Chemical Composition and Minerals Analysis of *Hippophae rhamnoides*, *Azadirachta indica*, *Punica granatum* and *Ocimum sanctum* Leaves

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**Abstract:** The chemical composition and minerals analysis of four different plant species viz., *Hippophae rhamnoides*, *Azadirachta indica*, *Punica granatum* and *Ocimum sanctum* leaves were studied. In the nutritional analysis the *Punica granatum* was found enriched source of ectin, crude fiber, sugar and vitamin C as compared with other targeted plant leaves. The fat contents was also found in valuable quantity i.e., 5.50% which is relatively same as in *Hippophae rhamnoides* while the value of protein and nitrogen were very low in comparison with other plants that is 3.53 and 0.56%. *Azadirachta indica* showed low level of acidity, total sugar, fiber and pectin i.e., 0.57, 10.0, 13.41 and 6.21%. The minerals analysis showed no remarkable change in the results. Although the maximum quantities of Fe is found in *Azadirachta indica* (0.0141%), Na in *Punica granatum* (0.75%), K in *Hippophae rhamnoides* (14.58%) and Ni was not found in any sample.

**Key words:** Plant leaves - Medicinal value - Chemical composition - Mineral analysis

**INTRODUCTION**

**Sea-Buckthorn:** The sea-buckthorn (*Hippophae rhamnoides*) is deciduous shrubs in the genus *Hippophae*, family Elaeagnaceae. The name sea-buckthorn is hyphenated here to avoid confusion with the buckthorns (*Rhamnus*, family Rhamnaceae). It is also referred to as sea-buckthorn, sand-thorn or sea-berry [1].

Phytochemical constituents of sea-buckthorn berries have potential value to affect inflammatory disorders, cancer or other diseases, although no specific health benefits. The fruit of the plant has high vitamin C content in a range of 114 to 1550 mg/100g with an average content (695 mg/100 g). The fruit also contains dense contents of carotenoids, vitamin E, amino acids, dietary minerals, β-sitosterol and polyphenols. Flavonols were found to be the predominating polyphenols while phenolic acids and flavan-3-ols (catechins) represent minor components. [2]. Preparations of sea-buckthorn oils are recommended for external use in the case of burns, bedsores and other skin complications. Internally, sea-buckthorn is used for the treatment of stomach and duodenal ulcers. Sea-buckthorn oil, juice or extracts from oil, juice, leaves and bark have been used successfully to treat high blood lipid symptoms, eye diseases, gingivitis and cardiovascular diseases such as high blood pressure and coronary heart disease. Different parts of sea-buckthorn have been used as traditional therapies for diseases [3]. Sea-buckthorn is an herbal remedy to relieve cough, aid digestion, invigorate blood circulation and alleviate pain. Bark and leaves may be used for treating diarrhea and dermatological disorders. For its haemostatic and anti-inflammatory effects, berry fruits are added to medications for pulmonary, gastrointestinal, cardiac, blood and metabolic disorders. Sea-buckthorn berry components have potential activity against cancer and dengue virus [4, 5].

**Neem:** Neem (*Azadirachta indica*) is a tree belongs to family Meliaceae. It is one of two species in the genus *Azadirachta*. In 1942, for the first time, three bitter compounds were extracted from Neem oil, which were named as, Nimbin, Nimbinin and Nimbidin. The seeds contain a complex secondary metabolite i.e., Azadirachtin [6]. Neem products have been observed to be antimalarial, anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative activities. Neem products are also used in selectively controlling pests in plants. It is considered a major
component in Ayurvedic and Unani medicine and is particularly prescribed for skin disease [7]. All parts of the tree are said to have medicinal properties (seeds, leaves, flowers and bark) and are used for preparing many different medical preparations. Part of the Neem tree can be used as a spermicidal [8]. Neem oil has been found to be an effective mosquito repellent. As neem products are cheap and non-toxic to higher animals and most beneficial insects, they are well-suited for pest control in rural areas. Neem leaf paste is applied to the skin to treatment and in a similar vein is used for measles and chicken pox sufferers. A mixture of Neem flowers are used as symbolic of sweet and bitter. Neem is deemed very effective in the treatment of scabies. The tender shoots and flowers of the Neem tree are used as vegetable in some countries of the world. Neem Gum is a rich source of protein.

**Pomegranate:*** The pomegranate (*Punica granatum*), is a fruit-bearing deciduous shrub or small tree. Pomegranate juice provides about 16% of an adult’s daily vitamin C requirement per 100 mL serving and is a good source of vitamin B₉ (pantothenic acid), potassium and polyphenols such as tannins and flavonoids [9]. Pomegranates are listed as high-fiber in some charts of nutritional value. That fiber, however, is entirely contained in the edible seeds which also supply unsaturated oils. The most abundant polyphenols in pomegranate juice are the hydrolyzable tannins called ellagitannins formed when ellagic acid binds with a carbohydrate. Punicalagins are tannins with free radical scavenging properties in laboratory experiments and with potential human effects [10-12].

The pomegranate has been extensively used as a source of traditional remedies. The rind of the fruit and the bark of the pomegranate tree are used as a traditional remedy against diarrhea, dysentery and intestinal parasites. The seeds and juice are considered a tonic for the heart and throat and classified as a bitter-astringent component under the Ayurvedic system and considered a healthful counterbalance to a diet high in sweet-fatty components [13], leading to clinical studies of pomegranate juice or fruit extracts for efficacy against several diseases. The effects of pomegranate extracts or juice consumption on diseases such as prostate cancer, prostatic hyperplasia, diabetes, lymphoma, rhinovirus infection, common cold, oxidative stress, in diabetic hemodialysis, atherosclerosis, coronary artery disease, infant brain injury, hemodialysis for kidney disease [14].

**Tulsi:** Tulsi (*Ocimum tenuiflorum*) is an aromatic plant belongs to family Lamiaceae which is native throughout the Old World tropics and widespread as a cultivated plant and an escaped weed [15, 16]. The Tulsi also contain sufficient quantity of antioxidants and fixed oil [17, 18]. Some of the main chemical constituents of Tulsi are; Oleanolic acid, Eugenol, Carvacrol, Linalool and β caryophyllene [19].

Tulsi is cultivated for and medicinal purposes and essential/fixed oils. One study showed that Tulsi can be an effective for diabetes treatment by reducing blood glucose levels and can also reduce significantly the total cholesterol levels [20]. Another study showed that Tulsi’s beneficial effect on blood glucose levels is due to its antioxidant properties [21]. Tulsi also shows some promise for protection from radiation and cataracts [22]. The fixed oil has demonstrated anti-hyperlipidemic and cardio-protective effects in rats fed a high fat diet [23]. Tulsi’s extracts are used for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria. Traditionally, Tulsi is taken in many forms: as herbal tea, dried powder, fresh leaf, or mixed with ghee. Essential oil extracted from Tulsi is mostly used for medicinal purposes and in herbal cosmetics and is widely used in skin preparations due to its anti-bacterial activity. The dried leaves of Tulsi have been mixed with stored grains to repel insects [24]. The other medicinal uses of Tulsi are; healing power, coughs, sore throat, respiratory disorders, kidney stone, heart disorder, children's ailments, stress, mouth infections, insect bites, skin disorders, eye disorders, etc.

**Aim and Objectives of the Present Study:** Plants or parts of the plants (medicinal plants) are well-known for their health curing potentials, while used in its original form, their crude extracts or purified chemical constituents isolated from them. The aim of the present study was to determine the chemical composition and mineral profile of the selected medicinal plants grown in Pakistan. Four medicinal plants namely, Sea-buckthorn (*Hippophae rhamnoides*), Neem (*Azadirachta indica*), Pomegranate (*Punica granatum*) and Tulsi (*Ocimum tenuiflorum*) were selected. The current work will provide new reference data of the chemical constituents of the selected medicinal plants, which could be used as base-line value. The present study will also be helpful regarding the standardization of materia medica.
MATERIALS AND METHODS

Chemicals and Reagents: Analytical grade chemicals and reagents and distilled water were used in the present study. n. Hexan (Purity: 96%, Scharlu Spain), Fehling’s Solution, Fehling A (copper sulphate solution), Fehling B (alkaline tartrate solution), Methylene blue indicator, Potassium oxalate, Lead acetate, Sodium hydroxide (NaOH) (Purity: 96%, Riedel-Dehaen Germany), Sulphuric acid (H₂SO₄) (95-97% Riedel-Dehaen Germany), Asbestos, Ethyl alcohol (Purity: 98%, Scharlu Spain), Celite (Fluka).

Instruments and Equipments: The following instruments and equipments were used during the present work. Top loading balance (Model: GP 3202, Sartorius AG Gottingen), Electrical Oven (Ontherm Designer Series Oven, Hutt City, New Zealand), Barnstead/ Electrothermal (UK), pH Meter (Model: pH 3110 Set 2, WTW, Germany), Digital Refractometer (Model: RX-1000, Atago, Japan), Electronic Dry Cabinet/Desiccating Cabinet (Model Dry 60 todays-instrument) and atomic absorption spectrophotometer (Model: 2000, Hitachi, Tokyo-Japan).

Selection and Sampling of Medicinal Plants: Four medicinal plants leaves namely, Sea-buckthorn, Neem, Pomegranate and Tulsi were selected for the present study. This is because of their use as medicine as well as food items. Sea-buckthorn (~2 kg) and pomegranate leaves (~1 kg) were provide by Mycotoxin Research Laboratory, PCSIR Laboratories Complex, Peshawar, Pakistan, which were originally collected from Skardu District, Pakistan. The Neem leaves (~4 kg) were collected from mature Neem plants grown at the Botanical Garden, Islamia College University, Peshawar, Pakistan. Similarly, Tulsi leaves (~3.5 kg) were also collected from mature Tulsi plants grown at the Botanical Garden, Islamia College University, Peshawar, Pakistan. The plants were identified and authenticated by Prof. Dr. Samin Jan, Department of Botany, Islamia College University, Peshawar, Pakistan.

Pre-Treatment of Plant Materials: The plant materials were washed with tap water and then with the distilled water for the removal of all possible dust particles or any other external body or contaminant. The plants materials were first shad-dried at room temperature and then grinded using grinding machine. After grinding, the powder form of the plant materials were obtained, which was packed in a plastic bottle, made it air tight and stored in an electrical desiccator till further study.

Chemical Analysis
Determination of Moisture: 2 g of powder plant materials was taken in a pre-weighed Petri dish and was completely dried in an oven at 100°C for 4 h. After the sample was completely dried, cooled in a desiccator and weighed again. The moisture contents (%) were determined using the following formula [24].

\[
\text{Moisture} \% = \frac{\text{Weight of Sample Taken (g)} - \text{Weight of Dried Sample (g)}}{\text{Weight of Sample Taken (g)}} \times 100
\]

Determination of Ash: 1 g of the dried powder plant materials was taken in a pre-weighed crucible and was completely dried in an oven at 100°C for 1 h. The sample was charred on low flame and then heated at 600°C in a muffle furnace until a white ash was obtained with constant weight. The crucible was then cooled in a desiccator and weighed again. The ash contents (%) were determined using the following formula [25].

\[
\text{Ash} \% = \frac{\text{Weight of the Sample After Ashing (g)}}{\text{Weight of Sample Taken (g)}} \times 100
\]

Determination of pH and Total Water-Soluble Solids: 5 g of the dried powder plant materials was added in 150ml distilled water and boiled for 30 min, then cooled to room temperature and filtered through Linen Cloth filter. The pH of the filtrate was determined using pre-calibrated digital pH-meter and total water-soluble solid contents by digital Refract meter [24].

Determination of Total Acidity: 10 g of the dried powder plant materials was added to in 300ml distilled water and boiled till the volume was reduced to 250 mL. The mixture was then cooled to room temperature and filtered through Linen Cloth filter. 20 mL of extract was poured in a titration flask, added to it few drops of phenolphthalein indicator and then titrated against 0.1 N NaOH solutions. The appearance of light pink coloration showed the end point. The total acidity (%) of the sample was determined using the following formula [24].

\[
\text{Total Acidity} \% = \frac{\text{Factor} \times \text{N} \times \text{Titer} \times \text{Dilution}}{\text{S}_w \times \text{S}_v} \times 100
\]

\[S_w\] is the weight of the sample taken (g) and \(S_v\) is the volume of the sample extract (20 mL) taken for analysis.
Determination of Fat: 2 g of the dried powder plant materials was taken in a Thimble and placed Soxhlet extractor. A dried and pre-weighed round-bottom flask (100 mL) was connected to the Soxhlet assembly containing 80 mL n-hexane. The assembly was heated in a heating mental for 8 h as mentioned in Section 2.4.6. The defatted sample was boiled for 30 min in 200 mL H₂SO₄ solution (0.255 N). After boiling, the mixture was then filtered through Linen Cloth filter and washed the residue with distilled water till obtained the acid free sample. This residue was again boiled using 200 mL NaOH solution (0.313 N). The mixture was filtered through a dried and pre-weighed Gooch crucible prepared with asbestos mat. The crucible along-with the samples was dried in an oven and was weighed and then ignited in a muffle furnace at 600°C for 4 h and weighed again. The crude fiber contents (%) were determined using the following formula [25].

\[
\text{Crude Fiber} = \frac{W_1 - W_2}{W_3} \times 100
\]

where, \(W_1\) is the weight of the dried sample (g), \(W_2\) is the weight of the ignited sample (g) and \(W_3\) is the weight of the sample taken (g) for analysis.

Determination of Crude Fiber: 2 g of the dried powder plant materials was taken in a Thimble and defatted using n-hexane as a solvent in a Soxhlet extractor for 8 h as mentioned in Section 2.4.6. The defatted sample was boiled in a beaker (500 mL) and few drops of methylene blue indicator were added. The NaOH solution (1 N) was added to it drop-wise till the appearance of light pink colour, then 2 mL of lead acetate solution (45%) was added to the mixture and after 10 min 2 mL of potassium oxalate solution (22%) was added to the same mixture. The mixture was then filtered through Whatman filter paper (No. 5) and marked as Filtrate-A. The Filtrate-A was taken in a burette. Fehling-A and Fehling-B solutions (each of 5 mL) were taken in two separate titration flasks and few drops of methyl blue were added as an indicator and then titrated against Filtrate-A till the appearance of dark blue color. The reducing sugar contents (%) were determined using the following formula [24];

\[
\text{Reducing Sugar} = \frac{\text{Factor} \times \text{Dilution}}{\text{Titer} \times 1000} \times 100
\]

where, Factor is obtained from Table. Dilution is the total volume of the mixture (250 mL) and Titer is the volume of the Filtrate-A used during titration (mL).

Determination of Total Sugar: For the determination of total sugar, 50 mL of Filtrate-A was taken in a titration flask (250 mL) and 5 g of citric acid and 50 mL distilled water were added into it. The mixture was boiled for 10 min to invert the sucrose and then cooled to room temperature. The mixture was then neutralized by drop-wise addition of 20% NaOH solution using phenolphthalein as an indicator till the appearance of light pink color. The light pink color was disappeared by drop-wise addition of HCl solution (1 N). This colorless mixture was taken in a burette and Fehling-A and Fehling-B solutions (each of 5 mL) were taken in two separate titration flasks and few drops of methylene blue were added as an indicator and then titrated against mixture till the appearance of dark blue color. The total sugar contents (%) were determined using the following formula [25].

\[
\text{Total Sugar} = \frac{\text{Factor} \times \text{Dilution}}{\text{Titer} \times 1000} \times 100
\]

where, Factor is obtained from Table [24], Dilution is the total volume of the mixture (250 mL) and Titer is the volume of the Filtrate-A used during titration (mL).
Determination of Non-Reducing Sugar: The non-reducing sugar contents (%) were determined by subtraction of reducing sugar from the total sugar using the following formula [34].

Non-reducing Sugar Contents (%) = Total Sugar (%) – Reducing Sugar (%)

RESULT AND DISCUSSION

Selection and Sampling of Medicinal Plants: Four medicinal plants namely, Sea-buckthorn (*Hippophae rhamnoides*), Neem (*Azadirachta indica*), Pomegranate (*Punica granatum*) and Tulsi (*Ocimum tenuiflorum*) were selected in the present study. As these plants are well-known for their medicinal values and at the same time they are also used as food items. The present study was therefore, conducted to determine the chemical composition and mineral profile of the selected medicinal plants grown in Pakistan.

Chemical Composition: The chemical composition of the Sea-buckthorn (*Hippophae rhamnoides*), Neem (*Azadirachta indica*), Pomegranate (*Punica granatum*) and Tulsi (*Ocimum tenuiflorum*) are given in Table 1.

### Table 1: Proximate analysis of medicinal plants.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Hippophae rhamnoides</th>
<th>Azadirachta indica</th>
<th>Punica granatum</th>
<th>Ocimum tenuiflorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture (%)</td>
<td>08.10±0.28</td>
<td>10.30±0.28</td>
<td>04.32±0.57</td>
<td>31.35±1.04</td>
</tr>
<tr>
<td>2</td>
<td>Ash(%)</td>
<td>07.12±1.73</td>
<td>08.31±1.52</td>
<td>05.14±0.13</td>
<td>14.21±1.50</td>
</tr>
<tr>
<td>3</td>
<td>Fat(%)</td>
<td>05.81±1.25</td>
<td>03.37±0.57</td>
<td>05.50±0.40</td>
<td>03.12±0.28</td>
</tr>
<tr>
<td>4</td>
<td>Pectin(%)</td>
<td>03.54±0.87</td>
<td>06.21±0.54</td>
<td>08.90±1.10</td>
<td>06.30±0.76</td>
</tr>
<tr>
<td>5</td>
<td>Crude Fiber(%)</td>
<td>17.31±2.08</td>
<td>13.41±0.45</td>
<td>63.10±1.00</td>
<td>16.81±1.25</td>
</tr>
<tr>
<td>6</td>
<td>Total Sugar(%)</td>
<td>27.29±1.23</td>
<td>10.00±0.98</td>
<td>054.3±2.70</td>
<td>27.23±1.92</td>
</tr>
<tr>
<td>7</td>
<td>Reducing sugar(%)</td>
<td>17.37±1.09</td>
<td>03.04±0.79</td>
<td>051.2±2.12</td>
<td>26.52±1.54</td>
</tr>
<tr>
<td>8</td>
<td>Non reducing sugar(%)</td>
<td>09.92±1.32</td>
<td>06.96±0.76</td>
<td>03.16±0.21</td>
<td>00.79±0.08</td>
</tr>
<tr>
<td>9</td>
<td>Total acidity (%)</td>
<td>01.96±0.15</td>
<td>00.57±0.15</td>
<td>051.2±2.12</td>
<td>26.52±1.54</td>
</tr>
<tr>
<td>10</td>
<td>Vitamin-C (mg/100g)</td>
<td>01.12±0.15</td>
<td>00.31±0.04</td>
<td>06.10±0.74</td>
<td>02.41±0.91</td>
</tr>
<tr>
<td>11</td>
<td>Protein (%)</td>
<td>11.06±0.41</td>
<td>08.93±0.31</td>
<td>03.53±0.03</td>
<td>04.93±0.03</td>
</tr>
<tr>
<td>12</td>
<td>Nitrogen(%)</td>
<td>01.77±0.06</td>
<td>01.42±0.05</td>
<td>00.56±0.01</td>
<td>01.63±0.09</td>
</tr>
<tr>
<td>13</td>
<td>Ph of 10% Sol</td>
<td>05.21±0.51</td>
<td>05.84±0.01</td>
<td>03.72±0.05</td>
<td>05.98±0.02</td>
</tr>
<tr>
<td>14</td>
<td>TSS 10% Sol</td>
<td>01.20±0.15</td>
<td>00.67±0.05</td>
<td>00.70±1.35</td>
<td>00.31±0.10</td>
</tr>
</tbody>
</table>

### Table 2: Macro-elements status of four medicinal plants

<table>
<thead>
<tr>
<th>Medicinal Plants (Botanical Name)</th>
<th>K</th>
<th>Na</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% ppm</td>
<td>ppm</td>
<td>% ppm</td>
<td>ppm</td>
</tr>
<tr>
<td><em>Hippophae rhamnoides</em></td>
<td>14.58</td>
<td>145800</td>
<td>0.09</td>
<td>900</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>9.0</td>
<td>90000</td>
<td>0.15</td>
<td>1500</td>
</tr>
<tr>
<td><em>Punica granatum</em></td>
<td>13.65</td>
<td>136500</td>
<td>0.75</td>
<td>7500</td>
</tr>
<tr>
<td><em>Ocimum tenuiflorum</em></td>
<td>14.55</td>
<td>145500</td>
<td>0.45</td>
<td>4500</td>
</tr>
</tbody>
</table>

### Table 3: Micro-elements status of four medicinal plants.

<table>
<thead>
<tr>
<th>Medical Plants (Botanical Name)</th>
<th>Ni</th>
<th>Cr</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% ppm</td>
<td>ppm</td>
<td>% ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td><em>Hippophae rhamnoides</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0063</td>
<td>63</td>
<td>0.0224</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.008</td>
<td>80</td>
<td>0.0927</td>
</tr>
<tr>
<td><em>Punica granatum</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0244</td>
<td>244</td>
<td>0.0141</td>
</tr>
<tr>
<td><em>Ocimum tenuiflorum</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0067</td>
<td>67</td>
<td>0.0546</td>
</tr>
</tbody>
</table>

As can be seen, the moisture and ash contents were in the range of 4.3 to 31.3 and 7 to 8.3, in which *Punica granatum* have the highest value of moisture and ash. The pH and total acidity of the 10% extract of all plants was found same. In the determination of fiber contents the sea buckthorn leaves contained maximum fiber contents as compared with other plants, that was 63%, while the other were in the range of 13 to 17%. The crude fat was extracted in hexane by hoxtlet apparatus in triplicate, the mean values tabulated in Table 1. The results showed that Tulsi and sea-buckthorn contained low and high levels of crude fat contents, respectively, while the rest of the plants contain intermediate values between these two. The maximum fat content was found in sea buckthorn and pomegranate, that is 5.81 and 5.50%. While 3.37 and 3.12% of crude fat were found in *Azadiractha indica* and *Ocimum tenuiflorum*. The pomegranate also enriched by sugar contents. The nitrogen and protein contents were determined in all the plant samples. The overall range of nitrogen contents were from 0.56 to 1.63% and 3.53 to 11.06%. In which sea-buckthorn have high value of protein and nitrogen.

**Minerals Contents:** Four macro-elements was reported in the minerals analysis viz., K⁺, Na⁺, Ca²⁺ and Mg²⁺. The concentration reported in the minerals analysis of these selected plants was in% (w/v) and ppm (mg/lit) on dry weight basis in Table 2. The value of K⁺, Mg²⁺ and Ca²⁺ was found high in Tulsi sample that is 14.55, 0.41 and 1.89%. While the highest value of Na⁺ was found in pomegranate that was recorded 0.75%. The five micro-elements were reported in the minerals analysis which are Ni, Cr, Fe, Zn and Cu. The concentration reported in the minerals analysis of these selected plants were in% (w/v) and ppm (mg/lit) on dry weight basis in Table 3. There is not found any remarkable change in micro elements of these plants while Ni was absent in all of the tested samples.

**REFERENCES**