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Research Article

Phytochemical investigation using GC/MS analysis and evaluation of antimicrobial and cytotoxic activities of the lipoidal matter of leaves of *Sophora secundiflora* and *Sophora tomentosa*

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ABSTRACT

This study aims at the investigation of the phytochemical composition, antimicrobial and cytotoxic activities of the lipoidal matter of leaves of *S. secundiflora* (Ortega) and *S. tomentosa* L. The saponifiable and unsaponifiable matter of *S. secundiflora* and *S. tomentosa* leaves were assessed using GC/MS analysis. Where, saponification of lipoidal matter of *S. secundiflora* and *S. tomentosa* leaves yielded 31.55%, 87.74% for unsaponifiable matter, and 19.66%, 38.70% for fatty acids methyl esters of both species, respectively. The dominant compounds in the unsaponifiable matter of *S. secundiflora* were *β*-amyrin acetate 55.20% and *α*-amyrin 9.73%. Whereas *n*-nonacosane 43.80% and 2-methyltriacontane 11.94% were the main components in *S. tomentosa*. In the saponifiable fraction, the content of saturated fatty acids identified in *S. tomentosa* 58.37% is higher than *S. secundiflora* 29.0%, while the percentage of unsaturated fatty acids identified in *S. secundiflora* 62.67% is higher than *S. tomentosa* 34.51%. Methyl linolenate 36.62% and methyl palmitate 40.02% are the major compounds in *S. secundiflora* and *S. tomentosa*, respectively. The lipoidal matters were evaluated *in vitro* for cytotoxic activity towards HCT-116 carcinoma cell line using the MTT assay with an IC_{50} value of 97.00 and 38.76 µg/mL for *S. secundiflora* and *S. tomentosa*, respectively. Using the technique of agar well diffusion, the lipoidal matter of *S. secundiflora* and *S. tomentosa* displayed moderate antimicrobial activity at conc. of 50 mg/mL.

Keywords: *Sophora secundiflora; Sophora tomentosa; cytotoxicity; antimicrobial; fatty acid methyl esters; GC/MS*.

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1. INTRODUCTION

Genus *Sophora* belongs to the family Fabaceae; comprises about 52 species **[1]**. This has a diverse array of pharmacological properties including cytotoxic, antimicrobial, antifungal, anti-diabetic, and anti-inflammatory and neuroprotective activities **[2-4]**. *Sophora secundiflora* (Ortega) Lag. ex DC [syn. *Calia secundiflora* (Ortega) Yakovlev] and reclassified as *Dermatophyllum secundiflorum* **[5, 6]** is a bushy plant that is spread throughout Africa, America, and Asia across southern Mexico **[7]**. Historically, the roots are used to treat inflammation and sore throat and as an

antipyretic, analgesic, antidote, antitumor, anti-parasitic, and diuretic as well **[8, 9]**. *Sophora tomentosa* L. is a shrub found all over China, Tanzania, Sri Lanka, and Queensland. Traditionally, it has medicinal importance as a remedy for cholera, diarrhea, and stomach disorders, also antidote after eating poisonous fish and other marine animals **[10, 11]**. Also, it was used for the treatment of hypertension in Taiwan folk medicine **[12]**. A myriad of active compounds including alkaloids, flavonoids, steroids, and triterpenoids compounds were isolated from the genus *Sophora* **[13-16]**.

This study intended to identify and compare the lipoidal matter of leaves of *S. secundiflora* and *S. tomentosa using* GC/MS analysis to widen the range of phytochemicals and biological investigations that were carried on *Sophora* members and to evaluate their cytotoxic and antimicrobial activities. This, to the best of our knowledge, is the first study of the phytochemical composition and evaluation of antimicrobial and cytotoxic activities of the lipoidal matter of *S. secundiflora* and *S. tomentosa* leaves.

2. MATERIAL AND METHODS

2.1. Plant material

Leaves of *S. secundiflora* were collected from El Zohreya Botanical Garden and leaves of *S. tomentosa* were collected from El Orman Botanical Garden, Giza, Egypt in December 2016. The taxonomic authentication was performed by the taxonomy specialist Terease Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture, Egypt. The identity was ascertained by DNA profiling performed by the authors **[17]**. Samples of the plant material were placed at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt with codes (PHG-P-SS-206) and (PHG-P-ST-207) for *S. secundiflora* and *S. tomentosa*, respectively.

2.2. Preparation of the lipoidal matter

The air-dried powder of leaves of *S. secundiflora* and *S. tomentosa* (130 g) were each independently exhaustively extracted with light petroleum ether (b.p. 60-80 °C) (3x250 mL) for 3 days. Both concentrates were evaporated individually under reduced pressure and produced petroleum ether extracts 4.12 g and 3.10 g (lipoidal matter), respectively **[18]**.

2.2. Preparation of the unsaponifiable matter

The prepared lipoidal matter of both plants was individually saponified by refluxing with 50 mL of 30% alcoholic KOH for 3 h followed by distillation of the alcohol under reduced pressure and dilution with 100 mL distilled water. The aqueous solution was extracted with diethyl ether (5 x 100 mL) in a separating funnel several times till complete exhaustion then, washed several times with distilled water till complete free alkalinity, anhydrous Na_2SO_4 used for dehydration. The extract was concentrated under reduced pressure to afford 1.30 g and 2.72 g of *S. secundiflora* and *S. tomentosa* unsaponifiable matter (USM), respectively. Both of them were kept in sealed containers for further investigation **[19]**.

2.3. Isolation of free fatty acids

Upon extraction of the unsaponifiable material, the aqueous alkaline layer left was acidified with 10% HCl gradually and the liberated fatty acids were extracted with diethyl ether (5 x1 00 mL) till exhaustion and then washed with distilled water until free of acidity, anhydrous Na_2SO_4 used for dehydration after that evaporated under reduced pressure to provide residue of total fatty acids 0.95 g and 1.38 g for *S. secundiflora* and *S. tomentosa*, respectively **[19]**.

2.4. Preparation of fatty acid methyl esters

The free fatty acid fractions of both *S.*

secundiflora and *S. tomentosa* were methylated by dissolving in 25 mL methanol, 2 mL concentrated H_2SO_4 and each mixture was refluxed for 3 h to produce fatty acid methyl esters. The methanolic solution was evaporated; the residue was diluted with 100 mL of distilled water and then extracted with ether (5 x 100 mL). Each of the combined ethereal extracts was washed with distilled water until neutral to litmus paper, anhydrous Na_2SO_4 used for dehydration, then evaporation under reduced pressure to provide fatty acid methyl esters (FAME) 0.81 g and 0.62 g for *S. secundiflora* and *S. tomentosa*. Both were kept in sealed vials for GC/MS analysis **[20-22]**.

2.5. GC/MS analysis

Shimadzu GCMS-QP2010 provided with RTX-5 fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, USA) with a split–splitless injector was used for recording mass spectra. The operating settings for the saponifiable fraction and the unsaponifiable matter analyses were adjusted according to the previous report **[21]**. The Wiley Registry of Mass Spectral Data, 8th edition, NIST Mass Spectral Library (December 2005), and previously published data were used to confirm the identity of the compounds **[23-30]**.

2.6. Cytotoxic activity

The cytotoxicity activity of the lipoidal matter of *S. secundiflora* and *S. tomentosa* was estimated using MTT assay against HCT-16 (human colon carcinoma cell line). The cell viability was expressed as a percentage of control and estimation of the concentration that induces 50% of maximum inhibition of cell proliferation (IC_{50}) from graphic plots of the dose-response curve for each concentration using Graphpad Prism software (San Diego, CA, USA) **[31-35]**.

2.7. Antimicrobial activity

The lipoidal matter of *S. secundiflora* and *S. tomentosa* at a concentration of 50 mg/mL were evaluated for their antimicrobial activity using the agar well diffusion technique against the Gram-positive bacteria *Bacillus subtilis* (RCMB 015(1) NRRL B-543), *Staphylococcus aureus* (RCMB 010010) and the Gram-negative bacteria *Escherichia coli* (ATCC 25955), *Pseudomonas aeruginosa* (NCIB-9016). Also, fungal strains *Candida albicans (*ATCC-10231) and *Aspergillus niger* (RCMB 0020080) according to the National Committee of Clinical Laboratory Standards (NCCLS) **[38, 39]**. The positive antibacterial and antifungal activities were estimated by the presence of measurable zones of inhibition for bacteria after 24 h incubation period and for fungi after 48 h. Gentamycin (4 µg/mL) and Ketoconazole (100 µg/mL) were used as positive reference antibiotics and antifungal drugs, respectively.

3. RESULTS

3.1. GC/MS analysis

Petroleum ether extract saponification of *S. secundiflora* and *S. tomentosa* leaves yielded 31.55% and 87.74% for unsaponifiable matter (USM), while 19.66% and 38.70% for fatty acids methyl esters (FAME), respectively. The lipoidal matter of both species *S. secundiflora* and *S. tomentosa* were qualitatively and quantitatively analyzed using GC/MS technique. The results revealed the existence of 9 compounds in the unsaponifiable matter (USM) of *S. secundiflora* while, 19 compounds in *S. tomentosa* accounting for 84.68% and 92.97%, respectively **(Table 1)**. In addition, a total of 15 and 31 compounds were specified in the saponifiable fraction of *S. secundiflora* and *S. tomentosa* accounting for 95.53% and 93.70%, respectively **(Table 2)**. The GC chromatograms are displayed in **(Fig. 1. and Fig. 2).** The structures of the main identified compounds of the lipoidal matter of both plants are illustrated in **(Fig. 3)**.

Table 1. Chemical composition of the unsaponifiable matter (USM) of the leaves of *S. secundiflora* (SS) and *S. tomentosa* (ST)

No.	Identified compound	R_t	Content%		RIexp.[(a)]	RIrep.[(b)]	Method of identification
			SS	ST			
1	Palmitic acid ethyl ester	35.29	-	1.08	1991	1993	KI, MS
2	Phytol	37.64	6.24	1.23	2118	2116	KI, MS
3	Linoleic acid ethyl ester	38.55	-	1.88	2168	2164	KI, MS
4	Oleic acid ethyl ester	38.64	-	3.38	2173	2180	KI, MS
5	*n*-Pentacosane	44.29	-	0.38	2497	2500	KI, MS
6	*n*- Heptacosane	47.41	0.33	5.55	2696	2700	KI, MS
7	*n*- Octacosane	48.86	-	1.36	2791	2800	KI, MS
8	*trans*- Squalene	49.40	-	0.49	2825	2833	KI, MS
9	*n*-Nonacosane	50.36	3.70	43.80	2887	2900	KI, MS
10	7,17-Dimethylnonacosane	51.62	0.41	1.33	2970	2970	KI, MS
11	2-Methyltriacontane	53.10	2.67	11.94	3057	3060	KI, MS
12	5-Methylhentriacontane	54.38	-	0.55	3145	3152	KI, MS
13	3,9-Dimethylhentriacontane	55.17	-	1.11	3196	3207	KI, MS
14	*β*-Sitosterol	55.49	-	1.32	3217	3220	KI, MS
15	*β*-Stigmasterol	56.02	3.13	5.05	3249	3248	KI, MS
16	2-Methyldotriacontane	56.19	-	1.35	3261	3260	KI, MS
17	Campesterol	57.07	3.27	4.73	3317	3305	KI, MS
18	*α*-Amyrin	57.90	9.73	2.16	3371	3376	KI, MS
19	*β*-Amyrin acetate	58.85	55.20	4.28	3434	3437	KI, MS
Total identified compounds			84.68%	92.97%			
Total hydrocarbons			7.11%	67.37%			
Total sterols			6.4%	11.1%			
Total terpenes			71.17%	8.16%			
Fatty acids methyl esters			-	6.34%			

a) $RI_{exp.}$: Retention index determined experimentally on a RTX-5 capillary column.
b) $RI_{rep.}$: Published retention indices

Compounds listed in order of their elution on RTX-5 GC column. Identification was based on a comparison of the compounds mass spectral data (MS) and retention indices (RI) with those of NIST Mass Spectral Library (2011), Wiley Registry of Mass Spectral Data 8th edition and literature.

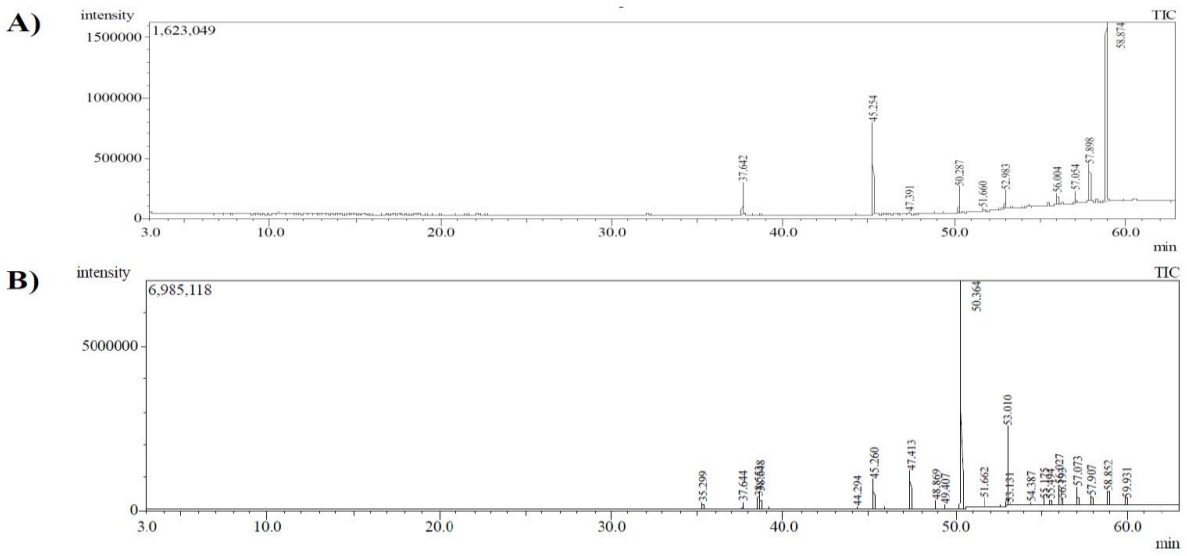

Fig.1. GC/MS chromatogram of the unsaponifiable matter of leaves of A) *S. secundiflora* and B) *S. tomentosa*

Table 2. Chemical composition of the saponifiable fraction (FAME) of the leaves of *S. secundiflora* (SS) and *S. tomentosa* (ST)

No.	Identified compound	R_t	Content%		RIexp.[(a)]	RIrep.[(b)]	Method of identification
			SS	ST			
1	Octanoic acid methyl ester	9.58	-	0.15	1118	1127	KI, MS
2	*trans*-2-Decenal	14.18	-	0.15	1256	1260	KI, MS
3	Decanoic acid methyl ester	16.50	-	0.12	1318	1325	KI, MS
4	Nonanoic acid, 9-oxo-, methyl ester	20.94	-	0.46	1438	1436	KI, MS
5	Octanedioic acid dimethyl ester	21.43	-	0.19	1452	1449	KI, MS
6	Methyl 9-oxodecanoate	24.02	-	0.19	1525	1515	KI, MS
7	Dodecanoic acid methyl ester	24.12	0.86	0.78	1528	1525	KI, MS
8	Nonanedioic acid dimethyl ester	25.00	-	1.17	1553	1550	KI, MS
9	Tridecanoic acid methyl ester	27.041	-	0.15	1625	1626	KI, MS
10	Myristic acid methyl ester	30.48	1.72	3.11	1727	1723	KI, MS
11	Undecanedioic acid dimethyl ester	31.24	-	0.24	1753	1750	KI, MS
12	*cis*-10-Pentadecenoic acid methyl ester	32.85	0.68	0.63	1808	1813	KI, MS
13	Pentadecanoic acid methyl ester	33.30	-	1.26	1825	1827	KI, MS
14	Dodecanedioic acid dimethyl ester	34.04	-	0.12	1853	1849	KI, MS
15	Palmitelaidic acid methyl ester	35.35	0.38	0.70	1922	1917	KI, MS
16	Palmitic acid methyl ester	36.17	17.06	40.02	1928	1927	KI, MS
17	Palmitoleic acid methyl ester	36.33	-	0.08	1940	1932	KI, MS
18	*cis*-10-Heptadecenoic acid methyl ester	37.89	-	0.32	1999	2009	KI, MS
19	Heptadecanoic acid methyl ester	38.54	0.71	1.40	2027	2029	KI, MS
20	Tetradecanedioic acid dimethyl ester	39.22	-	0.17	2057	2055	KI, MS
21	Linoleic acid methyl ester	40.289	22.12	8.94	2102	2098	KI, MS
21	Oleic acid methyl ester	40.530	-	22.87	2114	2113	KI, MS
22	Linolenic acid methyl ester	40.470	36.62	-	2111`	2108	KI, MS
23	Stearic acid methyl ester	41.037	3.73	5.71	2136	2135	KI, MS
24	Methyl (8*E*,11*E*)-8,11-octadecadienoate	42.403	-	0.18	2196	2196	KI, MS
25	Methyl 16-hydroxy-hexadecanoate	42.937	-	0.21	2220	2121	KI, MS
26	*n*-Nonadecanoic acid methyl ester	43.242	-	0.17	2233	2230	KI, MS
27	Eicosapentaenoic acid methyl ester	43.898	-	0.21	2262	2264	KI, MS
28	Eicosanoic acid methyl ester	45.502	0.52	2.07	2329	2333	KI, MS
29	Heneicosanoic acid methyl ester	47.651	-	0.32	2427	2424	KI, MS
31	2-Methyltetracosane	48.425	3.46	-	2461	2461	KI, MS
32	Docosahexaenoic acid methyl ester	48.562	2.87	-	2466	2470	KI, MS
33	Behenic acid, methyl ester	50.289	-	1.41	2542	2531	KI, MS
34	Nonadecanoic acid, methyl ester	51.090	2.43	-	2577	2573	KI, MS
35	Cerotic acid methyl ester	51.265	1.97	-	2585	-	MS
36	9-Octadecenoic acid methyl ester	53.440	-	0.20	2681	2689	KI, MS
Total identified compounds			95.53%	93.7 %			
Saturated fatty acid			29.0%	58.37 %			
Unsaturated fatty acid			62.67%	34.51%			
Others			3.86 %	0.82%			

a) $RI_{exp.}$: Retention index determined experimentally on a RTX-5 capillary column.

b) $RI_{rep.}$: Published retention indices

Compounds listed in order of their elution on RTX-5 GC column. Identification was based on a comparison of the compounds mass spectral data (MS) and retention indices (RI) with those of NIST Mass Spectral Library (2011), Wiley Registry of Mass Spectral Data 8th edition and literature.

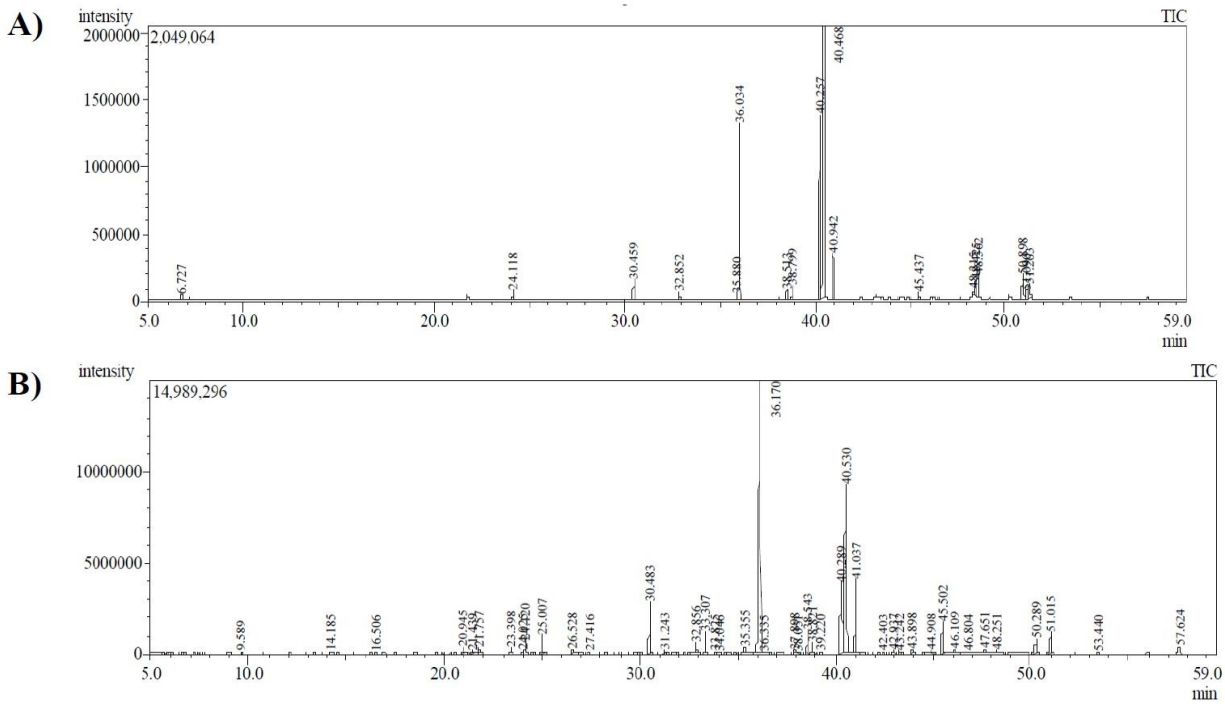

Fig.2. GC/MS chromatogram of the saponifiable fraction of leaves of A) *S. secundiflora* and B) *S. tomentosa*

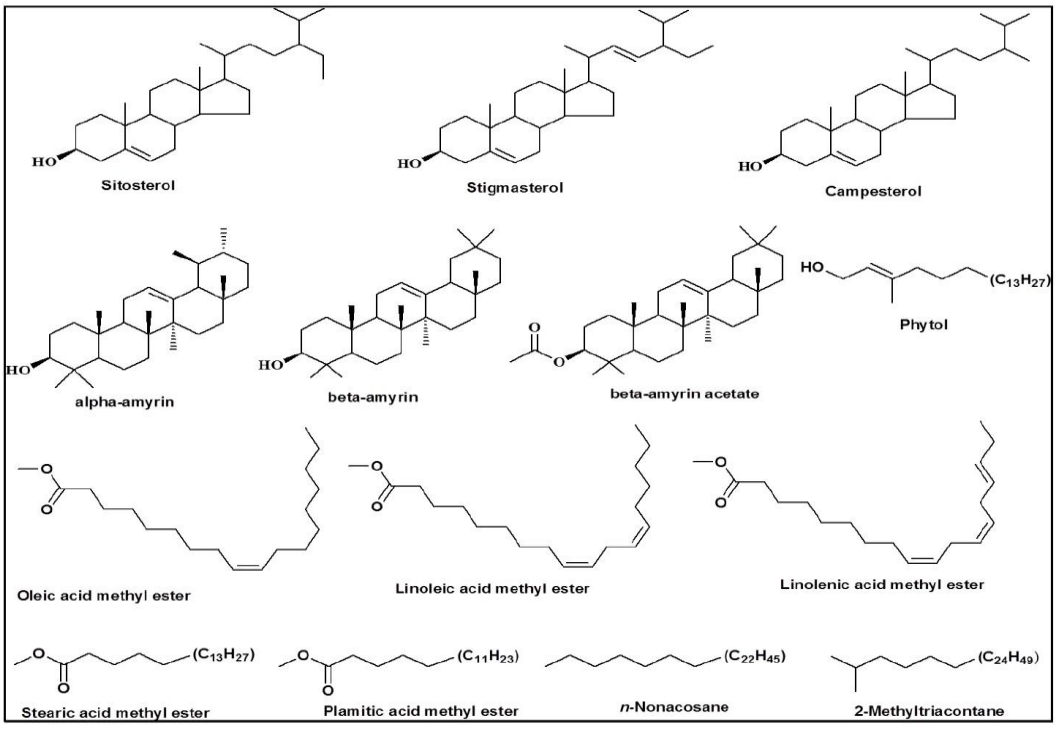

Fig.3. Structures of the main constituents of the lipoidal matter of the leaves of *S. secundiflora* and *S. tomentosa*

Total identified hydrocarbons in USM of *S. secundiflora* were 4 compounds, representing 7.11% of the total identified unsaponifiable compounds. Furthermore, 9 hydrocarbons were specified in *S. tomentosa* accounting for 67.37% of the total identified unsaponifiable compounds, mainly attributed to *n*-nonacosane accounting for 3.70% for *S. secundiflora* and 43.80% for *S. tomentosa*. Besides, 2-Methyltriacontane ($C_{31}H_{64}$) a monomethyl-branched alkane **[40]** accounting for 2.67% for *S. secundiflora* and 11.94% for *S. tomentosa*.

Three terpenes phytol, *α*-amyrin, and *β*-amyrin acetate were identified and constitute 71.17% of USM of *S. secundiflora* where *β*-amyrin acetate 55.20% is the major compound. Besides, the identified sterols; *β*-stigmasterol, and campesterol representing 6.4% of USM of *S. secundiflora*. Furthermore, the investigation of USM of *S. tomentosa* disclosed the presence of *β*-amyrin acetate 4.28% and *α*-amyrin 2.16%. Besides, three sterols were detected; *β*-stigmasterol, campesterol, and *β*-sitosterol representing 11.1%.

The percentage of identified fatty acids in *S. secundiflora* was 29.0% and 62.67% for saturated fatty acids and unsaturated fatty acids, respectively. While in *S. tomentosa* the percentage was 58.37% and 34.51% for saturated fatty acids and unsaturated fatty acids, respectively. Results of GC/MS analysis of the FAME showed that the major compound is methyl palmitate accounting for 17.06% for *S. secundiflora* and 40.02% for *S. tomentosa.* In the saponifiable fraction of *S. secundiflora*, linolenic acid methyl ester 36.62% and methyl linolenate 22.12% were detected. Furthermore, oleic acid methyl ester 22.87% and methyl linolenate 8.94% are the major unsaturated fatty acids in *S. tomentosa* saponifiable fraction. In the saponifiable fraction of *S. tomentosa*, different saturated and unsaturated fatty acids were identified including oleic acid methyl ester 22.84%, stearic acid methyl ester 5.71%, myristic acid methyl ester 3.11%, eicosanoic acid methyl ester 2.07%, behenic acid methyl ester 1.41% and pentadecanoic acid methyl ester 1.26%.

3.2. Cytotoxic activity

The cytotoxicity of the lipoidal matter was evaluated using HCT-116. The IC_{50} values are represented in **(Fig. 4)**. The highest cytotoxic activity was observed for *S. tomentosa* with an IC_{50} value of 38.76 µg/mL, while *secundiflora* with an IC_{50} value of 97.0 µg/mL.

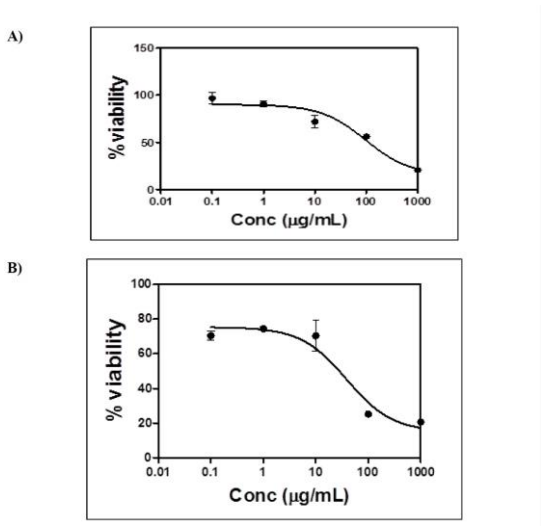

Fig.4. Cytotoxic activity on HCT-116 cell line of the lipoidal matter of the leaves of A) *S. secundiflora* and B) *S. tomentosa*

3.3. Antimicrobial activity

By using the agar well diffusion technique, the lipoidal matter of *S. secundiflora* and *S. tomentosa* leaves were screened for antimicrobial activity at conc. of 50 mg/mL against selected microbial strains. The average diameters of the growth inhibition zones are listed in **(Table 3)**. The zone of inhibition diameter was used for estimation of the antimicrobial activity where inactive when the zone of inhibition diameter <9 mm. The partial activity was reported with a zone of inhibition diameter ranged from 9 to 12. While

active when the zone of inhibition diameter range 13–18 mm and very active when the zone of inhibition diameter >18 mm **[41]**. Our results revealed that the lipoidal matter of *S. secundiflora* showed partial activity against *B. subtilis, Staph. aureus* and *E. coli* with inhibition zones of 10, 11, and 11 mm diameter, respectively. While the lipoidal matter of *S. tomentosa* was partially active against *Staph. aureus* and active against *E. coli* with inhibition zones of 9 and 14 mm diameter, respectively, and no activity was observed against *B. subtilis.* Both lipoidal matters showed no activity towards *Klebsiella pneumonia*, *Candida albicans*, and *Aspergillus niger*.

Table 3. Inhibition zones diameter (mm) of the tested extracts of *S. secundiflora and S. tomentosa* against the tested microbial strains

Name of pathogen	Pet. ether extract		Control	
	SS	ST	Ketoconazole	Gentamycin
Bacillus subtilis	**10**	**8**	-	26
Staphylococcus aureus	**11**	**9**	-	24
Escherichia Coli	**11**	**14**	-	30
Klebsiella pneumonia	NA	NA	-	21
Candida albicans	NA	NA	20	-
Aspergillus niger	NA	NA	16	-

NA= No activity, Inhibition zones diameter in mm.
Positive control for bacteria: Gentamycin (4 µg/mL)
Positive control for fungi: Ketoconazole (100 µg/mL)
Sample was tested at 50 mg/ml concentration
SS = S. secundiflora
ST = S. tomentosa

4. DISCUSSION

The GC/MS analysis of both lipoidal matters of *S. secundiflora* and *S. tomentosa* revealed the presence of bioactive components as phytol a cyclic diterpene and a member of branched-chain unsaturated alcohols with antioxidant activity related to antinociceptive activities **[42]**. α- and β-amyrins are pentacyclic triterpenes with antioxidant, antimicrobial, anti-inflammatory, and anticancer properties **[43, 44]**. Linolenic acid $C_{18:3}$ is a polyunsaturated fatty acid called omega-3 fatty acid relative to its three double bonds. It is essential for all mammals; its consumption might reduce heart disease mortality and has a preventative effect against cardiovascular diseases **[45, 46]**.

A recent previous study by the authors concerning the GC/MS analysis of the essential oil of flowers of *S. secundiflora* and *S. tomentosa*. The study reported the prevalence of fatty acid methyl and ethyl esters accounting for 4.63% and 2.72% of the total components in *S. secundiflora* and *S. tomentosa*, respectively. In *S. secundiflora* essential oil the following fatty acids are detected; methyl and ethyl palmitate, linolenic acid methyl and ethyl ester, linoleic acid ethyl ester, myristic acid methyl, and ethyl ester and lauric acid methyl ester. While in *S. tomentosa* essential oil; methyl palmitate and methyl linolenate were identified **[47]**.

Additionally, previous studies on genus *Sophora* reported that *S. alopecuroides* seed oil composed of five steroidal compounds account for 22.11% of the total components, they were identified using GC/MS analysis including 19-norpregn-4-ene-3, 20-dione, stigmastan-3-ol, 5-

chloro-, acetate, (3*β*, 5*α*), ergost-5-en-3-ol, (3*β*)-, stigmasterol, and *γ*-sitosterol **[48]**. While *β*-daucosterol was isolated for the first time by Bian *et al.*, **[49]**. In *S. alopecuroides* seeds, unsaturated fatty acids account for (88%) of the total fatty acids **[50]**.

Polyunsaturated fatty acids mainly palmitic, linoleic, oleic, and stearic acids were identified in *S. flavescens* and *S. japonica* seeds accounting for 86.47% and 90.49% of the total compounds, respectively **[51]**. Triterpenoids reported in roots of *S. flavescens* are purified and identified as lupeol, lupenone, monogynol B, *β*-amyrenol, soyasaponin I, and sophoraflavoside I, II, III, and IV **[52-54]**.

The highest cytotoxic activity was observed for *S. tomentosa* with an IC_{50} value of 38.76 µg/mL against HCT-116 that might be attributed to synergistic potentiation between plant components present in the lipoidal matter that may improve its biological effects **[55]**. Where 19 compounds were identified in the unsaponifiable matter of *S. tomentosa* accounting for 92.97%, and 31 compounds were identified in the saponifiable fraction of *S. tomentosa* accounting for 93.70%. According to our results, stigmasterol, sitosterol, and campesterol are basic phytosterols of plant cell membranes that are numerous in vegetable oils, nuts, seeds, and grains **[56]**. They are considered to have miscellaneous biological activities including anti-inflammatory, anti-oxidant, and anti-carcinogenic activities, and also their capacity of cholesterol-lowering **[57, 58]**. Many other reports have shown the phytosterols cytotoxicity on fast proliferating tumor cells as monocytic cells, colon adenocarcinoma cells, and hepatoma cells **[59, 60]**. Palmitic acid is the major saturated fatty acid in both lipoidal matters; it showed anti-inflammatory activity and selective cytotoxic activity towards the human leukemia cell line MOLT-4 **[61-62]**. Also, the presence of linolenic acid in the lipoidal matter decreased the growth of transplanted prostate, colon, and breast cancer cells *in vivo* **[63-66]**. Regarding the *in vitro* studies, it was able to inhibit growth and promote apoptosis of transplanted prostate, colon, and breast cancer cells. Nevertheless, linolenic acid-induced apoptosis of colon and breast cancer cells via a mitochondrial-mediated pathway **[67, 68]**.

The antimicrobial activity would be assigned to the existence of phytosterol and fatty acids. Sterols are membrane lipophilic components playing a key role in its fluidity and have numerous biological activities **[69]**. Furthermore, linoleic acid and linolenic acid have been reported for their antibacterial activities against *S. aureus* and *B. subtilis* **[70]**.

CONCLUSION

The present study indicated the existence of bioactive lipophilic compounds in *S. secundiflora* and *S. tomentosa* that make them a great source for natural health products. Hydrocarbons were the major components identified in *S. tomentosa* representing 67.37% of the total identified unsaponifiable compounds, mainly attributed to *n*-nonacosane accounting for 43.80%. While terpenoids were the major components identified in *S. secundiflora* representing 71.17% of USM, where, *β*-amyrin acetate 55.20% is the major compound. Methyl linolenate 36.62% is the major compound in the saponifiable fraction of *S. secundiflora*. While methyl palmitate 40.02% is the major compound in the saponifiable fraction of *S. tomentosa*. Antibacterial activity for both species was moderate and they didn't show an antifungal activity was observed on both tested fungal strains. *Sophora tomentosa* lipoidal matter showed higher cytotoxic activity towards HCT-116 with an IC_{50} value of 38.76 µg/mL, while *secundiflora* with an IC_{50} value of 97.0 µg/mL.

Sophora secundiflora and *S. tomentosa* are

worthy candidates for more comprehensive pharmacological and phytochemical studies owing to their prospect as a source of biologically active compounds.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study were included in the main manuscript

Competing interests

The authors declare that no competing interests exist

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Authors' contributions

Shaza H. Aly: conceptualization, data curation, writing the original draft. Dr. Ahmed M. Elissawy: validation, investigation, supervision, manuscript reviewing & editing. Prof. Omayma A. Eldahshan: visualization, supervision, manuscript reviewing & editing. Prof. Mohamed A. Elshanawany: visualization, supervision, manuscript reviewing & editing. Prof. Abdel Nasser B. Singab: visualization, supervision, manuscript reviewing & editing, project administration. All authors have read and approved the final manuscript.

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List of abbreviations

DMSO, Dimethyl sulfoxide; FAME, Fatty acids methyl esters; GC/MS, Gas chromatography and mass spectrometry; HCT-16, human colon carcinoma cell line; MOLT-4, Human T lymphoblast; acute lymphoblastic leukemia cell line; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RI, Retention index; NIST, National Institute of Standards and Technology; RPMI, Roswell Park Memorial Institute; USM, Unsaponifiable matter.

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