Research Journal of Pharmacognosy (RJP) 2(3), 2015: 43-47
Received: Nov 2014
Accepted: Jan 2015

Original article

Phytochemical evaluation and antioxidant activity of *Verbascum sublobatum* Murb. leaves

A.R. Shakeri[1*], A. Farokh[2]

[1]*School of Chemistry, College of Science, University of Tehran, Tehran, Iran*.
[2]*Department of Chemistry, University of Golestan, Gorgan, Iran.*

Abstract

Background and objectives: The genus *Verbascum*, with nearly 360 species, is one of the largest members of Schrophulariacea family. In the Flora of Iran, the genus *Verbascum* is represented by 43 species among them seventeen plants are endemic. *Verbascum* species are well known in folk medicine and are widely used for therapeutic purposes. *Verbascum sublobatum* Murb. grows wildly in north of Iran. Literature review has shown that there is no report on phytochemical investigation about *V. Sublobatum* leaves. In the present study, phytochemicals of the plants have been isolated and the antioxidant activity of the extracts from leaves of *Verbascum sublobatum* Murb. has been evaluated. **Methods**: Dried and powdered plant were extracted with 70% methanol and then partitioned by chloroform, ethyl acetate, and buthanol. The ethyl acetae fraction showed the strongest DPPH radical scavenging activity among the three fractions and was subjected to separation and identification. The separation and purification process were performed using various chromatographic methods. Structural elucidation was carried out on the basis of FT-IR, NMR and UV data. **Results:** The isolated compounds which had flavonoid structure, were identified as apigenin and luteolin. **Conclusion:** The isolated compounds have been previously reported from other species of *Verbascum* which demonstrates the chemotaxonomic significance of the isolated compounds.

Keywords: apigenin, flavonoid, luteolin, schrophulariacea, *Verbascum sublobatum*

Introduction

Plants of the genus *Verbascum* are rich sources of flavonoids and saponins and some of them are used in folk medicine as anesthetic and wound-healing agents in treatment of burns and tumors, in revealing toothache and inflammation of the eyes, and as an expectorant in chronic coughs [1]. The genus *Verbascum* L. (common name mullein), has been predominantly distributed in Asia, Europe, and North America [2,3]. The efficacy of mullein as a mild expectorant, demulcent and emollient is because of the presence of saponins and mucilage [4,5]; whereas its anti-inflammatory, antimicrobial and diuretic activity are believed to be connected with the phenolic compounds, flavonoids and phenylethanoids [6,7]. The genus *Verbascum* with 360 species [8] world wide and 43 species in Iran [9] belong to Verbasceae (Scrophulariaceae),

Available at: http://rjpharmacognosy.ir
[*]*Corresponding author: alireza.shakeri@ut.ac.ir*, Tel/Fax: +9821-66495291

is one of the largest genera of the family. Among the species distributed in Iran, 17 are endemic.
Verbascum sublobatum Murb, is one of these species which grows wildly in northern parts of Iran [10]. Several *Verbascum* species are known as antiseptic, antimalarial, astringent, demulcent, emollient, sedative, and narcotic and have been used for treatment of tumors, inflammations, migraine, asthma and spasmodic coughs in Europe, Asia and Northern America [11]. Phytochemical studies of *Verbascum* species have revealed the presence of saponins, iridoids and phenylethanoid glycosides, mono terpeneglucosides, neolignanglucosides, flavonoids, steroids and spermine alkaloids [12].
In the present study, isolation and identification of some flavonoids have been reported from the leaves extract of *Verbascum sublobatum*.

Experimental

General experimental procedures and materials
^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker Avance 400 DRX spectrometer with tetramethyl silane as the internal standard and chemical shifts have been reported in δ (ppm). Vacuum column chromatography was carried out using silica gel (35-70 mesh) obtain from Merck (Darmastadt, Germany). Silica gel 60 F_{254} pre-coated plates (Merck, Germany) were used for TLC. The spots were detected using UV at 254 and 366 nm after spraying with vanillin-H_2SO_4 reagent followed by heating. The FTIR spectra were recorded on a Perkinelmer spectrophotometer. The UV/VIS spectra were recorded on a PG Instrument Ltd UV/VIS spectrophotometer.
DPPH was purchased from Sigma-Aldrich (Steinheim, Germany). All reagents were of analytical grade.

Plant material
The leaves of *Verbascum sublobatum* Murb. were collected during the flowering stage from Golestan province (near Gorgan), Iran in June 2011 and were dried at room temperature. They were then identified at the Herbarium of Faculty of Science, Golestan University.

Extraction and isolation
The dried and powdered plant material (350 g) was exhaustively extracted with 70% MeOH using maceration method three times, each time at least for 48 h. The extract was the concentrated, dissolved in water and then filtered and partitioned with $CHCl_3$, EtOAc and *n*-BuOH.
The EtOAc fraction (2.7 g) was subjected to vacuum column chromatography on silica gel and was eluted with *n*-hexane-ethyl acetate in a gradient way by increasing polarity (60:40, 50:50, 40:60, etc., 50 mL each). Fractions were combined according to their TLC pattern.
Fraction A_3 (68 mg) was subjected to silica gel column chromatography (CC) with MeOH-$CHCl_3$ (1:9) as the eluent to obtain compound 1 (9 mg). Fraction A_4 (119 mg) was subjected to silica gel CC with using MeOH-$CHCl_3$ (1:4) as the mobile phase to obtain compound 2 (12 mg).

DPPH radical scavenging activity
The method described by Tepe *et al.* was used [13]. One mL of 500 μM DPPH in methanol was mixed with equal volume of the extract solution in phosphate buffer (pH 7.4). The mixture was slightly shaken and kept in dark for 20 min. The absorbance at 517 nm was monitored in presence and absence of different concentrations of the extracts.
Catechin was used as the standard. The antioxidative property of catechin is manifested particularly by its ability to inhibit and scavenge free radicals.

Results and Discussion

The order of antioxidant activity of different fractions of *Verbascum sublobatum* was as follows: chloroform fraction < buthanol fraction <ethyl acetate fraction < catechin. The EtOAc fraction showed the lowest IC_{50} value in DPPH radical scavenging activity among three fractions. The result showed that the compounds with relatively high antioxidant activity might be

present in this fraction. Therefore, the EtOAc fraction was subjected to further separation and identification.

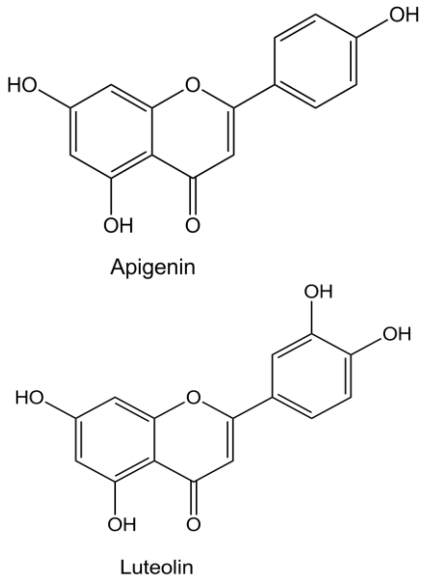

Figure 1. The structures of the purified compounds of *Verbascum sublobatum* Murb.

The pharmacological effects of phenolic compounds and flavonoids are linked to their ability to act as strong antioxidants and free radical scavengers, to chelate metals, and to interact with enzymes, adenosine receptors, and bio-membranes [14]. Among different fractions, the ethyl acetate fraction showed the lowest IC_{50} (15.2 µg/mL) indicating the highest scavenging activities whereas the chloroform fraction showed the least scavenging activity (EC_{50} 41.6 µg/mL) compared to catechin (IC_{50} 3.6 µg/mL). The ethyl acetate and butanol fractions showed reasonable radical scavenging activity. This might be due to the presence of high content of phenols, since polyphenols play an important role as antioxidants in living systems resulting from the presence of hydroxyl groups in *ortho*- and *para*- positions [15].

The EtOAc-soluble parts of the hydromethanolic extract of the leaves of *V. sublobatum* were subjected to vacuum column chromatography and CC and yielded known compounds including two flavonoids.

Apigenin and luteolin (figure 1) were identified from the leaves of *Verbascum sublobatum* Murb based on the spectroscopic spectra (^{1}H-NMR, ^{13}C-NMR and FT-IR) compared to literature [16,17]. To the best of our knowledge, there is no report about the isolation and structural elucidation of these compounds from the leaves of *Verbascum sublobatum* Murb. Literature rewires show that flavonoids have been intensively studied for their pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial and anticancer properties. Flavonoids are also common constituents of plants used in traditional medicine to treat a wide range of diseases [18]. Both apigenin, and luteolin, have shown various pharmacological activities. Apigenin, a common dietary flavonoid abundantly present in fruits and vegetables, is believed to possess preventive and therapeutic potential against cancers. The anti-proliferative property of apigenin has also been evaluated [19-20]. Dietary sources of luteolin include, for instance, carrots, peppers, celery, olive oil, peppermint, thyme, rosemary and oregano [21]. The ability of luteolin to inhibit angiogenesis, to induce apoptosis, to prevent carcinogenesis in animal models, to reduce tumor growth *in vivo* and to sensitize tumor cells to the cytotoxic effects of some anticancer drugs suggests that this flavonoid has cancer chemopreventive and chemotherapeutic potential [22]. luteolin possesses potent estrogenic activity at low concentrations, it could be a useful agent for hormone replacement therapy [23]. luteolin was found to inhibit the metabolism of carcinogens that generates active mutagens in live. Luteolin may also suppress angiogenesis by stabilizing hyaluronic acid, a neovascularization barrier [24-25]. Luteolin is also reported to inhibit NO production [26] and active oxygen species [27]. Those findings may be related to the anti-inflammatory and anti-allergic actions; however, most of them are *in vitro* findings. These two flavonoides have also been recognized as

neuroprotective and neurotrophic agents. Apigenin and luteolin protected the dopaminergic neurons probably by reducing oxidative damage, neuron inflammation and microglial activation along with enhanced neurotrophic potential [28]. In conclusion, the results of this research show that the main compounds of *Verbascum sublobatum* Murb. could be biologically and pharmacologically active flavonoids.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Yuldashev MP. Flavonids of the roots of *Verbascum songoricum*. *Chem Nat Compd*. 1996; 32(6): 925-931.

[2] Heywood VH. *Flowering plants in the world.* New York: Oxford University Press, 1993.

[3] Klimek B, Olszewska MA, Tokar M. Simultaneous determination of flavonoids and phenylethanoids in the flowers of *Verbascum densiflorum* and *V. phlomoides* by high-performance liquid chromatography. *Phytochem Anal*. 2010; 21:150-156.

[4] Klimek B. Hydroxycinnamoyl ester glycosides and saponins from flowers of *Verbascum phlomoides*. *Phytochemistry*. 1996; 43: 1281-1284.

[5] Willuhn G. *Verbasciflos*. In: *Herbal Drugs and Phyto-pharmaceuticals*. Wichtl M, Ed. Stuttgart: Medpharm, 2004.

[6] Rigano D, Formisano G, Basile A, Lavitola A, Senatore F, Rosselli G, Bruno M. Antibacterial activity of flavonoids and phenylpropanoids from *Marrubium globosum* ssp. *Libanoticum. Phytother Res.* 2007; 21: 395-397.

[7] Turker AU, Gurel LE. Common mullein (*Verbascum thapsus* L.): recent advances in research. *Phytother Res*. 2005; 19: 733-739.

[8] Judd WS, Campbell CS, Kellogg EA, Stevens PF. *Plant systematics: a phylogenetic approach*. Sinauer: Sunderland, 1999.

[9] Rechinger, KH. *Verbascum* L. in: *Flora Iranica*. Graz: Akademische Druck-U. Verlagsanstalt, 1981.

[10] Grieve M. *A Modern Herbal*. New York: Barnes and Noble Books, 1996.

[11] Tatli II, Akdemir ZS. Chemical constituents of *Verbascum* species. *J Pharm Sci*. 2004; 29: 93-107.

[12] Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chem*. 2005; 90: 333-340.

[13] Middleton EJ, Kandaswami C. *The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer*. In: Harborne JB, Ed. *The Flavonoids: Advances in research since 1986*. London: Chapman & Hall, 1994.

[14] Lapornik A, Prosek M, Wondra GA. Comparision of extracts prepared from plant byproducts using different solvents and extraction time. *J Food Eng*. 2005; 71: 214-222.

[15] Fathiazad F, Delazar A, Amiri R, Sarker SD. Extraction of flavonoids and identification of rutin from waste tobacco leaves. *Iran J Pharm Res.* 2006; 3: 222-227.

[16] Kang J, Li Z, Wu T, Jensen GS, Schauss AG, Wu X. Anti-oxidant capacities of flavonoid compounds isolated from acai pulp (*Euterpe oleracea* Mart.*). Food Chem*. 2010; 122: 610-617.

[17] Kim HP, Son KH, Chang HW, Kang SS. Anti-inflammatory plant flavonoids and cellular action mechanisms. *J Pharmacol Sci*. 2004; 96(3): 229-245.

[18] Chiang LC, Ng LT, Lin I, Kuo PL, Lin CC. Anti-proliferative effect of apigenin and its apoptotic induction in human HepG-2 cells. *Cancer Lett*. 2006; 237(2): 207-214.

[19] Yin F, Giuliano AE, Law RE, Van Herle AJ. Apigenin inhibits growth and induces G2/M arrest by modulating cyclin-CDK regulators and ERK MAP kinase activation

in breast carcinoma cells. *Anticancer Res*. 2001; 21(1A): 413-420.

[21] Lopez-Lazaro M. Distribution and biological activities of the flavonoid luteolin. *Mini-Rev Med Chem*. 2009; 9(1): 31-59.

[22] Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potentials for cancer prevention and therapy. *Curr Cancer Drug Tar*. 2008; 8(7): 634-646.

[23] Zand RS, Jenkins DJ, Diamandis EP. Steroid hormone activity of flavonoids and related compounds. *Breast Cancer Res Tr*. 2000; 62: 35-49.

[24] Buening MK, Chang RL, Huang MT, Fortner JG, Wood AW, Conney AH. Activation and inhibition of benzo(a)pyrene and aflatoxin B1 metabolism in human liver microsomes by naturally occurring flavonoids. *Cancer Res.* 1981; 41: 67-72.

[25] Huang MT, Wood AW, Newmark HL, Sayer JM, Yagi H, Jerina DM, Conney AH. Inhibition of the mutagenicity of bay-region diolepoxides of polycyclic aromatic hydrocarbons by phenolic plant flavonoids. *Carcinogenesis*. 1983; 4: 1631-1637.

[26] Nimnual AS, Taylor LJ, Bar-Sagi D. Redox-dependent down regulation of Rho by Rac. *Nat Cell Biol*. 2003; 5: 236-241.

[27] Robak J, Shridi F, Wolbis M, Krolikowska M. Screening of the influence of flavonoids on lipoxygenase and cyclooxygenase activity, as well as on nonenzymic lipid oxidation. *Pol J Pharmacol Pharm*. 1988; 40: 451-458.

[28] Patil SP, Jain PD, Sancheti JS, Ghumatkar PJ, Tambe R, Sathaye S. Neuroprotective and neurotrophic effects of apigenin and luteolin in MPTP induced Parkinsonism in mice. *Neuropharmacology*. 2014; 86:192-202.