• radical scavenging assay, reducing power assay and ferric reducing antioxidant potential), the cytotoxic (MTT assay on K562 cells) and the apoptotic activity (fluorescence microscopy assay).

Results: We found that the total flavonoid content of the PF, CF, EF, NF and H₂O is 25.2±0.4, 442.4±2.6, 746.8±3.9, 553.5±0.2 and 24.8±0 µg rutin equivalent/mg respectively. Obviously the EF had the highest content. The five soluble fractions from *S. chusanum* (L.) Ching showed significant reducing power activity and the total antioxidant capacity, and the activities were, in decreasing order, as follows: VC (positive control) > EF > NF > CF > WF > PF. All the five fractions exhibited a dose-dependent phenomenon on the antioxidant evaluation. In addition, the effect of the different fraction on the proliferation of K562 cells was as follows: EF > NF > CF > PF > WF. Correspondingly, the IC₅₀ value of the EF (150.2± 2.1 µg/ml) was found to be the lowest compared with the NF (185.2±3.5 µg/ml) and the CF (268.3±1.6 µg/ml), while the value of the other two fractions could not be detected. Correlation analysis suggested that flavonoid content present in the CF, EF and NF was responsible for high antioxidant and cytotoxic activity (P<0.05). Above all, the EF contained the highest amount of total flavonoid content and showed the maximum antioxidant and cytotoxic activity.

Conclusion: The five soluble fractions from *S. chusanum* (L.) Ching show multiple biological effects and the EF has the highest potential due to its antioxidant, cytotoxic and apoptotic activity.

P82: Phenolic profiles and antioxidant activities of exocarp, endocarp, and hypanthium of three pear cultivars grown in China

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ABSTRACT

Pear (*Pyrus communis* L.) is one of the most common and widely consumed fruits in the world. Fruits in general contain a wide array of dietary phytonutrients such as flavonoids, phenolic acids, carotenoids, and vitamins with strong antioxidant capacities. Among fruits, the pear is reported to contain a considerable amount of valuable compounds such as natural antioxidants and in turn, impart health-promoting effects to consumers.

Objective: This study aimed to investigate and analyze the phenolic composition and antioxidant activity of the exocarp, hypanthium, and endocarp of three different Chinese pear cultivars.

Methods: The phenolic composition of the exocarp, hypanthium, and endocarp from three pear cultivars: Jingbai, Korla and Crystal were measured by high-performance liquid chromatography method with diode array detection (HPLC-DAD), while antioxidant activities were investigated using DPPH, ABTS radical scavenging activity, and FRAP.

Results: The exocarp of the Jingbai pear had a relatively high chlorogenic acid content (0.691 mg kg^{-1}) while the hypanthium and endocarp had the high chlorogenic acid content of the three pear varieties. The exocarp of the Korla pear had the highest ferulic acid and rutin content. The exocarp, hypanthium, and endocarp of the Crystal pear had the highest content of chlorogenic acid of the three pear varieties. The Crystal pear presented the highest total phenolic and flavonoid contents and had the highest antioxidant activities in terms of FRAP. The Jingbai pear exocarp presented the highest ABTS value, $83.62 \pm 0.08\%$. The endocarp of the Crystal pear had the highest DPPH value, $87.30 \pm 0.03\%$.

Conclusion: Positive correlations were detected among chlorogenic acid, TPC, TFC, and FRAP in three pear cultivars grown in China. Provide scientific support to produce an enhanced value-added fruit and a source of a low-cost functional food.

P84: Chemical constituents and antibacterial properties of *Indocalamus latifolius* McClure leaves, the packaging material for “Zongzi”

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ABSTRACT

Objective: The glutinous rice dumpling named “Zongzi” in Chinese is a type of traditional food that is popular in East Asian countries. “Zongzi” is wrapped in the leaves of *Indocalamus latifolius* McClure as the packaging material. “Zongzi” has been characterized by a long shelf life since ancient times. In this research, we examined the phytoconstituents of *I. latifolius* McClure leaves in detail and assayed their antibacterial properties.

Methods: Dried *I. latifolius* McClure leaves were extracted with 95% aqueous ethanol. The crude extract was sequentially separated on a macroporous resin column and performed on medium-scale preparative performance liquid chromatography, and was further applied to a column chromatography system to yield 10 compounds. The structures and relative configurations of the compounds were determined by detailed spectroscopic analysis (HRESIMS, HSQC, HMBC, NOE and CD). All of the isolated compounds were screened for their antibacterial activities against two Gram(+) and two Gram(-) bacterial strains *in vitro*.

Results: Four new compounds, latifoliusine A (2), (7*S*,8*R*) syringylglycerol-8-*O*-4'-sinapyl ether 4-*O*- β -D-glucopyranoside (7), (7*S*,8*S*) syringylglycerol-8-*O*-4'-sinapyl ether 7-*O*- β -D-glucopyranoside (8) and (7*R*,8*S*) syringylglycerol-8-*O*-4'-sinapyl ether 7-*O*- β -D-glucopyranoside (10), along with six known compounds (1,3-6 and 9) were isolated from *I. latifolius* McClure leaves for the first time. Latifoliusine A (2) is a novel norsesquiterpenoid with a distinctive fragrance. The results of the antibacterial activity tests indicated that the 10 compounds had selective antibacterial properties. Apigenin 6-*C*- α -L-arabinopyranosyl-8-*C*- β -D-glucopyranoside (5) and apigenin 7-*O*,8-*C*-*di*-glucopyranoside (6) had antibacterial activities against all 4 bacterial strains, and more notably, these two compounds showed strong antibacterial activities against two food-contaminating bacteria, *S. aureus* and *E. coli*.

Conclusion: Since ancient times, the leaves of *I. latifolius* McClure have been used as a packaging material for food, and presently, they still play a unique role in producing “Zongzi” in China. The identification of the antibacterial compounds in the leaves of *I. Latifolius* McClure is important for helping us to understand the long shelf life of “Zongzi” as well as for exploring the potential of *I. latifolius* McClure leaves as a natural, healthy, and eco-friendly alternative packaging material for other applications.

P90: Flow cytometry in the study of anti-tumor activity of pennogenyl saponins from *Paris quadrifolia* L.

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ABSTRACT

Objective: Pennogenyl saponins are active compounds of many plant species from *Liliaceae* family. One of the important saponin-bearing genus from this family is *Paris*, widely occurring in the Orient. In Europe, the representative species of the genus is *Paris quadrifolia* L. The aim of this study was the estimation of anti-proliferative activity and explanation of the mechanism of action of the pennogenyl saponins isolated from *P. quadrifolia* rhizomes on human cervical cancer HeLa cells. The structure of **compound 1** is determined as pennogenin 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside and **compound 2** as pennogenin 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside (1).

Methods: In our study we investigated the cytotoxic activity of the pennogenyl saponins on the cells using MTT assay. To confirm saponins-induced cell apoptosis we performed the cell staining using annexin V and 7-amino-actinomycin (7-AAD). To determine the state of mitochondrial membranes of apoptotic HeLa cells after treatment the cells with the two saponins we used mitopotential dye (Merck Millipore, Germany). The cells in both experiments were analyzed by flow cytometry on the Muse Cell Analyzer (Merck Millipore). The system delivers high-performance cell analysis using miniaturized fluorescence detection and microcapillary technology.

Results: The two tested compounds show significant anti-proliferative effect on HeLa cells. The IC₅₀ values obtained in MTT assay for compounds 1 and 2 were 0.93 ± 0.15 μ g/ml and 0.55 ± 0.01 μ g/ml, respectively. The flow cytometry analysis shows that the two compounds induce apoptosis in a dose-dependent manner. We also observed mitochondrial dysfunction in the early stage of the cell apoptosis.

Conclusions: The two examined pennogenyl saponins have strong cytotoxic activities on the tumor cells. The compounds induce apoptosis and modulate mitochondrial membrane potential of HeLa cells. This indicates that mitochondria play a role on the pathway of cell death.

In our study, we used the Muse Cell Analyzer which can be a suitable tool to estimate the mechanism of anti-tumor action of active plant compounds.

Reference:

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P92: Structure-uptake/binding relationship of dietary polyphenols on human EA.hy 926 endothelial cells and its influence by the stability of polyphenols under cell culture conditions

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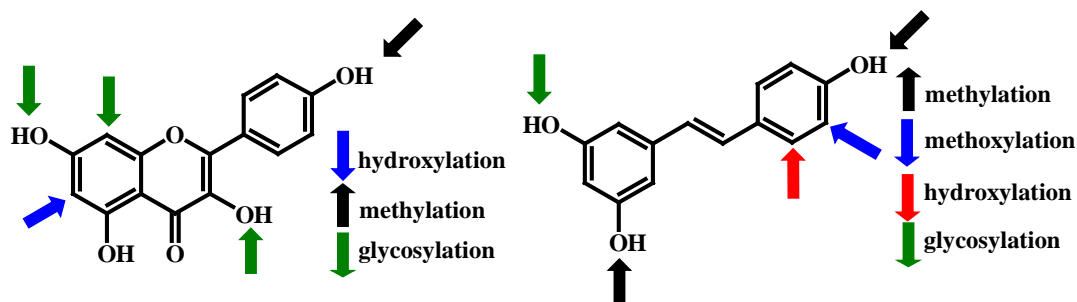
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ABSTRACT

Objective: Herein, the structure-uptake//binding relationship of dietary polyphenols on human EA.hy 926 endothelial cells was investigated in detail.

Methods: The uptake/binding percentages of polyphenols depend on their structure properties. Multi-hydroxyflavanones, multi-hydroxyflavones and isoflavones exhibited very low uptake by EA.hy926 human endothelial cells. Less-hydroxyflavanones and less-hydroxyflavones showed relative high uptake. The structure–stability relationship of polyphenols in DMEM culture revealed that the pyrogallol-type polyphenols were evidently instable. Therefore, the determined data of uptake percentages of these polyphenols on EA.hy926 human endothelial cells are not precise. For example, because of significantly unstable, the determined uptake data for quercetin, fisetin and myricetin couldn't reflect the real uptake results.

Results: The structure–stability relationship of polyphenols were determined as: 1) The glycosylation of polyphenols significantly reduced their cell uptake. 2) In most case, the methoxylation of polyphenols improved their uptake. 3) The hydroxylation of polyphenols affected their uptake depending on the type of polyphenols and the position and number of hydroxyl groups. 4) All the isoflavones tested were hardly absorbed by EA.hy926 human endothelial cells. 5) It is difficult to compare the structure-uptake relationship of tea catechins because of their instability.



Conclusions: The potential sites of polyphenols affecting the uptake percentages of polyphenols on EA.hy926 human endothelial cells are schematically illustrated. The up arrows represent increasing the uptake, the down arrows represent decreasing the uptake.

P93: New formulas used in gastrointestinal disorders – phytochemical evaluation

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ABSTRACT

The present study enabled the identification of the main compounds found in 10 different species and the preparation/completion of 9 new formulas (tea and tinctures association type) indicated in gastrointestinal disorders. The antioxidant properties were considered to belong to the bioactive compounds, such as flavonoids and phenolic acids, identified in the phytocomplex.

Objective: The aim of this study was to obtain new association preparations, either as tea species or as food supplements, from medicinal plants harvested from Romanian ecological cultures. Both the raw material and the final extracts were chemically assessed.

Methods: Qualitative and quantitative methods were used to investigate the MAPs. All extracts were obtained in accordance with the European Pharmacopoeia provisions. The amounts of total phenolics, flavonoids, essential oils and triterpens and/or phytosterols were measured (spectrophotometry, TLC, HPLC, GC-MS). The antioxidant activity was measured by ABTS (1) and DPPH methods.

Results: The rosmarinic acid was the main compound common for most of the formulas, formula 1 (*Centaurii herba, Millefolii flos, Menthae folium*) and formula 6 (*Foeniculi fructus, Menthae folium, Melissa folium, Calami rhizome*) containing the highest amounts (1.535 and 1.961 g/100g respectively). Moreover, these two combinations showed a rich content in rutin-type flavonoids (1.233 and 0.843 g/100g respectively). The GC-MS analysis indicated that formulas 1 and 8 (*Cynarae folium, Menthae folium, M. flos*) are rich in essential oil compounds, out of which *Millefolii aetheroleum* contains high amounts of chamazulene (29.69%). The essential oil isolated from *Melissae folium* is characterized by the presence of citral (34.91%), neral (28.24%) and citronellal (2.86%), whereas methol along with neoisomenthol (32.78%) and pulegone (12.42%) were the main compounds of *Menthae folium* used in our preparations. There are very good correlations between the total polyphenol contents and antioxidant activities of samples, for yarrow ABTS, DPPH 37.58±0.46 and 31.92±0.15 µM Trolox/g plant (DW).

Conclusion: The new formulas contain bioactive compounds with known therapeutic effects, thus allowing the completion of new products used as complementary medicines in gastrointestinal disorders. Such preparations can enrich the portfolio of the SME profile.

Reference:

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P95: Biotransformation of flavonoid conjugates with Omega 3 fatty acids and evaluations of their functionalities

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ABSTRACT

Bioactive natural compounds are produced in plants and animals for various metabolic purposes. Past and recent research has supported the health claims for various bioactive compounds, i.e. flavonoids and polyunsaturated fatty acids (PUFAs). These compounds have been (or have the potential to be) utilized as ingredients in nutraceuticals and pharmaceuticals, however, when extracted, they often do not possess the optimum physical properties suitable for the product development.

Objective: This study aimed to attach Omega 3 FAs to citrus fruit-derived flavonoids under lipase catalysis, in order to generate novel flavonoid/FA conjugates with desirable functionalities.

Methods: Grapefruit extracts, naringin, and neohesperidin dihydrochalcone were esterified with Omega 3 FAs derived from PUFA rich fish oil, catalysed by Novozyme 435 in acetone at 50 °C. The reaction mixture were then dried, and phase-separated with heptane and acetonitrile at 60° C to remove unreacted FAs. The unreacted flavonoids and flavonoids FA conjugates were dried and re-dissolved in acetone, then separated on silica gel chromatography under chloroform/methanol elution. The purified flavonoid conjugates alongside their original flavonoids were analyzed for antioxidant activities via DPPH scavenging assay and anti-peroxidation test via peroxide values measured during a one-week fish oil storage trial. VEGF (vascular endothelial growth factor) assay was conducted with 1, 10 and 100 µM of the flavonoids and their conjugates, respectively, and total VEGF levels were measured at 24 and 48 hours, respectively using ELISA assay.

Results: LC-MS analysis confirmed 85-89% conversions in the esterification between flavonoids and Omega 3 FAs. The main FAs esters formed were identified as esters of C20:5, C22:6, C22:5, and C18:1, consistent with the FAs profile found in the fish oil starting material. NMR analysis of the conjugates confirmed that the FA carbon chain was linked onto the primary -OH group on the glucose of the flavonoids. The functionality study demonstrated that flavonoid FAs conjugates had at least comparable (if not higher) antioxidant activity, anti-peroxidation activity and anti-angiogenic activity at all 3 concentrations tested.

Conclusion: This study has demonstrated the feasibility of biotransformation directly applied to natural product extracts, to generate a new class of bioactives – flavonoid FA conjugates- with desirable functionalities: PUFAs with built-in protection against peroxidation or lipophilic antioxidants.

P96: Stilbenoids-protein interaction

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ABSTRACT

Stilbenoids are phytoalexins that are activated when plants are stressed and are important polyphenols with the C6–C2–C6 structure. The typical natural stilbenoids are resveratrol and its derivatives. These compounds exist in foods and are widely consumed. The health effects of resveratrol and its derivatives depend on their bio-availability and bio-accessibility.

Objective: Biological properties of stilbenoids depend on their bioavailability. Stilbenoids-protein interactions are expected to modulate the bio-availability of stilbenoids. Herein, the molecular structure-affinity relationship, stability and antioxidant activity of non-covalent interaction between dietary stilbenoids and proteins were investigated.

Methods: High performance affinity chromatography (HPAC) and fluorescence measurements have been used to check the binding affinity between stilbenoids and human serum albumin (HSA). The influence of albumin-stilbenoids interactions on the free radical scavenging activity of stilbenoids were investigated by a DPPH assay.

Results: The affinities between stilbenoids and HSA based on fluorescence changes were determined as: piceatannol > resveratrol > pterostilbene > pinostilbene > oxyresveratrol > isorhapontigenin > piceid. The determined structure–affinity relationships of stilbenoids were: 1) a glycosylation significantly decreased the affinity; 2) a methylation and methoxylation reduced it; 3) the hydroxylation of stilbenoids affected the affinity depending on the position and number of hydroxyl groups. The protein binding (%) of stilbenoids analysed by HPLC with a HSA column revealed the order: pterostilbene (96.09%) > pinostilbene (93.61%) > oxyresveratrol (92.81%) > piceatannol (90.86%) > resveratrol (90.70%) > isorhapontigen (86.74%) > piceid (71.99%). The structure–protein binding relationships of stilbenoids can be described as: 1) a glycosylation significantly decreased the affinity; 2) a methylation enhanced the affinity and a methoxylation reduced it; 3) the hydroxylation of stilbenoids slightly improved the affinity. The influence of proteins on the antioxidant activity of stilbenoids was governed by the antioxidant assay, the structure characteristics of stilbenoids, as well as the proteins. The stilbenoids in a solution with HSA showed higher stability than that in human plasma. The glycosylation and methoxylation of OH moiety on stilbenoids enhanced the stability and the hydroxylation of stilbenoids decreased the stability.

Conclusion: The structure of stilbenoids has a significant influence on the affinities of stilbenoids-protein, which also play a significant role in the stability and antioxidant activity of stilbenoids.

P99: Anti-obesity effect of 5,7-dimethoxyflavone on 3T3-L1 adipocytes and high-fat diet-induced obese C57BL/6J mice

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ABSTRACT

The rise of obesity throughout the past decades has become a worldwide healthcare problem. Obesity is one of the metabolic diseases which is due to the abnormal growth of adipose tissue by imbalance of energy intake and expenditure. The outbreak of obesity is related to lifestyle, environmental, and genetic factors and is related to a variety of other metabolic diseases, including type 2 diabetes, hypertension, and cardiovascular disease.

Objective: In the present study, the anti-obesity effect of 5,7-dimethoxyflavone (DMF) was evaluated in 3T3-L1 adipocytes and high-fat diet (HFD)-induced obese C57BL/6J mice.

Methods: DMF, isolated from *Kaempferia parviflora* Wall. Ex Baker (*K. parviflora*), was used to investigate the accumulation of lipid droplets and triglyceride in 3T3-L1 adipocytes by Oil Red O staining. In addition, the anti-obesity effect of DMF (50 mg/kg/day for 6 weeks) was evaluated in HFD-induced mice. Regulation of adipogenic factors, lipid metabolism-related enzymes, and lipolysis-related proteins during adipogenesis were determined by Western Blot and Reverse transcriptase polymer reaction chain (RT-PCR) in 3T3-L1 adipocytes and fat tissue of obese mice.

Results: The accumulation of lipid droplets and triglyceride in adipocytes was potently and dose-dependently suppressed by DMF through inhibition of adipogenesis and lipid production. DMF markedly attenuated the levels of adipogenic transcription factors and lipid metabolism-related enzymes during adipogenesis. Additionally, DMF up-regulated lipolysis-related proteins but significantly reduced the level of adiponectin. During the *in vivo* experiment, the oral administration of DMF (50 mg/kg/day for 6 weeks) significantly decreased the body weight gain without affecting the food intake. DMF also improved serum levels such as low-density lipoprotein (LDL) cholesterol, total cholesterol levels, and triglyceride. Particularly, DMF showed a decrease in fat mass in epididymal and perirenal fat tissue as well as adipocyte hypertrophy in epididymal fat tissue through reducing the expression of adipogenic transcription factors. DMF also prevented HFD-induced non-alcoholic fatty liver by reducing triglyceride in liver tissue.

Conclusion: DMF might be a potential natural agent for attenuating obesity and metabolic syndromes caused by obesity.

P100: Computer-aided drug screening based on natural products

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ABSTRACT

Nowadays, there are more than 120 chemical substances sold as drugs in one or more countries that are made from plants, such as taxol and quinine. As an important source of biological active compounds and drug-like compounds, natural products play an increasing significant role in drug development. Discovery of new potential active-compounds from natural products for some particular disease targets, and identification of their pharmacological activity have become a promising way in drug development. However, because of the huge amount of data, the process takes a lot of time and money, which makes it difficult to achieve in reality. Computer-aided drug screening is a useful way to quickly find lead compounds, and we hope its use is helpful to solve these problems.

Objective: This study aimed to build two databases in pdbqt format, natural products database (NPD-pdbqt) and traditional Chinese medicines compounds database (TCMCD-pdbqt), and to discover potential functional compounds by applying computer-aided drug screening, which is useful to decrease the costs and time of drug discovery.

Methods: 1. Construction of NPD-pdbqt and TCMCD-pdbqt: As the source of NPD-pdbqt and TCMCD-pdbqt, we mainly collected the molecular structure information of natural products and traditional Chinese medicines from both domestic and international mainstream databases, such as ZINC database. Then convert the format of compounds structures to pdbqt format through Applied Chemistry Software openbabel.

2. Virtual screening based on natural products: Do structural analysis for the protein targets of diseases. In order to get the potential functional compounds of these targets, do virtual screening based on the constructed NPD-pdbqt and TCMCD-pdbqt by applying computer-aided virtual screening platform.

Results: We successfully constructed traditional Chinese medicines compounds database, which contains 8,445 kinds of active ingredients in traditional Chinese medicines and 33,765 molecular compounds, and natural products database, which contains 149,515 molecular compounds. The results of several virtual screening experiments based on these two databases indicate that they can effectively provide potential active compounds for the selected protein targets through virtual screening.

P103: Proteomic analysis of cyanobacterium *Nostoc flagelliforme* in response to pH shift

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ABSTRACT

Nostoc flagelliforme, former name *Nostoc commune* var. *flagelliforme*, is a terrestrial nitrogen-fixing cyanobacterium distributed on arid or semiarid steppes in many countries including Algeria, China, Czechia, Slovakia, France, Japan, Mexico, Mongolia, Morocco, Russia, Somalia and the USA.

Objective: This study aimed to identify protein candidates involved in alkali stress response in aquatic *Nostoc flagelliforme*.

Methods: Exponentially growing cells at pH 7.5 were harvested by centrifuging at 6000 xg for 5 min, and resuspended in fresh BG-11 medium adjusted to the specified pH, 7.5 and 9.0 respectively, using 5 mM Tris (Hydroxymethyl) aminomethane. Then harvest cells and extract fracture protein. The protein solution was used immediately for 2-DE (two-dimensional gel electrophoresis). Gel images were captured using the unis can m1600 scanner at a resolution of 600dpi and digitalized images were analyzed with the Image Master 2D platinum version 7.0 software (GE Healthcare). Excision of protein spots and sample preparation for MALDI-TOF analysis was done. NCBI (www.ncbi.nlm.nih.gov/BLAST/) and Cyanobase (<http://www.kazusa.or.jp/cyanobase/>) were used to search sequence information.

Results: Comparison of soluble proteins from the total fraction of cells grown on media set at pH 7.5 and 9.0 using 2-DE identify seven proteins, which showed significant changes in abundance. Among those proteins expression profiling at different external pH 7.5 and 9.0, two unknown proteins showed significant changes corresponding to pH shift, and provided ideal target for further studies in the mechanism of secretion polysaccharides (capsular and released polysaccharides). This study also identified three novel proteins, hitherto unknown in Cyanobacterium.

Conclusion: Among those proteins expression profiling at different external pH 7.5 and 9.0, two unknown proteins showed significant changes corresponding to pH shift, and provided ideal target for further studies in the mechanism of secretion polysaccharides (capsular and released polysaccharides). This study also identified three novel proteins, hitherto unknown in Cyanobacterium.

P104: Metabolic profiling of saponins in plant *in vitro* cultures of the Balkan endemic species *Saponaria stranjensis* D. Jord.

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ABSTRACT

Saponaria stranjensis D. Jord. (Caryophyllaceae) is a Balkan endemic plant under the protection of the Bulgarian biodiversity law with national conservation status: Vulnerable. Saponins are secondary metabolites, characteristic for most of the representatives of family Caryophyllaceae. These biologically active molecules have anti-inflammatory, expectorant, anti-cancer, anti-HIV, and anti-bacterial activity which makes saponins and saponin containing extracts and preparations pharmaceutically important.

Objective: This study aimed to identify saponin composition of *in vitro* cultivated plants, cell and adventitious root cultures from *S. stranjensis* using high resolution system LC/ESI-LTQ-Orbitrap-MS. Comparing the metabolite profiles of micropropagated plants, cell and organ cultures will allow assessment of their biosynthetic potential in order to create economically driven process and conservation of plant biodiversity.

Methods: Initiation of stem *in vitro* culture from Strandja soapwort was accomplished by successively sterilization of seeds with 70% ethanol and Domestos®. The micropropagation was achieved on hormone-free basal MSmedium with 30 g/L sucrose and 8 g/L agar. Saponins were profiled and identified using mass spectrometry techniques with negative ion detection LC/ESI-LTQ-Orbitrap-MS and accurate mass measurement in MS and MS² modes.

Results: Eighteen triterpenoid saponins with saponogenin from oleanane type were identified in *S. stranjensis in vitro* cultures. Fourteen of them were reported for the first time in representatives of genus *Saponaria*. Saponarioside A, saponarioside B, and two saponariosides with [M-H]⁻1861 and 1729 respectively which have been previously established as major saponins in *S. officinalis*, were also identified in Strandja soapwort. The widest variety of compounds with saponin nature was found in adventitious root culture followed by suspension culture but the ions intensity in the latter was significantly lower.

Conclusion: A collection from tissue and organ cultures, which is an approach for preservation of *S. stranjensis* has been established. *S. stranjensis* adventitious root culture has a potential to be utilize as a promising model system for the production of pharmaceutically valuable saponins.

Acknowledgements: The research was supported by L'Oreal-UNESCO "For Women in Science" Fellowship Program, 2014; Grand DFNI-BO2/14 of Ministry of Education, Bulgaria.

P105: Comparative determination of essential oil composition in the Bulgarian endemic plant *Achillea thracica* Velen. during the process of *ex situ* conservation

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ABSTRACT

Achillea thracica Velen. (Asteraceae) is a Bulgarian endemic under protection of the Bulgarian Biodiversity Law with national conservation status: critically endangered. The species is in the Bern Convention on the Conservation of the European Wildlife and Natural Habitats as "rare" (1979).

Objective: In the present work we aimed to assess *in vitro* cultivation of *A. thracica* and subsequent adaptation in *ex vitro* conditions and comparative determination of essential oil composition in *in situ*, *in vitro* and *ex vitro* cultivated plants.

Methods: *In vitro* shoot cultures were induced from ripe dry seeds, collected from *in situ* growing wild plant and sterilized with 70% ethanol. The micropropagation was achieved on basal MS-B5 medium with 30 g/L sucrose and 8 g/L agar. *Ex vitro* adaptation was accomplished in growth chamber with 100% survival. Air-dried aerial part of *in situ*, *in vitro* and *ex vitro* cultivated plants were separately subjected to hydro distillation using a Clevenger type apparatus. The essential oils were analyzed by Gas Chromatography – Mass Spectrometry (GC-MS).

Results: The GC-MS analysis of the essential oils resulted in the identification of 15, 20 and 48 compounds in *in situ*, *in vitro* cultivated and *ex vitro* adapted plants respectively, constituting 88.9%, 86.3% and 77.8% of the total oils. Geraniol, β -eudesmol, yomogi alcohol, cineole, and artemisia alcohol were the principal components comprising 80.7% of the oil of *in situ* plants. *In vitro* cultivated plants consist of mainly presilphiperfolan-9 α -ol and cineole representing 74.9% of the oil. The widest variety of compounds was found in the essential oil of *ex vitro* adapted plants where santolina alcohol, artemisia alcohol, β -eudesmol, and α -cadinol were the main components comprising 52.85% of the oil.

Conclusion: A collection from *in vitro* tissue and *ex vitro* cultures, which is an approach for preservation of *A. thracica* has been established. Different growth conditions affect significantly the composition of essential oils and probably the wide variety of compounds established in the oils from *ex vitro* and *in vitro* plants suggests their contribution during the process of adaptation and surviving in changing environmental conditions.

Acknowledgements: The research was supported by Grand DFNI-BO2/14 of Ministry of Education, Bulgaria.

P107: Black rice with Giant Embryo attenuate obesity-associated metabolic disorders in ob/ob mice

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ABSTRACT

Obesity associated metabolic disorders can lead to cardiovascular and renal dysfunctions. Identification of biological functions of major staple food, rice, against such common disorders is of particular interest. Among various rice varieties, black rice with giant embryo is rich in bioactives such as carotenoids, vitamin E and oryzanols.

Objective: This study aimed to determine biological functions of black rice with giant embryo against obesity and associated metabolic disorders such as hyperglycemia, dyslipidemia and hypertension *in vivo*.

Methods: C57BL/6J-ob/ob mice (ob/ob, n=55) and C57BL-6J (littermate, n=45) were randomly divided into three groups and fed with black rice with giant embryo (BRGE), white rice (WR), and AIN-93G (Control) diets for 14 weeks, respectively. Body weight and blood glucose were measured throughout the feeding period. Blood pressure was determined by non-invasive tail-cuff method, and body fat was assessed by dual-energy X-ray absorptiometry (DXA) at the final week. Blood serum and various tissues such as liver, kidney and adipose tissues were collected and being analyzed for lipid profiles and glucose by colorimetric assays and DNA damage by a single cell gel electrophoresis (comet assay).

Results: The total body fat of BRGE-fed mice was significantly lower than those of WR- and AIN-93G-fed mice ($p<0.05$), despite of no difference in body weights among these groups. The liver per body weights was significantly lower in BRGE-fed mice as compared with those of white rice- ($p<0.05$) and AIN-93G ($p<0.001$) fed mice. As expected, the ob/ob mice in control group had significantly higher blood glucose level than those of littermates. Among the ob/ob mice, the BRGE-fed mice had significantly lower blood glucose level than those of the control ($p<0.05$). In addition, the significantly elevated DNA damage in ob/ob mice as compared with the littermates were significantly attenuated by feeding of BRGE ($p<0.05$).

Conclusion: The bioactives of BRGE has promising functional activities against obesity as well as obesity-associated metabolic disorders.

Acknowledgements: Supported by the Rural Development Administration (PJ010059), Republic of Korea.

P109: Protective effect and mechanism of a combination of the water extract of *Salvia miltiorrhiza* and ligustrazine hydrochloride on hydrogen peroxide-induced oxidative injury in H9c2 cells

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ABSTRACT

Objective: To evaluate the protective effect of a combination of the water extract from *Salvia miltiorrhiza* and ligustrazine hydrochloride (FSL) in rat cardiac H9c2 cells against oxidative damage induced by H₂O₂ and to clarify its mechanism.

Method: The oxidative damage model was established by incubating cardiac H9c2 cells with H₂O₂. Cell viability, SOD activity, LDH, MDA and ROS contents were determined to evaluate the cardioprotective effects of FSL on H₂O₂-induced damage; the mitochondrial membrane potential ($\Delta\psi_m$) were analyzed, the cellular morphology was examined by AO/EB staining, and the anti-apoptosis effect was evaluated by determining the expressions of Bcl-2, Bax and Caspase-3 using quantitative real-time PCR (qRT-PCR) and Western blotting. The expression profiling alteration was examined by using Rat Genome 230 2.0 Array (Affymetrix Inc.) to determine the signaling pathways related to the anti-apoptosis effect.

Result: Compared with the model group, FSL not only decreased the release of LDH, MDA and ROS, but also increased the activity of SOD and cell viability ($p < 0.05$). The $\Delta\psi_m$ was significantly increased and the morphology was improved by FSL. Besides, Bcl-2 expression was increased in FSL pre-treatment group by 2.2 fold and Caspase-3 expression down-regulated by 3.1 fold. Microarray analysis showed differential expressions of 101 genes including genes involved in p53, Jak-STAT, mTOR, MAPK and PI3K/Akt pathways. Biggest up-regulation of expression was detected for Akt gene, in which a 6.83-fold up-regulation was observed.

Conclusion: FSL could protect cardiac H9c2 cells from H₂O₂-induced injury. The mechanism may involve strengthening the activities of SOD, scavenging extra ROS, attenuating the levels of MDA, maintaining mitochondrial membrane potential and preventing apoptosis by regulating Bcl-2 and Caspase-3 through the PI3K/Akt signaling pathway.

P110: The effect of *Clinacanthus nutans* root extracts on cell proliferation and expression of apoptotic genes

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ABSTRACT

Clinacanthus nutans Lindau is a medicinal plant that belongs to family Acanthaceae which has been used to treat inflammation, viral infection and herpes infection in Thailand. In Malaysia, it is claimed to be effective in treating various cancers.

Objective: This study was to investigate the ability of *C. nutans* root extracts in inhibiting the growth of MCF-7 cells, its effect on DNA fragmentation and apoptotic genes.

Methods: The roots of this plant were extracted separately using methanol and ethyl acetate. Anti-proliferative effects of these extracts on MCF-7 cells was examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. DNA fragmentation caused by plant extracts was evaluated through DNA extraction. The effects of *C. nutans* extracts on the expression of apoptotic-related genes (*BCL-2* and *BAX*) were evaluated via RT-PCR.

Results: MTT results showed that MCF7 cells were inhibited by these extracts at the IC₅₀ values of 35 µg/ml and 30 µg/ml for methanol and ethyl acetate extracts respectively. Although no formation of DNA laddering was observed in treated cells, RT-PCR results revealed down-regulation of anti-apoptotic gene (*BCL-2*) in the treated cells and no alteration of pro-apoptotic gene (*BAX*) in treated cells.

Conclusion: The results suggest that *C. nutans* root extracts promote apoptosis pathway by suppressing *BCL-2* expression while maintaining *BAX* expression in MCF-7 cells.

P111: Fungus *Leptosphaeria maculans*, an oilseed rape (*Brassica napus*) pathogen, as the source of bioactive carbohydrates

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ABSTRACT

The loculoascomycete *Leptosphaeria maculans* is a pathogen caused blackleg of Brassicas (1). Metabolism of this parasite is tightly bound with its plant host. Metabolites of *L. maculans* may enhance resistance of *B. napus* plants toward fungal infection (2). Biochemical composition of this fungus and its potentially bioactive compounds are still poorly investigated.

Objective: The goal of this study was evaluation of fungus *L. maculans*, an oilseed rape pathogen, as the source of bioactive compounds including carbohydrates.

Methods: Cultivated mycelium of *L. maculans* was fractionalised by subsequent extractions and fractions F1–5 were collected. The fraction F3 was then treated with amylase and/or pronase to remove ballast compounds. Obtained fractions were characterised by spectroscopic methods. Sugar composition was analyzed by total hydrolysis (0.5M TFA, 100 °C for 1 h) and GC-FID.

Results: The fraction F1a was composed of lipids. Mannitol was the major component of F1b and F2, while glucose and trehalose were found in smaller amounts. The fraction F3, which demonstrated potent elicitation activity, consisted mainly of α -glucan and proteins. Enzymatic hydrolysis of F3 led to purification of the D-galactofuranose containing oligosaccharides that could be responsible for elicitation activity. Finally, F4 and F5 were identified as cell wall polysaccharides, mainly β -glucans.

Conclusion: Fungus *L. maculans* was found to be a source of bioactive carbohydrates including mannitol, D-galactofuranose containing oligosaccharides and cell wall β -glucans.

Acknowledgements: Financial support from the Ministry of Education, Youth and Sport of the Czech Republic (project LD14093) is greatly acknowledged.

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P112: Pterostilbene inhibits lipid accumulation through cell cycle delay in 3T3-L1 adipocyte

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ABSTRACT

Obesity is caused abnormal or excessive fat accumulation that presents a risk to health such as high blood pressure, type 2 diabetes and atherosclerosis. However, recent studies have reported that resveratrol has numerous beneficial effect on cardio-protective, anti-cancer and obesity. Pterostilbene is a stilbenoid and structurally related to resveratrol. Pterostilbene have reported that anti-oxidant, anti-inflammatory, and anti-carcinogenic properties more than resveratrol.

Objective: In this study, we examined that pterostilbene inhibits lipid accumulation by cell cycle delay.

Methods: We investigated Cell viability of pterostilbene through XTT assay. Lipid accumulation of 3T3-L1 checked by using Oil-Red O staining. We experimented western blot to check down regulating obesity-related genes in protein level.

Results: Pterostilbene delayed cell cycle in the S phase which is related with delay of cell differentiation. Pterostilbene decreased the accumulation of lipid droplets. The expression of Peroxisome proliferator-activated receptor gamma (PPAR γ), CCAAT-enhancer-binding proteins (C/EBP α), and adipocyte protein 2 was decreased by pterostilbene. In additional, pterostilbene reduced lipogenesis (LPAAT0, lipin1, and DGAT1) and fatty acid synthesis (FASN, SREBP) factor in protein level dose dependent manner.

Conclusion: Our findings show that pterostilbene inhibits lipid accumulation by regulating adipogenic factors and delaying cell cycle.

P113: Inhibitory effects of dieckol on lipid accumulation and obesity-induced insulin resistance in db/db mouse

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ABSTRACT

Obesity is a physical condition in which excessive fat accumulates, resulting in certain health risks. This global health problem is a major risk factor for metabolic diseases such as type 2 diabetes, insulin resistance. Dieckol is a phlorotannin that can be found in *Ecklonia cava*, in *E. stolonifera* or *Eisenia bicyclis*. Recent research suggested that, dieckol has anti-thrombotic and profibrinolytic activities and hair growth effect.

Objective: Dieckol improves insulin sensitivity through suppression of obesity and lipid accumulation related factors.

Methods: Glucose uptake ability of dieckol was determined by glucose uptake assay and α -glucosidase assay. 3T3-L1 preadipocytes were treated with various concentrations of fucoxanthin for up to 8 days following standard induction of differentiation and investigated molecular events related with glucose uptake. 5 week old of db/db mouse were treated with rosiglytasone (10mg/ml), dieckol (30mg/kg), dieckol (120mg/kg) for 6 weeks after 1 week of acclimatization. Protein expressions of lipid accumulation, glucose uptake related factors were determined by western blotting.

Results: Our data shows that, glucose uptake activity of dieckol was increased by dose dependent manner. Also, dieckol activates glucose uptake related factors such as IRS-1, AKT, GLUT4 in 3T3-L1 and db/db mouse. Besides, dieckol suppressed mouse body weight and C/EBP α , PPAR γ , FABP4, the adipogenesis key factors in mouse. In mouse, dieckol suppressed body weight, OGTT and OMTT.

Conclusion: Dieckol inhibits adipogenesis and improves insulin sensitivity. Our data demonstrate that, dieckol may act as therapeutic agent for obesity and insulin resistance related metabolic syndromes.

P115: Cytotoxic activity of an active fraction of *Zhumeria majdae* Rech. F. & Wendelbo

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ABSTRACT

Breast cancer is the most common cancer among women worldwide. Numerous chemotherapy agents are used in cancer treatment which exhibit significant effects on malignant cells. However, high side effects and resistance of cancer cells limit the use of these drugs. Therefore, searching for novel agents that are more efficient and selective on cancerous cells is still an important research line. Many plants have been identified to be potent in the treatment of cancer and considerable efforts are being made to isolate bioactive components from medicinal plants for their possible utility in cancer therapy.

Objective: The present study was designed to determine the *in vitro* cytotoxic effect of an active fraction of *Z. Majdae* Rech. F. & Wendelbo (1-2), an Iranian native plant, on three human breast cancer cell lines.

Methods: MDA-MB-231(ER-), MCF-7(ER+) and T-47D (ER+) human breast cancer cell lines were treated with different concentrations of petroleum: diethyl ether (3:1) fraction of the ethanol extract of *Z. majdae*. After 72hours of incubation, the *in vitro* cytotoxicity was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Doxorubicin was used as a positive control. The results were presented as 50%-inhibitory concentration (IC₅₀).

Results: The results indicate that IC₅₀ values of the fraction are 0.566±0.003, 1.050±0.012 and 2.136±0.02 µg/ml against MDA-MB-231, MCF-7 and T-47D cells, respectively. IC₅₀ values of doxorubicin on the aforementioned were 0.095±0.026, 0.178±0.042 and 0.371±0.252, respectively.

Conclusion: The results conclusively imply that the active fraction of *Z. majdae* possesses a very potent cytotoxic activity. In addition, stronger cytotoxic effects on estrogen receptor negative cell (MDA-MB-231) compared to estrogen receptor positive cells (MCF-7 and T-47D), suggests that this fraction might have an estrogen independent mechanism for its cytotoxic effect.

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P116: Discovery of a hepatoprotective agent from the co-occurring substances of natural oleanolic acid crude drug

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ABSTRACT

Pharmaceutical preparations of oleanolic acid (OA) have been approved by CFDA in China as an adjuvant therapy for acute and chronic hepatitis. Co-occurring substances present in the tablets of OA and their hepatoprotective effects have not been reported.

Objective: To understand the hepatoprotective activity and the possible target of auriculatone.

Methods: The crude OA drug was separated by repeated column chromatography and the structures of the isolated compounds were characterized by spectral analysis. Cytotoxicity assay was evaluated *in vitro* against the human normal liver cell LO2 using the MTT method. The hepatoprotective effect was evaluated by testing the cell viability as well as the leakage of alanine transaminase (ALT), aspartate transaminase (AST), and glutathione (GSH) on the APAP-induced cell injury model. The potential target of auriculatone was revealed by using CDOCKER.

Results: 11 co-occurring trace compounds of OA were isolated and structurally characterized. Cytotoxicity tests showed that OA and some of its co-occurring compounds were non-toxic even at 1000 $\mu\text{mol/L}$. In addition, OA and its co-occurring compound auriculatone were found to promote the growth of hepatocytes at 500 $\mu\text{mol/L}$ and above. Moreover, auriculatone significantly reduces the hepatotoxicity induced by acetaminophen (APAP) at all the tested concentrations (0.1, 1, and 10 $\mu\text{mol/L}$). Besides, at 0.1 $\mu\text{mol/L}$, auriculatone was able to decrease the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), with potency comparable to 10 $\mu\text{mol/L}$ of the clinical drug N-acetylcysteine (NAC). However, auriculatone showed no effect on the GSH release induced by APAP. Through docking investigations between auriculatone and APAPhepatotoxicity-related targets, auriculatone showed the best binding mode in combining with CYP3A4 and the highest CDOCKER energies.

Conclusion: Auriculatone is a promising hepatoprotective agent worthy of further research and development. Auriculatone was supposed to protect hepatocytes from injury probably through preventing the metabolizing of APAP by CYP3A4 from producing toxic N-acetyl-p-benzoquinone imine (NAPQI).

P120: The evaluation of soil fertility systems on some physiological properties and quality of sage (*Salvia officinalis* L.) under different moisture conditions

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ABSTRACT

Sage (*Salvia officinalis* L.) is a popular medicinal plant which is widely used in food and pharmaceutical industries. It has been used in a variety of food preparations since ancient times. Sage essential oil is applied in the treatment of a large range of diseases such as those of the nervous system, heart and blood circulation, and respiratory.

Objective: An experiment was conducted during 2012 in Faculty of Agriculture, Tarbiat Modares University to study the response of sage to different nutrition systems under water scarcity.

Methods: For this purpose a randomized complete block design arranged in split plots with three replications were used. Three irrigation levels (irrigation after depletion of 40% available water (C), irrigation after depletion of 60% available water (MWD), irrigation after depletion of 80% available water (SWD)) and five different soil fertility systems (control (no fertilizer), chemical fertilizer (urea), nitrogen fixing bacteria (NFB), vermicompost (V), vermicompost (V)+ nitrogen fixing bacteria (NFB)) were allocated to main plots and sub plots respectively.

Results: Chlorophyll a and b declined with increasing of water stress, but sugar and proline increased. Results showed that the highest values of all measured traits were related to the vermicompost + nitrogen fixing bacteria treatment. Also, the maximum essential oil content and essential oil compounds were obtained at moderate stress and vermicompost + nitrogen fixing bacteria. Totally, 48 compounds were identified in essential oils of sage by means of GC-MS. 1, 8-cineol, α -thujone and camphor were the dominant essential oil compounds which indicated an increasing under moderate stress \times (V+NFB) treatments.

Conclusion: Drought stress have decreased photosynthesis pigments whereas have increased sugar and proline content. Vermicompost + nitrogen fixing bacteria treatments have increased photosynthesis pigments, sugar and proline content. The highest dominant essential oil compounds were observed in the moderate stress and vermicompost + nitrogen fixing bacteria treatment.

P126: Molecular cloning and expression of chalcone synthase gene from *Dendrobium officinale* Kimura et Migo

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ABSTRACT

Dendrobium Sw. is a species of perennial herb in the Orchidaceae family, flavonoids are important medicinal ingredients of them. Chalcone synthase is the first committed enzyme in the flavonoid biosynthesis.

Objective: This study was carried out to clone a chalcone synthase gene (CHS) from a medicinally important endangered orchid species *Dendrobium officinale* Kimura et Migo, followed by bioinformatics and expression analysis. To provide references for the study of its biological function.

Method: Reverse transcriptase polymerase chain reaction (RT-PCR) and Rapid amplification of cDNA ends (RACE) approaches were used to isolate the full-length gene. Characteristics of the molecular weight, conserved domain and subcellular localization of the deduced CHS protein were determined using a series of bioinformatics tools. The analyses of multiple alignment and phylogenetic tree were performed using DNASTAR 6.0 and MEGA 4.0, respectively. Quantitative Real-time PCR (q-PCR) was employed to examine the tissue specific expression patterns of CHS.

Results: A full-length cDNA encoding chalcone synthase, designated as DoCHS (GenBank accession No.KR078267), was identified from *D. officinale* Kimura et Migo. The gene comprised 1308 bp and the opening reading frame (ORF) of 1188 bp encoding a 395 amino acid polypeptide with a calculated molecular weight of 97.94 kilodaltons and an isoelectric point (pI) of 5.00. Phylogenetic analysis clearly showed that DoCHS was close to the second subgroup of CHS protein family in Orchidaceae. Expression analyses revealed that DoCHS was detected in all the studied tissues contains roots, stems, flowers and leaves. The transcripts of DoCHS were the abundant in flowers which were highly rich than in stems. Followed by leaves and almost no detection in roots.

Conclusion: The full-length cDNA of CHS gene was successfully cloned. The high expression level of DoCHS in *D. officinale* Kimura et Migo flowers and leaves suggested that the gene might play a vital regulatory role in flowers and leaves and lead to the high flavonoids contents in flowers and leaves, then the medicinal parts of the *D. officinale* Kimura et Migo will increase assuredly.

P128: Optimization of anti-oxidative activities from the roots of *Polygonum multiflorum* using response surface methodology

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ABSTRACT

Polygonum multiflorum is a one of the most famous traditional medicine in East Asian countries. This plant has been used as anti-aging, anti-cancer, anti-inflammatory, anti-allergy, anti-bacterial, hemostatic, spasmolytic, and analgesic properties. Previous phytochemical studies on this plant had led to the isolation of stilbenes, anthraquinones, flavonoids, and tannins.

Objective: The present study was undertaken to optimize the extraction conditions with maximum anti-oxidative activities using a response surface methodology, and further to purify anti-oxidative compounds from the roots of *P. multiflorum*.

Methods: The dried roots of *P. multiflorum* were extracted with MeOH, and partitioned with *n*-hexane, CH₂Cl₂, EtOAc, and *n*-BuOH, and water, successively. The EtOAc-soluble fraction was purified by several column chromatography including MPLC and preparative HPLC. The structures of the isolated compounds were elucidated on the basis of spectroscopic and spectrometry data such as ¹H, ¹³C-NMR, and ESI-MS. All of the compounds were evaluated for their DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging activity. Optimization of extraction conditions with maximum anti-oxidative activities was determined using response surface methodology (RSM). Moreover, quantitative analysis of major compound **1** was conducted.

Results: The structures of the isolated compounds were identified as (*Z*)-2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucopyranoside (**1**), (*E*)-2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucopyranoside (**2**), torachryson-8-*O*-β-D-glucopyranoside (**3**), and physcin-8-*O*-β-D-glucopyranoside (**4**). Of these, compound **1** showed potent anti-oxidative activity than the other compounds. The content of compound **1** was quantified by HPLC-DAD analysis. Under the optimal condition of the maximum anti-oxidative activities, the yield of ethanol extract reached 23.5% (extraction condition; solvent: EtOH, time: 60 min, and temperature: 30°C).

Conclusion: (*Z*)-2,3,5,4'-Tetrahydroxystilbene-2-*O*-β-D-glucopyranoside (**1**) was isolated as one of the active compounds from the roots of *P. multiflorum*. RSM analysis indicated that the optimal extraction condition showed 55.5% inhibition of DPPH radical scavenging activity.

P129: Isolation and structural characterization of Chinese date (*Ziziphus jujube* Mill.) polysaccharides

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ABSTRACT

Chinese date (*Ziziphus jujuba* Mill.) is an example of medicinal plant (1) that unfortunately is not common in Central and Eastern Europe. The fruits and other organs of *Z. jujuba* contain biologically active pectins (2, 3) and other polysaccharides (3) interesting for medicinal applications.

Objective: The main goal of this study was isolation and structure characterization of cell wall polysaccharides from fruits of Chinese date (*Z. jujuba* Mill.) as important dietary fibre components.

Methods: Dried fruits of Chinese date originated from State enterprise "Experimental field "Novokakhovskoe" KSAES NAAS", Nova Kakhovka, Ukraine. Fibres (total, soluble and insoluble) were estimated in the raw milled and dried material. The raw material was fractionalized by subsequent extractions with cold and hot water and 1M NaOH solutions yielding fractions F1–3. Fractions were characterized by FT-IR and NMR spectroscopy. The monosaccharide composition was determined by GC-FID.

Results: The contents of total, soluble and insoluble fibres (TF, SF and IF) in Chinese date fruits were found to be 14.5 % (TF), 3.8 % (SF) and 10.8 % (IF). Fractions F1 and F2 were identified mainly as pectins, F3 as hemicellulose with some amount of integrated proteins. Fractions F1 and F2 contained low methylated (LM) and slightly *O*-acetylated pectin (homogalacturonan, HG) and branched rhamnogalacturonan I (RG I) with β -galactan side chains.

Conclusion: Fruits of Chinese date (*Z. jujuba* Mill.) were found to be plentiful source of pectic compounds and hemicelluloses, which are the components of soluble and insoluble dietary fibres.

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P130: Identification of bioactive compounds and antimicrobial activity of rutabaga (*Brassica napus L. var. napobrassica*) sprouts and roots -a new example of functional food

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ABSTRACT

Sprouts of rutabaga are considered to be a novel type of functional food containing valuable bioactive compounds. However, nutritional value of rutabaga sprouts and roots has not been investigated thoroughly so far. An examination of various *Brassicaceae* species revealed a wide range of chemical compounds with biological activity such as glucosinolates, flavonoids, phenolic and fatty acids. Moreover, *Brassica* vegetables are natural products which may express chemopreventive activity. Despite this fact and, the question how this novel functional food affects the body has not been fully determined; especially the potential thyrotoxic effect of rutabaga sprouts needs to be evaluated further.

Objective: This study aimed to evaluate qualitative and quantitative composition of rutabaga sprouts and roots, and to determine their antibacterial and antifungal activity.

Method: Rutabaga sprouts were grown for 8, 10 and 12 days after seeding. Half of the culture was stored in natural conditions, while the rest was kept in darkness at all times. Roots were harvested in southern Poland.

HPLC method was used to identify phenolic compounds. LC/MS was employed to evaluate content of glucosinolates, and GC to determine a fatty acid profile. Antibacterial and antifungal activity was evaluated by an agar diffusion test using Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus* and yeast *Candida albicans*.

Results: The main phenolic compounds identified in rutabaga were hydrocaffeic, chlorogenic and gentisic acids, quercetrin, isoquercetrin, robininand morin. Among glucosinolates: progoitrin, glucoerucin, glucoiberin and sinigrin were detected. Dominating fatty acids were oleic, linoleic, alpha-linolenic and erucic acid. Antibacterial activity of prepared sprouts extracts was observed in all samples, however antifungal activity wasn't significant.

Conclusion: Concerning the unique chemical profile rutabaga sprouts can be recommended as an element of functional food.

P131: Comparison of polyphenol content and antioxidant activity of *Sanguisorba officinalis* rhizome originating from China and Poland.

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ABSTRACT

The *Sanguisorba officinalis* rhizome are well-known in European (PhEur) and Asian (TCM). They have been traditionally used for treatment of inflammatory and metabolic diseases, including diarrhea, chronic intestinal infections, duodenal ulcers and bleeding.

Objective: The aim of the study was to compare the *S. officinalis* rhizome from China and from Poland regarding their content of polyphenols and tannins, as well as to check the correlation between this substance and antioxidant activity.

Methods: The dried and crushed raw material was extracted with 70% acetone and fractionated with dichloromethane, diethyl ether, ethyl acetate and n-butanol. Obtained extracts and fractions were screened for total phenols and tannins. Free radical scavenging activity has been checked by DPPH and phosphomolybdenum assay.

Results: The strongest antioxidant activities were different for extracts and fractions from Polish rhizomes and from Chinese rhizomes. The strongest antioxidant activity had ethyl acetate fraction with EC₅₀ 6,16 µg/mL for the raw material from Poland, whereas for Chinese ethyl acetate fraction had EC₅₀ at the level of 10,30 µg/mL. Chinese raw material indicated the strongest activity in water fraction with EC₅₀ 6,22 µg/mL, but for ethyl acetate showed only EC₅₀ 10,30 µg/mL. Phosphomolybdenum assay revealed similar results. These results were strongly correlated with polyphenols and tannins amount. Ethyl acetate fraction from Polish *S. officinalis* revealed the highest amount of polyphenols (554,98 µg per mg fraction) and tannins (441,63 µg per mg fraction) among the others fraction from this raw material. For Chinese *S. officinalis*, the highest amount of polyphenols had water fraction (557,10 µg/mg) as similar for tannins (417,00 µg/mg). Correlation between the amount of polyphenols and EC₅₀ DPPH for whole extracts and fractions from Polish raw material was r=-0,79. Similar between tannins and DPPH r=-0,79. For Chinese the correlation was even stronger (for polyphenols and DPPH, r=-0,97, for tannins and DPPH, r=-0,91)

Conclusion: Despite the fact that both raw materials indicated strong antioxidant activity, the significant difference between the polyphenols and tannins content in each fraction was found.

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P132: 6-Gingerol inhibits lipid accumulation and TNF- α in 3T3-L1 adipocytes and RAW264.7 macrophages

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ABSTRACT

Obesity is associated with lipid accumulation leads obesity. Many researchers have been reported that, obesity causes metabolic syndroms and chronic inflammation. The obesity-induced inflammatory response is one of the metabolic diseases. And It has been reported that tumor necrosis factor- α (TNF- α) is an important modulator of a lipid accumulation and obesity-induced inflammation. 6-gingerol is one of the compounds in ginger. And 6-gingerol is a major compound in ginger. Recent research suggested that, 6-gingerol has anti-cancer, anti-inflammation and anti-obesity effects.

Objective: In this study, effects of 6-gingerol on anti-obesity and obesity-induced inflammatory responses the interaction between adipocytes and macrophages.

Methods: The suppressive effect of 6-gingerol, on lipid accumulation was investigated using 3T3-L1 cells. We performed Oil Red O for measuring lipid accumulation on 8day in 3T3-L1. And Cells were harvested and performed western blot and RT-PCR to investigate expression of adipogenic and lipogenic factors. We examined cytokine assay for measuring TNF- α and monocyte chemoattractant protein 1 (MCP-1) in co-culture system.

Results: Our results showed that, 6-gingerol significantly decreased the mRNA expression level of TNF- α , activation protein 2 (AP2) and CCAAT-enhancer-binding protein α (C/EBP α) by suppressing of peroxisome proliferator-activated receptors (PPAR γ) in 3T3-L1 adipocytes. Also, In a co-cultured model of 3T3-L1 adipocytes and RAW264.7 macrophages, 6-gingerol suppressed Nitric Oxide (NO) secretion which is related to inflammation. Additionally, we will investigate the proteins level.

Conclusion: We expect that the 6-gingerol may provide useful applications to reduce the chronic inflammatory properties of adipocytes.

P133: Amino acid at position 482 of human ATP-binding cassette transporter ABCG2 is crucial for camptothecin analogue recognition

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ABSTRACT

The human ATP-binding cassette transporter ABCG2 is one of the most important factors regulating the pharmacokinetics of a number of clinically important drugs in the body, and determines the success or failure of cancer chemotherapy. ABCG2-overexpressing cancer cells were selected with different DNA topoisomerase I inhibitors, including 7-ethyl-10-hydroxycamptothecin (SN-38).

Objective: We investigated the relationship between the amino acid moiety at position 482 in ABCG2 and the recognition of camptothecin (CPT) analogues by ABCG2.

Methods: We examined the resistance of control and [Arg⁴⁸²]-, [Gly⁴⁸²]-, or [Thr⁴⁸²]-ABCG2-expressing HEK-293 cells to 15 CPT analogues with various substitutions at positions 10 (X) or 11 (Y) using an MTT assay, by evaluating their growth inhibition (IC₅₀). Briefly, viable cells (2 × 10³ cells/well) were cultured for 72 h with the CPT analogues and then for 4 h with 500 µg/ml MTT. The resulting MTT-formazan was solubilized with 10% (w/v) SDS. The absorbances at 570 nm and 630 nm were measured as the test and reference wavelengths, respectively, after overnight incubation.

Results: After treatment with 15 CPT analogues, the MTT assay showed that the IC₅₀ value of SN-38 (X = OH, Y = H) in [Thr⁴⁸²]-ABCG2-expressing cells was about half that in [Arg⁴⁸²]- or [Gly⁴⁸²]-ABCG2-expressing cells. The IC₅₀ values of SN-398 (X = OH, Y = F) in [Gly⁴⁸²]- and [Thr⁴⁸²]-ABCG2-expressing cells were about half that in [Arg⁴⁸²]-ABCG2-expressing cells. The IC₅₀ values of both SN-392 (X = NH₂, Y = F) and SN-443 (X = CH₃, Y = F) in [Gly⁴⁸²]-ABCG2-expressing cells were about two-fold higher than that in [Arg⁴⁸²]-ABCG2-expressing cells, and the IC₅₀ value of SN-355 (X = H, Y = OH) in [Gly⁴⁸²]-ABCG2-expressing cells was about half that in [Arg⁴⁸²]-ABCG2-expressing cells.

Conclusion: These results suggest that the amino acid moiety at position 482 is crucial for the maintenance of the ABCG2 protein structure and its interactions with its substrates.