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Antimicrobial Potentials of Different Solvent Extracts of *Justicia landonoides and Plantago lanceolata* Against Standard and Drug Resistant Human Bacterial Pathogens

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Abstract: Scientists paid attention to search new antimicrobials against multidrug resistant pathogenic microbes' wide therapeutic potentials. The objective of this study was to determine antimicrobial potentials of extracts of *Justicia landonoides and Plantago lanceolata* against standard and drug resistant human microbial pathogens. Active antimicrobial compounds were extracted using three different solvents (acetone, methanol alnd water). The antimicrobial activities of crude extracts were determined using agar well diffusion assay and broth dilution methods. Penicillin, methicillin alnd amoxicillin were used as positive controls and water as negative control. Minimum inhibitory concentrations and minimum bactericidal concentrations of each crude extracts were also determined against test microorganisms using broth dilution method. Combined effects of crude extracts and commercial antibiotics were also determined. Crude extracts of *P. lanceolata* showed various degrees of antimicrobial activity towards each standard and drug resistant microbial pathogen with mean zone of inhibition ranges up to 26±3.60 mm against multidrug resistant *K. pneumoniae*. Of the different crude extracts, acetone extract of *J. landanoides* showed the highest mean zone of inhibition 55.3 ±3.05mm against *E. coli* (ATCC2592). Clinical isolate of *C. albicans* was inhibited by all extracts with mean diameter inhibition zone ranges up to 22±2.64 mm for water extract of *P. lanceolata*. Specifically, *S. aureus* (ATCC2923), *E. coli* (ATCC2592), *S. pneumoniae* (multidrug resistant), *K. pneumoniae* (multidrug resistant) alnd *S. boydii* (ATCC9289) have been shown MIC and MBC ranging from 6.25% to 25% for both plant extracts investigated in this study. The minimum bactericidal concentrations of *P. lanceolata* against on both Gram positive and Gram negative bacteria were ranges from 12.5 to 25%. According to this study, crude extracts of the plants under investigation have potential inhibitory effects against pathogenic microorganism.

Key words: Antibiotics · Crude Extract · Dilution Method · Drug Resistant · Inhibition Zone · Well Diffusion

INTRODUCTION

In ancient times people used spices and herbs in their food not only as flavoring agents, but also as folk medicine [1] and food preservatives [2] (Das, S. Anjeza, C. and Mandal, S. 2012; Gutierrez *et al*. 2008). In addition to flavoring agents, many herbs, spices also possess free radical scavenging antioxidant activities and antimicrobial activities like bacteriostatic and bactericidal effect up on pathogenic bacteria [3]. The current demands for medicinal plants and herbal medicinal products in both developed and developing countries is rising dramatically [4]. In developed countries this may be partly due to the dissatisfaction with conventional medicines and also due to the spread of multidrug resistant pathogenic bacteria. In developing countries, in addition to problem of multidrug resistant pathogenic bacteria, lack of medical doctors, shortage of pharmaceutical products and their unaffordable prices. Therefore herbal remedies are currently enjoying widespread popularity throughout the world including Ethiopia [5].

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Ethiopia is believed to be home for about 6,500-7,000 species of higher plants with approximately 12% endemic elements [6] (and hence it is one of the six plant biodiversity rich countries in Africa [7]. High concentrations of medicinal plants are found in the south and south western parts of Ethiopia with high degree of biological and cultural diversity [8]. But, it is faced with a problem of sustainability and continuity mainly due to the loss of taxa of medicinal plants [9].

Many investigations have been indicated that there was growing of drug resistant pathogens in different parts of the world [10]. Antibiotic resistant human pathogens, threat currently applicable drug effectiveness and significantly lead to high morbidity and mortality rates [11]. In order to face microbial resistance, new antibiotics are produced, but it is costly and resistance must be expected sooner.

To solve such kind of infectious disease with multidrug resistance challenges, search for potential medicinal plants is significant. Of those traditional medicinal plants, *Plantago lanceolata* and *Justicia landonoides* were considered to investigate their antimicrobial activity using scientific standard methods. *P. lanceolata* is species belongs to family Plantaginaceae, genus plantago and native in Europe and Asia, which has introduced to other temperate zones (Now naturalized throughout the world [12]. It has been used as an anesthetic, antiviral, anti-inflammatory, astringent, anti helmintic, analgesic, analeptic, antihistaminic, antirheumatic, antitumor, anti ulcer, diuretic and expectorant as well as hypotensive in traditional medicine [13,14]. It has also used as a fabric stiffener by a mucilage from the seed coats a good fiber is obtained from the leaves, accepted to be suitable for textiles. It is obtained by macerating the seed in hot water a sources for gold and brown dyes from the whole plant. It has been proven to contain mucilage, tannin and silic acid [15] for wide range of applications. Safe to treat various haemorrhage; also for bronchitis, cystitis, catarrh sinusitis, asthma and hay fever, gastritis and coughs moreover it encourages the repair of damaged tissue [16].

J. landonoides belongs to the genus Justicia which is the largest in the family consists of over 600 species and distributed throughout the tropics and subtropics. This genus is distributed in the edge of low land rainforest, montane forest, mixed evergreen and deciduous wood land, wooded grass land, river forest and other environments. The specimens from the flora area tend to be variable, while the species is said to be extremely variable outside the flora area, particularly in western Africa [17]. Traditionally *J. Landonoides* is widely used medicinal plants in Ethiopia, to best of our knowledge no previous work has been reported about the economical significant and its antimicrobial activities against pathogenic microorganisms. This is the basic reason that this study was focused on the antimicrobial activity of this plant.

Limited researches have been done on Ethiopian medicinal plants and few have been conducted scientifically by modern researches where their principal component has been analyzed and defined. In this study two Ethiopian medicinal plants: *P. lanceolata and J. landonoides* were selected, which are often and widely practiced by the society for healing effect during treatment of various diseases in the region. But there is no any scientific report on the antimicrobial activity of *J. landanoides.* Therefore, the main objective of this study was to investigate antimicrobial potentials of extracts of *P. lanceolata and J. landonoides* against standard and drug resistant human bacterial pathogens and fungus. Furthermore, the data generated in this study may used as a bench mark for further investigation particularly for *in vivo* study for treatment application.

MATERIALS AND METHODS

Study Area and Duration: The study was conducted in North Gondar, in northwest part of Ethiopia. According to 2006/07 reports above 45% of the population in the zone has access to health services facilities. According to the national population survey of 2010, the city has a population of approximately 231,977 people. Gondar has a latitude and longitude of 12°36'N 37°28'E with l annual elevation of 2135 meter above sea level. The study was conducted at University of Gondar, Department of Biotechnology (Microbiological laboratory) from July 1, 2012 to January 26, 2013.

Collection and Preparation of Plant Samples: Each plant species, *J. landanoides and P. lanceolata,* were collected and identified for further investigation in Addis Ababa University, Botanical Herbarium. The fresh leaves of each plant was washed three times with pure (sterilized) water, allowed it to air dry at room temperature under shade. The dried leaves were powdered with the help of pestle and mortar and store in sterile bottle at 4°C [18] for further analysis in this study.

Extraction of Plant Leaves Using Different Solvents: In this study, 20g of shade dried powder was loaded in 250 ml flask and mixed with 100 ml of 95% methanol, 95% acetone and water solvents for extraction and then shacked on a rotary shaker for three consecutive days. The extracts were filtered by passing through Whitman's filter paper No1 and further centrifuged at 5000 rpm for 15min. The solvent extracts were concentrated under reduced pressure using Rota Vapor and preserve at 4°C in air tight bottle for further investigation.

Test Microorganisms: Standard and multidrug resistant pathogenic bacteria were used for determination of antimicrobial activities of different extracts. In brief, the bacterial strains used to assess the antimicrobial properties of extracts included both standard and drug resistant clinical isolates of Gram positive and Gram negative strains*: Staphylococcus aureus* (ATCC2923), *Streptococcus pneumoniae* (ATCC49619), *Escherichia coli* (ATCC2592), *Shigella boydii* (ATCC9289), Methicillin resistant *Staphylococcus aureus* (MRSA), Multidrug resistant clinical isolate of *Streptococcus pneumoniae* (Resistant to tetracyclines, ampicilin and penicillin), *Klebsiella pneumoniae* (resistant to tetracycline, amoxicillin, ampicillin and cefriaxone) and *Escherichia coli* (Resistant to nalidixic acid, tetracyclines, amoxicillin and ampicillin) as well as *Candidia albicans* (clinical isolate). The strains were obtained from microbiology laboratory and Gondar University hospital. They were maintained on Sabrouraud-dextrose agar (for the fungal strain only) and Muller-Hinton Agar (MHA) test tube slant at 4°C and subcultured before use.

Preparation of Inoculums: The microbial stock cultures were maintained at 4°C on slopes of MHA. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Muller-Hinton broth and incubated without agitation for 24 h at 37°C. To 5 ml of Muller-Hinton Broth (MHB), 100µl of culture was inoculated and incubated till it reached the turbidity equal to 0.5 MacFarland standard solutions which is equivalent to 1.5×10^8CFU/ml [19].

Antimicrobial Activity Assay: The antimicrobial activity assay was done by agar well diffusion method. In short, antibacterial activities of the different extracts were determined by agar well diffusion assay on MHA medium. The MHA was melted and cooled to 48 - 50°C and poured into sterile Petri dishes to give a solid plate. Then standardized inoculums of 100 µl (1.5×10^8 CFU/ml, 0.5 McFarland) were added aseptically and streaked on the agar plate surface. Wells were prepared in the seeded agar plates with sterile cork borer (6 mm diameter). The test compound or crude extract (100 µl) were carefully dispensed into the wells. This was done in triplicate in parallel to different control antibiotics. Extracts were allowed to diffuse for about 2 h before incubation. Plates were incubated overnight at 37 °C. After overnight incubation, the plates were observed for the zone of inhibition and the diameter of the inhibition zone was measured. The presence of a zone of inhibition around each well was indicative of antibacterial activity. The diameters of zone of inhibition produced by the extract agents were compared with those produced by the commercial control antibiotics.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)/ Minimum Fungicidal Concentration (MFC): The minimum inhibitory concentration (MIC) of *J. landanoides and P. lanceolata* leave extracts were determined according to methods described by Shahidi [18] and Kabir *et al* [20]. Extracts were diluted to concentrations ranging from 6.25% to 25%. To each dilution of crude extracts, nutrient broth tubes were seeded with 100 µl of the pathogenic standard and resistant clinical bacterial inoculums and clinical isolate of the fungus. Negative control tubes with no bacterial and fungal inoculation were simultaneously maintained. Tubes were incubated aerobically at 37°C for 24 h. The lowest concentration of the extract that produced no visible growth (Turbidity) was recorded as the MIC. Dilutions showing no visible growth for the MIC were subcultured onto a fresh MHA plate and incubated at 37°C for 24 h for the determination of MBC and MFC. In brief, dilution showing no visible growth in the determination of MIC was streaked (subcultured) onto MHA and incubated for 24 h. The least concentration of the extract with no visible growth after incubation was taken as the MBC and MFC [21].

Determination of Synergetic Antimicrobial Activities of Crude Plant Extracts and Commercial Antibiotics: The crude extracts and standard antibiotics were diluted to (10% extracts of sample, 10%, amoxicillin, 10% tetracycline) as well as combination of extracts with clinically prescribed drugs were diluted to (10% extract plus 10% amoxicillin or tetracycline) to evaluate weather

synergetic effect was there or not. Then 100 µl of each crude extract and combination of commercial antibiotics were separately added into the wells of 6 mm to examine inhibition zone differences after incubation [22].

Data Analysis: The data were analyzed using SPSS version 16.0. Means and standard deviations of the triplicate analysis were calculated using analysis of variance (ANOVA) to determine the significance differences between the means using Duncan's Multiple range test ($P \leq 0.05$) when the F-test demonstrated significance. The statistically significant difference was determined as $P \leq 0.05$.

RESULTS

The Plant Extracts Yielded: Leaves of medicinal plants were extracted for the crude antibiotics with water, methanol and acetone. The crude extracts obtained from 20 g of plant leaf powder of *J. landanoides* were 13.10, 18.35 and 22.05% using acetone, methanol and water respectively. Out of 20 g *P. lanceolata* leaf powder, 17.10, 18.40 and 14.95% were extracted by acetone, methanol alnd water respectively.

Determination of Antimicrobial Activity of Plant Extracts:

Agar Well Diffusion Method: The inhibition zones obtained by each solvent extract of *P. lanceolata* and commercial antibiotics (control) against each tested microorganisms were compared using one way ANOVA and presented in Table 1 and 2. Inhibition zone showed by the crude extracts and commercial antibiotics were compared within the same row against tested microorganisms and inhibition zone among each different solvent extracts were compared within the same column to each tested bacteria and fungus.

The inhibition zone of acetone (16.3±3.21mm), methanol (17.3±2.51 mm) alnd water (16.3±1.52 mm) extract of *P. lanceolata* against *S. aureus* (ATCC2923) were statistically (P = 0.05) greater than inhibition zone of penicillin (8±.00mm) and methicillin (10±1.73 mm). However, with regard to different solvent extraction potential of *P. lanceolata*, there was no statistically significant (P = 0.05) difference among inhibition zone of acetone, methanol and water extracts against *S. aureus* (ATCC2923).

Interestingly, inhibition zone of each solvent extracts of *P. lanceolata* was significantly (P = 0.05) greater than inhibition zone of the control commercial antibiotics against MRSA Table 1 and *S. boydii* (ATCC9289) Table 2, but there was no statistical inhibition zone difference observed among the different extracts to the same bacteria.

P. lanceolata crude extracts of methanol (17±2.64 mm) and water (15.3±.57 mm) inhibition zones against *S. pneumoniae* (ATCC49619) were significantly (P = 0.05) greater than pencillin (10±1.00 mm) and methicillin (10±1.00 mm), but less than amoxicillin (20±1.00 mm). Of three different solvent extractions, methanol (17±2.64 mm) and water extracts (15.3±.57mm) were significantly (P = 0.05) greater than inhibition zone of acetone (10.7±1.15 mm) extracts on the same organism Table 1.

The antimicrobial potential of *P. lanceolata* crude extracts of acetone (10.3±0.57mm), methanol (10.3±0.57mm) and water (13.6±2.88mm) showed statistically (P = 0.05) greater inhibition zone than penicillin (0±.00 mm) and methicillin (0±.00 mm), but no statistical inhibition zone difference (P = 0.05) with that of amoxicillin (11±2.64mm) against drug resistant *S. pneumonia*. With regard to inhibition zone of each solvent extracts, there was no statistically significant ($P \geq 0.05$) antibacterial activity difference between each crude extracts against *S. pneumoniae* (drug resistant).

P. lanceolata crude extracts of acetone (12.7±.57 mm) and water (13.3±1.52 mm) inhibition zones were statistically ($P \leq 0.05$) less than inhibition zones of amoxicillin, but no statistical difference ($P \geq 0.05$) to inhibition zones of penicillin (13.6±2.76 mm) and methicillin (12.6± 1.57mm) against *E. coli* (ATCC2592). In addition, there was no statistical inhibition zone difference ($P \geq 0.05$) between methanol (15±.00 mm) extract from that of the control antibiotics, penicillin (13.6±2.76mm) and amoxicillin (17.6±.5.50 mm), but greater than from inhibition zone of methicillin (12.6± 1.57 mm). Likewise, among the solvent crude extracts, inhibition zone measured with methanol extract was statistically ($P \leq 0.05$) greater than inhibition zone of acetone (12.7±.57 mm) extract against *E. coli* (ATCC2592) Table 2.

The inhibition zone of acetone (11±1.00mm), methanol (11±1.00 mm) alnd water (14.6±2.51 mm) extracts were statistically ($P \leq 0.05$) greater than the standard antibiotics such as penicillin (0±.00 mm), methicillin (0±.00 mm) and amoxicillin (0±.00 mm), which had no any activity at all against *E. coli* (multidrug resistant) whereas from the different solvent extracts, inhibition zone with water (14.6±2.51 mm) extract was significantly ($P \leq 0.05$) greater than acetone (11±1.00 mm) and methanol (11±1.00 mm) extracts to the same resistant bacteria isolate.

Table 1: Comparison of inhibition zone among crude extracts of *P. lanceolata* using different solvents and also with commercial antibiotics against standard and drug resistant Gram positive bacteria

		Inhibition zone (mean ± s.d)				
			Positive controls			Negative control
Test Microorganisms	Solvents used for extraction	Crude extracts	P	Me	Am	Distilled water
S. aureus (ATCC2923	A	16.3±3.21 ^{c}	8±.00^{a}	10±1.73^{ab}	14±2.00^{bc}	0
	M	17.3±2.51 ^{c}	8±.00^{a}	10±1.73^{ab}	14±2.00^{bc}	0
	W	16.3±1.52 ^{c}	8±.00^{a}	10±1.73^{ab}	14±2.00^{bc}	0
MRSA	A	16.7±2.88^{c}	10±1.00 ^{b}	0±.00 ^{a}	11 ±2.00 ^{b}	0
	M	16±4.00^{c}	10±1.00 ^{b}	0±.00^{a}	11 ±2.00 ^{b}	0
	W	15.3±.57 ^{c}	10±1.00 ^{b}	0±.00^{a}	11 ±2.00^{b}	0
S. pneumoniae (ATCC4961)	A	10.7±1.15^{a}	10±1.00^{a}	10±.00^{a}	20±1.00^{c}	0
	M	17±2.64^{b}	10±1.00^{a}	10±.00^{a}	20±1.00^{c}	0
	W	15.3±.57^{b}	10±1.00^{a}	10±.00^{a}	20±1.00^{c}	0
S. pneumoniae (multidrug resistant)	A	10.3±.57^{b}	0±.00^{a}	0±.00^{a}	11±2.64 ^{b}	0
	M	10.3±.57^{b}	0±.00^{a}	0±.00^{a}	11±2.64 ^{b}	0
	W	13.6±2.88^{b}	0±.00^{a}	0±.00^{a}	11±2.64 ^{b}	0

Where: mean ± Standard deviation in triplicate, P= Penicillin, Me= Methicillin, Am= Amoxicillin, A= Acetone, M= Methanol, W= Water; Values are means of triplicate determination values within the same row and column followed by different superscripted letters are statistically different ($P \leq 0.05$) against each tested microorganisms

Table 2: Comparison of inhibition zone among crude extracts of *P. lanceolata* using different solvents and commercial antibiotics against standard and multidrug resistant clinical pathogenic Gram negative bacteria and *C. albicans* (clinical isolate)

		Inhibition zone (mean & sd)				
			Positive controls			Negative control
Test Microorganisms	Solvents used for extraction	Crude extracts	P	Me	Am	Distilled water
E. coli (ATCC2592)	A	12.7±.57^{a}	13.6±2.76^{a}	12.6± 1.57^{a}	17.6±.5.50^{c}	0
	M	15±.00^{bc}	13.6±2.76^{ab}	12.6± 1.57^{a}	17.6±.5.50^{c}	0
	W	13.3±1.52^{ab}	13.6±2.76^{a}	12.6±1.57^{a}	17.6±.5.50^{c}	0
E. coli (multidrug resistant)	A	11±1.00^{b}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0
	M	11±1.00^{b}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0
	W	14.6±2.51 ^{c}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0
S. boydii (ATCC9289)	A	16±6.08^{b}	10.7±0.68^{a}	10.3±1.57^{a}	11±1.00^{a}	0
	M	16.6±3.05^{b}	10.7±0.68^{a}	10.3±1.57^{a}	11±1.00^{a}	0
	W	15.3±.57^{b}	10.7±0.68^{a}	10.3±1.57^{a}	11±1.00^{a}	0
K. pneumoniae (multidrug resistant)	A	17±7.00^{b}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0
	M	26±3.60^{c}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0
	W	15.7±.57 ^{b}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0
C. albicans (clinical isolate)	A	8.7±1.15^{b}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0
	M	15.3±.57^{c}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0
	W	22±2.64^{d}	0±.00^{a}	0±.00^{a}	0±.00^{a}	0

Where: mean ± Standard deviation in triplicate, P= Penicillin, Me = Methicillin, Am = Amoxicillin, A= Acetone, M= Methanol, W = Water; Values are means of triplicate determination values within the same row and column followed by different superscripted letters are statistically different ($P \leq 0.05$) against each tested microorganisms.

The methanol (26±3.60 mm) extract of the same plant was statistically ($P \leq 0.05$) greater than acetone (17±7.00 mm) and water (15.7±.57 mm) extracts to the drug resistant *K. pneumonia*, where the control antibiotics were resistant at all.

Of the different solvent extracts tested against *C. albicans* (clinical isolate), inhibition zone (22±2.64 mm) with water extract was statistically ($P \leq 0.05$) greater than with that of acetone (8.7±1.15 mm) and methanol (15.3±.57 mm) extracts, alternatively inhibition zone of methanol extract was statistically ($P \leq 0.05$) greater than acetone extract, Table 2.

All extracts of *P. lanceolata* showed various degrees of activity against *S. aureus* (ATCC2923), MRSA, *S. boydii* (ATCC9289) and *K. pneumoniae* (clinical isolate) with mean diameter ranges from 15.3 mm to 26mm.

Table 3: Comparison of inhibition zone among crude extracts of *J. landanoides* and commercial antibiotics against standard and clinical isolated multidrug resistant pathogenic Gram positive bacteria

		Inhibition zone (mean ± s. d)				
			Positive controls			Negative control
Test Microorganisms	Solvents used for extraction	Crude extracts	P	Me	Am	Distilled water
S. aureus (ATCC2923)	A	20.7 ± 5.0^{c}	8±.00^{a}	10±1.73^{ab}	14±2.00^{b}	0
	M	22 ±3.46^{c}	8±.00^{a}	10±1.73^{ab}	14±2.00^{b}	0
	W	22.7±3.05^{c}	8±.00^{a}	10±1.73^{ab}	14±2.00^{b}	0
MRSA	A	16 ±2.00^{c}	10±1.00^{b}	0±.00^{a}	11 ±2.00^{b}	0
	M	17.3±3.05^{c}	10±1.00^{b}	0±.00^{a}	11 ±2.00^{b}	0
	W	18.7±2.30^{c}	10±1.00^{b}	0±.00^{a}	11 ±2.00^{b}	0
S. pneumoniae (ATCC49619)	A	25.3±9.01^{bc}	10±1.00^{a}	10±.00^{a}	20±1.00^{b}	0
	M	21.3±1.15^{b}	10±1.00^{a}	10±.00^{a}	20±1.00^{b}	0
	W	29.3±7.57^{c}	10±1.00^{a}	10±.00^{a}	20±1.00^{b}	0
S. pneumoniae (multidrug resistant)	A	12 ±2.00^{b}	0±.00^{a}	0±.00^{a}	11±2.64^{b}	0
	M	11.3±2.30^{b}	0±.00^{a}	0±.00^{a}	11±2.64^{b}	0
	W	22.7±3.05^{c}	0±.00^{a}	0±.00^{a}	11±2.64^{b}	0

Where: mean ± Standard deviation in triplicate, P= Penicillin, Me= Methicillin, Am= Amoxicillin, A= Acetone, M= Methanol, W= Water; Values are means of triplicate determination values within the same row and column followed by different superscripts of letters are statistically different ($P \leq 0.05$) against each tested microorganisms.

The inhibition zones obtained by each solvent extracts of *J. landanoides* and commercial antibiotics (control) as well as among the different solvent extracts, against each tested microorganisms were statistically compared using one way ANOVA, Table 3. Inhibition zone showed by the crude extracts of *J. landanoides* and commercial antibiotics were compared within the same row against tested microorganisms and inhibition zone among each different solvent extracts were compared within the same column in particular to each tested bacteria and fungus.

As a shown in the Table 3, there was no statistical ($P \geq 0.05$) inhibition zone difference obtained between each solvent crude extracts of *J. landanoides* against *S. aureus* (ATCC2923) and *MRSA.* However, all the crude extracts were much significant ($P \leq 0.,05$) greater inhibition zones than that of the control disc antibiotics to the same bacteria.

The inhibition zones of each solvent extracts of *J. landanoides* were statistically ($P \leq 0.05$) greater than the commercial antibiotics penicillin (10±1.00mm), methicillin (10±.00 mm) against *S. pneumoniae* (ATCC49619). However, there was no statistical difference ($P \geq 0.05$) observed between inhibition zones of acetone extract (25.3±9.01 mm) and amoxicillin (20±1.00 mm) and between methanol (21.3±1.15 mm) extract and amoxicillin (20±1.00 mm) against *S. pneumoniae* (ATCC49619). In respect to inhibition zone of the different solvent extracts of *J. landanoides,* water extract (29.3 ±7.57 mm) was significantly ($P \leq 0.05$) greater than methanol (21.3 ±1.15mm) crude extract, but there was no statistical difference ($P \geq 0.05$) with that of acetone (25.3±9.01 mm) extract against to the same bacterial strain Table 3.

Crude extract of water (22.7±3.05 mm) inhibition zone was significantly ($P \leq 0.05$) greater than acetone (12±2.00 mm) and methanol (11.3±2.30 mm) extracts as well as to the control antibiotics against *S. pneumoniae* (drug resistant). In comparison to the commercial antibiotics, there was no statistical inhibition zone difference ($P \geq 0.05$) between acetone (12±2.00 mm) and methanol (11.3±2.30 mm) extracts with that of amoxicillin (11±2.64mm), but greater than penicillin (0 ± 00mm) and methicillin (0±.00mm) against to the multidrug resistant *S. pneumonia*.

Justicia landanoides crude extract of acetone (55.3±3.05 mm) against *E. coli* (ATCC2592) was statistically ($P \leq 0.05$) greater than methanol (30±4.00 mm) and water (29.3±9.45 mm) extracts, there was no significant ($P \geq 0.05$) inhibition zone difference between methanol (30±4.00 mm) and water (29.3±9.45 mm) extracts against on the same organism. All these crude extracts were statistically ($P \leq 0.05$) shown wide inhibition zone than penicillin (13.6±2.76mm), methicillin (12.6± 1.57 mm) and amoxicillin (17.6±.5.50 mm) against *E. coli* (ATCC2592) Table 4.

The methanol (32±8.71 mm) extract showed significantly ($P \leq 0.05$) greater inhibition zone than acetone (15.3±1.15 mm) and water (22.7±3.05 mm) crude extracts against *S. boydii* (ATCC9289), in the other words, water extract was statistically ($P \leq 0.05$) greater than

Table 4: Comparison of inhibition zone among crude extracts of *J. landanoides* and commercial antibiotics against standard and clinical isolated multidrug resistant pathogenic Gram negative bacteria and *C. albicans* (clinical isolate)

		Inhibition zone (mean ± s. d)				
			Positive controls			Negative control
Test Microorganisms	Solvents used for extraction	Crude extracts	P	Me	Am	Distilled water
E. coli (ATCC2592)	A	55.3 ±3.05[d]	13.6±2.76[a]	12.6± 1.57[a]	17.6±.5.50[b]	0
	M	30 ±4.00[c]	13.6±2.76[a]	12.6± 1.57[a]	17.6±.5.50[b]	0
	W	29.3 ±9.45[c]	13.6±2.76[a]	12.6± 1.57[a]	17.6±.5.50[b]	0
E. coli (multidrug resistant)	A	22 ±10.39[b]	0±.00[a]	0±.00[a]	0±.00[a]	0
	M	34.7 ±2.30[c]	0±.00[a]	0±.00[a]	0±.00[a]	0
	W	22 ±3.46[b]	0±.00[a]	0±.00[a]	0±.00[a]	0
S. boydii (ATCC9289)	A	15.3 ±1.15[b]	10.7±0.68[a]	10.3±1.57[a]	11±1.00[a]	0
	M	32 ±8.71[d]	10.7±0.68[a]	10.3±1.57[a]	11±1.00[a]	0
	W	22.7±3.05[c]	10.7±0.68[a]	10.3±1.57[a]	11±1.00[a]	0
K. pneumoniae (multidrug resistant)	A	22 ±1.00[c]	0±.00[a]	0±.00[a]	0±.00[a]	0
	M	18 ±2.64[b]	0±.00[a]	0±.00[a]	0±.00[a]	0
	W	20.7±1.15[bc]	0±.00[a]	0±.00[a]	0±.00[a]	0
C. albicans (clinical isolate)	A	16.3 ±2.51[c]	nd	nd	nd	0
	M	11 ±1.00[b]	nd	nd	nd	0
	W	15.3 ±2.51[c]	nd	nd	nd	0

Where: mean ± Standard deviation in triplicate, P= Penicillin, Me= Methicillin, Am= Amoxicillin, A= Acetone, M= Methanol, W= Water, nd = not determined; Values are means of triplicate determination values within the same row and column followed by different superscripts of letters are statistically different ($P \leq 0.05$) against each tested microorganisms.

acetone extract to the same organism. All the different crude extracts were statistically ($P \leq 0.05$) much greater than inhibition zones of penicillin (10.7±0.68mm), methicillin (10.3±1.57mm) and amoxicillin (11±1.00mm) to the same standard strain, *S. boydii* (ATCC9289).

The inhibition zones of each solvent extracts of *J. landanoides* were statistically ($P = 0.05$) greater than the commercial antibiotics penicillin (0±00 mm), methicillin (0±.00 mm) and amoxicillin (0±.00 mm) against *K. pneumoniae* (multidrug resistant) and *E. coli* (multidrug resistant). Particularly with regard to the different solvent extracts, inhibition zone of acetone (22±1.00 mm) extract was significantly ($P \leq 0.05$) greater than that of methanol (18±2.64 mm) extract against *K. pneumoniae* (multidrug resistant). On the contrary, methanol extract (34.7±2.30 mm) was much significant ($P \leq 0.05$) than the other two crude extracts, acetone (22 ±10.39 mm) and water (22 ±3.46 mm) against *E. coli* (drug resistant) (Table 4).

The antimicrobial activity (Inhibition zone) of *J. landanoides* crude extracts of acetone, methanol and water against *C. albicans* (clinical isolate) were 16.3 ±2.51 mm, 11 ±1.00 mm and 15.3 ±2.51 mm, respectively. However, with regard to different solvent extractions, the inhibition zones showed by methanol (11±1.00 mm) extract was significantly ($P \leq 0.05$) less than acetone (16.3±2.51 mm) and water (15.3±2.51 mm) extracts of *J. landanoides* against *C. albicans* (clinical isolate).

Of the two medicinal plants, all solvent extracts of *J. landanoides* were shown the highest inhibition zone ranges from 29.3 mm (water extract) to 55.3 mm (acetone extract) against *E. coli* (ATCC2592) (Table 4). Alternatively, all extracts of *P. lanceolata* was also exhibited antibacterial activities of inhibition zone ranges from 11 mm to 14.6 mm, on the same bacterial strain Table 2.

In short, all leaf solvent extracts of the studied plants were shown great inhibition activity against the entire tested test microorganisms that have been implicated in infections, thus exhibited abroad spectrum of activities.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): MIC and the respective MBC values of all crude extracts against test microorganisms were performed using broth dilution method and streak plate method, respectively alnd are displayed on Table 5 and 6. Remarkably, all the test organisms which were inhibited by all extracts in agar well diffusion method also showed MIC and MBC raging from 6.25% to 50%. Specifically, *S. aureus* (ATCC2923), *E. coli* (ATCC2592), *S. pneumoniae* (multidrug resistant), *K. pneumoniae* (multidrug resistant) alnd *S. boydii* (ATCC9289) have been shown MIC and MBC ranging from 6.25% to 25% for both plant extracts investigated in this study.

Table 5: MIC and MBC determination of different concentration of *P. lanceolata* and *J. landanoides* crude extracts against Gram positive bacteria

Tested gram positive bacteria	Different solven t extractions	*P. lanceolata* crude extracts		*J. landanoides* crude extracts	
		MIC (%)	MBC (%)	MIC (%)	MBC (%)
S. aureus (ATCC2923)	A	25	25	6.25	12.5
	M	6.25	25	12.5	25
	W	12.5	25	6.25	12.5
MRSA	A	25	25	25	50
	M	12.5	12.5	12.5	25
	W	6.25	12.5	25	25
S. pneumoniae (ATCC49619)	A	6.25	12.5	25	50
	M	6.25	12.5	12.5	25
	W	6.25	6.25	6.25	12.5
S. pneumoniae (multidrug resistant)	A	25	25	25	25
	M	12.5	25	12.5	12.5
	W	12.5	25	6.25	12.5

Where: A= acetone, W= water, M=methanol

Table 6: MIC and MBC/MFC determination of different concentration of *P. lanceolata* and *J. landanoides* against Gram negative bacteria and *C. albicans* (clinical isolate)

	Different solvent extractions	*P. lanceolata* crude extracts		*J. landanoides* crude extracts	
		MIC (%)	MBC/MFC (%)	MIC (%)	MBC/MFC (%)
E. coli (ATCC2592)	A	12.5	12.5	6.25	6.25
	M	6.25	12.5	6.25	12.5
	W	6.25	12.5	6.25	12.5
E. coli (multidrug resistant)	A	25	25	25	50
	M	25	25	12.5	25
	W	6.25	12.5	25	25
S. boydii (ATCC9289)	A	6.25	12.5	25	25
	M	25	25	6.25	6.25
	W	6.25	12.5	12.5	25
K. pneumoniae (multidrug resistant)	A	25	25	12.5	25
	M	25	25	6.25	6.25
	W	12.5	25	12.5	25
C. albicans (clinical isolate)	A	25	25	6.25	25
	M	12.5	25	25	50
	W	25	25	12.5	25

Where: A= acetone, W= water, M=methanol

Acetone extract of *P. lanceolata* and *J. landanoides* showed the same MIC and MBC of 25% against the multidrug resistant *S. pneumonia*. Likewise, the same MIC and MBC of 25% were shown against clinical isolate of *K. pneumoniae* (multidrug resistant) with methanol extract of *P. lanceolata* alnd *J. landanoides*, respectively Table 6.

The minimum bactericidal concentrations of *P. lanceolata* against on both gram positive and gram negative bacteria was ranges from 12.5% to 25%, however, only MBC of water extract of *P. lanceolata* against *S. pneumoniae* (ATCC49619) was found to be 6.25%. MBC of each crude extract of *P. lanceolata* against *K. pneumoniae* (Multidrug resistant), *S. pneumoniae* (Multidrug resistant) alnd *S. aureus* (ATCC2923) was 25% and it was 12.5% against *E. coli* (ATCC2592). Minimum inhibitory concentration of crude extracts of *P. lanceolata* against each tested bacteria was vary from 6.25% to 25%, exceptionally, MIC of all the crude extracts against *S. pneumoniae* (ATCC49619) was 6.25% Table 5.

Water extract of *J. landanoides* was enough to inhibit the growth of *S. aureus* (ATCC2923), *S. pneumoniae* (ATCC49619) and *S. pneumoniae* (Multidrug resistant) at MIC of 6.25%, but in the rest tested pathogenic bacteria it was ranges from 12.5% to 25%. On the other hand, MIC of the different solvent extracts of the same plant against *E. coli* (ATCC2592) was 6.25% Table 5.

Table 7: Combination between methanol extract of *P. lanceolata* and pure antibiotics against pathogenic bacteria

Types of microorganisms	Pl (mm)	Am (mm)	T (mm)	Pl + Am (mm)	Pl + T (mm)
S. aureus (ATCC2923*	11	37	40	32	45
MRSA*	12	30	13	29	16
E. coli (ATCC2592)	11	22	28	16	25
E. coli (multidrug resistant)	9	0	0	17	15
S. pneumoniae (ATCC49619)	13	28	30	23	27
S. pneumoniae (multidrug resistant)	10	0	0	16	17
S. boydii (ATCC9289)	11	31	33	29	30
K. pneumoniae (multidrug resistant)	9	0	0	27	28

Where: Pl =Inhibition zone of 10% crude methanol extract of *P. lanceolata*, Am=Inhibition zone of 10% Amoxicillin, T=Inhibition zone of 10% Tetracycline, Pl+Am =Inhibition zone of 10% (*P. lanceolata* extract + Amoxicillin), Pl+T =Inhibition zone of 10% (*P. lanceolata* extract + Tetracycline), *= bacteria showed synergetic effect, - = not determined.

Table 8: Combination between water extract of *J. landanoides* and commercial antibiotics against pathogenic bacteria

Types of microorganisms	JL (mm)	Am (mm)	T(mm)	JL+ Am (mm)	JL+T (mm)
S. aureus (ATCC2923)	13	37	40	25	28
MRSA	13	30	13	18	11
E. coli (ATCC2592)	10	22	28	16	27
E. coli (multidrug resistant)	8	0	0	26	25
S. pneumoniae (ATCC49619)	11	28	30	25	28
S. pneumoniae (multidrug resistant)*	9	0	0	24	15
S. boydii (ATCC9289)	11	31	33	28	31
K. pneumoniae (multidrug resistant)	10	0	0	25	28

Where: JL=Inhibition zone of 10% crude water extract of *J. landanoides*, Am=Inhibition zone of 10% Amoxicillin, T=Inhibition zone of 10% Tetracycline, JL+Am=Inhibition zone of 10% (*J. landanoides* extract + Amoxicillin), JL+T=Inhibition zone of 10% (*J. landanoides* extract + Tetracycline), *= bacteria showed synergetic effect

MBC of methanol extracts of *J. landanoides* was 6.25% against gram negative bacterial isolates of *S. boydii* (ATCC9289) and *K. pneumoniae* (Multidrug resistant) as well as MBC of acetone was also 6.25% against *E. coli* (ATCC2592). MBC of *J. landanoides* crude extracts against each pathogenic gram positive bacteria was ranges from 12.5% to 50%, i.e. there was no MBC of 6.25% to other pathogenic bacteria strains.

The MIC of acetone extract of *J. landanoides and* methanol extract *of P. lanceolata* against *C. albicans* (clinical isolate) was 6.25% and 12.5%, respectively while the MBC of *all* extracts were 25%. Each crude extracts of *J. landanoides* were showed antifungal activities at different MIC values, as well as MFC of methanol extracts was 50% and the rest was MFC of 25% against *C. albicans* (clinical isolate) Table 6.

Evaluation of Synergetic Effect of Plant Extracts and Commercial Antibiotics: The antibiotics (tetracycline, amoxicillin) and their combination with *P. lanceolata* methanol extract were much active than the crude extract alone (Table 7). Among the antibiotics and their combination with methanol crude extracts, synergetic effect was obtained by combination of tetracycline and 10% crude extract which has been shown greater inhibition zone than the single antibacterial agents against *S. aureus* (ATCC2923) and MRSA with inhibition zone 45 mm and 16 mm, respectively. Although, 10% methanol extract of *P. lanceolata* alone inhibited the growth of bacteria and fungus, it couldn't show any synergetic effect when combined with 10% amoxicillin (Table 7).

Interaction between tetracycline with *J. landanoides* water extract was shown synergic effect against *S. pneumoniae* (multidrug resistant), *K. pneumoniae* (multidrug resistant) and *E. coli* (multidrug resistant) with amoxicillin and tetracycline (Table 8).

DISCUSSION

The development of multidrug resistant bacterial strains to many antibiotics by microorganisms has initiated the search for medicinal plants for new and effective antimicrobial constituents [23]. The advantage of medicinal plants being a source of active compounds in the management of infectious diseases has take the attention of scientist's all over the world [24, 25]. In this study, two medicinal plants, traditionally in practice for treatment of microbial infections, were tested for their antibacterial activity of the crude extracts against eight pathogenic bacterial strains and fungus (*C. albicans*). To best of our knowledge, there was no previous work reported on the antimicrobial activities of *J. landanoides*

investigated before this study and the results obtained from these plants may help as first hand information for further studies aimed at isolating and identifying the active components and brining them into *in-vivo*, as more researches were done on the antimicrobial and nematicidal activity of *P. lanceolata* [26].

Combination therapy between active plant extracts and antibacterial agents have become promising area where emerging of resistant bacteria managed and reduced [27]. In this finding, one solvent extract which has shown better antibacterial activity was selected from each plant and tested for synergism with known drugs. As investigated in this study, *P. lanceolata* methanol extract and *J. landanoides* water extract, were considered for combined effects against eight bacterial strains and specifically showed desirable synergistic effects against some strains (inhibition zone from 40 mm to 45 mm and from 13 mm to 16 mm against *S. aureus* (ATCC2923) and MRSA, respectively, as well as from 0 to 12 mm and from 0 to 15 mm against *S. pneumoniae* (multidrug resistant).

The result of this study has shown that all the standard and drug resistant isolates of bacterial and fungal strains were susceptible to all solvent extracts of tested plants with the least inhibition zone of 11 mm to largest inhibition zone of 55.3mm diameter in the agar well diffusion assay. In addition, all the tested bacterial strains including *C. albicans* (clinical isolate fungus) has shown MIC value ranging from 6.25 to 25% against the entire solvent extracts; the plants will provide effective ways to control microbial infections especially caused by those multidrug-resistant pathogens.

An extract is considered having antimicrobial activity with a specific solvent if it inhibits a particular microorganism at 50% concentration in the agar well diffusion assay [28]. Most extracted crude antibiotics from all plants showed strong activities than the controls used on the same tested pathogenic microorganisms. For instance, acetone extract of *J. landanoides* was active against *E. coli* (multidrug resistant) to (22 mm) inhibition zone where the control antibiotics (penicillin, methicillin, amoxicillin) have no any activity at all. Therefore, it is promising that the plants have antimicrobial substances with a potential to inhibit growth of tested pathogens in advance over clinically in use commercial antibiotics, especially if further processed into new drugs.

The antibacterial and antifungal activity of *P. lanceolata* leave Methanolic extract has been studied and it was not observed for the antibacterial or antifungal effects [29]. However, here in this study was shown effective antimicrobial activity to all tested pathogens, similarly the results obtained with plant extracts could be attributed to many factors (geographical location where plants grew or plant source, choice of solvent, choice of extraction method, antimicrobial test method, whether it is used fresh or dried alnd the quality of extract tested and test microorganisms), so that it is not much reliable to compare one research outcomes with publicized results or other works done before [30].

In the present study, three different solvents were used to extract compounds which are responsible for the antimicrobial activities. Unexpectedly, water extracts performed good antimicrobial activities and this shares the valuable techniques often practiced by traditional healers, usage of water to isolate important compounds for disease treatment. However, in many reports usage of water is not more accepted to extract compounds of antimicrobial activity or the extract had been showing weak antimicrobial effect [31] and it didn't perform well like acetone and methanol extracts [32]. However, in this study it was applicable and effective in equivalent to the other solvents used in both antimicrobial activity testing methods.

There were also variations in the antimicrobial activities of different solvent extracts of the same plant. For instance, antimicrobial activities of *P. lanceolata* methanol extracts dominate over acetone and water extracts towards the tested pathogens. It indicates, either the microbes might be relatively resistant to compounds of the plant which are extracted by acetone and water, or the bioactive constitutes may be miscible better in methanol than the other two.

In the present study, there were few results which are not parallel among methods used to determine antimicrobial activity (agar well diffusion and serial dilution). In the agar well diffusion assay, *J. landanoides* extracts slightly showed better antibacterial activities against drug resistant isolates than the standard strains, it is novel takes into consideration those clinical bacteria were resistant to the commercial antibiotics (penicillin, methicillin, amoxicillin). On the other outcomes, growths of standard bacterial strains were inhibited at low concentration (6.25%) than drug resistant isolates during serial dilution method. This can strengthen some reports [33] showed that comparison of methodologies (agar well diffusion and serial dilution) which are used to evaluate the antimicrobial activity may not usually give compatible results. In the other work, it was also deduced that some constituents may affect diffusible properties of the active compounds that are from crude plant extracts [34].

The antibacterial activity of *J. landanoides and P. lanceolata* extracts against those tested pathogens can robust scientific evidences of the effectiveness of medicinal plants used in the treatment of bacterial infections by the society. Some of the crude extracts which have relatively shown weak activity may be potent *in-vivo* due to immune- modulation of active compounds and its metabolic transformation into highly active intermediates when with drugs [35, 36], as few of them were proved for synergetic effect with commercial antibiotics. In addition to that, some of the weak antimicrobial activities manifested, in both diffusion and dilution methods, by plant extracts may be due to concentration of active components contained in the plant that might need further purification techniques.

Using of the plant extracts (*P. lanceolata* methanol extract and *J. landanoides* water extract) in combination with cell wall and protein synthesis inhibiting antibiotics might affect their antibacterial activities either by acting on the same target (i.e. cell wall) [37] or by increasing bacterial membrane permeability alnd acting on 30S bacterial protein synthesis [38]. For example, drug resistant *S. pneumoniae* was shown no susceptibility for tetracycline but in combination with the water extract of *J. landanoides,* inhibits the bacteria to 15 mm inhibition zone; therefore, this implies that the plant extracts may contain bioactive compounds that can enhance or reduce the efficiencies of commercial antibiotics.

The two crude leave extracts (*P. lanceolata* methanol and *J. landanoides* water) which demonstrated better activity during agar well diffusion assay and MIC/MBC determination against the tested pathogenic microorganisms were evaluated for synergism with commercial prescribed antibiotics. The standard antibiotics: amoxicillin and tetracycline were selected, because most of pathogenic bacteria were resistant to such commercial prescribed antibiotics and due to their different targets on bacteria. The need to examine the plant extracts for probability of synergy effect with antibiotics is in order to see or discuss if the combined effects of such crude extracts with standard antibiotics can bring about positive modification in the susceptibility of the test strains.

The overall synergetic activities of the plant extracts to both Gram positive and Gram negative bacterial strains, especially to those drug resistant clinical isolates, reveals that the therapeutic potentials of these plants when associated with pure drugs to control continually emerging resistant bacteria, which are becoming a threat to human health.

CONCLUSION

From the overall study, it can be concluded that, the results obtained confirm the therapeutic potentials of *J. landanoides* and *P. lanceola* leaf extracts, which are currently used by traditional healers for treatment of various diseases causing pathogen alnd affirm the traditional usage of these plants as an alternative medicine in the local community. The results can also provide baseline information for future studies about isolation, identification and characterization of the pure and active components which are responsible for antibacterial and antifungal activities of these plants and it has shown a promising potential for being used as starting material in new drug discovery. Detection of synergistic effect from association of antibiotic with crude plant extracts against clinical isolates leads to a conclusion that the plants contain compounds which can modify antibiotic resistant. Generally, outcomes of this research have been shown both plant extracts contain compounds that have antibacterial and antifungal activities and it is important to propose the plants in the control of resistant pathogens.

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