Pharmacognostical Evaluation, Phytochemical Analysis and Antioxidant Activity of the Roots of Achillea tenuifolia LAM.

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ABSTRACT

Introduction: Achillea L. (Asteraceae) contains about 100 species throughout the world with many therapeutic aspects. Achillea tenuifolia is one of the mentioned species that grows wildly in Iran. Methods: In this research the antioxidant activity of methanol and ethyl acetate extracts obtained from the roots of A. tenuifolia against free DPPH (2,2-diphenyl-1-picrylhydrazyl) radical together with the total phenol contents of extracts were assayed. Furthermore, preliminary phytochemical analysis of the above mentioned extracts and microscopic characterization of various plant tissues were determined. Results: The results showed that total phenol contents of methanol and ethyl acetate extracts were 59.4 ± 1 and 70.6 ± 3.8 (GAE µg/mg EXT), respectively. IC50 value for BHA, vitamin E, methanol and ethyl acetate in radical inhibition were calculated in the following order: 7.8, 14.2, 145.5 and 320 µg/mL. The scavenging capacity of methanol extract was higher than ethyl acetate extract. Preliminary phytochemical analysis indicated that both extracts contained sterols and terpenoids, nevertheless, tannins were detected in the methanol extract. Microscopic observations exhibited the presence of undeveloped cypsela, papillae stigma and elongated epidermal cells in the flower tissue, lanceolate leaflet with anomocytic stomata, cubic calcium oxalate prism and oil containing cells in the leaf parts, anomocytic stomata and cicatrix in the stem segments and finally sclereids, pitted and spiral vessels in the root tissue. Conclusion: Root extracts of A. tenuifolia mainly contain tannins, terpenes and sterols, and shows antioxidant activity not necessarily related to their total phenol content. Different plant tissues exhibited characteristic microscopic properties which make it distinguishable from other Achillea species.

Key words: Achillea tenuifolia, antioxidant activity, microscopy, preliminary phytochemical analysis

INTRODUCTION

Microscopy characterization of medicinal plants is a valuable procedure for identity and quality assessment of herbal ingredients. It is well accepted by all national and international regulatory authorities as one of the four primary methodologies for the identification of crude drug materials including macroscopic appearance, organoleptic characters, microscopic characteristics, and the presence or absence of chemical substances.[1-3] Identification of some specimens is very difficult, this is especially true for Achillea species which has different varieties, including wild and ornamentals.[1] The genus Achillea is well represented in Flora Iranica with about 100 species, 19 of them grow in Iran.[3] A. tenuifolia LAM. (Asteraceae) is distributed in the north and north-west of Iran. It is a perennial herbaceous plant, woody at base, with many stems and growing up to 25-90 cm.[4] Phytochemical investigations of Achillea species have exhibited bioactive components such as flavonoids, phenolic acids, coumarins, terpenoids, sterols, alkamides and volatile oils.[5-7] Many species of this genus have been used as traditional herbal remedies against fever, common cold, hemorrhage, pneumonia, rheumatic pain and digestive complaints. These are topically used for healing wounds and skin inflammation. In addition recent studies confirmed pharmacological and biological activities of this genus such as antioxidant, anti-tumor, anti-spasmodic, estrogenic, anti-spermatogenic, and treatment of alimentary tract disease.[6-8]

Literature review revealed that there is no report about the microscopy analysis of this species. Only chemical and physical characteristics of A. tenuifolia seed oil have been determined by gas chromatography which contains linoleic (69.4%) and oleic (14.5%) acids as the most abundant fatty acids. The antioxidant activity, total phenols and total flavonoids of the aerial parts of A. tenuifolia, together with the cytotoxicity evaluation of the mentioned extracts against the larvae of Artemia salina have been previously reported.

In the present study methanol and ethyl acetate extracts of A. tenuifolia have been reported for antioxidant activity, total phenol content assay, preliminary phytochemical investigation, and microscopic evaluation of various parts of the plant.

MATERIALS AND METHODS

Plant Material
All parts (leaves, stems, flowers and roots) of A. tenuifolia were collected from Qazvin province (1500 m) in June 2011, and identified by Mr. Yousef Ajani. A herbarium specimen (No. 1604) has been deposited at the Herbarium of Institute of Medicinal Plants, Jahade-Daneshgahi (ACECR), Karaj, Iran. The plant materials were cleaned and dried in shade at room temperature. Each part of the plant was separated and crushed for microscopic investigation.

Extraction
The powdered plant material was extracted (700 g) by maceration method in ethyl acetate and methanol, consequently, three times for each solvent at room temperature. The extracts were concentrated after removing the solvent by rotary evaporator and then lyophilized using a freeze dryer. The concentrated methanol and ethyl acetate extracts weighed as 2.7 and 1.91 g (on the basis of dry weight), respectively. The extracts were then kept in opaque containers under cold and dry conditions until assay.

Free Radical Scavenging
Free radical scavenging activity of the root extracts has been evaluated by 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) modified method. Free stable radical DPPH has been widely used to elucidate the free-radical scavenging of natural antioxidants. One mL of different concentrations of the extracts (100-500 µg/mL) was added to 2 mL of DPPH (4 x 10^-2 mg/mL in methanol). The absorptions at 517 nm were measured after 30 min. Free radical 50% inhibition (IC50) provided by extracts concentrations were determined from the plot of inhibition percentage against extract concentration. The assay was carried out in triplicate. Vitamin E and BHA were used as positive standards.

% Oxidation = \[\left(\frac{\text{Abs. sample} - \text{Abs. control}}{\text{Abs. blank}}\right) \times 100\]

% Inhibition = 100 – % Oxidation

Total Phenol Assay
Total phenolic contents were examined as GAE, expressed as µg GAE mg⁻¹ extract. Different concentrations of the root extracts (1 mL) were transferred to glass tubes, to which 5 mL Folin-Ciocalteu reagent (diluted 1:10) was subsequently added and incubated at room temperature for 10 min. Four milliliter of sodium bicarbonate (75 mg/mL) was added to the mixture and it was made up to 10 mL with distilled water. Each solution was incubated for 30 min at room temperature, and then its absorbance was measured at 765 nm. The sample absorbance was compared to gallic acid absorption. All determinations were carried out in triplicate and the mean values were presented (Figure 1).

![Figure 1: Gallic acid standard curve obtained from total phenol assay.](image-url)

**Statistical Analysis**
Comparisons between controls and extract antioxidation activity have been done in triplicate sets. The data were recorded as mean ± standard error and analyzed by SPSS (Version 11.5, SPSS Inc.). P values < 0.05 were regarded as significant.

**Preliminary Phytochemical Analysis**
In order to determine the various classes of natural compounds in the ethyl acetate and methanol extract of *A. tenuifolia*, preliminary screening tests for detection of alkaloids, flavonoids, sterols, tannins and terpenoids were carried out on the basis of those reported in the literature.[14]

**Microscopic Observations**
One gram of each tissue powder (leaf, flower, stem and root) of *A. tenuifolia* was separately boiled in potassium hydroxide solution (10%) in a beaker on heater for 30 seconds (or 1 minute) depending on the tissue hardness, and washed afterwards with distilled water three times. The powders were successively treated with sodium hypochlorite for bleaching and then washed with distilled water. The preparation was mounted in aqueous glycerin.[15] Photomicrographs were taken using Zeiss microscope attached with a digital camera. Photomicrographs of sections were taken at different magnifications depending upon the microscopic details to be observed.

**RESULTS**

**Radical Scavenging and Total Phenol Contents**
Values of IC₅₀ for radical scavenging in methanol and ethyl acetate extracts were calculated as 145.5 and 320 µg/mL, respectively. IC₅₀ in free radical inhibition for standard vitamin E (14.2 µg/mL) and BHA (7.8 µg/mL) were also measured (Figure 2). Total phenol contents were measured as 59.4 ± 1 and 70.6 ± 3.8 (GAE µg/ mg EXT) for the methanol and ethyl acetate extracts, respectively. The extent of DPPH inhibition of the methanol extract (100 µg/mL) showed the same activity as 10 µg/mL of vitamin E. The DPPH inhibition of ethyl acetate extract in 300 and 400 µg/mL were similar and they significantly displayed lower radical scavenging activity as compared to 500 µg/mL of this extract. However, the methanol extract indicated higher activity in comparison to ethyl acetate extract of the roots. DPPH inhibition for both of methanol (300 µg/mL) and ethyl acetate (300 µg/mL) extracts were plotted and compared with standard BHA and Vitamin E.

**Table 1: Qualitative phytochemical analysis of *A. tenuifolia* root methanol and ethyl acetate extracts**

<table>
<thead>
<tr>
<th>Test</th>
<th>Reagent</th>
<th>Observation</th>
<th>Methanol extracts</th>
<th>Ethyl acetate extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Meyers and Wagner reagent</td>
<td>Cream and orange precipitate</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>HCl plus Amylic alcohol</td>
<td>Reddish color</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl₃ 10%</td>
<td>Blue color</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Sterols</td>
<td>Sulfuric acid (conc.)</td>
<td>Reddish brown interface</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Glacial acetic acid plus</td>
<td>Reddish brown interface</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Figure 2: Evaluation of methanol and ethyl acetate extracts of *A. tenuifolia* compared to the standards BHA and Vitamin E, obtained from DPPH inhibitory assay.
and ethyl acetate (500 µg/mL) extracts were observed the same as 25 µg/mL of BHA (positive standard).

**Preliminary Phytochemical Analysis**

Phytochemical screening of both extracts revealed the presence of sterols and terpenoids. Tannin was also present in the methanol extract of the root (Table 1).

**Microscopic Observations**

Microscopic characterization of the plant flower was assessed, epidermis with oblong cells, undeveloped cypsela, papillae stigma and part of the style (Figure 3). In the leaf sample, the leaflet exhibited a lanceolate tip and consisted of the oil-containing cells, together with the cubic calcium oxalate prism (Figure 4). Upper epidermis made up of elongated cells and lower epidermis composed of slightly elongated cells with sinuous walls, but both epidermis of the leaf consisted anomocytic stomata (Figure 4). Stem epidermis demonstrated cicatrix and anomocytic stomata the same as the flower sample with abundant covering trichomes (Figure 5). Sclereids, pitted and spiral vessels were observed in the roots (Figure 6).

**DISCUSSION**

Although, the total phenol contents of ethyl acetate extract were higher than methanol extract, the latter showed 50% inhibition of DPPH in a lower concentration and was a better free-radical scavenger. Antioxidant activity of an extract can be the result of various active components such as peptides,
lanceolate shape. Anomocytic stomata were illustrated in both species, although upper epidermis of *A. tenuifolia* has elongated leaf cells without wavy cell walls. Stem sample of *A. tenuifolia* displays anomocytic stomata, cicatrix and covering trichome with elongated cell. Additionally, sclereids and vessels tissue can be observed in powdered plant roots.

**REFERENCES**


