SHORT COMMUNICATION

HPLC–DAD–ESI/MS profiles of bioactive compounds, antioxidant and anticholinesterase activities of Ephedra alata subsp. alenda growing in Algeria

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ABSTRACT

Ephedra (Ephedraceae) is used in medicine for various purposes as having, antioxidant, anticarcinogen, antibacterial, anti-inflammatory hepatoprotective, anti-obesity, antiviral and diuretic activities. In this study the aim was to investigate chemical constituents of Ephedra alata and understand the possible effects of those constituents in antioxidant activity and alzheimer’s disease essay. For this purpose, natural compounds from E.alata were characterized by LC–DAD–ESI-MS/MS using negative and positive ionization modes, while the bioactivity was assessed by acetylcholinesterase (AChE) inhibition study and determining of antioxidant activity; DPPH radical scavenging and β-carotene bleaching assays were used to assess the antioxidant potential. The proposed method of spectrometry provided tentative identification of 27 compounds including alkaloids and phenolic compounds as flavonoids. The methanolic extract showed high contents of total phenolic and exhibited an important antioxidant potential and demonstrated a potent inhibitory effect against acetylcholinesterase (IC\textsubscript{50}: 11,25 ± 0,25 μg/mL). The results showed that the plant possesses a therapeutic effect.

ARTICLE HISTORY

Received 20 August 2021
Accepted 21 December 2021

KEYWORDS

Ephedra alata subsp. alenda; flavonoids; phenolics; alkaloids; LC–DAD–ESI-MS/MS; antioxidant activity; acetylcholinesterase

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.2024184.

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1. Introduction

In living systems, oxidation is a basic part of the normal metabolic process and rapid production of free radicals may cause human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases (Pinton et al. 2012). Medicinal plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites (Zheng and Wang 2001; Cai et al. 2003).

*Ephedra* has been one of the best-known herbal remedies in Traditional Chinese Medicine for the treatment of allergies, bronchial asthma, chills, fever, flu, headache, colds hay, nasal congestion and central nervous system disorders (Bagheri-Gavkosh et al. 2009; Chebouat et al. 2014). In addition to the known components of the genus *ephedra* which are alkaloids (Al-Snafi 2017); phytochemical analyzes of *E. alata* have indicated the presence of phenolic compounds such as flavonoids, cardiac glycosides and reducing sugars (Jaradat et al. 2015). Therefore, the present study aims to characterize the phytochemical content and therapeutic value of the Algerian plant *E. alata* subsp. alenda.

The analytical technique used was the high-performance liquid chromatography-diode array detector with tandem mass spectrometry (HPLC–DAD–ESI-MS/MS), while the biological effect was evaluated by cholinesterase inhibition and antioxidant activities (DPPH• scavenging, β-carotene bleaching assays).

2. Results and discussion

2.1. Analysis of LC–MS/MS

The metabolic profiling of the aqueous-methanolic extract of *E. alata* subsp. alenda was performed by using HPLC-DAD-ESI-MS/MS. Figure S1 (Supplementary material) shows the base peak chromatograms (BPC) of *E. alata* alenda in the negative and positive ionization modes. Tables S1 and S2 (Supplementary material) showed a list of 27 compounds tentatively detected and characterized. These compounds were tentatively characterized by means of MS data, together with the interpretation of the observed MS/MS spectra in comparison with those found in the literature.

2.1.1. Negative ionization

This study showed that flavonoids are useful markers in *E. alata* alenda. Compound 04 corresponding to gallocatechin, this compound yielded [M-H]− at m/z 305. Its MS/MS fragmentations showed the major fragment at m/z 125 (Supplementary material, Table S1), which resulted from a dissociation at O1-C2 and C4-C10 (Supplementary material, Figure S2) (Yuzuak et al. 2018). The loss of Phloretin moiety occurred in peaks 10 and 13, both with m/z 435, which were characterized as Phloridzin isomer 1 and isomer 2, respectively (Zhao et al. 2014; Sut et al. 2019). Compound 14 was described as apigenin 6, 8-di C-glucoside (vicenin-2 isomer) with an [M−H]− at m/z 593 and typical C-glycosyl fragments at m/z 503, 473 and 383 (Ferreres et al. 2003; Brito et al. 2014).
Peaks 17, 21 and 25 gave the same pseudo molecular ion [M-H]⁻ at \( m/z \) 463 and have been tentatively assigned as Quercetin 3-O-glucoside isomers. Their MS/MS fragmentations showed the loss of Quercetin group by the fragment \( m/z \) 301 (Barros et al. 2013). Compound 15 corresponding to Catechin, this compound yielded [M-H]⁻ at \( m/z \) 289. Its MS/MS fragmentations showed the major fragment at \( m/z \) 151 corresponding the (B-ring of catechin + 76) (Supplementary material, Figure S3) (Cren-Olivé et al. 2000). Compound 22 presented a molecular ion signal at \( m/z \) 607 [M–H]⁻ and the observed fragmentation pattern was typical of diosmin (Zhang et al. 2011). Myricetin-3-O-glucoside was proposed for compound 23, this compound yielded a pseudo molecular ion [M-H]⁻ at \( m/z \) 479. It had a representative fragment at \( m/z \) 317 corresponding to Myricetin (Abu-Reidah et al. 2013).

Two other polar compounds were also represented by sinapic acid hexoside at \( m/z \) 385 and vanillic acid glucoside at \( m/z \) 329, which had in MS/MS fragmentation a major fragment at \( m/z \) 167 corresponding to vanillic acid molecule (Ammar et al. 2015).

2.1.2. Positive ionization

The Ephedra genus is known for its alkaloids, this is why a positive ionization study was necessary. Table S2 (Supplementary material) shows the list of 8 tentatively identified through LC/MS/MS/ESI (+). Compound 04 was characterized as norephedrine with \([M+H]^+\) ion at \( m/z \) 152. It had a representative fragment at \( m/z \) 91 corresponding to the deprotonated \( C_7H_7^+\). Ephedrine is proposed for compound 25, in MS/MS spectra the losses of water and methylamine gave the fragment ion at \( m/z \) 117, the MS/MS spectra gave also a major fragment ion at \( m/z \) 91 due to the loss of \( C_7H_7^+\) (Bijlsma et al. 2011). Methyl ephedrine appeared at 6,755 min with an [M–H]⁻ ion at 180, like the compounds mentioned above, its MS/MS spectra showed major fragment at \( m/z \) 91 which represented deprotonated \( C_7H_7^+\) (Food and Drug Administration et al. 2004). Compound 4 eluting at 4,214 min with an [M–H]⁻ ion at 164 is proposed as ephedrone (Bijlsma et al. 2011).

Positive mode revealed also the presence of other polar compounds such as cinnamic acid derivative, citropten, cyanidin rutinoside and Quercetin 3-O-rutinoside (Ammar et al. 2015).

2.2. Total phenolic and flavonoid contents

The results obtained of TPC, TFC of *E. alata* alenda extract are presented in Table S3 (Supplementary material). The total phenol content was significantly higher than total flavonoid. The amount of TPC and TFC reached to 358,06 ± 10,74 μg GAE/mg dry extract) and 22,93 ± 0,23 μg GAE/mg extract) respectively.

2.3. Antioxidant activity

The importance of conducing phytochemical studies, is to not only used for the chemical characterization but also important for correlating the chemical contents with specific functional properties. For these reasons, antioxidant activities were evaluated by using two methods; Diphenylpicrylhydrazyl radical (DPPH) scavenging and β-Carotene
bleaching assay. According to the results presented in Table S4 (Supplementary material); hydro-methanolic extract showed a powerful antioxidant activity in DPPH scavenging assay with IC_{50} value 12.25 \pm 1.09 \mu g/mL, which is lower compared with standard used BHT. Interestingly, the results presented in Tables S3 and S4 (Supplementary material) indicates that methanolic extract of Ephedra alata var. alenda contains large quantities of polyphenols. This suggests that these secondary metabolites may be responsible for the observed radical scavenging activities.

2.4. Acetylcholinesterase inhibitory activity

The inhibition of acetylcholinesterase (AChE) is considered the primary approach to treat Alzheimer’s disease (AD). Methanolic extract of Ephedra alata var. alenda showed a high potential to inhibit AChE as seen in Table S5 (Supplementary material), the inhibition IC_{50} value was 11.25 \pm 0.25 \mu g/mL which is close to that of Galanthamine standard.

2.5. Molecular modeling

Docking studies were performed in order to get insights into the possible binding mode of the identified alkaloids to hAChE (PDB: 4EY6). The obtained results are shown in Table S6 and Figures S5 and S6 (Supplementary material). As can be seen, all the studied compounds exhibit good affinity for the active site of hAChE with low binding energies ranging from –4.9 to –11.6 kcal/mol. Norephedrine and ephedrine form a favorable interaction at the anionic site with the residue TRP86. Ephedrine and methyl-ephedrine could bind favorably with the residue SER125. While 2-(5-ethyl-2-pyridine)ethyl form a hydrogen bond with the residue of the oxyanion hole SER203. This indicates that the studied alkaloids are potential inhibitors of hAChE, which may explain the promising activity of the methanolic extract of E. alata var. alenda.

3. Conclusion

More than 27 compounds were characterized using the HPLC-ESI-MS/MS method from of the methanolic extract of E. alata. Most of them presented flavonoids as O-glucoside isomers, Herbacetin 7-O neohesperidoside and Phloridzin isomers. This study showed also that alkaloids are useful markers of this species notably ephedrine and pseudoephedrine. This extract showed a good AChE inhibitory activity with an IC_{50} of 11.25 \pm 0.25 \mu g/mL in a quantitative assay. A strong antioxidant activity was demonstrated by the two used methods namely, DPPH (IC_{50} 12.25 \pm 1.09 \mu g/mL) and \beta-Carotene bleaching assay (IC_{50} 20.28 \pm 2.63 \mu g/mL). The docking study indicated that the identified alkaloids are potential inhibitors of AChE. The obtained results show that E. alata var. alenda is very rich in polyphenols and flavonoids (TPC, 358.06 \pm 10.74 \mu g of GAE/mg dry extract, TFC, 22.93 \pm 0.23 mg \mu g of GAE/mg dry extract), which explains the powerful antioxidant effect of the studied extract. It can be concluded that E. alata var. alenda is a rich source of beneficial phytochemicals and antioxidants.
Acknowledgments

The authors are grateful to the DGRSDT for the financial support.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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