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# UPLC-MS/MS Profiling and Antitumor Activity of *Silene succulenta* Forssk. Growing in Egypt

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**Abstract:** The present study aims to detect the phenolics and flavonoids constituents of *Silene succulenta* Forssk**.** belonging to Caryophyllaceae family, also screening of their antioxidant and cytotoxic activities. GC-MS analysis is one of our goals in this study. The phenolic and flavonoid profiles were carried out using Microplate assay. In addition, in vitro antioxidant activity was performed using DPPH assay while the cytotoxicity was summarized by SRB test. GC-MS analysis for n-hexane was carried out using gas chromatography-mass spectrometry. Also ultra-performance liquid chromatography coupled with hybrid triple time-of-flight mass spectrometry (UPLC-Triple TOF-MS/MS) was carried out to determine the different phytochemicals in total alcoholic extract. Total phenolic and flavonoids content in aerial parts of *S. succulenta* were found 20.86 mg/g and 8.06 mg/g respectively. Cytotoxicity of *n*-hexane fraction showed a high activity against Breast (MCF7) and Lung Carcinoma (A549) cell lines with $IC_{50}$ 15.5 and 22.6 µg /ml respectively. Total alcoholic extract and ethyl acetate fraction showed antioxidant activities with $IC_{50}$ 799.8 ± 45.45 and 749.9 ± 29.73 (ug/ ml) respectively. Finally, ninety six compounds were detected in *n*-hexane using GC-MS and comparison with standards. UPLC-Triple TOF-MS/MS showed the presence of different classes of secondary metabolites (flavonoids, coumarins, amino acids, anthocyanidines and alkaloids)

**Keywords:** Silene Succulent; Caryophyllaceae; Phytoecdysteroids; Triterpenoidal Saponins; Cytotoxicity.

## 1. INTRODUCTION

The importance of medicinal plants is growing exponentially since natural compounds are better tolerated and have fewer side effects when compared to chemicals or synthetic drugs[1].

The study of free radicals (atoms with unpaired electrons) such as ROS (reactive oxygen species) and RNS (reactive nitrogen species) is still important, atoms with unpaired electrons are formed as byproducts of some biological process and may affect positively or negatively on the body. From their positive effects energy production, cell growth regulation and phagocytosis, while their harmful effects are related to their damage of important biomolecules such as cell membrane, nucleic acids, lipids and proteins, these effects lead to the development of very dangerous diseases such as cancer, neurodegenerative disorders and aging. All harmful effects of free radicals can be balanced by endogenous and exogenous antioxidants which prevent the onset of oxidative stress. Uses of natural antioxidants such as polyphenolic compounds are still largely preferred over synthetic antioxidants which are suspected to promote harmful health effects[2].

Family Caryophyllaceae comprises 86 genera and 2200 species of annuals and perennial herbs. Most of its plants have high traditional and medicinal values for some diseases as gastrointestinal infection, diarrhea, cough, fever and upper respiratory tract infection[3].

The genus *Silene* is from an important genus in family Caryophyllaceae; as it contains more than 700 species, which have different classes of secondary metabolites; important classes of its secondary metabolites include phytoecdysteroids (which is similar to insect molting hormones), triterpenesaponins (with cleaning properties) and phenolic compounds[4].

Most of *Silene* species have high biological and pharmacological activities.

*S. multifida, S. acaulis* and *S. regia* produce beautiful flowers, so they have been cultivated as ornamental plants[5].The roots of *S. kumaonensis*, *S. latifolia*, *S. acaulis*, and *S. conoidea* have been

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traditionally used as soap as they are rich in saponins with cleaning properties, similar to other plants of the Caryophyllaceae[6, 7]. *Silene* also contains some edible species such as *S. cucubalis*, *S. vulgaris* and *S. acaulis*[8, 9]. A number of *Silene* species have been used in folk medicine as a diuretic, analgesic, antipyretic and emetic, also used for treatment of cold, inflammations, bronchitis and infections[4].

Phytochemical studies of genus *Silene* showed the presence of major constituents such as ecdysteroids, androsteroids, glycolipids, saponins, alkaloids and flavonoid glycosides (especially *C*-glycosides)[10]. *S. succulenta* Forssk., is a succulent perennial herb, distributed on the coastal sand and Mediterranean coast of Egypt. It is listed as a medicinal plant as its extracts are highly effective in inducing cells proliferation of macrophages[11] and its high content of saponin in root and aerial parts including quillaic acid and oleanolic acid[12].

## 2. MATERIALS AND METHODS

### 2.1. Plant material

The plant under investigation; *Silene succulent* Forssk. were collected in March 2019 from the northwestern coast, west of Matrouh (**GPS coordinates; 30.375277, 27.186111**) at flowering stage and were kindly established by Dr. Omran Ghaly, Head of Plant Taxonomy Unit, Desert Research Center, Egypt**.**

*1.1.1. Preparation of extracts*

Air dried powdered of *S. succulenta* leaves (1.315 kg) were macerated with 70% methanol at room temp. (4 x 3L, 1.5h each). The combined methanol extract was dried under reduced pressure at 50 °C. The sticky residue from total extract (176 g) was suspended in distilled water (500 ml) and partitioned by *n*-hexane (3 x 1L), followed by ethyl acetate (3 x 1L) then *n*-butanol (3 x 1L) to obtain 22.68 g hexane fraction, 7.58 g ethyl acetate fraction, 27.77 g butanol fraction and 98 g aqueous fraction.

### 2.2. Total phenolic, total flavonoids and antioxidant

Microplate assay used for screening phenolic and flavonoid content[13].

*2.2.1. Preparation of standards and sample Gallic acid standards for Total phenolic*

Stock solution of gallic acid of 1mg/ml was prepared in methanol, and serial dilutions were prepared in seven different concentrations of 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 µg/ml.

*Rutin standards for Total flavonoids*

Ruin stock solution of 1mg/ml was prepared in methanol, and standards were prepared using six concentrations of 1000, 500, 250, 150, 100, and 50 µg/ml.

*Sample preparation*

Solution of 5mg/ml of sample was prepared in methanol.

*Microplate reader analysis*

The results were recorded using microplate reader FluoStar Omega.

*Determination of Total phenolic content*

Each of the 7 standards and the sample were pipetted in the plate wells in 6 replicates. Measurement was performed at 630 nm.

*Determination of total flavonoida content*

Each of the 6 standards and the sample were pipetted in the plate wells in 6 replicates. Measurement was performed at 510 nm.

### 2.3. GC-MS analysis

The GC-MS analysis was carried out using gas chromatography-mass spectrometry Instrument[14]

### 2.4. High-resolution UPLC-Triple TOF-MS/MS analysis

The total alcoholic extract was analyzed using a Triple TOF 5600+ System (AB SCIEX, Canada) [15]**.**

### 2.5. Antioxidant activity

*2.5.1. Samples preparation*

*2.5.2. Initial screening step*

Solutions of the provided sample was prepared in concentrations of 1000 and 100 µg/mL in DMSO in order to identify a range within which the inhibitory concentration 50 (IC50) lies.

*2.5.3. IC50 determination*

Extracts that exceeded 50% inhibition in any of the initial screening step concentrations were serially diluted to provide 4 concentrations.

*2.5.4. Trolox standard preparation*

A stock solution of 100µM concentration of trolox was prepared in methanol from which 7concentration were prepared including 50, 40, 30, 20, 15, 10 and 5 µM.

*2.5.5. DPPH Assay*

DPPH-(2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging assay was carried out according to the reported method [2].

*2.5.6. Microplate reader analysis*

The results were recorded using microplate reader FluoStar Omega.

*2.5.7. Data analysis*

Data was analyzed using *Microsoft Excel*® and the IC50 value was calculated using Graph padPrism 5® by converting the concentrations to their logarithmic value and selecting nonlinear inhibitor regression equation (log (inhibitor) vs. normalized response – variable slope equation) [16]

**2.6. Cytotoxic activity**

Sulphorhodamine-B (SRB) assay was carried out to determine the cytotoxic activity of five fractions of *Silene succulenta* which are aqueous extract (1), hexane fraction (2), Butanol fraction (3), Ethyl acetate fraction (4), Total extract (5).This method reported by Vichai and Kirtikara, 2006[17].

*2.6.1. Human tumor cell lines*

The tumor cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Human tumor carcinoma cell lines (A549, MCF-7) used in this study were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.).

*2.6.2. Chemicals*

The used chemicals are obtained from:

A- **Sigma Aldrich Chemical Co.,** St. Louis, Mo, U.S.A., was the source of the following chemicals: Dimethylsulphoxide (DMSO), RPMI-1640 medium, trypan blue, Fetal Bovine Serum, Penicillin/ Streptomycin antibiotic and Trypsin-EDTA.

B- **Applichem**, Germany was the source of Tris buffer.

All used chemicals and reagents are of highest analytical grade.

*2.6.3. Calculation*

The percentage of cell survival was calculated as the following:

Surviving fraction = O.D. (treated cells)/ O.D. (control cells).

The $IC_{50}$ values (the required concentrations to produce 50% inhibition of cell growth) were also calculated.

## 3. RESULT AND DISCUSSION

**3.1. Total phenolic and total flavonoids**

*3.1.1. Total phenolic*

Average absorbance at 630 nm= 0.1946 (Standard Deviation = 0.015339). After substitution in the linear regression equation; the total phenolic were 104.3µg/ml (Standard Deviation = 7.66974)

**Total phenolics (Gallic Acid Equivalent) = 20.86 mg / g Extract (Standard Deviation = 1.533).**

*3.1.2.Total flavonoids*

Average Absorbance at 510 nm = 0.053333(Standard Deviation =0.00432). By substitution in the linear regression equation, the total flavonoids were 40.33µg/ml (Standard Deviation 4.320)**.**

**Total flavonoids (RutinEquivalent) 8.06 mg / g Extract (Standard Deviation = 0.864)**

**3.2. GC-MS analysis**

GC-MS analysis of n-hexane fraction lead to detection of ninety sex compounds and RT and area % of each compound represented in **Table 1 (**Supplementary Table 1) **Palmitic acid is the major component (area =6.41 with RT=31.38)**

RT; retention time

RTT*; Relative Retention Time (RT for each / RT of the highest AREA)

**3.3. UPLC-Triple TOF-MS/MS analysis**

The retention times observed molecular weight, and fragment ions for each metabolite and their identities are presented in Table 2 Supplementary Table 2**.**

UPLC-Triple TOF-MS/MS analysis of total alcoholic extract showed identification of 37 compounds: among these compounds 15 flavonoids, 3 coumarins, 6 amino acids and one alkaloid.

**Compound 1**: (RT= 1.28 min.) showed a $[M^-H]^-$ peak at m/z 477.12, therefore identified as Isorhamnetin-3-O-glucoside[18]. **Compound 7** (RT= 6.03 min.) showed $[M^-H]^-$ peak at m/z623.16 and fragmentation showed rutinoside moiety at m/z 308, so it is identified as Isorhamnetin-3-O-rutinoside[18]. **Compound 2** (RT= 1.19 min.) showed a $[M^-H]^-$ peak at m/z 317.05, so identified as Myricetin[19]. **Compound 3, 5, 9** (RT= 5.55, 5.9 and 6.89 min. respectively) showed $[M^-H]^-$ peak at m/z 447.09, **compound 6** showed $[M+H]^+$ peak at m/z 449.1(RT= 5.98 min.) fragmentation of compounds 3, 5, 6, 9 showed the aglycone part of luteolin which is indicated by loss of one glucose moiety $[M-H-162]^-$, also m/z ($[M^-H]$ -19) indicated that the sugar attached to C-8, also loss of water molecule indicates attachment of sugar moiety to -6.

**3.4. Antioxidant Activity**

*3.4.1. Preliminary screening*

From table 3(supplementary table 3), the IC50 was found to be:

1) Above 1000 µg/mL in samples hexane and butanol.

2) between 1000 and 100 µg/mL in samples ethyl acetate and total alcohol extract.

*3.4.2. IC50 determination*

Table 4(supplementary table 4) showed $IC_{50}$ of antioxidant activity of total alcoholic extract and ethyl acetate fraction of *Silene succulent (*749.9 ± 29.73 and 799.8 ± 45.45 respectively). This result may be due to phenolic content of alcoholic extract.

Figure 1, 2 showed inhibition effect of different dilution of Trolox as standard, total alcoholic extract and ethyl acetate fraction.

So, compound 5 identified as luteolin, compound 3 identified as Luteolin-8-C-glucoside, compound 6 is luteolin -6-C-glucoside and compound 9 identified as Luteolin-7-O-glucoside[20]. **Compound 15** (RT= 22.47 min.) showed $[M^-H]^-$ peak at m/z 609.14, identified as Luteolin-3', 7-di-O-glucoside[20]. **Compound 4, 8, 13** (RT= 5.79, 6.8, 7.9 min. respectively) showed fragment of kaempferol aglycone, compound 4 showed loss of neohesperidose; *m/z* $[M - H - 308]^-$ so compound 4 identified as Kaempferol-7-neohesperidoside[c], compound 8 showed *m/z* $[M - H - 132]^-$ which indicates loss of arabinose, compound 13 showed *m/z* $[M - H - 176]^-$ corresponding to loss of glucuronoide moiety, so it is identified as Kaempferol-3-Glucuronide[18]. **Compound 12** (RT= 7.74 min.) showed $[M^-H]^-$ peak at m/z at 431.09, also it showed $[M-H-162]^-$ corresponding to loss of a glucose moiety. Compound 12 identified as apigenin-7-O-glucoside[20]. **Compound 11** (RT= 7.74 min.) showed $[M+H]^+$ peak at m/z 609.18, $[M+H- 146]^+$; corresponding to loss of rhamnose moiety, it also showed loss of rutinoside $[M+H-308]^+$, so compound 11 identified as diosmin (diosmetin-7-O-rutinoside)[20]. **Compound 10** (RT= 7.68 min.) showed $[M^-H]^-$ peak at m/z 611.19, identified as Neohesperidin dihydrochalcone.

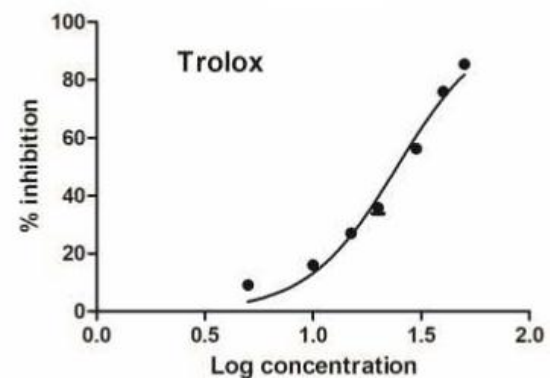


**Figure 1:** Curve of Trolox with different concentrations

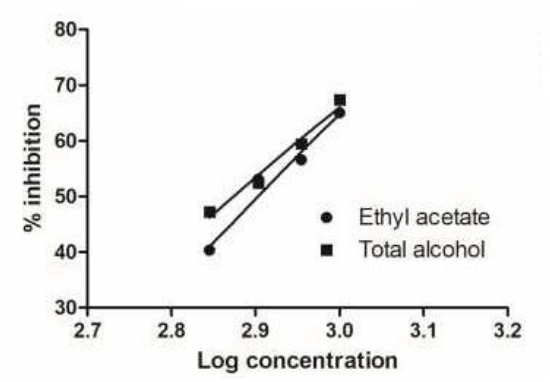


**Figure 2:** Curve of total alcoholic extract and ethyl acetate fraction with different concentrations

### 3.5. Cytotoxic Activity

*n*-Hexane fraction Showed higher biological activity against Lung and Breast Carcinoma cell lines as showed in table 5(Supplementary table 5) this may be attributed to its high content of fatty acids.

## 4. CONCLUSION

Cytotoxicity of *n*-hexane fraction of *Silene succulenta* Forssk. showed a high activity against Breast (MCF7) and Lung Carcinoma (A549) cell lines, this result may be attributed to its high content of fatty acids. On the other hand *n*-hexane fraction showed no effect with DPPH assay compared to alcoholic extract which is rich with phenolic compounds. So the non-polar fraction of *Silene succulenta* Forssk can be used for the development of natural drugs against breast and lung cancer.

**Supplementary Materials:**

**Tables** 1, 2, 3, 4, 5

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**Author Contribution:** Sarah A. Badawy contributed to data collection and performed the practical chromatographic section under supervision of other authors. Abd-Elsalam I. Mohammad and Salwa A. Abu El Wafa and Ahmed R. Hassan contributed to the design of the the practical chromatographic section. Abd-Elsalam I. Mohammad and Salwa A. Abu El Wafa designed the research, analyzed the data and interpreted the results. They also contributed equally to critically drafting and revising the manuscript. All authors have read and approved the final manuscript.

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