Antibacterial Activity Evaluation of Selected Medicinal Plants in Comparison with Some Standard Antibiotics

Demisse Dakone¹*, Gizachew Zeleke²

¹Central Ethiopia Environment and Forest Research Center (CEE-FRC), P.O. Box, 24536 (1000), Addis Ababa, Ethiopia
²Hawassa Environment and Forest Research Center (HE-FRC), P.O. Box, 1832 (1000), Hawassa, Ethiopia

ABSTRACT
Ethnopharmacological relevance: An emerging of antibiotic resistance strains of bacteria brings most serious public health problems. It is therefore, important to look for more effective, safer and less toxic alternative options of treatment. Development of new antibacterial agents from plant extract is among the proposed solutions to overcome this problem. Aim of the study: To study the antibacterial activity evaluation of Leucas aspera (Wild.) Link. (L. aspera), Solanum incanum L. (S. incanum), and Hydnora johanis A.B. (H. johanis) against Escherichia coli (E. coli), Salmonella typhimurium (S. typhimurium), Staphylococcus aureus (S. aureus) and Enterococcus feacalis (E. feacalis) in comparison with GEN (Gentamicin), CIPRO (Ciprofloxacin), PCN (Penicillin), Ampicillin (AMP) and TCN (Tetracycline). Materials and Methods: Following plant material collection and extraction; disc diffusion method was used for antibacterial activity test. Results: The plants showed a promising broad spectrum of activity against Gram-negative (Escherichia coli and Salmonella typhimurium) and Gram-positive (Staphylococcus aureus and Enterococcus feacalis) test bacteria with growth inhibition zone values ranging from 7.40±0.6-16.70±0.4 mm. Compared with standard antibiotics; most active crude extracts were showed comparative antimicrobial effect as do penicillin, ampicillin and tetracycline. Acetone extract of H. johanis on E. coli (16.70±0.4) and ethyl acetate extract of S. incanum on S. typhimurium (16.0±2.0) were demonstrated promising activity than the activity demonstrated by PCN, AMP and TCN. Ethanol extract of L. aspera on S. aureus (16.40±1.1) were also displayed better activity than the activity of AMP. Conclusions: Over all, the present investigation proves the scientific basis for the traditional use of L. aspera, S. incanum and H. johanis as antibacterial agent for the treatment of infections caused by E. coli, S. typhimurium, S. aureus and E. feacalis.

Keyword: Antibacterial activity, Medicinal plants, Pathogenic bacteria, Standard antibiotics.

INTRODUCTION
The increasing global trend of multidrug resistance among Gram’s-positive and Gram’s-negative bacteria raises difficulty in their management. This permanently emerging resistance poses major challenges to health care system resulting in increased risks of death, length and the cost of hospitalization and the cost on healthcare systems¹. This impact of pathogens in developing countries is particularly large due to relative unavailability of medicines and the emergence of widespread drug resistance along with appearance of undesirable side effects of certain antibiotics².

Traditional medicine is the sum total of all the knowledge, beliefs and practices that are used in prevention, diagnosis and elimination of physical, mental and social imbalance that exclusively rely on practical experiences and observation³. The most common traditional medicine is the use of medicinal plants. Medicinal plant in the context of traditional medicine can be defined as any plant which contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs⁴. Syed and Rajeev⁵ also defined medicinal plant as any plant in which one or more of its organs containing substances that can be used for therapeutic properties.

According to the World Health Organization, greater than 80% of the total world’s population depends on the traditional medicine in order to satisfy their primary health care needs⁶. The organization estimates approximately over 21,000 plant species has been used for their medicinal purpose though out the world⁷. World health organization also observed that the majority of the populations in the developing countries are still relying on this herbal medicine to meet their health need⁸.

Numerous studies shown that, medicinal plants are source of diverse nutrient and non-nutrient molecules⁹. It was mentioned that about 25% of modern medicines are developed from plants sources used traditionally¹⁰. There for their usefulness in the development of the modern medicine is extensive. This is due to the presence of wide variety of plant bioactive compounds such as alkaloids, tannins, flavonolid, terpanoids, phenolic compounds steroids, resins and other secondary metabolites. Significantly; these compounds act on different systems of animals through interfering in the metabolism of microbes infecting them¹¹. Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide¹². However, antibiotics have always

¹Author for Correspondence: demissedakone2005@gmail.com
played a major role in treating them. Nevertheless, due to the acquired resistance of the pathogens against certain antibiotic drug resistance bacteria have increased all over the world. This has created immense clinical problem in the treatment of infectious diseases. Therefore, the limited number of drugs available for their treatment and emerging resistance permanently encourage the search for alternatives with low cost and low toxicity. Among traditional therapies, plant extracts overcomes most likely the above mentioned antibiotic disadvantages.

In Ethiopia, there are many plant species (6500-7000) reported that had been used for traditionally treating infectious diseases. Out of these medicinal plants, 12% are endemic to Ethiopia. Nearly 80% of human and more than 90% of livestock population in the country depend on this traditional medicine to meet their health care needs. At present, the demand on these medicinal plants is increasing due to their use in traditional medicine, pharmaceutical industries, cosmetic fields and agribusiness, and for the quality of their essential oil.

Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. Evidently, there was no previous research work carried out on antibacterial activity evaluation of selected medicinal plants from Arba Minch Zuria Woreda in comparison with some standard antibiotics, where traditional medicine is extensively employed in daily life for curative, preventive and promotive health care design. To my knowledge, I have not also found literature providing comparative antibacterial screening test of the plants like S. incanum (root extract), H. johanis (root extract) and L. aspera (leaf extract) against E. coli, S. typhimurium, S. aureus and E. feacalis in in comparison with GEN, CIPRO, PCN, AMP and TCN. Therefore, this paper reports antibacterial activity evaluation of some medicinal plants from Arba Minch Zuria Woreda, southern Ethiopia in comparison with some standard antibiotics to confirm their traditional medicinal uses scientifically.

### MATERIALS AND METHODS

The plant was collected in January 2015 from Arba Minch Zuria Woreda, Southern Ethiopia. Geographically, Arba Minch Zuria Woreda lies in between 05°39’36” to 06°12’2” N and 37°24’36” to 37°33’2” E at an altitude of 1100-1950 m above sea level. The mean annual rainfall in the Woreda is 930 mm. Average maximum and minimum temperature is about 33.3 and 17.4 °C, respectively. The rainfall distribution has a bimodal nature with the first and second rainfall during April to May and September to October, respectively. The area falls within the latitudes 30°00’–34°15’S and longitudes 22°45’–30°15’E. It is bounded by the Lake Abaya and Chamo in the east.

The plant parts used for this research were S. incanum (root), H. johanis (root) and L. aspera (leaf). Selected parts of the plant were collected from their natural habitat and transported to the Arba Minch University for their taxonomic identification. Identities of the specimens were confirmed taxonomically by transport to the Arba Minch University for their taxonomic identification. Identities of the specimens were confirmed taxonomically by taxonomic identification.

### Results and Discussion

#### Table 1: Sensitivity zone of inhibition of commercial standard antibiotics.

<table>
<thead>
<tr>
<th>Standard antibiotics</th>
<th>Inhibition zone against tested bacteria (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25.0±0.0</td>
</tr>
<tr>
<td>Penicillin</td>
<td>12.0±1.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>12.4±0.6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>8.7±0.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>21.0±1.0</td>
</tr>
</tbody>
</table>

- - No inhibition zone; * - Dimethyl sulfoxide (DMSO), Negative control

#### Table 2: Zone of inhibition (mm) against different bacterial strains by plants extracts

<table>
<thead>
<tr>
<th>Plants</th>
<th>Parts tested</th>
<th>Test organisms</th>
<th>Inhibition effect by extract (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. incanum</td>
<td>Root</td>
<td>E. coli</td>
<td>Acetone: 14.0±1.0, Ethanol: 8.40±0.8, Chloroform: - , Ethyl acetate: 10.40±1.6, DMSO*: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. typhimurium</td>
<td>Acetone: 13.70±0.4, Ethanol: 11.33±1.6, Chloroform: - , Ethyl acetate: 16.0±2.0, DMSO*: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
<td>Acetone: 15.40±0.6, Ethanol: 14.70±4.6, Chloroform: 7.40±1.6, Ethyl acetate: - , DMSO*: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. feacalis</td>
<td>Acetone: 7.40±0.6, Ethanol: 10.70±0.6, Chloroform: 8.70±0.6, Ethyl acetate: 15.0±0.0, DMSO*: -</td>
</tr>
</tbody>
</table>

- - No inhibition zone; * - Dimethyl sulfoxide (DMSO), Negative control

#### Table 3: Zone of inhibition (mm) against different bacterial strains by plants extracts.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Parts tested</th>
<th>Test organisms</th>
<th>Inhibition effect by extract (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. aspera</td>
<td>Leaf</td>
<td>E. coli</td>
<td>Acetone: 11.0±1.0, Ethanol: - , Chloroform: - , Ethyl acetate: 11.0±1.0, DMSO*: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. typhimurium</td>
<td>Acetone: 11.40±1.5, Ethanol: - , Chloroform: - , Ethyl acetate: 13.0±1.0, DMSO*: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
<td>Acetone: 16.40±1.1, Ethanol: - , Chloroform: - , Ethyl acetate: 12.40±1.3, DMSO*: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. feacalis</td>
<td>Acetone: 14.0±1.0, Ethanol: - , Chloroform: - , Ethyl acetate: 10.70±1.4, DMSO*: -</td>
</tr>
</tbody>
</table>

- - No inhibition zone; * - Dimethyl sulfoxide (DMSO), Negative control
confirmed by a taxonomist and dried samples were deposited at the botanical Herbarium in Arba Minch University.

Preparation of plant extract

The plant parts such as root extract of *S. incanum* and *H. johanis* and leaf extract of *L. aspera* were used for the *in vitro* antibacterial screening test. The selected plant parts were collected brought to lab, washed in running tap water; surface sterilized with 70% alcohol and then rinsed with sterile distilled water before use. After which it was cut into smaller size and shade dried at room temperature for about three days without exposing it to direct sun light. Shade dried plant material was then milled into powder using a mechanical grinder. Preparation of crude extracts was made as to that of Ijeh17. Accordingly, 100g of powdered extract of each selected sample was successively extracted using maceration technique in five solvent systems (aqueous, chloroform, ethanol, acetone and ethyl acetate) within 72 hours. The macerates of each plant were filtered using Whatman filter paper No1. Each of the filtrates was then concentrated at reduced pressure using rotary evaporator and subjected to antibacterial activity test.

Evaluating antibacterial activities

Sample collection and maintenance

The antibacterial activity of the plant crude extract was studied for a broad range of microorganisms. That is Gram’s positive including, *S. aureus* (ATCC25923) and *E. faecalis* (ATCC29212) as well as Gram’s negative bacteria including *E. coli* (ATCC25922) and *S. typhimurium* (ATCC13311). All strains are obtained from the Arba Minch Regional Laboratory. For each test organism, viability test was checked by growing each strain on nutrient broth. Then after, they were sub cultured on nutrient agar medium and incubated at 37 °C for 24 hours. The microorganisms were then maintained on sterile nutrient agar slants in refrigerator (at 4 °C) and used as stock culture when it was required.

Preparation of suspension culture

Fresh culture was obtained by sub-culturing the microorganisms from stock culture on nutrient agar slants and incubated for 24 hours at 37 °C. Then after, a cell suspension of each organism was freshly prepared by transferring isolated colonies selected from 24 hours agar plate in to a broth and suspension turbidity was adjusted to a 0.5 McFarland turbidity standard (1×10⁸ cfu/ml) in sterile saline solution. The solution was then diluted 1:20 to yield 5×10⁵ cfu/ml¹⁹.

Preparation of test solution

For each crude extract test solution was prepared to carry out their antibacterial activity test. This were prepared by dissolving 100 mg of each of the crude extracts in 1ml of dimethyl sulfoxide (DMSO) to achieve final concentration of 100 mg/ml solution of test sample.

Screening antibacterial activities of plant extract

Antibacterial activity screening test was done by the disc diffusion assay. The bacterial suspension (5×10⁵ cfu/ml) was spread over the 90 mm Petri dishes containing Muller Hinton agar using a sterile cotton swab. Fifty μl of each test solutions was then applied onto the surface of six mm diameter sterile discs (Whatman filter paper No. 3) and allowed to diffuse for five minutes. Then after, it was placed on the surface of the previously inoculated Muller Hinton agar in Petri dishes and the plates were then kept in an incubator at 37 °C for 24 hours. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the disc in mm with ruler¹⁹.

Screening antibacterial activity of standard antibiotics

The antibacterial activity was also analyzed with five commercially available standard antibiotics. Ciprofloxacin, PCN, AMP, TCN and GEN disc were used for comparative study.

RESULTS AND DISCUSSIONS

According to WHO2⁰ the relationship between susceptibility of bacterial strains against standard antibiotics applied and the nearest inhibition zone diameter can be classified as follows: For GEN, if the diameter is ≤
Table 4: Zone of inhibition (mm) against different bacterial strains by plants extracts.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Parts tested</th>
<th>Test organisms</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Aqueous</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>DMSO*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. johanis</td>
<td>Root</td>
<td>E. coli</td>
<td>16.70±0.4</td>
<td>13.70±0.4</td>
<td>13.0±1.0</td>
<td>-</td>
<td>15.0±0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. typhimurium</td>
<td>15.40±0.8</td>
<td>8.0±0</td>
<td>13.40±0.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
<td>-</td>
<td>7.70±0.60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. faecalis</td>
<td>10.70±0.60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- No inhibition zone; * Dimethyl sulfoxide (DMSO), Negative control

12.0 mm: resistant; between 13.0-14.0 mm: intermediate and ≥ 15.0 mm: the bacteria is susceptible, for AMP ≤ 11.0 mm: resistant, between 12.0-13.0 mm: intermediate and ≥ 14.0 mm: the bacteria is susceptible, for CIPRO ≤ 15.0 mm: resistant, between 16-20.0 mm: intermediate and ≥ 21.0 mm: the bacteria is susceptible, for TCN ≤ 14.0 mm: resistant, between 15.0-18.0 mm: intermediate and ≥ 19.0 mm: the bacteria is susceptible and for PCN, if the diameter is ≤ 14.0 mm: resistant and ≥ 19.0 mm: the bacteria is susceptible.

From this it can be deduced that, S. aureus was susceptible to all standard antibiotics except AMP. Enterococcus faecalis was also susceptible to GEN and CIPRO but resistant to PCN, AMP and TCN. The strain of E. coli and S. typhimurium was resistant to PCN, AMP and TCN but susceptible to GEN (Table 1). This may arise from the fact that microorganisms are developing resistance to many drugs.

When the activities of S. incanum was compared with these standard antibiotics, most of its active crude extract have showed better antibacterial activity against E. coli (8.4±0.8-14.0±1.0) than all standard antibiotics but least activity than GEN and CIPRO (21.0±1.0-25.0±0.0). Aqueous and ethanol active crude extracts of S. incanum was showed lower antibacterial activity against S. aureus than all standard antibiotics but superior activity than AMP (Table 1 and 2). On the other hand, acetone active crude extracts of the plant has showed advanced antimicrobial activity than TCN and poorer activity than GEN and CIPRO against E. faecalis. Similarly ethanol and ethyl acetate active crude extracts were exhibited significant antimicrobial activity on S. typhimurium than PCN, TCN and AMP but insignificant activity than CIPRO and GEN (Table 1 and 2). In cause of active aqueous extract, superior activity was detected on S. typhi than AMP and TCN. In the same way, active crude aqueous extract was displayed better sensitivity towards E. coli than PCN, TCN and AMP (Table 1 and 2).

Similarly, when potential activity of each antibiotics were compared with several solvent extract of L. aspera, active ethanol and ethyl acetate extracts was revealed better antimicrobial activity against E. coli in comparisons with PCN, AMP and TCN but lower activity than GEN and CIPRO (Figure 1). Although, these extracts were showed more potent activity than PCN (except ethanol extract), AMP and TCN on S. typhimurium, but not as much active as GEN and CIPRO. All antibiotics except AMP were showed fairly better activity against S. aureus than ethyl acetate and ethanol active extract. Likely, the ethanol active extract of the plant was showed superior antibacterial activity than AMP, TCN and PCN against E. faecalis. Whereas, ethyl acetate active extract demonstrated superior antibacterial activity on E. faecalis than TCN only (Table 1 and 3).

When compared, all active crude extract of H. johanis except chloroform extract were demonstrated enhanced activity on E. coli than PCN, AMP and TCN but minor activity than GEN and CIPRO (Table 1 and 4). Its acetone and aqueous active crude extracts were showed superior activity on S. typhimurium compared with PCN, AMP and TCN but minor activity on E. faecalis when compared with GEN and CIPRO. In comparison to all standard antibiotics sensitivity values, the inhibitory zone concentration of ethanol solubilized extracts of H. johanis appear to be not significant on S. aureus but studies have shown that it is useful to carry out susceptibility test on both standard and clinical isolates to establish sensitivity of the test organisms.

Antimicrobial activities of plant were supported with the presence of different bioactive compounds detected in the extract including flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. Comparison of such antimicrobial potency of the plant extract and antibiotics cannot be drown from this result because a higher sensitivity may be caused by a highly active compounds present in a quite small amounts or by a substance of comparatively low activity but present in a high concentration of plant extract. Thus to proof this fact it has to be stressed that these extract need further analysis of bioactive compounds.

Preventative or treatment therapies for complication caused by pathogens S. typhimurium, E. faecalis, S. aureus and E. coli. Thus; the relative comparative evidence in comparison with standard antibiotics and observed antibacterial activities of the crude extract from these traditional medicinal plants could support scientifically their traditional use for the treatment of infections caused by S. typhimurium, E. faecalis, S. aureus and E. coli.

AUTHORS’ CONTRIBUTIONS
Demisse Dakone involved in designing the experiment, analyzed data and manuscript write up. Gizachew Zeleke

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was involved in data collection, analysis and drafting the manuscript.

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CONFLICT OF INTEREST
Authors declare that there is no conflict of interest.