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Original Research

Essential Oil Composition and Radical Scavenging Activity of *Paeonia Daurica* Subsp. *macrophylla* Root

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Abstract

Paeonia daurica subsp. *macrophylla* (*Paeonia*cea) is a perennial plant growing in Iran. In Persian traditional medicine, *Paeonia officinalis* [Ood-e-Saleeb] have been used for treatment of some diseases especially for epilepsy and brain disorders. Based on initial phytochemical evaluations of *Paeonia* spp. roots, steroidal and terpenoid compounds were identified. Hydro distilled essential oil of the roots of P. *daurica* subsp. *macrophylla* collected from north of Iran, was investigated using gas chromatography-mass spectrometry (GC-MS). Altogether, five constituents, forming 96.02% of the total oil composition were identified. Major constituents of the essential oil were salicylaldehyde (20.32%), beta-pinene-oxide (13.35%) and thymol acetate (61.12%). Antioxidant effect of the ethanolic (80%) extract was measured using DPPH free radicals. According to the results, IC50 of the extract was 25.2 µg/ml, which can be assessed as strong antioxidant effect using standard Antioxidant Activity Index.

Keywords: Antioxidant, DPPH, Essential oil composition, *Paeonia daurica* subsp. *macrophylla*

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Introduction

Plants belong to *Paeonia genous* (peony) have been known for beautiful appearance and also the important pharmacological effects which have been attributed to its roots. *Paeonia* is the only genus in the family *Paeonia*ceae. *Paeonia daurica* subsp. *macrophylla* (Syn.: *Paeonia wittmannianna*) is one of the two species growing in the north parts of Iran [1]. *Paeonia officinalis* known as "*Oode-Salee*b" in Persian traditional medicine has been used in treatment of some diseases especially brain illnesses such as epilepsy, nightmare, tremor, paralysis and uterine complications [2]. The roots of several peony species have also been used as medicinal plants in some other countries as an analgesic, a sedative, an anti-inflammatory agent and as a remedy for female genital diseases. *Paeonia* species are a rich source of several bioactive compounds comprising monoterpenoids, triterpenoids, flavonoids, phenols, and tannins [3, 4]. The main monoterpenoids like paeonilide, paeoniflorin and benzoylpaeoniflorin demonstrated anti aggregatory and anticoagulative activities. Anti-inflammatory, sedative and analgesic, hypoglycemic, anti-osteoporotic effects also have been reported with paeoniflorin and various components of peony species [4].

There have been several reports on the anti-oxidative effect of *Paeonia* species of Chinese origin. P. *lactiflora* and P. *suffruticosa* was reported to have high antioxidant capacity and DPPH free radicals scavenging activity [5, 6].

In this study, gas chromatography/mass spectrometry (GC/MS) is used for the chemical analysis of the essential oil composition obtained by hydro-distillation of the root of P. *daurica* subsp. *macrophylla* collected from north of Iran. Furthermore, preliminary phytochemical evaluation and DPPH radical scavenging activity of root ethanol extract of this plant were examined.

Methods

Chemicals

2, 2-diphenyl 1-picrylhydrazyl (DPPH; Fluka, Switzerland); Butylatedhydroxylanizole (BHA), ethanol and methanol (Merck, Germany) were purchased.

Plant material

The roots of P. *daurica* subsp. *macrophylla* were collected from Alborz mountains in Mazandaran province in June 2015. A voucher specimen of the plant (66-20-THE) was deposited in the herbarium of the faculty of pharmacy, Tehran University of Medical Sciences (Figure 1).

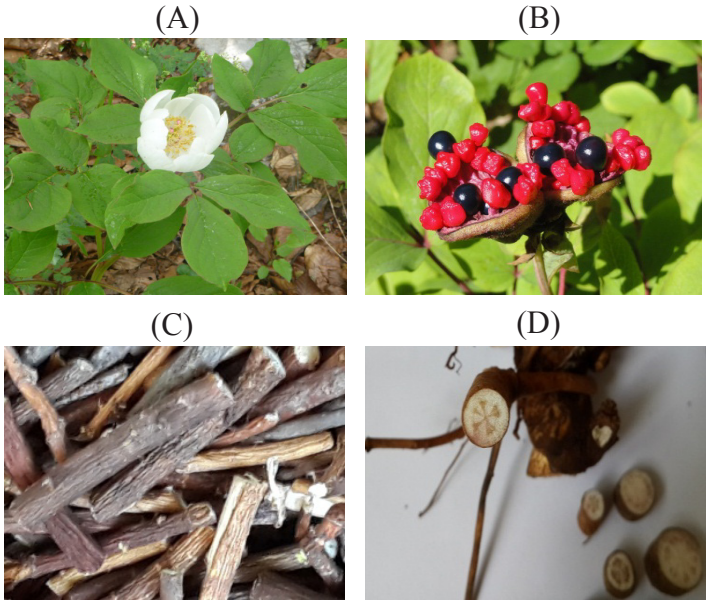


Figure 1: Morphology of *Paeonia daurica* ssp. *macrophylla*; (A) flower, (B) fruits, (C) and (D) root

Extraction

The collected parts were dried in the shade. 200 grams of dried roots were powdered with a grinder and macerated in 80% aqueous ethanol for three days at room temperature. The excessive ethanol and water were evaporated with a rotary vacuum evaporator (60 rpm at 40 °C). Crude extract was stored at 4ºC until use.

Phytochemical evaluation

Phytochemical analysis in order to screen tannins, alkaloids, flavonoids, sterols and triterpens was done according to Wagner and Bladt (1996) [7].

Microscopic evaluation

Microscopic evaluation of the powder of P. *daurica* ssp. *macrophylla* root was studied to find the structural characteristics of this plant sample [8].

Antioxidant determination of DPPH assay

The hydro alcoholic extract was evaluated for its free radical scavenging activities using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method according to Brand Williams et al. (1995). Different concentrations (25, 50, 100 µg/ml) of sample solutions (1 mL) in methanol were added to DPPH methanol solution (2 ml, 40 µg/mL). BHA (100 µg/ml) was used as positive control. After 30 min, the absorbance was measured at 517 nm. All tests were carried out in triple replicate. Percentage of radical scavenging activity of sample was calculated according to the following equation: Inhibition% = [(A0–As)/A0] × 100 that A0 is the absorbance of the control and As is the absorbance of the sample. Half maximal inhibitory concentration (IC50) value (indicate the concentration of the sample (mg/mL), required to scavenge 50% of DPPH) was calculated from the plotted graph of scavenging activity versus the concentration of extract, using linear regression analysis [9].

Isolation of the volatile constituents

The air-dried roots (150 g) were submitted for 4 h to hydro-distillation to afford 0.15 ml of oil, (i.e. yield 0.1 %). The derived oil was dried over anhydrous sodium sulphate and stored at 4 ºC for GC and GC/MS analysis.

Chemical analysis

GC/MS analysis was carried out in a HP (Agilent Technology): 6890 Network GC System, equipped with a capillary column HP-5MS (DB-5) 30 m × 0.25 mm; film thickness, 0.25 µm; temperature program, 50-300 °C at a rate of 3 °C/ min. Helium (99.999 %) was used as carrier gas at a flow rate of 1 ml/min. The percentage compositions of the identified compounds were computed from the GC peak areas. The oils were analyzed by GC/MS using a Hewlett Packard 5973 mass selective detector connected to a HP 6890 gas chromatograph. The volatile constituents were analyzed using GC/MS technique. The identification of components was based on direct comparison of their mass spectra with those of Wiley and NBS Libraries (Massada, 1976) and those described by Adams (2001) [10, 11].

Results and discussion

Phytochemical screening of hydro alcoholic extract

Preliminary qualitative analysis was performed on total extract of P. *daurica* subsp. *macrophylla*. Results showed that saturated and unsaturated sterols and triterpenoids are the main compo nent of the extract.

Microscopic evaluation

The root powder microscopy showed different tissues such as fibers and parenchyma with cluster crystals of calcium oxalate and scalariform vessels (Figure 2).

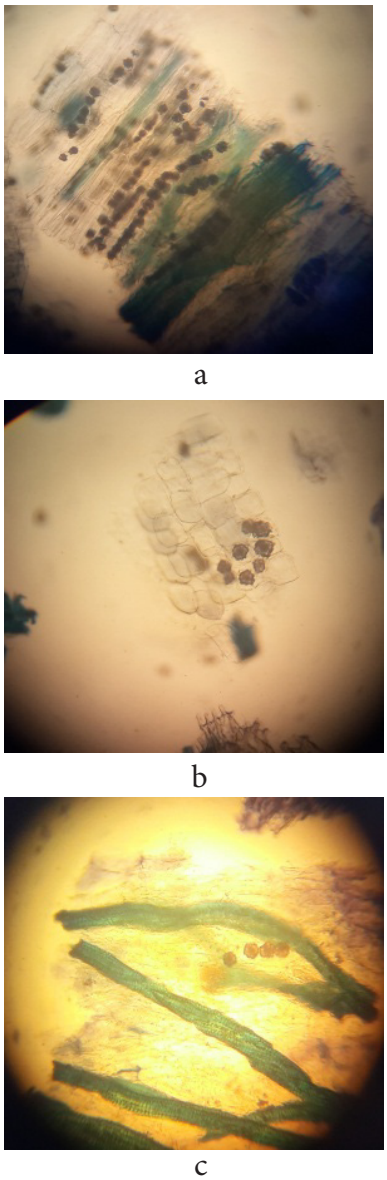


Figure 2: Microscopic appearances of Paeonia daurica ssp. macrophylla root; a: fibers with cluster crystals of calcium oxalate, b: parenchyma containing cluster crystals, c: scalariform vessel

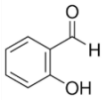
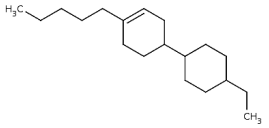
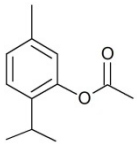
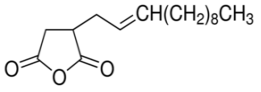
Antioxidant assay

Antioxidant potential of the ethanol extract of the root sample of P. *daurica subsp macrophylla* was determined by its radical scavenger activity against DPPH. Our results demonstrate this medicinal plant showed a potent anti-radical activity against DPPH (IC50= 25.2 µg/ml) compared to the BHA IC50=7.9 µg/ml.

GC/MS *analysis of essential oil*

The essential oil of the P. *daurica* subsp. *macrophylla* root was observed as yellow color liquid and were obtained in yield of 0.1 (v/w). Volatile compounds of the sample was identified and demonstrated in Table 1.

Table 1. Volatile composition of essential oil from root of *Paeonia daurica* subsp. *macrophylla*

Number	Name	Structure	(%) Percent
1	Salicylaldehyde		20.32
2	Beta-pinene oxide		13.35
3	4-ethylcyclohex-)4-yl)-1-pentyl-cyclohexene		1.16
4	Thymol acetate		61.12
5	Dodecenyl succinic anhydride		0.07
Non terpene compounds	-	-	21.55
Oxygenated monoterpene	-	-	74.47
Total identified	-	-	96.02

Reviewing the root essential oil compounds from other species and sub-species of this genus has determined that salicylaldehyde is one of the main components of all studied species while the principal compound identified in this research were thymol acetate (61.12%) and beta pinene oxide (13.35%) that have never been reported in essential oil of other species of *Paeonia*.

Essential oil compositions and antioxidant potentials of fourteen ethanol (75%) root extracts prepared from twelve taxa of the genus *Paeonia*, from Anatolian areas were evaluated. The major components were identified as salicylaldehyde (10%–94.4%),*cis*-myrtanal(5.5%–59.7%),and methyl salicylate (2%–52.2%). The results of detailed study showed great compositional variation in all samples oil in which salicylaldehyde, *Cis*-myrtanal, and methyl saliciylate were the major components. Salicylaldehyde was the main compounds in *P*. cf. *mascula* subsp. *mascula* samples of central and northeastern Anatolia, *P. x kayae, P. daurica, P. mascula* subsp. bodurii, *P. peregrina* of northwestern Anatolia, *P. turcica*, and *P. wittmanniana*, while methylsalicylate was the highest component only in *P. arietina. Cis-myrtanal* was the major com-

pound in *P. tenuifolia* (59.7%), P. *peregrina* of west Anatolia (45.7%), and *P. cf. officinalis* (42.2%) [12].

Chemical analysis of the volatile constituents obtained from the roots of two Greek endemic taxa: *P. clusii* Stern subsp. *Clusii* and *P. parnassica* Tzanoud., and one subspecies, *P.mascula* L. subsp. *hellenica* by GC/MS showed that the major volatile components of the roots of *P. clusii* subsp. *clusii* were salicylaldehyde (32.57%), paeonol (32.52%) and benzoic acid (7.70%), of *P. parnassica* were salicylaldehyde (58.43%) and methyl salicylate (24.72%) and of *P.mascula* subsp. *hellenica* were salicylaldehyde (74.70%) and methyl salicylate (5.22%) [13].

Salicylaldehyde, the main compound in the essential oils of the Greek and some of the Turkish *Paeonia species*, has not been reported from Chinese peony roots. Conversely, benzoic acid was the dominant component in the essential oils of two Chinese *Paeonia species* (*P. lactiflora* and *P. suffruticosa*). Also, benzoic acid and its monohydroxy-, dihydroxy- and trihydroxy-derivatives were observed by GC/MS analysis of acidic fractions obtained from *Paeonia peregrina* and *Paeonia tenuifolia* roots growing in Bulgaria [6, 3].

Conclusion

The results of this study showed that *Paeonia daurica* subsp. *macrophylla* roots with terpenoid compounds and sterols showed high antioxidant potency. Also, the presence of thymol acetate, salicylaldehyde and beta pinene oxide can be a good guide for future studies. Considering the therapeutic effects of various species of *Paeonia* genus worldwide, phytochemical studies and evaluation of the therapeutic effects of *Paeonia* species in Iran are needed.

Conflict of Interest

None.

Acknowledgment

None.

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