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# Effects of plant age, nitrogen fertilization and development stage at harvest on the content of phenolic diterpenes in cultivated sage (Salvia officinalis L.)

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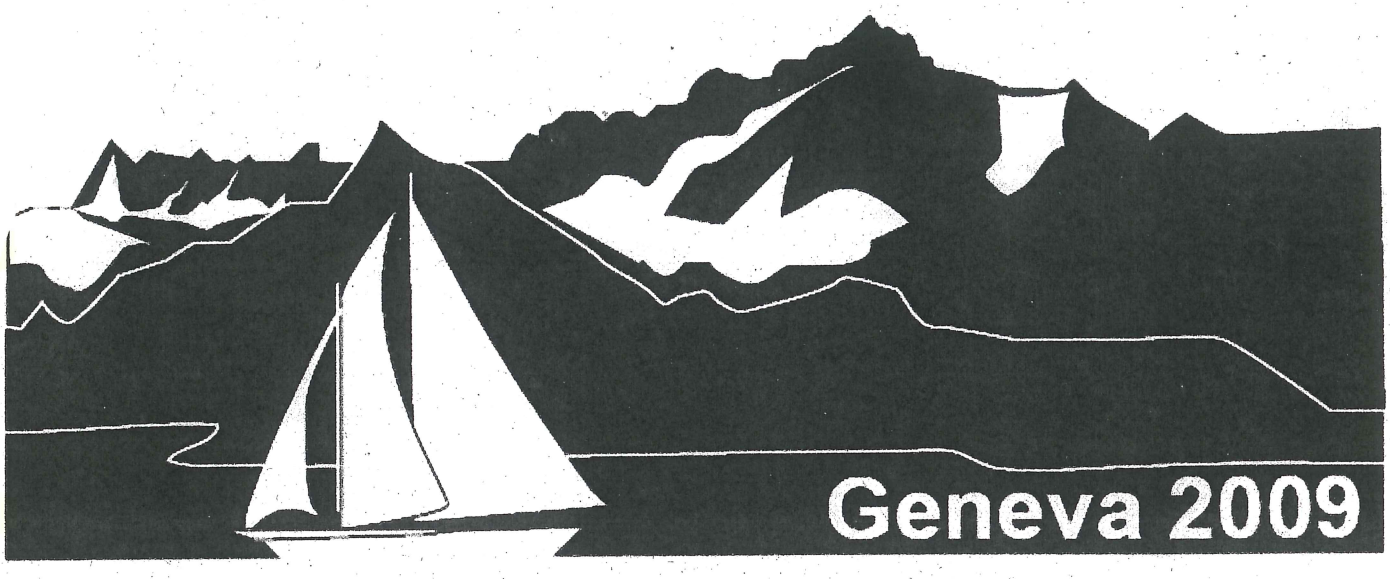


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# Book of abstracts
# Addendum

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identification and automated analysis, such as X-ray Diffractometry (XRD) and XRF, 5 out of 7 Sal Ammoniac samples were found to be imitations. The five imitations contained sodium chloride, ferric ions and calcium sulfate. Sal Ammoniac is usually made from natural sources and the main purpose of processing is to remove dust and other impurities. Each ten-gram sample of Sal Ammoniac was dissolved in hot water with or without acetic acid and filtered through filter paper. The refined Sal Ammoniac was then dried in a dry oven at 55°C. Sal Ammoniac includes a small amount of NaCl, but processing reduced the sodium (Na) content. When Sal Ammoniac was dissolved in 5% acetic acid solution, all of the Na was removed. We suggest that recrystallization with 5% acetic acid solution is effective as a Standard Processing Method for Sal Ammoniac. *Acknowledgements : This work was supported by the Second Stage of Brain Korea 21 project in 2009, Oriental Medical Science Center and KFDA.* **References**: [1] The Korean Herbal Pharmacopoeia 2008. [2] Shuirong Deng et al. (1997) Journal of Chinese Medicinal Materials 20:77-78.

PG71

### The marker profiling of botanical *Holarrhena antidysenterica* (bark) wall extracts

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Our study attempted to develop the marker profile of *Holarrhena antidysenterica* bark extracts which is used as antidysentery remedy in the Indian system of medicine. Preliminary phytochemical analyses of the ethanolic (by maceration) and aqueous (by Soxhlet) extracts of the bark of *Holarrhena antidysenterica showed* presence of alkaloids, phenols, tannins and flavonoids as major active constituents. Thin layer chromatography with Toluene: Ethyl acetate: Diethyl amine in the ratio of 6.5:2.5:1 as mobile phase yielded six distinct bands with $R_f$ ranging from 0.32 to 0.81. The band at $R_f$ 0.81 when scanned spectrophotometrically gave an overlapping spectrum with that of a marker compound, conessine. This was further confirmed by HPLC and HPTLC studies. The peaks in IR spectrum of this band show presence of functional groups of conessine. The study provides a stage and preliminary data on the chemical fingerprinting of this medicinally important plant for future studies related to quality control of the plant which may prove to be useful in its commercial exploitation. **References**: [1] Aman, D. et al. (2008) J. Ethnopharmacol. 46:391-394. [2] Ballal, M. et al (2001) Indian J. Pharmacol. 33:392-393.

## Topic H: Prevention of metabolic diseases by medicinal plants and nutraceuticals

PH48

### Anti-inflammatory effect of tablets with Salvia extract using carrageenan air pouch model

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Inflammation studies using air pouch models with carrageenan injection revealed increased vascular permeability, and exudates formation bearing huge numbers of neutrophils. In present work, we used this experimental model to investigate anti-inflammatory effects of Salvia tablets (SV) and reference drugs ST. SV tablets represent combination of dry extracts of *Salvia officinalis* (12.5 mg) and its ether oil (2.4 mg). Male Wistar rats (120–150 g) were anaesthetized in $CO_2$ chamber, and 15 ml of sterile air was injected into the subcutaneous tissue of the back at six days prior to drug treatment, and 3 days later, the pouches were reinforced with 7.5 ml sterile air. At day six, 3 ml of 0.5 % carrageenan in 0.9% NaCl was injected into the pouches to induce a local inflammatory process. Tablets were administered orally in starch gel in doses of 50, 100, 150, and 250 mg/kg of extract or into the air pouch in doses 5, 10, 15, and 25 mg/kg of extract immediately after injection of carrageenan. Control group was administrated with 1% starch gel. After 6 h, rats were killed by $CO_2$ exposure and pouches washed thoroughly with 10 ml of 0.9% NaCl. Exudates fluids were centrifuged at 1860 rpm for 10 min at 4 °C and frozen for future analysis. Our results suggest that SV preparation administered to Wistar rats has significant anti-inflammatory effects when assessed with a carrageenan-induced inflammation model. The anti-inflammatory effects of SV after oral administration of single dose and injection of single dose were dose related and increased in the order 100 < 200 < 150 < 50 mg/kg and 20<10<5<15 mg/kg for oral administration and injection respectively. After oral administration of 50 mg/kg of SV decrease of neutrophils in 1.9 times, and TNFα in 3.2 times comparing to control were observed. After injection of 15 mg/kg of SV decrease of neutrophils in 1.6 times comparing to control was observed, and TNFα was similar to that of control group. These findings offer additional pharmacological information on the therapeutic efficacy of tablets with Salvia extract.

PH49

### Evaluation of aqueous extract of *Psidium guajava* L. budding leaves on improvement of symptoms in STZ-induced SD rats

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Diabetes mellitus (DM) is one of the major diseases that human being eagerly wants to overcome. In 2002, DM was the fourth of top ten causes of death in the Taiwan. The aqueous extract of *Psidium guajava* L. budding leaves (PE) were tested on streptozotocin (STZ)-induced diabetic Sprague-Dawley (SD) rats. Rats (mimic DM blood sugar level > 230 mg/dL) were orally administered 100, 300, 1000 mg PE/kg body weight, respectively. PE decreased plasma sugar but increased insulin levels in a dose-dependent manner. Amylase level was significantly elevated by PE 1000 mg/kgbw ($p<0.05$). This study was undertaken to examine the function of three antioxidant scavenger enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). SOD and CAT activities were significant increased in STZ rats ($p<0.05$), whereas GSH-Px activities were decreased. H&E stains showed that pancreatic islets and function of β-cells were partially restored by PE treatment. Results indicate that PE may alternatively act as an anti-hyperglycemic through regeneration of β-cell in STZ-induced DM rats. **References**: [1] Mythili, M.D. et al. (2004) Microsc. Res. Tech. 63: 274-281.

PH50

### Effects of plant age, nitrogen fertilization and development stage at harvest on the content of phenolic diterpenes in cultivated sage (*Salvia officinalis* L.)

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Sage (*Salvia officinalis*, Lamiaceae) has been shown to have antimicrobial, antiviral, anti-inflammatory, antioxidative, anti-diabetic and neuroprotective effects. These pharmacological properties are mainly ascribed to its content of flavonoids, phenolic acids, and not least to its content of phenolic diterpenes [1, 2]. The aim of the present study was to investigate how the concentration of diterpenes depends on plant age (cultivation year), the plant development stage during the growth season and on the nitrogen fertilization of the crop. Two sage cultivars were grown in field experiments in 2007 and 2008 with three different levels of nitrogen (N) fertilization (0, 100 and 200 kg applied N/ha). Another sage cultivar planted in 2006 was harvested at three different development stages in 2007 (before, during, and after flowering). In all harvests fresh weight yield and dry matter (DM) were monitored. From dichloromethane extracts of the aerial parts the diterpenes carnosol, carnosic acid, 12-*O*-methylcarnosic acid, 20-hydroxyferruginol, 20-deoxocarnosic acid, and 20-deoxocarnosol were isolated by CC and prep. HPLC and identified by ESI-MS and 1D- and 2D-NMR. Diterpenes were quantified in frozen plant material using a three-step extraction procedure and subsequent analysis by LC-PDA using carnosol as authentic standard. The total content of phenolic diterpenes was found to vary between 7.9 ± 0.7 mg/g DM and 15.8 ± 3.3 mg/g DM. Significant differences between years (plant age) were found for the concentrations of all diterpenes averaged over nitrogen fertilization and cultivar. A tendency for the highest content of phenolic diterpenes was found for medium nitrogen application (100 kg/ha), but only the content of carnosol was significant. No significant differences in

diterpene concentrations for plant development stage during the growing season were found. **References**: [1] Poeckel, D. et al. (2008) Biochemical Pharmacology 76, 91-97. [2] Kavvadias, M. et al. (2003) Planta Med. 69, 113-117.

PH51

### Modulators of human peroxisome proliferator-activated receptor γ (hPPARγ) from selected medicinal plants have potential use in the prevention and/or treatment of insulin resistance

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The metabolic syndrome constitutes a set of metabolic risk factors that predisposes individuals to a number of diseases such as type 2 diabetes (T2D). Insulin resistance (IR) is one of these metabolic risk factors that can lead to the development of T2D. IR can be treated with insulin sensitizing drugs such as thiazolidinediones, which are full agonists of PPARγ, and hence may induce severe side effects. Partial PPARγ agonists maintain the beneficial effect on IR without inducing severe side effects. Recently, a general screening for potential partial PPARγ agonists was carried out on several plant extracts. The most active extracts were those of elderflower (*Sambucus nigra*), purple coneflower (*Echinacea purpurea*), sage (*Salvia officinalis*), and buckwheat (*Fagopyrum tataricum*) [1]. Active metabolites were isolated by bioassay-guided fractionation using a PPARγ transactivation assay. Fatty acids (FAs) such as linoleic and α-linolenic acid were identified as PPARγ agonists from all investigated plant extracts. Alkamides are structurally similar to FAs and a new alkamide, hexadeca-2*E*,9*Z*,12*Z*,14*E*-tetraenoic acid isobutylamide, isolated from purple coneflower was able to activate PPARγ and stimulate glucose uptake in adipocytes [2]. Polyphenols are widespread in both elderflowers and buckwheat but only naringenin was able to activate PPARγ [3]. From sage the diterpene 12-*O*-methylcarnosic acid was found to activate PPARγ. The present data suggests that common FAs are responsible for the PPARγ activity of plant extracts together with specific groups of secondary metabolites such as alkamides and flavonoid aglycones, whereas phenolic acids and flavonoid glycosides are not PPARγ agonists. The present investigations also show that plants contain compounds, which can be used in the management of IR and T2D. **References**: [1] Christensen KB et al. (2009) Phytother. Res. in press. [2] Christensen KB et al. (2009) J. Nat. Prod. 72:933–937. [3] Christensen KB et al. (2009) Phytother. Res. accepted.

PH52

### An extract from purslane herb might modulate glucose metabolism through multiple mechanisms

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The purslane herb (*Portulaca oleracea* L.) is a very common plant used worldwide as a food ingredient. It is traditionally used in folk medicine for a variety of ailments, in Near East purslane is treasured as an aid against diabetes. Animal studies confirm that purslane herb has antidiabetic properties [1-3].

To investigate the efficacy of the herb for this indication, a hydroalcoholic extract from purslane herb, Portusana™ (EFLA®308), was tested *in vitro* in three different assays related to glucose metabolism. Firstly, a glucose rapid-uptake assay was performed in Caco-2 cells to model glucose uptake through the intestinal wall. The extract significantly decreased the absorption of glucose compared to the control. Reduced glucose absorption through the intestine may contribute to a hypoglycaemic effect. Secondly, to assess whether the extract could affect glucose disposal in metabolically active cells, a glucose uptake assay was conducted in differentiated 3T3-L1 adipocytes as a model for adipose tissue. Portusana dose-dependently stimulated the uptake of glucose, both in presence and absence of insulin. These results show that the extract may enhance glucose disposal in adipocytes. Thirdly, the ability to modulate the nuclear receptor PPARγ was investigated in HEC-1B cells, since PPARγ is an important molecular target for antidiabetic activity. Portusana showed agonistic as well as antagonistic activity on PPARγ, clearly suggesting that the constituents in the extract are able to bind and to activate PPARγ. In conclusion, the findings from these *in vitro* studies suggest that Portusana™ may affect different mechanisms related to the pathophysiology of diabetes mellitus. **References**: [1] Eskander, E.F., Won Jun, H. (1995) Egypt. J. Pharm. Sci 36, 1-6: 331-342. [2]. Shen, L., Lu, F. (2003) CJIM 9:289-292. [3] Sinha B.P. et al. (1962) Seyler's Zeit: 274-275.

PH53

### Antioxidant effect of *Ziziphus vulgaris*, *Portulaca oleracea*, *Berberis integerima* and *Gundelia tournefortti*

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Free radicals especially reactive oxygen species can produce some disorders by damaging biomolecules like Deoxyribonucleic Acid (DNA), proteins, and membrane lipids. Lipid peroxidation in Low-Density Lipoprotein (LDL) and membranes of hepatocytes are involved in atherosclerosis and liver disease respectively. Non-enzymatic glycosylation of proteins is involved in complications of diabetes. We studied the antioxidant effects of some plants, namely, *Ziziphus vulgaris*, *Portulaca oleracea*, *Berberis integerima*, *Gundelia tournefortti* on the above mentioned reactions. Ethanolic and water extracts of the mentioned plants were prepared in three different concentrations:2.5, 5 and 10 µg/ml. Hepatocytes of rat were exposed to tert-butyl hydroperoxide (TBH). The amount of Alanine Aspartate Aminotransferase (AST) released from membrane lipid peroxidation was also measured in presence and absence of the plant extract. Glycosylation. The changes of hemoglobin and red blood cell hemolysis were measured in the presence and absence of the extract. The percent of oxidation inhibition was compared with that in control subjects. Tukey test was used and significance determined at $p<0.001$.The results showed the highest extent of glycosylation inhibition of hemoglobin was due to Gundelia and Berberis by 33.33% and 28.3% respectively. Portulaca, Gundelia and Berberis decreased AST release from hepatocytes by 69.7% and 45% and 59% respectively and Berberis decreased AST release from hepatocytes by 64%. The highest extent of hemolysis inhibition of red blood cell was due to Ziziphus, Gundelia and Berberis by 67%, 56% and 43% respectively. This study showed that the plants have an antioxidant effect and they can be probably used as an antioxidant in food supplement in diabetic and liver disease patients.

## Topic I Cosmetics, flavours and aromas

PI42

### Volatile compounds of three *Thymus sipyleus* subspecies from different sites in Turkey

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The genus Thymus (Lamiaceae) is represented by 38 species and 64 taxa, 20 of which are endemic in Turkey. Volatile compounds isolated by microdistillation from nine samples of *Thymus sipyleus* Boiss. subsp. *sipyleus* var. *sipyleus*, *T. sipyleus* Boiss. subsp. sipyleus Boiss. var. davisianus Ronniger, and *T. sipyleus* Boiss. subsp. *rosulans* (Borbas) Jalas were analysed by GC and GC/MS. *T. sipyleus* subsp. *sipyleus* var. *sipyleus* was collected from 3 regions in Elmali, Antalya. 1,8-Cineole (11.2%) and p-cymene (21.8%), α-terpineol (38.6%) and carvacrol (20.5%), 1,8-cineole (11.6%) and carvacrol (18.2%) were identified as major compounds in three samples respectively. *T. sipyleus subsp. sipyleus var. davisianus* was collected from *3* regions in Saklikent, Antalya. Geranial (30.3%) and neral (19.6%), 1,8-cineole (31.1%) and β-caryophyllene (14.6%), and α-terpineol (19.8%) and geranial (11.1%) were the main compounds in the samples respectively. *T. sipyleus subsp. rosulans* was collected from 3 regions in Gazipaşa, Antalya. β-Caryophyllene (14.2%) and intermedeol (13.2%), 1,8-cineole (11.6%) and α-terpineol (35.4%), α-pinene (18.4%) and β-caryophyllene ( 8.9%) were the major compounds of the samples respectively. Thymol is the main compound in most *Thymus* species in Turkey. According to our study, chemical polymorphisms have been found among the *T. sipyleus* subspecies.

PI43

### Essential oil composition of *Matricaria chamomilla* var. *chamomilla* and var. *recutita* from Turkey

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