Ethnopharmacological evaluation and antioxidant activity of some important herbs used in traditional medicines

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ABSTRACT
OBJECTIVE: To document the indigenous knowledge and evaluate the antioxidant activity of medicinal herbs used for treatment of various diseases.

METHODS: The ethnomedicinal data were collected through semi-structured interviews of local informants along with collection of herbarium specimens. The antioxidant activity was evaluated by using 1-diphenyl-2-picryl-hydrazyl radical scavenging assay.

RESULTS: A total of 12 important medicinal herbs were evaluated for ethnomedicinal uses and antioxidant activity. Medicinal plant diversity showed that Solanaceae was the dominating family (3 spp.), followed by Asteraceae and Lamiaceae (both 2 spp.). Leaves (31%) were most frequently used in preparation of traditional medicines, and the most recommended mode of preparation was powder (28%). The antioxidant results revealed that the highest activity was found in Atropa acuminata and Crocus sativus.

CONCLUSION: The results of this study proved that A. acuminata and C. sativus can be the excellent sources of antioxidant compounds. A sustainable use and conservation of the valuable natural resource greatly promote the development of functional food additives and cosmetics.

INTRODUCTION
Recently, the rising prices of synthetic drugs and the associated harmful effects, such as human toxicity, environmental pollution and carcinogenic behavior, have enhanced the interest in medicinal plants used as a re-emerging health aid and for the synthesis of new plants derived drugs. For several decades, plants have been used in nutrition, medicine and cosmetics with less or no harmful effects. World Health Organization estimated that more than 80% population of the developing countries depends on traditional medicinal plants for primary health care, livelihood improvement and income generation. Plant derived drugs are generally nontoxic, effective at low concentrations, easily affordable and environment friendly. It has been reported that some fruits, vegetables and herbs are effective for certain chronic diseases because they may show their bactericidal, antiviral, analgesic, anti-inflammatory, anti-carcinogenic, and/or antioxidant actions. Traditional medicines have a long history of serving the people all over the world. The use of medicinal plants...
to maintain public health and treat diseases in many countries with cultures of different nations is highly prevalent. Nowadays, bioactive phytochemicals found in plants have gained significant consideration among the food industries because these compounds retard the lipids degradation, prevent microbial deterioration and improve the food quality. Several natural compounds discovered from the medicinal plants, such as alkaloids, terpenoids, flavonoids, phenolic acids, lignans, tannins, quinones, coumarins, etc. exhibit significant antioxidant and other activities. Thus, medicinal plants have become a focal point to improve the present and future health needs. However, it is the need of the times to search new sources and compounds of specific antioxidants for determined objectives. Therefore, the present study is designed in an attempt to evaluate the ethnopharmacological importance and antioxidant activity of the selected herbs used in traditional medicines.

MATERIALS AND METHODS

Ethnobotanical data collection
Different field surveys were conducted to compile the traditional knowledge of the local communities about the use of medicinal plants for treatment of various diseases. During the field survey, the protocol for ethnobotanical data documentation was followed. Traditional remedial information were obtained through semi-structured interviews, questionnaires, and group discussions with the local inhabitants and traditional healers. During the course of study, 56 key informants (44 local inhabitants and 12 traditional health practitioners) of different age groups, gender, education and experience, who have sufficient traditional knowledge of useful indigenous medicinal plants, were interviewed. The informants were selected with the help of local administrators. The selected informants belonged to three age categories (up to 30, 30-60 and above 60). Interviews were made in local language (Pahari). The authors were familiar with the local language, which allowed to present the accurate data and real picture of the community that could be lost during the translation. The questionnaire includes information about the local name, part(s) used, mode of utilization and therapeutic uses of the plants, and the ethnographical information of the informants. The collected specimens were pressed, dried, preserved, and mounted on herbarium sheets. Voucher numbers were assigned and submitted in Herbarium of Pakistan (ISL), Department of Plant Sciences, Quaid-i-Azam University Islamabad.

Antioxidant activity
The plant materials were collected from the resource base areas of the country during 2013-2014, and authentically identified by Dr. Mushtraq Ahmad (Plant Taxonomist, Quaid-i-Azam University Islamabad, Pakistan) after comparing with the available flora. Seeds of different plants were also procured from different herbal shops. The collected plant parts were washed, cleaned and dried in shade. The dried materials were ground into fine powder by using electric blender. Ten grams of the ground plant material was mixed in 100 mL of methanol with constant stirring on orbital shaker at 150 rpm for 24 h, and centrifuged at 10,000 rpm for 10 min. Then, the mixture was filtered through Whatman filter paper No. 1, and the filtrate was concentrated by rotary evaporator. All the chemicals used were of the highest analytical grades.

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay: The DPPH free radical scavenging activity of the extracts was determined by following the standard protocol with some modifications. 2 mL of 0.004% DPPH solution was added in 200 µL of plant extracts at varying concentrations. The mixtures were vortex-mixed and incubated in dark for 30 min at room temperature. The optimal density (OD) was measured at 517 nm. Methanol was used as blank, and ascorbic acid as the positive reference. The percentage scavenging activity of DPPH was estimated with the formula:

Scavenging activity (%) = (A_Control – A_sample / A_Control) × 100. The IC_{50} (concentration providing 50% inhibition of DPPH radicals) was calculated by linear regression analysis.

Statistical analysis
All the measurements were made three times, and the results obtained were expressed as mean ± standard deviation (SD). Statistical analyses were performed using Minitab (Version 17, Minitab Incorporation, USA). The data was analyzed by One-way Analysis of Variance test and the means of significant differences were separated using Fisher’s Least Significant Difference test at the 0.05 level of probability. To calculate the IC_{50} values, linear regression analysis was carried out using Microsoft Office Excel 2007 (Microsoft Corporation, USA).

RESULTS

Medicinal plant diversity
The present study reported twelve important medicinal herbs used for treatment of various human ailments. The documented medicinal herbs along with local names, part(s) used, modes of preparation and therapeutic uses are shown in Table 1. Medicinal plant diversity showed that the plant family contributing higher number of medicinal species was Solanaceae (3 spp.), followed by Asteraceae and Lamiaceae (both 2 spp.) (Figure 1). Leaves (31%) were most frequently used in preparation of traditional medicines, followed by seeds (26%) and flowers (13%) (Figure 2).
The most commonly-used preparation of herbal medicines prescribed by traditional healers was powder (both 14%) (Figure 3).

**DPPH radical scavenging activity**

In the present study, the antioxidant activity of the twelve medicinal herbs was evaluated by using DPPH radical scavenging assay. Acorric acid (positive control) showed the highest antioxidant activity. The results revealed that the lowest area values (highest activity) were recorded for Atropa acuminata, Crocus sativus, Carthamus tincotorius and Picrorhiza kurroa, while the highest area values (lowest activity) were recorded for Ocimum basilicum, Allium cepa, Solanum nigrum and Xanthium strumarium (Figure 4). Based on DPPH scavenging assay, IC_{50} values of the plant ex-
tracts were respectively 4.41 for Atropa acuminata, 06 for Crocus sativus, 29 for Carthamus tinctorius and 40.16 µg/mL for Picrorhiza kurroa. There was no significance difference ($P > 0.05$) between the IC$_{50}$ values of A. acuminata and C. sativus (Figure 5).

**DISCUSSION**

Ethnobotanical knowledge and traditional use of medicinal plants have been widely acknowledged all over the world. The tradition of using plants for medicinal purposes is still alive in rural population. Majority of the local communities depend on a variety of indigenous medicinal plants to cure various ailments because the modern health care facilities are inaccessible to them. Several chemicals and synthetic drugs are available in pharmaceutical market with different side effects; while the side effects of some traditional medicines are minimal. The free radical reactions in disease pathology are well known and are involved in many acute and chronic disorders in human beings, such as atherosclerosis, diabetes, aging, immunosuppression and neurodegeneration. Environmental stress enhances the creation of reactive oxygen species (ROS), which damage the cell membranes, chloroplast pigments and nucleic acid. ROS accumulation is induced by the imbalance between ROS and the inherent antioxidant capacity. Under the normal conditions, the levels of ROS are controlled by enzymatic antioxidants. However, under the environmental stress, the level of ROS rises, which stimulates the activity of antioxidant enzymes. Antioxidants fight against free radicals and protect us from various chronic diseases, such as cardiovascular disease, atherosclerosis, cancer and the aging process. To identify the natural antioxidants, many studies have been conducted, with the aim to replace the synthetic antioxidants for food and drugs. Plants are the natural source of antioxidant compounds which have free radical scavenging effects and the herbs are used in many industries as a source of medicine, food, and for fragrance and cosmetics. It has been reported that some crude extracts of herbs are rich in...
phenolics and have attained more attention in food industry because of their antioxidant activities. DPPH scavenging activity depends on the electron donation ability of natural products which reacts with the DPPH free radical. When DPPH solution is mixed with a substance that can donate a hydrogen atom or transfer electron, the free radical character can be neutralized and the DPPH solution decolorized (non-radical). With the increasing percentage of the free radical inhibition, radical scavenging activity increases. The change of color from violet to yellow and the decrease in absorbance of the stable radical DPPH were measured for testing the concentrations. In the present study, the radical scavenging capacity of methanolic extracts of important medicinal herbs were tested using the radical scavenging method. The stable free radical DPPH was oxidized and de-colored differently at different extract concentrations, using ascorbic acid as the standard. An inhibition was shown in all the extracts, with IC50 value indicating the antioxidant potential of plant extracts. Ascorbic acid has the maximum antioxidant activity (96.02). The highest inhibition was found for A. acuminate and C. sativus (61.33% and 62.51%, respectively. IC50 value for ascorbic acid is 0.246; while methanolic extracts of A. acuminate and C. sativus have IC50 value of 4.41 and 06 respectively, with no significant difference to that of the standard ascorbic acid. O. basilicum and A. cepa seeds were the samples with the lowest antioxidant activities (Table 2). Finally, the antioxidant activity in the twelve samples tested were in the following order of A. acuminate > C. sativus > C. tinctorius > P. kurroa > D. stramonium > S. chirayita > L. royleana > N. sativa > X. strumarium > S. nigrum > A. cepa > O. Basilicum, suggesting mean that A. acuminate and C. sativus have great potential for development of various ingredients used as the antioxidants.

In conclusion, the rural people have rich knowledge of medicinal plants. A sustainable use and conservation of the valuable natural resource can greatly promote the development of functional food additives and cosmetics. And for the discovery of new drugs to improve healthcare system, the medicinal plants with high antioxidant activities should be screened for pharmacological studies to obtain the valuable phytochemical compounds.

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The authors are thankful to the Higher Education Commission (HEC), Pakistan to the local informants for their help in collection of the ethnobotanical information of medicinal plants.

REFERENCES


4 Hoareau L, DaSilva EJ. Medicinal plants: a re-emerging food industry because of their antioxidant activities. DPPH scavenging activity depends on the electron donation ability of natural products which reacts with the DPPH free radical. When DPPH solution is mixed with a substance that can donate a hydrogen atom or transfer electron, the free radical character can be neutralized and the DPPH solution decolorized (non-radical). With the increasing percentage of the free radical inhibition, radical scavenging activity increases. The change of color from violet to yellow and the decrease in absorbance of the stable radical DPPH were measured for testing the concentrations. In the present study, the radical scavenging capacity of methanolic extracts of important medicinal herbs were tested using the radical scavenging method. The stable free radical DPPH was oxidized and de-colored differently at different extract concentrations, using ascorbic acid as the standard. An inhibition was shown in all the extracts, with IC50 value indicating the antioxidant potential of plant extracts. Ascorbic acid has the maximum antioxidant activity (96.02). The highest inhibition was found for A. acuminate and C. sativus (61.33% and 62.51%, respectively. IC50 value for ascorbic acid is 0.246; while methanolic extracts of A. acuminate and C. sativus have IC50 value of 4.41 and 06 respectively, with no significant difference to that of the standard ascorbic acid. O. basilicum and A. cepa seeds were the samples with the lowest antioxidant activities (Table 2). Finally, the antioxidant activity in the twelve samples tested were in the following order of A. acuminate > C. sativus > C. tinctorius > P. kurroa > D. stramonium > S. chirayita > L. royleana > N. sativa > X. strumarium > S. nigrum > A. cepa > O. Basilicum, suggesting mean that A. acuminate and C. sativus have great potential for development of various ingredients used as the antioxidants.

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REFERENCES


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Table 2 Antioxidant activity of methanolic extracts of the plants studied (x ± s)

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Parts investigated for activity</th>
<th>DPPH radical scavenging ability</th>
<th>Mean IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allium cepa</td>
<td>Seeds</td>
<td>44±8a</td>
<td>413±35</td>
</tr>
<tr>
<td>Atropa acuminate</td>
<td>Leaves</td>
<td>61±5a</td>
<td>4±1</td>
</tr>
<tr>
<td>Carthamus tinctorius</td>
<td>Flowers</td>
<td>60±8a</td>
<td>29±4b</td>
</tr>
<tr>
<td>Crocus sativus</td>
<td>Flowers</td>
<td>62±6a</td>
<td>06±2a</td>
</tr>
<tr>
<td>Datura stramonium</td>
<td>Leaves</td>
<td>56±2a</td>
<td>145±5</td>
</tr>
<tr>
<td>Lallemantia royleana</td>
<td>Seeds</td>
<td>47±11a</td>
<td>298±15</td>
</tr>
<tr>
<td>Nigella sativa</td>
<td>Seeds</td>
<td>35±3a</td>
<td>35±13</td>
</tr>
<tr>
<td>Ocimum basilicum</td>
<td>Seeds</td>
<td>29±4a</td>
<td>127±13</td>
</tr>
<tr>
<td>Picrorhiza kurroa</td>
<td>Rhizomes</td>
<td>61±4a</td>
<td>40±4</td>
</tr>
<tr>
<td>Solanum nigrum</td>
<td>Leaves</td>
<td>42±1a</td>
<td>37±11</td>
</tr>
<tr>
<td>Swertia chirayita</td>
<td>Leaves</td>
<td>56±2a</td>
<td>208±7</td>
</tr>
<tr>
<td>Xanthium strumarium</td>
<td>Leaves</td>
<td>41±6a</td>
<td>35±12</td>
</tr>
<tr>
<td>Standard drug</td>
<td></td>
<td>96±5a</td>
<td>0.24±0.1</td>
</tr>
</tbody>
</table>

Notes: data are expressed as mean ± SD. The superscript letters (a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z) are significantly different (P < 0.05) as determined using the Fisher’s LSD test. DPPH: 1,1-diphenyl-2-picryl-hydrazyl.

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