PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF VERBASCUM BLATTARIA L.

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PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF VERBASCUM BLATTARIA L. (Abstract): Aim: The HPLC-ESI-Q-TOF-MS phytochemical profile and in vitro antioxidant activity of extracts obtained from the aerial parts of Verbascum blattaria were investigated. Material and methods: Phytochemical screening was performed by a hyphenated LC-MS technique, whereas total phenolic content was assessed by Folin-Ciocalteu method. Antioxidant activity was evaluated using four different in vitro tests: hydroxyl and DPPH radicals scavenging, ferrous ion chelating and ferric ion reducing assays. Results and discussion: A total of 17 secondary metabolites (carboxylic acids, flavonoids, iridoids, phenylethanoids and saponins) were identified in V. blattaria extracts. Among the four samples, n-butanol extract was the most potent scavenger of the hydroxyl and DPPH radicals, with EC50 values of 224.14 and 591.67 µg/mL, respectively. Additionally, the same extract exhibited an EC50 of 193.51 µg/mL in the ferric ion reducing assay. Conclusions: The current work allowed concluding that n-butanol extract of V. blattaria is the most promising in relation to antioxidant potential; the in vivo significance of its antioxidant effects needs to be further evaluated. Keywords: VERBASCUM BLATTARIA, IRIDOIDS, ANTIOXIDANT, HYDROXYL RADICAL, LC-MS.

With more than 350 species worldwide distributed, Verbascum L. is the largest genus of Scrophulariaceae family (1). In the traditional folk medicine, the roots, leaves and flowers have been used since ancient times mainly as remedies for respiratory tract disorders, such as bronchitis, cough, tuberculosis or asthma (2). Verbascum species (mulleins) attracted researchers’ attention in the past 30 years, as they were found to be a very rich source of bioactive constituents (iridoids, phenylethanoids, saponins, flavonoids) with confirmed anti-inflammatory, antioxidant, antimicrobial or cytotoxic properties (3). Despite the fact that numerous mullein species have been already scientifically approached (1), new species are continuously discovered, whilst some known ones are currently very little investigated. It is the case of Verbascum blattaria L. (moth mullein), for which literature is not very abundant. In one study, 11 iridoid glycosides have been identified by thin-layer chromatography (4), whereas in another one, four iridoid and flavonoid glycosides have been isolated (5). The aim of this study was
to perform the phytochemical screening of various extracts obtained from the aerial parts of *V. blattaria* using different polarity solvents and evaluate for the first time their *in vitro* antioxidant activities.

**MATERIAL AND METHODS**

**Plant material**

The aerial parts of *Verbascum blattaria* L. were collected in Răscăieți (Ștefan Vodă County, Republic of Moldova) in June 2014. The plants were dried, milled to powder and deposited in the Department of Pharmacognosy, Faculty of Pharmacy, “Grigore T. Popa” University of Medicine and Pharmacy, Iasi, Romania (voucher specimen VB2806/2014).

**Extraction**

Five grams of powdered plant material were subjected to ultrasound extraction for three cycles of 30 minutes each, with 50 mL of solvent. Extracts were filtered, combined and dried under reduced pressure. *n*-Hexane (HE, 0.015 g, yield: 0.15%), ethyl acetate (EAE, 0.025 g, yield: 0.26%), *n*-butanol (BE, 0.19 g, yield: 3.80%) and aqueous (AQE, 0.840 g, yield: 16.8%) extracts resulted.

**HPLC-ESI-Q-TOF-MS analysis**

LC-MS analysis was performed using an Agilent 1260 HPLC equipped with a G1312C binary pump, G1322A degasser, G1316A column oven, G1315 D diode array detector (DAD), G1320B autosampler and a G6530B quantum-time-of-flight-mass spectrometer (Q-TOF-MS) with an electrospray ionization (ESI) source. Separation was carried out on a Zorbax RP-18 (150 × 2.1 mm, 3.5 μm) column. The mobile phase consisted of: solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). A linear gradient varying from 0% B (0 min) to 55% B (40 min) was used, at a flow rate of 0.2 mL/min. Analyses were performed in negative ionization mode with the additional parameters: nitrogen gas flow rate 12 L/min; nebulizer pressure 40 psig; vaporizer temperature 300°C; capillary voltage 4000 V; fragmentor 140 V; skimmer 65 V; 100–1700 m/z; auto-tandem MS (MS/MS) acquisition mode; collision induced dissociation 20 eV.

**Total phenolic and flavonoid contents**

Total phenolic and flavonoid contents were estimated using Folin-Ciocalteu assay (6) and the method of Ozsoy *et al.* (7), respectively. Results were expressed as mg gallic acid equivalents (GAE)/g extract for total phenolics and mg catechin equivalents (CE)/g extract for flavonoids.

**In vitro antioxidant assays**

DPPH radical scavenging and ferric ion reducing assays were performed as described in Aprotosoaie *et al.* (8), hydroxyl radical scavenging as in Cretu *et al.* (9) and ferrous ion chelating assay as in Luca *et al.* (10). DPPH radical-scavenging activity (%) was calculated according to the following equation: % = \(\frac{\text{absorbance of DPPH solution} - \text{absorbance of DPPH solution with sample after 5 min}}{\text{absorbance of the DPPH solution}}\) × 100. Ferrous ion chelating and hydroxyl radical scavenging activities (%) were calculated as follows: % = \(\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}}\) × 100.

**Statistical analysis**

All experiments were conducted in triplicate. The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Student’s *t* test; *p* < 0.05 was considered statistically significant. The efficient concentration 50 (EC₅₀) values were calculated by linear interpolation between absorbances above and below 0.5000 in ferric ion reducing assay and activities above and below 50% in all other tests.
RESULTS AND DISCUSSION

HPLC-ESI-Q-TOF-MS analysis

The four extracts of *V. blattaria* by using different polarity solvents (*n*-hexane, ethyl acetate, *n*-butanol and water) were subjected to a rapid HPLC-ESI-Q-TOF-MS analysis to systematize the information regarding their phytochemical composition. Two compounds (verbascoside and luteolin-7-*O*-glucoside) were identified by additionally injecting the corresponding commercially available standards. The other constituents were tentatively identified by comparing their MS data with those reported in the literature (11) and METLIN database (12). A total of 17 secondary metabolites were identified (fig. 1), grouped into several classes: carboxylic acids (compounds 1–5), flavonoids (compounds 7 and 10), iridoids (compounds 6, 9, 11–15), phenylethanoids (compound 8) and saponins (compounds 16 and 17). Acylated derivatives of 6-*O*-rhamnosylcatalpol, such as *p*-coumaroyl acetyl 6-*O*-rhamnosylcatalpol and *cinnamoyl* acetyl 6-*O*-rhamnosylcatalpol, were the most abundant iridoids. This subcategory of compounds is known to be well represented in *Verbascum* species, with more than 30 congeners reported up to date (1). Moreover, verbascoside and luteolin-7-*O*-glucoside are among the most frequent constituents, being identified in almost all investigated mullein species (1). Several noteworthy qualitative differences between the four types of *V. blattaria* extracts were observed. In HE (the least polar extract) very few metabolites were identified, whereas AQE showed the highest number of polar constituents (aliphatic carboxylic acids, such as malic acid, citric acid, quinic acid). EAE and, especially, BE contained metabolites with medium polarity (including here iridoids, phenylethanoids and saponins) that are known to possess intrinsic antioxidant, cytotoxic and anti-inflammatory properties (1).

![Fig. 1. Base peak chromatograms of extracts obtained from the aerial parts of *Verbascum blattaria*. 1 – caffeic acid glucoside (Rt 1.8 min); 2 – malic acid (Rt 2.3 min); 3 – citric acid (Rt 2.6 min); 4 – quinic acid (Rt 3.2 min); 5 – hydroxyvaleric acid glucoside (Rt 5.4 min); 6 – ajugol (Rt 10.0 min); 7 – apigenin pentoside (Rt 22.2 min); 8 – verbascoside (Rt 23.2 min); 9 – *p*-coumaroyl 6-*O*-rhamnosylcatalpol (Rt 24.0 min); 10 – luteolin-7-*O*-glucoside (Rt 25.0 min); 11, 12 – *p*-coumaroyl acetyl 6-*O*-rhamnosylcatalpol isomers (Rt 27.3 and 28.9 min); 13 – di-*p*-coumaroyl 6-*O*-rhamnosylcatalpol (31.1 min); 14 – cinnamoyl acetyl 6-*O*-rhamnosylcatalpol (Rt 32.6 min); 15 – *p*-coumaroyl diacetyl 6-*O*-rhamnosylcatalpol (33.2 min); 16 – mulleinsaponin IV (Rt 38.4 min); 17 – ilwensisaponin C (Rt 39.4 min).]
Phytochemical profile and antioxidant activity of *Verbascum blattaria* L.

**Total phenolic and flavonoid contents**

Total phenolic content of *V. blattaria* extracts varied between 1.53±0.21 mg GAE/g in HE and 52.41±1.13 mg GAE/g in BE. No significant differences were observed between total phenolic content in EAE and AQE (tab. I). These values were very similar to those reported in the aqueous extracts of *V. phlomoides* (51.3±0.3 mg GAE/g) and *V. thapsus* (52.3±0.3 mg GAE/g), but lower than those found in various extracts of other *Verbascum* species (74.1±0.4 –135.0±2.1 mg GAE/g) (13).

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPC (mg GAE/g extract)</th>
<th>TFC (mg CE/g extract)</th>
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<tbody>
<tr>
<td>HE</td>
<td>1.53±0.21a</td>
<td>–</td>
</tr>
<tr>
<td>EAE</td>
<td>43.90±1.86b</td>
<td>11.11±0.21a</td>
</tr>
<tr>
<td>BE</td>
<td>52.41±1.13c</td>
<td>26.34±0.32b</td>
</tr>
<tr>
<td>AQE</td>
<td>43.88±1.18b</td>
<td>9.66±0.38c</td>
</tr>
</tbody>
</table>

AQE aqueous extract; BE n-butanol extract; CE catechin equivalents; EAE ethyl acetate extract; GAE gallic acid equivalents; HE n-hexane extract; values in each row with different superscripts are significantly different (p < 0.05).

With regard to flavonoid content, BE possessed the highest amount (26.34±0.32 mg CE/g), whereas EAE and AQE had contents 2.3–2.8 times lower. No flavonoids were quantified in HE (tab. I). Mihailovic *et al* (13) reported a flavonoid content of 10.2±0.2–53.4±0.2 mg rutin equivalents/g in *V. nigrum*, *V. phlomides* and *V. thapsus*, whilst Karamian and Ghasemlou (14) showed that *V. nudicaule*, *V. sinuatuam* and *V. speciosum* contained 4.83±0.13–5.77±0.23 mg rutin equivalents/g.
**DPPH free radical scavenging activity**

*V. blattaria* extracts showed moderate scavenging activity against DPPH radical. At the highest tested concentration (667 µg/mL), EAE, BE and AQE scavenged 34.29±1.07%, 52.78±1.31% and 32.25±1.28% of the radical, respectively. At the same concentration, HE had very weak scavenging effects (3.59±0.28%). With respect to the EC$_{50}$ values, BE was the most active extract (591.67±25.58 µg/mL). This activity was, however, lower than that reported in literature for the methanolic and aqueous extracts of *V. thapsus*, *V. phlomoides* and *V. nigrum* (EC$_{50}$ = 60.7±1.7–257.0±4.6 µg/mL) (13).

**Hydroxyl radical scavenging activity**

Among the four extracts of *V. blattaria*, BE was the most active, with a hydroxyl scavenging activity of 71.61±1.34% at 470 µg/mL. At the same concentration, EAE and AQE were two times less efficient (31.22±0.93% and 26.91±1.81%, respectively), whereas HE was almost inactive (Fig. 3). EC$_{50}$ value was possible to be subtracted only for BE (224.14±10.35 µg/mL). As compared to the *n*-butanol extract of *V. ovalifolium* with a reported EC$_{50}$ of 490.49±2.57 µg/mL (11), BE of *V. blattaria* was a more efficient hydroxyl radical scavenger. However, the methanolic and water extracts of *V. thapsus*, *V. nigrum* and *V. phlomoides* generated lower EC$_{50}$ values (3.3±0.7–171.6±5.0 µg/mL) (13).

**Ferrous ion chelating activity**

The extracts obtained from *V. blattaria* exhibited poor ferrous ion chelating effects. At the concentration of 8 mg/mL, the most active extract, BE (11.69±1.29%), was followed by EAE (10.56±0.94%), AQE (8.17±0.77%) and HE (2.37±0.29%) (Fig. 4). Using a slightly different experimental protocol, Karamian and Ghasemlou (14) found that the methanolic extracts of *V. nudicaule*, *V. sinuatum* and *V. speciesum* exhibited chelating activities of 19.06±8.80%, 14.44±4.89% and 5.42±0.72%, respectively, at 1 mg/mL.

**Ferric ion reducing activity**

EAE and BE catalyzed the reduction of iron (III) to iron (II) in a linear dose-dependent manner, the absorbance (700 nm) increasing from 0.1725±0.0106 and 0.3153±0.0114, respectively, at 30 µg/mL, to 0.5949±0.0076 and 1.0611±0.0306, respectively, at 240 µg/mL (R$^2$=0.9999 and 0.9945, respectively). At the highest tested concentration, HE and AQE showed an absorbance of only 0.1633±0.0170 and 0.1344±0.0100, respectively (Fig. 5). Therefore, EC$_{50}$ values were calculated only for EAE (EC$_{50}$=193.51±6.68 µg/mL) and BE (EC$_{50}$=73.59±3.48 µg/mL). These values suggest that BE was approximately 2.5-fold more efficient in reducing iron (III). At a similar concentration (250 µg/mL), the methanolic extract of *V. thapsus* showed an absorbance of 1.162 at 700 nm, very close to that of BE (13). However, as compared to *n*-butanol extract of *V. ovalifolium* with a reported EC$_{50}$ of 15.56±0.47 µg/mL (11), BE of *V. blattaria* was 4.5 times less active.
CONCLUSIONS
In the current work, four extracts obtained from the aerial parts of *V. blattaria* by extraction with different polarity solvents were phytochemically screened by HPLC-ESI-Q-TOF-MS. Base peak chromatograms, together with evaluation of total phenolic and flavonoid contents, suggested that ethyl acetate, *n*-butanol and aqueous extracts contained the main metabolites responsible for the potential health benefits of this species. Indeed, *in vitro* antioxidant activity allowed concluding that *n*-butanol was the most potent DPPH and hydroxyl radicals scavenger, as well as the most efficient ferric ion reducing agent. In most tests, ethyl acetate, and aqueous extracts exhibited similar antioxidant activities, but significantly lower. Therefore, solvents with medium polarities, such as *n*-butanol, might have the ability to extract a higher number of mullein bioactive constituents, as compared to non-polar (*n*-hexane, ethyl acetate) and polar (water) solvents.

REFERENCES


12. ***METLIN:*** The original and most comprehensive MS/MS metabolite database. [https://metlin.scripps.edu/landing_page](https://metlin.scripps.edu/landing_page).


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**CIPROFLOXACIN - ALLY OR ENEMY?**

Aortic dissection is the most common fatal disease process of the aorta and is preceded in most cases by the aneurysm formation. Ciprofloxacin is one of the most often used antibiotic and it is prescribed to treat a broad spectrum of bacterial infections, including infected aortic aneurysm. Results of recent clinical studies revealed that treatment with fluoroquinolone is associated with an increased risk of acute aortic events. In a recent study, LeMaire *et al.*, have induced a sporadic aneurysm formation in mice with high-fat diet and low-dose angiotensin infusion, the effects of ciprofloxacin on smooth muscle cells and frequency of acute aortic events were studied. The results were stunning, incidence of aortic dissection and rupture was almost 10 times higher in the group with ciprofloxacin and this outcome was linked to the increased level and activity of matrix metalloproteinase. Thus, this drug should be used with caution in patients with aortic dilatation and those at high risk for acute aortic events (Scott A. LeMaire, Lin Zhang, Wei Luo, *et al*. Effect of Ciprofloxacin on Susceptibility to Aortic Dissection and Rupture in Mice. *JAMA Surg*. doi:10.1001/ jamasurg. 2018.1804).