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

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**Abstract**

*Background and objectives:* The genus *Verbascum*, with nearly 360 species, is one of the largest members of Schrophulariaceae 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, schrophulariaceae, *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),
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, monoterpeneglucosides, 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*  
1H-NMR and 13C-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 (Darmstadt, Germany). Silica gel 60 F254 pre-coated plates (Merck, Germany) were used for TLC. The spots were detected using UV at 254 and 366 nm after spraying with vanillin-H2SO4 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 CHCl3, 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 A3 (68 mg) was subjected to silica gel column chromatography (CC) with MeOH-CHCl3 (1:9) as the eluent to obtain compound 1 (9 mg). Fraction A3 (119 mg) was subjected to silica gel CC with using MeOH-CHCl3 (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 IC50 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 flavonoids 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


