Evaluation of Plants Extracts for Proximate Chemical Composition, Antimicrobial and Antifungal Activities

Ghosia Lutfullah, Hina Tila, Arshad Hussain and Abid Ali Khan

Centre of Biotechnology and Microbiology, University of Peshawar 25120, KPK, Pakistan

Food Technology Center, PCSIR Laboratories Complex, Jamrud Road, University Town, Peshawar 25000, KPK, Pakistan

Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, KPK, Pakistan

Abstract: Agro-industrial wastes such as peels of Aloe vera, Cucumis sativus, Citrus paradisi, Citrus aurantium, Punica granatum and leaves of Olive europaea were evaluated for their proximate analysis, antimicrobial and antifungal activities. The results of proximate analysis indicate that all the plants contained some important nutrients such as crude fiber, crude fats and brix. In comparative assessment of above plants species the results indicated that O. europaea have shown higher concentration of the crude fiber (22.0±0.03%), crude fats (15.6±0.07%), however P. granatum has shown the higher concentration brix (2.5±0.03). The dry crude extracts were tested against the pathogenic bacteria i.e., Bacillus cereus, Xanthomonas species, Clostridium species, Staphylococcus aureus, Salmonella heidelberg, Salmonella typhi, Klebsiella pneumoniae and Escherichia coli. All the extracts inhibited the growth of E. coli and S. aureus. While in comparative assessment the extract of Punica granatum have shown higher inhibition of Klebsiella pneumoniae with 25±0.07 mm. The dry crude extracts were also tested against the pathogenic fungi i.e., Aspergillus niger, Aspergillus flavus, Alternaria alternate and Penicillium digitatum. All the extracts inhibited the growth of A. niger and P. digitatum however, in comparative assessment the extract of P. granatum have shown higher inhibition of A. niger and C. digitatum with 12±0.02 and 12±0.10 mm respectively.

Key words: Plant Peels · Proximate Analysis · Antibacterial Activities · Antifungal Activities

INTRODUCTION

Infectious diseases caused by pathogenic microorganisms are a serious threat for human health. While the condition is worse in developing countries due to unavailability of antimicrobial drugs and the resistance of microorganisms to the available drugs [1]. Therefore, for the last two to three decades, the side effects associated with certain antibiotics and the problem of drug resistance [2], has increased the need to discover new chemical metabolites from plants which should have the potential to inhibit the microorganisms and will have no side effects to human health [3].

The potential of plant extracts such as an antibacterial or antifungal is attributed to their biological activities, which are due to their phytochemical contents [4]. Which are found in fruits, vegetables and grains can work against the cellular damage due to oxidative stress and prevent many chronic diseases to occur [5]. The use of herbal medicines is very old for the treatment of many chronic diseases including enteritis however, extract of many herbal plants such as ginger, basil, cinnamon, curry, garlic and mustard have shown antimicrobial activities [6]. While extract of Chinese cassia and chives inhibit the growth of many bacteria including Escherichia coli therefore, it is used as preservative for the storage of milk,
juices and meat for long time [7]. The peel extract of *Citrus sinensis* inhibited the growth of different pathogenic bacteria i.e., *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella epidermis*, *Shigella flexineri* and *Pseudomonas aeruginosa* [8].

The extract of *Aloe vera* leaf has been evaluated for different biological activities like immunomodulatory, wound healing, antiviral, hypertensive, antidiabetic and antineoplastic activities and the extract has remained very active during every activity [9]. Research has shown that the extract of *Aloe vera* is also effective in treating septicemia and liver toxicity [10]. Another member of *Citrus* genus, *Citrus aurantium* (sour orange) has been used widely as root stock. As the name indicates, sour oranges are highly bitter due to presence of compounds called neohesperidin. The secondary metabolites, limonoids, are found in all citrus fruits. In clinical trials, these compounds have shown to inhibit human colon cancer and induce apoptosis of carcinogenic cells in human breasts [11].

Citrus industry yields large amounts of seeds and peel residues which are about half of the total fruit weight. The phenolic content of citrus fruit peels (lemon, oranges and grapefruit) were 15% more than that of peeled fruit [12]. Due to these compounds citrus fruits exhibit potent anti-inflammatory and blood clot inhibition properties [13]. Citrus fruits are important as anti-depressant and anti-malignant and pomegranate may have a significant role against cardiovascular diseases and malignancy, from centuries, its juice has been used against internal parasites and as astringent for remedy of diarrhea [14]. *Olea europaea* (olive) leaf has been used for thousands of years in certain pathological conditions like malaria and other associated fevers. Several studies have been suggested that olive leaves have the potential to decrease blood pressure and enhance flow of blood in coronary arteries [15]. Moreover, the extract of olive leaves inhibit growth of *Helicobacter pylori*, *Campylobacter jejuni* and *Staphylococcus aureus* [16].

Cucumber is known to contain cucurbitacins compounds which are cytotoxic, laxative, anti-inflammatory, anti-fertility and fight malignancy. The pulp tissue of cucumber fruit has been used against xerosis, warts and skin peeling [17]. The main objectives of the present study was to evaluate the peels of *Aloe vera*, *Cucumis sativus*, *Citrus paradisi*, *Citrus aurantium*, *Punica granatum* and leaves of *Olive europaea* for their nutritional value along with antibacterial and antifungal activities.

**MATERIALS AND METHODS**

**Sample Collection:** The samples such as peels of *Punica granatum* (pomegranate), *Citrus paradisi* (grape fruits), *Citrus aurantium* (sour oranges), *Cucumis sativus* (cucumber), *Aloe vera* and leaves *Olive europaea* (Olive) were purchased from the local market of Peshawar, KPK, Pakistan. The peels and leaves were washed, dried and stored in cold dark conditions.

**Chemicals:** Chemicals used were Barium chloride, Hydrochloric acid, Dimethyl sulfoxide, Methanol, Nutrient broth, Nutrient agar, Sulphuric acid Sodium hydroxide and n-hexane. All the chemicals were purchased of Merck, Germany

**Determination of Proximate Composition:** Determination of moisture, total ash, total acidity, crude fiber, crude fat, total and reducing sugars were determined by standard method [18-19].

**Antimicrobial Activity**

**Preparation of Extracts:** The plant peels samples were washed to remove dust residue and dried in vacuum oven at 40 °C to lower the moisture content. The samples were grinded using electrical grinder up to 20 mesh sized and 50 g of each sample was extracted with 200 mL methanol, overnight at room temperature using an orbital shaker. The sample mixture was then filtered through Whatman No. 1 filter paper to remove the extract residues. The residues were re-extracted twice with 200 mL of the same solvent and extract was collected. The extract was then concentrated to dryness at 42 °C in a rotary evaporator. The crude dried extracts were stored in a refrigerator until further analysis. Sample extracts (200 mg) were dissolved in 10 mL of DMSO to obtain a concentration of 20 mg/mL. The extracts will be used in further study.

**Microorganisms:** Eight strains of bacteria were used; four of them were gram positive namely, *Bacillus cereus*, *Xanthomonas species*, *Clostridium species* and *Staphylococcus aureus* and four gram negative bacteria namely, *Salmonella heidelberg*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Escherichia coli*. However; four fungi were used in this study namely, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternate* and *Penicillium digitatum*. All the identified bacterial and fungal species were collected from the cultures collection unit of Food Microbiology Laboratory, PCSIR Laboratories Complex Jamrud Road Peshawar Pakistan.
Antibacterial Assay: The McFarland standard was prepared by adding 0.5 mL of 0.048 M BaCl₂ to 99.5 mL 0.36 N H₂SO₄. Barium sulfate turbidity standard (4 to 6 mL) was taken in screw capped test tube and was used to compare the turbidity [20, 21]. Streptomycin were used as standard drug.

Preparation of Inoculum: Nutrient broth medium was prepared by dissolving 8 g/L. The pH was adjusted to 7 and it was then autoclaved. Autoclaved nutrient broth in test tubes was inoculated with a single colony from previously identified cultures. The test tubes were incubated for 24 hours at 37 °C. The turbidity of these bacterial cultures was adjusted according to McFarland turbidity standard 1.5 x 10⁶ colony forming unit (CFU) per mL by adding sterile physiological saline.

Preparation of Seeded Agar Plates: Nutrient agar medium was prepared by adding 20 mg/L of nutrient agar in distilled water. It was also autoclaved after adjusting pH at 7.0. Nutrient agar was allowed to cool up to 45 °C. Petri plates were prepared by addition of nutrient agar and seeded with about 0.02 mL of inoculum from nutrient broth medium of bacteria. The Petri plates were then allowed to solidify at room temperature. Wells were punched with the help of a cork borer (6 mm).

Measurement of Zone of Inhibition: 50 µL of plant extracts were added to the wells. Each well was named according to type of extract. Antibiotic disc was used as a standard and DMSO was used as negative control in each plate. Petri plates were incubated for 24 hours at 37°C. The zone of inhibition for each bacterial strain was measured around each well. Plates were prepared in triplicates for each extract.

Antifungal Assay
Preparation of Fungal Strain: Deionized water (10 mL) was added to plates having 7 days old growing spores of experimental fungi. The surface was scratched carefully with sterilized loop to pass the spores into the deionized water. The spore suspension (1.5 x 10⁶ CFU/mL) were transferred to 40% glycerol.

Preparation of Seeded Agar Plates: Potato dextrose agar was prepared by dissolving 39 g in one liter distilled water. The media was autoclaved at 121°C for 15 minutes. Potato dextrose agar was allowed to cool at room temperature. It was then poured into Petri plates and allowed to solidify. Fungus suspension (1 mL) of all the species were transferred on to the plates from 40% glycerol. A sterile glass spreader was moistened in the wells. Positive control (meconazole) and negative control (DMSO) were also placed. The plates were incubated at 28°C for 2 days [21].

Statistical Analysis: After triplicate results the data were subjected to statistical analysis of variance and the significance of the difference of the results between means was determined. The values were expressed as means ± SE. P-values < 0.05 were considered significant [22].

RESULTS AND DISCUSSION
Proximate Composition: The proximate composition of plants A. vera, C. sativus, C. paradisi, C. aurantium, P. granatum peels and O. europaea leaves is shown (Table 1). The ash content of peels of A. vera, C. sativus, C. paradisi, C. aurantium, P. granatum and O. europaea leaves were 2.7±0.05 %, 2.2±0.08 %, 9.01±0.11 %, 8.73±0.09 %, 5.6±0.06 % and 4.12±0.13 % respectively. C. paradisi showed highest ash content among the samples.

Moisture content of peels of A. vera, C. sativus, C. paradisi, C. aurantium, P. granatum peels and O. europaea leaves was found 88.21±0.03 %, 90.29±0.07 %, 45.60±0.05 %, 30.40±0.02 %, 62.51±0.06 % and 52.29±0.12 % respectively. Crude fiber of peels of A. vera, C. sativus peel, C. paradisi peel, C. aurantium peel, P. granatum peels and O. europaea leaves were 7.5 %, 19.0 %, 18.0 %, 13.5 %, 20 % and 22 % respectively. The crude fat was found 2.2±0.11 %, 2.6±0.09 %, 3.8±0.04 %, 2.0±0.14 %, 9.0±0.12 % and 15.6±0.07 % for peels of A. vera, C. sativus, C. paradisi, C. aurantium peels, P. granatum peels and O. europaea leaves respectively. The brix value of peels of A. vera, C. sativus, C. paradisi, C. aurantium peels, P. granatum and O. europaea leaves were 0.6±0.02 %, 0.5±0.04 %, 1.0±0.01 %, 0.7±0.03 %, 2.5±0.03 % and 1.0±0.04 % respectively. The pH values of peels of A. vera, C. sativus, C. paradisi, C. aurantium peels, P. granatum peel and O. europaea leaves were 5.45±0.01, 6.86±0.03, 5.38±0.02, 5.23±0.04, 4.15±0.03 and 7.17±0.05 respectively.
Table 1: Proximate composition of different plant parts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ash (%)</th>
<th>Moisture (%)</th>
<th>Crude fiber (%)</th>
<th>Crude fat (%)</th>
<th>Brix (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera peel</td>
<td>0.70±0.05</td>
<td>82.1±0.03</td>
<td>07.5±0.05</td>
<td>2.2±0.11</td>
<td>0.6±0.02</td>
<td>5.45±0.01</td>
</tr>
<tr>
<td>Cucumis sativus peel</td>
<td>0.20±0.08</td>
<td>90.29±0.07</td>
<td>19±0.03</td>
<td>0.6±0.09</td>
<td>0.5±0.04</td>
<td>6.86±0.03</td>
</tr>
<tr>
<td>Citrus paradisi peel</td>
<td>09.01±0.11</td>
<td>45.60±0.05</td>
<td>13.5±0.08</td>
<td>03.8±0.04</td>
<td>1.0±0.01</td>
<td>5.38±0.02</td>
</tr>
<tr>
<td>Citrus aurantium peel</td>
<td>08.73±0.09</td>
<td>30.40±0.02</td>
<td>18.0±0.06</td>
<td>02.0±0.14</td>
<td>0.7±0.03</td>
<td>5.23±0.04</td>
</tr>
<tr>
<td>Punica granatum peel</td>
<td>05.60±0.06</td>
<td>62.51±0.06</td>
<td>20.0±0.08</td>
<td>09.0±0.12</td>
<td>2.5±0.03</td>
<td>4.51±0.03</td>
</tr>
<tr>
<td>Olive europea leaves</td>
<td>04.12±0.13</td>
<td>52.92±0.12</td>
<td>22.0±0.03</td>
<td>15.6±0.07</td>
<td>1.0±0.04</td>
<td>7.17±0.05</td>
</tr>
</tbody>
</table>

Values are expresses as mean ± S.D of triplicate data

Table 2: Anti-bacterial activity of different plants part extracts

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Aloe vera peel</th>
<th>Cucumis sativus peel</th>
<th>Citrus paradisi peel</th>
<th>Citrus aurantium peel</th>
<th>Olive europea leaves</th>
<th>Punica granatum peel</th>
<th>Streptomycin (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>6±0.02</td>
<td>8±0.01</td>
<td>10±0.11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15±0.05</td>
</tr>
<tr>
<td>Salmonella heidelberg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18±0.04</td>
<td>20±0.01</td>
<td>15±0.05</td>
<td></td>
</tr>
<tr>
<td>Eschrechia coli</td>
<td>14±0.04</td>
<td>11±0.02</td>
<td>15±0.15</td>
<td>17±0.06</td>
<td>12±0.02</td>
<td>23±0.04</td>
<td>25±0.05</td>
</tr>
<tr>
<td>Xanthomonas spp.</td>
<td>-</td>
<td>-</td>
<td>13±0.03</td>
<td>19±0.10</td>
<td>10±0.11</td>
<td>23±0.13</td>
<td>25±0.05</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>05±0.14</td>
<td>10±0.04</td>
<td>19±0.08</td>
<td>10.4±0.03</td>
<td>07±0.21</td>
<td>17±0.11</td>
<td>20±0.05</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>09±0.06</td>
<td>18±0.21</td>
<td>11±0.09</td>
<td>09±0.03</td>
<td>-</td>
<td>25±0.05</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13±0.02</td>
<td>14±0.07</td>
<td>-</td>
<td>13±0.06</td>
<td>25±0.07</td>
<td>28±0.05</td>
<td></td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>17±0.03</td>
<td>22±0.04</td>
<td>20±0.02</td>
<td>-</td>
<td>-</td>
<td>25±0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are expresses as mean ± S.D of triplicate data

Antibacterial Activity: The antibacterial activity of extracts of different plants part is presented (Table 2). A. vera peel extract showed growth inhibition of B. cereus, E. coli, S. aureus, S. typhi, K. pneumoniae and Clostridium spp. with zone of inhibition 6±0.02 mm, 14±0.04 mm, 5±0.14 mm, 9±0.06 mm, 13±0.02 mm and 17±0.03 mm, respectively. The A. vera peel extract was efficient in inhibiting the growth of both gram positive and gram negative bacteria. The growth of S. aureus was also inhibited by A. vera peel extract as reported earlier by Agaray and his co-workers, with zone of inhibition 4 mm [23]. The in vitro antibacterial activity of A. vera peel extract was efficient against E. coli and vibrio spp, reported by. It has been reported that Aloe spp. extracts possessed antibacterial activity against both gram positive and negative bacteria like S. aureus, K. pneumoniae and E. coli. Their organic extracts (ethanolic or petroleum ether) were more effective as compared to aqueous extracts [24].

The extract of C. sativus peel inhibited the growth of B. cereus, E. coli, S. aureus and K. pneumoniae. The zone of inhibitions for these bacteria was 8±0.01 mm, 11±0.02 mm, 10±0.04 mm and 14±0.07 mm, respectively. The cucumber peel extract was ineffective against Xanthomonas spp, S. typhi S. heidelberg and Clostridium spp. Previous study regarding the antimicrobial activity of C. sativus peel extract also showed inhibition of growth against E. coli, S. aureus and K. pneumoniae [25].

The C. sativus extract was as effective as streptomycin against K. pneumoniae. Zone of inhibition observed by both C. sativus and streptomycin was 14 mm. C. paradisi peel extract showed bacteriocidal activity against B. cereus, E. coli, Xanthomonas spp, S. aureus, S. typhi and Clostridium spp. with mean value of zone of inhibition 10±0.11 mm, 15±0.15 mm, 13±0.03 mm, 19±0.08 mm, 18±0.21 mm and 23±0.04 mm, respectively. The extract did not inhibit the growth of S. heidelberg and K. pneumoniae. The peel extract of C. aurantium was effective against E. coli, Xanthomonas spp, S. aureus, S. typhi and Clostridium spp. with zone of inhibitions 17±0.06 mm, 19±0.10 mm, 10.4±0.03 mm, 21±0.03 mm and 20±0.02 mm, respectively. The highest zone of inhibition was observed by Clostridium spp. followed by Xanthomonas spp.

Citrus fruits are known for their flavonoid contents which have many beneficial effects on human health. One of their important functions is the prevention of cancer. Naringinin is a flavonoid glycoside, mostly present in grape fruit and sour oranges peels [26]. The antimicrobial activity of citrus peels could be attributed to these flavonoids.
and Xanthomonas spp., S. aureus, the growth of Salmonella heidelberg extract did not inhibit the growth of Clostridium spp. [27]. was ineffective against the rest of fungi. The antifungal activity of different some plants which are used to be discarded as waste, Antifungal Activity: plants part is also presented (Table 3). Peel extract of A. niger A. aurantium olive leaves were effective in stopping or delaying the growth inhibition against A. niger, A. flavus, A. alternaria Penicillium digitatum with mean values of zone of inhibition 8±0.05 mm, 5±0.04 mm, 10±0.08 mm and 11±0.21 mm, respectively. The inhibition of mycelial growth of these fungi has also been documented by essential oils (EOs) of C. paradisi peel [29].

C. aurantium peels extract was effective in inhibiting A. niger and Penicillium digitatum with zone of inhibition as 7±0.08 mm and 11±0.05 mm, respectively. The EOs of C. aurantium peels at different concentrations 10, 25 and 50 µL was effective in inhibiting A. niger, A. flavus and A. parasiticus [30]. O. europaea showed antifungal activity only against A. niger and penicillium digitatum with zone of inhibition 6±0.08 mm and 9±0.03 mm, respectively. It was ineffective against the rest of fungi. P. granatum possessed antifungal activity against A. niger and A. alternate and penicillium digitatum with mean values of zone of inhibition as 12±0.02 mm, 11±0.03 mm and 12±0.10 mm, respectively. Antifungal activity of P. granatum was due to the presence of antifungal compounds such as punicalagin, kaemperol, granatin, catechin, galocatechin, querectin and castagalagin [31].

CONCLUSIONS

This study was conducted to see if the wastes of plant could be used as a source of nutrients and their extracts as a possible source of natural antimicrobials. Therefore, it is concluded that the peels and leaves of some plants which are used to be discarded as waste, possess efficient antimicrobial activities against the tested microorganisms. It is now important to carry out further work on the isolation of antimicrobial compounds from the fruit peels and it is suggested to use as food supplement.

REFERENCES


