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Assessment of the Antimicrobial Activities of Leaf Extracts of *Leonotis ocymifolia* and Root Extracts of *Plumbago zeylanica* Against Pathogenic Bacteria and *Candida albicans*

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Abstract: Many infectious diseases are treated with herbal medicine throughout the history of mankind. Currently, drugs from plant origin are continued to be an effective therapeutic agents throughout the world; with herbal remedies having great impact in the improvement of primary health care in developing countries. Therefore, the objective was to characterize the anti-microbial activity of crude extracts of leaves of *L. ocymifolia* and roots of *P. zeylanica* against some standard and clinically isolated pathogenic bacteria and *C. albicans*. The leaves of *L. ocymifolia* and matured root of *P. zeylanica* L. was collected aseptically and dried under shade area in an open air at room temperature. The dried material was powdered with a mechanical grinder; then extracted with different solvents. Antimicrobial activity was determined against gram positive and gram negative pathogenic bacteria and *C. albicans*. The Minimum inhibitory concentration and minimum bactericidal (fungicidal) concentration of antimicrobial samples were determined using standard methods. The clinical and standard bacteria showed sensitivity to one or two of *L. ocymifolia* extracts with inhibition zones ranging from 10.33 ± 0.58 mm to 16.67 ± 0.58 mm. Inhibition by methanol extract was statistically ($P \leq 0.05$) greater than both Amoxicillin and Penicillin positive control drugs against *E. coli* ATCC and clinical isolates. However, no significant ($P \geq 0.05$) difference was observed against *L. ocymifolia* acetone extract with Amoxicillin and Penicillin positive control drugs for MRSA. Off all the root extracts tested, both methanol and acetone extract showed antimicrobial activity against all of the bacterial species investigated which indicates its wide spectrum of activity while aqueous extract of *P. zeylanica* induces inhibition of growth for all bacterial species except *K. pneumoniae* clinical isolate. The crude extracts of different solvent preparations had shown an interesting profile of antibacterial activity against clinical isolates which show multi drug resistance.

Key words: Antimicrobial activity · Inhibition zone · Medicinal plants, Pathogen,

INTRODUCTION

The history of plant based medicine is dated back to primordial mankind who used plants for the treatment of a range of diseases in addition to their food, shelter and aromatic values. The evidence for the use of medicinal plants for the preparation of anti infection has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old [1]. The document comprises 12 steps for drug preparation referring to over 250 various plants. Ayruveda of Indus civilization around 2500 BC, Arabian medicine of Mesopotamia, Chinese and Tibetan medicine of yellow river civilization of China and Kampo of the Japanese used plants as therapeutic agents for the treatment and prevention of disease. Some of the plants were still in use for instance, *Ephedra, Rhei rhisoma*, *Podophyllum,* etc [1,2].

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Currently, the development of multi-drug resistant strains of bacteria has been increasing. This worsens the occurrence of bacterial infections that cannot be treated with conventional antimicrobial agents. Moreover, the new generation antibiotics are less available and are expensive for resource poor countries including Ethiopia. Because of these reasons, a number of efforts should be made to discover new antimicrobial agents from different sources like micro-organisms, animals and plants [3-5]. Plants have diverse traditional applications for the treatment of different alignments in humans and animals including as antimicrobial agents [4, 6-8]. They have been used as anti infective's over centuries in Ethiopia were investigated for their antibacterial activity at different times [9]. There is no report for antibacterial activity of *L. ocymifolia* and *P. zeylanica* in the country. Therefore, the present study may be important in providing direction for finding novel antimicrobial agents from the herbs such as *L. ocymifolia* and *P. zeylanica* that can be used for future treatment of multi-drug resistant pathogens. The main objective of this study is to characterize the anti-microbial activity of crude extracts of leaves of *L. ocymifolia* and roots of *P. zeylanica* against some standard and clinically isolated pathogenic bacteria and *C. albicans*.

MATERIALS AND METHODS

Study Area: The study was conducted in Amhara Regional state, North Gondar Zone, Gondar city. Gondar city is one of the earliest cities in Ethiopia and located in North West part of Ethiopia. It is found at 738 Km away from Addis Ababa, the capital city of Ethiopia. It is particularly located at latitude and longitude of 12°36´N 37°28´E with an elevation of 2133 meters above sea level.

Plant Material Collection: The plant material for the current study was collected in the rainy season particularly between June and August 2013. The leaves of *C. macrostachyus* and *L. ocymifolia* were collected in the garden of University of Gondar and around Gondar kebele's. The root of *P. zeylanica* was collected from the trees growing around Shire Endaslassie district woreda Asgedestinbla kebele mayshak, Tigray region.

Preparation of Plant Material Extracts: In the present study, the leaves and the roots were dried in an open air at room temperature in shadow. The dried leaves and root were powdered with a mechanical grinder (KM20); the powder was stored in a sterile bottle at room temperature under dark place. The dried powder of the plant parts (leaves of *L. ocymifolia* and roots of *P. zeylanica*) was extracted with 95% methanol, 99% acetone and distilled water by maceration of each part (20g/100mL) in 250 mL Erlenmeyer flask in the respective solvents and shaked on an incubator shaker (Edmund Buhler GMBH) at room temperature for three days [10, 11]. Then the solution was filtered (using Whatman no.1 filter paper) into previously washed, dried, labeled and weighed beakers. The filtrate was concentrated by evaporation in an oven at 40°C and stored in dark bottles in refrigerator below 4°C. The antibacterial activities of the crude extract of the plants were tested against selected gram positive and gram negative test pathogenic bacteria and fungus (*C. albicans)*.

Inoculum Preparation for Antimicrobial Testing: Bacterial strains used were; *E. coli* (ATCC 2592), *E. coli* clinical isolate (multidrug resistant for Ampicillin, Amoxicillin, Tetracycline and Naldistic acid), *S. pneumonia* (ATCC 49619), *S. pneumonia* clinical isolate (resistant for Ampicillin and Penicillin) *S. aureus* (ATCC 2923), *S. boydii* (ATCC 9289), *K.pneumonia* clinical isolate (multidrug resistant for Ampicillin, Amoxicillin, Tetracycline and Cefotaxime (CTX) and Methicillin resistant *Staphylococcus aureus* (MRSA) from bacteria and *C. albicans* for anti-fungus test.

The standard strains as well as the clinical isolates were obtained from stocks of culture collections maintained at University of Gondar teaching hospital, Microbiology Laboratory. The bacteria were maintained in triptone Soya broth (UNI-CHEM) with 20% glycerol and kept at a temperature below 4°C. Sub-culturing was done on nutrient agar (UNI-CHEM) and Sabouraud Dextrose agar (DYNAMICRO), respectively, during the experiment. Two to three isolated overnight cultured colonies of each organism were transferred aseptically by sterilized wire loop into 5 ml sterile normal saline in a test tube and mixed thoroughly, using vortex mixer (GEMMY Model: VM-300p), for uniform distribution and the turbidity was compared with 0.5 McFarland standard.

Determination of Antimicrobial Activity

Agar Well Diffusion: Muller Hinton agar was prepared according to the manufacturers recommendation by dissolving the required amount of the powder in distilled water and boiled to mix thoroughly and and then autoclaved at 121oC for 15 minutes.

The sterilized media was aseptically poured into sterile Petri-plates in a laminar air flow and allowed to solidify at room temperature for 30 min. Bacterial suspension was prepared to a density of 1.5 x 10^8 cells/mL by comparing to 0.5 McFarland standards in sterile normal saline. Aliquots of the organism (100µL) were aseptically transferred to Muller Hinton agar (OXOID) using a micropipette and seeded evenly by using sterilized cotton swab. On each plate, equi-distant wells were prepared with a 6 mm diameter sterilized, cork borer, 10 mm from the edge of the plate. Hundred micro liter of each plant extract (500 mg/ml) was aseptically introduced into a respective agar wells. Amoxicillin (30 µg/mL) and Erythromycin (15 µg/mL) for gram negative bacteria and Amoxicillin (30 µg/mL), Penicillin (10 µg/mL) and Methicillin (5 µg/mL) for gram positive bacteria; were used as positive controls, while distilled water was used as negative control. This was followed by allowing the agar plate on the bench for 1 h for pre-diffusion consequently incubated at 37°C overnight and clear inhibition zones around the wells were measured and compared with inhibition zones generated by positive control drugs.

The formation of clear inhibition zone of >7 mm diameters around the wells was regarded as significant susceptibility of the organisms to the extract [12]. The experiment was performed in replicate. Experiments that gave contradicting results were repeated for the third time to minimize experimental error. Those extracts showing any inhibition at all were noted for further tests for the quantitative assessment of their activity.

Determination of Minimum Inhibitory Concentration (MIC): MIC of the preparations was determined for extracts that showed >7 mm diameter inhibition zone according to methods described by Shahidi [13] and Akinyemi *et al*, [11]. The test was performed using macro-tube dilution method where, the extract solution (500 mg/ml) was serially diluted to 250 mg/mL, 125 mg/mL and 62.5 mg/ mL, concentrations, respectively, with sterilized distilled water. The extract was; then aseptically introduced to a tube containing sterilized and specified volumes of Nutrient broth no. 2 (UNI-CHEM). The inhibition of growth was determined by comparing with negative and positive control tubes which contains pure and inoculated nutrient broth, respectively, after 24 hrs incubation at 37°C. The minimum concentration that inhibited growth was considered as MIC value of the extract.

Determination of the Minimum Bactericidal and Fungicidal Concentration (MBC/MFC): MBC/MFC was determined by sub-culturing the test tubes that does not show bacterial growth from the results obtained in MIC determination. The organisms were sub-cultured on to Nutrient agar (UNI-CHEM) and incubated at 37°C for 24 h, after which the presence of viable cells were seen. The test tubes were taken one less or greater than the MIC value reading and streaked on nutrient agar media (UNI-CHEM). A tube that yields no single bacterial colony was taken as MBC value [14].

The synergetic effects of the crude extracts of the plants with tetracycline and Ampicillin was determined for *E. coli*, *K. pneumonia* and *S. pneumonia* using agar well diffusion method. For the test 20% of Tetracycline and Ampicillin was prepared from 500mg of the drugs. Equal volume of the prepared solution was mixed with 50% crude methanol extract of *P. zeylanica* to achieve final concentration of 100 mg/mL and 250 mg/mL of the drug and crude extract respectively and then the synergetic effect of the mixture was assessed [15, 16].

Data Analysis: The experimental data was collected in Biotechnology Laboratory, University of Gondar and the collected data was analyzed using SPSS version 16.0 software. Means and standard deviations of the triplicates analysis were calculated using analysis of variance (ANOVA) to determine the significance difference between the means followed by Duncan's multiple range test ($p \leq 0.05$) and the statistically significant difference was defined as ($P \geq 0.05$).

RESULT

The leaves of *L. ocymifolia* and roots of *P. zeylanica* (20g each) were extracted with three solvents namely methanol, acetone and distilled water as described in material and methods. The percentage yield for the two plants in the present study was ranged from 4.56 to 20.69%. The maximum yield was obtained with methanol and water extracts (4.14g and 2.14g), respectively. The least amount of extract was found with acetone (0.91g). The reason for the different percentage of extracts obtained with different solvents may be due to the differences in polarity and solubility of the samples in the solvents used.

Table 1: Comparison of inhibition zones methanol, acetone and water crude extracts of L. ocymifolia within and against positive control drugs for gram positive bacteria.

		Inhibition zones (mm) ± SD			
Organism		Solvent extract	Amoxicillin	Penicillin	Methicillin
S. aureus ATCC	MeOH	14.67 ± 0.58^{a}	20.00 ± 1.00^{b}	22.33 ± 1.53^{c}	11.00 ± 1.00^{d}
	Act	15.33 ± 0.58^{a}	20.00 ± 1.00^{b}	22.33 ± 1.53^{c}	11.00 ± 1.00^{d}
	Aq	0.00^{a}	20.00 ± 1.00^{b}	22.33 ± 1.53^{c}	11.00 ± 1.00^{d}
MRSA	MeOH	16.00 ± 1.00^{a}	10.33 ± 1.53^{b}	12.67 ± 1.53^{c}	0.00^{d}
	Act	11.67 ± 0.58^{bc}	10.33 ± 1.53^{b}	12.67 ± 1.53^{c}	0.00^{d}
	Aq	12.33 ± 0.58^{a}	10.33±1.53^{b}	12.67 ± 1.53^{a}	0.00^{c}
S. pneumoniae ATCC	MeOH	15.00 ± 1.00^{a}	12.33 ± 1.53^{b}	11.67 ± 1.53^{b}	11.67 ± 0.58^{b}
	Act	12.00 ± 1.00^{a}	12.33 ± 1.53^{a}	11.67 ± 1.53^{a}	11.67 ± 0.58^{a}
	Aq	0.00^{a}	12.33 ± 1.53^{b}	11.67 ± 1.53^{b}	11.67 ± 0.58^{b}
S. pneumoniae (clinical)	MeOH	10.33 ± 0.58^{a}	7.33 ± 0.58^{b}	0.00^{c}	8.67 ± 0.58^{d}
	Act	11.33 ± 0.58^{a}	7.33 ± 0.58^{b}	0.00^{c}	8.67 ± 0.58^{d}
	Aq	0.00^{a}	7.33 ± 0.58^{b}	0.00^{a}	8.67 ± 0.58^{c}

MeOH= methanol, Act= acetone, Aq= aqueous. Values are means of triplicate determinations. Values within the same row followed by different supper scripts (letters a, b, c) are significantly different at ($P \leq 0.05$).

Table 2: Comparison of inhibition zones methanol, acetone and water crude extracts of L. ocymifolia within and against positive control drugs for gram negative bacteria and fungus.

		Inhibition zones (mm) ± SD		
Organism		Solvent extract	Amoxicillin	Erythromycin
K. pneumoniae (clinical)	MeOH	16.00 ± 1.00^{a}	0.00^{b}	9.00 ± 1.00^{c}
	Act	0.00^{a}	0.00^{a}	9.00 ± 1.00^{b}
	Aq	0.00^{a}	0.00^{a}	9.00 ± 1.00^{b}
S. boydii ATCC	MeOH	13.33 ± 0.58^{a}	18.67 ± 1.53^{b}	20.67 ± 1.53^{c}
	Act	11.00 ± 1.00^{a}	18.67 ± 1.53^{b}	20.67 ± 1.53^{c}
	Aq	0.00^{a}	18.67 ± 1.53^{b}	20.67 ± 1.53^{c}
E. coli ATCC	MeOH	16.67 ± 0.58^{a}	8.67 ± 0.58^{b}	14.00 ± 1.00^{c}
	Act	14.33 ± 0.58^{a}	8.67 ± 0.58^{b}	14.00 ± 1.00^{a}
	Aq	0.00^{a}	8.67 ± 0.58^{b}	14.00 ± 1.00^{c}
E. coli (clinical)	MeOH	13.00 ± 1.00^{a}	0.00^{b}	0.00^{b}
	Act	12.67 ± 0.58^{a}	0.00^{b}	0.00^{b}
	Aq	0.00^{a}	0.00^{a}	0.00^{a}
Fungus			Kanamycin	
C. albicans (clinical)	MeOH	10.67 ± 0.58^{a}	21.00 ± 1.00^{b}	
	Act	0.00^{a}	21.00 ± 1.00^{b}	
	Aq	0.00^{a}	21.00 ± 1.00^{b}	

MeOH= methanol, Act= acetone, Aq= aqueous Values are means of triplicate determinations. Values within the same row followed by different supper scripts (letters a, b, c) are significantly different at ($P \leq 0.05$).

Antimicrobial Activity Testing: The antimicrobial testing was done by agar well diffusion technique and the results obtained were recorded in Table 1 and 2. The clinical and standard strains showed sensitivity to *L. ocymifolia* preparations either in methanol, acetone or water extracts with inhibition zone size ranging from 10.33 ± 0.58 mm to 16.67 ± 0.58 mm. From the preparations methanol extract showed greater inhibition zone than acetone. The comparison of *L. ocymifolia* crude preparation by each of the solvents used, show differences between the methanol and acetone extracts (Table 1).

In this study, significant difference was observed in between methanol and acetone extracts except to *S. aureus* ATCC and *E. coli* clinical isolates, in contrast methanol and acetone extract showed significantly ($P \leq 0.05$) high antibacterial activity than the aqueous extract against the tested microbes (Table 1). Significant difference ($P \geq 0.05$) was seen in the bactericidal activity of *L. ocymifolia* methanol extract when compared to acetone and water extract against *K. pneumoniae* clinical isolate, hence, methanol extract is greater inhibition zone than the other two solvent extracts.

Table 3: Comparison of inhibition zones of methanol, acetone and water crude extracts of P. zeylanica within and against positive control drugs for gram positive bacteria.

		Inhibition zones (mm) ± SD			
Organism		Solvent extract	Amoxicillin	Penicillin	Methicillin
S. aureus ATCC	MeOH	40.33 ± 0.58[a]	20.00 ± 1.00[b]	22.33 ± 1.53[c]	11.00 ± 1.00[d]
	Act	41.00 ± 1.00[a]	20.00 ± 1.00[b]	22.33 ± 1.53[c]	11.00 ± 1.00[d]
	Aq	29.00 ± 1.00[a]	20.00 ± 1.00[b]	22.33 ± 1.53[c]	11.00 ± 1.00[d]
MRSA	MeOH	36.00 ± 1.00[a]	10.33 ± 1.53[b]	12.67 ± 1.53[b]	0.00[c]
	Act	36.67 ± 1.53[a]	10.33 ± 1.53[b]	12.67 ± 1.53[b]	0.00[c]
	Aq	20.33 ± 1.53[a]	10.33±1.53[b]	12.67 ± 1.53[b]	0.00[c]
S. pneumoniae ATCC	MeOH	21.00 ± 1.00[a]	12.33 ± 1.53[b]	11.67 ± 1.53[b]	11.67 ± 0.58[b]
	Act	44.00 ± 1.00[a]	12.33 ± 1.53[b]	11.67 ± 1.53[b]	11.67 ± 0.58[b]
	Aq	17.00 ± 1.00[a]	12.33 ± 1.53[b]	11.67 ± 1.53[b]	11.67 ± 0.58[b]
S. pneumoniae (clinical)	MeOH	21.67 ± 0.58[a]	7.33 ± 0.58[b]	0.00[c]	8.67 ± 0.58[d]
	Act	25.00 ± 1.00[a]	7.33 ± 0.58[b]	0.00[c]	8.67 ± 0.58[d]
	Aq	16.67 ± 0.58[a]	7.33 ± 0.58[b]	0.00[c]	8.67 ± 0.58[d]

MeOH= methanol, Act= acetone, Aq= aqueous. Values are means of triplicate determinations. Values within the same row followed by different supper scripts (letters a, b, c, d) are significantly different at $P \leq 0.05$.

The table compares the results of crud extracts of *L. ocymifolia* with positive control drugs and the numbers in the same column and row designated with different letters as a, b, c, d are significantly different. The result of this study shows that, inhibition of bacterial growth by leaves of *L. ocymifolia* methanol extract was significantly ($P \leq 0.05$) greater than both Amoxicillin and Penicillin positive control drugs in contrast, considerable difference was not observed between acetone extract, Amoxicillin and Penicillin positive control drugs against MRSA. With regard to *S. pneumoniae* clinical isolate, both methanol and acetone preparations exhibited significantly greater inhibition zone ($P \geq 0.05$) than that of the positive control drugs.

The antimicrobial activity of *L. ocymifolia* extract with positive control drugs against gram negative bacteria was presented in Table 2. The results of methanol extract shows significantly ($P \leq 0.05$) high activity against *K. pneumoniae* clinical, *E. coli* standard and clinical strains when compared to the positive control drugs, while significantly ($P \geq 0.05$) lower activity of methanol and acetone extract was observed against *S. boydii* (ATCC) compared to the standard drugs. The acetone extract demonstrated significantly higher activity against *E. coli* clinical isolate when compared to positive control drugs Amoxicillin and Erythromycin.

On the other hand, this study focused on the antimicrobial action of *P. zeylanica* root extracts. Out of the entire root extracts tested, both methanol and acetone extract showed antimicrobial activity against all of the bacterial species investigated, indicating its broad spectrum of activity. The aqueous extract of *P. zeylanica* induces inhibition of growth for all bacterial species except *K. pneumoniae* clinical isolate. From the results obtained, methanol and acetone root extracts showed significant ($P \leq 0.05$) difference in the antibacterial activity against *S. aureus* ATCC and MRSA than aqueous extract. The comparison of *P. zeylanica* root crude extracts against standard positive control drugs for gram positive bacteria was indicated in Table 3; generally all extracts showed significantly higher ($P \geq 0.05$) inhibition zones when compared to the positive control drugs used against gram positive bacteria's.

Roots of *P. zeylanica*, methanol and acetone extracts were shown significantly ($P \leq 0.05$) greater inhibition zone than the positive control drugs Erythromycin and Amoxicillin against *K. pneumoniae* clinical isolate. Inhibition by methanol and acetone preparation was statistically significant ($P \geq 0.05$) greater than both Amoxicillin and Erythromycin positive control drugs against *E. coli* ATCC. In contrast no significant ($P \geq 0.05$) difference was observed between aqueous extract and Erythromycin positive control drug against *E. coli* standard isolate. The antibacterial activity demonstrated by Amoxicillin and Erythromycin was significantly low ($P \leq 0.05$) as compared to the crude solvent extracts against standard and drug resistant gram negative bacteria tested.

With regard to antifungal activity, the highest activity 30.67 ± 0.58 mm diameter of zone of inhibition was observed for *P. zeylanica* root methanol extract, 29.33 ± 1.15 mm for acetone extract both of which are significantly ($P \leq 0.05$) greater than positive control drug Kanamycin which was showed 21 ± 1.00 mm of zone of inhibition and 13.33 ± 1.15 mm against aqueous extracts. Only the methanol extract of *L. ocymifolia* has showed

Table 4: Comparison of inhibition zones methanol, acetone and water crude extracts of P. zeylanica within and against positive control drugs for gram negative bacteria and fungus.

		Inhibition zones (mm) ± S D		
Organism		Solvent extract	Amoxicillin	Erythromycin
K. pneumoniae (clinical)	MeOH	11.67 ± 1.15[a]	0.00[b]	9.00 ± 1.00[c]
	Act	14.00 ± 1.15[a]	0.00[b]	9.00 ± 1.00[c]
	Aq	0.00[a]	0.00[a]	9.00 ± 1.00[b]
S. boydii ATCC	MeOH	35.00 ± 1.00[a]	18.67 ± 1.53[b]	20.67 ± 1.53[b]
	Act	28.33 ± 1.53[a]	18.67 ± 1.53[b]	20.67 ± 1.53[b]
	Aq	29.33 ± 1.15[a]	18.67 ± 1.53[b]	20.67 ± 1.53[b]
E. coli ATCC	MeOH	17.33 ± 0.58[a]	8.67 ± 0.58[b]	

Table 5: MIC and MBC (MFC) value for leave extracts of L. ocymifolia for the tested pathogenic microorganisms

	MIC &MBC mg/mL					
	Methanol		Acetone		Aqueous	
Organism	MIC	MBC	MIC	MBC	MIC	MBC
G. positive	125	250	125	250	ND	ND
Staph. aureus ATCC						
MRSA	125	250	125	500	250	500
Strep. pneumonia ATCC	62.5	250	125	250	ND	ND
Strep. pneumonia (clinical)	250	500	250	500	ND	ND
G. negative	250	500	250	500	ND	ND
K. pneumonia (clinical)						
Shiegella boydii ATCC	125	250	125	250	ND	ND
E. coli ATCC	125	250	125	500	ND	ND
E. coli (clinical)	125	500	250	500	ND	ND
Fungus	250	MFC 500	ND	ND	ND	ND
C. albicans (clinical)						

*ND-not determined

anti-candidal effect with inhibition zones of 10.67 ± 0.58 mm. The anticandidal effect of *L. ocymifolia* extracted by methanol was shown significantly ($P \leq 0.05$) high activity when compared to acetone and aqueous extracts.

Determination of MIC and MBC (MFC) of the Plant Extracts: Regarding *L. ocymifolia,* methanol extract the smallest minimum inhibitory concentration was found to be 62.5 mg/mL for *S. pneumoniae* ATCC 49619. The value for methanol extract with 125 mg/mL which represents 55.56 % was the highest which is followed by 250 mg/mL with 22.22% and the acetone extract showed the smallest inhibition concentration of 125 mg/mL which was 55.56 % for the tested organisms. From the extracts of *L. ocymifolia* only the methanol extract showed antifungal activity at a concentration of 250 mg/mL. The MBC (MFC) value for *L. ocymifolia* methanol and acetone extracts was found 250 mg/mL for standard bacteria and around 500 mg/mL for clinical isolates.

The MICs and MBCs (MFC) of the extracts of *P. zeylanica* showed varying degrees of effectiveness. The methanol and acetone extract had MIC values ranging from 62.5 to 250 mg/mL for the standard as well as clinical isolates. The aqueous extract exhibited MIC values ranging from 125 to 250 mg/mL among the tested organisms.

The value 62.5 mg/mL was recorded in methanol and acetone extracts among *S. aureus* ATCC 2923, *S. pneumoniae* ATCC 49619, *S. boydii* ATCC 9289 and fungus *C. albicans* clinical isolate which represents 44.4% and 55.56% of the concentrations used respectively. The methanol extract of *P. zeylanica* with a concentration of 125 mg/mL (44.4%) was recorded for MRSA*, E. coli* ATCC, *K. pneumoniae* and *S. pneumoniae clinical isolates* and 250 mg/mL (11.11%) was observed for *E. coli* clinical isolate. The MIC and MBC of the test results for *L. ocymifolia* and *P. zeylanica* are tabulated in Tables 5 and 6.

Table 6: MIC and MBC (MFC) value for roots extracts of P. zeylanica for the tested pathogenic microorganisms

	MIC &MBC mg/mL					
	Methanol		Acetone		Aqueous	
Organism	MIC	MBC	MIC	MBC	MIC	MBC
G. positive	62.5	62.5	62.5	125	250	125
S. aureus ATCC						
MRSA	125	250	62.5	125	125	250
S. pneumonia ATCC	62.5	125	62.5	125	125	250
S. pneumonia (clinical)	125	250	125	250	250	500
G. negative	125	250	250	500	ND	ND
K. pneumonia (clinical)						
Shiegella boydii ATCC	62.5	125	62.5	125	250	250
E. coli ATCC	125	250	125	250	125	250
E. coli (clinical)	250	500	125	250	250	500
Fungus	62.5	MFC 125	62.5	MFC 125	125	MFC250
ND-not determined						

DISCUSSION

The use of different parts of plants as a source of treatment for various illnesses has been employed since prehistoric times to treat human and animal diseases. Several countries still rely on plants and herbs as the main sources of traditional medicine for primary health care. *L. ocymifolia* and *P. zeylanica* and other species of plants are widely employed in the traditional herbal medicine because of their multiple practices in the treatment of different diseases. The scientific evaluation studies on the effectiveness of these plants *in vitro* on infectious microbes may give acceptance to the ethno botanical use of the plant parts in the treatment of infectious disease and alleviate problems that accompanied with infection due to pathogenic bacteria and fungus [17]. The leaves of *L. ocymifolia* and roots of *P. zeylanica* crude extracts with the solvents methanol, acetone and water were investigated.

Currently various infections caused by bacteria and fungi are become a serious health problems of human beings [18]. The common bacterial pathogens included in this study were: *S. aureus* (a wound infecting pathogen), *S. pneumoniae* (causative agent for lung inflammation), *E. coli* (causes gastroenteritis, urinary tract infections, neonatal meningitis), *K. pneumoniae* (causes destructive changes to the lungs through inflammation), *S. boydii* (causes diarrhea) and *C. albicans* a causal agent of opportunistic oral and genital infections in humans [19]. Most strains of these pathogenic bacteria are acquired multi drug resistance against most of commonly prescribed antimicrobial agents: As a result treatments of such pathogenic infections are a severe current dispute which needs intensive investigation to have better treatment options [20].

In spite of their cell membrane permeability difference, the results of the current study shown that, the methanol and acetone extract of *P. zeylanica* and *L. ocymifolia* exhibited a broader spectrum of antimicrobial activity on gram negative and gram positive bacterial strains. The demonstrated higher bactericidal activity of *P. zeylanica* and *L. ocymifolia* extract against standard and clinical isolates of gram positive and gram negative bacteria may be clinically useful in treating infections generally attributable to the tested organisms.

In the present study: leaves of *L. ocymifolia* extracted by methanol and acetone were shown strong antibacterial activity against most of the bacterial strains used. This finding also demonstrates the fact that the plant might contain important compounds that have used for the treatment of infectious diseases caused by microbes tested in the present study.

Methanol and acetone extract of *L. ocymifolia* was shown minimum inhibition zones at a concentration of 500 mg/mL against *S. pneumoniae* clinical isolate and *S. boydii* (ATCC), respectively. The methanol extract was also shown minimum inhibition zones for fungus *C. albicans;* in contrast no inhibition was seen with the acetone extract for *K. pneumoniae* and *C. albicans* in agar well diffusion method. Except for MRSA, the aqueous extract of *L. ocymifolia* was not shown any activity against the test organisms.

The present study showed that *S. pneumoniae* and *K. pneumoniae* clinical isolate had a higher MIC value which can be converted that a higher concentration of the *L. ocymifolia* extract is required to inhibit the organism's growth, while *S. pneumoniae* ATCC have a lower MIC value and would require a very low extract concentration to inhibit its growth. The MBC value of the extract was high for *E. coli, S. pneumoniae* and *K. pneumoniae*

clinical isolates which were drug resistant. The other bacteria test organisms have an intermediate MIC and MBC values for *L. ocymifolia* methanol and acetone extracts. This could be explained that higher concentrations of the extract above those used in the experiment may be required to inhibit the growth of these organisms. Hence, the *L. ocymifolia* extract was bacteriostatic to *E. coli, S. pneumoniae* and *K. pneumoniae* clinical isolate at the concentration used but was bactericidal against other tested pathogenic organisms [21].

In the other hand, the results obtained indicated that methanol, acetone and aqueous, *P. zeylanica* root extracts were inhibited the growth of the isolates used. All the organisms tested were susceptible to both methanol, acetone and water extracts at different concentrations of root extract of *P. zeylanica* with the exception of *K. pneumoniae* which was not shown susceptibility to the aqueous extract. This shows that the extract contains substance that can inhibit the growth of pathogenic microorganisms under investigation.

The results of this study was shown that the extracts of *P. zeylanica* has been exhibited varied antimicrobial activities against the tested pathogenic organisms including both gram positive and gram negative bacterial strains and fungal strain, which may be indicative of the presence of broad spectrum antibiotic compounds in this extract. This may be an immense advantage in fighting the hazard of antibiotic resistant pathogens in recent times.

The anti candidal effects of the crude extracts of *P. zeylanica* and methanol extracts of *L. ocymifolia* was important in using these plants for the treatment of such infection. Previous studies on the antibacterial and antifungal activity conducted on different plants have showed significant activity of organic (like methanol, ethyl acetate, acetone, etc) extracts against different bacteria and fungi [22]. This provides support to the present study in which organic solvents are a better solvent for extraction and isolation of phytochemicals having antimicrobial activity [23]. The present study was shown that no or moderate activity of water extract to the organisms tested which was in agreement with earlier reports that indicate use of organic solvents is always better for extraction and isolation of antibacterial compounds [24]. This might be due to the insoluble nature of active compounds in water or there might be side reactions that decrease the activity of the compounds. As it was known that the society traditionally uses the plant material without diluting with water water.

CONCLUSION

The most medically important microbial and other infections for which Ethiopian traditional medicinal plants employed include diarrhea, stomach ache, skin disorder, syphilis, gonorrhea, rabies and others. Although there is information regarding the prolonged and ordinary local use of the traditional medicinal plants *L. ocymifolia* and *P. zeylanica*; large gaps has been exist in providing scientific evidences.

The results of this study suggested that crude extracts of different solvent preparations of *P. zeylanica* and *L. ocymifolia* have shown an interesting profile of antibacterial activity against clinical isolates which show multi drug resistance and reference strain of the tested pathogenic organisms. This finding can indicate the presence of bioactive compounds in *P. zeylanica* and *L. ocymifolia* responsible for the observed activity. The preliminary results of this investigation indicates that *P. zeylanica* root have high potential of antimicrobial activity than *L. ocymifolia*. Although the *in vitro* antibacterial activity of these plant extracts does not yet justify their use in the treatment of infections caused by the tested pathogenic bacteria and fungus, these results confirm the use of these medicinal plants in the treatment of infection caused by the aforementioned bacteria and fungus.

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