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Bioactive Properties of Oil and Methanol Extracts of *Pimpinella Aurea* Dc.

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ABSTRACT

The present study was conducted to evaluate the in vitro antimicrobial and antioxidant properties of essential oil and methanol extracts from aerial parts of *Pimpinella aurea*. Antimicrobial activity of the *P. aurea* was tested against 10 bacteria and fungi by disk diffusion method, whereas the extract remained inactive. Minimal Inhibitory Concentration (MIC) values of each active sample were determined. The antioxidative potential of the samples were evaluated using inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid assay. The polar subfraction of the methanol extract was able to reduce the stable free radical DPPH with an IC_{50} of 108 ±0.5 µg/ml, which was higher than that of synthetic antioxidant butylated hydroxyl toluene (BHT) with IC_{50}=19.8 ± 0.5 µg/ml. In contrast the nonpolar subfraction showed major effectiveness in β-carotene/linoleic acid assay with 65.87% inhibition. The amounts of total phenolic compounds were also determined in this study.

Key words: *Pimpinella aurea*; Antimicrobial Activity; Antioxidant Property; Phenolic Compounds.

Introduction

Essential oils and extracts of many plants have antioxidant and antimicrobial properties which are used in food, cosmetic and pharmaceutical industries (Reische *et al*., 1998). As synthetic drugs have been developed in recent century, medicinal herbs have been abolished. After many scientific researches, it is obvious that synthetic drugs have many undesired side effects, and these are not compatible with human nature. So plants can be used as natural additives to replace synthetic ones. For this reason scientists and researchers are concentrating again on plants and medicinal herbs to minimize these effects. The first step in scientific research on these plants includes extraction, recognition, evaluation, measurement and control of the effective compounds of them (Jantova *et al*., 2000; Dorman and Deans, 2000; Dang *et al*., 2001). It should be mention that Iran contains a rich, unique and wealth plant flora due to various climate condition and soil type.

Pimpinella aurea DC.(Apiaceae) is one of the 20 species, growing in different regions of Iran, and also distributed in Iraq, Afghanistan, Greece, Pakistan, Turkey and Syria (Mozafarian, 1996). The essential oils from the fruits of some *Pimpinella* species are also valuable in perfumery and in medicine (Ernst, 1989; Simon *et al*., 1984). *Pimpinella* species extracts and essential oils are known to have a high content of phenylpropanoid derivatives (Baser *et al*., 1997). These components isolated from *P. aurea* show various bioactivities such as antigermination, insecticidal, week antitumor, antimalarial, antimicrobial and antifungal (Demiric *et al*., 2005; Tabanca *et al*., 2005; Tabanca *et al*., 2003; Kleiman *et al*., 1988). *P. aurea* is used in animal feeds to increase milk production in eastern and southeastern Turkey (Tabanca *et al*., 2006). Furthermore, literature survey of *P. aurea* revealed that no researcher has yet reported antimicrobial and antioxidant properties of essential oil and methanol extract from aerial parts of this plant. Therefore, it is worth conducting an investigation on the in vitro antimicrobial and antioxidant activities of essential oil and methanol extract from aerial parts of *P. aurea*.

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Materials and methods

Plant Material

Materials for extraction were aerial parts of *Pimpinella aurea* DC. collected from Kashan area (Isfahan Province, central Iran) at an altitude of ca. 1985 m in July 2007 which were dried in the shade at room temperature. The voucher specimens of the plant were deposited in the Herbarium of Kashan Botanical Garden, Research Institute of Forests and Rangelands, Kashan, Iran.

Preparation of the extracts

41 g of the aerial parts of *Pimpinella aurea* DC. was Soxhlet-extracted with MeOH for 8 h (Sokmen *et al*., 1999). The extract was concentrated and yielded 6.30 g (15.36%) of a crude extract. The prepared extract was suspended in chloroform and water to obtained 3.3 g (11.27%) polar and non-polar 0.92 g (3.14%) extracts. The extract was concentrated using rotary evaporator at a maximum temperature of 50 ºC and kept in dark at +4 °C until used.

Isolation of the essential oil

Air-dried herbal parts of the plant were subjected to hydrodistilation using a Clevenger apparatus for 3.5 h according to the method recommended in the European Pharmacopoeia (Maisonneuve, 1975). The yellow-colored essential oil was obtained in the yields of 0.30% (v/w) and dried over anhydrous sodium sulfate and stored in vial at low temperature (4 ºC) until used.

Antioxidant activity

DPPH assay

Radical scavenging activity (RSA) of *P. aurea* essential oil and extracts was determined using the stable free radical, 2,2'-diphenylpicrylhydrazyl (DPPH˙) by a slightly modified DPPH˙ radical scavenging activity assay (Sarker *et al*, 2006). It is a widely used reaction based on the ability of antioxidant molecule to donate hydrogen to DPPH˙ which consequently turns into an inactive form. Briefly, a 10 mg/mL stock solution of each essential oil and extract in methanol was prepared. Dilutions are made to obtain concentrations ranging from 1 to 5×10^{-10} mg/mL. Diluted solutions (2 mL each) are mixed with 2 mL of a freshly prepared 80 µg/mL DPPH˙ methanol solution and allowed to stand for 30 min in dark at room temperature for any reaction to take place. The ultraviolet (UV) absorbances of these solutions are recorded at 517 nm using a blank containing the same concentration of oil or extract without DPPH. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated form the graph plotted of inhibition percentage against sample concentration. Tests were carried out in triplicate.

β-Carotene/linoleic acid assay

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. The method described by Tepe *et al*. (2005) was used with a slight modification. A stock solution of *β*-carotene and linoleic acid was prepared with 0.5 mg of *β*-carotene in 1 mL chloroform, 25 µL of linoleic acid and 200 mg Tween 40. The chloroform was evaporated under vacuum and 100 mL of aerated distilled water was then added to the residue. 300 µL of each extract was added to 2.5 mL of the previous mixture. The samples were dissolved in DMSO. The test tubes were incubated in hot water (50 °C) for 2 h, together with two blanks, one containing the antioxidant BHT as a positive control and the other with the same volume of DMSO instead of the extracts. In the test tube with BHT, the yellow color is maintained during the incubation period. The absorbance was measured at 470 nm. Antioxidant capacities (Inhibition percentage, I%) of the tested solutions were calculated using the following equation:

$$I\% = (-\beta\text{carotene content after 2 h assay/initial } \beta\text{-carotene content}) \times 100$$

Tests were carried out in triplicate. Percent inhibitions of the samples were compared with that of positive and negative standards.

Assay for total phenols

Total phenolic constituents of the aforesaid extracts of *P. aurea* were determined by literature methods involving Folin-Ciocalteu reagent and gallic acid as standard (Chandler and Dodds, 1983; Slinkard and Singleton, 1997). Extract solution (0.1 ml) containing 1000 µg of extract was taken in a volumetric flask; 46 ml of distilled water and 1 ml Folin-Ciocalteu reagent were added and the flask was thoroughly shaken. After 3 min, 3 ml of a solution of 2% Na_2CO_3 were added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for all standard gallic acid solutions (0–1000 mg 0.1 ml^{-1}) and a standard curve was obtained with the equation given below:

$$\text{Absorbance} = 0.0012 \times \text{Gallic acid } (\mu g) + 0.0033$$

Antimicrobial activity

Microorganisms

The extracts will be individually tested against a panel of microorganisms, including *Aspergillus* niger ATCC 16404, *Candida albicans* ATCC 10231*, Escherichia coli* ATCC 10536*, Klebsiella pneumoniae* ATCC 10031, *Proteus vulgaris* PTCC 1182, *Pseudomonas aeruginosa* ATCC 27853*, Salmonella paratyphi-A serotype* ATCC 5702, *Shigella dysenteriae* PTCC 1188, *Staphylococcus aureus* ATCC 29737, *Staphylococcus epidermidis* ATCC 12228. Microorganisms were provided by Iranian Research Organization for Science and Technology (IROST).

Antimicrobial Screening

The *in vitro* antimicrobial activity of the samples was evaluated by the disc diffusion method, using mueller hinton agar for bacteria, sabouraud dextrose agar and potato dextrose agar for yeast and fungi respectively (Wayne, 1999). Briefly, a suspension of the tested microorganism (0.1ml of 10^8 CFU) was spread on the solid media plates. Filter paper discs (6mm in diameter) were impregnated with 10 µl of the oil or the 30 mg/ml extracts (300 µg/disc), then placed on the inoculated Plates. These plates were incubated at 37 °C for 24 h (for bacteria), and at 30 °C for 48 h (for yeast) and 72 h (for mould). Gentamicin (10 µg/disc) and Tetracycline (30 µg/disc) were used as positive controls for bacteria and Nystatin (100 I.U.) for fungi.

Microwell dilution assay

Minimal Inhibitory Concentration (MIC) values were measured by the microdilution broth susceptibility assay recommended by NCCLS (Wayne, 1999). The inocula of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The samples were dissolved in 10% dimethylsulfoxide (DMSO) and diluted to the highest concentration (5 mg/ml) and (30 mg/ml) for extract and essential oil respectively to be tested. Then serial two-fold dilutions were made in a concentration range from 7.8 to 500 mg/ml and 0.94 to 30 mg/ml for extract and essential oil respectively in 10 ml sterile test tubes containing brain heart infusion broth (BHI) for bacteria and sabouraud dextrose broth for yeast. MIC values of the essential oil against microorganisms were determined based on a micro-well dilution method. The 96-well plates were prepared by dispensing into each well 95 µl of BHI broth (for bacteria) and sabouraud dextrose (for yeast) and 5 µl of the inoculums. A hundred µl aliquot from the stock solutions of the samples initially prepared at the concentration of 5 mg/ml and 30 mg/ml for extract and essential oil respectively, was added into the first wells. Then, 100 µl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl of BHI broth without compound and 5 µl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µl. After incubation at 37°C for 24 h (for bacteria) and at 30 °C for 48 h (for yeast), the MIC values were determined. All experiments were performed in duplicate.

Results and Discussion

Results

A hydrodistilled oil from different part of *P. aurea* (Apiaceae) produced from dried aerial parts collected from Kashan area, were provided. Percent yield of oils were 0.31%.

Amount of total phenols

There are many ways to estimate the total amount of phenolic compounds persent in plant tissue, but it is important to keep in mined that none of these methods will detect all phenolic compounds. One of them is based on the absorbance values of the methanol extract reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents.

In this regard, the amount of total phenolics in the methanol extract of the polar subfraction of *P. aurea* was estimated as 48.56±0.63 µg/mg (4.8%, w/w).

Antioxidant activity

The antioxidant activity of *P. aurea* polar and nonpolar subfraction of methanolic extract and essential oil from aerial part was initially determined using the DPPH test and β-Carotene/linoleic acid assay. The extracts, essential oil and positive control (BHT) concentration providing 50% inhibition in free radical generation (DPPH) (IC_{50}) is given in Table 1. The polar subfraction of methnolic extract exhibited major effectiveness in DPPH assay (IC50 = 108.18 ± 0.87), which was higher than that of synthetic antioxidant, BHT, (IC_{50}=19.8 ± 0.5 µg/ml). In contrast the nonpolar subfraction showed high antioxidant activity in β-Carotene/linoleic acid assay with 65.87% inhibition (Table 1). The samples exhibited les antioxidant capacity than the positive control in both test.

Table 1: Antioxidant activity of essential oil and polar and nonppolar subfraction of *P. aurea* DC. methanolic extract and BHT measured by the DPPH assay and by the β-Carotene/linoleic acid assay.

Sample	DPPH test IC_{50}(µg/ml)	β-Carotene/linoleic acid test (%)
Polar subfraction	108.18±0.87	50.14
Non-polar subfraction	549.95±6.21	65.87
Essential oil	na	5.98
BHT (positive control)	19.72±0.80	87.59na, not active

Antimicrobial activity

The antimicrobial activities of *P. aurea* essential oil and extracts against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameter and MIC values. The results presented in Tables 2 and 3 exposed moderate antimicrobial activity against some microorganisms tested. Although, the antibacterial effect of another species of this family with the moderate antimicrobial activity has been reported (Tepe *et al*., 2006; Ponce *et al*., 2003), there is no previous report on evaluation of this plant against these set of microorganisms.

Table 2: Antimicrobial activity expressed as inhibition zone diameter (mm)[a] against some yeast strains of the essential oil from *P. aurea* DC. extract, essential oil and positive Control.

Sample Bacteri name	Polar subfraction	Non-polar subfraction	Essential oil	Gentamisine	Tetracycline	Nystatin (100 I.U/disc)
A. niger	na	na	na	nt	nt	27
C. albicans	na	na	na	nt	nt	32
E. Coli	na	na	na	20	20	nt
K. pneumoniae	na	na	10	22	22	nt
P. vulgaris	na	na	10	23	20	nt
P. aeruginosa	na	na	na	23	8	nt
S. paratyphi	na	na	na	nt	nt	nt
Sh. dysenteriae	na	na	15	18	25	nt
S. epidermidise	na	na	na	33	39	nt
S. aureus	na	na	16	21	24	nt[a]

Diameter of inhibition zone including disc diameter of 6 (mm).
na, not active
nt, not tested.

Discussion

The polar subfraction of the methanol extract was able to reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) concentration with an IC_{50} of 108.18±0.87 µg/ml, which was higher than that of synthetic antioxidant, BHT, (IC_{50}=19.72±0.8 µg/ml). The low antioxidant activity in DPPH assay is related to amount of total phenolic compounds of the plant. Since, this plant has no considerable amounts of phenolic compound in the polar subfraction of methanolic extract, hence, it could be considered as moderate natural antioxidant for different applications. In β-Carotene/linoleic acid assay the plant inhibition capacity of conjugated diene hydroperoxide formation arising from linoleic acid oxidation was measured (Tepe *et al*., 2005). The nonpolar subfraction of methanolic extract showed major effectiveness in this test. The essential oil doesn't exhibited strong antioxidative activity in two methods employed in this study.

Table 3: Antimicrobial activity expressed as minimum inhibitory concentration (MIC)[a] against some yeast strains of the essential oil from *P. aurea* DC. extract, essential oil and positive Control.

Sample Bacteri name	Polar subfraction	Non-polar subfraction	Essential oil	Gentamisine (10 µg/disc)	Tetracycline (30 µg/disc)	Nystatin (100 I.U/disc)
A. niger	na	na	na	nt	nt	0.031
C. albicans	na	na	na	nt	nt	0.125
E. Coli	na	na	na	0.5	0.5	nt
K. pneumoniae	na	na	>30	0.25	0.25	nt
P. vulgaris	na	na	>30	0.5	0.125	nt
P. aeruginosa	na	na	na	0.5	0.5	nt
S. paratyphi	na	na	na	nt	nt	nt
Sh. dysenteriae	na	na	>30	0.5	0.25	nt
S. epidermidise	na	na	na	0.5	0.25	nt
S. aureus	na	na	>30	0.5	0.25	nt

[a]MIC, minimum inhibitory concentration values are given as mg/ml
na, not active
nt, not tested.
[a] Diameter of inhibition zone including disc diameter of 6 (mm).
na, not active
nt, not tested.

The antimicrobial test results showed that the essential oil of *P. aurea* exhibited moderate antimicrobial activity against some tested microorganisms, whereas the extract remained inactive. These results showed low antimicrobial activity in comparison with the positive control. Indeed, more than 30 mg/ml of the essential oil was necessary to inhibit *K. pneumoniae, P. vulgaris, Sh. Dysenteriae* and *S. aureus* growth.

Conclusion

Antioxidative and antimicrobial properties of the essential oils and various extracts from many plants are of great interest in both academia and industry due to their possible uses as natural additives to replace synthetic ones. In this respect, the present study was designed to evaluate the *in vitro* antioxidant properties and antimicrobial activity of *P. aurea* extract and essential oil against set of microorganisms. The results presented here can be considered as the first information on the antioxidant and antimicrobial properties of this plant. As a result, it could be concluded that the *P. aurea* may be useful as a moderate antioxidant and antimicrobial agent following extensive investigation. Particularly, the extract of *P. aurea* may be potentially useful source of natural antioxidant principles.

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References

Baser, K.H.C., N. Tabanca, N. Kirimer, E. Bedir, I.A. Khan and D.E. Wedge, 1997. Recent advances in the chemistry and biological activities of the Pimpinella species of Turkey. Pure and Applied Chemistry, 79: 539-556.

Chandler, S.F, and J.H. Dodds, 1983. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of Solanum lacinitum. Plant Cell Reports, 2: 105-107.

Dang, M.N., M. Takacsova, D.V. Nguyen and K. Kristianova, 2001. Antioxidant activity of essential oils from various species. Nahrung, 45: 64-66.

Demiric, N.B., N. Kirimer, K.H.C. Baser, E. Bedir, I.A. Khan and D.E. Wedge, 2005. Gas chromatographic–mass spectrometric analysis of essential oils from *Pimpinella aurea, Pimpinella corymbosa, Pimpinella peregrine* and *Pimpinella puberula* gathered from Eastern and Southern Turkey. Journal of Chromatography A, 1097: 192-198.

Dorman, H.J.D. and S.G. Deans, 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88: 308-316.

Ernst D., 1989. Biotechnology in agriculture and foresty. Medicinal and aromatic plants II. Ed., Bajaj Y.P.S., Springer-Verlag, Berlin, Germany, 7: 381-397.

Jantova, S., L. Nagy, L. Ruzekova, D. Grancai, 2000. Antibacterial activity of plant extracts from the families fabaceae, olaceae, philadelphaceae, rosaceae and staphyleaceae. Phytotherapy Research, 14: 601-603.

Kleiman, R., D.R. Plattner and D. Weisleder, 1988. Antigermination activity of phenylpropenoids from the genus pimpinella. Journal of Natural Products, 52: 249-256.

Maisonneuve, S.A., 1975. European Pharmacopoeia. Sainte-Ruffine, 3: 68-80.

Mozafarian, V., 1996. A Dictionary of Iranian Plant Names. Farhange Moaser, Tehran: 412.

Ponce, A.G., R. Fritz, C. del Valle and S.I. Roura, 2003. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard, Journal of Lebensm.-Wiss. U.-Technol. 36: 679-684.

Reische, D.W., D.A. Lillard, and R.R. Eitenmiller, 1998. Antioxidants in food lipids. In Chemistry, nutrition and biotechnology, Eds. Akoh C. C. and D. B. Min. New York: Marcel Dekker: 423-448.

Sarker, S.D., Z. Latif and A.I. Gray, 2006. Natural products isolation. New Jersey, USA: Humana Press Inc., 2th Ed: 20.

Simon, J.E., A.F. Chadwick and L.E. Cracker, 1984. Herbs, an indexed bibliography. Elsevier, Amsterdam, The Netherlands: 1971-1980.

Slinkard, K. and V.L. Singleton, 1997. Total phenol analyses: automation and comparison with manual methods. American Journal of Enology and Viticulture, 28: 49-55.

Sokmen, A., B.M. Jones and M. Erturk, 1999. The in vitro antibacterial activity of Turkish plants. Journal of Ethnopharmacology, 67: 79-86.

Tabanca, N., E. Bedir, D. Ferraira, D. Slade, D.E. Wedge, M.R. Jacob, S.I. Khan, I.A. Khan, N. Kirimer and K.H.C. Baser, 2005. Bioactive constituents from Turkish *Pimpinella* species. Chemistry and Biodiversity, 2: 221-232.

Tabanca, N., E. Bedir, N. Kirimer, K.H.C. Baser, S.I. Khan, M.R. Jacob and I.A. Khan, 2003. Antimicrobial compounds from *Pimpinella* species growing in Turkey. Planta Medica, 69: 933-938.

Tabanca, N., B. Demiric, T. Ozek, N. Kirimer, K.H.C. Baser, E. Bedir, I.A. Khan, and D.E. Wedge, 2006. Gas chromatographic–mass spectrometric analysis of essential oils from *Pimpinella* species gathered from central and northern Turkey. Journal of Chromatography A, 1117: 194-205.

Tepe, B., D. Daferera, A. Sokmen, M. Sokmen and M. Polissiou, 2005. Antimicrobial and antioxidant activities of essential oil and various extracts of *Salvia tomentosa Miller* (Lamiaceae). Food Chemistry, 90: 333-340.

Tepe, B., H.A. Akpulat, M. Sokmen, D. Daferera, O. Yumrutas, E. Aydin, M. Polissiou, and A. Sokmen, 2006. Screening of the antioxidative and antimicrobial properties of the essential oils of Pimpinella anisetum and *Pimpinella flabellifolia* from Turkey. Food Chemistry, 97: 719-724.

Wayne, PA., 1999. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Susceptibility Testing, 9th International Supplement, M100-S9.