

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

Demisse Dakone<sup>1\*</sup>, Gizachew Zeleke<sup>2</sup>

<sup>1</sup>Central Ethiopia Environment and Forest Research Center (CEE-FRC), P.O. Box, 24536 (1000), Addis Ababa, Ethiopia

<sup>2</sup>Hawassa Environment and Forest Research Center (HE-FRC), P.O. Box, 1832 (1000), Hawassa, Ethiopia

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### 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* (Willd.) Link. (*L. aspera*), *Solanum incanum* L. (*S. incanum*), and *Hydnora johannis* A.B. (*H. johannis*) against *Escherichia coli* (*E. coli*), *Salmonella typhimurium* (*S. typhimurium*), *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*) 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 faecalis*) 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. johani* 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. johannis* as antibacterial agent for the treatment of infections caused by *E. coli*, *S. typhimurium*, *S. aureus* and *E. faecalis*.

**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 systems<sup>1</sup>. 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 antibiotics<sup>2</sup>.

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 observation<sup>3</sup>. 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 drugs<sup>4</sup>. Syed and Rajeev<sup>5</sup> 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 needs<sup>6</sup>. The organization estimates approximately over 21,000 plant species has been used for their medicinal purpose though out the world<sup>7</sup>. 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 need<sup>8</sup>.

Numerous studies shown that, medicinal plants are source of diverse nutrient and non-nutrient molecules<sup>9</sup>. It was mentioned that about 25% of modern medicines are developed from plants sources used traditionally<sup>10</sup>. 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, flavonoid, 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 them<sup>11</sup>.

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide<sup>12</sup>. However, antibiotics have always

\*Author for Correspondence: demissedakone2005@gmail.com

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

Standard antibiotics	Inhibition zone against tested bacteria (mm)				
	<i>E. coli</i>	<i>S. typhimurium</i>	<i>E. faecalis</i>	<i>S. aureus</i>	DMSO*
Ciprofloxacin	25.0±0.0	29.0±0.0	32.7±2.6	29.4±0.6	-
Penicillin	-	12.0±1.0	12.0±1.0	16.7±0.6	-
Ampicillin	-	-	12.4±0.6	9.0±0.6	-
Tetracycline	-	8.7±0.0	-	25.0±0.0	-
Gentamicin	21.0±1.0	25.0±1.0	22.0±1.0	27.0±0.0	-

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

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

Plants	Parts tested	Test organisms	Inhibition effect by extract (mm)					DMSO*
			Acetone	Ethanol	Aqueous	Chloroform	Ethyl acetate	
<i>S. incanum</i>	Root	<i>E. coli</i>	-	14.0±1.0	8.40±0.8	-	10.40±1.6	-
		<i>S. typhimurium</i>	-	13.70±0.4	11.33±1.6	-	16.0±2.0	-
		<i>S. aureus</i>	-	15.40±0.6	14.70±4.6	7.40±1.6	-	-
		<i>E. faecalis</i>	7.40±0.6	10.70±0.6	8.70±0.6	-	15.0±0	-

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

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

Plants	Parts tested	Test organisms	Inhibition effect by extract (mm)					DMSO*
			Acetone	Ethanol	Aqueous	Chloroform	Ethyl acetate	
<i>L. aspera</i>	Leaf	<i>E. coli</i>	-	11.0±1.0	-	-	11.0±1.0	-
		<i>S. typhimurium</i>	-	11.40±1.5	-	-	13.0±1.0	-
		<i>S. aureus</i>	-	16.40±1.1	-	-	12.40±1.3	-
		<i>E. faecalis</i>	-	14.0±1.10	-	-	10.70±1.4	-

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

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<sup>2</sup>. 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<sup>13</sup>.

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<sup>14</sup>. 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<sup>15</sup>.

Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use<sup>13</sup>. 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. faecalis* 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<sup>16</sup>. 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

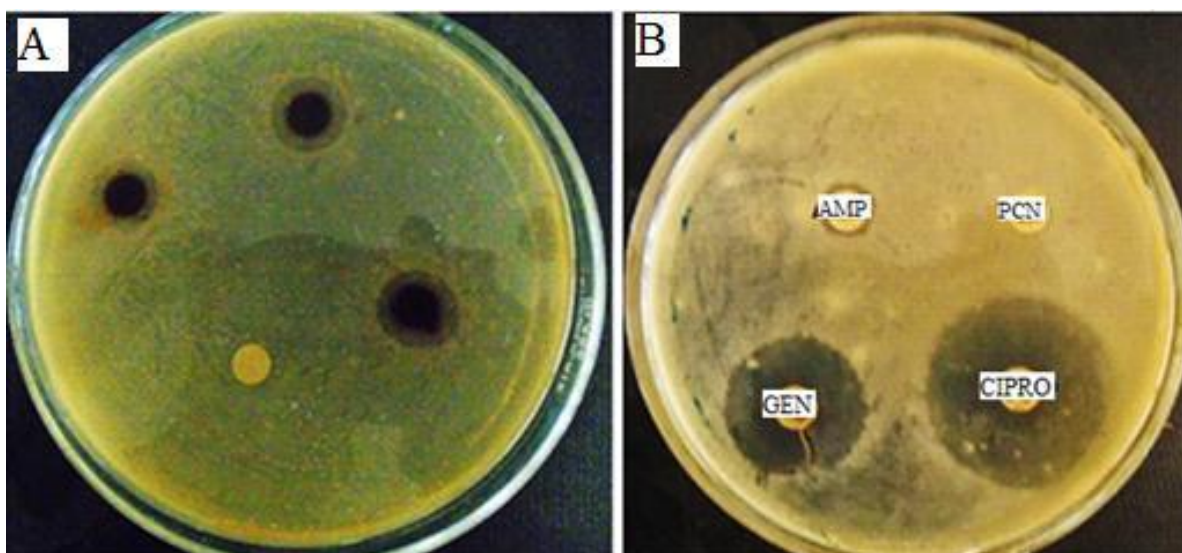


Figure 1: (A) Antibacterial activities of *L. aspera* leaf extract and (B) standard antibiotics on *E. coli*

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 Ijeh<sup>17</sup>. 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 ( $1 \times 10^8$  cfu/ml) in sterile saline solution. The solution was then diluted 1:20 to yield  $5 \times 10^5$  cfu/ml<sup>18</sup>.

#### 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 ( $5 \times 10^5$  cfu/ml) was spread over the 90 mm Petri dishes containing Muller Hinton agar using a sterile cotton swab. Fifty  $\mu$ l 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 ruler<sup>19</sup>.

#### 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 WHO<sup>20</sup> 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  $\leq$

Table 4: Zone of inhibition (mm) against different bacterial strains by plants extracts.

Plants	Parts tested	Test organisms	Inhibition effect by extract (mm)					
			Acetone	Ethanol	Aqueous	Chloroform	Ethyl acetate	DMSO*
<i>H. johanis</i>	Root	<i>E. coli</i>	16.70±0.4	13.70±0.4	13.0±1.0	-	15.0±0	-
		<i>S. typhimurium</i>	15.40±0.8	8.0±0	13.40±0.6	-	-	-
		<i>S. aureus</i>	-	7.70±0.60	-	-	-	-
		<i>E. faecalis</i>	10.70±0.6	-	-	-	-	-

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

12.0 mm: resistant; between 13.0-14.0 mm: intermediate and  $\geq 15.0$  mm: the bacteria is susceptible, for AMP  $\leq 11.0$  mm: resistant, between 12.0-13.0 mm: intermediate and  $\geq 14.0$  mm: the bacteria is susceptible, for CIPRO  $\leq 15.0$  mm: resistant, between 16-20.0 mm: intermediate and  $\geq 21.0$  mm: the bacteria is susceptible, for TCN  $\leq 14.0$  mm: resistant, between 15.0-18.0 mm: intermediate and  $\geq 19.0$  mm: the bacteria is susceptible and for PCN, if the diameter is  $\leq 14.0$  mm: resistant and  $\geq 19.0$  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* ( $8.4\pm 0.8$ - $14.0\pm 1.0$ ) than all standard antibiotics but least activity than GEN and CIPRO ( $21.0\pm 1.0$ - $25.0\pm 0.0$ ). 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

#### Conclusions

In conclusion, as demonstrated by results in this study, each solvent extract of the plants had showed significant antibacterial activities against to one or more bacterial strains. Up on comparison with five commercial standard antibiotics the antibacterial potentials of the extracts on tested bacteria were relatively found comparative or better than three antibiotics such as AMP, PCN and TCN. Hence, there is considerable evidence that their extracts, have the potential to be developed into agents that can be used as

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<sup>21</sup>.

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 drawn 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<sup>21</sup>.

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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