Chemical constituents from *Silene schimperiana* Boiss. belonging to Caryophyllaceae and their chemotaxonomic significance

Ismail A. Hussein[1,2], Radhakrishnan Srivedavyasasri[1], Atef A. El-Hela[2], Abd-Elsalam I. Mohammad[2], Samir A. Ross[1,3*].

[1]National Center for Natural Products Research, University of Mississippi, University, MS 38677,USA.

[2]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo 11371, Egypt.

[3]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Telephone: +1-6629151031; Email: sross@olemiss.edu; Fax: +1-662-915-7989.

Abstract

Phytochemical investigation of *Silene schimperiana* Boiss. ethanolic extract led to the isolation of fifteen compounds (**1-15**). The isolated compounds were identified by their NMR, MS spectral data analyses and comparing with published data as: vanillic acid (**1**), ferulic acid (**2**), caffeic acid (**3**), ethyl ferulate (**4**), apigenin (**5**), hesperetin (**6**), diosmetin (**7**), luteolin (**8**), kaempferol (**9**), quercetin (**10**), ecdysterone (**11**), hesperedin (**12**), diosmin (**13**), kaempferol-3-*O*-rutinoside (**14**) and rutin (**15**). The lack of chemical and biological investigations on this plant encouraged us to carry out the above-mentioned work.

Key words: *Silene schimperiana*; Flavonoids; Ecdysterone; Phenolic acids; *E. coli*

Introduction:

Family Caryophyllaceae is broadly known for planting herbs however their therapeutic significance is scantily known (Chandra and Rawat, 2015). Most of Caryophyllaceae plants are utilized for some common ailments as cold, cough, fever, diarrhea, throat infection, and gastrointestinal infection (Chandra and Rawat, 2015). Caryophyllaceae species are known for their rich content in bioactive metabolites, such as triterpene saponins, flavonoids, phytoecdysteroids and oligosaccharides (Golea et al., 2017, Cheikh-Ali et al., 2019).

The genus *Silene* (family Caryophyllaceae) comprises more than 700 species of annuals, biennials, and perennials which are mainly distributed in temperate zones of the Northern Hemisphere of Eurasia and America, but also in Africa (Mamadalieva et al., 2014). *Silene* is one of the genera of blooming plants on the planet consisting of about 750 species. Most of these species are distributed in Mediterranean region (Bağcıand Biçer, 2015).

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 (Hosny et al., 1993). The endemism ratio of *Silene* L. is 13.8 % in Egypt. *Silene* produces a diversity of secondary metabolites, many of them are important for the plants as defense compounds against herbivores and microbes (Mamadalieva et al., 2014). Also, *Silene* species have antiviral, antimicrobial, antioxidant, cytotoxic, hepatoprotective and insecticidal activity (Mamadalieva et al., 2014).

Phytochemical investigations of *Silene* species have revealed that many components from this genus are highly bioactive. More than 400 compounds have been isolated (Mamadalieva, 2012). The most prominent compounds in *Silene* species are the phytoecdysteroids, which have a similar chemical structure to molting hormones of insects (Mamadalieva et al., 2014).

In continuation of search for bioactive secondary metabolites from Egyptian plants (Hussein et al., 2019), we chose *Silene schimperiana* Boiss. for investigation, due to lack of phytochemistry reports.

Experimental:

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. CD_3OD, DMSO-d_6 and C_5D_5N were used as solvents, and TMS was used as an internal standard. FTMS-ESI was analyzed on Thermo Orbitrap Fusion (Thermo Scientific). The sample was

analyzed in the positive and negative mode of ionization. Mass was analyzed in Orbitrap (mass error on the instrument <2 ppm).

Plant material

The plant materials *Silene schimperiana* Boiss. were collected in April 2014 from Saint Catherine, South of Sini- Egypt (GPS coordinates are 28.336711, 33.944774) at flowering stage and were kindly established by Prof. Dr. Ibrahim El Garf, Prof. of Botany and taxonomy, Faculty of Science, Cairo University. A voucher specimen (C.S. # 0912-915) were deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt.

Phytochemical studies

The air-dried powdered aerial parts (300 g) *S. schimperiana* were macerated with 70 % ethanol (3 x 1.5 L) at room temperature. The combined ethanolic extract was concentrated under *vacuo* at 50 °C to yield 28 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 7.5 g of hexane fraction, 5.3 g ethyl acetate fraction and 10 g of *n*-butanol fraction.

The ethyl acetate fraction was subjected to fractionation over Silica gel column using *n*-hexane: ethyl acetate gradient as mobile phase to afford 10 sub fractions (SSE1 - SSE10) (100% :0 % to 0 %: 100%). These sub fractions were subjected to further purification using Sephadex LH-20 to afford 10 compounds. Fraction SSE2 yielded 5 mg of vanillic acid (**1**); fraction SSE3 yielded 5.9 mg of ferulic acid (**2**) and 7 mg of caffeic acid (**3**); fraction SRE4 yielded 10 mg of ethyl ferulate (**4**); fraction SSE6 yielded 8 mg of apigenin (**5**); fraction SSE7 yielded 10 mg of hesperetin (**6**) and 9 mg of diosmetin (**7**); fraction SSE8 yielded 20 mg of luteolin (**8**) and 17.8 mg of kaempferol (**9**); finally fraction SSE9 yielded 15.4 mg of quercetin (**10**).

The *n*-butanol fraction was subjected to fractionation on Silica gel using methylene chloride: methanol as mobile phase (100% :0 % to 0 %: 100%) to afford 10 sub fractions (SSB1 - SSB10). Further purification of these sub fractions on Sephadex LH-20 to afford 5 compounds. Fraction SSB3 yielded 30 mg of ecdysterone (**11**); fraction SSB5 yielded 30 mg of hesperedin (**12**) and 17 mg of diosmin (**13**); fraction SSB7 yielded 10 mg of kaempferol-3-*O*-rutinoside (**14**); finally fraction SSB9 yielded 13 mg of rutin (**15**).

Antimicrobial, and antimalarial assays

The extracts and isolated compounds were screened for antimicrobial, and antimalarial activities at concentration 200 and 20 µg/mL using the reported methods (Bharate et al., 2007; Radwan et al., 2009; Ma et al., 2004; Manohar et al., 2014).

Results and discussion

Phytochemical investigation of the ethanolic extract of dried aerial part of *S. schimperiana* led to the isolation of fifteen compounds (**1-15**, figure **1**). The isolated compounds were identified by their NMR, MS spectral data as vanillic acid **[1]** (Zeid et al., 2009), ferulic acid [**2**] (El-gizawy et al., 2017), caffeic acid **[3]** (Tošović, 2017), ethyl ferulate [**4**, which could be possibly an artifact] (da Silva et al., 2015), apigenin [**5**] (da Silva et al., 2015), hesperetin [**6**] (Maltese et al., 2009), diosmetin **[7]** (Hu et al., 2017), luteolin [**8**] (da Silva et al., 2015), kaempferol [**9**] (Aisyah et al., 2017), quercetin [**10**] (Liu et al., 2010), ecdysterone [**11**] (Fang et al., 2017), hesperidin [**12**] (Maltese et al., 2009), diosmin [**13**] (Gopalakrishnan et al., 2015), kaempferol-3-*O*-rutinoside [**14**] (Dehaghani et al., 2017) and rutin [**15**] (Fathiazad et al., 2010). All the isolated compounds were reported for first time from this plant. This is the first report on isolation of secondary metabolites from *S. schimperiana*.

Crude ethanolic extract and isolated compounds (**1-15**) of *S. schimperiana*, were tested for their antimicrobial and antiplasmodial activities. Only crude extract exhibited moderate activity against *E. coli* at a concentration of 200 µg/mL with IC_{50} 40.58 µg/mL (standard: Meropenem IC_{50} 13.31 µg/mL at a concentration of 100 µg/mL).

Plant chemotaxonomic significance

In genus *Silene*, major constituents are ecdysteroids, androsteroids, flavonoid glycosides (especially C-glycosides), alkaloids, saponins and glycolipids (Mamadalievia et al., 2014, Olennikov 2019, Olennikov and Chirikova 2019, Olennikov and Kashchenko 2019, Saatov et al., 1987, Seo et al., 2020). In this study, four phenolic acids (**1**–**4**), ten flavonoids (**5**-**10**, **12**-**15**) and one ecdysteroid (**11**) were isolated from *S. schimperiana*. Notably, this is the first isolation study to report all these compounds from this species. Based on literature search, we found that compound **9** was isolated from *S. diclinis* (Lag) M Laínz and *S. littorea* Brot., compound **10** isolated from *S. littorea* Brot which they are belonging to *Silene* genus (Mamadalievia et al., 2014), compound **7** glycoside derivative isolated from *S. conoidea* L. (Ullah et al, 2019). Also, compound **11** is a member of ecdysteroids family which considered as chemotaxonomical marker for genus *Silene* (Zibareva et al.,

2009; Mamadalievia et al., 2014). Compounds **1**-**6**, **8**, **12**-**15** were first time to be reported from genus *Silene*.

On the other hand, it is notable to indicate some of these compounds had been isolated before from plants belong to Caryophyllaceae family. Compounds **1**, **2** & **14** were isolated from *Dianthus caryophyllus* L. (Al- snafi, 2017); compound **8** was isolated from *Lychnis flos-cuculi* L. (López-Lázaro, 2009); compounds **3** & **15** were isolated from *Gypsophila sphaerocephala* L. (Altay et al., 2018) and compound **4** was isolated from *Fortunella japonica* Swingle (El-gizawy & Hussein, 2017). Also, compound **5** was isolated from *Stellaria media* Linn. (Sharma et al., 2012) compounds **6** & **12** were isolated from *Citrus sinensis* (L.) Osbeck (pro sp.) (Lahmer et al, 2015) and compound **13** was isolated from citrus fruits (Kawaii et al, 2000).

In summary, the present study certainly enriches the chemical diversity and provides evidence for plant chemotaxonomic studies of *S. schimperiana*.

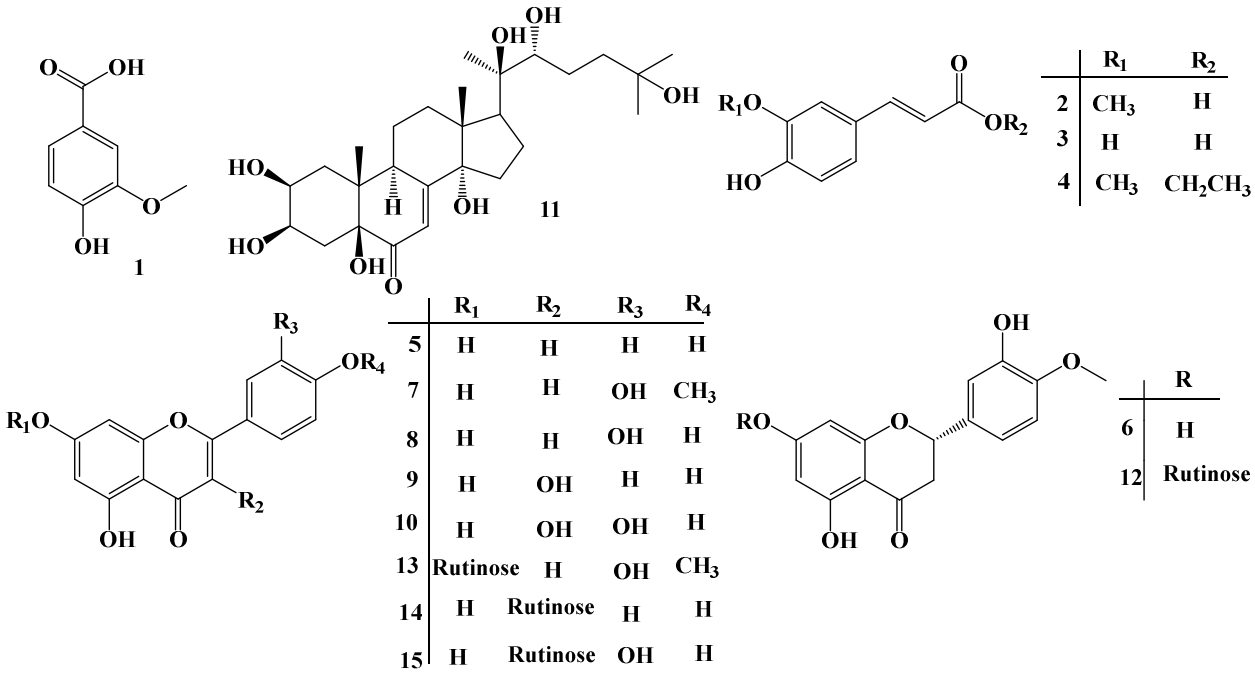

Figure 1: Chemical structure of the isolated compounds

Acknowledgements

The project was supported by Egyptian government, National Center for Natural Product Research, USA and in part by the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6060-6-015. The authors wish to thank Dr. Ibrahim El Garf for identification of plant material.

References

Aisyah, L.S., Yun, Y.F., Herlina, T., Julaeha, E., Zainuddin, A., Nurfarida, I., Hidayat, A.T., Supratman, U., Shiono, Y., 2017. Flavonoid compounds from the leaves of *Kalanchoe prolifera* and their cytotoxic activity against P-388 murine leukemia cells. Nat. Prod. Sci., 23, 139-445. https://doi.org/10.20307/nps.2017.23.2.139

Al-Snafi, A. E., 2017. Chemical contents and medical importance of *Dianthus caryophyllus*-A review. IOSR. J. Pharm., 7(3), 61-71. DOI: 10.9790/3013-0703016171

Altay, A., Degirmenci, S., Korkmaz, M., 2018. In vitro evaluation of antioxidant and anti-proliferative activities of *Gypsophila sphaerocephala* (Caryophyllaceae) extracts together with their phenolic profiles. J. Food Meas. Charact., 12(4), 2936-2945. https://doi.org/10.1007/s11694-018-9909-y

Bağcı, Y., Biçer, H., 2015. Analysis of anatomical and morphological characters of the *Silene cappadocica* Boiss. &Heldr. and *Silene spergulifolia* Bieb.(Caryophyllaceae) species. Sci, Papers Ser, B, Hortic., 59, 293-302. NO DOI

Bharate, S.B., Khan, S.I., Yunus, N.A., Chauthe, S.K., Jacob, M.R., Tekwani, B.L., Khan, I.K., Singh, I.P., 2007. Antiprotozoal and antimicrobial activities of O-alkylated and formylated acylphloroglucinols. Bioorg, Med, Chem., 15, 87-96. DOI: 10.1016/j.bmc.2006.10.006

Chandra, S., Rawat, D.S., 2015. Medicinal plants of the family Caryophyllaceae: a review of ethno-medicinal uses and pharmacological properties. Integr, Med, Res., 4, 123-131. https://doi.org/10.1016/j.imr.2015.06.004.

Cheikh-Ali, S., Farman, M., Lacaille-Dubois, M. Semmar, N., 2019. Structural organization of saponins in Caryophyllaceae. Phytochem. Rev., 18**,** 405–441. https://doi.org/10.1007/s11101-019-09600-8.

da Silva, L.A., Faqueti, L.G., Reginatto, F.H., dos Santos, A.D., Barison, A., Biavatti, M.W., 2015. Phytochemical analysis of *Vernonanthura tweedieana* and a validated UPLC-PDA method for the quantification of eriodictyol. Rev, Bras, Farmacogn., 25, 375-381. https://doi.org/10.1016/j.bjp.2015.07.009

Dehaghani, Z.A., Asghari, G., Dinani, M.S., 2017. Isolation and Identification of Nicotiflorin and Narcissin from the Aerial Parts of *Peucedanum aucheri* Boiss. J.A.S.T.A.,.7, 45-51. DOI: 10.17265/2161-6256/2017.01.007

El-gizawy, H.A., Hussein, M.A., 2017. Isolation, Structure Elucidation of Ferulic and Coumaric acids from *Fortunella japonica* Swingle leaves and their Structure Antioxidant activity relationship. Free Radicals and Antioxidants, 7, 23-30. DOI: 10.5530/fra.2017.1.4

Fang, L., Li, J., Zhou, J., Wang, X., Guo, L., 2017. Isolation and purification of three ecdysteroids from the stems of *Diploclisia glaucescens* by high-speed countercurrent chromatography and their anti-inflammatory activities *in vitro*. Molecules, 22, 1310. doi: 10.3390/molecules22081310

Fathiazad, F., Delazar, A., Amiri, R., Sarker, S.D., 2010. Extraction of flavonoids and quantification of rutin from waste tobacco leaves. Iran J. Pharm., 20, 222-227. DOI: 10.22037/IJPR.2010.680

Golea, L., Benkhaled, M., Lavaud, C., Long, C., Haba, H., 2017. Phytochemical components and biological activities of *Silene arenarioides* Desf. Nat. Prod. Res., 31, 2801-2805. doi: 10.1080/14786419.2017.1294174.

Gopalakrishnan, V., Pillai, S.I., Subramanian, S.P., 2015. Synthesis, spectral characterization, and biochemical evaluation of antidiabetic properties of a new zinc-diosmin complex studied in high fat diet fed-low dose streptozotocin induced experimental type 2 diabetes in rats. Biochem. Res. Int., 2005, Article ID 350829, 11 pages. https://doi.org/10.1155/2015/350829

Hosny, A.I., El Hadidi, M.N., Shamso, E., 1993. Taxonomic studies of Silenoideae (Caryophyllaceae) in Egypt: Systematic revision of the genus Silene L. Taeckholmia, 14, 1-36 NO DOI.

Hu, J., Ma, W., Li, N., Wang, K.J., 2017. Antioxidant and Anti-Inflammatory Flavonoids from the Flowers of Chuju, a Medical Cultivar of *Chrysanthemum Morifolim* Ramat. J. Mex. Chem., 61, 282-9. DOI: 10.29356/jmcs.v61i4.458

Hussein, I.A., Srivedavyasasri, R., El-Hela, A.A., Mohammad, A.I., Ross, S.A., 2019. Antimicrobial secondary metabolites from *Silene rubella* growing in Egypt. JBPR., 8, 81-84. https://doi.org/10.32553/jbpr.v8i6.694

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M., Koizumi, M., Ito, C., Furukawa, H. 2000. Quantitative study of flavonoids in leaves of Citrus plants. J. Agric. Food Chem., 48, 3865-3871. DOI: 10.1021/jf000100o

Lahmer, N., Belboukhari, N., Cheriti, A., Sekkoum, K., 2015. Hesperidin and hesperitin preparation and purification from *Citrus sinensis* peels. Der Pharm. Chem., 7, 1-4. NO DOI

Liu, H., Mou, Y., Zhao, J., Wang, J., Zhou, L., 2010. Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities. Molecules. 15, 7933-7945. doi: 10.3390/molecules15117933

López-Lázaro, M., 2009. Distribution and biological activities of the flavonoid luteolin. Mini Rev. Med. Chem. 9, 31-59. DOI: 10.2174/138955709787001712

Ma, G., Khan, S.I., Jacob, M.R., Tekwani, B.L., Li, Z., Pasco, D.S., Walker, L.A., Khan, I.A., 2004. Antimicrobial and antileishmanial activities of hypocrellins A and B. Antimicrob. Agents Chemother. 48, 4450-4452. DOI: 10.1128/AAC.48.11.4450-4452.2004

Maltese, F., Erkelens, C., van der Kooy, F., Choi, Y.H., Verpoorte, R., 2009. Identification of natural epimeric flavanone glycosides by NMR spectroscopy. Food Chem., 116, 575-579. https://doi.org/10.1016/j.foodchem.2009.03.023

Mamadalieva, N.Z., 2012. Phytoecdysteroids from Silene plants: distribution, diversity and biological (antitumour, antibacterial and antioxidant) activities. B. Latinoam. Caribe. Pl., 11, 474-497. NO DOI.

Mamadalieva, N.Z., Lafont, R., Wink, M., 2014. Diversity of secondary metabolites in the genus Silene L.(Caryophyllaceae)—structures, distribution, and biological properties. Diversity, 6(3), 415-499. https://doi.org/10.3390/d6030415

Manohar, S., Tripathi, M., Rawat, D.S., 2014. 4-aminoquinoline based molecular hybrids as antimalarials: an overview. Curr. Top. Med. Chem., 14, 1706-1733. DOI: 10.2174/1568026614666140808125728

Olennikov, D.N., 2019. Ecdysteroids, Flavonoids, and Phenylpropanoids from *Silene nutans*. Chem. Nat. Compd., 55, 127–130. https://doi.org/10.1007/s10600-019-02632-8.

Olennikov, D.N., Chirikova, N.K., 2019. C-Glycosyl Flavones from Two Eastern Siberian Species of Silene. Chem. Nat. Compd., 55, 642–647. (https://doi.org/10.1007/s10600-019-02768-7.

Olennikov, D.N., Kashchenko, N.I., 2019. Glycosylflavones from *Silene armeria* and *S. compacta*. Chem. Nat. Compd., 55, 934–936. https://doi.org/10.1007/s10600-019-02851-z.

Radwan, M.M., Rodriguez-Guzman, R., Manly, S.P., Jacob, M., Ross, S.A., 2009. Sepicanin A—a new geranyl flavanone from *Artocarpus sepicanus* with activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Phytochem. Lett., 2, 141-143. doi: 10.1016/j.phytol.2009.04.002

Saatov, Z., Gorovits, M.B., Abubakirov, N.K., 1987. Phytoecdysteroids of plants of the genus Silene XIII. Ecdysterone 20,22-monoacetonide from *Silene scabrifolia* . Chem. Nat. Compd., 23, 643. https://doi.org/10.1007/BF00598705.

Seo, C., Shin, H.S., Lee, J.E., Jung, Y.W., Kim, J.K., Kwon, J.G., Jeong, W., Choi, C.W., Oh, J.S. and Hong, S.S., 2020. Isolation and structure elucidation of siliendines A–D, new β-carboline alkaloids from *Silene seoulensis*. Phytochem. Lett., 36, 58-62. https://doi.org/10.1016/j.phytol.2020.01.010

Sharma, A., Arora, D., 2012. Phytochemical and pharmacological potential of genus Stellaria: a review. J. Pharma. Res., 5(7), 3591-3596. NO DOI.

Tošović, J., 2017. Spectroscopic features of caffeic acid: Theoretical study. Kragujevac. J. Sci., 39, 99-108. DOI: 10.5937/KgJSci1739099T.

Ullah, F., Ayaz, A., Saqib, S., Zaman, W., Butt, M.A., Ullah, A. 2019. *Silene conoidea* L.: A review on its systematic, ethnobotany and phytochemical profile. Plant Sci. Today, 6, 373-382. DOI: https://doi.org/10.14719/pst.2019.6.4.571

Zeid, A.H., Hifnawy, M.S., Mohammed, R.S., 2009. Phenolic compounds and biological activities of *Dichrostachys cinerea* L. Med. Aromat. Plant. Sci. Biotechnol., 3, 42-49. NO DOI.

Zibareva, L.N., 2009. Phytoecdysteroids of Caryophyllaceae Juss. Contemp. Probl. Ecol., 2, 476-488. DOI: 10.1134/S1995425509050154.