Antimicrobial and antioxidant activities and total phenolic content of
*Tanacetum polycephalum* Schutz.Bip. as a folkloric herb in South western Iran

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Medicinal plants and their essential oils are potentially useful sources of antioxidant and antibacterial compounds. *Tanacetum polycephalum* Schutz.-Bip belonging to the family Asteraceae is an important folkloric medicinal plant in Chaharmahal and Bakhtiari province, South western Iran. In this study, total phenolic content, antioxidant and antibacterial activities of the essential oil from the aerial parts of *T. polycephalum* collected from 6 different natural habitats of Chaharmahal va Bakhtiari province were investigated. The total phenolic content of the essential oil was determined by Folin–Ciocalteu method and antioxidant activity using α, α ‐ diphenyl ‐ β ‐ picrylhydrazyl (DPPH) method was tested. In addition, the antibacterial activity of the essential oils against 6 bacteria, including *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, *Listeria monocytogenes*, *Streptococcus agalactiae* and *Salmonella typhimurium* was tested by agar disc diffusion assays. Results of this study indicated that total phenolic contents ranged between 0.06 to 0.16 mg GAE/gm DWE. In addition, results indicated that the essential oils from different populations of *T. polycephalum* exhibited radical scavenging activity. The results of this study confirmed the essential oils from different populations of *T. polycephalum* exhibited marked antibacterial activity. This finding suggests that the herb can be served as a valuable source of natural antioxidants and antibacterial for further isolation and purification.

**Keywords:** *Tanacetum polycephalum*, Biological activity, Essential oil

**IPC Int. CL:** C11B 9/00, A23L 1/222, C09K 15/00, C12M, C12N

Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth¹⁻³.¹⁴ There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases⁴. In addition, many herbal and some common medicinal plants are good antioxidant compounds. Many of the biologically active substances found in plants, including phenolic compounds (flavonoid and phenolics) are known to possess potential antioxidant properties.⁶ Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals. Free radicals have been implicated in the etiology of several major human ailments including cancer, cardiovascular diseases, neural disorders, diabetes and arthritis⁷. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating and catalytic metals and by acting as oxygen scavengers⁸. However, numerous studies have been published on the antioxidant and antimicrobial activities of plant compounds⁹⁻¹³.

The genus *Tanacetum* (syn. *Chrysanthemum*), belonging to the family Asteraceae, is distributed over Europe and west Asia and consists of about 150- 200 species¹⁴,¹⁵. *T. polycephalum* Schultz-Bip. is a taxonomically complex species, in which Rechinger recognized seven subspecies in *Flora Iranica*¹⁶. *T. polycephalum* as an important medicinal plant in traditional medicine of Iran (*Unani* medicine) have
been used to treat many disorders\textsuperscript{17,18}. According to recent studies, the essential oils and extracts of \textit{T. polycephalum} have biologically activity, including antibacterial\textsuperscript{19,20,21}, antifungal\textsuperscript{21,22}, antioxidant\textsuperscript{19}, antiallergic, anticancer, anti-irritant, antiseptic, anesthetic, analgesic, and disinfective effects\textsuperscript{23}.

Some earlier works have been reported on the essential oils of various \textit{Tanacetum} species from all over the world\textsuperscript{24,25,26}. This genus is rich in essential oils, bitter substances, and sesquiterpene lactones\textsuperscript{27}. Pervious chemical investigations on different species of \textit{Tanacetum} have shown the presence of flavonoids\textsuperscript{28} and essential oils\textsuperscript{29,30,31}.

To our knowledge, there are no published reports on total phenolic content, antibacterial and antioxidant activities of \textit{T. polycephalum}. The main aim of the present study was to evaluate the content of phenolic compounds, antioxidant and antibacterial activities of essential oils of wild populations of \textit{T. polycephalum} from Chaharmahal va Bakhtiari province, Iran.

**Material and methods**

**Plant material**

The aerial parts (up to ~ 5 cm, 0.05-0.2 kg) of wild populations of \textit{T. polycephalum} were collected from Chaharmahal va Bakhtiari province, South western Iran at the early flowering stage on April-June 2011. The samples of the plants were identified and voucher specimens were deposited at the Herbarium of I A U, Shahrekord Branch, Shahrekord, Iran (Voucher specimen No. 1357).

Soil physical and chemical characteristics such as pH, EC, texture, and OC were taken from a soil-sampling from different locations of Chaharmahal va Bakhtiari province (Table 1). The pH is determined using a glass and reference electrode with a pH meter on a 1:1 suspension (Metrohm AG, 691). The EC is determined using with an EC meter on a 1:1 suspension (Jenmay 4020). Also the texture and organic carbon of soil is determined.

**Essential oil preparation**

Harvested flowering aerial parts (leaves and flowers) were dried at room temperature for one week. Dried plant material were powered (100gm) and subjected to hydro-distillation (1000 ml distilled water) for 3 hrs using a Clevenger-type apparatus. Samples were dried with anhydrous sodium sulphate and kept in amber vials at 4°C until chromatographic analysis.

**Determination of total phenolic content (TPC)**

The total phenolic content in each essential oil was determined using the Folin–Ciocalteu method. Briefly, 0.5 ml of the sample were mixed with 2.5 ml of Folin–Ciocalteu’s phenol reagent and kept for 5 min at 37°C. Then 2 ml of saturated Na\textsubscript{2}CO\textsubscript{3} (7.5%) was added, and the mixture was brought to 10 ml with the addition of deionized-distilled water. The mixture was maintained at room temperature in the dark for 120 min and then the absorbance of the mixture was measured at 765 nm against a reagent blank using a UV–Vis spectrophotometer (Shimadzu Corp., Japan). Gallic acid equivalent (GAE) was used as the reference standard and total phenolic content was expressed as mg of GA equivalents per gram of each essential oil on dry basis.

**Antioxidant test**

The DPPH radical scavenging activity of essential oils was determined using the method proposed by Hung \textit{et al.}\textsuperscript{32} The essential oils at concentrations of 16 to 500 µg/ml were mixed with an equal volume of 0.2 mM methanol solution of DPPH. The disappearance of the DPPH after 30 min of incubation at room temperature was determined spectrophotometrically at 515 nm. Ethanol was used to zero the spectrophotometer. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC\textsubscript{50}) was calculated graphically and the percentage inhibition was calculated according to the equation:

<table>
<thead>
<tr>
<th>Region</th>
<th>pH</th>
<th>E.C. (dS/m)</th>
<th>O.C. (%)</th>
<th>Clay %</th>
<th>Silt %</th>
<th>Sand %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biregan</td>
<td>7.18</td>
<td>1.098</td>
<td>0.995</td>
<td>28</td>
<td>29</td>
<td>43</td>
</tr>
<tr>
<td>Saratshniz</td>
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<td>2.010</td>
<td>1.677</td>
<td>23</td>
<td>49</td>
<td>28</td>
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<tr>
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<td>0.980</td>
<td>1.131</td>
<td>33</td>
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<td>24</td>
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<tr>
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<td>0.903</td>
<td>1.170</td>
<td>32</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Tang-e-sayad</td>
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<td>0.810</td>
<td>0.527</td>
<td>26</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>Dezak</td>
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<td>1.016</td>
<td>1.287</td>
<td>16</td>
<td>50</td>
<td>34</td>
</tr>
</tbody>
</table>
% inhibition = \left( \frac{A_{C(0)} - A_{A(t)}}{A_{C(0)}} \right) \times 100

Where $A_{C(0)}$ is the absorbance of the control at 
$t = 0$ min and $A_{A(t)}$ is the absorbance of the
antioxidant at $t = 30$ min. The food preservative
butylhydroxyanisole (BHA) was used as positive
control.

Antimicrobial test

Four gram-positive bacteria (Staphylococcus
aureus, Listeria monocytogenes, Bacillus cereus and
Streptococcus agalactiae) and two Gram-negative
bacteria (Proteus vulgaris and Salmonella
typhimurium) obtained from Food Microbiology
Laboratory, Veterinary Medicine Faculty, I A U, Iran
and identified using conventional morphological as
well as biochemical tests. Stock cultures of bacteria
were kept in 20% glycerol PBS (phosphate buffered
saline) at 70°C. Active cultures were generated by
inoculating 100 µl of the thawed microbial stock
suspensions into 5ml nutrient broth (Merck,
Germany) followed by overnight incubation at 37°C.
An initial bacterial suspension containing 10^7
CFU/ml was made from the flask broth culture. Subsequent
dilutions were made from the above suspension,
which were then used in tests.

The disc diffusion method was used with some
modification to determine rate of inhibition growth
of bacteria by plant essential oils. BHI agar (Merck,
Germany) was used to prepare the culture medium
and autoclaved at 115°C for 10 min. Briefly, plates
(8 cm diameter) were prepared with 10 ml agar
inoculated with 1 ml of each bacterial suspension.
Sterile paper discs (6 mm in diameter) were
impregnated with 60 µl of dilutions of known
essential oils concentrations (100µg/disc) and
incubated at 35°C for 18 hrs. The essential oils were
dissolved in dimethyl sulfoxid (DMSO, 15 µl)
before the test for antimicrobial activity. Standard
antibiotic discs (6 mm diameter) of tetracycline and
streptomycin (10 µg) were used as positive controls.
Bacterial growth inhibition was determined as the
diameter of the inhibition zones around the discs
(mm). The growth inhibition diameter was an average
of three measurements, taken at 3 different directions.
All tests were performed in triplicate.

Statistical analyses

The data was statistically analyzed using
one-way ANOVA by the program SPSS (17.0), and
comparison of the means of the main constituents of
essential oils evaluated by Duncan’s multiple range
test at $p<0.05$ level.

Results and discussion

Total phenolic content

Total phenolic content was determined
spectrometrically according to the Folin–Ciocalteau
method and calculated as gallic acid equivalents
(GAE). A significant difference ($p \leq 0.01$) for total
phenolic content among the essential oils of different
populations of T. polypephalum was found (Fig. 1).
The highest total phenolic content was obtained
from the essential oil of the Hiregan population
(0.16 GAE/g DWE). The lowest total phenolic
content was obtained from the essential oil of the
Sar-teshniz population (0.063 GAE/gm DWE)
(Fig. 2). There are no reports in assessment of
total phenolic content of essential oil and extract of
T. polypephalum, but about other species of
Tanacetum, Farzam et al.33 reported that the amount
of total phenolic of methanol extract of T. fruticulosum
was 0.760 mg GAE/gm. In addition, Changqing

![Fig. 1—Total phenolic content of the essential oils of Tanacetum polypephalum](image1)

![Fig. 2—Antioxidant activity of the essential oils of Tanacetum polypephalum](image2)
et al.\textsuperscript{34} reported that the amount total phenolic content of the extract of \textit{T. parthenium} was measured in 21.21 µg GAE/mg DWE.

The composition and quantity of the phenolics vary significantly according to different intrinsic and extrinsic factors, such as species, plant genetics and cultivar, soil and growing conditions, maturity state and harvest conditions\textsuperscript{35}.

**Antioxidant test**

Antioxidant activity is very important in counteracting the deleterious role of free radicals in foods and biological systems. DPPH method is widely reported for screening of antioxidants and for determining comparative antioxidant effectiveness\textsuperscript{36}. The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The effect of antioxidants on DPPH is based on their ability to donate a hydrogen atom to DPPH, thus converting the radical into a stable molecule\textsuperscript{37}. The lower IC\textsubscript{50} value indicates a stronger ability of the essential oils to act as a DPPH scavenger while the higher IC\textsubscript{50} value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction\textsuperscript{38}.

In our study, the antioxidant activity of essential oils of the various populations of \textit{T. polycephalum} was expressed as IC\textsubscript{50} with value between 1.18 to 7.6 mg/ml that indicating the essential oils acts as moderate DPPH scavenger (Fig. 3). Significant differences (\(p< 0.01\)) in DPPH values were found in antioxidant activity of different populations of \textit{T. polycephalum}. The antioxidant activity of the \textit{Tanacetum} species were reported by numerous investigators\textsuperscript{17,39,40,41,42}. These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. Therefore, the phenolic compounds are the major group contributing to the antioxidant activity of vegetables, fruit, cereals and other plant-based materials. The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metallic chelating potential\textsuperscript{43,44}. Atoui \textit{et al.}\textsuperscript{45} mention that the antioxidant activity of phenolic is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

**Antibacterial test**

The antibacterial activity of essential oil from the various populations of \textit{T. polycephalum} was tested against the 6 pathogenic bacteria by using the disc diffusion method. Results indicate that the essential oils used in this study inhibited development of bacteria at different ratios. Generally, the antimicrobial activity of essential oils of \textit{T. polycephalum} against the studied bacteria was found as moderate to good (Table 2).

Previous investigations on different species of \textit{Tanacetum} have shown the antibacterial effects of this genus\textsuperscript{46,47}. The results indicated a broad spectrum activity against both Gram-positive and Gram-negative bacteria. Gram-negative bacteria were more resistant to the essential oils. Presence of an outer membrane in Gram-negative bacteria may explain this resistance effect\textsuperscript{48}.

<table>
<thead>
<tr>
<th>population</th>
<th>(L.\ m)</th>
<th>(S.\ a)</th>
<th>(S.\ Ag)</th>
<th>(B.\ c)</th>
<th>(S.T.M)</th>
<th>(P.\ v)</th>
</tr>
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<tbody>
<tr>
<td>Biregan</td>
<td>18</td>
<td>26</td>
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<tr>
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</tr>
<tr>
<td>Hiregan</td>
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</tr>
</tbody>
</table>

\(L.\ m\): \textit{Listeria monocytogenes}; \(S.\ a\): \textit{Staphylococcus aureus}; \(S.\ Ag\): \textit{Staphylococcus agalactie}; \(B.\ c\): \textit{Bacillus cereus}; \(S.\ T.\ M\): \textit{Salmonella tiphimorium} and \(P.\ v\): \textit{Proteus vulgaris}. 

Fig. 3—Antibacterial activity of the essential oils from \textit{Tanacetum polycephalum} against six bacteria by disc diffusion method.
Conclusion

The results of this study demonstrate that there are differences in the phenolic compounds contents, and antibacterial and antioxidant activities of essential oils of different populations of *T. polycephalum*. The total phenol contents were related to the antioxidant activities of essential oils. In conclusion, we might say that our results further support the view that some medicinal plants are promising sources of natural antioxidants and antibacterial components. Considering the antioxidant and antibacterial effects of *T. polycephalum*, there is a potential benefit of using the essential oil of this plant in combination with classic antioxidant and antibacterial agents to improve the antioxidant and antibacterial effects of the classic compound and consequently reduce the side effects of the compound. Further, study should be carried out to isolates the active compounds for pharmaceutical and industrial purposes.

References


