Antibacterial Activity and Comparison of the Volatile Oils of *Tanacetum tenuisectum* (Boiss.) Podl. Obtained by Three Different Methods of Extraction

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**Abstract**

The essential oils obtained by hydrodistillation (HD), steam distillation (SD) and solvent free microwave extraction (SFME) from the stems and flowers of *Tanacetum tenuisectum* (Boiss.) Podl., which is endemic to Iran, were analyzed by combination of GC and GC/MS. Camphor (26.91%, 27.23% and 25.52%), borneol (12.61%, 11.48% and 7.62%) and 1,8-cineole (7.93%, 13.23% and 11.26%) were the main constituents of the HD, SD and SFME oils of *Tanacetum tenuisectum* respectively.

All three oils were rich in regard to monoterpenes and small percentage of sesquiterpenes and non terpenoid compounds.

Antibacterial activity of the essential oil of the plant was determined against six Gram positive and Gram negative bacteria. The results showed that this oil was active against all of the tested bacteria.

**Keywords:** *Tanacetum tenuisectum*; Compositae; Essential oil; Hydrodistillation; Steam distillation; Microwave extraction; Antibacterial activity.

**Introduction**

The genus *Tanacetum*, which is an important member of the Compositae family, is widespread in Europe and Western Asia and consists about 150-200 species. The flora of Iran comprises 26 species of *Tanacetum* of which 12 are endemic (1, 2).

Some members of this genus have traditionally been used as a spicy additive for food, in cosmetics and as herbal remedies due to their biologically active compounds (3). Especially *Tanacetum parthenium* has been used since ancient times for a variety of medicinal proposes, and recently has gained considerable prominence due to its ability of alleviating the symptoms of migraine, arthritis and psoriasis, and to inhibition of blood platelet aggregation (4).

According to recent studies, essential oils and extracts of members of the genus *Tanacetum* exhibit anti-inflammatory (5-7) anticancer (8) antibacterial (9, 10) antiviral (11) antifungal (12) insecticidal (13) and antiprotozoal effects (14, 15).

This genus is also found to contain sesquiterpene lactones (16, 17) a large group of molecules with several biological activities (18, 21).

Previous chemical investigations on different Iranian species of *Tanacetum* have shown that they possess sesquiterpene lactones (22, 23) and essential oils (24-32).

As part of our ongoing work on the chemical analysis of volatile obtained from wild plants of
Iran, we report the composition of the volatile oils of *Tanacetum tenuisectum* obtained by hydrodistillation, steam distillation and solvent free microwave extraction with its antibacterial activity.

**Experimental**

**Plant material**

The stems and flowers of *Tanacetum tenuisectum* (syn. *Pyrethrum tenuisectum* Boiss., *Chrysanthemum gauvae* Borm., and *Chrysanthemum tenuisectum* (Boiss.) Parsa), which is endemic to Iran, was collected from Rameh area, Garmser, Province of Semnan, Iran in July 2013, during the flowering stage.

Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

**Isolation of the essential oils**

**Distillation**

Air-dried ground stems and flowers of the plant were separately subjected to hydrodistillation and steam distillation using a Clevenger type apparatus for 3h. The obtained essential oils were dried over anhydrous sodium sulfate and after filtration, stored at 4ºC until tested and analyzed. The yield was found to be 0.2% and 0.3% (w/w), respectively.

**Solvent Free microwave extraction**

SFME extraction was performed in a Milestone ETHOS 1600 batch reactor, which is a multimode microwave reactor operating at 2455 MHZ with a maximum delivered power of 1000 W, variable in 10 W increments. The dimensions of the PTFE-coated cavity are 35×35×35 cm. During the experiment time, temperature, pressure, and power were controlled using the ‘easy-WAVE’ software package. Temperature was monitored with the aid of a shielded thermocouple (ATC-300) inserted directly in to the sample container.

In a typical SFME procedure, 250 g of dry stems and flowers of *T. tenuisectum* were moistened prior to extraction by soaking in water for 1 h, then draining off the excess water. This step is essential to give the stems and flowers the initial moisture. Moistened stems and flowers were next placed in a reactor without any added solvent or water. The essential oil is collected, dried with anhydrous sodium sulfate and stored at 0ºC until used.

**Gas chromatography**

GC analysis was performed on Schimadzu 15A gas chromatograph equipped with a split/splitless injector (25ºC) and a flame ionization detector (250ºC). Nitrogen was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (50m×0.2×mm, film thickness 0.32µm). The column temperature was kept at 60ºC for 3 min and then heated to 220ºC with 5ºC/ min rate and kept constant at 220ºC for 5 min. Relative percentage amount were calculated from peak area using Schimadzu C-R4 Achromatopac without the use of correction factors.

**Gas chromatography - mass spectrometry**

Analysis was performed using a Hewlett-Packard 5973 with a HP-5MS column (30m×0.25× mm, film thickness 0.25 µm). The column temperature was kept at 60ºC for 3 min and programmed to 220ºC at a rate of 5ºC/min and kept constant at 220ºC/min for 5 min. The flow rate of Helium as carrier gas with (1 mL/min).

Mass spectrometry was taken at 70 eV. The retention indices for all the components were determined according to the Van Den Door method, using n-alkanes as standards (33).

The compounds were identified by (RRI, DB5) with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra (34, 35).

**Antibacterial assay**

The antibacterial activity of the essential oil from the aerial parts of *Tanacetum tenuisectum* was evaluated by disc diffusion method using Mueller- Hinton Agar (36). The antibacterial activity of the essential oil of the plant was tested against three Gram- positive and three Gram- negative bacteria.

The Gram-positive bacteria included *Staphylococcus aureus* ATCC 25923,
Bacillus subtilis ATCC 9372, and Bacillus creus ATCC 6633, and the Gram-negative bacteria included Klebsiella pneumoniae ATCC 27736, Enterobacter aerogenes ATCC 49469, and Escherichia coli ATCC 25922. The bacteria were obtained from the Iranian Research Organization of Science and Technology.

A serial dilution of the oil was prepared in Mueller-Hinton Broth for bacteria. The oil was diluted by the water and ethanol solvents. The solvents, at an appropriate concentration were also used as a negative control. The standardized suspension of bacteria was incubated in to each tube. The tubes were incubated at 37ºC for 24 h. The lowest oil concentration, where there was no visible growth, was the Minimum Inhibitory Concentration (MIC) when compared to control.

To determine the Minimum Bactericidal Concentration (MBC), for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and incubated on Muller-Hinton Agar by streaking. Plates incubated with bacteria were then incubated at 37ºC for 24 h. After incubation, the concentration at which no visible growth was seen was noted as MBC for bacteria. All the experiments were carried out in triplicate and mean calculated.

Results and Discussion

The identified volatile components and their peak area percentages of the stems and flowers of Tanacetum tenuisectum obtained by hydrodistillation, steam distillation and solvent free microwave extraction are given in Table 1. The components are listed in order of their elution on the DB-5 column.

As it is shown from the Table 1, about 96.87% (34 components) of the hydrodistilled oil, 91.32% (46 constituents) of the steam distilled oil and 95.1% (44 components) of the solvent free microwave extraction oil of T. tenuisectum were identified.

The main components in three oils were camphor (26.91%, 27.23% and 25.52%), borneol (12.61%, 11.48% and 7.62%) and 1,8-cineole (7.93%, 13.23% and 11.26%), respectively. Other notable constituents were in hydrodistilled oil hexadecanoic acid (7.30%), carotol (6.58%) and γ-eudesmol (5.75%); in steam distilled oil camphene (5.44%), hinesol (4.35%) and (E)-sequilavandulol (4.24%) and in solvent free microwave extraction oil hinesol (6.86%), hexadecanoic acid (6.55%) and (E)-sequilavandulol (4.89%).

According to these results, the composition of the three oils show significant similarity for the concentration of the main components. All three oils were rich in regard to monoterpenes (55.14%, 68.69% and 55.97%, respectively), while the sesquiterpenes fraction was (27.27%, 13.89% and 24.47%, respectively). The nonterpenoid fraction was relatively small, representing 14.46%, 8.74%, and 14.66%, respectively.

In our previous investigations on Tanacetum genus we have identified essential oil compositions of T. balsamitha, T. polycephalum, T. khorassanicum, T. paradoxum, T. tabrisianum, T. elburensis and T. persicum (24-28). The dominant compound in T. balsamitha was carvone (68.0%) (24).

Camphor (18.2% and 13.9%) and 1,8-cineole (17.0% and 18.6%) were found to be the major components of the oil of T. polycephalum and T. lingulatum, respectively (25, 29).

The major constituents of the aerial parts of T. khorassanicum were (E)-myroxide (19.8%), camphor (16.4%), isopulegon (13.4%) and 1,8-cineole (11.4%) (26).

Water distilled oil obtained from the aerial parts of T. paradoxum and T. tabrisianum have been the subject of our previous studies. The major components were camphor (23.8%), lavandulyl acetate (19.1%), lavandulol (15.9%) and 1,8- cineole (13.2%) in the former oil, caryophyllene oxide (12.0%) and spathulenol (10.3%) in the latter (27). Water- distilled oils from the aerial parts of T. elburensis and T. persicum growing wild in Iran were investigated.

The main constituents of the oil of T. elburensis were menthylisovalerate (20.0%) and 1,8 cineole (16.6%). The oil of T. persicum was characterized by higher amounts of borneol (24.3%), menthyl acetate (17.3%), isobornyl 2-methyl butyrate (16.0%) and artemosoloxide D (14.3%) (28).

The oils obtained by hydrodistillation of the leaves and flowers of T. dumosum growing wild in Iran were investigated. The main constituents
Table 1. Comparative chemical composition (%) of *Tanacetum tenuisectum* oil obtained by HD, SD and SFME.

| No. | Compounds          | RI  | HD(%) | SD(%) | SFME(%) |
|-----|--------------------|-----|-------|-------|---------|
| 1   | Hexanal            | 800 | -     | 0.24  | -       |
| 2   | Tricyclene         | 924 | -     | 0.37  | -       |
| 3   | α-Thujene          | 928 | -     | 0.19  | t       |
| 4   | α-Pinene           | 935 | -     | 1.51  | t       |
| 5   | Camphene           | 951 | 0.70  | 5.44  | 3.92    |
| 6   | β-Pinene           | 981 | -     | 1.04  | 0.68    |
| 7   | Mesitylene         | 994 | -     | 0.33  | t       |
| 8   | p-Cymene           | 1024| t     | 1.84  | 1.17    |
| 9   | 1,8-Cineole        | 1033| 7.93  | 13.23 | 11.26   |
| 10  | γ-Terpinene        | 1062| -     | 0.42  | t       |
| 11  | Linalool           | 1096| 0.73  | 0.62  | t       |
| 12  | Camphor            | 1141| 26.91 | 27.23 | 25.52   |
| 13  | cis-Chrysanthenol  | 1160| 0.82  | -     | 1.09    |
| 14  | Pinocarvone        | 1162| -     | 0.32  | t       |
| 15  | Borneol            | 1165| 12.61 | 11.48 | 7.62    |
| 16  | Terpin-4-ol        | 1177| 1.14  | 0.99  | 0.59    |
| 17  | Naphthalene        | 1183| -     | 0.25  | t       |
| 18  | α-Terpineol        | 1188| 1.20  | 0.84  | 0.58    |
| 19  | Myrtenal           | 1191| -     | 0.22  | t       |
| 20  | Myrtenol           | 1193| -     | 0.24  | t       |
| 21  | trans-Carveol      | 1217| 0.64  | 0.78  | 0.54    |
| 22  | cis-Chrysanthenyl acetate | 1260| t     | 0.23  | t       |
| 23  | Bornyl acetate     | 1285| 1.66  | 1.40  | 1.12    |
| 24  | 2- methyl Naphthalene | 1292| -     | 1.00  | 0.86    |
| 25  | 1- methyl Naphthalene | 1306| -     | 0.63  | t       |
| 26  | α-Terpiny acetate  | 1350| -     | 0.3   | -       |
| 27  | Decanoic acid      | 1352| 2.03  | -     | 0.19    |
| 28  | α-Copaene          | 1376| -     | 0.27  | -       |
| 29  | 2,6-dimethyl Naphthalene | 1379| -     | -     | 0.57    |
| 30  | (E)-β-Damascenone  | 1380| -     | t     | -       |
| No. | Compounds* | RP* | HD(%) | SD(%) | SFME(%) |
|-----|------------|-----|-------|-------|--------|
| 31  | 1,7- dimethyl Naphthalene | 1397 | -     | -     | 0.44   |
| 32  | 2,7- dimethyl Naphthalene | 1400 | -     | 0.54  | -      |
| 33  | 1,3- dimethyl Naphthalene | 1415 | -     | 0.46  | -      |
| 34  | β-Caryophyllene           | 1418 | -     | -     | 0.73   |
| 35  | Neryl acetone             | 1431 | 0.80  | -     | 0.88   |
| 36  | isobutyl-n-Butyrate       | 1471 | 1.12  | -     | -      |
| 37  | Pentadecane               | 1500 | -     | 0.17  | -      |
| 38  | β-Bisabolene              | 1509 | -     | -     | 0.56   |
| 39  | Elemol                    | 1546 | -     | -     | 0.41   |
| 40  | (E)- Nerolidol            | 1564 | 1.07  | 0.36  | 1.37   |
| 41  | (2)-dehydroApofarnesol    | 1568 | 0.70  | -     | 0.77   |
| 42  | Spathulenol               | 1574 | 2.75  | 1.11  | 1.37   |
| 43  | Caryophyllene oxide       | 1581 | 3.43  | 1.40  | 2.01   |
| 44  | Carotol                   | 1594 | 6.58  | -     | -      |
| 45  | Tetradecanal              | 1610 | 1.30  | -     | 1.23   |
| 46  | (E)- isoEugenol acetate   | 1611 | -     | 0.39  | -      |
| 47  | γ-Eudesmol                | 1630 | 5.75  | -     | -      |
| 48  | (E)- Sesquilavandulol     | 1632 | -     | 4.24  | 4.89   |
| 49  | β- Caryophylla-4(12),8(13)dien-5-ol | 1636 | 1.87 | - | 0.87 |
| 50  | Hinesol                   | 1638 | -     | 4.35  | 6.86   |
| 51  | β-Eudesmol                | 1646 | -     | 1.58  | 2.33   |
| 52  | α-Eudesmol                | 1652 | 1.95  | -     | -      |
| 53  | α-Cadinol                 | 1653 | -     | -     | 1.41   |
| 54  | 1(5),3-Aromadenedriene    | 1660 | -     | -     | 0.51   |
| 55  | 14- hydroxy-9-epi-β- Caryophyllene | 1664 | 0.97 | 0.27 | -     |

| No. | Compounds*                                      | RP* | HD(%) | SD(%) | SFME(%) |
|-----|------------------------------------------------|-----|-------|-------|--------|
| 56  | Khusinol                                        | 1674 | -     | 0.31  | -      |
| 57  | (z)-Nerolidol acetate                           | 1675 | 0.79  | -     | -      |
| 58  | 1,6-dimethyl-4-(1-methylethyl) Naphthalene      | 1685 | -     | 0.30  | 0.98   |
| 59  | Germacrone                                      | 1693 | 0.86  | -     | -      |
| 60  | 2-Pentadecanone                                 | 1696 | -     | -     | 0.61   |
| 61  | (E)- Neroliol acetate                           | 1712 | 0.55  | -     | -      |
Comparison of the Volatile Oils of Tanacetum tenuisectum (Boiss.).

| Compound                        | Retention Index | Relative Intensity | Specific Intensity |
|---------------------------------|-----------------|--------------------|--------------------|
| (E,E)- Farnesol                 | 1722            | -                  | 0.38               |
| Tetradecanoic acid              | 1771            | 0.61               | 0.50               |
| Octadecane                      | 1800            | t                  | 0.21               |
| Hexadecanal                     | 1806            | -                  | 0.64               |
| 6,10,14-trimethyl-2-Pentadecane | 1872            | 0.80               | 0.44               |
| Nonadecane                      | 1900            | 0.67               | 0.18               |
| Hexadecanoic acid               | 1973            | 7.30               | 2.76               |
| Eicosane                        | 2000            | 0.63               | -                  |
| Henicosane                      | 2100            | -                  | 0.34               |

| Compartment                      | Retention Index | Relative Intensity | Specific Intensity |
|----------------------------------|-----------------|--------------------|--------------------|
| Monoterpene hydrocarbons         | -               | 0.70               | 10.81              |
| Oxygenated monoterpenes          | 54.44           | 57.88              | 49.20              |
| Sesquiterpene hydrocarbons       | -               | 0.27               | 1.29               |
| Oxygenated sesquiterpenes        | 27.27           | 13.62              | 23.18              |
| Other compounds                  | 14.46           | 8.74               | 14.66              |
| Total                            | 96.87           | 91.32              | 95.1               |

Note: *Compounds listed in order of elution from HP-5 MS column; Retention indices to C8 - C24 n-alkanes on HP-5 MS column; t = trace ( < 0.1% ).

of the leaves oil were borneol (27.9%), bornylacetate (18.4%) and 1,8-cineole (17.5%), while the main components of the flower oil were isobornyl-2-methylbutanoate (41.1%) and trans-linalyl oxideacetate (11.9%) (30).

**Aerial parts of T. sonbolii, endemic in Iran, contained α- cadinol (35.3%), globulol (20.1%) and 1,8-cineole (8.6%) as major constituents** (31).

Camphor (30.2%), (z)-chrysanthenylacetate (26.5%) and α- farnesene (11.1%) were found to be the major components from the root oil of *T. parthenium* from Iran. (32).

The dominant compound in the flower and stem oils of *T. chilophyllum*, from Turkey, was camphor (17.3% and 10.4%) respectively, while root oil of the plant was characterized with hexadecanoic acid (37.5%) (37).

The dominant compound in the oil of *T. nitens* and *T. argenteum* from Turkey was 1,8 cineole (27.57%) and α- pinene (27.86%) respectively (38).

Baser et al. reported the oils from the flowers of *T. zahlbruckneri* and flowers and stems of *T. tabrisionum* from Turkey.

The flower oil of *T. zahlbruckneri* was characterised by germacreneD (29.7%) and spathulenol (12.0%), 1,8-Cineole (17.6% and 22.5%) and hexadecanoic acid (10.3% and 8.0%) were the major constituents of the flower and stem oil of *T. tabrisionum* respectively (39).

The flower, stem and leaf oils of *T. densum* from Turkey, were characterized with camphor (30.9%, 25.7% and 27.7%, respectively) (40). Lavandulol (21.5%) and 1,8-cineole (15.2%) were identified as major components in the oil of *T. gracile* from Ladakh Himalaya (India) (41).

Results of the antibacterial activity of the essential oil of *Tanacetum tenuisectum*...
Table 2. Antibacterial activity of essential oil of Tanacetum tenuisectum.

| Microorganisms                  | Inhibition zone (mm) | MIC*  | MBC*  |
|--------------------------------|----------------------|-------|-------|
| Staphylococcus aureus (ATCC 5923) | 11                   | 625   | 1250  |
| Escherichia coli (ATCC 25922)   | 13                   | 625   | 1250  |
| Klebsiella pneumoniae (ATCC 27736) | 16                   | 625   | 1250  |
| Entobacteria aerogenes (ATCC 49469) | 16                   | 625   | 625   |
| Bacillus subtilis (ATCC 9372)    | 13                   | 625   | 625   |
| Bacillus creus (ATCC 6633)      | 16                   | 1250  | 2500  |

Note: *Minimum inhibitory concentration; **Minimum bactericidal concentration.

MIC and MBC of compounds are indicated in µg/mL.

are shown in Table 2.

Anti bacterial activity was determined against six bacteria.

The oil has shown maximum zone of inhibition against *Klebsiella pneumoniae*, *Entobacteria aerogenes* and *Bacillus creus*. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Entobacteria aerogenes* and *Bacillus subtilis* were the most sensitive bacteria to the essential oil (having MIC value 625 µg/mL).

*Entobacteria aerogenes* and *Bacillus subtilis* have a minimum bactericidal concentration (MBC value 625 µg/mL). 1,8- Cineole and camphor are well-known chemicals having antibacterial potentials (42, 43). The antibacterial effects of borneol were also reported (44). As a result of these findings, antibacterial activity of *T. tenuisectum* oil could be attributed to 1,8 cineol, camphor and borneol. The present study confirms that there is a positive correlation between the chemical content of the oils and their antibacterial activities.

Acknowledgment

We are grateful to Dr. V. Mozaffariam (Research Institute of Forests and Rangelands, Tehran) for his helpful assistance in botanical identification.

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