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Brief communication

Chemical composition and antioxidant activity of essential oils and solvent extracts of *Ptychotis verticillata* from Morocco

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ABSTRACT

The objective of this study was to characterize the chemical composition of the essential oil and extracts of *Ptychotis verticillata*. The antioxidative activities of this species were also evaluated to suggest it as a new potential source of natural antioxidants. Analysis of the chemical composition of *P. verticillata* essential oil from Morocco was carried out using GC and GC–MS. The oil was dominated by phenolic compounds (48.0%) with carvacrol (44.6%) and thymol (3.4%) as the main compounds. Plant phenolics constitute one of the major groups of components that act as primary antioxidant free radical terminators. The amounts of total phenolics and flavonoids in the solvent extracts (diethyl ether and ethyl acetate) were determined spectrometrically. Furthermore, the antioxidant activities of the essential oil and extracts were determined using a DPPH test system. The DPPH scavenging activity of extracts increased in the order ethyl acetate > ascorbic acid > diethyl ether > essential oil. Finally, a relationship was observed between the antioxidant activity potential and total phenolic and flavonoid levels of the extract.

1. Introduction

Phenolic compounds are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step (Agraval, 1989). This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups (Havsteen, 2002). Polyphenolic compounds are also known for their ability to prevent fatty acids from oxidative decay (Fecka et al., 2007). The oxidation is caused by the rancidity of unpreserved aliments rich in unsaturated fatty acids (Li et al., 2008). Furthermore, many synthetic antioxidant components (BHA and BHT) have shown toxic and/or mutagenic effects; therefore, plant antioxidants are suggested as an interesting alternative. Numerous studies exhibited a strong relationship between total phenolic content and antioxidant activity in fruits, vegetables, and medicinal plants (Dorman et al., 2003; Velioglu et al., 1998). Flavonoid constituents possess a wide spectrum of chemical and biological activities, including radical scavenging properties (Shimoi et al., 1996). Indeed, Shimoi et al. (1996) reported that plant flavonoids that show antioxidant activity *in vitro* also function as antioxidants *in vivo*. Malkowski (2006) showed the role of these compounds in the defense mechanism against oxidative stress from oxidizing agents and free radicals.

The scavenging of reactive oxygen species (ROS) is a possible mechanism of action for antioxidant compounds (Havsteen, 2002; Matkowski, 2008; Peterson and Dwyer, 1998). ROS may be the causative factor involved in many human degenerative diseases, and antioxidants are known to have some degree of preventive and therapeutic effects on these disorders. The ROS hydrogen peroxide caused lipid peroxidation and DNA oxidative damage in cells (Nordberg and Arner, 2001; Pervaiz and Clement, 2007). Moreover, high levels of ROS such as superoxide and hydrogen peroxide are observed in various cancer cells (Ushio-Fukai and Nakamura, 2008). Small molecular weight antioxidants are considered possible protection agents that reduce oxidative damage in the human body when the internal enzymatic mechanisms fail or are inadequately efficient (Halliwell, 1995).

Ptychotis verticillata Nûnkha (Apiaceae family), otherwise known as *Ammoides pusilla* (Brot.) Breistr., *Psychotis ammoides* Koch (Bellakhdar, 1997), *Ammoides verticillata* Briq. and *Petroselium ammoides* Rchb. fil. (Wehmer, 1931; Quezel and Santa, 1963), is an aromatic herbaceous species (10–35 cm tall) widespread in northern Africa (Quezel and Santa, 1963).

In Morocco, this species is currently used in folk medicine as a febrifuge and for its antispasmodic, antiseptic and antidiabetic properties (Bnouham et al., 2007, 2010). Bekhchi and Abdelouahid (2004) showed the antimicrobial and antifungal properties of

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essential oils from *P. verticillata*. Moreover, the antimicrobial activity of essential oil was studied using the agar diffusion test on eight strains of bacteria and against fungus and yeast. A twofold oil solution showed important antimicrobial activity (Laouer et al., 2003). These authors was also described the effect of *P. verticillata* oil on the growth of two pathogenic bacteria (*Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *mosprunorum*) using agar diffusion test (Laouer et al., 2004). While the essential oil composition has only been the subject of a few investigations, the oils were found to be rich in thymol (Bellakhdar, 1997). Indeed, the essential oil of the aerial parts of plant was analyzed using both GC and GC/MS. The results revealed no less than 46 constituents, among which thymol (44.5%), γ-terpinene (32.9%), and *p*-cymene (13.5%) were the most abundant. More recently, Bnouham et al. (2007, 2010) have reported the antidiabetic and antihyperglycemic activities of the water extract from this species. The toxicology tests used in this paper suggested no adverse effect of the use of this plant.

In the present work, the antioxidant activity of essential oil and extracts of *P. verticillata* were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test system. Antioxidants react with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the free radical scavenging potential of the sample/antioxidant, and it has been found that antioxidants such as cysteine, glutathione, ascorbic acid, tocopherol, and polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.) reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their donating ability (Blois, 1958). The aim of this work is to evaluate the antioxidative properties of the essential oil and extracts of *P. verticillata*. Additionally, the total phenolic and flavonoid contents of diethyl ether and ethyl acetate extracts and the chemical composition of the essential oil have been determined.

2. Materials and methods

2.1. Plant material

The aerial parts of *P. verticillata* were harvested in May 2009 (full bloom) from Ahfir, Morocco. Voucher specimens were deposited in the herbarium of Mohamed 1st University, Oujda, Morocco.

2.2. Essential oil isolation

Fresh vegetal material was water distillated (3 h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia (Council of Europe, 1996). The essential oil yields were 2% (w/w). The oils were dried over anhydrous sodium sulfate and then stored in sealed glass vials at 4–5 °C prior to analysis.

2.3. Preparation of the extracts

Boiling water extracts (100 mL) of plant samples obtained under reflux conditions (hydrodistillation process) were extracted three times (3 × 20 mL) with organic solvents (diethyl ether and ethyl acetate). Water extract residues were then extracted by boiling acidified water (2 N HCl) prior to liquid–liquid extraction. The diethyl ether and ethyl acetate extracts were filtered and concentrated under vacuum to obtain two extracts in yields of 0.15% and 0.33% (w/w), respectively. The organic solvent extracts were dried over anhydrous sodium sulfate and then stored in sealed glass vials at 4–5 °C prior to analysis. Each extraction was performed in triplicate.

2.4. GC analysis

GC analysis was carried out using a Perkin-Elmer Autosystem XL GC apparatus (Waltham, MA, USA) equipped with a dual flame ionization detection (FID) system and the fused-silica capillary columns (60 m × 0.22 mm I.D., film thickness 0.25 μm) Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol). The oven temperature was programmed from 60 to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. Samples were injected in the split mode (1/50) using helium as a carrier gas (1 mL/min) and a 0.2 μL injection volume of pure oil. Retention indices (RI) of compounds were determined relative to the retention times of a series of *n*-alkanes (C_5–C_{30}) (Restek, Lisses, France) with linear interpolation using the Van den Dool and Kratz (1963) equation and software from Perkin–Elmer.

2.5. GC–MS analysis

Samples were analyzed with a Perkin-Elmer turbo mass detector (quadrupole) coupled to a Perkin–Elmer Autosystem XL equipped with the fused-silica capillary columns Rtx-1 and Rtx-wax. Carrier gas: helium (1 mL/min), ion source temperature: 150 °C, oven temperature programmed from 60 to 230 °C at 2 °C/min and then held isothermally at 230 °C (35 min), injector temperature: 280 °C, energy ionization: 70 eV, electron ionization mass spectra were acquired over the mass range 35–350 Da, split: 1/80, injection volume: 0.22 μL of pure oil.

2.6. Identification of essential oil constituents

Identification of individual components was based (i) on comparison of calculated RI, on polar and apolar columns, with those of authentic compounds or literature data (König et al., 2001; National Institute of Standards and Technology, 2008); and (ii) on computer matching with commercial mass spectral libraries (Adams, 2001; König et al., 2001; National Institute of Standards and Technology, 1999) and comparison of mass spectra with those of our own library of authentic compounds or literature data (Adams, 2001; König et al., 2001).

2.7. Determination of total phenolic contents

Total phenolic contents of the extracts were determined using Folin–Ciocalteu reagent according to the method previously reported by Slinkard and Singleton (1977), using caffeic acid as a standard, and as modified by Li et al. (2008). 200 μL of the dilute extract solution containing 40 μg of the extract was added to 1 mL of Folin-Ciocalteu reagent (diluted in distillated water). After 4 min, 800 μl of Na_2CO_3 (75 mg/mL) solution was added and the mixture was allowed to stand for 45 min at room temperature. At the end of the incubation, the absorbance was measured at 760 nm. The same procedure was also applied to the standard solutions of caffeic acid, and a standard curve was obtained. The concentrations of phenolic compounds expressed as μg caffeic acid equivalent per mg of extract

Table 1
Essential oil composition of *Ptychotis verticillata*.

N[a]	Components	RI *l*[b]	RI *a*[c]	RI *p*[d]	%[e]	Identification
1	α-Thujene	924	923	1031	0.2	GC, CG–MS
2	α-Pinene	936	931	1028	1.0	GC, CG–MS
3	Sabinene	973	966	1123	2.2	GC, CG–MS
4	β-Pinene	978	972	1113	0.7	GC, CG–MS
5	Myrcene	987	982	1160	1.3	GC, CG–MS
6	*p*-Cymene	1015	1015	1268	9.4	GC, CG–MS
7	Limonene	1025	1025	1203	18.4	GC, CG–MS
8	Cineole-1,8	1024	1025	1210	1.4	GC, CG–MS
9	γ-Terpinene	1051	1052	1244	9.5	GC, CG–MS
10	Linalol	1086	1084	1513	0.2	GC, CG–MS
11	Borneol	1150	1151	1691	0.2	GC, CG–MS
12	Terpinen-4-ol	1164	1163	1592	0.3	GC, CG–MS
13	α-Terpineol	1176	1175	1686	0.2	GC, CG–MS
14	Carvacryl methyl ether	1226	1227	1540	0.2	GC, CG–MS
15	Thymol	1267	1266	2153	3.4	GC, CG–MS
16	Carvacrol	1278	1279	2169	44.6	GC, CG–MS
17	α-Terpinyl acetate	1335	1334	1668	0.7	GC, CG–MS
18	Geranyl acetate	1362	1364	1751	4.7	GC, CG–MS
19	Caryophyllene oxyde	1578	1569	1967	0.3	GC, CG–MS
	Total identified				98.9	
	Monoterpene hydrocarbons				33.3	
	Phenolic components				48.2	
	Oxygenated Monoterpenes				6.9	
	Oxygenated Sesquiterpenes				0.3	

[a] The numbering refers to elution order on apolar column (Rtx-1).
[b] RI *l* = retention indices on the apolar column of literature (König et al., 2001; National Institute of Standards and Technology, 2008).
[c] RI *a* = retention indices on the apolar column (Rtx-1).
[d] RI *p* = retention indices on the polar column (Rtx-Wax).
[e] Relative percentages of components (%) are calculated on GC peak areas on the apolar column (Rtx-1); Values expressed are means of three parallel measurements.