ANTIMICROBIAL SECONDARY METABOLITES FROM *SILENE RUBELLA* GROWING IN EGYPT

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**ABSTRACT:**

Phytochemical investigation of the ethanolic extract of dried aerial part of *Silene rubella* led to the isolation of eighteen compounds (1-18). The isolated compounds were identified by their NMR, and MS spectral data as ecdysterone (1), apigenin (2), diosmetin (3), kaempferol (4), luteolin (5), myricetin (6), quercetin (7), isovitexin (8), vicenin 2 (9) rutin (10), (R)-naringin & (S)-naringin (11 & 12), chlorogenic acid (13), betulinic acid (14), oleanolic acid (15), ursolic acid (16), D-pinitol (17), and spinasterol (18). This is the first report on isolation of chemical entities from this plant. Crude ethanolic extract of *S. rubella*, exhibited antimicrobial activity against *Escherichia coli* with 

IC₅₀ 48.85 µg/mL. Oleanolic acid (15) exhibited good activity against *E. coli* with IC₅₀ 15.78 µg/mL. Oleanolic acid (15) and betulinic acid (14) exhibited potent antibacterial activity against *Vancocmycin resistant Enterococcus* (VRE), with IC₅₀ 6.36 and 7.51 µg/mL, respectively.

**Keywords:** *Silene rubella*; antimicrobial; *Vancomycin resistant Enterococcus* (VRE); *Escherichia coli* (E. coli); Oleanolic acid; Betulinic acid

**INTRODUCTION:**

The genus *Silene* L. (Caryophyllaceae) is one of the largest genera of flowering plants consisting about 700 species, commonly known as campion and catchfly¹¹. These species are mainly distributed in Europe, Asia and Northern Africa¹¹,². In Egypt, 29 species of *Silene* are distributed in the Mediterranean, Suez and Aqaba Gulfs, coastal plains in Sinai, the Nile Valley, Oases and Gebel Elba massive³. The endemism ratio of *Silene* L. is 13.8 % in Egypt.

*Silene* genus includes a number of cultivated species and widespread weeds. *S. acaulis*, *S. multifidi* and *S. regia* have been cultivated as ornamental plants⁴. The roots of several *Silene* species, such as *S. latifolia*, *S. acaulis*, *S. kumaonensis*, and *S. conoidea* are rich in saponins with detergent properties, and traditionally used as a soap substitute for washing clothes similar to other plants of the Caryophyllaceae⁵-¹⁰.

A number of *Silene* species have been used in traditional medicine to treat inflammations, bronchitis, cold infections and also used as a diuretic, antipyretic, analgesic, and emetic¹¹. Previous studies on six wild *Silene* species (*S. alba*, *S. conoidea*, *S. dichotoma*, *S. italic*, *S. supina*, and *S. vulgaris*), had shown potent activities as antimicrobial, antioxidant anti-inflammatory and analgesic¹²,¹³.

The genus *Silene* is rich in diverse chemical compounds, such as phytocdysteroids¹⁴, anthocyanidins, N-containing compounds¹⁵, triterpene saponins¹⁶, terpenoids, benzenoids, flavonoids¹⁷, sterols, and vitamins¹⁸,¹⁹. Many of the *Silene* secondary metabolites are important as defense compounds for the plants against herbivores and microbes²⁰.

In continuation of search for bioactive secondary metabolites from African plants²¹-²³, *S. rubella* was chosen for investigation, due to lack of
phytochemistry reports. Ecdysteroids were only identified by using TLC\cite{24}.

**Results and Discussion**

Phytochemical investigation of the ethanolic extract of dried aerial part of *S. rubella* led to the isolation of eighteen compounds (1-18, figure 1). The isolated compounds were identified by their NMR, MS spectral data as ecdysterone (1)\cite{25}, apigenin (2)\cite{26}, diosmetin (3)\cite{27}, kaempferol (4)\cite{28}, luteolin (5)\cite{28}, myricetin (6)\cite{29}, quercetin (7)\cite{30}, isovitexin (8)\cite{31}, vicenin 2 (9)\cite{32}, rutin (10)\cite{30}, R-naringin & S-naringin (11 & 12)\cite{33}, chlorogenic acid (13)\cite{34}, betulinic acid (14)\cite{35}, oleanolic acid (15)\cite{36}, ursolic acid (16)\cite{36}, D-pinitol (17)\cite{37} and spinasterol (18)\cite{38}. All the isolated compounds were reported for first time from this plant.

Crude ethanolic extract and isolated compounds (1-18) of *S. rubella*, were tested for antimicrobial and antiplasmodial assays. Ethanolic extract exhibited antimicrobial activity against *E. coli* at a concentration of 200 µg/mL with IC\(_{50}\) 48.85 µg/mL. Only compounds 4,14,15 were exhibited significant activities. Oleanolic acid (15) exhibited moderate activity against *E. coli* at a concentration of 20 µg/mL with IC\(_{50}\) 15.78 µg/mL (standard: Meropenem IC\(_{50}\) 13.31 µg/mL at a concentration of 100 µg/mL). Betulinic and oleanolic acids (14, 15) exhibited potent antibacterial activity against VRE at a concentration of 20 µg/mL, with IC\(_{50}\) 7.51 and 6.36 µg/mL (standard: Ciprofloxacin IC\(_{50}\) >10 µg/mL at a concentration of 10 µg/mL). Kaempferol (4), exhibited moderate activity against *Plasmodium falciparum* D6 IC\(_{50}\) 3.34 µg/mL. (standard: Chloroquine IC\(_{50}\) 13.6 ng/mL)

**Conclusion**

Crude ethanolic extract of *S. rubella*, exhibited antibacterial activity against *E. coli* with IC\(_{50}\) 48.85 µg/mL. Phytochemical investigation of the ethanolic extract of dried aerial part of *S. rubella* led to the isolation of eighteen compounds. This is the first report of isolation of chemical entities from this plant. Oleanolic acid (15) isolated from it exhibited moderate activity against *E. coli* with IC\(_{50}\) 15.78 µg/mL. Betulinic and oleanolic acids (14, 15) exhibited potent antibacterial activity against VRE with IC\(_{50}\) 6.36 and 7.51 µg/mL.

**Experimental Section**

**General**

A Bruker model AMX 500 MHz and 400 MHz spectrometers operating on a standard pulse system collected \(^1\)H and \(^13\)C NMR spectra. The instrument ran at 500 and 400 MHz in \(^1\)H and 125 to 100 MHz in \(^13\)C. CDCl\(_3\), CD\(_3\)OD, DMSO-\(d_6\), and C\(_6\)D\(_6\)N were used as solvents, and TMS was used as an internal standard. HRMS-ESI was done on Thermo Orbitrap Fusion (Thermo Scientific). Sample was analyzed in the negative and positive mode of ionization. Mass was analyzed in Orbitrap (Voltage – 4300, Mass error on the instrument <2 ppm).

**Plant material**

The plant materials *S. rubella* L. were collected in April 2015 from middle Delta (Ekhnawy - Tanta - Egypt) at flowering stage and were kindly established by Prof. Dr. Ibrahim El Garf Prof. of Botany and taxonomy, Faculty of Science, Cairo University. Voucher specimens (C.S. # 0912-914) were deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt.

**Phytochemical studies**

The air-dried powdered aerial parts (1000 g) *S. rubella* were macerated with 70 % ethanol (3 x 5 L) at room temperature. The combined ethanolic extract was concentrated under vacuum at 50°C to yield 35 g residue. The concentrated alcoholic extract was then suspended in distilled water (500 ml) and partitioned with n-hexane (3 x 1 L), followed by ethyl acetate (3 x 1 L) and finally with n-butanol (3 x 1 L) to afford 6 g of hexane fraction, 5 g ethyl acetate fraction and 10 g of n-butanol fraction.

The ethyl acetate fraction was subjected to fractionation on vacuum liquid chromatography (VLC) on Silica gel using n-hexane: ethyl acetate as mobile phase afforded 10 sub fractions (SRE1 - SRE10) (100%-0% to 0%-100%). These sub fractions were subjected to further purification using Sephadex LH-20 to afford 11 compounds. Fraction SRE1 yielded 7 mg of spinasterol (18), SRE2 yielded 16 mg of apigenin (2) and 20 mg of diosmetin (3), SRE3 yielded 16.5 mg of kaempferol (4) and 14 mg...
of luteolin (5), SRE5 yielded 8 mg of betulinic acid (14), 6.5 mg of oleanolic acid (15) and 10 mg of ursolic acid (16). SRE7 yielded 14 mg of quercetin (7), SRE8 yielded 16 mg of myricetin (6), finally SRE9 yielded 12 mg of chlogenic acid (13).

The n-butanol fraction was subjected to fractionation on VLC over Silica gel using methylene chloride: methanol as mobile phase (100 %: 0 % to 0 %: 100 %) afforded 10 sub fractions (SRB1 - SRB10). Further purification of these sub fractions on Sephadex LH-20 to afford 7 compounds. SRB1 yielded 6 mg of isovitexin (8) and 100 mg D-pinitol (17), SRB4 yielded 60 mg of ecdysterone (1), SRB8 yielded 20 mg of vicenin 2 (9) and 18 mg of rutin (10), SRB9 yielded 20 mg of (R)-naringin & (S)-naringin (11 & 12).

Antimicrobial, and antimalarial assays

The extracts and isolated compounds were screened for antimicrobial, and antimalarial activities at 200 and 20 μg/mL concentration using the reported methods [39-42].

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