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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), chlrogenic 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 IC_{50} 48.85 µg/mL. Oleanolic acid (15) exhibited good activity against *E. coli* with IC_{50} 15.78 µg/mL. Oleanolic acid (15) and betulinic acid (14) exhibited potent antibacterial activity against *Vancomycin resistant Enterococcus* (VRE), with IC_{50} 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 catchfly[1]. These species are mainly distributed in Europe, Asia and Northern Africa[1, 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 massive[3]. 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 plants[4]. 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 Caryophyllaceae[5-10].

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 emetic[11]. Previous studies on six wild *Silene* species (*S. alba*, *S. conoidea*, *S. dichotoma, S. italica, S. supina*, and *S. vulgaris*), had shown potent activities as antimicrobial, antioxidant anti-inflammatory and analgesic[12, 13].

The genus *Silene* is rich in diverse chemical compounds, such as phytoecdysteroids[14], anthocyanidins, N-containing compounds[15], triterpene saponins[16], terpenoids, benzenoids, flavonoids[17], sterols, and vitamins[18, 19]. Many of the *Silene* secondary metabolites are important as defense compounds for the plants against herbivores and microbes[20].

In continuation of search for bioactive secondary metabolites from African plants[21-23] *S. rubella* was chosen for investigation, due to lack of

phytochemistry reports. Ecdysteroids were only identified by using TLC[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**)[25], apigenin (**2**)[26], diosmetin (**3**)[27], kaempferol (**4**)[28], luteolin (**5**)[28], myricetin (**6**)[29], quercetin (**7**)[30], isovitexin (**8**)[31], vicenin 2 (**9**)[32], rutin (**10**)[30], *R*-naringin & *S*-naringin (**11** & **12**)[33], chlorogenic acid (**13**)[34], betulinic acid (**14**)[35], oleanolic acid (**15**)[36], ursolic acid (**16**)[36], D-pinitol (**17**)[37] and spinasterol (**18**)[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_3OD, DMSO-d_6 and C_5D_5N 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 *vacuo* 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 chlrogenic 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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