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 \(\beta\)-amyrin acetate 55.20% and \(\alpha\)-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.

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, antiparasitic, 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 Terese 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) (3 x 250 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$_2$SO$_4$ 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 x 100 mL) till exhaustion and then washed with distilled water until free of acidity, anhydrous Na$_2$SO$_4$ 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.
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secundiflora and S. tomentosa were methylated by dissolving in 25 mL methanol, 2 mL concentrated H₂SO₄ 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 Na₂SO₄ 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 (IC₅₀) 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<sub>t</sub> | Content% | R<sub>exp.</sub><sup>a</sup> | R<sub>rep.</sub><sup>b</sup> | Method of identification |
|-----|------------------------------------------|---------------|----------|-----------------|-----------------|--------------------------|
| 1   | Palmitic acid ethyl ester                | 35.29         | -        | 1.08            | 1991            | 1993                     | KL, MS                   |
| 2   | Phytol                                   | 37.64         | 6.24     | 1.23            | 2118            | 2116                     | KL, MS                   |
| 3   | Linoleic acid ethyl ester                | 38.55         | -        | 1.88            | 2168            | 2164                     | KL, MS                   |
| 4   | Oleic acid ethyl ester                   | 38.64         | -        | 3.38            | 2173            | 2180                     | KL, MS                   |
| 5   | *n*-Pentacosane                          | 44.29         | -        | 0.38            | 2497            | 2500                     | KL, MS                   |
| 6   | *n*-Heptacosane                          | 47.41         | 0.33     | 5.55            | 2696            | 2700                     | KL, MS                   |
| 7   | *n*-Octacosane                           | 48.86         | -        | 1.36            | 2791            | 2800                     | KL, MS                   |
| 8   | *trans*-Squalene                         | 49.40         | -        | 0.49            | 2825            | 2833                     | KL, MS                   |
| 9   | *n*-Nonacosane                           | 50.36         | 3.70     | 43.80           | 2887            | 2900                     | KL, MS                   |
| 10  | 7,17-Dimethylnonacosane                  | 51.62         | 0.41     | 1.33            | 2970            | 2970                     | KL, MS                   |
| 11  | 2-Methyltriacontane                      | 53.10         | 2.67     | 11.94           | 3057            | 3060                     | KL, MS                   |
| 12  | 5-Methylhentriacontane                   | 54.38         | -        | 0.55            | 3145            | 3152                     | KL, MS                   |
| 13  | 3,9-Dimethylhentriacontane               | 55.17         | -        | 1.11            | 3196            | 3207                     | KL, MS                   |
| 14  | β-Sitosterol                             | 55.49         | -        | 1.32            | 3217            | 3220                     | KL, MS                   |
| 15  | β-Stigmasterol                           | 56.02         | 3.13     | 5.05            | 3249            | 3248                     | KL, MS                   |
| 16  | 2-Methyldotriacontane                    | 56.19         | -        | 1.35            | 3261            | 3260                     | KL, MS                   |
| 17  | Campesterol                              | 57.07         | 3.27     | 4.73            | 3317            | 3305                     | KL, MS                   |
| 18  | α-Amyrin                                 | 57.90         | 9.73     | 2.16            | 3371            | 3376                     | KL, MS                   |
| 19  | β-Amyrin acetate                         | 58.85         | 55.20    | 4.28            | 3434            | 3437                     | KL, MS                   |

Total identified compounds: 84.68% SS, 92.97% ST
Total hydrocarbons: 7.11% SS, 67.37% ST
Total sterols: 6.4% SS, 11.1% ST
Total terpenes: 71.17% SS, 8.16% ST
Fatty acids methyl esters: 6.34%

<sup>a</sup> RI<sub>exp.</sub>: Retention index determined experimentally on a RTX-5 capillary column.
<sup>b</sup> R<sub>rep.</sub>: 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 8<sup>th</sup> 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<sub>t</sub> | Content% | Method of identification |
|-----|-------------------------------------------------|---------------|----------|--------------------------|
|     |                                                 |               | SS       |                          |                         |
| 1   | Octanoic acid methyl ester                      | 9.58          | 0.15     | 1118                    | 1127 | KL, MS |
| 2   | trans-2-Decenal                                 | 14.18         | 0.15     | 1256                    | 1260 | KL, MS |
| 3   | Decanoic acid methyl ester                      | 16.50         | 0.12     | 1318                    | 1325 | KL, MS |
| 4   | Nonanoic acid, 9-oxo-, methyl ester             | 20.94         | 0.46     | 1438                    | 1436 | KL, MS |
| 5   | Octadecenoic acid dimethyl ester                | 21.43         | 0.19     | 1452                    | 1449 | KL, MS |
| 6   | Methyl 9-oxodecanoate                           | 24.02         | 0.19     | 1525                    | 1515 | KL, MS |
| 7   | Dodecanoic acid methyl ester                    | 24.12         | 0.86     | 1528                    | 1525 | KL, MS |
| 8   | Nonanedioic acid dimethyl ester                 | 25.00         | 1.17     | 1553                    | 1550 | KL, MS |
| 9   | Tridecanoic acid methyl ester                   | 27.041        | 0.15     | 1625                    | 1626 | KL, MS |
| 10  | Myristic acid methyl ester                      | 30.48         | 1.72     | 1727                    | 1723 | KL, MS |
| 11  | Undecanedioic acid dimethyl ester               | 31.24         | 0.24     | 1753                    | 1750 | KL, MS |
| 12  | cis-10-Pentadecenoic acid methyl ester          | 32.85         | 0.68     | 1808                    | 1813 | KL, MS |
| 13  | Pentadecanoic acid methyl ester                 | 33.30         | 1.26     | 1825                    | 1827 | KL, MS |
| 14  | Dodecanedioic acid dimethyl ester               | 34.04         | 0.12     | 1853                    | 1849 | KL, MS |
| 15  | Palmitelaidic acid methyl ester                 | 35.35         | 0.38     | 1922                    | 1917 | KL, MS |
| 16  | Palmitic acid methyl ester                      | 36.17         | 17.06    | 1928                    | 1927 | KL, MS |
| 17  | Palmitoleic acid methyl ester                   | 36.33         | 0.08     | 1940                    | 1932 | KL, MS |
| 18  | cis-10-Heptadecanoic acid methyl ester          | 37.89         | 0.32     | 1999                    | 2009 | KL, MS |
| 19  | Heptadecanoic acid methyl ester                 | 38.54         | 0.71     | 2027                    | 2029 | KL, MS |
| 20  | Tetradecanoic acid dimethyl ester               | 39.22         | 0.17     | 2057                    | 2055 | KL, MS |
| 21  | Linoleic acid methyl ester                      | 40.289        | 8.94     | 2102                    | 2098 | KL, MS |
| 22  | Oleic acid methyl ester                         | 40.530        | 22.87    | 2114                    | 2113 | KL, MS |
| 23  | Linolenic acid methyl ester                     | 40.470        | 36.62    | 2111                    | 2108 | KL, MS |
| 24  | Stearic acid methyl ester                       | 41.037        | 5.71     | 2136                    | 2135 | KL, MS |
| 25  | Methyl (8,11E,14Z)-octadecadienoate             | 42.403        | 0.18     | 2196                    | 2196 | KL, MS |
| 26  | Methyl 16-hydroxy-hexadecanoate                 | 42.937        | 0.21     | 2220                    | 2121 | KL, MS |
| 27  | n-Nonadecanoic acid methyl ester                | 43.242        | 0.17     | 2233                    | 2230 | KL, MS |
| 28  | Eicosapentaenoic acid methyl ester              | 43.898        | 0.21     | 2262                    | 2264 | KL, MS |
| 29  | Eicosanoic acid methyl ester                    | 45.502        | 0.52     | 2239                    | 2333 | KL, MS |
| 30  | Heptacosanoic acid methyl ester                 | 47.651        | 0.32     | 2427                    | 2424 | KL, MS |
| 31  | 2-Methyltetrascanoate                           | 48.425        | 3.46     | 2461                    | 2461 | KL, MS |
| 32  | Docosahexaenoic acid methyl ester               | 48.562        | 2.87     | 2466                    | 2470 | KL, MS |
| 33  | Behenic acid, methyl ester                      | 50.289        | 1.41     | 2542                    | 2531 | KL, MS |
| 34  | Nonadecanoic acid, methyl ester                 | 51.090        | 2.43     | 2577                    | 2573 | KL, MS |
| 35  | Cerotic acid methyl ester                       | 51.265        | 1.97     | 2585                    | -    | MS    |
| 36  | 9-Octadecanoic acid methyl ester                | 53.440        | 0.20     | 2681                    | 2689 | KL, 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) \( R_{exp} \) : Retention index determined experimentally on a RTX-5 capillary column.  
b) \( R_{rep} \) : 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\textsubscript{31}H\textsubscript{64}) a monomethyl-branched alkane \[40\] accounting for 2.67% for *S. secundiflora* and 11.94% for *S. tomentosa*.

Three terpenes phytol, α-amyрин, and β-amyрин acetate were identified and constitute 71.17% of USM of *S. secundiflora* where β-amyрин 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-amyрин acetate 4.28% and α-amyрин 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\textsubscript{50} values are represented in (Fig. 4). The highest cytotoxic activity was observed for *S. tomentosa* with an IC\textsubscript{50} value of 38.76 μg/mL, while *secundiflora* with an IC\textsubscript{50} value of 97.0 μg/mL.

![Fig.4](image)

**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          |
|----------------------------------|--------------------|------------------|
| *Bacillus subtilis*              | SS 10, ST 8        | Ketoconazole 26  |
| *Staphylococcus aureus*          | SS 11, ST 9        | Gentamycin 24    |
| *Escherichia Coli*               | SS 11, ST 14       |                  |
| *Klebsiella pneumonia*           | NA, NA             |                  |
| *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, stigmaster-3-ol, 5-
chloro-, acetate, (3β, 5α), ergost-5-en-3-ol, (3β)-, 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 IC₅₀ 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 monocyctic 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 IC₅₀ value of 38.76 μg/mL, while *secundiflora* with an IC₅₀ 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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