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Antioxidant lipoxygenase inhibitors from the leaf extracts of *Simmondsia chinensis*

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ABSTRACT

Objective: To isolate and identify chemical constituents with antioxidant and lipoxygenase inhibitory effects of the ethanolic extract of *Simmondsia chinensis* (Jojoba) leaves. **Methods:** The alcoholic extract was subjected to successive solvent fractionation. The antioxidant active fractions (chloroform, ethyl acetate and aqueous fractions) were subjected to a combination of different chromatographic techniques guided by the antioxidant assay with DPPH. The structures of the isolated compounds were elucidated on the basis of spectroscopic evidences and correlated with known compounds. The antioxidant activity was assessed quantitively using DPPH and β—carotene methods. The inhibitory potential against enzyme lipoxygenase was assessed on soybean lipoxygenase enzyme. **Results:** Ten flavonoids and four lignans were isolated. Flavonoid aglycones showed stronger antioxidant and lipoxygenase inhibitory effects than their glycosides. Lignoid glycosides showed moderate to weak antioxidant and lipoxygenase inhibitory effects. **Conclusions:** A total of 14 compounds were isolated and identified from *Simmondsia chinensis*; 12 of them were isolated for the first time. This is the first report that highlights deeply on the phenolic content of jojoba and their potential biological activities and shows the importance of this plant as a good source of phenolics in particular the flavonoid content.

1. Introduction

Phytochemicals have been of great interest as a source of natural antioxidants used for food flavouring, food preservation, health promotion and cosmetics since they are environmentally more friendly and safer for consumption than their synthetic counterparts[1]. Plants are known for their health protective effect mostly due to their phenolic components which have strong antioxidant activity against the reactive oxygen species. Reactive oxygen species induce oxidative damage to biomolecules like nucleic acids, lipids, proteins and carbohydrates. This damage leads to many diseases such as cancer, rheumatism, cirrhosis, arteriosclerosis and diabetes[1].

Lipoxygenases are the key enzymes in the biosynthesis of leukotrienes which play an important role in several

inflammatory diseases such as cancer, arthritis, asthma and allergic diseases. Therefore, lipoxygenase inhibitors may be of many medicinal benefits in prevention of these inflammatory cases[2].

Jojoba [*Simmondsia chinensis* (Link) C. Schneider (*S. chinensis*), family Simmondsiaceae], (Syn. *Buxus chinensi*s Link; *Simmondsia californica* Nutt.), is known as coffee berry, wild hazel and goat nut[3]. It is a woody, evergreen perennial shrub, reaching about 3 m in height. It is a dioecious plant, growing in desert and semi−desert areas, native to Southwestern USA and Northwestern Mexico. Cultivation of jojoba has been established in several countries predominantly in Argentina, Peru, Australia, Israel, Palestinian Authority and Egypt due to its high economic value. It has been introduced to Egypt in 1991 and cultivated by seeds from USA[4]. Jojoba is used by Native Americans for widespread medicinal purposes and is a folk remedy for cancer, cold, dysuria, obesity, parturition, sore throat warts and wounds[5,6]. Jojoba is well known for its seed oil (liquid wax esters) which mainly composed of straight chain monoesters in the range of $C_{40}–C_{44}$[7]. Jojoba oil is used in cosmetics in a number of skin care products, mainly as a moisturizer, as well as in hair conditioners and as lubricant[8]. Research studies suggest that the seed oil has an anti−inflammatory effect[9], antioxidant[10] and promote wound healing properties and can be used as a remedy, mainly for skin disorders[6].

The meal (the seed plant material after removing of the oil) is rich in protein 29%–30% in addition to group of cyanogenic glycosides (cyanomethylenecyclohexyl glycosides), simmondsin and it′s derivatives, simmondsin−2′−ferulate, demethylsimmondsin, didemethylsimmondsin, 4,5−didemethyl−4−*O*−α−D−glucopyranosyl simmonsin[11–14]. These toxicants could be removed and the meal could be used as livestock feed ingredient[15]. Simmondsins have insecticidal, antifeedant and antifungal activities[16].

Furthermore, some carbohydrates were isolated and identified from jojoba meal as 4−β−galactobiose, 4−β−galactotriose, 1D−2−*O*−α−D−galactopyranosyl−chiro−inositol, D−pinitol, galactinol, sucrose, 5−α−D−galactopyranosyl−D−pinitol and 2−α−D−galactopyranosyl−D−pinitol[12].

In the course of our ongoing research activities toward the isolation of antioxidants and lipoxygenase inhibitors from plants growing in Egypt either wild or cultivated, we had the opportunity to work on the leaf of *S. chinensis*. Through phytochemical investigation of jojoba extracts, few research works were reported and only few flavonoids were isolated from jojoba pericarp. These flavonoids include quercetin 3,3′−dimethyl ether, isokaempferide and quercetin 3−methylether[17]. For jojoba leaf, there are very few hits describing the leaf extract activity[18], and this is the first study investigating in details the chemistry of the constituents as well as their potential biological activities.

The aim of the present study was to assess the antioxidant and lipoxygenase inhibition activities of the phenolic compounds isolated by bioassay−guided fractionation using the 1,1−diphenyl−2−picrylhydrazyl (DPPH*) assay from the leaf extract of *S. chinensis*. The data provide evidence for the utilization of *S. chinensis* as a rich bioactive source of natural antioxidants.

2. Materials and methods

2.1. Apparatus and chemicals

UV spectra were obtained on a Cary 50 spectrophotometer. NMR spectra were obtained on a Bruker Avance DRX600 spectrometer or a Bruker Avance DRX400 spectrometer. HRESIMS measurements were obtained on a Bruker micro TOF mass spectrometer. Column chromatography was performed using a silica gel (Kieselgel 60 Å, 40−63 µm mesh size, Fluorochem, UK), resin HP−20 (Diaion, Japan), ODS−A (YMC) and size exclusion chromatography (Sephadex LH−20, 25−100 mm mesh size, SIGMA, Germany). Thin layer chromatography (TLC) was done using pre−coated silica gel 60 F254 (0.25 mm, ALUGRAM® SIL G/UV$_{254}$, Macherey−Nagel, Germany) and RP−18 F254S plates (0.25 mm, Merck, Germany). Reversed phase high performance liquid chromatography (HPLC) were carried out using an Agilent Chromatorex Zorbax SB C18 (5 µm) semi−preparative column (9.4 mm× 250 mm) and an Agilent Chromatorex Eclipse XDB C3 (5 µm) semi−preparative column (9.4 mm× 250 mm) on an Agilent 1200 Series separations module equipped with Agilent 1200 Series diode array and/or multiple wavelength detectors, controlled using ChemStation Rev.9.03A and Purify version A.1.2 software. All flash chromatography was performed on Sepacore Flash Chromatography System (Buchi Labortechnik AG, Netherlands). β−carotene, and DPPH were purchased from Sigma−Aldrich Chemical Co. (St. Louis, USA). Butylated hydroxytoluene (BHT) was obtained from Aldrich Chemical Co., Gillingham, Dorset, UK. D−glucose, L−rhamnose, L−arabinose and D−galactose were obtained from El−Nasr Pharmaceutical and Chemical Co., Egypt (Adwic) and Sigma Aldrich Chemicals. Soybean Lipoxygenase (1.13.11.12) type I−B and linoleic acid were purchased from Sigma (St. Louis, MO, USA).

2.2. Plant material

The leaves were collected in June 2009 from organically cultivated plant, Wadi−Assiut, Assiut, Egypt and authenticated by Prof. Dr. Abdel−Aziz Fayed, professor of plant taxonomy, Botany Dept., Faculty of Science Assiut. A voucher specimen was deposited in Pharmacognosy Department Herbarium (No. 2009SC), Faculty of Pharmacy Assiut University, Assiut, Egypt.

2.3. Extraction and isolation

A total of 500 g of the air−dried powdered leaves were

extracted by maceration (48 h×3) and percolation with 70% ethyl alcohol (EtOH) till complete exhaustion (3L×3). The alcoholic extract was concentrated and the solvent free residue (47 g, 9.4%) was mixed with 500 mL of distilled H_2O, and subjected to successive solvent fractionation with *n*–hexane, chloroform, ethyl acetate and water till complete exhaustion in each case to give hexane fraction (21 g), chloroform fraction (4 g), ethyl acetate fraction (6 g) and aqueous fraction (14 g). The chloroform and ethyl acetate fractions were subjected to normal silica gel column using CHCl$_3$–MeOH mixtures in a manner of increasing polarities. Each fraction of 50 mL was collected and monitored on TLC (silica gel) using CHCl$_3$–MeOH (90:10) and CHCl$_3$–MeOH–H$_2$O [(80:20:2) and (70:30:3)] as solvent systems and 20% v/v H_2SO_4 in EtOH and/or 0.2% DPPH in MeOH as spraying agents. Similar fractions on TLC were combined and subjected to HPLC (Agilent Chromatorex Zorbax SB C18, 5 μm, 9.4 mm×250 mm) using a gradient of 5%–100% CH_3CN–H_2O over 45 min to give three compounds from chloroform fraction; (1) (23.8 mg), (2) (11.4 mg), (3) (6.5 mg); and three compounds from ethyl acetate (4) (13.6 mg), (5) (32.4 mg) and (6) (18.2 mg).

The aqueous fraction (14 g) was subjected to Diaion–HP$_{20}$ column chromatography using H_2O and MeOH (5 L each). The methanolic elute was concentrated under reduced pressure to yield a fraction (10.6 g). The methanolic fraction was subjected to flash chromatography on silica gel column using CHCl$_3$–MeOH mixtures in a manner of increasing polarities. Ninety fractions (20 mL each) were collected and monitored on TLC (silica gel) using CHCl$_3$–MeOH–H$_2$O [(80:20:2), (70:30:3) and (55:40:5)] as well as on RP–C18 using H_2O–MeOH [(70:30), (50:50) and (30:70)] as solvent systems and 20% v/v H_2SO_4 in EtOH and/or 0.2% DPPH in MeOH as spraying reagents.

Similar fractions on TLC were combined to yield nine groups. Group 2, 4, 5 and 6 were subjected to gel filtration chromatography using a Sephadex LH–20 column with CHCl$_3$–MeOH (1:1) followed by HPLC (Agilent Chromatorex Eclipse XDB C3, 5 μm, 9.4 mm×250 mm) using a gradient of 5%–80% CH_3CN–H_2O over 40 min to give eight compounds. Identified compounds are (7) (19.1 mg), (8) (26.3 mg) from group 2; (9) (20.8 mg) and (10) (16.5 mg) from group 4; (11) (7.5 mg), (12) (12.3 mg) from group 5; and finally (13) (14.8 mg) and (14) (24.7 mg) from group 6.

2.4. Acid hydrolysis of isolated flavonoid glycosides

Isolated flavonoid glycosides (3 mg) were refluxed in 2 mol/L HCl (5 mL) for 2 h. After cooling, the reaction mixture was extracted with Et$_2$O (2 mL×3). The ethyl acetate fraction containing aglycone and the aqueous fraction containing sugars units were concentrated to dryness. The aglycones were identified by co–TLC with authentic samples, while sugars were identified by comparison with standards using descending paper chromatography, with BuOH: AcOH: H_2O (4:1:5) as the mobile phase, the spots were detected by spraying with aniline phthalate followed by heating at 110 °C[19].

2.5. Acid hydrolysis of isolated lignan glycosides

A solution of lignan glycosides (each 3 mg) in MeOH (5 mL) containing 1 mol/L HCl (4 mL) was refluxed for 4 h, concentrated under reduced pressure, and diluted with H_2O (8 mL). The solution was extracted with EtOAc to obtain the aglycone, while the glycone was obtained from the aqueous part after concentration. In all the cases the glycone could be identified as D–glucose by paper chromatography using BuOH: AcOH: H_2O (4:1:5) as mobile phase and aniline phthalate as spraying agent with heating at 110 °C[20].

2.6. Antioxidant activity

2.6.1. β–carotene bleaching method

Spectrophotometric assay according to the method of Lelono *et al.*[21] and Duymuş *et al.*[22] was carried out for quantitative determination. The β–carotene (0.2 mg), linoleic acid (20 mg) and Tween 20 (200 mg) were mixed in 0.5 mL of chloroform. The solvent was subsequently removed at 40 °C in a vacuum evaporator and the mixture was diluted with 50 mL of triply distilled, oxygenated water. Aliquots (4 mL) of this emulsion were transferred into test tubes, to which were then added with 0.2 mL aliquots of test samples in ethanol. BHT was used for comparative purposes. A control containing 0.2 mL of ethanol and 4 mL of above emulsion was also prepared. The test tubes were covered with aluminium foil and placed in a water bath at 50 °C. Absorbances for all samples at 470 nm were recorded at zero time (t=0 min). Measurement of absorbance was continued until the colour of β–carotene disappeared in the control reaction (t=180 min) at 15 min intervals. The above mixture without β–carotene served as blank. All determinations were carried out in triplicate. Antioxidant activity was expressed as percentage inhibition relative to the control using the equation[21,22]:

$$A_A(\%)=100\left[1-(A_0-A_t)/(A_0^0-A_t^0)\right]$$

Where A_A is the antioxidant activity, A_0 and A_0^0 are the absorbance values measured at zero time of incubation for the test sample and control respectively. A_t and A_t^0 are the corresponding values at the end of the reaction time (180 min).

2.6.2. DPPH radical scavenging assay

Firstly, radical scavenging activity of the isolated compounds against stable DPPH* was performed with a rapid TLC screening method using 0.2% DPPH in MeOH. About 30 min after spraying, the active compounds appeared as yellow spots against purple background.

In a second experiment, spectrophotometric assay was carried out according to the method of Abdel–Mageed[23]. Briefly, 2.0 mL of a wide range of concentrations (2.5–120 μmol/L) of test sample (in MeOH) was added to 2.0 mL of 100 μmol/L of methanolic solution of DPPH. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min

in dark. When DPPH• reacts with an antioxidant compound, it changes its colour from deep–violet to light–yellow which is measured at 517 nm on a UV/visible light spectrophotometer. Absorption of blank sample containing the same amount of MeOH without sample and DPPH solution was prepared and measured daily. The experiment was carried out in triplicate, using BHT as a positive control. The percentage reduction of the DPPH, Q, referred to inhibition or quenching was calculated by the following formula[24–27]:

Q (% inhibition)=[(A$_B$–A$_A$)/A$_B$]×100

Where A$_B$ is absorption of blank sample (t=0 min); A$_A$ is absorption of tested extract solution (t=30 min).

2.7. Lipoxygenase inhibition assay

Lipoxygenase–inhibiting activity was conveniently measured by slight modification of the spectrometric method developed by Tappel[23]. The reaction mixture containing 160 µL of 100 mmol/L sodium phosphate buffer (pH 8.0), 10 µL of test compound solution and 20 µL of lipoxygenase solution was mixed and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 µL linoleic acid (substrate) solution, with the formation of (9Z, 11E)–(13S)–13–hydroperoxyoctadeca– 9,11–dienoate. The change of absorbance at 234 nm was followed for 6 min. Test compounds and the control were dissolved in methanol. All the reactions were performed in triplicate in 96–well micro–plate in SpectraMax Plus 384 (Molecular Devices, USA). The IC$_{50}$ values were then calculated using the EZ–Fit Enzyme kinetics program (Perrella Scientific Inc., Amherst, USA). The percentage (%) inhibition was calculated as follows; (E–S)/E–100, where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound[23].

3. Results

The phytochemical investigation for the leaf extract guided by the antioxidant assay using DPPH method[24,25] lead to isolation and identification of group of phenolic compounds including quercetin and isorhamnetin type flavonoids in free aglycone and glycoside forms as well as lignoid glycosides.

Chromatographic fractionation of the chloroform, ethyl acetate and aqueous fractions, and active DPPH fractions using a combination of flash, gel filtration and HPLC guided by the antioxidant assay with β–carotene afforded ten flavonoids and four lignans. Their structures were elucidated by extensive 1D, 2D nuclear magnetic resonance analysis, accurate mass measurements and by comparing with the reported data.

The ten known flavonoids were identified as quercetin 3′–methyl ether (isorhamnetin) (1)[28], quercetin 3–methylether (2)[17], quercetin 3,3′–dimethyl ether (3)[17], quercetin (4)[28], isorhamnetin 3–O–glucoside (5)[29], quercetin 3–O–glucoside

(6)[30], isorhamnetin 3–O–rutinoside (7)[31], quercetin 3–O–rutinoside (8)[32], isorhamnetin 3–O–(2″,6″–di–O–α–L–rhamnopyranosyl)–β–D–glucopyranoside (Typhaneoside) (9) [33], isorhamnetin 3–O–(2″,6″–di–O–α–L–rhamnopyranosyl)–β–D–galactopyranoside (10)[34], and four lignans as (+)–lyoniresinol 4,4′–bis–O–β–D–glucopyranoside (11)[20], (+)–lyoniresinol 4,9′–bis–O–β–D–glucopyranoside (12)[35], 5,5′–dimethoxylariciresinol 4,4′–bis–O–β–D–glucopyranoside (salvadoraside) (13)[36] and syringaresinol diglucoside (eleutheroside E) (14)[37]. All physical and spectral data of these compounds were in agreement with the respective published data.

The quantitative estimation of antioxidant activity for isolated compounds was carried out using β–carotene bleaching and DPPH assay methods. Results from DPPH method (Table 1) showed that flavonoid free aglycones (1–4) have stronger antioxidant activity than flavonoid glycosides which is in good consistency with the results obtained from β–carotene bleaching method. The strongest antioxidant activity was observed with quercetin (4) [IC$_{50}$=7.4 (DPPH) and 15.1 (β–carotene) µmol/L] superior to those of BHT (positive standard) [IC$_{50}$=16.1 (DPPH) and 28.7 (β–carotene) µmol/L]. Lignoid glycosides (11–14) showed weak to moderate activity and maximum activity was observed with compound (12) [IC$_{50}$=94.3 (DPPH) and 178.4 (β–carotene) µmol/L] compared with those of BHT.

Table 1

Antioxidant and lipoxygenase inhibition activities for isolated compounds (1–14).

Compound	DPPH	β–carotene bleaching	lipoxygenase
	IC$_{50}$ (µmol/L)	IC$_{50}$ (µmol/L)	IC$_{50}$ (µmol/L)
1	15.3	36.6	44.7
2	11.6	24.5	23.2
3	42.8	69.3	35.4
4	7.4	15.1	5.6
5	131.3	>200.0	>250.0
6	78.1	104.2	237.6
7	185.8	>200.0	Inactive
8	143.9	>200.0	Inactive
9	>200.0	>200.0	Inactive
10	>200.0	>200.0	Inactive
11	121.7	>200.0	43.3
12	94.3	178.4	48.5
13	167.5	>200.0	136.2
14	>200.0	>200.0	149.1
Luteolin	–	–	4.3
BHT	16.1	28.7	–

The enzyme inhibition assay for isolated compounds was evaluated on soybean lipoxygenase enzyme. For flavonoids, the free aglycones exhibited strong promising activity. The highest activity was observed in quercetin (4) with IC$_{50}$ of 5.6 µmol/L. Luteolin was used as a reference. The lignoid glycosides (11–14) showed moderate to weak inhibitory action. Compounds (11) and (12) showed comparable moderate effect with IC$_{50}$ of 43.3 and 48.5 µmol/L respectively.

4. Discussion

For the antioxidant effect, it is obvious that antioxidant activity of flavonoids is decreased by methylation and/or glycosylation at 3 and/or 3′–OH groups. This can be explained by understanding the mechanisms for antioxidant activity of flavonoids. There are various antioxidant mechanisms for flavonoids, one of them is the presence of structural features as ortho–dihydroxy groups (usually on B ring), α,β–unsaturated carbonyl moiety and α– and/or β–hydroxyketone groups, which are responsible for chelating properties or enhancement of the radical stabilization after the initial oxidation steps[38]. The lack of a catechol moiety on B ring and a free hydroxyl group at C–3 in flavonoid glycosides may account for its low ability as antioxidant. For lignans, the antioxidant activity is improved by the presence of phenolic hydroxyl groups.

For the lipoxygenase inhibitory effect, the activity was dramatically suppressed by introducing sugars to 3–OH as seen in flavonoid glycosides (5–10). This latter effect was plausible from the higher hydrophilicity of the glycoside which is expected to give rise to a lower affinity towards the lipophilic active site of lipoxygenase. These results are in good agreement with the previously published data[39] dealing with structural activity relationship of flavonoids to inhibit 15–lipoxygenases which confirmed that introduction of sugar moiety to flavonoid aglycone diminish its lipoxygenase inhibition activity. In the other words, increasing the hydrophilicity of flavonoids by introducing sugars decreases their antioxidant and lipoxygenase inhibition activities. Lignans exhibited moderate to weak inhibitory action and more studies are required to investigate their structural activity relationship.

Lipoxygenase is involved in arachidonic acid metabolism, generating various biologically active leukotrienes that play important role in inflammation. Therefore, lipoxygenase are potential target for the rational drug design and discovery of mechanism–based inhibitors for the treatment of variety of disorders such as bronchial asthma, inflammation, cancer and autoimmune diseases[40].

In conclusion, 14 compounds, including 10 flavonoids and 4 lignans, were isolated from *S. chinensis*, in which 12 of them (1, 4–14) were recorded for the first time. The antioxidant and lipoxygenase–inhibiting activities were assessed for isolated compounds. Flavonoids free aglycones (1–4) showed strong antioxidant and lipoxygenase–inhibiting activities. The presence of a sugar moiety in the flavonoid appears to dramatically depress the antioxidant and lipoxygenase–inhibiting activity. Lignans showed variable moderate to weak antioxidant and lipoxygenase–inhibiting activities and the highest activity was observed with compounds 11 and 12 with comparable values.

This paper is considered to be the first report that highlights deeply on the phenolic content of jojoba and their biological activities. Most of previous work was focusing on its seed oil and neglecting other constituents. In this report, we mentioned the importance of this plant as a good source of phenolics in particular the flavonoid content. These data may change the view of research concerning this plant and also help pharmaceutical industry as well as folk medication practitioners who use this plant as a source of curing diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

References

[1] Khasawneh MA, Elwy HM, Fawzi NM, Hamza AA, Chevidenkandy AR, Hassan AH. Antioxidant activity, lipoxygenase inhibitory effect and polyphenolic compounds from *Calotropis procera* (Ait.) R. Br. *Res J Phytochem* 2011; **5**(2): 80–88.

[2] Czapski GA, Czubowicz K, Strosznajder RP. Evaluation of the antioxidative properties of lipoxygenase inhibitors. *Pharmacol Rep* 2012; **64**(5): 1179–1188.

[3] Phillips SJ, Comus PW. *A natural history of the Sonoran Desert*. Ewing, USA: University of California Press; 2000, p. 256–257.

[4] El–Baz EE, El–Dengawy EF, El–Shahat SE, El–Hassan EM. Studies on some morphological aspects of jojoba [*Simmondsia chinensis* (Link) Schneider] under Egyption conditions. *J Agric Sci Mansoura Univ* 2009; **34**: 10575–10586.

[5] Bloomfield F, Bernardi M. *Jojoba and yucca (miracle plants)*. London: Ebury Press; 1985.

[6] Ranzato E, Martinotti S, Burlando B. Wound healing properties of jojoba liquid wax: an *in vitro* study. *J Ethnopharmacol* 2011; **134**(2): 443–449.

[7] Vrkoslav V, Urbanova K, Cvacka J. Analysis of wax ester molecular species by high performance liquid chromatography/ atmospheric pressure chemical ionisation mass spectrometry. *J Chromatogr A* 2010; **1217**(25): 4184–4194.

[8] Pazyar N, Yaghoobi R, Ghassemi MR, Kazerouni A, Rafeie E, Jamshydian N. Jojoba in dermatology: a succinct review. *G Ital Dermatol Venereol* 2013; **148**(6): 687–691.

[9] Habashy RR, Abdel–Naim AB, Khalifa AE, Al–Azizi MM. Anti–inflammatory effects of jojoba liquid wax in experimental models. *Pharmacol Res* 2005; **51**(2): 95–105.

[10] Ibrahim HM, Abou–Arab AA, Abu Salem FM. Antioxidant and antimicrobial effects of some natural plant extracts added to

lamb patties during storage. *Grasas Y Aceites* 2011; doi:10.3989/gya.066510.

[11] Van Boven M, Busson R, Cokelaere M, Flo G, Decuypere E. 4-Demethyl simmondsin from *Simmondsia chinensis*. *Ind Crops Prod* 2000; **12**(3): 203-208.

[12] Van Boven M, Leyssen T, Busson R, Holser R, Cokelaere M, Flo G, et al. Identification of 4,5-Didemthyl-4-*O*-α-D-glucopyranosylsimmondsin and pinitol α-D-galactosides in jojoba seed meal (*Simmondsia chinensis*). *J Agric Food Chem* 2001; **49**(9): 4278-4283.

[13] Lein S, Van Boven M, Holser R, Decuypere E, Flo G, Lievens S, et al. Simultaneous determination of carbohydrates and simmondsins in jojoba seed meal (*Simmondsia chinensis*) by gas chromatography. *J Chromatogr A* 2002; **977**(2): 257-264.

[14] Bellirou A, Bouali A, Bouammali B, Boukhatem N, Elmtili BN, Hamal A, et al. Extraction of simmondsin and oil in one step from jojoba seeds. *Ind Crops Prod* 2005; **21**(2): 229-233.

[15] Bouali A, Bellirou A, Boukhatem N, Hamal A, Bouammali B. Enzymatic detoxification of jojoba meal and effect of the resulting meal on food intake in rats. *Nat Prod Res* 2008; **22**(7): 638-647.

[16] Abbassy MA, Abdelgaleil SA, Belal AS, Rasoul MA. Insecticidal, antifeedant and antifungal activities of two glucosides isolated from the seeds of *Simmondsia chinensis*. *Ind Crops Prod* 2007; **26**(3): 345-350.

[17] El-Shamy AM, Shehata AH, Sanad OA, El-Halawany AM, Abd El-Latif HA. Biologically active flavonoids from *Simmondsia chinensis* (Link) Schneider growing in Egypt. *Bull Fac Pharm Cairo Univ* 2001; **24**: 152-158.

[18] Yarmolinsky L, Zaccai M, Ben-Shabat S, Huleihel M. Anti-herpetic activity of *Callissia fragrans* and *Simmondsia chinensis* leaf extracts *in vitro*. *Open Virol J* 2010; **4**: 57-62.

[19] Han N, Huang T, Wang YC, Yin J, Kadota S. Flavanone glycosides from *Viscum coloratum* and their inhibitory effects on osteoclast formation. *Chem Biodivers* 2011; **8**(9): 1682-1688.

[20] Yang XW, He HP, Du ZZ, Liu HY, Di YT, Ma YL, et al. Tarennanosides A-H, eight new lignan glucosides from *Tarenna attenuata* and their protective effect on H$_2$O$_2$-induced impairment in PC12 cells. *Chem Biodivers* 2009; **6**(4): 540-550.

[21] Lelono RA, Tachibana S. Bioassay-guided isolation and identification of antioxidative compounds from the bark of *Eugenia polyantha*. *Pak J Biol Sci* 2013; **16**(16): 812-818.

[22] Duymuş HG, Göger F, Başer KH. *In vitro* antioxidant properties and anthocyanin compositions of elderberry extracts. *Food Chem* 2014; **155**: 112-119.

[23] Abdel-Mageed WM, Backheet EY, Khalifa AA, Ibraheim ZZ, Ross SA. Antiparasitic antioxidant phenylpropanoids and iridoid glycosides from *Tecoma mollis*. *Fitoterapia* 2012; **83**(3): 500-507.

[24] Ibraheim ZZ, Abdel-Mageed WM, Dai H, Guo H, Zhang L, Jaspars M. Antimicrobial antioxidant daucane sesquiterpenes from *Ferula hermonis* Boiss. *Phytother Res* 2012; **26**(4): 579-586.

[25] Wagner M, Abdel-Mageed WM, Ebel R, Bull AT, Goodfellow M, Fiedler HP, et al. Dermacozines H-J isolated from a deep-sea strain of *Dermacoccus abyssi* from Mariana Trench sediments. *J Nat Prod* 2014; doi:10.1021/np400952d.

[26] Abdel-Mageed WM, Milne BF, Wagner M, Schumacher M, Sandor P, Pathom-aree W, et al. Dermacozines, a new phenazine family from deep-sea dermacocci isolated from a Mariana Trench sediment. *Org Biomol Chem* 2010; **8**(10): 2352-2362.

[27] Yu Z, Wang XS, Hu ZH. Dammarane-type saponins from *Ziziphus jujube*. *J Asian Nat Prod Res* 2014; **16**(2): 200-205.

[28] Hassan RA, Tawfik WA, Abou-Setta LM. The flavonoid constituts of *Leucaena leucocephala*. Growing in Egypt, and their biological activity. *Afr J Tradit Complement Altern Med* 2013; **11**(1): 67-72.

[29] Wang DM, Pu WJ, Wang YH, Zhang YJ, Wang SS. A new isorhamnetin glycoside and other phenolic compounds from *Callianthemum taipaicum*. *Molecules* 2012; **17**(4): 4595-4603.

[30] Liu H, Mou Y, Zhao JL, Wang JH, Zhou LG, Wang M, et al. Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities. *Molecules* 2010; **15**: 7933-7945.

[31] Bhattacharya S, Christensen KB, Olsen LC, Christensen LP, Grevsen K, Færgeman NJ, et al. Bioactive components from flowers of *Sambucus nigra* L. increase glucose uptake in primary porcine myotube cultures and reduce fat accumulation in *Caenorhabditis elegans*. *J Agric Food Chem* 2013; **61**(46): 11033-11040.

[32] Nenaah G. Antimicrobial activity of *Calotropis procera* Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. *World J Microbiol Biotechnol* 2013; **29**(7): 1255-1262.

[33] Liu JY, Yu HS, Feng B, Kang LP, Pang X, Xiong CQ, et al. Selective hydrolysis of flavonoid glycosides by *Curvularia lunata*. *Chin J Nat Med* 2013; **11**(6): 684-689.

[34] Yasukawa K, Sekine H, Takido M. Two flavonol glycosides from *Lysimachia fortunei*. *Phytochemistry* 1989; **28**(8): 2215-2216.

[35] Sun J, Yu J, Zhang PC, Tang F, Yue YD, Yang YN, et al. Isolation and identification of lignans from Caulis Bambusae in Taenia with antioxidant properties. *J Agric Food Chem* 2013; **61**(19): 4556-4562.

[36] Ohtani K, Yamasaki K, Tanaka O. Lignan glycosides from stems of *Salvadora persica*. *Phytochemistry* 1992; **31**(7): 2469-2471.

[37] Wang Z, Zhang L, Sun Y. Semipreparative separation and determination of eleutheroside E in *Acanthopanax giraldii* Harms. by high-performance liquid chromatography. *J Chromatogr Sci* 2005; **43**(5): 249-252.

[38] Lago JH, Toledo-Arruda AC, Mernak M, Barrosa KH, Martins MA, Tibério IF, et al. Structure-activity association of flavonoids in lung diseases. *Molecules* 2014; **19**(3): 3570-3595.

[39] Sadik CD, Sies H, Schewe T. Inhibition of 15-lipoxygenases by flavonoids: structure-activity relations and mode of action. *Biochem Pharmacol*. 2003; **65**(5): 773-781.

[40] Azhar-Ul-Haq, Malik A, Anis I, Khan SB, Ahmed E, Ahmed Z, et al. Enzymes inhibiting lignans from *Vitex negundo*. *Chem Pharm Bull (Tokyo)* 2004; **52**(11): 1269-1272.