Antimicrobial and Phytochemical Screening of Methanol Extracts of Three Medicinal Plants in Ethiopia

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Abstract: Medicinal plants play a key role in the development and advancement of modern studies by serving as a starting point for the development of novelties in drug. The World Health Organization (WHO) estimates that about 80% of the world’s people relay chiefly on traditional medicine, mostly of plant origin to meet their primary health care needs. In Ethiopia medicinal plants, Calpurnea aurea, Hypericum revolutum and Peterollobium stellatum traditionally used to treat amoebic dysentery, stomachache and respiratory diseases, respectively. The current study was conducted to investigate the antibacterial activity these plants against bacterial isolates. In vitro antibacterial activity against Salmonella typhi, Salmonella paratyphi, Salmonella typhimurium, Shigella species, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli was performed. The tests were carried out using the agar well diffusion method at 250 mg/mL concentration of the crude extracts. The minimum inhibitory concentrations (MICs) of the extracts were determined against these microorganisms using micro dilution. These plant extracts exhibited minimum inhibitory concentration values ranging 125 mg/mL to 250 mg/mL against some bacterial isolates. Among these three plant species; P. stellatum was found to be the most active against S. typhi, Salmonella paratyphi, P. aeruginosa, S. aureus and E. coli. Out of seven clinical bacterial isolates tested, P. aeruginosa and E. coli were found to be more sensitive to the plant extracts than other bacterial isolates. The results indicated the potential of these herbal drugs in treating bacterial infections.

Keywords: Antibacterial Activity • Medicinal Plants • Crude Extracts • Infectious Diseases • Calpurnea aurea • Hypericum revolutum • Peterollobium stellatum

INTRODUCTION

Infectious diseases represent a continuous and increasing threat to human health and welfare. They are the major cause for enormous morbidity and mortality in all parts of the world, although developing countries are carrying the major part of the burden [1, 2]. In addition to the usual infectious diseases such as malaria, tuberculosis, anthrax, brucellosis or HIV, incidences of nosocomial and opportunistic infections have risen dramatically. The number of infections caused by new, reemerging or drug-resistant pathogens is growing day to day and the increased proportion of hospitalized patients with immunodeficiency has resulted in an increase of severe and invasive infections [3].

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The majority of infectious diseases have been preventable (e.g. by vaccines), readily treatable (e.g. by antibiotics) or effectively managed using fairly simple, low cost measures such as ensuring adequate sanitation and educational interventions [4]. All these interventions, however, have not protected the world from the emergence of new diseases and the re-emergence of old ones [5]. The unsatisfactory management of the whole infectious diseases particularly bacterial infections throughout the world, which allows partially treated and relapsed patients to become sequentially resistant that play a significant role in the development of antibiotic resistance. Moreover, in recent years, the number of new antibiotics licensed for human use in different parts of the world has been lower than in the recent past. There has been also less innovation in the field of antibiotic discovery research and development. In order to fill such gaps, new antimicrobial agents are urgently needed and research programs for alternative therapeutics should be encouraged. It has been suggested that the best available in vitro indicator of possible therapeutic activity is the early microbicidal activity of medicinal plants [6].

According to WHO, herbal medicines serve the health needs of about 80% of the world’s population, especially for millions of people in the vast rural areas of developing countries. In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of the cultural beliefs and attitudes [7-11].

In Ethiopia, about 800 species of plants, which occur throughout the country’s diverse highlands and lowland areas, are used in the traditional health care system to treat nearly 300 physical and mental disorders and remains to be the main resource of treatment for a large majority of other diseases conditions [10, 12]. (What man No. 1 filter paper, What man Ltd., England).

Traditionally, the root and bark of Calpurnea aurea used for the treatment of amoebic dysentery, stomachache [13], the leaves of Hypericum revolutum used for the treatment of stomach problems [14-15] and fresh roots of Pterolobium stellatum are chewed for medicinal purposes [15]. Therefore, the present study was carried out to examine the antibacterial activity of methanolic extracts of leaves of C. aurea and H. revolutum and roots of P. stellatum, growing in Ethiopia against a wide range of pathogenic bacteria.

MATERIALS AND METHODS

Chemicals and Drugs: Methanol (Research LAB Fine Chemical Industries, India) and Dimethyl sulfoxide (SIGMA®) were obtained from School of Pharmacy, Addis Ababa University. Gentamicin sulphate (Working standards) was kindly supplied by the Department of Quality Control, Drug Administration and Control Authority (DACA), Addis Ababa, Ethiopia.

Media: Mueller Hinton agar (BBL® USA) was used for antimicrobial sensitivity screening and for the determination of minimum inhibitory concentration. The media was prepared and treated according to manufacturer’s guidelines.

Test Microorganisms: All the test strains (Salmonella typhi, Salmonella paratyphi, Salmonella typhimurium, Shigella species, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli) were clinical isolates obtained from human clinical samples.

Collection and Preparation of the Plant Material: The leaves of C. aurea were collected from Addis Ababa and the leaves of H. revolutum and roots of P. stellatum were collected from Menagesha Forest, Oromia District, West of Addis Ababa in November 2010. The identity of each plant specimen was confirmed at the National Herbarium, Addis Ababa University where a voucher specimen was deposited. The plant materials were dried in an open air protected from direct exposure to sunlight. The dried plant materials were separately powdered to suitable size.

Preparation of Methanol Extracts: This was conducted according to Alanis et al., [16]. From each powdered plant material, 200 grams were extracted with methanol 80% by maceration and left at room temperature for 72 h with frequent agitation and the resulting liquid was filtered (What man No. 1 filter paper, What man Ltd., England). Extraction was repeated three times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using rota vapor (BUCHI Rota-vapor R-205, Switzerland) at temperature not more than 40°C. The aqueous residue was then placed in a vacuum oven at 40 °C for about a week to remove the water. The resulting dried mass was then powdered, packed into a glass vial and stored in desiccators until use.

Antibacterial Sensitivity Testing: It was carried out according to the methods of Cheesbrough [17] and Ojo et al., [18]. In brief: a concentration of 250 mg/ml of the plant extracts was prepared from the stock solution and antimicrobial sensitivity testing was done by agar well diffusion assay. Cultures of each organism (Bacterial
suspensions of $1.0 \times 10^8$ colony-forming units (CFU) per mL were inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and evenly spread over the entire surface of agar plate to obtain uniform inoculums. Wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile borer. About 50 µL of the 250 mg/ml 80% methanol extract of each plant was dispensed into respective wells and 120 µg Gentamicin was used as a positive control. Dimethyl sulfoxide (DMSO) was used as negative control. The set up was incubated for 24 h at 37°C. After 24 h incubation, the zones of inhibition were measured using a ruler and the results reported in millimeters (mm). All the tests were run in triplicates and the average result was taken.

**Determination of Minimum Inhibition Concentration (MIC):** MIC was determined using the agar dilution method. The MIC was evaluated on plant extracts that showed antibacterial activity in the agar well diffusion assay on any organism. This test was performed at five concentration of each extract (250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL and 15.75 mg/mL) employing doubling serial dilutions of plant extract in nutrient broth up to the fifth dilution. Overnight incubated suspension of each organism in nutrient broth was prepared (inoculums size of $1.0 \times 10^8$ cfu/mL) and 50 µL was added to all the test tubes and preparations were incubated at $37^\circ$C for 24 h. After incubation, using a sterile cotton swab, suspension of each tube was inoculated on nutrient agar to see if bacterial growth was inhibited or not. Growth of bacteria on solid media indicated that particular concentration of the extract was unable to inhibit the bacteria. The MIC was defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation [18-20].

**RESULTS**

The species of plants have not shown significant variation in percentage yield in 80% methanol extraction by maceration. The highest yield was recorded for *C. aurea* (16%) and the lowest yield was observed for *P. stelatum* which was 10% (Table 1).

Zone of inhibition of bacterial growth by different plant extracts are shown in Table 2. *S. typhimurium* was not inhibited by any of the plant extracts examined at the concentrations tested. Of all the plant extracts tested, 80% methanol extract of *P. stellatum* showed antibacterial activity against most of the bacterial species investigated indicating its wide spectrum of activity while extract of *C. aurea* and *H. revolutum* did not induce inhibition of growth of most bacterial species. The extracts of these plants inhibited the growth of *P. aeruginosa* and *E. coli*. The extract from *H. revolutum* also showed antibacterial activity against the growth of *Shigella* spp. The maximum zone of inhibition of 16 mm was recorded for extract of *P. stellatum* against *E. coli* (Table 2).

**Table 1:** Yield percentage of plant extracts using maceration with methanol 80%.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Parts used</th>
<th>% Yield (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calpurnea aurea</td>
<td>Leaves</td>
<td>16</td>
</tr>
<tr>
<td>Hypericum revolutum</td>
<td>Leaves</td>
<td>12.2</td>
</tr>
<tr>
<td>Pterolobium stellatum</td>
<td>Roots</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 2:** Antibacterial activity of 80% methanol extracts of medicinal plants using agar well diffusion method

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th><em>C. aurea</em></th>
<th><em>H. revolutum</em></th>
<th><em>P. stellatum</em></th>
<th>Gentamicin</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhi</em></td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td><em>S. paratyphi</em></td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td><em>Shigella</em> spp</td>
<td>9</td>
<td>12</td>
<td>11</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>10</td>
<td>10</td>
<td>16</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>10</td>
<td>16</td>
<td>16</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

All the values are mean ± Standard Error of Mean of three determinations -No zone of inhibition detected
The MIC obtained using broth dilution method for different plant extracts are shown in Table 3. No inhibition was recorded for S. typhimurium for all plant extracts tested at all concentrations. The extracts of P. stellatum and C. aurea were effective against E. coli and H. revolutum against Shigella spp. (MIC = 125 mg/mL).

The anti-bacterial activity of the extracts of the investigated plants appears to be due to the presence of secondary metabolites such as terpenoids (Identified in all species), saponins (found in P. stelatum and C. aurea), tannins (found in P. stelatum and H. revolutum), alkaloids (Identified only in C. aurea) and flavonoids (found only in H. revolutum) (Table 4).

**Table 3: Minimum inhibitory concentration (MIC) of plant extracts against different bacterial isolates (mg/ml)**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Plant species</th>
<th>C. aurea</th>
<th>H. revolutum</th>
<th>P. stellatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhi</td>
<td>250</td>
<td>-</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>S. paratyphi</td>
<td>-</td>
<td>-</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>-</td>
<td>125</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>250</td>
<td>250</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>125</td>
<td>-</td>
<td>125</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4: Phytochemical components of the crude plant extracts**

<table>
<thead>
<tr>
<th>Phytochemicals content</th>
<th>Pterlobium stelatum</th>
<th>Calpurnia aurea</th>
<th>Hypericum revolutum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: + = Present- = Absent

The finding of the current study with regard to P. stellatum revealed that the 80% methanol extracts was active against different bacterial species. As of our knowledge there is no report on the antibacterial activity and phytochemical constituents of this plant extracts. The antibacterial activity of the root extracts of this plant can be attributed to the action of the phytochemical compounds (terpenoids, saponins and tannins) investigated in this study. The extract showed strong antibacterial activity against E. coli in the current study. The fact that the extracts of the plants are showing different efficacy against some species of bacteria could be due to extraction ability of active ingredients responsible for antibacterial activity by 80% methanol and presence of different secondary metabolites responsible for antibacterial activity in those plants. On the other hand, the extract from the same plant species has shown variable activity against different bacterial species presumably because of difference in sensitivity of the microorganisms to specific active ingredients present in the plant.

**DISCUSSION**

Infectious diseases of bacterial origin, such as S. aureus, Salmonella spp., Shigella spp., etc, constitute the major cause of morbidity and/or mortality in developing countries like Ethiopia [21]. With the emergence of HIV, the negative role of this micro-flora has even become worse as they facilitate the infection rate by the virus or by significantly reducing the onset time of AIDS. Nowadays, there are very few, if any, antibiotics to which these micro-organisms have not developed resistance. The situation is further compounded by the lack of patient compliance to antibiotic regimen and by the exorbitant costs of the antibiotics. The preliminary results of the present study, therefore, not only confirms the justifiable use of those plants in the traditional health care system against some of the micro-organisms of public health importance but also reflects the hope for development of effective chemotherapeutic agents in the future from plants.

Groups of phytochemical compounds commonly implicated for antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, triterpenoids, different essential oils, saponins, saponin glycosides and phenols [22-23]. Presence of alkaloids, terpenoids, saponins, tannins and flavonoids in the crude extracts the plants in the current study could be linked to their activities against the growth of microorganisms. Previous works have shown that Hypericum species contains alkaloids, flavonoids, tannins, saponins and anthraquinones [24]. The antibacterial activity of the extract from H. revolutum could be explained by the presence of tannins, flavonoids and terpenoids in the current study. The mechanism of action of tannins is based on their ability to bind proteins thereby inhibiting cell protein synthesis. Flavonoids are a diverse group of plant secondary metabolites, present almost ubiquitously in higher plants, often at relatively high concentrations. They have a wide range of biological activities that stem largely from their ability to bind to proteins [24]. Previous study has also shown that quinolizidine alkaloids, lectins, non-protein amino acids and tannins are the major components of C. aurea [8]. The antibacterial activity of this plant extract against P. aeruginosa and E. coli in the current study might be due to the presence of alkaloids which is in agreement with the previous study [25-26].
Among these three plants; extract of *P. stellatum* exhibited antibacterial activity against most organisms tested, followed by *H. revolutum*. The *in vitro* finding is not always dependable, plants which are effective *in vitro* might not work when used *in vivo* and some plants which showed little or no effect *in vitro* study might also be effective when evaluated in animals due to various factors that affect or favor the release of active ingredients in animal bodies. Therefore, further detailed *in vitro* and *in vivo* evaluation of these medicinal plants should be carried out.

In conclusion the results of this study have shown that 80% methanolic extracts of leaves of *C. aurea* and *H. revolutum* and roots of *P. stellatum* have great potential as antibacterial agents in the treatment of infectious diseases. Further detailed investigation of the active components of the plant for the exact mechanism of action will contribute greatly to the development new pharmaceuticals.

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REFERENCES


