Journal of Food and Drug Analysis, Vol. 15, No. 3, 2007, Pages 249-257 藥物食品分析 第十五卷 第三期

Chemical and Biological Study of the Residual Aerial Parts of *Sesamum indicum* L.

AMAL EL-SAYED KHALEEL[1]*, MARIAM HUSSAIN GONAID[1], RAMZIA ISMAIL EL-BAGRY[2], AMANY AMEEN SLEEM[3] AND MARWAN SHABANA[1]

[1.] *Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, 11562, Egypt*
[2.] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, 11562, Egypt*
[3.] *Department of Pharmacology, National Research Center, Dokki, Giza, Egypt*

(Received: August 30, 2006; Accepted: April 24, 2007))

ABSTRACT

The chemical composition of the residual aerial parts of the Egyptian crop of *Sesamum indicum* L. after collection of the seeds, was studied. Sesamin, sesamolin, stigmasterol, β-sitosterol and stigmasterol-3-O-β-D-glucoside were isolated from the petroleum ether fraction of the alcoholic extract. Ferulic acid, rhamnetin, verbascoside, kaempferol-3-O-β-D-glucuronide and mequelianin (quercetin-3-O-β-D-glucuronide) were isolated from the butanol fraction. The content of the major constituents, namely sesamin and sesamolin, were determined by HPLC method. The alcoholic extract as well as its petroleum ether and butanol fractions were tested *in vivo* for the antioxidant, antihyperglycaemic and anticoagulant activities. The alcoholic extract exhibited a higher activity than its petroleum ether and butanol fractions in all the tested biological activities.

Key words: *Sesamum indicum*, residual aerial parts, lignans, flavonoids, sterols, antioxidant, anticoagulant, antihyperglycaemic activity

INTRODUCTION

Many phytochemical investigations on the chemical constituents of *S. indicum* L. have been traced in literature. These include lignans and lignan glycosides[1-5] as well as sterols[6] from the seeds, phenylethanoid glycosides[7] from the whole plant and phenolic acids from the leaves and seeds[8,9]. Several reports concerning the chemical composition of the plant cultivated in Egypt were also found, on the components of the unsaponifiable matter of sesame oil[10], the fatty acid composition of the seed[11] and determination of the moisture, protein, fat, carbohydrates, ash and fiber content of the seed[12]. However, no reports concerning studying the residual aerial parts has been found in the literature. Therefore, it was of interest to study the economical value of the residual aerial parts of the plant after collection of the seeds (about 65% of the Egyptian sesame crop), based on the present chemical and biological studies, as well as, to carry out a quantitative determination of the main active constituents in a trial to standardize the extract of the residual aerial parts.

It has been shown that free radicals cause oxidative degeneration of biomolecules such as membrane lipids, proteins and nucleic acids. Therefore, antioxidants that quench free radicals may play a significant role in the prevention of numerous degenerative diseases[13]. In fact, natural antioxidants have captured the interest of consumers and scientists of the medical and pharmaceutical industries due to their antitumor, antimutagenic as well as anticarcinogenic properties[14]. Therefore, it was deemed necessary to test the antioxidant property of the studied residual aerial parts. The main sesame lignans in sesame oil, sesamin and sesamolin were found to possess no appreciable antioxidant activity *in vitro*, while sesamolin inhibited lipid peroxidation in rat liver and kidney[15]. In addition, both lignans as well as sesaminol di- and triglucosides exhibited lower DPPH scavenging activity than their crude extracts[16]. Therefore, the crude extracts rather than the isolated compounds were investigated *in vivo* to determine their ability to act as antioxidants. Sesame seeds had a reductive effect on plasma glucose concentration[17]. So, the antihyperglycaemic effect of the residual aerial parts was studied. One of the uncovered biological activities, the anticoagulant activity, of the extracts was also studied.

MATERIALS AND METHODS

I. *Materials*

Seeds of the brown variety of *S. indicum* L. (Giza 24) were purchased from Ministry of Agriculture and cultivated in a special farm in Salhia, Egypt. Samples of the residual aerial parts after collection of the seeds were

* Author for correspondence. Tel: +202-3714729;
Fax: +202-5320005; E-mail: amaalkhaleel@yahoo.com

collected in September 2004 and powdered. Silica gel 60 (230-400 mesh ASTM, Machery Nagel, Germany), silica gel H for vacuum liquid chromatography (Merck), sephadex LH-20 (Pharmacia) and RP-18 (Merck) were used for column chromatography. UV spectra were measured on a Schimadzu 265 spectrophotometer, in methanol and after addition of different shift reagents. ^{1}H- and ^{13}C-NMR spectra were recorded on Jeol TMS Route (300 MHz) and (75 MHz) in DMSO-d_6 and $CDCl_3$ using TMS as internal standard. Male rats of albino Sprague Dawely strain weighing 130-150 g and male mice (20-25 g) from the animal house colony of the National Research Center, Dokki, Giza, Egypt, were kept on standard laboratory diet with the following composition: 1% vitamin mixture, 4% mineral mixture, 10% corn oil, 20% sucrose, 10.5% casein pure (95%), 0.2% cellulose, 54.3% starch and water was provided *ad Lib*. Alloxan was purchased from Sigma Co. Cairo, Egypt, vitamin E (dl α-tocopheryl acetate) from Pharco Pharmaceutical Co. Egypt, metformin, from CID, Pharmaceutical Co. Egypt, Glutathione kit from Wak. Co. Germany and glucose reagent kit from BioMérieux, France.

II. *Preparation of Crude Extracts*

The air-dried residual aerial parts (1.5 kg) were extracted by cold maceration with alcohol 95% (20 L) to give 110 g resinous residue. The residue was dissolved in water and fractionated successively with petroleum ether and *n*-butanol saturated with water to give 28 g and 57 g respectively.

III. *Thin-layer Chromatography*

Thin-layer chromatography was performed on Silica gel GF_{254} precoated plates (Machery Nagel, Germany). The spots were visualized under UV light at λ 254 nm before and after spraying with $AlCl_3$ (flavonoids and phenylethanoids) or after spraying with *p*-anisaldehyde spray reagent (lignans and sterols).

IV. *Investigation of the Petroleum Ether Fraction*

The petroleum ether fraction (15 g) was chromatographed on a vacuum liquid chromatography column (VLC, Si gel H, 120 g, 10 × 4 cm). Gradient elution was carried out using petroleum ether-chloroform mixture with increasing polarity and fractions of 250 mL were collected. Column fractions were combined on the basis of their TLC patterns into three main fractions (A-C). Fr A (10% $CHCl_3$/petroleum ether, 1.3 g) was rechromatographed on a Si gel column (20 g, 20 × 1.5 cm) using hexane-ethyl acetate step gradient and fractions 20 mL each were collected. Fr A-1 (6% EtOAc/hexane) on concentration gave inseparable mixture of compounds **1** and **2** as white needle crystals (51 mg). Fr A-2 (6% ethyl acetate in hexane) gave on crystallization from MeOH 70 mg of white crystals (**1-3**). The previous mixture was dissolved in acetone and precipitated with methanol. Acetone supernatant was purified on small silica column using petroleum ether-ethyl acetate (8:2) to give compound **3** (30 mg).

Fr B (25% $CHCl_3$/petroleum ether) was concentrated to give a mixture of compounds **3** and **4** (250 mg), which on rechromatography on a Si gel 60 column (10 g, 10 × 1.5 cm, EtOAc/petroleum ether) gave B-1 and B-2. Fr B-1 (3% EtOAc/petroleum ether) gave white needle crystals of compound **4** (200 mg), while fr B-2 (EtOAc) gave white amorphous powder of compound **5** (20 mg).

V. *Preparation of the Unsaponifiable Fraction of the Petroleum Ether Extract*

One gram of petroleum ether extract was saponified by heating under reflux with 10 mL of 10% alcoholic KOH and 4 mL of benzene for 4 hr.

VI. *GLC Analysis of the Unsaponifiable Matter*

Two microliter of the chloroformic solution (10%) of the unsaponifiable matter as well as the sitosterol mixture (compounds **1&2**) were injected separately into Hewlett Packard HP 6890 Gas Liquid Chromatograph (GLC) with flame ionization detector (FID) on a HP-5, 5% phenyl methyl capillary column (30 m × 530 μm i.d., 50 μm film thickness). The oven temperature was programmed at 8°C/min from 80°C to 250°C, and then isothermally for 20 min. Nitrogen, hydrogen and air-flow rates were 30 mL/min, 30 mL/min and 300 mL/min, respectively. Detector and injection temperatures were 300 and 250°C. The identification of the sterols was accomplished by comparing the retention times with reference materials.

VII. *Thin Layer Chromatographic Investigation of the Unsaponifiable Matter*

The unsaponifiable matter was investigated using TLC precoated plates. The plates were developed using chloroform/benzene/methol (60/40/1), sprayed with *p*-anisaldehyde spray reagent and heated. The identification of the various compounds was achieved by comparison of their R_f values with reference standards as well as with those reported by Mohamed & Awatif[10].

VIII. *HPLC Analysis of Sesamin and Sesamolin*

Sesamin and sesamolin were analyzed with an Agilent 1100 series (PP 5500) HPLC (USA). Serial dilutions of both sesamin and sesamolin were dissolved in acetonitrile. The unsaponifiable matter of the petroleum ether extract was dissolved in acetonitrile and filtered and 20-μL samples were injected. Reversed phase separations were performed with a Zorbax ODS column (5

μm, 250 × 4.6 mm). The chromatography was operated with a mobile phase of 50% acetonitrile in water at a flow rate of 1 mL/min. The amount of each compound present was determined by the peak area of UV absorbance at 300 nm. A standard calibration curves for both of sesamin and sesamolin were obtained and their concentration in the unsaponifiable matter was calculated from the regression equation[18]: Y = bX + C where Y = absorbance, b = slope, X = concentration and C = intercept. Results of HPLC determination of both substances were shown in Table 1 and Figure 2.

IX. *Investigation of the n-Butanol Fraction*

n- BuOH fraction (35 g) was chromatographed on a VLC column (Si gel H, 160 g, 12.5 × 4 cm) using chloroform-ethyl acetate mixture with increasing polarity and fractions of 1 L each were collected. Column fractions were combined on the basis of their TLC patterns and four main fractions (A-D) were obtained.

Fraction A (25% $CHCl_3$/EtOAc, 0.6 g) was filtered on sephadex LH-20 column (30 × 1.5 cm), eluted with methanol and fractions of 3-5 mL were collected. Fraction A-1 (0.3 g) was chromatographed on 12 g Si gel 60 column (13 × 1.5 cm) eluted with chloroform and further purified on RP-18 column eluted with 20% MeOH/H_2O to give 10 mg of needle crystals of compound **6**.

Fraction B (100% ethyl acetate, 2.1 g) was chromatographed on sephadex LH-20 column (30 × 1.5 cm) eluted with methanol and fractions of 10 mL each were collected to give 15 mg of compound **7**.

Fraction C (25% MeOH /EtOAc, 19.5 g) was chromatographed on a polyamide column (10 × 4 cm, H_2O/MeOH). Fraction C-1 (H_2O/MeOH, 0-25%, 10 g) was chromatographed on a VLC column (Si gel H, 50 g, EtOAc/MeOH) and purified by rechromatography on Si gel columns eluted with 15% MeOH/$CHCl_3$ and $CHCl_3$/MeOH/H_2O (35:8:1) to give 150 mg of compound **8**.

Fraction D (75% MeOH/EtOAc 1.3 g) was chromatographed on a silica gel column (60 g, 15 × 3 cm, ethyl acetate/methanol/water, 100/16.5/13.5) and on several sephadex LH-20 columns eluted with methanol/water mixtures to afford compounds **9** (12 mg) and **10** (10 mg).

The yield of the isolated compounds from 1-10 are shown in Table 2.

X. *LD_{50} Study Experiment*

Determination of LD_{50} of the total alcoholic extract, petroleum ether and *n*-butanol fractions of the residual aerial parts was performed in mice by the oral administration of the tested extracts[19].

XI. *Antioxidant Activity*

The antioxidant activity of the total alcoholic extract, petroleum ether and *n*-butanol fractions of the residual aerial parts was determined by measuring the glutathione level[20] in blood of alloxan-induced diabetic rats[21] upon administration of each extract for one week. Thirty six rats were divided into six groups (six animals each). One group was kept as a negative control while diabetes was induced in the other groups, according to method described by Eliasson and Samet[21] using a dose of 150 mg alloxan/kg b. wt. by a single intraperitoneal injection followed by overnight fast. Blood samples were collected from the retro-orbital venous plexus of each rat and the blood glucose level was measured[22] to confirm induction of diabetes using BioMérieux kits. Diabetic rats were divided into 5 groups. The first group was kept untreated. Three groups were given the plant extracts in an oral dose of 100 mg/kg b. wt. for 7 days. The last group received the reference drug (vitamin E, 7.5 mg/kg b. wt.).

At the end of the experiment, blood samples were obtained and blood glutathione level was measured using glutathione kit [Elman's reagent, 5,5-dithio bis-(2-nitrobenzoic acid), to yield a stable yellow color which can be measured colorimetrically at 412 nm]. The intensity of the yellow color developed is related to the amount of glutathione in blood. Results are shown in Table 3.

XII. *Antihyperglycaemic Activity*

Adult rats were induced diabetics as described above[21]. Hyperglycaemia was assessed after 72 hr by measuring the blood glucose level[22]. Animals were divided into five groups, each group of 10 animals. The first group is the diabetic group received saline and served as positive control. Three groups received a daily dose of 100 mg/kg body weight of the total alcoholic extract, petroleum ether and *n*-butanol fractions of the residual aerial parts respectively for 2 months. The fifth group received metformin (150 mg/kg body weight) as a reference drug. Blood glucose levels were determined at one and two months' intervals after overnight fast. Blood samples were collected and serum was isolated by centrifugation and used for colorimetric estimation of glucose using BioMérieux test reagent kit. Results are shown in Table 4.

XIII. *Anticoagulant Activity*

This activity was evaluated for the total alcoholic extract, petroleum ether and *n*-butanol fractions of the residual aerial parts using the whole blood clotting time and prothrombin time[23]. Twenty-four rats were divided into four groups each of six animals. The first group was kept as a control received 1 mL of saline. The other three groups received the tested extracts at a dose of 100 mg/kg body weight orally. After 24 hours the whole blood clotting time and prothrombin time were measured by the capillary method. Blood samples were obtained from the retroorbital venous plexus with 0.11 mole/liter

trisodium citrate capillaries. The whole clotting time was measured. For prothrombin time, thromboplastin with calcium capillaries (BioMérieux) were used. The results are recorded in Table 5.

XIV. *Statistical Analysis*

Statistical analysis was done using the student's *t* test.

RESULTS AND DICUSSION

I. *Chemical Study*

Compounds **1** and **5** (Figure 1) gave positive Liebermann's and Salkoviski's testes indicating their steroidal nature. In addition, compound **5** gave positive reaction with Molisch's test indicating its glycosidic nature. The physical and spectral data of compound **1** were identical with those published for β-sitosterol[24]. The mass spectrum of compound **1** revealed the presence of a molecular ion peak at m/z 414 in addition to the characteristic peaks at m/z 396, 329, 273, 303 and 255. ^{1}H-NMR spectrum showed the presence of C-24 ethyl sterol nucleus by the characteristic 6 methyl groups at δ 0.69-1.26 ppm. Moreover, a signal at δ 3.53 (1H, m) corresponding to H-3 and a signal at δ 5.36 (1H, br. s) ppm corresponding to the olefinic proton at H-6 were also displayed.

The presence of stigmasterol (compound **2**) with β-sitosterol was deduced from both of ^{1}H-NMR spectrum and mass spectrometry[24]. The characteristic two equivalent doublets of doublets at δ 5.05 and 5.18 ppm assigned to H-23 and H-22 of stigmasterol (**2**) were obvious in ^{1}H-NMR spectrum, but the integration of the peaks leads to the conclusion that both compounds **1** and **2** are present as a mixture. The presence of the peaks at m/z 412, 394, 369, 271 and 301 in the mass spectrum of compound **1** supported the previous conclusion. This was also confirmed by the GLC analysis of the mixture.

The percentage of both sterols was determined in the unsaponifiable matter using GLC analysis and was found to be 5.5 & 8.1 % for β-sitosterol and stigmasterol, respectively. Both sterols were reported in the oil of the seeds of four *Sesamum* species[6].

The mass spectrum of compound **5** revealed the presence of a molecular ion peak at m/z at 412 calculated for [M-hexose]. ^{1}H-NMR spectrum showed the characteristic signals of stigmasterol nucleus in addition to an anomeric proton at δ 4.22 ppm (1 H, d, J = 7.5 Hz) which indicated the attachment of the sugar moiety with β-linkage. From ^{13}C-NMR, compound 5 was identified as stigmasterol-3-O-β-D-glucoside[24], which is reported for the first time in genus *Sesamum*.

The obtained physical and spectral data of compounds **3** and **4** were found identical with the reported data for sesamolin[25] and sesamin[26], respectively. Both were previously reported as the main sesame lignans in the seed oil[16]. However, they are reported for the first time in the residual aerial parts.

^{1}H-NMR spectrum of compound **3** displayed two quartets at δ 2.97 and 3.31 ppm (J = 8.5 Hz), each assigned to one methine proton (H-5 and H-1 respectively), two pairs of methylene protons at δ 3.65 (dd, J = 8.5, 9) and 4.45 (t, J = 8.9 Hz) for H-8 and 3.97 (d, J = 9Hz) and 4.14 (dd, J = 8.5, 9 Hz) for H-4 and two benzylic protons at δ 4.41 (d, J = 8.4) and 5.51 (s) assigned to H-6 and H-2 respectively. In addition, two singlet signals at δ 5.93 and 5.96 ppm assigned to the two methylenedioxy protons and two ABX systems at δ 6.51-6.89 assigned to H-6'', H-2'', H-5'' and H-5', H-6', H-2' respectively were also displayed[25].

The mass spectrum of compound **4** displayed M^+ peak at m/z 354. ^{1}H-NMR spectrum of compound **4** showed a multiplet at δ 3.04 ppm integrated as 2 protons assigned to H-1 and H-5, a doublet doublet at δ 3.86 ppm (J = 9, 3.8 Hz) integrated as 2 protons assigned to Hα-4 and Hα-8, a doublet doublet at δ 4.23 ppm (J = 9, 6.6 Hz) integrated as 2 protons assigned to Hβ-4 and Hβ-8, a doublet at δ 4.71 ppm (J = 4.2 Hz) integrated as 2 protons assigned to H-2 and H-6, a singlet at δ 5.94 ppm integrated as 4 protons assigned to 2 oxymethelene units and a multiplet at δ 7.80 ppm integrated as 6 protons assigned to the six aromatic protons 2', 5', 6', 2'', 5'' and 6''[26].

Further confirmation of compounds **3** and **4** was carried out by comparing their retention times in HPLC to those previously reported[27].

^{1}H-NMR spectrum of compound **6** displayed an ABX system at δ 6.78 (1 H, d, J = 8.1), 7.07 (1H, dd, J = 8.1, 1.8) and 7.27 (1H, d, J = 1.8), a pair of *trans*-olefenic protons at δ 6.34 and 7.47 ppm with a large coupling constant (J = 15.9), in addition to a singlet at 3.8 integrated as three protons assigned to a methoxy group. By direct comparison on TLC with authentic, compound **6** (Figure 1) was identified as ferulic acid that was previously reported in leaves and seeds[8,9].

The UV spectral data of **7** showed the characteristic features of a flavonol aglycone, with an orthodihydroxy group in ring B, free OH groups on C-5 and C-3 and absence of free OH at 7-position. ^{1}H-NMR spectrum of **7** showed two doublets at δ 6.41 and 6.55 ppm (J = 2.1) assigned to H-6 and H-8, respectively. An ABX system was displayed at δ 6.82-7.37 ppm assigned to H-5', H-6' and H-2'. In addition to a singlet at δ 3.72 ppm integrated as three protons assigned to the methoxy group on C-7. Therefore, compound **7** (Figure 1) was identified as rhamnetin[28] which was reported for the first time in genus *Sesamum*.

^{1}H-NMR spectrum of compound **8** showed the presence of (*E*)-caffeic acid and 3,4-dihydroxy phenylethanol moieties confirmed by the six aromatic proton signals between δ 6.47-7.02 ppm for two ABX systems, two olefinic protons (AB system, d, J = 15.5 Hz) at δ 6.19 and 7.45, a benzylic methylene at δ 2.67 (2H, t, J = 7.5) and two non-equivalent protons at δ 3.87 and 3.67 (each

1H, m). Additionally two doublets of anomeric protons were observed at δ 4.35 (d, J = 7.8) and 5.02 (d, J = 0.9) and a doublet at 0.95 (3H, d, J = 6.3) indicating its diglycosidic structure with rhamnose and glucose which was confirmed by ^{13}C-NMR of the compound. The significant deshielding of H-4' of the glucose (δ 4.71, t, J = 9.3 Hz) confirmed that the caffeoyl residue was attached to C-4' of glucose. A downfield shift of C-3' of glucose at 79.1 in ^{13}C-NMR indicated that rhamnose is attached to C-3' of glucose. The data of compound **8** were in agreement with the published data for verbascoside (aceteoside) which was isolated previously from the whole plant cultivated in Japan[7].

Compounds **9** and **10** (Figure 1) were identified as kaempferol-3-O-β-D-glucuronide and mequelianin[29] which were reported for the first time in genus *Sesamum*. The UV shifts and ^{1}H-NMR spectra of compound **9** was in agreement with a quercetin skeletal pattern. Additionally, the sugar fraction of the hydrolysis product of **9** was D-glucuronic acid. The identity of D-glucuronic

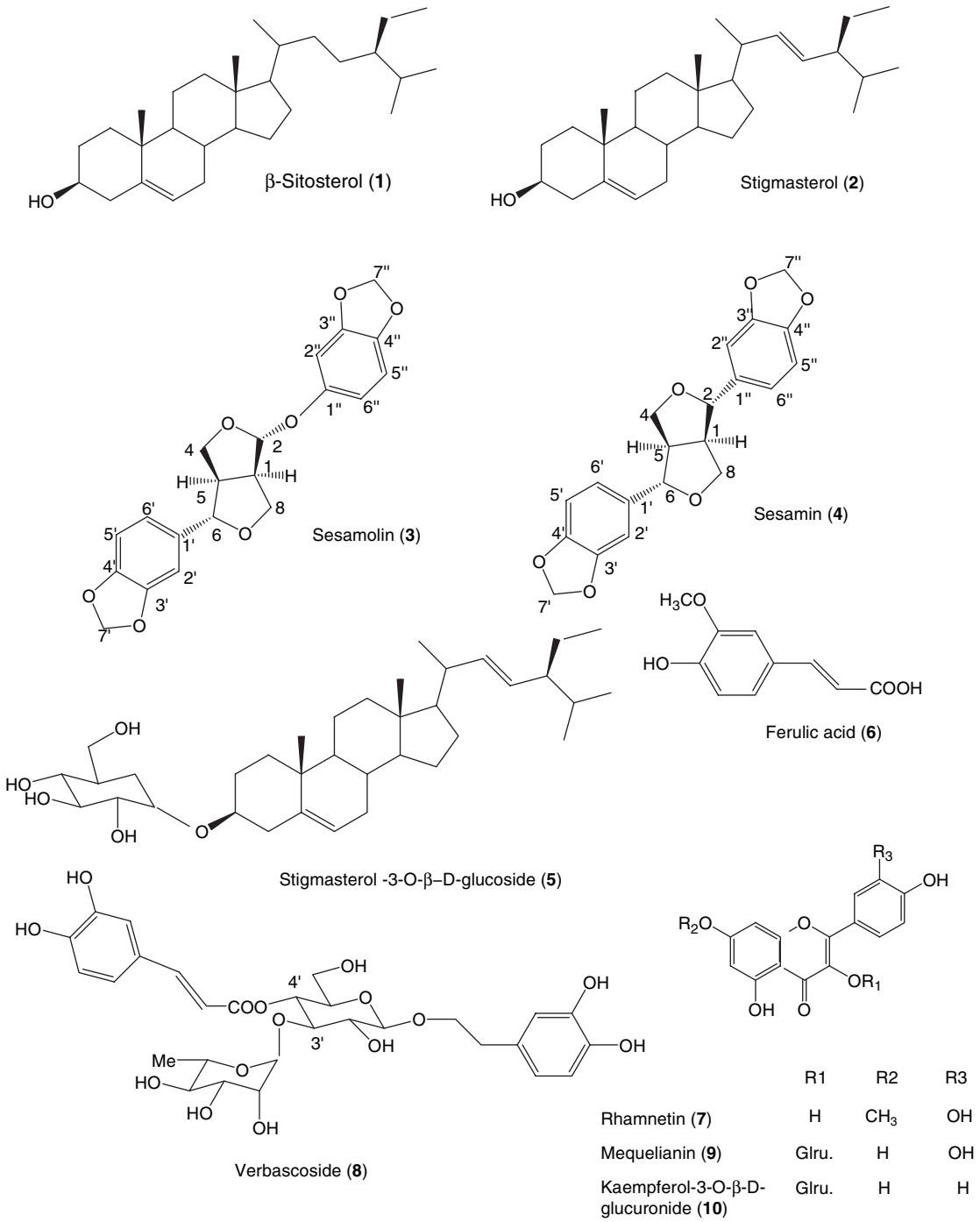

Figure 1. The isolated compounds from the residual aerial parts of *Sesamum indicum* L.

acid was confirmed by ^{13}C-NMR, where C-6'' was found at δ_C 171.93. Therefore, **9** was identified as miquelianin (quercetin-3-O-β-D-glucuronide). ^{1}H-NMR spectrum of compound **10** showed the characteristic signals of kaempferol nucleus in addition to the anomeric sugar proton at δ_H 5.46, J = 7.2 Hz which suggests the presence of β-linked sugar moiety. PC investigation of the sugar fraction of the hydrolysis product of **10** indicated the presence of D-glucuronic acid.

Qualitatively, TLC indicated that the unsaponifiable matter contained 6 major components with different intensities. The separation was comparable to that described previously[10] except the absence of the minor unknown spots reported at R_f 0.16, 0.77 and 0.88. The phytosterols were observed separately as three spots, demethyl sterols (R_f 0.32), monomethylsterols (R_f 0.38) and dimethylsterols (R_f 0.43). Moreover, sesamin (R_f 0.50) and sesamolin (R_f 0.69) were displayed as two major spots enclosing a smaller spot of toccopherol (R_f 0.56).

HPLC analysis of sesamin and sesamolin revealed a good linear response within the range of 2-28 μg for sesamin and 2-13 μg for sesamolin (r = 0.9991 and 0.9983 for sesamin and sesamolin respectively). A good separation of sesamin and sesamolin in the tested extract was obtained as shown in Figure 2. Data of the statistical evaluation of HPLC determination of sesamin and sesamolin are shown in Table 1.

This provides a sensitive, precise and reliable method for the quality control of the residual aerial parts extract. This method is less time-consuming than that reported by Chang *et al*., 2002[27] for the determination of sesamin and sesamolin in sesame seed. Also, it provides a better resolution of both compounds in the studied extract than the method described by Mohamed & Awatif[10]. The unsaponifiable matter contains approximately equal amounts of sesamolin (11.40%) and sesamin (11.32%). Meanwhile the reported values in the oil samples of the seed of the same brown variety were 302 mg and 227 mg/100g oil indicating a higher percentage for sesamin[10].

Table 1. Results of HPLC determination of sesamin and sesamolin in the unsaponifiable matter of the petroleum ether extract of the residual aerial parts of *Sesamum indicum* L.

Item[a]	Sesamin	Sesamolin
X'	99.34	101.38
SD	±2.669	±1.868
RSD	±2.687	±1.843
V	7.121	3.489
SEM	±1.19	±0.311
n	6	6
LOD	0.71	0.22
LOQ	2.37	0.72
RSD% (interday)	3.89	1.32
RSD% (intraday)	1.62	0.34

[a]X': mean percentage recovery; SD: standard deviation; RSD: relative standard deviation; V: variance; SEM: mean standard error; n: number of measurements; LOD: low limit of detection; LOQ: low limit of quantification; RSD%: relative standard deviation percentage.

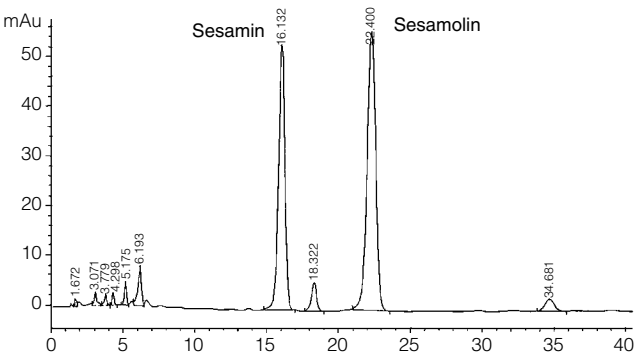

Figure 2. HPLC chromatogram of unsaponifiable matter of the pet. ether extract of the residual aerial parts of *Sesamum indicum* L. with ODS (RP-18) column.

Table 2. The yield of the isolated compounds from the different fractions of *Sesamum indicum* L.

Isolated compounds	Amount of isolated extract (mg/g)	
	Petroleum ether extract	*n*- Butanol extract
Sitosterol mixture	3.4	–
Sesamolin	2	–
Sesamin	13.3	–
Stigmasterol -3-O-β-D-glucoside	1.3	–
Ferulic acid	–	0.29
Rhamnetin	–	0.43
Verbascoside	–	4.3
Miquelianin	–	0.34
Kaempferol-3-O-β-D-glucuronide	–	0.29

II. *Pharmacological Study*

LD_{50} of the total alcoholic extract of the residual aerial parts and both of the petroleum ether and *n*-butanol fractions were found to be 8.9, 8.4 and 8.1 g/kg b. wt., respectively.

Free radical formation and oxidative stress may act as a common pathway to diabetes itself and to its later complications. Hyperglycaemia is accompanied with decrease in glutathione level. However, the reduced levels of glutathione in the diabetic rats were significantly restored by the alcoholic, pet. ether and butanol extracts (compared with the diabetic control group). The total alcoholic extract is the most potent; it showed a

comparable activity to that of vitamin E (Table 3) at the tested dose level. The stronger activity of the total alcoholic extract over its fractions might be attributed to a synergistic effect of the lignans present mainly in the pet. ether fraction, and the phenolic compounds present in the butanol fraction. Sesame lignans[15], rhamnetin[30], ferulic acid[31], verbascoside[32] and mequelianin[33] were reported to possess antioxidant activity.

The tested extracts showed a reductive effect on the blood glucose level of diabetic rats (Table 4). The total alcoholic extract showed a more powerful effect than its fractions. The potency of the total alcoholic extract is 77.4% of metformin after 8 weeks of administration at the tested dose level. Blood sugar reduction may be due to possible inhibition of free radicals and subsequent inhibition of tissue damage induced by alloxan[34]. β-Sitosterol[35] and ferulic acid[31] were reported to possess hypoglycaemic activity. Therefore, they may be responsible at least partly for the hypoglycaemic activity of the alcoholic extract.

Taken together, these findings suggest that the alcoholic extract may be useful in alleviating oxidative stress and attenuating the hyperglycemic response associated with diabetes.

Also, the total alcoholic extract as well as both of its petroleum ether and *n*-butanol fractions (dose of 100 mg/kg body weight) possesses a significant anticoagulant effect (Table 5). The three extracts increased the time required for coagulation of rat blood in comparison with the control group. It is noteworthy to mention that, this is the first report on the anticoagulant activity of the plant under investigation. The pronounced activity of each extract leads us to spot light on a new anticoagulant effect of this plant.

The total alcoholic extract seems to be more effective in all the tested biological properties than its corresponding fractions which may be due to a synergistic effect of its constituents. The total alcoholic extract is of high safety margin as shown by comparing the effective dose with its LD_{50}.

Finally, it is confirmed in this study that the residual aerial parts of *Sesamum indicum* L., which were considered as a waste product, constitutes about 65% of the Egyptian sesame crop, are of medicinal importance. The alcoholic extract of the residual aerial parts is more effective than its petroleum ether and *n*-butanol fractions in the tested biological activities. The more powerful effect of the alcoholic extract over its fractions may add another advantage due to the low costs of processing it. This leads us to suggest the use of the alcoholic extract rather than its fractions as an alternative natural antioxidant, antihyperglycaemic and anticoagulant. The extract could be standardized through a sensitive, precise and reliable HPLC method based on the determination of

Table 3. Effect of the residual aerial parts of *Sesamum indicum* L. extracts on blood glutathione level

Group	Blood glutathione (mg %)	Change from control (%)
Normal	36.4 ± 9	–
Diabetic	23.1 ± 0.4[a]	36.5
Vitamin E	36.1 ± 0.7[b]	0.8
Total alcoholic extract	35.6 ± 0.8[b]	2.19
Petroleum ether fraction	31.7 ± 0.5[b]	12.9
n-Butanol fraction	33.2 ± 0.6[b]	8.7

The extracts were given in a dose of 100 mg/kg body weight. Vitamin E (7.5 mg/kg body weight) was used as a standard antioxidant drug. Male albino rats (n = 6) were used.
[a]Statistically significant different from control at $p < 0.01$.
[b]Statistically significant different from diabetic at $p < 0.01$.

Table 5. The effect of the residual aerial parts of *Sesamum indicum* L. extracts on clotting and prothrombin times

Group	Clotting and prothrombin time after 24-hr administration	
	Clotting time (sec)	Prothrombin time (sec)
Control	42.8 ± 1.7	14.8 ± 0.3
Total alcohol extract	85.4 ± 2.1*	27.3 ± 0.7*
Petroleum ether fraction	79.1 ± 0.6*	23.9 ± 0.4*
n-Butanol fraction	74.3 ± 0.4*	24.6 ± 0.8*

The extracts were given in a dose of 100 mg/kg body weight. Male albino rats (n = 6) were used.
*Statistically significant from control group at $p < 0.01$.

Table 4. The effect of the residual aerial parts of *Sesamum indicum* L. extracts on blood glucose level

Time	Fasting blood glucose level (mg/dl)								
	Diabetic	Alc. extract		Pet. ether fraction		*n*-Butanol fraction		Metformin	
	M ± SE	M ± SE	Change (%)	M ± SE	Change (%)	M ± SE	Change (%)	M ± SE	Change (%)
Zero	247.9 ± 8.2	251.2 ± 12.6	–	241.2 ± 10.5	–	254.7 ± 8.1	–	249.6 ± 9.8	–
4 weeks	256.4 ± 11.3	179.4* ± 6.2	28.6	193.1* ± 8.2	19.9	184.3* ± 7.2	27.6	132.4* ± 5.1	47
8 weeks	261.5 ± 12.8	124.6* ± 4.3	50.4	156.3* ± 7.4	35.2	143.4* ± 6.1	43.7	87.1* ± 3.2	65.1

Extracts were administered orally (100 mg/kg body weight) for 8 weeks. Metformin (150 mg/kg body weight) was used as a standard antihyperglycaemic agent. Male albino rats (n = 10) were used. The change (%) was compared with zero time.
*Statistically significant from zero time at $p < 0.01$.

sesamin and sesamolin. This study may participate, at least partly, in the solution of the problem of eliminating wastes and decreasing the pollution which may be produced during this process.

REFERENCES

1. Beroza, M. and Kinman, M. L. 1955. Sesamin, sesamolin, and sesamol content of the oil of sesame seed as affected by strain, location grown, ageing, and frost damage. J. Am. Oil Chem. Soc. 32: 348-350.
2. Osawa, T., Nagata, M., Namiki, M. and Fukuda, Y. 1985. Sesamolinol, a novel antioxidant isolated from sesame seeds. Agric. Biol. Chem. 49: 3351-3352.
3. Katsuzaki, H., Kawasumi, M., Kawakishi, S. and Osawa, T. 1992. Structure of novel antioxidative lignan glucosides isolated from sesame seed. Biosci. Biotech. Biochem. 56: 2087-2088.
4. Katsuzaki, H., Kawakishi, S. and Osawa, T. 1993. Structure of novel antioxidative lignan triglucoside isolated from sesame seed. Heterocycles 36: 933-936.
5. Katsuzaki, H., Kawakishi, S. and Osawa, T. 1994. Sesaminol glucosides in sesame seed. Phytochemistry 35: 773-776.
6. Kamal-Eldin, A., Appelqvist, L. A., Yousif, G. and Iskander, G. M. 1992. Seed lipids of *Sesamum indicum* and related wild species in Sudan. Part 3. The sterols. J. Sci. Food Agric. 59: 327-334.
7. Suzuki, N., Miyase, T. and Ueno, A. 1993. Phenylethanoid glycosides of *Sesamum indicum*. Phytochemistry 34: 729-732.
8. Duke, J. A. 1992. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, Fl. CRC Press. http://www.ars-grin.gov/cgi-bin/duke/farmacy2.pl
9. Kato, M. J., Chu, A., Davin, L. B. and Lewis, N. G. 1998. Biosynthesis of antioxidant lignans in *Sesamum indicum* seeds. Phytochemistry 47: 583-591.
10. Mohamed, H. M. A. and Awatif, I. I. 1998. The use of sesame oil unsaponifiable matter as a natural antioxidant. Food Chem. 67: 269-276.
11. Ragab, A. I. 1989. Oil content and fatty acids, evaluation and correlation studies for some sesame (*Sesamum indicum* L.) mutants. International Conference for Statistics, Computer Science, Social and Demographic Research. 14th. March 25-30. v7. pp. 117-128. Cairo, Egypt.
12. Shahin, A., Authman, M. A. and Hashem, H. 1991. Production of Halva tahinia from sunflower (*Helianthus annuus*) seeds; I- Chemical evaluation of sunflower and sesame seeds. Egypt. J. Food Sci. 19: 179-187.
13. Rice-Evans, C. and Diplock, A. T. 1993. Current status of antioxidant therapy. Free Radic. Biol. Med. 15: 77-96.
14. Shahidi, F., Wanasudara, P. K. P. D. and Hong, C. 1992. Antioxidant activity of phenolic compounds in meat model systems. In "Phenolic Compounds in Food and Their Effects on Health". ACS symposium series. Vol. I. p. 507. Ho, C. T., Lee, C. Y. and Haung, M. T. eds. American Chemical Society. Washington, DC, U. S. A.
15. Kang, M., Naito, M. Tsujihara, N. and Osawa, T. 1998. Sesamolin inhibits lipid peroxidation in rat liver and kidney. J. Nutr. 128: 1018-1022.
16. Shyu, Y. and Hwang, L. S. 2002. Antioxidative activity of the crude extract of lignan glycosides from unroasted Burma black sesame meal. Food Res. Int. 35: 357-365.
17. Takeuchi, H., Mooi, L. Y., Inagaki, Y. and He, P. 2001. Hypoglycemic effect of a hot-water extract from defatted sesame (*Sesamum indicum* L.) seed on the blood glucose level in genetically diabetic KK-Ay mice. Biosci. Biotechnol. Biochem. 65: 2318-2321.
18. Miller, J. C. and Miller, J. N. 1993. Statistics for Analytical Chemistry. 3rd ed. P. 128. Ellis Horwood. London, U. K.
19. Karber, G. 1931. Determination of LD_{50}. Pathol. Pharmacol. 162: 480-496.
20. Beutler, E., Duron, O. and Kelly, B. 1963. Improved method for the determination of blood glutathione J. Lab. Clin. Med. 61: 882-888.
21. Eliasson, S. G. and Samet, T. M. 1969. Alloxan induced neuropathies. Lipid changes in nerve and root fragments. Life Sci. 8: 493-498.
22. Trinder, P. 1969. Estimation of serum glucose and triglycerides by enzymatic method. Am. Clin. Biochem. 6: 24-32.
23. Kaneka, J. and Carnetius, C. E. 1971. Clinical Biochemistry of Domestic Animals. Vol. 2. 2nd ed. p. 194. Saunders Company. W. P. Philadelphia.
24. Soliman, F. M., Shehata, A. H., Khaleel, A. E. and Ezzat, S. M. 2004. Lipids and coumarins of *Foeniculum vulgre* Mill. and *F. dulce* DC. Egypt. J. Biomed. Sci. 14: 148-166.
25. Kang, S. S., Kim, J. S., Jung, J. H. and Kim, Y. H. 1995. NMR assignments of two furofuran lignans from sesame seeds. Arch. Pharm. Res. 18: 361-363.
26. Iida, T., Nakano, M. and Ito, K. 1982. Hydroperoxysesquiterpene and lignan constituents of *Magnolia kobus*. Phytochemistry 21: 673-675.
27. Chang, L., Yen, W. Huang, S. C. and Duh, P. 2002. Antioxidant activity of sesame coat. Food Chem. 78: 347-354.
28. Mabry, T. J., Markham, K. R. and Thomas M. B. 1970. The systemic identification of flavonoids. Springer-Verlag, New York. Heidelberg, Berlin.
29. Khaleel, A. E. M. 2005. Phenolics and lipids of *Carum Carvi* L. and *Coriandrum sativum* L. flowers. Egypt. J. Biomed. Sci. 18: 35-47.
30. Torres, R., Faini, F., Modak, B., Urbina, F., Labbe, C. and Guerrero, J. 2006. Antioxidant activity of coumarins and flavonols from the resinous exudate of *Haplopappus multifolius*. Phytochemistry 67: 9874-9877.
31. Ohnishi, M., Matuo, T., Tsuno, T., Hosoda, A., Nomura, E., Taniguchi, H., Sasaki, H. and Morishita, H. 2004.

Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-Ay mice. Biofactors 21: 315-319.

32. Harput, U. S., Saracoglu, I., Inoue, M. and Ogihara, Y. 2002. Phenylethanoid and irridoid glycosides from *Veronica persica*. Chem. Pharm. Bull. 50: 869-871.
33. Yang, H., Protiva, P., Cui, B., Ma, C., Baggett, S., Hequet, V., Mori, S., Weinstein, I. B. and Kennelly, E. J. 2003. New bioactive polyphenols from *Theobroma grandiflorum* ("cupuacu") J. Nat. Prod. 66: 1501-1504.
34. Dhanabal, S. P., Sureshkumar, M., Ramanathan, M. and Suresh, B. 2005. Hypoglycemic effect of ethanolic extract of *Musa sapientum* on alloxan induced diabetes mellitus in rats and its relation with antioxidant potential. J. Herb Pharmacother. 5: 7-19.
35. Ivorra, M. D., Paya, M. and Villar, A. 1989. A review of natural products and plants as potential antidiabetic drugs. J. Ethnopharmacol. 27: 243-275.