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The *in vitro* antioxidant and antibacterial activities of *Tanacetum pinnatum* boiss. grown in Iran

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This study was designed to examine the *in vitro* antioxidant and antibacterial activities of the aerial parts of *Tanacetum pinnatum* Boiss. GC and GC-MS analysis of the essential oil resulted in the identification of 25 compounds, representing 98.7% of the oil content. The main components in the oils were camphor (23.2%), *α*-pinene (8.5%), camphene (7.7%), 1,8-cineole (7.3%), *β*-eudesmol (5.8%) and caryophyllene oxide (5.6%). The possible antioxidant and antibacterial activity of the samples was studied using the DPPH and the *β*-carotene-linoleic acid assays and the disc agar diffusion test, respectively. In general, the nonpolar extract of *T. pinnatum* exhibited the greatest antioxidant activity in the DPPH test system. The essential oil displayed the highest antioxidant activity in the *β*-carotene-linoleic acid assay; it showed the best antibacterial activity against *Staphylococcus aureus.*

Keywords: *Tanacetum pinnatum*, antibacterial activity, camphor, *α*-pinene, antioxidant activity.

1. INTRODUCTION

The *Tanacetum* genus is represented in Iran's flora by 26 species including 12 endemics [1, 2]. Plants belonging to the *Tanacetum* genus are reputed to have excellent medicinal values, and a large number of sesquiterpenoids and sesquiterpene lactones, which are typical constituents of these drugs, have been isolated from *Tanacetum* species. These compounds might be partly or wholly responsible for the effect exhibited by the plants. Since the Middle Ages the plant *T. parthenium* has been used in the treatment of migraine, asthma, rheumatism and gynecological problems [3]. Previously, sesquiterpene lactones have been identified in the aerial parts of *T. polycephalum* [4]. The essential oil of *T. vulgare* has displayed antibacterial activity [5-7] and its sesquiterpene lactones [8] *T. pinnatum***,** *T. khorassanicum* and *T. fruticulosum* have been the subject of our previous studies [9-11]. Sesquiterpene lactones in *T. indicum* var. tuneful [12], and *T. argyrophyllum* [13], and terpenoid constituents in the oils of *T. cilicium*, *T. corymbosum* and *T. macrophyllum* have been reported. Anticoagulant and antifibrinolytic properties of these oils have also been reported [14-22]. Many applications, including food preservation, pharmaceuticals, alternative medicine and natural therapies, related to the antimicrobial and antioxidant activities of plant oils and extracts have been reported [23]. 25 components have been identified in the oil of *T. pinnatum,* in which camphor (23.2%), *α*-pinene (8.5%) and camphene (7.7%) were the main components [24]. The extract of *T. pinnatum* exhibited the greatest antioxidant activity in the DPPH test system. In the *β*-carotene-linoleic acid test system the highest antibacterial effect was displayed against *Staphylococcus aureus*.

2. EXPERIMENTAL

Plant Mmaterial

The samples of *T. pinnatum* were collected during the flowering stage from Khoramabad, Province of Lorestan, Iran, in June 2008. Voucher specimens were deposited at the Herbarium of the Research Center of Lorestan, Khoramabad, Iran.

Oil isolation

The aerial parts of *T. pinnatum* (200 g) were subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. After decanting and drying of the oils over anhydrous sodium sulfate, the corresponding oils were isolated in yields of 0.35% (w/w).

Preparation of methanol extracts

The essential oil (5 μg) was dissolved in a minimum amount of methanol. The obtained

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sample was used in the antibacterial and antioxidant activity tests.

Analysis

GC analysis of the oils was performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless injector (250°C). Nitrogen was used as carrier gas (1 mL/min), and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 μm). The column temperature was maintained at 60°C for 3 min, then increased to 220°C with a rate of 5°C/min and kept at 220°C for 5 min.

GC/MS analysis was performed using a Hewlett-Packard 6890/5973 instrument with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was maintained at 60°C for 3 min, then programmed to 220°C at a rate of 5 °C/min, and kept at 220°C for 5 min. The flow rate of the helium carrier gas was 1 mL/min. MS was taken at 70 eV.

Identification of the constituents of each oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature for authentic samples [25, 26]. Relative percentage amounts were calculated from the peak areas using a Shimadzu C-R4A Chromatopac without correction factors.

Antibacterial Activity

A collection of four microorganisms was used, including the Gram-positive bacteria *Staphylococcus aureus* (PTCC 1113), *Staphylococcus epidermidis* (PTCC 1349), *Staphylococcus saprophyticus* (PTCC 1379) and the Gram-negative bacteria *Erichia coli* (PTCC 1330) and *Pseudomonas aeruginosa* (PTCC 1310) identified by the Research Centre of Science and Industry, Tehran, Iran.

The microorganisms (obtained from enriched culture of the microorganisms in 1 mL of Mueller-Hinton broth incubated at 37 °C for 12 h) were cultured on Mueller-Hinton agar medium.

The following method was used to measure the antibacterial activity: 40 μL of diluted essential oil (40 μL oil in 2 mL DMSO 10%) were added to 200 μL of a microbial suspension (1 loop from the medium in physiological serum that corresponded to a 0.5 McFarland standard) in well 1 of a microplate, and 100 μL from this well were added to a 100 μL microbial suspension in well 2, and thiscontinued until 8 wells in the microplate were filled. The microplates were incubated at 37°C for 24 h.

Antioxidant activity

DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay usually involves hydrogen atom transfer reaction, but, based on kinetic data, electron transfer mechanism has also been suggested for this assay [27, 28]. The radical-scavenging activity (RSA) of *T. pinnatum* essential oils was determined using a published DPPH radical-scavenging activity assay method [29, 30] with minor modifications. The decrease in absorbance at 517 nm was measured on a Perkin-Elmer spectrophotometer for all samples. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without antioxidant (control sample) was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution.

The inhibition percentage of free radical DPPH (I%) was calculated as follows:

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

where A_{blank} is the absorbance of the control sample (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentage against sample concentration. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

β-Carotene/linoleic acid bleaching assay

Antioxidant activity was determined by measuring the inhibition of volatile organic compounds and conjugated diene hydroperoxides arising from linoleic acid oxidation. The method described by Miraliakbari and Shahidi (2008) [30] was used with slight modifications. 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 of Tween 40. The chloroform was evaporated under vacuum and 100 ml of aerated distilled water were then added to the residue. The samples (2 g/l) were dissolved in DMSO and 350 μl of each sample solution were added to 2.5 ml of the above mixture in test tubes. The test tubes were incubated in a water bath at 50 °C for 2 h, together with two blanks, one containing the antioxidant BHT as a positive control and the other containing the same volume of DMSO instead of the extracts. The test tube with BHT maintained its yellow color during the incubation period. The absorbances were

measured at 470 nm on an ultraviolet spectrometer. Antioxidant activities (inhibition percentage, I%) of the samples were calculated using the following equation:

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

where $A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance of β-carotene remaining in the samples after 2 h and $A_{\text{initial }\beta\text{-carotene}}$ is the absorbance of β-carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as means ± SD of triplicates.

3. RESULTS AND DISCUSSION

Chemical composition of essential oils

The percentage chemical compositions of the essential oil from the aerial parts of *T. pinnatum* Boiss. are listed in Table 1. 25 components representing 98.7% of the oil were identified. The main components of the oil were: camphor (23.2%), α-pinene (8.5%) and camphene (7.7%), 1,8-cineole (7.3%), β-eudesmol (5.8%), and caryophyllene oxide (5.6%).

Table 1. Percentage composition of the oils in the aerial parts of *Tanacetum pinnatum*

Compound	RI*	%
α-Thujene	931	0.6
α- Pinene	939	8.5
Camphene	953	7.7
β-Pinene	980	1.1
p-Cymene	1026	4.2
1,8- Cineole	1033	7.3
γ-Terpinene	1062	0.5
Linalool	1098	4.5
2,6-Dimethyl phenol	1102	1.1
Ocimene	1129	4.6
Camphor	1143	23.2
Borneol	1165	4.6
Terpin-4-ol	1177	1.2
cis-Pinocarveol	1183	1.8
Dihydro carveol	1192	0.6
Myrtenol	1193	1.8
Methyl chavicol	1195	1.0
Dihydro myrcenol acetate	1215	2.6
Sabinene hydrate acetate	1219	0.7
cis-Carveol	1229	1.3
Carvacrol	1356	3.4
Caryophyllene oxide	1581	5.6
α-Muurolol	1645	3.2
β-Eudesmol	1649	5.8
Hexadecanoic acid	1970	1.8
Total		98.7

Other notable constituents in the oil from the aerial parts of the plant were: ocimene (4.6%), borneol (4.6%), *p*-cymene (4.2%) and linalool (4.5%). One of the studies [31] compared the composition of essential oils from *T. argyrophyllum* L. and *T. parthenium* L, determined by GC/MS. In our experiments, the α-pinene content was 8.5% for *T. pinnatum*, which complies with the above-mentioned results.

Camphor is usually externally applied to relieve arthritic and rheumatic pains. It is also used in steam vaporizers to control coughs by producing a local anesthetic action to the throat and to loosen congestion due to colds [32, 33].

Both enantiomers of camphor are found in nature, but the (−)-form is less common compared to the (+)-form. Although these two enantiomers have similar camphoraceous odor [32], little is known about their biological activity. According to Ravid *et al*. [33], enantiomerically pure (−)-camphor (100%) was found in *T. parthenium*, while *T. vulgare* was rich in (+)-camphor (75%). Enantioselective analysis can easily differentiate between these two common *Tanacetum* oils. Enantiomerically pure (−)-camphor (100%) was also detected in *T. armenum* and *T. haradjani* leaf oils [34]. α-Pinene enantiomers are widespread in the nature. α-Pinene is used in fragrance industry as a starting material for the synthesis of terpineols, borneol and camphor [32]. (+)-α-Pinene has a slight minty-terpene odor while (−)-α-pinene has a coniferous odor. Yassaa and William recently reported that (+)-α -pinene was the major enantiomer in the *Pinus sylvestris* chemotypes [33].

Determination of antioxidant activity with the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity of the essential oil of *T. pinnatum* was determined by the scavenging method using two different test systems, namely DPPH and β-carotene/linoleic acid. The antioxidant activity of volatile compounds was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH. The degree of discoloration indicates the free radical scavenging potentials of sample/antioxidant and it has been found that known antioxidants such as cysteine, glutathione, ascorbic acid, tocopherol and polyhydroxy-aromatic compounds reduce DPPH by their hydrogen donating ability [36]. In the present study, the weakest radical scavenging activity was exhibited by the essential oil (568.1±4.3 μg ml^{-1}). The antioxidant activity of the essential oil was superior to that of all samples tested with an IC_{50} value of 151.9± 0.6 μg ml^{-1}. On the other hand, none of the samples showed activity as strong as the positive control BHT (88.4 ± 0.4 μg ml^{-1}). Particularly, synergistic effects of

Table 2. Antibacterial activity of the oils from the aerial parts of *Tanacetum pinnatum* based on the dilution method using four reagents. Values represent the mean diameter of the inhibitory zone (mm)

Bacterial Species	Gram +/–	Plant oils	Gentamicin	Penicillin	Sefazolin	Norfloxacin
Staphylococcus aureus PTCC 1113	+	24.2	0.0	0.0	15.7	0.0
Staphylococcus epidermidis PTCC 1349	+	32.7	30.3	21.0	30.3	31.0
Staphylococcus Saprophyticus PTCC 1379	+	29.7	0.0	25.0	20.0	11.0
Escherichia coli PTCC 1330	–	9.3	19.0	0.0	17.7	28.3
Pseudomonas aeruginosa PTCC 1310	–	15.4	15.6	0.0	15.3	30.3

phenolic acids e.g., rosmarinic acid and polyphenols, as well as other chemicals such as flavonoids could be also taken into account for the radical scavenging activity observed in methanol extracts [37].

In the *β*-carotene/linoleic acid model system, *β*-carotene undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of *β*-carotene and linoleic acid, which generates free radicals. The linoleic acid free radical formed upon abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated *β*-carotene molecules. As a result, *β*-carotene is oxidized and broken down in part; subsequently the system loses its chromophore and characteristic orange color, which is spectrophotometrically monitored. The % inhibition capacity of the essential oil (88.4±1.2) was found to be superior to that of the sample which is nearest to the inhibition capacity of the positive control BHT (96.2 ± 0.9).

The auto-oxidation of linoleic acid without volatiles and methanol extracts accompanies the rapid increase of peroxides. According to Farag *et al*. (1989)[38], there is a relationship between the inhibition of hydroperoxide formation and the presence of phenolic nuclei in the essential oils and extracts. The antioxidative effectiveness of natural sources has been reported to be mostly due to phenolic compounds [39].

Antibacterial activity

The results of the antibacterial screening showed that *T. pinnatum* oil was active against the Gram-positive bacteria *Staphylococcus aureus* (PTCC 1113), *Staphylococcus epidermidis* (PTCC 1349) *Staphylococcus saprophyticus* (PTCC 1379) (36, 23 and 22 mm diameter respectively). The same oil showed inhibitory activity against the Gram-negative bacteria *Escherichia coli* (PTCC 1330) while *Pseudomonas aeruginosa* (PTCC 1310) (36, 31 and 29 mm diameter respectively) had only moderate inhibitory activity against *Escherichia coli* (9.3 mm diameter). The results of the antibacterial screening showed that *T. pinnatum* oil was insensitive against Gram-positive and Gram-negative bacteria except *Escherichia coli* Gram-negative bacteria, for which it had only moderate activity (14.0 mm diameter) (Table 2).

4. CONCLUSIONS

The purpose of this research was to study the *in vitro* antioxidant and antibacterial activities of *Tanacetum pinnatum* Boiss. grown in Iran. The essential oil is bactericidal for certain strains tested. The antioxidant activity of *T. pinnatum* may help in preventing oxidative damages in the human body, such as lipid peroxidation, associated with cancer and diabetes. The essential oil of *T. pinnatum* exhibited greatest antioxidant activity in the DPPH and *β*-carotene–linoleic acid test systems. The oil of *T. pinnatum* exhibited the highest antibacterial activity against *Staphylococcus aureus*. The essential oil of *T. pinnatum* may be used in perfumery.

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АНТИОКСИДАНТНО И АНТИБАКТЕРИАЛНО ДЕЙСТВИЕ *IN VITRO* НА *TANACETUM PINNATUM* BOISS., РАСТЯЩО В ИРАН

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(Резюме)

Това изследване е предназначено да провери антиоксидантното и антибактериално действие *in vitro* на надземната част на *Tanacetum pinnatum* boiss., растящо в Иран. В резултат на анализ чрез GC и GC-MS на етеричното масло са идентифицирани 25 съединения, представляващи 98.7% от съдържанието на маслото. Основните компоненти на маслото са камфор (23.2%), *α*- пинен (8.5%),%), камфен (7.7%), 1,8-цинеол (7.3%), *β*-еудесмол (5.8%) и кариофиленен оксид (5.6%). Възможното антиоксидантното и антибактериално действие на пробите е изследвано чрез DPPH и *β*- каротин-линолева киселина и дискова дифузия в агар, съответно. По принцип неполярният екстракт на *T. pinnatum* показа най-голяма антиоксидантна активност при тестовата система с DPPH. Етеричното масло прояви най-висока антиоксидантна активност при изследванията с *β*-каротин-линолева киселина, то показа най-добро антибактериално действие срещу *Staphylococcus aureus.*