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Chemical Composition, Antibacterial and Antioxidant Activity of the Essential Oil of *Tanacetum polycephalum* Schutz. Bip

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Abstract: The chemical composition of the essential oil obtained by hydrodistillation from *Tanacetum polycephalum* was analysed by GC and GC/MS and 39 compounds constituting 94.02% of the oil were identified, the major components being borneol (28.30%), β-pinene (10.10%), α-pinene (6.5%), camphene (6%), α-terpineol (5.16%) and 1,8-cineol (5.10%). The essential oil exhibited remarkable bactericidal activity against *Staphylococcus aureus*, *Shigella flexnery*, *Salemonella thyphi*, *Staphylococcus epidermidis*, *Staphylococcus saprophiticus*, *Psudomonas aeroginosa* and *Escherchia coli*. The antioxidant activity of the essential oil was evaluated using the DPPH test and 5-lipoxygenase test. The antioxidant activity show that, in reduction of the stable radical DPPH, the highest activity was obtained with the essential oil and with Trolox (IC_{50} = 12.4 and 8.9 μg mL^{-1}, respectively).

Key words: *Tanacetum polycephalum*, oil, antioxidant, antibacterial, borneol

INTRODUCTION

The essential oil and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and preservation of foods from the toxic effects of oxidants. Particularly, the antimicrobial and antioxidant activities of plants oils and extracts have formed the basis of many applications, including low and processed food preservation, pharmaceuticals and alternative medicine and natural therapies (Lis-Balchin and Deans, 1997). Moreover, they offer an effective way to prevent the development of various off-flavours and undesirable compounds that result from lipid peroxidation in foods (Wang *et al*., 1998). Because of the possible toxicities of the synthetic antioxidants, butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT), increasing attention has been directed toward natural antioxidants (Namiki, 1990).

Steam distillated oils obtained from the flowers and leaves of *T. polycephalum* were already investigated. The main components of the oil of flowers were camphor (59.1%), camphene (14.9%) and 1,8-cineol (10.1%), wherease the leaves oil comprised mainly camphor (53.5%), bornyl acetate (12.1%), camphene (10.9%), 1,8-cineol (7.8%) and borneol (6.1%) (Nori-Shargh, *et al*., 1999). Camphor (18.2%), 1,8-cineol (17.0%), carveol (9.1%), trans-isopulegone (8.0%) and α-thujone (6.1%) as major constituents also are reported of the aerial parts oil of this plant (Mojab and Nickavar, 2006).

The genus *Tanacetum* (Compositae) is represented by 26 species in the flora of Iran, 12 of them are endemic (Mozaffarian, 1996). *Tanacetum polycephalum* are used in folk medicine to treat many disorders (Zargari, 1996), therefore, it seem interesting to investigate its biological activity and chemical analysis.

Present study deals with the analysis, antimicrobial and antioxidant activity of the essential oil of *Tanacetum polycephalum* grown wild in West of Iran.

MATERIALS AND METHODS

The aerial parts of *Tanacetum* species were collected during flowering period (May 2005) from Aleshtar, Lorestan province, Iran. The specimens were then subjected to hydrodistillation using a Cleavenger-type apparatus for 2.5 h subsequent to decanting and drying over anhydrous sodium sulfate.

GC analysis: GC analysis was performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless injector and a flame ionization detector at 250°C. N_2 was used as a carrier gas (1 mL min^{-1}) and a DB-5 type was utilized as the capillary (50 m×0.2 mm, film thickness 0.32 μm). Temperature within the column for 3 min was retained at 60°C, after that the column was heated at a rate of 5°C min^{-1} until it reached at 220°C and maintained in this condition for 5 min. The percentage of relative amounts were calculated from peak area using a shimadzu C-R4A chromatopac without applying correction factors.

GC/MS analysis: The analysis of the essential oil was performed using a Hewlett-packard 5973 with a HP 5MS column (30 m×0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60°C or 3 min and programmed to reach 220°C at rate of 5°C min^{-1} and stayed steady at 220°C for 3 min. The components of each oil were then identified by comparison of their mass spectra and Retention Indices (RI) with those given in literature and those authentic samples (Adams, 1995).

Antimicrobial activity : The antimicrobial tests were carried out by using the following microorganisms: *Staphylococcus aureus* PTCC 1113, *Staphylococcus epidermidis* PTCC 1349, *Staphylococcus saprophyticus* PTCC 1379 (Gram-positive bacteria), *Salmonella typhi* PTCC 1185, *Shigella flexneri* PTCC 1234, *Escherichia coli* PTCC 1330, *Pseudomonas aeroginosa* PTCC 1310 and *Klebsiella pneumoiae* PTCC 1053 (Gram-negative bacteria) identified by Iranian Research organization for Science and Technology (IROST).

Microorganisms (obtained from enrichment culture of the microorganisms in 1 mL of Muller-Hinton broth, incubated at 37°C for 12 h) were cultured on Muller Hinton Agar medium. After drilling wells on medium oils dissolved in hexane and 40 μL from solutions in each well was poured. After incubation at 37°C during 24 h, incubation zone diameter was measured.

Antioxidant activity

Inhibition of lipid peroxide formation: Free radical scavenging activity was evaluated by the 5-lipoxygenase test in sample and in positive controls. The activity of the enzyme was assayed spectrophotometrically according to Holman. This method was modified by Sud'ina *et al.* (1993).

The assay mixture (1 mL) contained: 10 mM linoleic acid, the sample (or the same quantity of solvent as reference) and 50 mM sodium phosphate, pH 6.8. This mixture was maintained at 20°C for 20 min.

Subsequently, 0.18 μg mL^{-1} commercial 5-lipoxygenase was added to mixture and formation of hydroperoxides from linoleic acid was observed spectrophotometrically at 235 nm at 20°C.

DPPH assay: Radical scavaging activity was determined by spectrophotometric method based on the reduction of an ehanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Mellors and Tappel, 1966). Tests were carried out in triplicate.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxlic acid), BHT (butylated hydroxytoluene) and ascorbic acid were used as positive controls and purchased from Merk company.

Hydroxyl radical scavenging: Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The attack of the hydroxyl radical on deoxyribose leads to TBARS formation (Kunchardy and Rao, 1990). Various concentration of the extracts were add to reaction mixture containing 3.0 mM deoxyribose, 0.1 mm $FeCl_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2 and 20 mM phosphate buffer (pH 7.4), making up a final volume of 3.0 mL. The reaction mixture was incubated at 37°C for 1 h. The formed TBARS were measured by the methods given elsewhere (Ohkawa *et al.*, 1979). On mililitre of thiobarbituric acid, TBA (1%) and 1.0 mL trichloroacetic acid, TCA (2.8%) were added to test tubes and incubated at 100°C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate. Inhibition (I) of deoxyribose degradation in per cent was calculated in following way:

$$I = (A_0 - A_1/A_0)\times 100$$

Where A_0 is absorbance of control reaction (containing all reagents except the test compound) and A_1 is the absorbance of the test compound.

RESULTS AND DISCUSSION

The yield of essential oil obtained by hydrodistillation from dried plant material was 0.45%. The composition of the volatile oil of *Tanacetum polycephalum* is listed in Table 1. Thirty-nine constituents, representing 94% of the total components in the oil, have been identified in the essential oil extracted from the aerial parts of this plant. There was a high content of monoterpene compounds including borneol (28.30%), β-pinene (10.10%), α-pinene (6.5%), camphene (6.04%), α-terpineol (5.16%) and 1,8-cineol (5.10%) as major components. Among the identified sesquiterpenes in the oil spathuenol (4.17%) and caryophyllene oxide (2.33%) were most abundant. The studied by us is different from the other Iranian samples (Nori-Shargh, *et al.*, 1999; Mojab and Nickavar, 2006). According to Nori-Shargh *et al.* (1999) camphor (59.1%), camphene (14.9%) and 1,8-cineol (10.1%) in flowers oil and camphor (53.5%), bornyl acetate (12.1%), camphene (10.9%), 1,8-cineol (7.8%) and borneol (6.1%) in the leaves oil were the main components of essential oil of this plant, wherease Mojab and Nickavar (2006) are reported camphor (18.2%), 1,8-cineol (17.0%), carveol (9.1%), trans-isopulegone (8.0%) and α-thujone (6.1%) as major

Table 1: Composition of the aerial parts oil of *Tanacetum polycephalum* Schutz. Bip

Compound	RI	%	Compound	RI	%
Tricyclene	927	0.15	Geranial	1269	0.19
α-thujene	930	0.10	Bornyl acetate	1285	4.05
α-pinene	935	6.50	Lavandulyl acetate	1292	0.35
Camphene	954	6.04	Geranyl acetate	1379	1.58
β-pinene	974	10.1	Cis-jasmane	1392	0.36
1,8-cineol	1027	5.10	Neryl acetone	1429	0.10
γ-terpinene	1059	1.32	β-selinene	1484	0.53
Trans sabinene hydrate	1064	1.40	Bornyl isovalerate	1491	2.29
Terpinolene	1085	0.24	δ-cadinene	1526	1.30
Cis-sabinene hydrate	1097	0.44	Nerolidol	1574	2.35
Camphor	1143	1.42	Spathuleol	1589	4.17
Borneol	1166	28.27	Caryophylene oxide	1596	1.33
Terpinene-4-ol	1175	1.25	E-sesqui-lavandulol	1606	1.36
α-terpineol	1187	5.16	Octadecane	1799	0.65
Myrtenol	1194	0.81	Banzyl banzoate	1863	0.30
Trans-carveol	1219	0.57	Hexadecanoic acid	1923	0.74
Cuminal	1240	0.18	Octadeca-1-ol	2082	0.34
Carvone	1242	0.64	Phytol	2135	0.18
Geraniol	1255	1.89	Tricosane	2300	0.10
Cis-chrysanthyl acetate	1262	0.17			

Table 2: Antibacterial activity of *Tanacetum polycephalum* oil

Microorganism	Essential oil	Genta.	n-hexane
Staphylococcus aureus PTCC1113	45	12	-
Staphylococcus epidermidis PTCC 1349	29	20	-
Staphylococcus saprophyticus PTCC 1379	21	15	-
Salmonella typhi PTCC1185	31	14	-
Shigella flexneri PTCC 1234	52	12	-
Escherichia coli PTCC 1330	25	15	-
Pseudomonas aeruginosa PTCC 1310	33	15	-
Klebsiella pneumoiae PTCC1053	41	11	-

-: Not determined

Table 3: Effect of *Tanacetum polycephalum* essential oil and positive controls on the *in vitro* free radical (DPPH and hydroxyl) and lipid peroxidation generation

	IC_{50} (μg mL $^{-1}$)		
Sample	DPPH	Hydroxyl	Lipid peroxidation
Essential oil	12.4	1.7	14.8
Ascorbic acid	94.5	nt	31.2
BHT	74.6	33.1	23.6
Trolox	8.9	0.1	6.7

Nt: Not tested

constituents of the aerial parts oil of *T. polycephalum*. On the other hand, in the present study camphor was detected only in low amount wherease is a abundant compound. These differences might have been derived from harvest time, extraction method, locality, climatic and seasonal factors.

The essential oil of *T. polycephalum* remarkably inhibited the growth of the tested bacteria. The oil was shown to possess the strongest antibacterial activity particulary against *Salemonella typhi* (52 mm), *Staphylococcus aureus* (45 mm) and *Klebsiella pneumoniae* (41 mm) corresponding to the largest inhibition zone diameters (Table 2). The antibacterial activities of *T. polycephalum* can be attributed to the presence of high concentration monoterpene compounds. The antimicrobial activities of borneol (an oxygenated monoterpene) as major compound have been previously reported (Dorman and Deans, 2000). Other than the main compounds, β-pinene, α-pinene, camphene and 1,8-cineol as well as other minor constituents of the essential oil of *T. polycephalum* have antibacterial activity (Sivropoulou *et al*., 1997; Sur *et al*., 1991).

The antioxidant activity of *T. polycephalum* essential oil was determined using three *in vitro* assays: Scavenging effect on DPPH and hydroxyl radicals and inhibition of lipid peroxide radicals formation. The antioxidant activity of the test sample, expressed as IC_{50} (μg mL^{-1}), was compared with activity of know antioxidants such as ascorbic acid, BHT and Trolox. The data collected in Table 3 show that, in reduction of the stable radical DPPH, the highest activity was obtained with the essential oil and with Trolox (IC_{50} = 12.4 and 8.9 μg mL^{-1}, respectively).

Hydroxyl radical scavenging assay was not performed with ascorbic acid since this compound was already present in the test medium. Hydroxyl radical scavenging of the essential oil was better than BHT (IC_{50} = 1.7 and 33.1 μg mL^{-1}, respectively) but lesser than Trolox (IC_{50} = 0.1 μg mL^{-1}). In the inhibition of lipid peroxidation the IC_{50} values obtained showed that the essential oil antioxidant activity were higher than ascorbic acid and BHT (IC_{50} = 31.2 and 23.6 μg mL^{-1}, respectively) and less than that of Trolox (IC_{50} = 6.7 μg mL^{-1}).

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