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Identification, occurrence and activity of quinazoline alkaloids in *Peganum harmala*

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ABSTRACT

Peganum harmala L. is a medicinal plant from the Mediterranean region and Asia currently used for recreative psychoactive purposes (Ayahuasca analogue), and increasingly involved in toxic cases. Its psychopharmacological and toxicological properties are attributed to quinazoline and β-carboline alkaloids. In this work three major quinazoline alkaloids were isolated from *P. harmala* extracts and characterized as peganine (vasicine), deoxypeganine (deoxyvasicine) and a novel compound identified by HPLC-DAD-MS and NMR as peganine β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (peganine glycoside). Peganine appeared in flowers and leaves in high levels; high amounts of deoxypeganine and peganine were found in immature and green fruits whereas peganine and peganine glycoside accumulated in high amount in dry seeds reaching up to 1 and 3.9% (w/w), respectively. Roots and stems contained low amount of quinazolines. Seeds extracts containing both quinazoline and β-carboline alkaloids potently inhibited human monoamine oxidase (MAO)-A. However, quinazoline alkaloids did not contribute to MAO inhibition that was due to β-carbolines, suggesting that MAO-related psychoactive or toxic actions do not arise from quinazolines. Quinazoline alkaloids were poor radical scavengers in the ABTS assay whereas seed extracts had good activity. Quinazoline alkaloids are known to exert bronchodilator and abortifacient actions, and could contribute to such effects reported in *P. harmala*.

1. Introduction

Peganum harmala L. (*Zygophyllaceae*) is a perennial herbaceous plant native to arid parts of North Africa, Mediterranean Sea, Middle East, Pakistan, India and China, and introduced and naturalized in the Southwest USA, South Africa and Australia. It has been traditionally used for medicinal purposes as a remedy against syphilis, fever, hysteria, malaria, neuralgia, parkinsonism, rheumatism, colic, asthma and eye complaints (Abdelfattah et al., 1995; Achour et al., 2012; Astulla et al., 2008; Berrougui et al., 2006; Elbahri and Chemli, 1991; Farouk et al., 2008; Im et al., 2009; Khlifi et al., 2013; Monsef et al., 2004; Shahverdi et al., 2008). The extracts of *P. harmala* exhibit fungicidal, bactericidal, anti-inflammatory and antitumor activity (Bensalem et al., 2014; Lamchouri et al., 2013; Sobhani et al., 2002; Song et al., 2004). Their seeds have been used as an incense, spice or condiment with narcotic, aphrodisiac, stimulant, sedative, emmenagogue, abortifacient and emetic properties. The inadequate use of *P. harmala* often triggers toxic effects and numerous cases of toxicity have been already reported in animals and humans. Intoxications produce paralysis, euphoria, convulsions, hallucinations, hypothermia, cardiovascular alterations, digestive problems (nausea, vomiting) and abortion (Achour et al., 2012; Mahmoudian et al., 2002; Yuruktumen et al., 2008). Nowadays, *P. hamala* is also being increasingly used for recreational purposes owing to its psychoactive and narcotic properties (Frison et al., 2009; Herraiz et al., 2010; Kikuchi et al., 2010). Thus, a number of websites contain information on the recreational use of *P. harmala* seeds as a substitute of *Banisteriopsis caapi* in an attempt to imitate Ayahuasca (yaje), a hallucinogenic beverage used by Amazonian tribes (http://www.imaginaria.org/a_yage.htm; http:/leda.lycaeum.org/?ID=360 (Brierley and Davidson, 2012; Brush et al., 2004; Frison et al., 2009). These preparations exhibit potent psychopharmacological effects by inhibiting human monoamine oxidase (MAO) (Herraiz

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et al., 2010), which may trigger adverse interactions (Bakim et al., 2012).

Peganum harmala contains β-carboline and quinazoline alkaloids which are responsible for the toxicological and pharmacological effects of the plant (Beyer et al., 2009; Herraiz et al., 2010; Pulpati et al., 2008). β-Carbolines are bioactive substances that exhibit activity on the central nervous system (CNS) through interaction with brain receptors and inhibition of MAO and kinases (El Gendy et al., 2012; Herraiz, 2016; Herraiz and Galisteo, 2015; Herraiz and Guillen, 2011; Rommelspacher and Wernicke, 2012; Ruben et al., 2015). Quinazoline alkaloids (i.e. peganine or vasicine) exhibit antimicrobial, antiprotozoal and antileishmanial activities and exert bronchodilator and hypotensive actions (Duraipandiyan et al., 2015; Khaliq et al., 2009; Roja et al., 2011). They are also strong stimulants of uterine contractions and abortifacient agents (Gupta et al., 1977). These alkaloids occur in the leaves of *Adhatoda vasica (Acanthaceae)*, an Indian plant widely used to treat respiratory ailments (Avula et al., 2008; Laakso et al., 1990; Nepali et al., 2013). Previous studies in *P. harmala* have generally focused on β-carboline alkaloids with much less attention given to quinazolines (Bensalem et al., 2014; Hemmateenejad et al., 2006; Herraiz et al., 2009). However, additional work is currently needed on quinazolines because the biological actions of *P. harmala* including the inhibition of MAO could arise in part from these alkaloids (Algorta et al., 2009; Iligner and Matusch, 2005; Liu et al., 2015a). In a previous study, we have isolated and characterized β-carboline alkaloids in *P. harmala* as potent inhibitors of MAO (Herraiz et al., 2010). The current work was aimed to study quinazoline alkaloids. It reports the characterization, content and distribution of these compounds in the plant. Three major quinazoline alkaloids are identified as peganine (vasicine), deoxypeganine (deoxyvasicine), and a novel compound characterized as peganine glycoside whose occurrence was previously suggested (Herraiz et al., 2009). The activity of these quinazolines on MAO enzymes and as radical scavengers (antioxidants) was subsequently evaluated in order to shed light on their potential contribution to pharmacological and toxicological effects of *P. harmala*.

2. Material and methods

Peganum harmala L. *(Zygophyllaceae)* plants were collected in Barcience, Toledo (Spain) from May to December and different parts of the plant: roots, leaves, stems, flowers, immature capsules, green capsules (fruits), and dry seeds (from brown to black colour) conveniently separated and used for isolation, identification and quantification of quinazoline alkaloids. Recombinant human monoamine oxidase-A and B were obtained from Gentest BD Biosciences (Woburn, MA, USA). Kynuramine, 4-hydroxyquinoline, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and the Mosher's reagents (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (*S*-MTPA-Cl) and (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (*R*-MTPA-Cl) were obtained from Sigma. HPLC grade acetonitrile, methanol and dimethyl sulfoxide (DMSO) were from Scharlau (Spain) and dichloromethane from Merck (Germany). NMR spectra were obtained in a 500 MHz Varian and a 300 MHz Bruker apparatus and accurate mass spectra obtained using a high resolution triple quadrupole mass spectrometer (HR-MS/MS) (Agilent 6520 accurate-mass Q-Tof) working under electrospray positive ionization mode (ESI+) with nitrogen gas at 15 L/min; capillary voltage at 4.0 kV; collision energy at 20 eV; temperature at 340 °C and fragmentor voltage at 45 or 90 V.

2.1. Extraction and isolation of quinazoline alkaloids from *P. harmala*

Seeds, roots, flowers, leaves, stems and fruits (capsules) were grinded and aliquots (0.2–0.5 g) homogenized in 0.6 M $HClO_4$ + methanol (1:1) (20 mL) using an ultraturrax homogenizer and subsequently centrifuged (10,000 rpm, 10 min). The supernatant was pooled and homogenization in 0.6 M $HClO_4$-methanol repeated twice with the residue. These extracts were used to analyse quinazoline alkaloids and for MAO inhibition and antioxidant studies. For isolation of quinazoline alkaloids (i.e. peganine and peganine glycoside), *P. harmala* dry seeds (2 g) were grinded and added to milli-Q water (20 mL), homogenized using an ultraturrax homogenizer and centrifuged (10,000 rpm, 10 min). This operation was repeated twice with the residue and supernatants pooled and then evaporated in vacuum using a rotary evaporator (53 °C). The concentrated sample (2 mL) was loaded into a chromatography column packed with octadecyl silica (C_{18}) and eluted with acidified milli-Q water (0.01% v/v, formic acid) with fractions monitored by RP-HPLC (280 nm, Zorbax-C18 column) and RP-HPLC-MS. Quinazoline compounds were eluted in two main fractions, the first one containing peganine and the second peganine glycoside. These fractions were adjusted to pH 10 with NH_4OH (10% v/v) and extracted with CH_2Cl_2. Peganine (11 mg) was isolated from the organic phase in the first fraction and peganine glycoside (23 mg) was isolated from the aqueous phase in the second fraction after evaporation in vacuum (53 °C) and a nitrogen stream. Deoxypeganine was isolated from green fruits (immature capsules) (0.5 g) homogenized in 0.6 M $HClO_4$-methanol (1:1) by successive injections into RP-HPLC, with the chromatographic peak corresponding to deoxypeganine collected at the end of the detector. Collected fractions were extracted with CH_2Cl_2 in acidic media (pH 2), and aqueous phase basified (pH 10) and extracted with CH_2Cl_2. After evaporation of organic solvent, deoxypeganine hydrochloride (1.8 mg) was isolated following addition of methanol-HCl. In addition, peganine was also isolated from green fruits that were homogenized in 0.6 M $HClO_4$-methanol (1:1) with the chromatographic peak corresponding to successive RP-HPLC injections collected at the end of the detector. The fractions were adjusted to pH 10, extracted with CH_2Cl_2 and after evaporation afforded peganine. Peganine and deoxypeganine were also isolated from flowers and immature capsules extracts, respectively following the same procedure. The purity of compounds was higher than 95% in HPLC. The spectroscopic data are as follows and structures are in Fig. 2.

2.1.1. Peganine (vasicine) (1,2,3,9-tetrahydropyrrolo[2,1-b] quinazolin-3-ol) ($C_{11}H_{12}N_2O$)

^{1}H NMR ($CDCl_3$, 300 MHz): 7.19 (m, 2H), 7.06 (m, 1H), 6.93 (br d, J = 7.3 Hz, 1H) (5-, 6-, 7- and 8-H), 4.91 (dd, J = 7.8, 6.3 Hz, 1H, 3-H), 4.67 (s, 2H, 9-H), 3.50 (m, 1H, 1-H_A), 3.37 (m, 1H, 1-H_B), 2.46 (m, 1H, 2-H_A), 2.15 (m, 1H, 2-H_B). HPLC-MS (ESI): $(M + H)^+$ at *m/z* 189. ESI-HRMS (90 V): 189.1019 $(M + H)^+$ (obtained) (calculated for $C_{11}H_{13}N_2O$ $(M + H)^+$: 189.1028). Spectroscopic data agree with previous results (Gao et al., 2008; Joshi et al., 1994; Khaliq et al., 2009). Peganine from *P. harmala* seeds was derivatized with Mosher's reagent (*S*-MTPA-Cl and *R*-MTPA-Cl) in CH_2Cl_2 and pyridine to form diastereoisomers and injected into RP-HPLC (Novapak C18 and Zorbax SB-C18 columns).

2.1.2. Peganine (vasicine) glycoside [peganine β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside; 3-[(6-O-β-D-glucopyranosyl)-β-D-glucopyranosyl)oxy]peganine; 3-O-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]peganine] ($C_{23}H_{32}N_2O_{11}$)

^{1}H NMR (D_2O, 500 MHz): 7.28 (dd, J = 7.8, 7.6 Hz, 1H, 6-H), 7.22