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SHORT COMMUNICATION

Chemical constituents and HRESI-MS analysis of an Algerian endemic plant - *Verbascum atlanticum* batt. - extracts and their antioxidant activity

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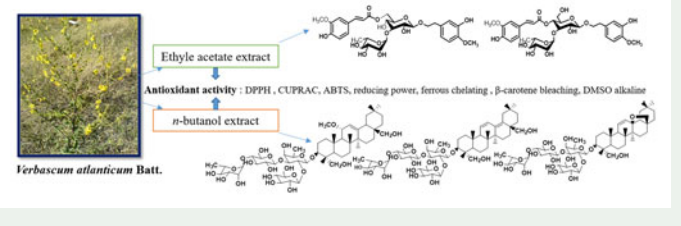
ABSTRACT

This is the first report on the phytochemistry and antioxidant activity of ethyl acetate and *n*- butanol extracts from an Algerian endemic plant *Verbascum atlanticum* Batt. (Scrophulariaceae). Both extracts were subjected to a phytochemical study by semi-preparative HPLC, which led to the isolation and identification of nine compounds: methyl linolenate (1), methyl linoleate (2), Phytol-1(3), Martynoside (4), Isomartynoside (5), *Cis*-martynoside (6), Ilwensisaponin C (7), Ilwensisaponin B (8), Ilwensisaponin A (9). In addition, the fractions from both extracts were analysed by LC-UV-MS and HRESI-MS. This later revealed the presence of eight other metabolites by using a comparison with known microbial metabolites data. Finally, both extracts were estimated for their phenolic and flavonoid contents as well as the evaluation of their antioxidant activity using five different assays DPPH, CUPRAC, reducing power, β-carotene bleaching and superoxide DMSO alkaline. The results showed that the ethyl acetate extract had the most antioxidant effect.



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1. Introduction

In this work, we investigated *Verbascum atlanticum* Batt. an Algerian endemic plant belonging to *Verbascum* genus (Benaissa et al. 2018). This genus is widely used in folk medicine to treat respiratory problems, eczema, and other types of inflammatory skin (Tatli et al. 2008). In addition, the isolated compounds from plants of this genus have several properties such as antioxidant activity (Ramunno et al. 2006). It is represented by 400 species distributed through the world (Sotoodeh et al. 2018), and it is well known for its variety of iridoids, saponosides and phenyl glycosides (Frezza et al. 2018) which are valuable for the taxonomic evaluation of this genus. In the present study, the ethyl acetate (VAA) and *n*-butanol (VAB) extracts of *V. atlanticum* were subjected to semi-preparative HPLC separation, this step led to the isolation and identification of nine compounds; those compounds are identified by NMR and comparison of spectral values with literature data. LC-UV-MS and HRESI-MS analysis of samples extracts allowed in the identification of eight other compounds from seven fraction where the DAD (UV − Vis) spectra, retention time, and positive and negative mass spectra of the samples are compared to the corresponding UV-LC-MS data of known microbial metabolites stored in the proprietary database library of Fundación MEDINA, Spain (Martín et al. 2014). Finally, both extracts were submitted to quantitative analysis as well as the evaluation of their antioxidant activity using different *in vitro* model assays.

2. Results and discussion

Both (VAA) and (VAB) extracts of *V. atlanticum* were chromatographed on silica gel column to give VAA1, VAA15 and VAB fractions respectively. The semi-preparative HPLC of subfractions VAA1-2, VAA15-3 and fraction VAB11, led to the isolation and identification of nine compounds named: methyl linolenate (1) (Chabert et al. 2006), methyl linoleate (2) (Huang et al. 2015), phytol-1(3) (Alarif et al. 2010), martynoside (4) (Khodaie et al. 2018), isomartynoside (5) (Caliş et al. 1984), *cis*-martynoside (6) (Skrzypek et al. 1999), ilwensisaponin C (7) (Caliş et al. 1993), Ilwensisaponin B (8) (El-Sayed et al. 2008), Ilwensisaponin A (9) (Caliş et al. 1993) (Figure 1). The chromatograms of isolated compounds (Figures S1, S2, and S3) are reported in the Supplementary Material. The spectroscopic data (NMR and MS) of all obtained compounds from our experiments were in good agreement with those reported in literature and are also reported in the Supplementary Material. In addition, all sub fractions obtained from chromatographic separation of both extracts of *V. Atlanticum* were submitted to LC-UV-MS analysis. This step permitted to choose nineteen samples which were injected in HR-ESI-MS. This later allowed to identifying eight other which are reported in (Table S1). These bioactives microbials metabolites were included in in the proprietary database library of Fundación MEDINA, Spain (Martín et al. 2014). The identification of phenylpropanoid glycosides (4), (5), (6) and saponin glycosides (7), (8) and (9) which were previously identified in *Verbascum* species is very interesting from the chemotaxonomic aspect of *Verbascum* genus (Frezza et al. 2018). On the other hand, the presence of these compounds is very important under the ethnopharmacological standpoint.

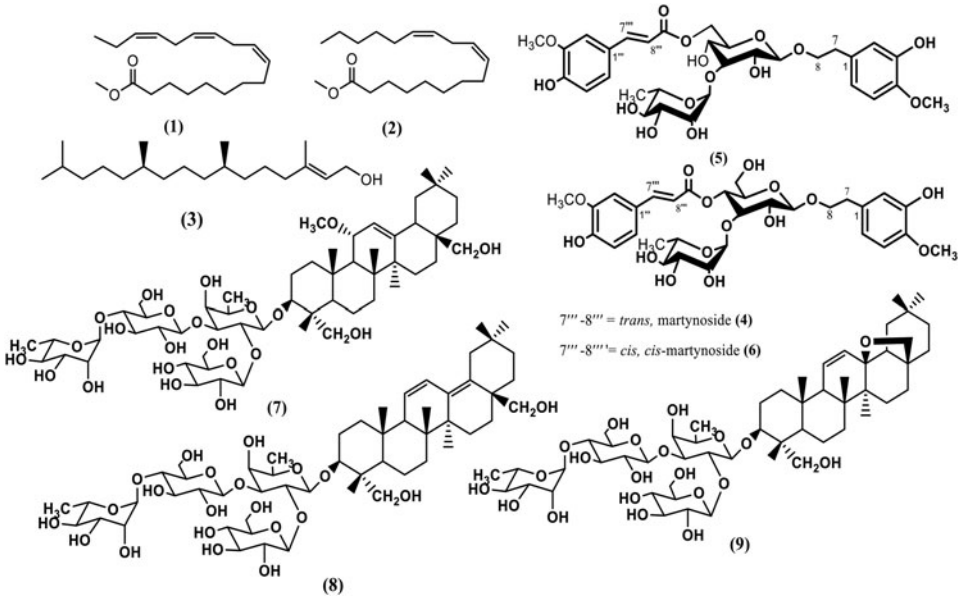

Figure 1. Identified compounds of VAA and VAB extracts.

2.1. Quantitative analysis

Both extracts were rich in phenolic and flavonoid contents. The (VAB) extract exhibited the highest value in polyphenol (416.84 ± 2.0 mg PEs/g extract) while (VAA) presented the highest value in flavonoid content (96.54 ± 3.64 mg QEs/g extract) (Table S2).

2.2. Antioxidant activity

It is suitable to use multiple methods of evaluation of antioxidant activity because of the difference in the mechanisms of action of antioxidants (Huang et al. 2005).The antioxidant potential of (VAA) and (VAB) extracts from the aerial parts of *V. Atlanticum* Batt. was assessed using five methods including radical scavenging assays (DPPH, CUPRAC, Reducing power, β-carotene bleaching as well as Alkaline DMSO assay) and the results are shown in (Table S3). All antioxidant methods used in the present study showed that the (VAA) has the higher activity results in comparison with (VAB) extract. The results of DPPH scavenging capacity showed that the (VAA) extract (IC_{50}:19.94 ± 0.06μg/mL) was active compared to standard compounds BHT (IC_{50}: 12.99 ± 0.41μg/mL), α-tocopherol (13.02 ± 5.17μg/mL) and ascorbic acid (13.94 ± 2.81μg/mL). In addition, the (VAA) extract exhibited the highest cupric reducing antioxidant capacity (IC_{50}: 11.90 ± 0.71μg/mL) in comparison with standards: ascorbic acid and α-tocopherol (IC_{50}: 12.43 ± 0.09; 19.92 ± 1.46 μg/mL respectively) and moderate compared with BHA (IC_{50}: 3.64 ± 0.19μg/mL). However, The (VAA) was found to be the best reducing agent ($A_{0.50}$: 23.81 ± 0.16μg/mL) in comparison with the (VAB) extract, better than α-tocopherol ($A_{0.50}$: 34.93 ± 2.38μg/mL), moderate compared with BHA and ascorbic acid ($A_{0.50}$: 7.99 ± 0.87; 6.77 ± 1.15μg/mL respectively). As known, the β-carotene bleaching method reveals the percentage of inhibition of lipid peroxidation. In this assay, the (VAA)

extract (IC_{50}:18.80 ± 0.65 μg/mL), was more active than ascorbic acid (IC_{50}: 52.59 ± 1. 98 μg/mL), but is lower than BHT, BHA and α-tocopherol (IC_{50}: 1.05 ± 0.01; 0.90 ± 0.02; 1.79 ± 0.03μg/mL respectively). The results of Superoxide DMSO alkaline assay were also presented in (Table S3). The (VAA) and (VAB) extracts (IC_{50}: 5.04 ± 0.21, 6.46 ± 0. 34μg/mL respectively) exhibited the highest inhibitory activity, even higher than all antioxidants standards used such as ascorbic acid and α-tocopherol (IC_{50}: 7.59 ± 1.16; 31.52 ± 2.22μg/mL respectively).

3. Conclusion

To our knowledge, this is the first report on the phytochemical study and antioxidant activity of *Verbascum atlanticum* Batt. extracts. From ethyl acetate and *n*-butanol extracts, nine compounds were isolated and identified using semi-preparative HPLC separation and NMR spectroscopic data from which six are known as taxonomic markers of *Verbascum* genus. In addition, eight other compounds were characterized by HRESI-MS analysis. The entire identified compounds are new for this species and known by their several biological activities. The EtOAc extract of *V. Atlanticum* exhibited the highest flavonoid content and the highest antioxidant effect in all tests.

Disclosure statement

No potential conflict of interest was reported by the authors.

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