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Phytochemical and Biological Study of Some Constituents of *Asparagus sprengeri* Regel., Growing in Egypt.

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ABSTRACT

Background: *Asparagus sprengeri* Regel. is an ornamental small shrub native to coastal southeastern South Africa. The main objective of this study was the phytochemical and biological evaluation of ethanolic extract of non-flowering aerial parts of *Asparagus sprengeri* Regel., growing in Egypt. **Methods:** The investigation included analysis of the lipoidal compounds in the unsaponifiable and fatty acids fractions by GC/MS. Quantitative estimation of polyphenols, flavonoids and saponins by colourimetric methods. HPLC analysis of polyphenolic compounds was undergone, in addition to LC/MS of the saponins. Isolation of flavonoids was undergone using chromatographic methods and the identification was accomplished on the basis of their spectral data. **Results:** The unsaponifiable fraction was composed mainly of hydrocarbons (52.99%) and alcohols (31.54%). Moreover, the fatty acids fraction was composed of saturated (52.12%) and unsaturated (21.61%) ones. Determination of total polyphenols (9.83%) and flavonoids (1.87%) was undergone. HPLC analysis of polyphenols revealed that flavonoids were detected in higher amounts than phenolic acids. Extraction, isolation and identification of three flavonoids (kaempferol, quercetin and genistin) was achieved. Saponins (2.44%) were determined, while LC/MS of the *n*-butanol fraction indicated the presence of sprengerinin A,B,C&D. The ethanolic (70%) extract exhibited moderate antioxidant (IC_{50} = 56 µg ml^{-1}) and potent cytotoxic activities. The most potent activity was that on colon cell line (IC_{50} = 3.73 µg ml^{-1}), followed by the breast cell line (IC_{50} = 10.20 µg ml^{-1}). **Conclusion:** Thus, non-flowering aerial parts of *Asparagus sprengeri* Regel. could be considered as a potential source for different bioactive metabolites.

KEYWORDS: *Asparagus sprengeri* Regel.; lipoidal matter; polyphenols; saponins; antioxidant; cytotoxic

1. INTRODUCTION

Plants of genus *Asparagus* (family Asparagaceae) are rich sources for steroidal saponins[1] that have various health-promoting properties[2]. A large number of *Asparagus* species have long been used in folk medicine as *Asparagus adscendens* Roxb. and *Asparagus racemosus* Willd. The roots of *Asparagus adscendens* Roxb. are used as galactogogue, antidiarrhoeal and in treatment of dysentery[3]. Meanwhile, *Asparagus racemosus* Willd. is used as galactogogue, anti-inflammatory, antimicrobial and immunomodulator[4]. However, *Asparagus sprengeri* Regel. was known as an ornamental small plant, native to coastal southeastern South Africa. To the best of our knowledge, few reports have been traced on *Asparagus sprengeri* Regel. phytoconstituents. Few saponins have been isolated *viz.* diosgenin and yamogenin from the roots and aerial parts[5], whereas sprengerinin A, B, C and D were identified in the roots [6]. Phytochemical investigation of *Asparagus sprengeri* grown in Egypt, led to identification of 14 hydrocarbons in the unsaponifiable fraction in which n-eicosane and n-hexacosane constituted to be major components, in addition to cholesterol. Nine fatty acids were detected, where myristic and linoleic acids were the main constituents. Investigation of the flavonoidal fraction resulted in isolation and identification of apigenin, dihydroquercitin, naringenin and apigenin-7-O-glucoside[7]. So, it was interesting for the authors to carry out this work to further investigate its phytochemical constituents and biological activities.

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2. MATERIALS AND METHODS

2.1. Plant material

The non-flowering aerial parts of *Asparagus sprengeri* Regel. (Figure 1) were collected from plants growing along Elkanater Elkhairya from June to Septemeber (2008-2010). The plant was authenticated by Mrs. Therese Labib, botanical consultant of the

Orman botanical garden, Giza, Egypt and verified by Dr. Reem Sameer, Ass. Prof., Botany department, Faculty of Science, Cairo University. A voucher specimen (Asp/AS/11-9/13) was kept in the herbarium of the department of medicinal plants and natural products (NODCAR).

Figure 1. The non-flowering aerial parts of *Asparagus sprengeri* Regel.

2.2. Preparation of plant extracts for phytochemical and biological evaluations:

One kilogram of the air-dried powdered non-flowering aerial parts were exhaustively extracted with *n*-hexane using Soxhlet, then refluxed with 70% ethanolic solution (3 hrs x 3 times). The extracts were concentrated under reduced pressure. The obtained residues of the *n*-hexane (33g) and 70% ethanolic (68g) extracts were separately kept at -4 °C in amber light tightly sealed glass containers for investigation.

A stock solution (10 mg ml^{-1}) of the ethanolic extract was prepared, using absolute ethanol, to be used in the determination of total polyphenols, flavonoids, HPLC analysis of polyphenolic compounds and *in vitro* antioxidant activity.

Different fractions were afforded from the ethanolic extract by subjecting it to successive extraction with dichloromethane, ethyl acetate and *n*-butanol. The fractions were evaporated under reduced pressure to dryness. The obtained residues were kept in sealed vials at low temperature to be subjected to phytochemical screening[8] and chromatographic techniques to identify and/or isolate the major constituents.

2.3. Preparation of unsaponifiable and fatty acid methyl esters fractions

One gram of the *n*-hexane extract was refluxed with 50 ml of N/2 ethanolic potassium hydroxide. The ethanol was distilled off and the aqueous layer was diluted to twice its volume with water, then exhaustively extracted with diethylether. The ethereal extract was washed with distilled water, dehydrated over anhydrous sodium sulphate and then the diethylether was distilled off. The residue obtained was the unsaponifiable fraction[9-10].

The remaining aqueous solution was acidified with sulphuric acid (10%) and the liberated fatty acids were extracted several times with diethylether. The ethereal extract was washed with distilled water, dehydrated over anhydrous sodium sulphate and diethylether was distilled off.

The fatty acids were converted to methyl esters by refluxing with 50 ml of absolute methanol and 1.5 ml conc. sulphuric acid. The methanol was distilled off and the residue was solubilized with distilled water then extracted with diethylether. The ethereal extract was washed with distilled water, dehydrated over anhydrous sodium sulphate and concentrated to dryness. The afforded residue was fatty acids methyl esters fraction[9-10].

2.4. Phytochemical evaluation of the extracts:

2.4.1. GC/MS of unsaponifiable and fatty acid methyl esters fractions

GC/MS analyses of the unsaponifiable and fatty acid methyl esters fractions were performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 mass spectrometric detector. The column used was an HP-5MS capillary column (30m× 320µm×0.25µm film thickness). Helium was the carrier gas with a flow rate of 1 ml min^{-1}. A volume of 1µl was injected in a splitless mode. The total run time was 40 minutes. In case of unsaponifiable fraction temperature programming was as follows: the initial oven temperature was maintained at 60°C for 3 min and then raised to 300°C at a rate of 8°C min^{-1} and injector temperature was 230 °C. In case of fatty acid methyl esters fraction, the initial oven temperature was maintained at 100°C for 3 min and then raised to 260°C at a rate of 8°C $min^{-1}$and injector temperature was 150 °C.

ESI/MS spectrum was recorded at 70 eV and mass range was 50 to 500 m/z. Quantitization was based on the peak area integration. Individual peaks were identified by comparing their Kovat´s indices (KI) and mass spectra with Wiley library and NIST databases, in addition to those in the available literature[11].

2.4.2. Quantitative determination of total polyphenols:

Total polyphenolic content was determined by using Folin-Ciocalteu reagent method[12]. To 1 ml of stock solution, 2ml of Folin-Ciocalteu reagent were added followed by 2 ml of sodium carbonate (20%) and the volume was made up to 10 ml with distilled water. The contents

were mixed and allowed to stand for 30 minutes. Absorbance of the solutions was measured at 765 nm using a UV spectrophotometer (Unicam, UK). The total polyphenolic content was expressed as gallic acid, using a standard calibration curve (10-35 µg ml^{-1}). The experiment was repeated in triplicate and the readings were mean values.

2.4.3. Quantitative determination of total flavonoids:

Total flavonoid content was determined according to Atanassova et al. (2011)[13] with slight modifications. To 1 ml of the stock solution, 4 ml of distilled water and 0.3 ml of 5% $NaNO_2$ solution were added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ solution was added. After further 2 minutes, 2 ml of 1N NaOH were added then the volume was completed to 10 ml with distilled water and mixed. The absorbances of the solutions were measured at 510 nm against blank (7.4 ml distilled water + 0.3 ml 5% $NaNO_2$ + 0.3 ml 10% $AlCl_3$ + 2 ml 1N NaOH) using a spectrophotometer. The total was flavonoid content was expressed as quercetin, using a standard calibration curve (5-75 µg ml^{-1}). The experiment repeated in triplicate and the readings were mean values.

2.4.4. HPLC analysis of polyphenolic compounds

The ethanolic (70%) extract of non-flowering aerial parts was subjected to HPLC analysis[14]. A volume of 10 ml of the stock solution was evaporated to dryness under vacuum. The residue was dissolved in methanol (5 ml) and the volume was adjusted to 10 ml with methanol. One ml of the methanolic extract was filtered through a 0.4µm membrane filter into the sampler vial for injection in HPLC (Agilent 1200 series), equipped with column compartment, quaternary pump, degasser, autosampler and UV detector. Analysis was performed using a Hypersil-BDS-C_{18} (4.6 x 250 mm) and separations were done in isocratic mode, using methanol: water: phosphoric acid (100:100: 2 v/v/v) at a flow rate of 1 ml min^{-1}, with an injection volume of 20 µl and UV detection at 270 nm. Quantitative determination was done against a number of flavonoids and phenolic acids in order to identify the compounds present in the plant. Each determination was carried out in triplicate.

2.4.5. Extraction and isolation from ethyl acetate fraction

The phytochemical screening and TLC investigation of the different fractions of the non-flowering aerial parts revealed the presence of polyphenolic compounds in the ethyl acetate fraction. The fraction (15g) was chromatographed on a polyamide column (450 g). Elution started with distilled water, followed by gradient addition of methanol until reaching 100% methanol. Fractions of 150 ml were collected and concentrated under reduced pressure, monitored by paper chromatography using the mobile phase S_1: *n*-butanol : glacial acetic acid : water (upper phase); 4:1:5 (v/v/v) as solvent system and fractions exhibiting the same chromatographic pattern were pooled together. Fractions (32-33) were further subjected to sephadex LH-20 column chromatography using methanol to yield two compounds, which were then purified by preparative TLC (dichloromethane: methanol, 8: 2) to afford compound **1** (10mg) and **2** (15 mg). Meanwhile, fractions (113-122) were subjected to paper chromatography using S_1 solvent system to afford compound **3** (10mg) which was further purified by recrystallization using methanol. Structural elucidation of the isolated compounds was performed via co-chromatography, UV spectroscopy and confirmed by their spectral data (ESI/MS and ^{1}H NMR).

2.4.6. Quantitative determination of total saponins

Determination of total saponins was performed as stated by Chen et al. (1996)[15] adopting few modifications. Pulverized sample (5g) was hydrolysed using conc. hydrochloric then neutralized with saturated lime water, filtered and the filtrate was evaporated to dryness under reduced pressure then extracted with petroleum ether (40-60 °C). The organic extract was diluted with petroleum ether to 250 ml, then 1 ml was heated with 0.2 ml of vanillin (5%) in acetic acid and 0.8ml of perchloric acid at 75 °C for 15 min before cooling in an ice-bath. Acetic acid was then added to a volume of 10 ml and after 30 minutes, the absorbance was measured at 530 nm. Total saponin content was determined as diosgenin using its standard calibration curve (0.05-0.2 µg ml^{-1}).

2.4.7. LC/MS of n-butanol fraction

According to the phytochemical tests of the different fractions, *n*-butanol fraction proved to be rich in saponins and thus it was subjected to identification of these compounds by using LC/MS. HPLC was achieved using an Accela 1200 LC equiped with Hypersilgold (Phenomenex, 50×2 mm, 2.1 µm) preceded by a C_{18} security guard cartridge Gemini C_{18} (Phenomenex, 4 x 3 mm, 5 µm) and a photodiode array detector; scanning from 200 to 400nm and heated electrospray ionization (HESI); ion spray voltage was 300 V. All analyses were performed at 30 °C with a flow rate of 250 µl min^{-1}. The sample volume injected was 20 µl. The mobile phase consisted of solution A (Acetonitrile) and solution B (0.1% formic acid), in a ratio 90% solution A: 10% solution B (isocratic elution). The heated capillary was maintained at 370 °C. The samples were individually tuned for each target analyte by direct injection of the individual solution (1 mg ml^{-1}) then the compounds were ionized for mass spectrometry detection by positive ion electrospray ionization in the range *m/z* 100 - 1000**.** Firstly, a full-scan mass spectrum to acquire data on ions in the *m/z* range, then an MS/MS experiment was performed in which data-dependent scanning was carried out on protonated molecules of the compounds.

2.5. In vitro biological evaluation of the extracts

2.5.1. Antioxidant activity by DPPH

The *in vitro* antioxidant activity was studied by the decolourization

of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) methanolic solution[16]. A volume of 10 ml of the ethanolic (70%) stock solution was evaporated to dryness under vacuum and the residue was redissolved in 10 ml of methanol. Three aliquots (0.1, 0.2, 0.3 ml) were separately used, then 5 ml of methanolic DPPH (20 mg ml^{-1}) were added to each and the volume was separately adjusted to 10 ml with methanol. The mixture was shaken, left to stand for 30 min and the reduction in colour was measured at 517 nm using a spectrophotometer. A control solution containing all reagents except the tested sample was treated similarly. The inhibition percentage (I%) was calculated using the following equation:

1(%)= 1- (Abs sample /Abs control) x 100

where: Abs sample = absorbance of tested extracts, Abs control = absorbance of control solution. Finally, the inhibition percentages were plotted versus respective concentrations (µg ml^{-1}) and IC_{50} (concentration needed to cause 50% inhibition) was calculated from the graph.

2.5.2. Cytotoxic activity

The potential cytotoxicity of the ethanolic (70%) extract (5-50 µg ml^{-1}), was tested using the method of SKehan et al. (1990)[17] on three cell lines: liver (HepG2), breast (MCF7) and colon (HCT116) cancer. The study was done at the National Cancer Institute, Cairo University. The cells were plated in 96-multiwell plate (10^4 cells $well^{-1}$) and after 24 hrs, inoculation of the different concentrations of the extract was performed. Incubation of the monolayer cells with the extract was done for 48 hrs at 37 °C and in an atmosphere of 5% CO_2. The cells were then fixed, washed and stained with sulforhodamine B stain (Sigma Aldrich). Excess stain was washed with acetic acid, tris EDTA buffer was added to each well and the optical density was measured in an ELISA reader. Percentage of viable cells was calculated as follows:

Percentage of viable cells = (Optical density of treated cells/ Optical Density of control cells) x 100

The relation between the surviving fractions and sample concentrations were plotted to get the survival curves and IC_{50} (the 50% cytotoxic concentration) for each extract was recorded.

3. RESULTS AND DISCUSSION

3.1. GC/MS of unsaponifiable and fatty acid methyl esters fractions

From GC/MS analysis of the unsaponifiable fraction of the non-flowering aerial parts (Table 1), 37 components were identified representing (89.28%) of the *n*-hexane extract yield.

Table 1. GC/MS analysis of the unsaponifiable fraction of *Asparagus sprengeri* non-flowering aerial parts

Peak no.	KI	Name of identified compound	Mol. formula	Mol. ion peak (M^+)	Yield (%)
1	1100	Undecane	$C_{11}H_{24}$	156	3.54
2	1146	2-Methyl decahydronaphthalene	$C_{11}H_{20}$	152	0.03
3	1149	1,3-Dimethylbutylcyclohexane	$C_{12}H_{24}$	168	0.1
4	1156	1,3-Pentanediol,2,2,4trimethyl	$C_8H_{18}O_2$	146	1.14
5	1200	Dodecane	$C_{12}H_{26}$	170	28.22
6	1214	Undecane,2,6-dimethyl	$C_{13}H_{28}$	184	9.68
7	1299	Naphthalene,1-methyl	$C_{11}H_{10}$	142	0.41
8	1370	1-Undecanol	$C_{11}H_{24}O$	172	0.08
9	1400	Tetradecane	$C_{14}H_{30}$	198	0.1
10	1406	2-Tetradecene	$C_{14}H_{28}$	196	1.87
11	1451	(5E)-6,10-Dimethyl-5,9-undecanedien-2one	$C_{13}H_{22}O$	194	0.35
12	1466	2,6-Di-tert-butylbenzoquinone	$C_{14}H_{20}O_2$	220	0.1
13	1470	Acenaphthylene	$C_{12}H_8$	152	0.43
14	1517	P-Cresol,2,6-di-tert-butyl	$C_{15}H_{24}O$	220	0.08
15	1521	Phenol,2,4-bis(1,1dimethylethyl)	$C_{14}H_{22}O$	206	2.01
16	1538	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro,4,4,7a-trimethyl	$C_{11}H_{16}O_2$	180	0.36
17	1593	1-Hexadecene	$C_{16}H_{32}$	224	2.73
18	1600	Hexadecane	$C_{16}H_{34}$	226	0.08
19	1700	Heptadecane	$C_{17}H_{36}$	240	0.12
20	1802	Bakkenolide A(+)	$C_{15}H_{22}O_2$	234	0.17
21	1805	1-Octadecene	$C_{18}H_{36}$	252	2.45
22	1840	Neophytadiene	$C_{20}H_{38}$	278	0.77
23	1848	2-Pentadecanone,6,10,4-trimethyl	$C_{18}H_{36}O$	268	1.55
24	1900	Nonadecane	$C_{19}H_{40}$	268	0.07
25	1949	Isophytol	$C_{20}H_{40}O$	296	0.27
26	2007	3-Eicosene	$C_{20}H_{40}$	280	0.1
27	2100	Henicosane	$C_{21}H_{44}$	296	0.42
28	2122	Phytol	$C_{20}H_{40}O$	296	29.41
29	2286	Palmitaldehyde,diallylacetal	$C_{22}H_{42}O_2$	338	0.13
30	2300	Tricosane	$C_{23}H_{48}$	324	0.32
31	2351	1-Henicosanol	$C_{21}H_{44}O$	312	0.64
32	2500	Pentacosane	$C_{25}H_{52}$	352	0.32
33	2600	Hexacosane	$C_{26}H_{54}$	366	0.23
34	2700	Heptacosane	$C_{27}H_{56}$	380	0.19
35	2800	Octacosane	$C_{28}H_{58}$	394	0.21
36	2900	Nonacosane	$C_{29}H_{60}$	408	0.39
37	3000	Triacontane	$C_{30}H_{62}$	422	0.21
		Hydrocarbons			52.99
		Alcohols			31.54
		Ketones			2.26
		Aldehydes			0.13
		Esters			0.17
		Phenols			2.09
		Quinone			0.1
		Total identified compounds			89.28

Major components were phytol (29.41%), dodecane (28.22%) and undecane, 2, 6-dimethyl (9.68%). The identified compounds were classified as hydrocarbons (52.99%), alcohols (31.54%), ketones (2.26%), phenols (2.09%), esters (0.17%), aldehydes (0.13%) and quinones (0.10%). Thus, *Asparagus sprengeri* Regel. could be considered a potential source of phytol that has proved to exhibit central and peripheral antinociceptive and *in vitro* antioxidant activity[18], in addition to its effect as antimicrobial against

Staphylococcus aureus [19], inhibition of the teratogenic activity of retinol [20] and in the treatment of rheumatic arthritis [21].

On the other hand, GC/MS analysis of fatty acid methyl esters fraction Table (2) revealed the presence of 27 identified fatty acids (73.73%), in addition the saturated fatty acids (52.12%) represented a greater amount than the unsaturated fatty acids (21.61%).

Table 2. GC/MS analysis of the fatty acid methyl esters of *Asparagus sprengeri* non-flowering aerial parts

Peak no.	KI	Name of identified compound	Mol. formula	Mol. ion peak (M+)	Yield (%)
1	1309	Capric acid	$C_{11}H_{22}O_2$	186	0.37
2	1509	Lauric acid	$C_{13}H_{26}O_2$	214	1.55
3	1511	Azelaic acid)dimethyl ester(	$C_{11}H_{20}O_4$	216	0.78
4	1680	Myristic acid	$C_{15}H_{30}O_2$	242	2.43
5	1714	Undecanedioic) acid dimethyl ester(	$C_{13}H_{24}O_4$	244	0.17
6	1807	Pentadecanoic acid	$C_{16}H_{32}O_2$	256	0.91
7	1854	Ferulic acid	$C_{11}H_{12}O_4$	208	0.28
8	1885	Palmitoleic acid	$C_{17}H_{32}O_2$	268	0.5
9	1926	Palmitic acid	$C_{17}H_{34}O_2$	270	15.8
10	1941	Cyclo propaneoctanoic acid,2-hexyl	$C_{18}H_{34}O_2$	282	0.18
11	2021	Margaric acid	$C_{18}H_{36}O_2$	284	1.59
12	2092	Linoleic acid	$C_{19}H_{34}O_2$	294	17.8
13	2138	Stearic acid	$C_{19}H_{38}O_2$	298	2.59
14	2101	6,9,12-Octadecatrienoic acid	$C_{19}H_{32}O_2$	292	0.36
15	2105	Linolenic acid	$C_{19}H_{32}O_2$	292	1.74
16	2221	Nonadecanoic acid	$C_{20}H_{40}O_2$	312	0.54
17	2302	11-Eicosenoic acid	$C_{21}H_{40}O_2$	324	0.49
18	2335	Arachidic acid	$C_{21}H_{42}O_2$	326	2.82
19	2430	Heneicosanoic acid	$C_{22}H_{44}O_2$	340	0.77
20	2483	Erucic acid	$C_{23}H_{44}O_2$	352	0.44
21	2502	Behenic acid	$C_{23}H_{46}O_2$	354	5.33
22	2574	Tricosanoic acid	$C_{24}H_{48}O_2$	368	2.46
23	2637	Docosanoic acid,2-hydroxy	$C_{23}H_{46}O_3$	370	0.19
24	2731	Lignoceric acid	$C_{25}H_{50}O_2$	382	9.17
25	2822	Pentacosanoic acid	$C_{26}H_{52}O_2$	396	1.34
26	2934	Cerotic acid	$C_{27}H_{54}O_2$	410	2.72
27	2943	24-methyl hexacosanoic acid	$C_{28}H_{56}O_2$	424	0.41
		Total identified fatty acids			73.73
		Total saturated fatty acids			52.12
		Total unsaturated fatty acids			21.61

The major identified saturated fatty acids were palmitic (15.80%), lignoceric (9.17%) and behenic (5.33%) acids. Linoleic acid (17.80%) was not only found to be the predominant among the unsaturated fatty acids, but of the total fatty acids content as well, thus, *Asparagus sprengeri* Regel. could be reported as a promising and cheap source for such valuable fatty acid. Linoleic acid, an omega-6 essential fatty acid, is necessary as an anti-inflammatory, for healthy cardiovascular system[22], in treatment of postmanupausal symptoms[23] and improving dry eye syndrome[24].

3.2. Phytochemical evaluation of polyphenolic compounds:

Determination of polyphenolic and flavonoidal contents in the non-flowering aerial parts revealed that the total polyphenolic content was found to be 9.83% calculated as gallic acid equivalents, while the total flavonoid content was found to be 1.87% calculated as quercetin. HPLC analysis was carried out to detect some of the phenolic acids and flavonoids, in the studied plant, against the available authentic compounds. The relative concentrations of the detected phenolic acids and flavonoids were calculated using peak area integration. From Table(3), it was concluded that the phenolic acids identified in the ethanolic (70%) extract of the non-flowering aerial parts were: caffeic acid (0.51 mg %), chlorogenic acid (0.15 mg %) and gallic acid (0.01 mg %).

Table 3. HPLC results of polyphenolic compounds in the ethanolic (70%) extract of the non-flowering aerial parts of *Asparagus sprengeri* Regel.

Peak No.	Retention time (min)	Name of compound	Concentration (mg %)
1	2.49	Gallic acid	0.01
2	3.25	Chlorogenic acid	0.15
3	3.78	unidentified	NA
4	4.13	Caffeic acid	0.51
5	4.35	unidentified	NA
6	4.68	unidentified	NA
7	5.33	unidentified	NA
8	5.46	unidentified	NA
9	6.02	Hyperoside	0.11
10	6.58	Kaempferol	0.17
11	7.01	unidentified	NA
12	7.51	unidentified	NA
13	7.97	unidentified	0.28
14	8.4	unidentified	NA
15	10.81	unidentified	NA
16	12.25	Naringenin	3.96
17	19.73	Quercetin	0.12

On the other hand, the identified flavonoids were: naringenin (3.96 mg %), kaempferol (0.17 mg %), quercetin (0.12 mg %) and hyperoside (0.11 mg %). From these results, it was clear that the major phenolic acid was caffeic acid, while naringenin proved to be the predominant identified flavonoid in the extract. Detection of naringenin is in agreement with Hassan et al. (2014)[7] who isolated and identified naringenin from the plant.

The phytochemical screening and TLC investigation of the successive fractions proved the presence of polyphenolic compounds in the ethyl acetate fraction of the ethanolic extract, which was subjected to fractionation resulting in the isolation of kaempferol (**1**), quercetin (**2**) and genistin (**3**). This is the first time for isolation of these compounds from the non-flowering aerial parts of

Asparagus sprengeri Regel., although they were previously detected in other *Asparagus* species[25-26].

Compound 1: 10 mg, yellow needle crystals, m.p. 276-278°C, soluble in ethyl acetate and methanol, practically insoluble in petroleum ether. It gave positive colour reaction for flavonoids, and negative colour reaction for carbohydrates and/or glycosides. R_f = 0.85 (dichloromethane : methanol; 8 : 2). It showed yellow fluorescence under UV which intensified with ammonia vapour indicating that it is a flavonol. ESI/MS: molecular ion peak at m/z 286.25 in agreement with the molecular formula $C_{15}H_{10}O_6$. ^{1}HNMR: 6.19 (1H, br.s, H-6), 6.42 (1H, br.s, H-8),6.9 (2H, d, *J*= 9Hz, H-32 , H- 52), 8.11(2H,m, H-22 , H-62).

Compound 2: 15 mg, yellow needle crystals, m.p. 310 - 316°C, soluble in ethyl acetate and methanol, practically insoluble in petroleum ether. It gave positive colour reaction for flavonoids, and negative colour reaction for carbohydrates and/or glycosides. R_f = 0.8 (dichloromethane : methanol; 8 : 2). It showed yellow fluorescence under UV which intensified with ammonia vapour indicating that it is a flavonol. ESI/MS: molecular ion peak at m/z 302 in agreement with the molecular formula $C_{15}H_{10}O_7$ and fragment ions at m/z 285, 275, 257, 247, 228, 201, 165, 154, 49, 136, 110 and 70. ^{1}HNMR: 6.19 (1H,d, *J*=2.1, H-6), 6.4 (1H,d, *J*=2.1, H-8), 6.9 (1H,d, *J*=8.4, H-52), 7.62 (1H,dd, *J*=2.1,2.2, H-62), 7.7 (1H,d, *J*=2.1, H-22).

Compound 3: 10 mg, off-white powder, m.p. 254-258°C, soluble in acetone and methanol. It gave positive colour reaction for flavonoids, carbohydrates and/or glycosides. R_f = 0.6 (*n*-butanol: acetic acid: water; 3:1:1). It showed yellow fluorescence under UV which changed to blue with ammonia vapour indicating that it is an isoflavone. ESI/MS: molecular ion peak at m/z 432.4 in agreement with the molecular formula $C_{21}H_{20}O_{10}$ and fragment ions at m/z, 307, 187.1, 165.2, 161.1, 149, 129.2, 119, 111.2, 97.15, 71.15, 57, 55 and 50. ^{1}HNMR: 5.8(1H,s, H-2),5.76(1H,s, H-22 &H-62), 5.69 (1H,br.s, H-32 & H-52), 4.97(1H,br.s, H-8), 4.38(1H,m,H-12 2), 3.42(1H,s, H-62 2), 3.36(1H,m, H-32 2), 3.31(1H,s, H-22 2).

3.3. Phytochemical evaluation of saponins:

The content of saponins in the non-flowering aerial parts was calculated using the calibration curve of diosgenin and represented 2.44%, on dried weight basis of the plant. These results proved to be high compared to other plants that are known to be rich sources of diosgenin *viz. Foeniculum vulgare* (0.529-1.27% w/w)[27], *Dioscorea alata* (0.13%)[28] and *Balanites aegyptica* (1.09-2.22%)[29].

According to the phytochemical screening of the successive extracts, the *n*-butanol fractionof non-flowering aerial parts of *Asparagus sprengeri* Regel. was found to be rich in saponins. Results of LC-MS analysis (Table 4) revealed the presence of the major saponin glycosides; Sprengerinin A, B, C& D. The mass spectral data of peak 1, showed an $(M+1)^+$ peak at 709.69, as well as fragment ions at 576.29 $[M-(Xyl-OH)]^+$, 396.09 $[M-(Xyl-Glu)]^+$, 415.22 (Diosgenin+1) and 353.01 [Diosgenin+1-(C_3H_9O)] revealing the compound to be sprengerinin A. Peak 2 was characterized to be sprengerinin B with a parent ion at a $(M+1)^+$ 723.02 and fragment ions at 577.22 and 397.50 which were obtained due to loss of rhamnose plus OH $[M-(Rha-OH)]^+$ and rhamnose plus glucose $[M-(Rha-Glu)]^+$ in addition to 415.20 (Diosgenin+1) and 300.15 [Diosgenin+1-($C_6H_{10}O_2$)]. Additionally, peak 3 was identified as sprengerinin C based on comparison of $(M+1)^+$ parent ion (855.62) with that reported by Sharma et al. (1983)[6] and fragment ions at 722.30, 709.03 and 576.19 representing elimination of xylose plus OH $[M-(Xyl-OH)]^+$, rhamnose plus OH $[M-(Rha-OH)]^+$ and xylose plus rhamnose $[M-(Xyl-Rha)]^+$, respectively. Also, the diagnostic fragment of the aglycone; 415.16 (Diosgenin+1) was found and 300.10 [Diosgenin+1-($C_6H_{10}O_2$)]. The presence of two rhamnose moieties and one glucose linked to a diosgenin aglycone, in the structure corresponding to peak 4 was identified by the parent ion at $(M+1)^+$ 869.18 and fragment ions at 723.61$[M-(Rha-OH)]^+$, 577.02 $[M-(2Rha-OH]^+$, 415.49 (Diosgenin+1) and 355 [Diosgenin+1-(C_3H_7O)] proving the compound to be sprengerinin D. The identifications are based on the reported data[6]. These findings emphasized for the first time the presence of Sprengerinin A, B, C& D in the aerial parts as well as in the roots as previously reported by Sharma et al. (1983)[6].

Table (4): Mass spectral data of *n*-butanol fraction of *Asparagus sprengeri* Regel. non-flowering aerial parts

Peak no.	Identified compound	Molecular ion peak (reported)	$(M+1)^+$	Diagnostic fragments	Main fragment (aglycone)
1	Sprengerinin A	708	709.69	576.29 $[M-(Xyl-OH)]^+$ 396.09 $[M-(Xyl-Glu)]^+$ 353.01 [Diosgenin+1- (C_3H_9O)]	415.22 (Diosgenin)
2	Sprengerinin B	722	723.02	577.22 $[M-(Rha-OH)]^+$ 397.50 $[M-(Rha-Glu)]^+$ 300.15 [Diosgenin+1- ($C_6H_{10}O_2$)]	415.20 (Diosgenin)
3	Sprengerinin C	854	855.62	722.30 $[M-(Xyl-OH)]^+$ 709.03 $[M-(Rha-OH)]^+$ 576.19 $[M-(Xyl-Rha)]^+$ 300.10 [Diosgenin+1- ($C_6H_{10}O_2$)]	415.16 (Diosgenin)
4	Sprengerinin D	868	869.18	723.61$[M-(Rha-OH)]^+$ 577.02 $[M-(2Rha-OH]^+$ 355 [Diosgenin+1- (C_3H_7O)]	415.49 (Diosgenin)

3.4. *In vitro* biological evaluation of the extracts:

The free radical scavenging activity of the ethanolic (70%) extract of the non-flowering aerial parts was estimated by decolourization of methanolic DPPH. The quenching of the free radical was evaluated spectrophotometrically at λ 517 nm against the absorbance of the DPPH solution (negative control) and silymarin (positive control). The ethanolic extract showed a good radical scavenging activity (IC_{50}= 56 µg ml^{-1}) which was comparable to that of silymarin (IC_{50}=31.7 µg ml^{-1}). This result is in agreement with Hassan et al. (2014)[7] that reported the antioxidant activity exhibited by the methanolic extract of the plant (IC_{50}=114 µg ml^{-1}).

From (Table 5), it was clear that the ethanolic (70%) extract of the non-flowering aerial parts of *Asparagus sprengeri* Regel. showed potent cytotoxic activity on the liver, breast and colon carcinoma. The most potent activity was that on colon cell line (IC_{50} = 3.73 µg ml^{-1}), followed by the activity on breast cell line (IC_{50}=10.2 µg ml^{-1}) and a weak activity was exerted by the extract on liver carcinoma (IC_{50}=39.4 µg ml^{-1}).

Table (5): Cytotoxic activity of the ethanolic extract of non-flowering aerial parts of *Asparagus sprengeri* Regel. on HEPG2, MCF and HCT116 cell lines.

Concentration (µg ml^{-1})	Surviving fraction (HEPG2)	(MCF7)	(HCT116)
0	1	1	1
62.5	0.22	0.42	0.35
125	0.14	0.41	0.28
250	0.32	0.41	0.24
500	0.42	0.44	0.28
	IC_{50} (µg ml^{-1})		
-	39.4	10.2	3.73

The cytotoxic activity was reported by Ji et al. (2012)[30] in *Asparagus* species, who related the activity to the presence of saponins in these plants. To the best of our knowledge, this is the first time to investigate the cytotoxic activity of *Asparagus sprengeri* ethanolic extract.

4. CONCLUSION

The present study revealed the presence of numerous biologically active constituents *viz.* polyphenols, saponins, hydrocarbons, phytol and fatty acids, in *Asparagus sprengeri* Regel. growing in Egypt. So this plant could be treated as a new cheap and available source for these bioactive metabolites, as raw material for phytopharmaceuticals. Also, it showed potent antioxidant and cytotoxic activities, thus clinical trials should be done to support the previous investigations and to facilitate production of new drugs from it.

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