



In-Vitro ANTIBACTERIAL ACTIVITY OF *Plantago Lanceolata* AGAINST SOME SELECTED STANDARD PATHOGENIC BACTERIAL

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ABSTRACT

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The leaves of *Plantago lanceolata* have been used for centuries to treat diseases. Currently there is an increasing incidence of multiple antibiotic resistances in microorganisms which is a major threat. Widespread overuse of antibiotics is one led to increasing clinical resistance of previously sensitive microorganisms and the emergence of previously uncommon infections. Thus all these calls for an urgent need to search new, effective and safe anti-bacterial agents. The objective of this study was to determine antibacterial potentials of extracts of *P. lanceolata* against standard pathogens bacteria. Active antibacterial compounds were extracted using three solvents. The antibacterial sensitivity activities of crude extracts were determined using agar well diffusion assay. MIC and MBC of each crude extracts were also determined using broth dilution method. The test showed various degrees of antibacterial activity towards each standard pathogenic microorganism with mean zone of inhibition ranges up to 18 ± 2 mm against *S. typhi*. The plant extract was showed as low 3 ± 1 mm to high 18 ± 2 mm diameter inhibition zone. Chloroform extract have the least inhibition zone while the ethanol have highest inhibition zone. The extract value of MIC was showed at the ranges of 12.5mg to 50mg while the MBC were showed at the range of 25mg to 50mg. The result of this study showed that leaves of *P. lanceolata* have considerable antibacterial potential. The claimed efficacy could be attributable to antibacterial activity of its components but its mode of action is unclear.

Contribution/Originality: This study contributes in the existing literature by providing basic information for other researchers regarding the antibacterial efficacy potential of *P. lanceolata* leaves crude extract. Therefore, even though it needs further studies, leaves of *P. lanceolata* may be considered as a potential option to antibiotic regimens.

1. INTRODUCTION

Herbal medicine has contributed lot in the healthcare system of human since the birth of civilization. Medicinal plants have been and still remain the main sources of treatments in modern as well as in traditional system of medicine (Khalil *et al.*, 2007; Sengul *et al.*, 2009). Numerous chemical substances present in the plant possess important therapeutic properties in the treatment of human disease (Shittu and Akor, 2015). The main secondary metabolites present in plants are Phenolic compounds, which are essential components of human diet and well known in their antimicrobial activities (Klančnik *et al.*, 2010). The indiscriminate use of commercial antibacterial drugs commonly administered in the treatment of infectious diseases is one of the main reasons for the increasing incidence of multiple antibiotic resistances in microorganisms in recent years (Oyeyipo and Onasoga, 2015). Thus, the potential of herbal medicine in treating diseases has attracted many scientists in finding solution to the

problems of multiple resistances to the existing standard synthetic antibiotics (Shittu and Akor, 2015). *Plantago lanceolata* L, Ribwort plantain (English), is a perennial herb, often with several tufted shoots and flowering stems growing to 40 cm high from a rosette of leaves. Leaves are mostly erect but sometimes also flat on the ground. Flowers heads are cylindrical spikes with small green flowers and creamy white anthers and filaments. Habitat: On roadsides, weed in crop and abandoned fields, waste ground and pastures, 1200-3200 m altitudes. It grows in most of Ethiopia and Eritrea. Native in Europe and Asia now naturalized throughout the world. Plantain herb contains 2-6.5% mucilage composed of polysaccharides, 6.5% tannins, iridoid glycosides, including 0.3-2.5% aucubin and 0.3-1.1% catalpol with over 1% silicic acid. Also present are phenolic carboxylic acids (protocatechuic acid), flavonoids (apigenin, luteolin) and significant amounts of minerals, Zinc and Potassium.

Plantain is internally used for catarrhs of the respiratory tract and inflammatory alterations of the oral and pharyngeal mucosa, for sore throats, as a laxative, common cold, cough, and bronchitis, inflammation of the mouth and pharynx. It is also used externally for inflammatory reactions of the skin and tendency to infection and as a hemo-styptic. In Ethiopian traditional medicine, the leaves are used against trachoma. The whole plant is used for taeniasis, leaf powder (mixed with leaf powder from *Ajugaremot*a), for rheumatism, juice for wounds and inflammations. Honeybees visit the flowers for pollen only. More uses of plantain: for treating poison ivy, sun burn, laryngitis and hemorrhoids. Ethiopian Traditional Medicine: Common Medicinal Plants in Perspective). More uses of Plantain in Ethiopia: Leaf for Taeniasis, trachoma, rheumatism (Natural Database for Africa, 2011).

2. MATERIALS AND METHODS

2.1. Plant Collection and Identification

The leaves of *P. lanceolata* were collected in January, 2017 from garden of University of Gondar, Ethiopia. The plant leaves were identified by botanist Mr. Abiyu Eniyew at Botanical sciences laboratory and herbarium of Department of Biology in the University and the voucher specimen PLS No. 0109/17 was deposited. Then, the fresh and healthy sample collected in a polythene bag was transported and kept in the dark areas at room temperature in Microbiology laboratory until it dries and processed.

2.2. Chemicals and Reagents

All solvents; ethanol, hydro-alcohol and chloroform, used for extraction in this study were obtained from Department of Biology laboratory equipment, chemicals and reagent store, University of Gondar.

2.3. Test bacterial strains

Standard pathogenic strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Streptococcus pyogenes* were obtained from University of Gondar teaching Hospital and maintained on nutrient Agar medium for further experiments.

2.4. Antibacterial agents

Commercial antibiotic, Gentamicin, was used as positive control and to compare with antibiotic activities of leaves of *P. lanceolata* at different dose.

2.5. Preparation of Plant extract

Plant leave materials was washed with running tap water and distil water respectively without squeezing, air dried in shade area at room temperature in Microbiology laboratory and powdered with powdering machine. The 100 gram powdered plant material was soaked separately in 1000 ml (i.e. 1:10 ratio) of each solvent of ethanol, hydro-alcohol and chloroform and stayed on shaker for 72hrs until complete extraction of the bioactive material achieved. At the end of 72hrs, each extract was filtered through Whatman No.1 filter paper and the extraction

solvent was evaporated and the extract was concentrate at room temperature. The dry extracts were stored in pre-weighed screw cup bottles and the yields of extracts were kept in refrigerator at 4°C until use. The concentration (Dose) needed for testing was prepared by homogeneously mixing 6.25mg, 12.5mg, 25mg and 50mg of extract with 100microlitre of distilled water separately.

2.6. Preparation of Media

Muller-Hinton agar media and agar disc diffusion method was used for antibacterial susceptibility test. It was prepared according to manufacturer instruction by mixing with distilled water. Then it was covered by aluminum foil, Mixed by magnetic stirrer and heated simultaneously to melt the agar. After melting it was autoclaved in 15 psi pressure at 121°C temperature for 15 minute. Next to autoclaving the medium was allowed to cool up to 50°C temperature inside laminar air flow hood. A100 ml diameter Petri plates were washed, dried, autoclaved and then dried inside laminar air flow hood. Final 20 ml slightly cool medium was poured in to Petri-plate.

2.7. Preparation of 0.5 McFarland Standards

McFarland 0.5 turbidity standard was prepared by adding 0.5 ml of a 1.175% (w/v) barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) solution to 99.5 ml of 1% (v/v) sulfuric acid.

2.8. Susceptibility Test

Antibacterial activity of plant crude extract was evaluated by Disc Diffusion Susceptibility method using the methodology of Aida *et al.* (2001). Nutrient broth with bacterial culture was inoculated and incubated at 37°C for 24hrs before the test. Bacterial suspensions were prepare by taking 3-4 same well isolate colonies from Muller-Hinton agar and transferred in to 4 ml Sterile Normal Saline Solution. The suspensions turbidity was made until comparable to 0.5 McFarland standards. From these suspensions a volume of 100 µl was add on the surface of previously solidified MHA. The suspension was streaked on MHA surface and uniformly distributed under aseptic condition. This was achieved by rotating the Petri-plate to have equal distribution of cultures over MHA surface.

On each plate, equidistance wells were made with a 6 mm diameter sterilize borer 20 mm from the edge of the plate. Hundred micro liter of each plant extracts with 50mg, 25mg 12.5mg and 6.25mg was aseptically introduced as test dose and water as negative control and Gentamicin as positive control was introduced in to separate well then all plates was incubated at 37°C for 24 hr. The tests were performed in triplicate. After 24 hrs, the inhibition zone of all plates were measured and compared to one another with statistical software.

2.9. Determination of Minimum Inhibitory Concentrations (MICs)

Minimum inhibitory concentration is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2001). The MICs of plant extracts was determined using Muller-Hinton broth. In this test, 100 micro-liter of distilled water was mixed with 50mg, 25mg, 12.5mg and 6.25mg extract solution. Broth containing test tubes was tightly closed, arranged in test tube rank and autoclaved under 15 psi pressures at 121°C for 15 min. The broths were allowed to cool until the temperature is equitable to room temperature. Each of the different concentration extracts and 100 micro liters of the test bacteria were aseptically introduced. The inhibition of growth was observed after 24hr incubation at 37°C. The presence of growth was evaluated by comparing with the negative control, positive control and culture containing test tubes.

2.10. Determination of the Minimum Bactericidal Concentrations (MBCs)

The MBC is the lowest concentration of antimicrobial agent that will prevent the growth of an organism after subculture on to antibiotic-free media (Sonibare *et al.*, 2016). Broth containing test tubes that didn't show any

bacterial growth at MIC was used to determine MBC. Small volumes of these broths are streaked on to the surface of MHA medium by sterile wire loop. The medium was incubated at 37°C for 24 hr. The least concentration of plant extracts that effectively inhibit bacterial growth on the agar plate was recorded as MBC of the extracts.

2.11. Data Decoding and Analysis

The data collection instrument was experimental through basic laboratory technique. Data's like susceptibility was analyzed using SPSS software package version 20.00. Microsoft Excel was employed for analysis of MIC and MBC.

3. RESULT

3.1. Antibacterial Susceptibility to Plant Extracts

The antibacterial effects of crude extract of *P. lanceolata* showed effective bacterial growth inhibition against all tested bacteria (Table 1). The chloroform extract has shown the least inhibition zones against both *E. coli* and *S. pyogenes* isolate (3±1 mm with 6.25mg) whereas the highest inhibition zone (9±1mm with 50mg) was recorded against *P. aeruginosa*. Ethanol extract has shown the highest inhibition zone against isolates of *S. typhi* (18±2mm with 50mg) and the least growth inhibition zone (5±1mm with 6.25mg) against *S. aureus* strain. Similarly, the maximum inhibition zone for hydro-alcohol extract was seen against *S. pyogenes* (14.6±6mm with 50mg) and the minimum was against *S. aureus* (3.6±6mm with 6.25mg). *S. typhi* has revealed higher inhibition susceptibility of (18±2.00 mm with 50mg). There was no any inhibition zone found for all tested bacteria with negative control.

Table-1. sensitivity test mean growth inhibition zones of *P. lanceolata* against standard Bacteria

Extract	Dose (per100µl)	Gram Positive		Gram Negative			
		<i>S. pyogenes</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>	<i>E. coli</i>
Chloroform	6.25mg	3±1	4.3±0.7	4.6±0.5	3.3±0.6	4±1	3±1
	12.5mg	3.7±1	4.6±0.5	4.9±1	4±2	4.4±1	3.6±1
	25mg	5±1	6±1	7±1	6±1	6±1	5.6±1
	50mg	7±1	8±1	9±1	7±1	8.3±1	7.6±1.5
Ethanol	6.25mg	5.3±1	5±1	7.6±1	7.3±2	8.3±1	8.6±2
	12.5mg	8.6±1	7.6±2	9±2	10.6±1	12±1	9.2±1
	25mg	15±1	12±1	14±2	15±1	16.3±0.6	12±1
	50mg	17.3±1	14±2	16±2	18±2	17.3±0.5	15±1
Hydro-Alcohol	6.25mg	5.6±1	3.6±0.5	5±1	4.3±1	4±1	8.3±1
	12.5mg	6.6±1	8.6±2	6±1	7±0.5	6.6±2	9.4±2
	25mg	8±1	9.3±1	7.6±1	9±1	8±1	10.6±0.5
	50mg	14.6±1.5	11.3±1	11.6±1	11.3±1	9.3±0.6	12±1
Positive	P1-P6	13±2	14.2±2	15.3±1.5	15.3±2	16.5±1.5	14.2±3
Negative	N1-N6	0	0	0	0	0	0

Source: In-vitro lab experiment data

3.2. Determination of MICs

The MICs of different concentrations of hydro-alcohol, ethanol and chloroform extract were assessed as shown in table below (table 2). The MIC values of plant extracts against tested bacteria have shown a range of 12.5mg - 50mg. Almost half of the tested bacteria showed MIC value at 50mg. Three of tested bacteria (*S. aureus*, *S. pyogenes* and *S. dysenteriae*) have shown similar MIC value of 50mg. The lowest MIC was with Ethanol against *S. typhi* (12.5mg).

Table-2. The MIC values the three extracts.

Extract	<i>S. pyogenes</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>S. dysenteriae</i>	<i>E. coli</i>
Chloroform	50 mg	50 mg	25 mg	25 mg	50 mg	25 mg
Ethanol	25 mg	25 mg	12.5 mg	25 mg	50 mg	25 mg
Hydro-alcohol	50 mg	25 mg	50 mg	50 mg	50 mg	25 mg

Source: In-vitro lab experiment data

3.3. Determination of MBCs

As that of MIC the extracts were showed a range of MBC values 25 mg to 50mg (**table 3**). Chloroform extract showed the MBC activity at 25mg against *S. pyogenes*, *E. coli*, *S. typhi* and *P. aeruginosa* and at 50 mg against *S. dysenteriae* and *S. aureus*. Regarding hydro alcohol extract, the MBC activities were with 50mg against all tested organisms except *S. aureus* which is 25mg.

Table-3. The MBC values of the three extracts against tested organism

Extract	<i>S. pyogenes</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>S. dysenteriae</i>	<i>E. coli</i>
Chloroform	25 mg	50 mg	25 mg	25 mg	50 mg	25 mg
Ethanol	25 mg	25 mg	25 mg	50 mg	50 mg	25 mg
Hydro alcohol	50 mg	25 mg	50 mg	50 mg	50 mg	50 g

Source: In-vitro lab experiment data

4. DISCUSSION

Ethno-botanical investigations have been found to offer important clues in the identification and development of traditionally used medicinal plants in to modern drugs (Kuetee *et al.*, 2008; Kuetee, 2010). Plants have continued to play a dominant role as a source of medicinal compounds in the maintenance of human health since ancient times (Tchana *et al.*, 2014). To the best of our knowledge, the *in vitro* antibacterial activity of *P. lanceolata* is being reported for the first time. This study has shown that the leaves of *P. lanceolata* have considerable potential in the fight against microbial infections. The entire tested organisms were sensitive to the extract. As it has been confirmed with several studies, the secondary metabolites derived from plants such as alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes have substantial antibacterial activities (Kuetee *et al.*, 2008; Kuetee, 2010) so the tested extracts in this experiment may contain the abovementioned secondary metabolites.

As a result, in this study chloroform extract has shown the lowest inhibition zone against *E. coli* and *S. pyogenes* organism and the highest inhibition zone was seen in *P. aeruginosa*. The observed difference in antibacterial activities of extracts between the aforementioned pathogenic bacteria may be attributed to the difference in the outer membrane of isolates and may be due to the concentration difference of the secondary metabolite in the extract (Olila and Opuda-Asibo, 2001). Gram-negative bacteria possesses high permeability barrier for numerous antibiotic molecules (Marthe *et al.*, 2014) similarly for these extracts, thus the tested extracts has shown lowest inhibition zone in almost all the Gram-negative bacteria during this experiment. Their periplasmic space also contains enzymes, which are capable of breaking down foreign molecules (Duffy and Power, 2001) and appears to be less susceptible to plant extracts than gram-positive bacteria. Furthermore, the weak antibacterial activities of this studied plants could be due to the resistance features of the studied bacterial strains against this plant extracts (Marthe *et al.*, 2014). In comparison among ethanol, chloroform and hydro alcohol, ethanol showed slightly higher inhibition zone. A similar result in other study, even though the plant is different, indicated that ethanol was found to be the most effective solvent that highly reduced the radial growth of the tested pathogens than other solvents (Abera *et al.*, 2011). Furthermore, the inhibition zone difference between tested Gram-negative and positive may be due to the different natures of the strain. Gram-negative bacteria cell envelope (made up of lipopolysaccharide), which restricts access to the membrane more than in Gram-positive bacteria (Saroat *et al.*, 2012). However, their effects on at least one bacterial species could justify their use in traditional medicine in the treatment of microbial infections (Kuetee, 2010). One of the well accepted and used criteria for measuring the susceptibility of microorganisms to inhibitors is MIC. However, temperature, inoculum size, and type of microorganisms are among the factors those affect the obtained MIC value (Jean-Bosco *et al.*, 2016). The extracts are considered to possess significant activity when they have MIC below 100 µg/mL (Kuetee, 2010). The highest MIC and MBC values is an indication that either the plant extracts are less effective on some bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC and MBC values for other bacteria is an indication of the

efficacy of the plant extract (Oyeyipo and Onasoga, 2015). The MIC and MBC result showed in this experiment may suggest the bactericidal effects of the plant extract (Jean-Bosco *et al.*, 2016). According to several studies on the MIC and MBC, the Gram-negative bacteria were more resistant than the Gram-positive (Saroat *et al.*, 2012). The MIC value of all tested extract in this experiment ranged from 12.5mg to 50mg. The observed difference may be due to the difference of the nature of the solvent and due to genetic differences between the bacteria (Jean-Bosco *et al.*, 2016). In the present study, MBC values were determined by sub culturing the samples having dilution values that didn't show any bacterial growth which was greater or equal extract concentration with MIC values as described in other researches (Mitiku *et al.*, 2014). As that of MIC the extracts were showed a range of MBC values 25mg to 50mg. These consequences suggest that *P. lanceolata* leaves used contain bioactive components. The activity of the plant against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant (Vaghasiya and Chanda, 2007). Nowadays, most pathogenic organisms are becoming resistant to antibiotics (Chandarana *et al.*, 2005). To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Medicinal plant leaves have been reported to be a good source of natural antioxidants and antimicrobial (Dillard and German, 2000). Thus, *P. lanceolata* could become promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

5. CONCLUSION

The overall results obtained from the present study has confirmed the therapeutic potentials of *P. lanceolata* leaf extracts, which are currently used by traditional healers for treatment of various diseases causing pathogen and affirm the traditional usage of these plants as an alternative medicine in the local community. This study also indicated that ethanol extract of this plant has highest capability to antibacterial activity followed by hydro-alcohol and chloroform extracts. Generally, outcomes of this research have shown the tested plant extracts contain compounds that have antibacterial activities and it is important to propose the plants in the control of some selected pathogens. Furthermore, the results can also provide baseline information for further studies.

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