Antibacterial and antioxidant activity analysis of some wild medicinal plants

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Abstract
The present study was performed for the analysis of antimicrobial and antioxidant properties of wild edible plants: *Amaranthus thunbergii*, *Caralluma edulis*, *Allium astrosanguineum*, *Rumex patientia* and *Portulaca oleracea*. Which were collected from the arid region of South Waziristan Agency (FATA), Pakistan. The experiment was performed for antibacterial activity for both aqueous and methanolic extracts of selected plants against the three bacteria: *Clavibacter michiganensis*, *Ralstonia solanacearum* and *Erwinia carotovora*. It was observed that the aqueous extract of plants were not effective against these bacteria while the methanolic extract of plants showed light to moderate activity. The antioxidant activities of different fraction of the methanolic extracts were indicated in the range of 69.08-84.89%. From the current study, it may be concluded that the selected plants have the potential of antimicrobial and antioxidant properties, which play a key role in controlling a variety of diseases caused by various pathogens of bacteria and by the oxidation of free radical in the body.

Keywords: Wild edible plants, Antimicrobial activity, Antioxidant activity

Introduction
Antimicrobial agents are substances that kill microorganisms or inhibit their growth. They are widely employed to cure bacterial diseases. Antimicrobial agents that reversibly inhibit growth of bacteria are called bacteriostatic whereas those with irreversible lethal action on bacteria are known as bactericidal [1]. Ideally, antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting the microbial membrane structure and function, or blocking metabolic pathways through inhibition of key enzymes [2].

Antioxidants are essential substance which has power over the capacity to defend the body from reparation caused by complimentary fundamental induced oxidative stress. Plants, which are sources of Phytochemicals with strong antioxidant action, have fascinated a huge deal of attention in recent years. Antioxidants, which inhibit the oxidation of organic molecules, are very important, not only for food preservation, but also for the defense of living systems against oxidative stress [3]. Phenolic antioxidants interrupt the propagation of the free radical autoxidation chain by causative a hydrogen atom from a phenolic hydroxyl group, with the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes [4].

Wild plants contain potential bio-molecules both in organic and inorganic combinations [5]. They are vital, inexpensive and lucrative source of vitamins, antioxidants, fiber, minerals and other nutrients for many economical deprived natives. These plants have high nutraceutical value and are used for a wide range of ailments and have the potential to protect human body from cancer, diabetes, inflammatory and cardiovascular diseases. Literature survey revealed that considerable work has been done on the organic constituents, however, little attention has been paid to the attributes of inorganic elements which plays a very important role in the formation of the active chemical constituents and thus responsible for nutritional as well as therapeutic properties [6]. A direct correlation between the elemental content of wild plants and their nutraceutical ability is not yet understood in term of modern scientific perspectives. Further, the utilization of such plants is limited due to the existence of some lethal and anti-nutritional factors viz. oxalates, tannins, alkaloids and saponins, however, important deviation
was observed in conditions of anti-nutritional factors amongst the undomesticated food plants [8, 9]. However, more than a few researchers reported that lots of conventional and predictable plants had the similar sort of these anti-nutritional factors. Nevertheless, these bio-molecules examination would elevate the consciousness about the development of these wild plants as food and medication. The present study documented 5 wild plants used as food and medicinal plants collected from different mountainous packets of South Waziristan Agency of Pakistan. The present studies were initiated to examine. The Biological activities like Antibacterial activity and Antioxidant activity of wild edible plants: *Portulaca oleracea*, *Rumex patientia*, *Amaranthus thunbergii*, *Caralluma edulis* and *Allium astrosaugeum*.

**Materials and Methods**
The trial on Analysis of Biological activities of selected wild edible plants (Table.1) from South Waziristan Agency (FATA), Pakistan was conducted in the different laboratories viz. Biochemistry lab Hazara University, Mansehra and Plant pathology lab, University of Agriculture Peshawar, Pakistan from November, 2014 to March, 2015.

**Collection and Identification of Plant Material**
The selected wild edible plants were collected from South Waziristan Agency (FATA), Pakistan in October 2014. The healthy and disease free edible plants having fully matured leaves were collected. The identification of selected wild edible plants was confirmed with the help of local people and technically from the department of weed science at the University of Agriculture, Peshawar. The details of each plant species, with respect to their local names, parts used and status are elaborated in Table 1.

The collected samples of plants were shaded dried at room temperature and ground into fine powder by using Thomas-Wiley laboratory Mill model-104. The powder placed temporarily in airtight polythene bags until further analysis.

**Table 1: Wild edible plants and pattern of local use**

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family Name</th>
<th>Local Name</th>
<th>Parts used</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus thunbergii</em></td>
<td>Amaranthaceae</td>
<td>Ranzakka</td>
<td>Areal Parts</td>
<td>Wild</td>
</tr>
<tr>
<td><em>Caralluma edulis</em></td>
<td>Asclepiadaceae</td>
<td>Pamankai</td>
<td>Areal Parts</td>
<td>Wild</td>
</tr>
<tr>
<td><em>Allium astrosaugeum</em></td>
<td>Alliaceae</td>
<td>Shezyee</td>
<td>Areal Parts</td>
<td>Wild</td>
</tr>
<tr>
<td><em>Rumex patientia</em></td>
<td>Polygonaceae</td>
<td>Zundaa</td>
<td>Areal Parts</td>
<td>Wild</td>
</tr>
<tr>
<td><em>Portulaca oleracea</em></td>
<td>Potulacaceae</td>
<td>Terwikai</td>
<td>Areal Parts</td>
<td>Wild</td>
</tr>
</tbody>
</table>

**Antioxidant Activity**
The antioxidant activity was performed by DPPH radical scavenging assay. The electron donation capacities of the corresponding fractions and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-pircylylhydrazyl (DPPH). All Analysis was carried out in triplicate according to the standard procedure. Briefly, a 1mM solution of the DPPH radical solution in methanol was prepared and 1ml of this solution was mixed with 3ml of sample solutions in methanol (containing 20 to 100g) and control (without sample). The solution was stood for 30 min, in dark the absorbance value was monitored by using spectrophotometer at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows:

\[
\% \text{ DPPH free radical scavenging} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100
\]

**Antimicrobial test of selected plants**

**Plant extract**
Aqueous and methanolic extracts were used for antimicrobial study. Ten grams of each powdered plant sample (Areal parts of the tested plants) were taken in 100ml distilled water and 100ml in methanol in labeled conical flasks respectively. The solutions were placed for 72 hours at room temperature. After that the extract were first filtered and squeezed through four layers of muslin cloth and then the supernatant was again filtered by filter paper. This filtrate was kept in labeled tubes in the refrigerator at 4 °C and later used for antimicrobial tests.

**Organism tested**
The pathogenic bacterial cultures: *Ralstonia solanacearum*, *Erwinia carotovora* and *Clavibacter michiganensis* were obtained from Department of Plant Pathology, University of Agriculture Peshawar, Khyber Pakhunhwa, Pakistan. The fresh culture of each bacterial stock culture was prepared in a PDA medium at 37 °C each for 24hours.

**Testing the Antimicrobial activity**
The Antimicrobial activities of the selected wild plant extract were tested by using the well diffusion method mentioned by [11].

A 0.6 ml each bacterial Stock suspension was thoroughly mixed with 100ml of sterile Potato dextrose agar in a sterile beaker and then 20 ml of the inoculated Potato Dextrose agar containing bacterial suspension were poured into sterile petri dishes. The agars were left for 5min to set and cooled. Each plate containing a bacterial suspension, five wells was bored by a sterile cork borer having width 6mm and the agar discs were removed. These wells were filled with 100μl of different concentration (10ppm, 50ppm, 75ppm and 100ppm) of each plant extract by using a microtiter-pipette and allowed to diffuse at room temperature for 1 hour. The plates were then incubated in the upright position at 37 °C for 24 hours. Three replicates were carried out for each extract against each of the test organism. Methanol and water were used as positive control and Antibiotic; Streptomycin (2mg/ml) was used as negative control [11]. After 24 hours the zone of inhibition was measured in millimeter.

**Results and Discussions**
It has been reported that the wild plants are the most abundant and cheapest source of food for human community and they are also used for medicinal purpose. The present study was designed to document the edibility of the wild plants and promoting the ethnobotanical awareness of the local community of the selected area. The selected wild edible plants were investigated for their therapeutic potential. It was found that these selected wild plants were a rich source of essential biomolecules. The result showed that these plants provide food either used raw or cooked and also herbal medicines. The general purposes of plants include dietary...
diversification, food supply, flavoring agent or spices, the increase nutraceutical potential of diet and for various human ailments. Results of our study revealed that Amaranthus thunbergii, Caralluma edulis, Allium astrosanguineum, Rumex patientia and Portulaca oleracea have medicinal importance for local community.

Antioxidant Activity: In the living organism's biochemical reaction takes place by the energy produced by oxidation. However, the production of oxygen inside the body having free radicals and other oxygen reactive species cause fatality of cell and injury of tissue of the body. The damage of cell due to free radicals in oxidation may be related to aging and diseases, such as diabetes, atherosclerosis, inflammation, cancer, and cirrhosis reported by [11-13]. It is reported that all living bodies have antioxidant defense and repair systems to protect the body against oxidative damage caused by free radicals, but these mechanisms are insufficient to avoid the trash entirely stated by [11-13]. The antioxidant supplements or foods having antioxidants property can protect the body from free radical stress and can prevent the body from a large number of diseases. Plants give us nutrients and energy, but the contributing factors are due to the presence of antioxidants like ascorbic acid, tocopherol and carotenoid. Plants are the rich source of phenolic substances which are a group of phytonutrients that exercise high antioxidant properties. Phenolic substances can be classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoid reported by [11-14]. The maximum Antioxidant activity was observed by the methanolic fraction of Amaranthus thunbergii, Rumex patientia, Allium astrosanguineum, Portulaca oleracea and Caralluma edulis (83.64, 82.55, 78.27, 76.09 and 75.85% respectively at the tested concentration of 10 ppm. At 20 ppm the activity was observed 76.405, 84.35, 83.33, 79.05 and 75.38% respectively. The activity of the five different plants at the tested concentration of 40 ppm was found 76.94, 84.65, 83.1, 78.89 and 77.88% respectively. All extracts showed lesser activity as compared to the standard ascorbic acid that showed 92.44% (Table 2). DPPH radical scavenging activity at concentration 80 ppm of the plants found that Rumex patientia has maximum (81.38%) absorbance as compared to the other four plants which were observed with a little difference among each other as Caralluma edulis 78.66% showed less activity than standard Ascorbic acid and Rumex patientia but having high activity from Portulaca oleracea 76.71%, Amaranthus thunbergii 75.39% and Allium astrosanguineum 72.04% which has minimum DPPH radical scavenging activity as compared to the others. While at 160 ppm, the antioxidant activity was found in order of: Caralluma edulis 84.8% > Allium astrosanguineum 80.68% > Amaranthus thunbergii 74.45% > Portulaca oleracea 74.14% > Rumex patientia 69.08% respectively. All showed lower activity than standard Ascorbic acid that was 96.35% (Table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>10 ppm</th>
<th>10 ppm</th>
<th>10 ppm</th>
<th>20 ppm</th>
<th>40 ppm</th>
<th>80 ppm</th>
<th>160 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthus thunbergii</td>
<td>83.64</td>
<td>76.40</td>
<td>76.94</td>
<td>75.39</td>
<td>74.45</td>
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<td></td>
</tr>
<tr>
<td>Caralluma edulis</td>
<td>75.85</td>
<td>75.38</td>
<td>77.88</td>
<td>78.66</td>
<td>84.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allium astrosanguineum</td>
<td>78.27</td>
<td>83.33</td>
<td>83.10</td>
<td>72.04</td>
<td>80.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rumex patientia</td>
<td>82.55</td>
<td>84.35</td>
<td>84.65</td>
<td>81.38</td>
<td>69.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portulaca oleracea</td>
<td>76.09</td>
<td>79.05</td>
<td>78.89</td>
<td>76.71</td>
<td>74.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>91.24</td>
<td>91.31</td>
<td>92.44</td>
<td>95.16</td>
<td>96.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: DPPH radical scavenging activities of various fractions of wild edible plants

The present study showed that Amaranthus thunbergii, Caralluma edulis, Allium astrosanguineum, Rumex patientia and Portulaca oleracea can be taken either in cooked or raw form in order to reduce the risk of various types of diseases that are caused by oxidation stress.

Antimicrobial activity: The plant species are suitable source of antimicrobial agents which act as an alternative strategy for the production of safe and reliable phytomedicines against pathogenic infections. The plant based antimicrobial agents have enormous therapeutic property while show less or no side effects to the human beings [15] Lwu MW, reported that phytochemicals such as alkaloids, saponins, flavonoid and phenolic compounds are present in plants which are responsible for many biological activities. Antibacterial activity studies of aqueous and methanolic extracts of selected plants: Amaranthus thunbergii, Caralluma edulis, Allium astrosanguineum, Rumex patientia and Portulaca oleracea are summarized in Tables: (3). The Methanolic extracts of four plants Caralluma edulis, Allium astrosanguineum, Rumex patientia and Portulaca oleracea were the most effective against gram positive bacteria; Clavibacter michiganensis, Ralstonia solanacearum and Erwinia carotovora. While the aqueous extract of all the tested plants and also the methanolic extract of Amaranthus thunbergii showed no activity against the tested bacterial species under the similar experimental condition Table (3). The absence of antibacterial activity of plants in aqueous extract was also reported by [16]. It was found that the methanolic extract of Caralluma edulis showed highest zone of inhibition (22mm and 18mm) against gram negative bacteria; Ralstonia solanacearum and Erwinia carotovora respectively, while 14mm were observed against gram positive bacteria; Clavibacter michiganensis Table (3). Allium astrosanguineum was found most effective against Erwinia carotovora and showed the maximum inhibition zone (11mm) whereas minimum zone of inhibition (9mm- 7mm) was found against Clavibacter michiganensis and Ralstonia solanacearum. Among the tested plants against the three bacteria, Rumex patientia was found with moderate antimicrobial activity from others. Rumex patientia showed less activity (8mm) against Ralstonia solanacearum while no activity against Erwinia carotovora and Clavibacter michiganensis. The result was compared with the result of [17] that determined the antibacterial activity of one species of the same Rumex family; Rumex vesicarius L. against six bacteria: E.coli, Pseudomonas aeruginosa, staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae and Streptococcus pyogenes and observed the zone of inhibition: 2mm, 6.7mm, 7.2mm, 5.6mm, 11.7mm and 6.8mm respectively. Portulaca oleracea were the second most effective plants among the five tested plants that showed maximum activity against the tested bacteria. The maximum zone of inhibition
in pair (22mm and 17mm) was determined against Erwinia carotovora and Clavibacter michiganensis respectively, whereas minimum zone of inhibition (15mm) was found against Ralstonia solanacearum Table (3). Bekkyaraj S, also observed the same result, 20mm and 15mm of the methanolic extract of Portulaca oleracea against both Gram-positive organisms; Bacillus subtilis and Staphylococcus aureus respectively, and 18mm against Gram-negative bacteria: Pseudomonas aeruginosa reported that the Portulaca oleracea has several phytochemicals and significant antifungal properties against Aspergillus species. Results showed that Portulaca oleracea L., Caralluma edulis L. Amaranthus thunbergii L. And Allium astrosanguineum extract possesses a broad spectrum of antimicrobial activity against a panel of bacteria responsible for the most common bacterial diseases.

Table 3: Data of Antibacterial activities (mm) of selected wild edible plants

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>A.thunbergii C. edulis Al. astrosanguineum R. patientia Portulaca oleracea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stand</td>
</tr>
<tr>
<td>Clavibacter michiganensis (-)</td>
<td>18</td>
</tr>
<tr>
<td>Ralstonia solanacearum (+)</td>
<td>0</td>
</tr>
<tr>
<td>Erwinia carotovora (-)</td>
<td>25</td>
</tr>
</tbody>
</table>

AE= Aqueous extract and ME= Methanol extract Gram (+) bacteria and Gram (-) bacteria, PC= positive control Stand= Standard

Conclusion

The results of the present studies revealed that all these edible plants have the potential of antimicrobial and antioxidant properties, which play a vital role in controlling various diseases caused by various pathogens of bacteria and by the oxidation of free radical in the body. The antioxidant supplements or foods containing antioxidants can protect the human community from oxidative stress and can promote a large number of diseases. These plants give us nutrients and energy, but the contributing factors of free radicals are due to the presence of ascorbic acid, tocopherol and carotenoid. These plants are the rich source of phenolic substances which are a group of phytonutrients that exercise high antioxidant properties. It is recommended that the selected plants have the potential of antimicrobial and antioxidant properties, which play a vital role in controlling various diseases caused by various pathogens of bacteria and by the oxidation of free radical in the body.

References


