Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.18, 2014

Invitro Antimicrobial Activity of *Plumbago Zylanica L.*

Yohannes Weldemariam Getahun (Corresponding author)
Department of Chemistry, Adigrat University, P.O. Box 50, Adigrat, Ethiopia
E-mail: jonygsfusw@gmail.com

Afework Mulugeta,
Department of Public Health, Mekelle University, P.O. Box 231, Mekelle, Ethiopia
E-mail: afework.mulugeta@gmail.com

Gebremedhin Gebremariam
Department of Chemistry, Mekelle University, P.O. Box 231, Mekelle, Ethiopia
E-mail: ggchem74@gmail.com

Abstract
Beside the search for new plants; Plants investigated to have medicinal value for particular diseases has been also checked for extra pathogens and further in other biological activities in a search for efficient and effective drug. In this study the in vitro antimicrobial capacity of *Plumbago zeylanica L.* root extracts were investigated against seven bacterial strains and one fungal strain. Antimicrobial activity was done employing Agar disc diffusion and Minimum inhibitory concentration (MIC) was determined involving broth dilution method. The chloroform crude extracts as well as fractions were more potent than ethanolic extracts. From MIC findings *S. entritidis, S. aureus* , *E.coli* were withdrawn from growth at 0.2mg/mL, 1mg/ml and 2mg/ml respectively. In addition, nine pure compounds were isolated and one (**PFr_8**) inhibited a bit higher than the standard drugs.
Keywords: *Plumbago zeylanica L*, antimicrobial activity, minimum inhibitory concentration (MIC), fractionation, invitro test

1. Introduction

Extensively more than 85% of deaths attribute to acute respiratory infections, diarrhoeal diseases, measles, AIDS, malaria, and tuberculosis.[1] Of these deaths; about one-half are due to infectious diseases. [2, 3]

A study by WHO on conventional antimicrobial drugs such as benzyl pencilin, gentamicin, metronidazole, ampicilin, phenoxy methyl pencilin, and ciprofloxacin revealed that those drugs are no longer very effective due to continually emerging resistance by the pathogens. As people use antimicrobials for treatment of common diseases such as acute respiratory infections antibiotic, resistance is becoming a growing public health concern. [1]

The species *plumbago zeylanica* is very known traditional medicinal plant.[4] Traditionally in India, it has been used against fever and malaria and tumor, [5] against diarrhoea, dyspepsia, piles, and skin diseases including leprotic lesions. In Nepal as an antiviral medicine, in Taiwanese folk medicine for anti-Helicobacter activity, in Assam for family planning and birth control and permanent sterilization.[6, 7, 8, 9]In DR Congo and Gabon, the pounded root is applied to treat itch. In Zambia a root decoction with boiled milk is swallowed to treat inflammation in the mouth, throat, and chest. In southern Africa, a paste of the root in vinegar, milk and water is used to treat influenza and black water fever. *Plumbago zeylanica L.*root cooked with meat in soup is consumed in Zimbabwe as an aphrodisiac, and it also helps digestion. A root infusion is taken orally to treat shortness of breath. In Madagascar the roots are applied as a vesicant, while in Mauritius and Rodrigues a root decoction is used to treat diarrhoea and dyspepsia.[8, 9, 10, 11]

Studies on crude extracts of *plumbago* species have shown antibacterial, antifungal activities against selected organisms infectious to human as well as anthelmintic, antimalarial, analegesic, anti-inflamatory and antioxidant activities. [10, 11, 12, 13, 14, 15, 16]

The plant is also proved to be rich in active metabolites such as flavonoids, saponins, naphthoquinone, linoleic acid, terpenoids and palmitic acid. [7, 15, 16, 17, 18]

Plumbago zeylanica L. known by its local name Aftuh (in Tigrigna) and Amera (in Amharic) is a shrub widely distributed in the West and Northwest parts of Ethiopia at 1500–2200m above sea level. Traditionally local healers in Ethiopia use powdered bark, root or leaves to treat gonorrhea, syphilis, tuberculosis, rheumatic pain and swellings wound.[19, 20]

Research works which screen plants for chemoterapautic significance in their crude extracts are much more times appear in the literature than those done in a fractionation system. The former ones are simple and cheaper. Thus, less worthed than the later ones. *Plumbago zeylanica L* is widely found in Asia particularly in China and India with medical value as antimutagenic,[6] antihelicobacter pylori,[7,] anti inflammatory,[8, 17, 21] analegesic activity, [22] nephroprotective activity,[23]for cytotoxic effects,[8] larvicidal ,[8, 24] as inhibitor of growth,

invasion of homene – refractory prostate cancer.[9, 25, 26] Plumbago zeylania shows higher antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* compared to standard Streptomycin[12] and significantly higher antioxidant activity compared to the standard Quercetin. It also show antimicrobial activity against bacterial species such as *Klebsiella pneumonia*, *Serratia marcescens*, *Bacillus Subtilis*[27], *Proteus vulgaris*, *Pseudomonas aeruginosa*[12, 13] *Micrococcus luteus*,[27] *Vibrio cholera* and fungal species such as, *Curvularia lunata*, *Colletotrichum corchori, Candida albicans* and *Fusarium equisetai*.[13] The It has been serving as potent plant for '*Mich*' mean allergy in the target area Tigiray, northern Ethiopia. However, the *Plumbago zeylanica L.* for infectious dieases is less known. The aim of this study is to foster the multidiamentional use of the plant by integrating its antimicrobial efficacy against pathogenic microorganisms. Besides pure compounds are isolated which could be new to the science, geographical variation is also a matter that can lead to different findings to those done outside Ethiopia.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and solvents

Petrolium ether, sodium sulphate anhydrous (Na_2SO4), sodium chloride, barium sulphate($BaSO_4$) bariumchloride($BaCl_2$) concentrated sulphuric acid, ammonia solution, muller hinton agar, nutrient agar, potato dextrose agar, nutrient broth, cyclohexane, diethyl ether, chloroform, dichloromethane, carbontetrachloride, ethyl acetate, acetone, methanol and ethanol.

2.1.2. Instruments and equipments

Electrical shaker, soxhlet extractor set up, Ultra violet – visible light source rotary evaporator (laborata 4000, Heithbad bath, 230, 50/60 Hz), separatory funnel, vacuum pump, oven, incubator, autoclave and fridge.

2.1.3. Test organisms

Bacteria: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Corno bacterium pyogenous*, *Salmonella typhimerum*, *Salmonella entritidis*, *Streptococci bovines*.

Fungi: *Aspergillus niger*

2.2. Methods

2.2.1. Plant Material collection

Fresh *Plumbago zeylanica L.* roots were collected from Shire Endasslassie - in the month – June - August/ 2013. Plant sample was then send to Addis Ababa University for botanical identification and then Voucher specimen was deposited at the National Herbarium of Addis Ababa University with voucher specimen number B (003).

2.2.2. Extraction

Before extraction root of *Plumbago zeylanica L.* was shade-dried at room temperature for ten days; crushed in to powder using mortar and pestle and then stored in a bottle prior to use.

The dried and powdered root material (156g) was extracted in 800ml chloroform for 36hrs at once using Soxhlet extraction. This was done thirteen times to obtain enough samples. Root powder of *Plumbago zeylanica* (238g) was also extracted by maceration in 1.5 liters of ethanol for three day on an electrical shaker (shake speed 220 at room temperature). Both the extracts were filtered using What man No1 filter paper and the filtrate was concentrated by rotary evaporator at room temperature and further with vacuum pump in Desiccator. [28, 29]

2.2.3. Antimicrobial activity

2.2.3.1. Microbial test cultures and growth conditions

Bacterial strains were maintained on Nutrient Agar (NA) petri dishes at 4 ^oC, while the fungus were rehersed on Potato Dextrose Agar (PDA) petri dishes. The fresh cultures were obtained by growing the test strains overnight at 37 ^oC for bacteria while fungi were grown at 28 ^oC for 48 hours. Individual colonies was then picked up and streaked on to another plate for further use.

Antifungal and antibacterial in-vitro assays were done using disc diffusion. All the procedures were done according to Clinical Laboratory Standard Institute Standards Procedures and Quality Control as described by (Lalitha, 2006) and (Atlas, 1997).[30]

2.2.3.2. Media preparation

Mueller Hinton Agar (MHA) (OXOID, UK) was used for bacteria bioassay while PDA(OXOID, UK) was used for fungi. MHA was prepared by dissolving 38g in 1000ml of distilled water and brought to boil to completely dissolve. Sterilization was achieved by autoclaving at 121 ^oC for 15 minutes. PDA was prepared by dissolving 65g in 1 liter of distilled sterilized water, brought to boil to ensure complete dissolution. The media was autoclaved at 121^oC for 15 minutes to ensure sterilization. The medias were dispensed (20ml) onto the pre-sterilized petri dishes yielding uniform depths. They were then covered and allowed to cool and solidify at room temperature. Filter paper discs (6mm diameter) were prepared and sterilized by autoclaving at 121 ^oC for 15 minutes.[31, 32]

2.2.3.3. Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard was used. Two solutions were prepared in separate flasks. Solution A was made up by

diluting 1.75g of $BaCl_2.H_2O$ to 100ml with distilled water to obtain 0.048M $BaCl_2$. In solution B (0.36 N H_2SO_4) 10ml of H_2SO_4 (Analar grade, sp. gr. 1.84) was made up to 100ml with distilled water. A 0.5ml aliquot of 0.048M $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.36N H_2SO_4 (1% v/v) with constant stirring to maintain a suspension. The Barium Sulfate suspension was then transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing and diluting the bacterial inoculums. The tubes were tightly sealed and stored in the dark at room temperature. The barium sulfate turbidity standard was vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance.[30, 31]

2.2.3.4. Preparation of crude extracts and isolated fractions for bioactivity test

Exactly 0.2g of crude chloroform extract was dissolved in 2ml chloroform to get 100mg/ml concentration. This was then serially diluted to obtain 50mg/ml, 25mg/ml, 10 mg/ml and 5mg/ml concentrations. This procedure was repeated for ethanol extracts using ethanol as a solvent for dilution. Similar dilution procedure was applied for the fractions corresponding to their yield. All of the fractions were prepared in two to three fold dilutions.

It should be clear that for those samples in which organic solvents were used for dilution, when antimicrobial test was done filter paper disks after impregnated in the stock solution was left to dry in flat glass (to let the solvent evaporated) and then it was sprayed with sterile distilled water.

2.2.3.5. Antimicrobial activity assay

Agar disc diffusion method for anti bacterial and antifungal screening and broth dilution method for determination of minimum inhibitory concentration was applied as described else were with little modification.[30]

2.2.3.5.1. Agar disc diffusion method

Disc diffusion method was employed in the preliminary antimicrobial screening of both the crude organic extracts and bioassay guided isolated compounds. Test strains suspension of 0.5 McFarland was prepared from fresh cultures using normal saline. The plates were aseptically streaked with the test microorganism using a sterile swab and allowed to dry for few minutes. Sterile 6 mm diameter filter paper discs were impregnated with stock solutions (at concentrations 5, 10, 25, 50, to 100mg/ml). Using sterile forceps the discs were placed aseptically on the inoculated agar plates. The plates were then incubated for 24 hours at 37 ^oC for bacteria and at 28 ^oC for fungi. The experiments were carried out in triplicates. Presence of a clear circular zone around the sample impregnated disc was used as an indicator of activity. The results (mean values, n = 3) were recorded by measuring zones diameter in millimeters with the help of calipers. Disc impregnated with the solvent used (chloroform, ethanol and mixture of ethyl acetate and n-hexane) was included as negative controls. For comparative purposes standard drugs gentamycin (30µg/disc) and amoxacilin (30µg/disc) for antibacterial and clotrymazole (40µg/disc) for antifungal were included as positive controls in the assays respectively. [31, 32]

2.2.3.5.2. Minimum inhibitory concentration (MIC)

The extracts, which showed fsuperior antibacterial activity in the agar disc diffusion method, were subjected to the MIC assay. The minimum inhibitory concentration (MIC) of the extracts was determined for each of the test organisms in triplicates. To a 0.5 ml of varying concentrations of the extracts (20.0, 15.0, 10.0, 5.0 and 1.0mg/ml), 2ml of nutrient broth was added (so the extracts were dilute by a factor of 5). Therefore the final concentrations were 4, 3, 2, 1, 0.2, and 0 mg/ml as a control), and then a loopful of the test organism previously adjusted to 0.5 McFarland turbidity standard[32, 33, 34] for bacteria was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotic (Streptomycin 500µg/mL). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37°C for 24h period. After incubation the tubes were then examined for bacterial growth by observing the turbidity present in the tubes. [33, 34]

2.2.4. Statistical analysis

All experiments were carried out in triplicate with their mean values and standard deviations by formula.

2.2.5. Fractionation

2.2.5.1. Thin layer chromatography

Samples were chromatographed on silicagel G (Precoated TLC plates (20 x 20 cm, 0.2 mm), Silicagel, 60 F_{254}, MERK, Germany) using a series of solvents. Solvents in pure and in combination with other solvents were run in five development chambers. R_f values were calculated for each spot.

2.2.5.2. Column chromatography

Approximatly1.5g of *Plumbago zeylanica L* root extracts was applied to a silica gel chromatography column (63-200µm, 102 x 525 mm, 70-230 mesh size). Elution of the column was then performed using mixtures of hexane/EtOAc in a series of three linear gradient steps. Step 1 consists of 100/0 to 90/10 using 1600mL and step 2 consisting of 90/10 to 75/25 using 400 mL. While, step 3 consist of 75/25 to 0/100 with total volume of 400 mL. Column eluate was collected in 10-mL test tubes. Fractions were recombined based on TLC Rf values. [35] Only chloroform was also used as an eluent for column chromatography.

3. Results and Discussion

3.1. Yield of plumbago zeylanica root powder

Maceration with ethanol and Soxhlet extaction of the root with chloroform gave 3.31% w/w and 0.82% w/w. Percentage yield and the colour of the extracts was in agreement with colour description and extractive value given. [35]Though, the yields were within the range, it was very small.

3.2. Antimicrobial activity

3.2.1. Chloroform crude extract

Table 1. Antimicrobial zone of inhibition (mm) of crude chloroform extracts of *Plumbago zeylanica L.*

Micro organisms	Concentrations(mg/ml)					Gent	Amox	Clotr
	100	50	25	10	5	30µg/disk	30µg/disk	40µg/disk
Bacterial strains								
S. aureus	20.67±0.26	17.33±0.47	11.66±0.13	10±0.08	8±0.55	22.23±0.76	19.59±0.59	--------
P. aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--------
E.coli	18.43±0.24	14.96±0.41	10.83±0.28	9.60±0.43	7.00±0.04	22.67±0.57	18±0.34	--------
C. pyogenousa	16.01±0.11	12.68±0.18	9.40±0.14	9.35±0.24	8.33±0.30	20.06 ±0.36	19.86±0.70	--------
S. typhimerum	17.04±0.14	11.33±0.11	6.67±0.28	7.67±0.14	6.67±0.17	20.34±0.65	19±0.87	--------
S. entritidis	21.49±0.26	19.04±0.15	12.7±0.09	9.83±0.25	7.33±0.16	20.01±0.95	21±0.87	--------
S. bovines	7.33±0.22	6.51±0.36	6.35±0.31	6.02±.01	0.00	14.43±0.24	18.00±0.42	--------
Fungal strain								
A. niger	19.50±0.21	16.00±0.42	12.00±0.15	8.02±0.04	8.00±0.06	--------	-------	21.47±0.76

- *S. aureus = Staphylococcus aureus, P. arenoginosa = Pseudomonas aeruginosa, E. coli = Escherichia coli, C. pyogenous = Corno bacterium pyogenous, S. typhimerum = Salmonella typhimerum, S. entritidis = Salmonella entritidis, S. bovines = Streptococci bovines, A. niger = Aspergilus niger*, Gent = Gentamycin (+ve control), Amox = Amoxycilin (+ve control), Clotr = Clotrymazole (+ve control).
- Results were reported as mean ± Standard deviation of three replicate experiments.

Chloroform extracts of *Plumbago zeylanica L* were tested against seven pathogenic bacterias and one pathogenic fungus at five different concentrations. The highest inhibition was recorded in *Salmonella entritidis* (21.49mm) followed by *Staphylococcus aureus* (20.67mm), *Escherichia coli* (18.43mm), *Salmonella typhimerum* (17.04mm), *Corno bacterium pyogenous* (16.01mm), *Streptococci bovines* (4.33mm) at 100mg/ml (Table 1). The species *Pseudomonas aeruginosa* was completely resistant at all concentrations even to the standard drugs. Similar results were recorded in descending concentration with insignificant diversion. However, *Streptococci bovines* was not affected at 5mg/ml. Concerning antifungal activity; *Aspergilus niger* was inhibited at all concentrations with highest activity recorded to be (19.5mm) at100mg/ml.

Some of these findings were in agreement with similar study by Jeyachandran, R. etal.[12] In the study chloroform extracts of *Plumbago zeylanica L.* showed 16.7±0.14mm zone of inhibition against *Escherichia coli*, 14.3±0.04mm against *Salmonella typhi*. There were also variations in some data. Unlike the present findings *P. arenoginosa* had some what low sensitivity and *S. aureus* had lower zone of inhibition.

3.2.1.1. Minimum Inhibitory Concentration (MIC)

Table 2. Minimum inhibitory concentration of *Plumbago zeylanica L.* chloroform extract

Microorganisms	MICs of extract (mg/mL)
S. entritidis	0.2
S. aureus	1
E.coli	2

- Results were reported as mean ± standard deviation of three replicate experiments

To their greater activity they show than the ethanolic extracts MIC was done for chloroform extracts only. Broth dilution method was used for this purpose and the microorganisms which were most susceptible to chloroform extracts were selected. About 0.2mg/mL was the point at which *S. entritidis* was withdrawn from growth. *S. aureus* was blocked from growth at 1mg/ml and *E.coli* was cleared in the test tube with 2mg/ml (Table 2).

3.2.2. Ethanolic crude extract

Table 3. Antimicrobial zone of inhibition of crude Ethanol extracts of *Plumbago zeylanica L.*

Micro organisms	Concentrations(mg/ml)					Gent	Amox	Clotr
	100	50	25	10	5	30µg/disk	30µg/disk	40µg/disk
Bacterial strains								
S. aureus	12.00±0.11	10.00±0.0	9.10±0.16	8.36±0.21	7.00±0.03	22.23±0.76	19.59±0.57	--------
E.coli	13.52±0.03	10.31±0.4	8.91±0.21	8.56±0.10	7.60±0.01	22.67 ±0.65	18.00±0.34	--------
S. entritidis	8.3±0.05	7.0±0.04	6.8±0.03	6.6±0.02	6.40±0.01	20.01±0.95	21.00±0.87	--------
Fungal strains								
A. niger	19.3±0.24	15.2±0.14	15.00±0.25	10.00±0.42	8.40±0.22	--------	--------	21.47±0.76

- *S. aureus* = *Staphylococcus aureus*, *E.coli* = *Escherichia coli*, *S. entritidis* = *Salmonella entritidis*, *A. niger* = *Aspergilus niger*, Gent = Gentamycin (+ve control), Amox = Amoxycilin (+ve control), Clotr = Clotrymazole (+ve control).
- Results were reported as mean ± standard deviation of three replicate experiments.

The microorganisms which were sensitive to chloroform extracts were selected purposely in antimicrobial activity test of ethanolic extracts. Unlike in the chloroform extracts, *E.coli* recorded the highest inhibition (13.52 mm) followed by *S. aureus* (12mm) and least inhibition were observed for *S. entritidis* (Table 3). The sensitivity of *A. niger* was comparable with that of chloroform extracts and the standard with the highest inhibition of 19.3mm at 100, 15.2mm at 50, 15mm at 25, 10 at 10 and 8.4 at 5mg/ml. But compared to the chloroform extracts the ethanolic extracts show lesser activity in the bacterial organisms. This particular finding compromise to those reported in sensitivity of *E.coli* and *S. aureus* to the plant extracts but less in zone of inhibition at corresponding concentrations. Similarly, unlike to that report *E.coli* more sensitive than *S. aureus* as recorded here. [27, 36]

3.2.3. Antimicrobial activity of the fractions

3.2.3.1. Chloroform crude extract fractions

Table 4. Antimicrobial zone of inhibition of n hexane – ethyl acetate (F_1-F_7) and chloroform (F_8) fractions of chloroform crude extracts of *Plumbago zeylanica L.*

Treatment groups	Concentrations(mg/ml)	Microorganisms		
		S. aureus	E.coli	S. entritidis
PF_1	100	20.27±0.52	12.06±0.92	16.54±0.31
	50	6.5±0.34	7.10±0.34	8.0±0.22
	25	0.0026	0.00±0.66	7.00±0.12
MF_2	100	18.26±0.65	26.20±0.57	20.24±0.32
	50	16.08±0.37	20.34±0.89	14.02±0.15
	25	6.53±0.46	10.29±0.46	14.92±0.24
PF_3	100	0.00	16.51±0.57	11.37±0.26
	50	0.00	16.43±0.29	11.85±0.23
	25	0.00	14.00±0.19	10.67±0.06
MF_4	100	0.00	20.04±0.26	11.50±0.06
	50	0.00	18.85±0.24	8.00±0.24
	25	0.00	14.5±0.21	7.05±0.64
PF_5	100	20.19±0.65	11.12±0.14	18.06±0.21
	50	17.22±0.48	11.25±0.16	7.36±0.35
	25	9.18±0. 24	0.00	6.69±0.26
PF_6	100	16.41±0.16	12.58±0.13	8.27±0.14
	50	12.33±0.24	11.11±0.11	8.08±0.16
	25	12.10±0.28	10.00±0.65	8.24±0.21
PF_7	100	8.73±0.46	17.00±0.57	10.28±0.35
	50	6.5±0.28	12.64±0.52	7.28±0.14
	25	6.5±0.49	8.5±0.34	0.00±0.01
PF_8	100	23.03±0.37	15.16±0.81	22.0±0.06
	50	18.29±0.25	11.55±0.48	16.49±0.21
	25	11.07±0.49	8.25±0.96	12.14±0.08
Gentamycin	30µg/disk	22.23±0.76	22.67±0.57	20.01±0.95
Amoxycilin	30 µg/disk	19.59±0.59	18.00±0.34	21.00±0.87
Canamycin	30 µg/disk	18.79±0.46	19.28±0.64	21.24±0.65

- *S. aureus* = *Staphylococcus aureus*, *E.coli* = *Escherichia coli*, *S. entritidis* = *Salmonella entritidis*
- **PF_7=** pure(**P**) fraction(**F**) Number seven(**7**) throughout the text

- **MF_2=** mixture(**M**) fraction(**F**) Number two(**2**) throughout the text
- Results were reported as mean ± standard deviation of three replicate experiments.

The activity of *Plumbago zeylanica L* seems to be better in some of the individual isolated compounds than the crude extracts. For example, **PF_8** recorded 23.03mm against *S. aureus* and 22.00mm against *S. entritidis* at 100mg/ml. Relatively the inhibition of **PF_1** against *S. aureus* (20.27mm), *S. entritidis* (16.54mm); **PF_3** against *E.coli* (16.51mm); **PF_5** againt *S. aureus*, (20.19mm), *S. entritidis* (18.06mm); **PF_6** against *S. aureus* (16.41mm), **P F_7** against *E.coli* (17.00mm) were promising. Inhibition zones were uniform in decreasing order with respect to decrease in concentration. Zones of inhibition were good at 25mg/ml (11.07, 8.25 and 12.14mm) in case of **PF_8** against the three microorganisms in that order. Similarly **PF_3** inhibited 14.00mm and 10.67mm against *E. coli* and *S. entritidis* respectively. **PF_6** inhibited -12, 10 and 8mm against *S. aureus*, *E.coli* and *S. entritidis* respectively. But low zones of inhibition were recorded for **PF_1** against *S. entritidis* (7mm) while, **PF_5** againt *S. entritidis* (6.69mm) and for **PF_7** 6.50 mm against *S. aureus*.

S. aureus was found to be resistant to **PF_1** at 25mg/ml and to **PF_3** at all concentrations. E. coli was resistant to **PF_1** and **PF_5** at 25mg/ml and *S. entritidis* to **PF_7** at 25mg/ml.

The **MF_2**, a mixture of two compounds, recorded highest zone of inhibition (26. 20mm) than individual compounds of its components and all treatment groups in this activity against E. coli. **MF_2** also showed better inhibition than its components against *S. entritidis* (20.24) at 100mg/ml. This was true at 50mg/ml compared to its individual components **PF_1** and **PF_3** . The activity of **MF_4** against *S. aureus* was totally diminished than individual components with null zone of inhibition at all concentrations. However, better zone of inhibition was observed against *E. coli* than its components at all concentration. Other results were comparable except **MF_4** exhibited less zone of inhibition (11.50mm) at 100mg/ml than **PF_5** (18.06mm) at the same concentration for *S. entritidis* (Table 4).

3.2.3.2. Ethanolic crude extract fractions

Table 5. Antimicrobial zone of inhibition of n hexane – ethyl acetate (**PF_A- PF_C**) fractions of ethanolic crude extracts of *Pumbago zeylanica L.*

Treatment groups	Concentrations(mg/ml)	Microorganisms		
		S. aureus	E.coli	S. entritidis
P F_A	100	12.23±0.25	12.46±0.18	8.00±0.45
	50	8.54±0.36	11.41±0.16	7.02±0.01
	25	6.55±0.24	6.53±0..05	7.05±0.1
PF_B	100	8.85±0.11	7.13±0.03	8.72±0.32
	50	6.54±0.26	7.06±0.2	7.50±0.16
	25	6.55±0.27	6.50±0.20	7.05±0.24
PF_C	100	14.59±0.65	10.54±0.44	7.07±0.15
	50	6.53±0.48	0.00	6.05±0.13
	25	6.56±0.65	0.00	0.00
Gentamycin	30µg/disk	22.23±0.76	22.67±0.57	22.09±0.95
Amoxycilin	30 µg/disk	21.59±0.59	20.00±0.54	21.00±0.87
Canamycin	30 µg/disk	18.79±0.46	19.28±0.64	21.24±0.65

- *S. aureus = Staphylococcus aureus*, *E.coli = Escherichia coli*, *S. entritidis = Salmonella entritidis*
- Results were reported as mean ± standard deviation of three replicate experiments.

Antimicrobial activity of fractions from ethanolic crude extracts were less active than fractions of chloroform extracts. Here the highest inhibition observed was 14.59mm by **PF_C** at 100mg/ml against *S. aures*. The next was 12mm endorsed to **PF_A** at 100mg/ml against *S. aures* and *E. coli*. Least inhibition (6.5mm) was observed by **PF_A** and **PF_B** at 25mg/ml against *S. aures* and *E. coli* correspondingly and by **PF_C** against *S. aures*. On the other hand, *E. coli* was resistant at 50 and 25mg/ml and *S. entrtidis* at 25mg/ml only to **PF_C**.

4. Conclusion

Especially for infectious diseases, there was no as an immediate remedy as plants and plant derived products in human history. Based on the traditional knowledge this study investigated the antimicrobial activity of *Plumbago zeylanica L.* hoping to add value for the local medicinal knowledge of the plant and better efficacy than those reported before. Significant zone of inhibition was recorded from both the crude as well as fractions. More over we have able to find out three to four fractions which showed promising results. The data we put here is an in vitro and for sure unless they are supported by invivo assays it will not be confidential. We also recommend additional works on characterization and structural elucidation of the active compounds isolated here.

Acknowledgements
We are grateful to the researchers and collegues who participated the research work. Wish to thank miss Adeline Letard (guest lecturer from France) for her supervision and technical assistance during the work, Hadush Ashebrom, technical assistance of organic chemistry research laboratory, department of chemistry, Mekelle University. Esmail Abdu technical assistance of undergraduate organic chemistry laboratory, Alem, technical assistance of Analytical chemistry, Department of Chemistry, Mekelle University. The of Chemistry, Mekelle University, The Department Veterinary Medicine, Mekelle University, the Department of Biology, Mekelle University.

References

1. Snowe, O. J., Harkin, T., Kennedy, E. M. (April 2004). Antibiotic Resistance. Report to Congressional Requesters. United States General Accounting Office Washington, D.C. 20548
2. Iwu, M., R. Duncan, A., O. Okunji, C. (1999). Perspectives on new crops and new uses: New Antimicrobials of Plant Origin. J. Janick (ed.), ASHS Press, Alexandria, VA. P 457 – 462
3. Chaudhary, S., Negi, A., Dahiya, V. (2010). The Study of in vitro Antimicrobial Activity and Phytochemical Analysis of Some Medicinal Plants in Chamoli Garhwal Region. *Journal of Pharmacognocy*, 2, 481–485
4. Pant, M., Lal A., Rana, S., Rani, A. (2012). Plumbago Zeylanica L.: A Mini Review. *International Journal of Pharmaceutical Applications,* 3, 399 - 405
5. Chitravadivu, C., Bhoopathi, M., Balakrishnan, V., Elavazhagan, T., Jayakumar, S. (2009). Antimicrobial Activity of Laehiums Prepared by Herbal Venders, South India. American-Eurasian. *American-Eurasian Journal of Scientific Research,* 4, 142-147
6. Aqil, F., Zahin, M., Ahmed, I. (2008). Antimutagenic Activity of Methanolic Extracts of Four Ayurvedic Medical Plants. *Indian Journal of Experimental Biology*, 46, 668-672
7. Wang, Y. C., Tung-Liang Huang, L. T. (2005). Anti-Helicobacter Pylori Activity of Plumbago zeylanica L. *FEMS Immunol Med Microbiol*, 43, 407–412
8. Zaha, E., Khalaf, A. N., Shakya, K. A., Al-othman, A., Farah, H.(2008). Antioxidant Activity of Some Common Plants. *Turk. Journal of Biology*, 32, 51-55
9. Aziz, H. M, Dreckschmidt, E. N., Verma, A. (2008). Plumbagin, a Medicinal Plant–Derived Naphthoquinone, Is a Novel Inhibitor of the Growth and Invasion of Hormone- Refractory Prostate Cancer. *Cancer Research*,Chem Abst, 68, 9024 – 9032
10. Nile, H. S., Khobragade, N. C. (2010). Antioxidant Activity and Flavonoid Derivatives of Plumbago Zylanica. *Journal of Naural Products*, 3, 130 -133
11. Mungwini, Y. (1753). Prota =1(1): Medicinal plants/Plantes médicinales 1 Record =isplay. *Protologue*. Sp. pl. 1: 151
12. Jeyachandran, R., Mahesh, A., Cindrella, L., Sudhakar, S., Pazhanichamy, K. (2009). Antibacterial Activity of Plumbagin and Root Extracts of *Plumbago Zeylanica* L. *ACTA BIOLOGICA CRACOVIENSIA Series Botanica*, 51, 17–22
13. Rahman, S. M., Anwar, M. N. (2007). Antimicrobial Activity of Crude Extract Obtained from the Root of *Plumbago zeylanica*. *Bangladesh Journal of Microbiology*, 24, 73-75
14. Mittal, V., Sharma K. S. (2010). An *In vitro* Antimicrobial Activity of Callus And Root Extracts of *Plumbago Zeylanica* Linn. In Various Test Microorganism. *International Journal of Pharmaceutical Sciences Review & Research,* 5, 1- 4
15. Beg, Z. A., Ahmad, I. (2000). Effect of *Plumbago zeylanica L.* Extract and Certain Curing Agents on Multidrug Resistant Bacteria of Clinical Origin. *World Journal of Microbiology and Biotechnology*, 16, 841- 844
16. Paiva, D.R.S., Marques, S.D.S., Figueiredo, R. M., Kaplan, C.A.M. (2003). Plumbaginales: A Pharmacological Approach. *Recebido para publicação em*, 10, 98 –105
17. Arunachalam, D. K., Velmurugan, P., Balaji, R. (2010). Anti-inflammatory and Cytotoxic Effects of Extract from Plumbago Zeylanica. *African Journal of Microbiology Researh*, 4, 1239 - 1245.
18. Rout, R. J., Kanungo, S., Das, R., Sahoo1, L. S. (2010). In Vivo Protein Profiling and Catalase Activity of *Plumbago zeylanica L. Natural and Science*, 8, 87- 90
19. Mungwini Y. (1753) Prota =1(1): Medicinal plants/Plantes médicinales 1 Record =isplay. Protologue. Sp. pl. 1: 151
20. Gopinath S., Muralidharan S., Rajan S., and Danaphal P.S. (2009). Simultaneous Estimation of Plumbagin and Embelin by Reverse Phase-High Performance Liquid Chromatographic Method. *Der. Pharmacia. Letters,* 1, 135-142
21. Checker, R., Sharma, D., Sandur, K. S., Subrahmanyam, G., Krishnan, S., Poduval, B. T. and Sainis, B. K.

(2010). Plumbagin Inhibits Proliferative and Inflammatory Responses of T Cells Independent of ROS Generation But by Modulating Intracellular Thiols. *Journal of Cell Biochemistry*, 110, 1082–1093

22. Mittal, V., Sharma, S. K., Kaushik, D., Khatri, M. and Tomar, K. (2010). A comparative Study of Analgesic Activity of *Plumbago zeylanica* Linn. Callus and Root extracts in Experimental mice. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,*1, 830-836
23. Khan, M., Kori, K. K.S., Kumar, P., Setty, R.S. and Rajendra, S. V. (2006). Antioxidant and Nephroprotective Activity of Leaves of *Plumbago zeylanica* (*Linn*.). *Indian Journal of Pharmacy,* Chem. Abst, 583131
24. Maniafu, B., Wilber, L., Ndiege, O.I., Wanjala, C.C. and Akenga, A.T. (2009). Larvicidal Activity of Extracts From Three Plumbago Species Against Anopheles Gambiae. *Mem. Inst. OswaldoCruz. Rio de Janeiro*, 104, 813-817
25. Ahmad, A., Banerjee, S., Wang, Z., Kong, D. and Sarkar, H.F. (2008). Plumbagin-Induced Apoptosis of Human Breast Cancer Cells Is Mediated by Inactivation of NF-kB and Bcl-2. *Journal of Cellular Biochemistry*, 105, 1461–1471
26. Nazeem, S., Azmi, S.A., Hanif, S., Ahmad, A., Mohammad, M.R., Hadi, M.S. and Kumar, S. K. (2009). Plumbagin Induces Cell Death Through a Copper-redox Cycle Mechanism in Human Cancer Cells. *Mutagenesis*, Mutagenesis, 1– 6
27. Mittal, V. and Sharma K. S. (2010). An *In vitro* Antimicrobial Activity of Callus And Root Extracts of *Plumbago Zeylanica* Linn. In Various Test Microorganism. *International Journal of Pharmaceutical Sciences Review & Resear,* 5, 1- 4
28. Wang, Y.C., Tung-Liang HLT. (2005). Anti-Helicobacter Pylori Activity of Plumbago zeylanica L. *Immu. Med. Microbiol. Chem.* Abst, 43, 407–412
29. Aladesanmi, A.J., Iwalewa, E.O., Adebajo, A.C., Akinkunmi, E.O., Taiwo, B.J., Olorunmola, F.O. and Lamikanra A. (2007). Antimicrobial and Antioxidant Activities of Some Nigerian Medicinal plants. *African Journal of Traditional, Complementary, and Alternative Medicines*, 4, 173 – 184
30. Lalitha, M. K. (2004). Manual on Antimicrobial Susceptibility Testing. Department of Microbiology. Christian Medical College, Vellore, Tamil Nadu.; p 3-11
31. Seyydnejad, M. S., Niknejad, M., Darabpoor, I. and Motamedi, H. (2010). Antibacterial Activity of Hydroalcoholic Extract of Callistemon citrinus and Albizia lebbeck. *American Journal of Applied Sciences*, 7, 13-16
32. Shahverdi, A., Iranshahi, M., Mirjani, R., Jamalifar, H., Amin, G. and Shafiee, A.(2005). Bioassay- Guided Isolation and Identification of an Antibacterial Compound from Ferula persica var. Persica roots. *DARU*, 13, 17 – 19
33. El-Mahmood, A. M. and Ameh , J. M.(2007). In vitro antibacterial activity of *Parkia biglobosa* (Jacq.) root bark extract against some microorganisms associated with urinary tract infections. *African Journal of Biotechnology*, 6, 1272-1275
34. Ert, M., Kati, H., Yayli, N. and Demürbaú, Z. (2006). Antimicrobial Properties of Silene multifida (Adams) Rohrb. Plant Extracts. *Turk . Journal of Biology*, 30, 17-21
35. Borhade, S.P., Deshmukh, T.A., Patil, V.R. and Khandelwal K.R. (2014). Pharmacognostic and Phytochemical Investigations of *Plumbago zeylanica* Linn. Root. *Journal of Pharmacognocy and Phytochemistry,* 2, 83-88
36. Renuga, G.A. and Thandapani B. (2013). Evaluation on anti microbial potential of root extracts *Plumbago zeylanica* L against human intestinal microflora. *International Journal of Pharmaceutical Sciences Review and Research*,4, 146-158

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