STUDIES ON THE IN VITRO ANTIRADICAL ACTIVITY, PHENOL AND FLAVONOID CONTENTS OF SAUDI MEDICINAL PLANTS OF THE FAMILY ASTERACEAE HAVING XANTHINE-INHIBITOR ACTIVITIES

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ABSTRACT
To search the new sources of antioxidants, the phytochemical content (total phenolic and total flavonoids) and the antiradical potential of the leaves of Artemisia herba-alba, Achillea fragrantissima and Xanthium strumarium (members of Asteraceae) with known xanthine-oxidase inhibitor activity, were evaluated. Phytochemical analysis of the methanolic leaf extracts of all the three plants revealed the existence of alkaloids, phenol, flavonoids, tannins, terpenoids and carbohydrates. Total phenol and flavonoid content was highest in Artemisia herba-alba and Achillea fragrantissima. Artemisia herba-alba showed maximum total antioxidant capacity and DPPH antiradical activity. However, nitric oxide and hydrogen peroxide scavenging activities were recorded highest for the methanolic extracts of Achillea fragrantissima than the other two members. In conclusion, the Saudi medicinal plants of the family Asteraceae are rich source of bioactive phytochemicals and could be an immense source of medicinally important natural antiradical compounds.

KEYWORDS: Asteraceae, Artemisia herba-alba, Achillea fragrantissima, Xanthium strumarium, Antiradical activity, Nitric oxide scavenging activity.

INTRODUCTION
Accumulating evidence have revealed that the pathogenesis of several diseases such as cardiovascular diseases, atherosclerosis, arthritis, asthma, ischemia and reperfusion injury, central nervous system injury, gastritis, cancer, inflammatory joint diseases and AIDS could
be linked to the spontaneous and induced generation of active oxygen species (AOS).\textsuperscript{[1,2]}
Among AOS, superoxide radicals (O\textsubscript{2} \textsuperscript{-}), hydroxyl radical (OH) and peroxyl radicals (H\textsubscript{2}O\textsubscript{2}) are very reactive and may elicit the peroxidation of lipid membrane by oxidizing the cellular thiols.

Gout with a worldwide occurrence is an inflammatory condition associated with hyperuricemia. Xanthine-xanthine oxidase system that catalyzes the oxidation of xanthine and hypoxanthine into uric acid produces superoxide radicals too. Strategies that suppress the level of superoxide radicals may not only lower the incidence of hyperuricaemia and gout but also ease the inflammation. However, the drug used to treat gout may result in a several life threatening conditions such as allergic reaction, hepatitis and nephropathy. Therefore, the search for a substitute that could lower the severity of gout related conditions with minimal side effects is worthwhile. With the burgeoning new discoveries in the field of herbal medicine, the plant-based antioxidants have gained the popularity which it has never attained in the past.

The plant family Asteraceae is the largest angiospermic family, with over 1,600 genera and 23,000 individual species. The plants of this family are dominant in Tabuk, the northern province of Saudi Arabia. Several members of the Asteraceae family have significant medicinal, ornamental, and economic values. \textit{Artemisia herba-alba, Achillea fragrantissima} and \textit{Xanthium strumarium} belongs to the family Asteraceae, are known to possess antimicrobial, antiviral, anti-diabetic activities, besides these they can also help to cure the symptoms of asthma, bronchitis and rheumatic inflammation. Additionally, they are known to have xanthine-oxidase inhibitor activity, which could treat the hyperuricaemia and can alleviate the inflammation\textsuperscript{[3,4]}. Therefore, the present study was aimed to evaluate the comparative antiradical efficiency and phytochemical constituents of \textit{Artemisia herba-alba, Achillea fragrantissima} and \textit{Xanthium strumarium}, the members of the family Asteraceae with known xanthine-inhibitor activities.

**EXPERIMENTAL**

**Plant collection**

The plants were collected from Tabuk, the Northern Province of Saudi Arabia (latitude $28^\circ 22' 59''$ N; $36^\circ 34' 59''$ E, altitude 773m). The region is bounded by Red sea on the west to the Hufa depressions in the east. The plant materials were authenticated by Dr. Mohammad Nasir Khan from Department of Biology, University of Tabuk. Collected plants were dried in
shade under dark. Air-dried leaf samples were ground to a fine powder (80 mesh) using an electric blender and stored in a clean labeled air-tight containers.

**Plant extracts**

100 g powdered leaf sample of each plant were extracted with methanol for 24 h by using soxhlet apparatus. The extracts were separated from the solids by filtration with Whatman No. 1 filter paper. The remaining solids were be extracted twice with the same methanol and extracts combined. The extracts were concentrated under reduced pressure at 45 °C, in a rotary evaporator (EYELA, Tokyo, Japan) and kept in a refrigerator at 4 °C until analyzed.

**Preliminary phytochemical screening**

The Leaf extracts of *Artemisia herba-alba*, *Achillea fragrantissima* and *Xanthium strumarium* were administered to different chemical tests for the analysis of bioactive phytoconstituents such as alkaloids, phenol, flavonoids, steroids, terpenoids, tannins, saponins, anthroquinones, carbohydrates and cardiac glycosides following the methods described by[^5] and[^6].

**Total Phenol**

The content of total phenols was estimated by the Folin-Ciocalteu method with little modification of.[^7] From each sample, 0.5 mL of methanolic extract was added to 2.5 mL of Folin-Ciocalteu reagent and 2 mL of sodium carbonate 1 M. The tubes were incubated at 45°C for 30 min. The absorbance of total phenolics was measured at 765 nm using Hewlett Packard, UV/visible light. Total phenolics content was expressed as mg gallic acid equivalents per g dry weight.

**Total Flavonoids**

Total flavonoid content in the methanolic extracts was measured spectrophotometrically following the method of.[^8] 4 ml of water was added to 1mL (500mg/mL) extract or standard catechin solution and 0.3 mL of 5% NaNO₂. After keeping it for 5 min, 0.3 mL 10% AlCl₃ was added. To this mixture, after 6 min 2 mL 1M NaOH was added and the total volume was made up to 10 mL with water. The solution was thoroughly mixed and the absorbance was recorded against a prepared reagent blank at 510 nm. Total flavonoid content of was expressed as catechin equivalents in mg per g dry weight.
Total antioxidant capacity
Total antioxidant capacity was determined by the method described by.\(^9\) 0.1 ml of methanolic plant extract was added to 1 mL of reagent solution (0.6 mol/L sulfuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The tubes were heated at 95 °C for 90 min. The absorbance of each solution was recorded at 695 nm against a blank. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW).

Determination of DPPH free radical scavenging
The free radical scavenging capacity of methanolic extracts of different plants was recorded using DPPH method as described by.\(^{10}\) 5mL of 0.004% freshly prepared methanolic solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) were added to 50 µl of different concentrations of sample. After 30 min in the dark at room temperature, the absorbance was recorded spectrophotometrically against a blank at 517nm. DPPH free radical scavenging activity was expressed as the percentage inhibition.

Nitric oxide scavenging activity
To estimate nitric oxide radical inhibition activity, method described by\(^{11}\) was followed with little modifications. Briefly, sodium nitropruside (5mM, pH 7.4) in phosphate buffer saline was mixed with 3mL of different concentrations of methanolic plant extracts and incubated at 25 °C for 150 min. From this incubated solution, 0.5 ml was taken and mixed with 0.5mL Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]. After 30 min of incubation, absorbance was recorded at 540 nm. A standard solution of ascorbic acid was treated in the same way with the Griess reagent as a positive control. Nitric oxide scavenging activity was expressed as the percentage inhibition.

Hydrogen peroxide scavenging activity
The ability of the extracts to scavenge hydrogen peroxide was estimated based on the method of.\(^{12}\) A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Plant extracts in methanol were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was noted after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide.
Ascorbic acid was used as positive control. Hydrogen peroxide scavenging activity was expressed as the percentage inhibition.

RESULTS AND DISCUSSION

Phytochemical assay

Methanolic leaf extracts of *A. herba-alba*, *A. fragrantissima* and *X. strumarium* were analysed qualitatively for the presence of different bioactive phytoconstituents. As in Table 1, the analysis revealed the presence of alkaloids, phenol, flavonoids, terpenoids, tannins and carbohydrates. However, steroids were absent in all the three studied plants.

Table I: Phytochemical analysis of leaves of *Artemisia herba-alba*, *Achillea fragrantissima* and *Xanthium strumarium* members of the family Asteraceae.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Artemisia herba-alba</th>
<th>Achillea fragrantissima</th>
<th>Xanthium strumarium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

As in Fig.1, methanolic leaf extracts of *Artemisia herba-alba*, *Achillea fragrantissima* and *Xanthium strumarium*, showed appreciable amount of phenolic compounds. The total phenol content of the investigated plant differed greatly. Highest content of phenol was found in the leaves of *Artemisia herba-alba* (53.7± 3.7 mg GAE/g DW) which was 54.8 % and 29.4% more than *Achillea fragrantissima* (41.5± 2.6 mg GAE/g DW) and *Xanthium strumarium* (34.7±1.6 mg GAE/g DW), respectively. According to[13] and[14] the content of phenol is hugely influenced by environmental factors and genotypic variations, selection of the parts, time of the sampling and analytical methods. We have noted a higher amount of total phenol in these three members of the family asteraceae than that of other asteraceae members such as
Matricaria recutita\textsuperscript{[15]} and Tanacetum vulgare\textsuperscript{[16]} but lower than the Helichrysum and Centaurea species.\textsuperscript{[17, 18]}

Among polyphenolic groups, flavonoids are the one of the highly diverse group of compounds in higher plants. Flavonoids have been acclaimed for exemplary pharmacological and biological actions that include anti-microbial and anti-inflammatory activities among several others.\textsuperscript{[19]} Maximum level of flavonoids were recorded in the Achillea fragrantissima (23.8± 0.7 mg CE/g DW) which was 30\% and 73.2\% higher than Artemisia herba-alba (18.3± 0.7 mg CE/g DW) and Xanthium strumarium (13.7± 0.4 mg CE/g DW). However, the values noted for the total flavonoid content for the plants which we studied was higher than the flavonoid contents of the Anthemis arvensis, Artemisia compestris, Artemisia herba-alba and Artemisia arboresens, the members of the Asteraceae as reported by.\textsuperscript{[20]}

**Antioxidant capacity**

We carried out the phosphomolybdenum assay to determine the antioxidant capacity of methanolic leaf extracts of Artemisia herba-alba, Achillea fragrantissima and Xanthium strumarium. This method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green Mo (V) complexes with a maximal absorption at 695 nm.\textsuperscript{[9]} The methanolic extract of Artemisia herba-alba had the maximum antioxidant activity with a value of 127.5 mg GAE/g DW. The minimum antioxidant capacity was recorded in the methanolic extract of Xanthium strumarium with a value of 74.7 mg GAE/g DW (Fig.1). Taga et al. \textsuperscript{[21]} suggested that phytochemical attributes such as flavonoids, carotenoids and cinnamic acid derivatives may contribute to the total antioxidant capacity. Furthermore, we have noted that antioxidant capacity followed a similar trend to that of total phenol contents of investigated plants. It confirms our assumption that the phenols present in the methanolic extracts are linked to the antioxidant capacity. Our results are in accordance with the,\textsuperscript{[22]} who also noted a high correlation between the total antioxidant capacity and the total phenol content in Synedrella nodiflora, a member of the family Asteraceae.
Fig. I. Total phenol, flavonoid and antioxidant capacity of methanolic extracts of leaves of *Artemisia herba-alba*, *Achillea fragrantissima* and *Xanthium strumarium* members of the family Asteraceae.

**DPPH**

The free radical scavenging activities of methanolic extracts of three members of Asteraceae at different concentrations were assessed by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method. DPPH is a stable free radical that accepts an electron or hydrogen radical that get converted in to yellow-coloured diphenylpicrylhydrazine, a diamagnetic molecule. The reduction capacity of DPPH is estimated by the decrease in the absorbance at 517 nm induced by antioxidants. As shown in Fig. 2-A, the DPPH radical scavenging activities of methanolic leaf extracts of different plants shown to occur in a dose-dependent manner. The concentration required to inhibit 50% radical-scavenging activity (IC$_{50}$) was established from the results of a series of concentrations evaluated. A lower IC$_{50}$ value corresponds to a larger scavenging activity. The rank order of potency showed that the ascorbic acid was 6-, 9.3- and 11-fold more powerful than the *Artemisia herba-alba*, *Achillea fragrantissima* and *Xanthium strumarium*. The scavenging effect of methanolic extracts and standard ascorbic acid on the DPPH radical expressed as IC$_{50}$ values was in the following order: *Artemisia herba-alba* > *Achillea fragrantissima* > *Xanthium strumarium* (Table 1). The substantial antioxidant activity of *A. herba-alba*, *A. fragrantissima* and *X. strumarium* could be assigned to the presence of sesquiterpene lactones and flavonoids in the methanolic extracts of these plants.
We have noted a higher DPPH antiradical activity as revealed by lower IC$_{50}$ value for all the studied members of the Asteraceae in comparison to *Doronicum hookeri* Hook f. (Asteraceae) which was 217µg/ml.$^{[23]}$

**Table II: IC$_{50}$ value (µg/ml) of DPPH radical, nitric oxide (NOX) and hydrogen peroxide (H$_2$O$_2$) scavenging activities of the methanolic extracts of leaves of Artemisia herba-alba, Achillea fragrantissima and Xanthium strumarium members of the family Asteraceae.**

<table>
<thead>
<tr>
<th>Medicinal Plants</th>
<th>DPPH</th>
<th>NOX</th>
<th>H$_2$O$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisia herba-alba</td>
<td>43.5</td>
<td>59.6</td>
<td>54.1</td>
</tr>
<tr>
<td>Achillea fragrantissima</td>
<td>66.9</td>
<td>51.4</td>
<td>44.1</td>
</tr>
<tr>
<td>Xanthium strumarium</td>
<td>79.2</td>
<td>73.2</td>
<td>62.9</td>
</tr>
</tbody>
</table>

**Nitric Oxide**

Nitrite and peroxynitrite anions are generated when oxygen reacts with the excess nitric oxide that may acts as free radicals.$^{[24]}$ In the present study the methanolic extracts of the plants competes with oxygen to react with nitric oxide and thus inhibits the generation of the anions in a dose dependent manner. As in Fig. 2-B, the highest nitric oxide scavenging activity among the analyzed plant extracts were displayed by *A. fragrantissima* (IC$_{50}$= 51.4 µg/ml) followed by *A. herba alba* (IC$_{50}$= 59.6 µg/ml) and *X. strumarium* (IC$_{50}$= 73.2 µg/ml) (Table 1). It should be noted that nitric oxide scavenging activity of ascorbic acid was 9.6-, 8.3- and 11.8- fold more than *A. herba alba, A. fragrantissima* and *X. strumarium*. The IC$_{50}$ value for nitric oxide scavenging activity of the methanolic extracts of these plants was comparable to the other members of the family Asteraceae *Xanthium strumarium*.$^{[25]}$ and.$^{[23]}$

**Hydrogen peroxide scavenging activity**

As in Fig. 1-C, the methanolic extracts aerial parts of all the plants investigated, have shown a concentration dependent hydrogen peroxide scavenging activity. Highest hydrogen peroxide scavenging activity was recorded for *A. fragrantissima* followed by *A. herba-alba* and *X. strumarium* (Fig. 1-C). *A. fragrantissima* have profound level of flavonoids, terpenids, lignans, amino acid derivatives, fatty acids and alkamides. According to,$^{[26]}$ H$_2$O$_2$ scavenging activity of these methanolic extracts could be assigned to the presence of active constituents that that may donate electrons to H$_2$O$_2$ and thereby neutralizing it to water. The IC$_{50}$ value of hydrogen peroxide scavenging activity of *A. herba-alba, A. fragrantissima* and *X. strumarium* was 8-, 6.6- and 9.4-fold lower than IC$_{50}$ value of ascorbic acid. Hydrogen
peroxide is not very reactive to the biomolecules, but, its ability to cross the biological membrane and serve as a precursor for potentially toxic hydroxyl radical makes it deleterious.\textsuperscript{[27]} The hydroxyl radical is an extremely reactive free radical capable of damaging almost every molecule found in living cells.\textsuperscript{[28]} The \( IC_{50} \) value for hydrogen peroxide scavenging activity of \textit{Helichrysum pedunculatum}\textsuperscript{[29]} was higher than the methanolic extracts of the aerial parts of plants studied. Lower \( IC_{50} \) value corresponds to higher scavenging activity.

![Graph showing scavenging activities of DPPH radical (A), Nitric oxide (B) and Hydrogen peroxide (C) of methanolic leaf extracts of leaves of \textit{Artemisia herba-alba}, \textit{Achillea fragrantissima} and \textit{Xanthium strumariumi} members of the family Asteraceae.]

**Fig. II.** Scavenging activities of DPPH radical (A), Nitric oxide (B) and Hydrogen peroxide (C) of methanolic leaf extracts of leaves of \textit{Artemisia herba-alba}, \textit{Achillea fragrantissima} and \textit{Xanthium strumariumi} members of the family Asteraceae.
CONCLUSIONS
In this study, the qualitative and quantitative evaluation of phytochemical and related total antioxidant capacity and antiradical activity of three Saudi medicinal plants were carried out. Results showed that these plants are rich phenols, flavonoids, terpenoids and tannins. This justifies the use of these plants by the ancient people to treat inflammatory symptoms. Among these plants, *Artemisia herba-alba* and *Achillea fragrantissima* are found to be highly encouraging as they have significantly higher antioxidant capacities. It signify that the bio-active phytocconstituents from the current studied Asteraceae plants might serve as a potential therapeutic agent capable of arresting the damage caused by reactive oxygen species. However, before arriving to such conclusion *in-vivo* tests are necessary to confirm the use of these species in therapeutic practice.

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REFERENCES


