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Antibacterial Activities of Selected Medicinal Plants against Multi-Drug Resistant Bacteria Isolated from Urine Samples of Catheterized Patients

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Abstract

Background

The current practice of prescribing antibiotics to treat UTIs is empirical and nonsensical in most resource-limited countries. The difficulties in the use of culture and DST for patients with UTIs, the irrational use of antibiotics, prolonged time usage, and the availability of a few drug classes are boosting the emerging of antibiotic resistance. Therefore, assessing and evaluating activities of traditional medicinal plants against such infectious organisms is critical.

Methodology

Three plant leaves were collected and extracted using the standard cold extraction methods and the yield was obtained. The extracted ingredients were then subjected to Multidrug Resistant (MDR) UTI causing bacteria isolated from catheterized patients to determine their antibacterial activity. MIC and MBC values were also carried out.

Results

Leaves of *Lannea fruticosa* gave the highest yield in all the extracts in its aqueous extract (22.6%), chloroform extract (7.6%), ethanol-aqueous extracts (19.04%). From the isolated organisms *E. coli* (0.83), *P. aeruginosa* (0.75), *P. mirabilis* (0.83) had highest MAR INDEX and were exclusively selected for the study. The aqueous extract of *Lannea fruticosa* showed the highest activity against both *P. aeruginosa* and *P. mirabilis* which was 20 mm and 19.5 mm of inhibition zone respectively. The MIC values of aqueous extracts of *Lannea fruticosa* against *P. mirabilis* and *P. aeruginosa* was at 1.953 mg/ml and the highest MBC value was recorded at 15.86 mg/ml in the ethanol-aqueous extract of *Malva parviflora* against *P. aeruginosa*.

Conclusion

Generally, all plant extracts revealed a good antibacterial effect with a very remarkable inhibition zone against the isolated organisms even better than some antibiotics supplemented to the patients. This significant result may be due to the active phytochemical compounds the plants contain. Therefore, evaluating the activities of these medicinal plants on *in vivo* activities and further toxicological studies will be beneficial as it will help in formulating effective antibiotics against infectious organisms.

Keywords: Antimicrobial activity; Drug-resistant bacteria; Catheterized patients; Medicinal plants; MIC; MBC

Introduction

Urinary Tract Infections (UTIs) are among the most common infectious diseases all over the world. Urinary tract infections encompass ranges of morbidity including pyelonephritis and cystitis which are characterized by the existence of microorganism in urinary tract [1]. Urinary tract infections are the major public health problems affecting millions of people every year. Worldwide, it is estimated that about 150 million people are diagnosed with UTI each year [2]. Generally, it is believed that about 35% of healthy individuals suffer from symptoms of UTI at some stages in their lives [2]. To treat such infections, different antibiotics were developed. These antibiotics have saved the lives of millions of people and have contributed to the major gains in life expectancy over the last many years. However, the clinical efficacy of many existing antibiotics is being threatened by the emergence of Multidrug-Resistant (MDR) pathogens the recent appearance of strains with reduced susceptibility as well as, undesirable side effects of certain antibiotics [3,4]. The effect is highly pronounced in the third world as the costly replacement drugs for treating the highly resistant infectious diseases are unaffordable [5]. Currently, bacterial resistance to many antimicrobials is being a global problem

and calls to scientists to discover new antibacterial agents. To solve this problem, researchers are usually focusing on natural products to develop better medications against multidrug-resistant microbial strains [6]. Usually, catheterized patients are at high most risk for developing UTIs due to the indwelling of catheter and difficulties in urination because of anatomical problems. Moreover, the inappropriate use of antibiotics and urinary catheter in such patients results in urinary tract colonization and infection with resistant bacteria and is an important cause of morbidity and mortality in these populations [7].

A wide variety of secondary metabolites, which are used either directly as precursors or as compounds in the pharmaceutical industry are produced by plants. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids and gums which are capable of producing definite physiological action on the body [8]. It is expected that other than plant used by antibiotics, plant extracts showing target sites will be more active against drug-resistant microbial pathogens [8]. The antimicrobial potency of plants is believed to be due to the presence of phytochemicals they contain [9]. Based on the WHO report 80% of the communities living in the developing countries relies heavily on the use of traditional medicinal plants to treat most infectious diseases [10]. Recently different studies have been published on the antimicrobial activities of different plants extracts [11,12]. Recently, it is concluded that the phytochemicals of the medicinal plants have the ability to stop the growth of Multidrug-Resistant organisms [13].

The prevalence of the UTIs in the community, the difficulties in the use of culture for isolating the causative organisms and performing Drug susceptibility test for the use of the appropriate treatment in most resource-limited settings like Eritrea, contribute to increasing the antibiotic resistance in most of the patients suffering from UTIs. This is most notably seen in patients with recurrent UTIs and catheterized patients. Therefore, the current study is aimed to investigate the efficacy of the traditional medicinal plants which are commonly used to treat UTIs in the community. The current study will provide baseline information to further investigate these plants In vitro in order to investigate new biologically active compounds for the production of novel drugs. This study will provide scientific validation to the traditional knowledge of using medicinal plants against infectious human diseases.

Methodology

Study design

The study was an experimental study designed to evaluate the antimicrobial activities of selected traditional medicinal plants against multi-drug resistant bacteria isolated from urine specimens of catheterized patients.

Study population and study setting

The current study was conducted in Denden Hospital located in Asmara a capital city of Eritrea. This hospital is serving to Eritrean war disabled veteran patients. From the total patients nursed at the hospital, twenty-four patients were randomly selected and enrolled in the study. These patients are followed in the hospital and one of their urine samples is sent to the microbiology department of the National health laboratory for culture and DST on a monthly basis. Then after, the appropriate treatment is prescribed based on the culture and DST results obtained. The catheter also changed in every two weeks and in any case or leakage is observed.

Urine sample collection

The patients enrolled in the study were informed on how to collect the biological specimens prior to the collection and all the standard procedures for sample collection were followed. As all of the patients were chronic, the indwelling catheter used is replaced every 2 weeks. Then, the urine specimens for culture were collected through the replacement catheter to avoid a urine specimen contamination with organisms growing in the catheter biofilms. The urine samples were collected early in the morning in sterilized, clean, dry, leak-proof containers which are prepared for the purpose. After voiding the first stream to avoid contamination and to prevent from the biofilms created in the catheter, an average volume of 30 ml of the urine sample was collected in each container from each patient. The samples were then transported in an icepack box immediately to the National microbiology department, National health laboratory for further process.

Isolation and identification of bacteria from catheterized UTI infected patients

Twenty four urine samples which were initially obtained from Denden Hospital were processed in the Microbiology department of National Health Laboratory in Asmara, Eritrea within thirty minutes of sample collection. First, the urine samples from the UTI patients were cultured in the Nutrient agar and incubated for 24 hr at 37°C. After overnight incubation, the characteristics of the microorganisms such as morphology, shape, size, number of colonies were assessed for the plates with bacterial growth. Subsequently, subculturing was done for the Medias with more than one colonial growth. Furthermore, the gram staining procedure was performed to differentiate the organisms as gram positive or gram negative. Following gram staining, selective and differential agar medium which include Nutrient agar, MacConkey agar, Mannitol salt agar and Chocolate agar were used in the present study and they were incubated for 24 hrs at 37°C prior to reading. Final identification of bacteria was done on the basis of biochemical testing such as Indole production test, Citrate utilization test, TSI (Triple sugar iron) test, Urease activity test, MR and VP test.

Collection and identification of medicinal plants

Leaves of three different plants which are used by traditional healers were collected from different places of Eritrea during the period of study, May 2018. The leaves of these plants (*Chenopodium murale, Malva parviflora and Lannea furticosa*) were obtained from the vicinity of Asmara and remote areas of the country, which were then used for the present experimental study. The plants were:

Malva parviflora is an annual or perennial herb that is native to Northern Africa, Europe and Asia and is widely naturalized elsewhere. Common names include cheeseweed, cheeseweed mallow, Egyptian mallow and locally known as Lhtit. Traditionally it is used in the treatment of some inflammatory disorders. It has antioxidant and antipyretic activity. It can also be used as a poultice on swelling running sores and boils [14].

Chenopodium murale (Chenopodiaceae) is an erect, annual herb with 30-60 cm in height with broad angular leaves. It is locally known as Hamli kubo which has a wide application in folk medicines as an

anthelmintic, stomachic, antispasmodic, diaphoretic, emmenagogue, for the pain of amenorrhea as an abortifacient and for the relief of asthma, catarrh, and migraine. The seeds are edible, and the shoots, stalks, and leaves can be eaten as greens [15].

Lannea fruticosa is a 3-10 m tree which is found in Tropical Africa, Sub-Saharan areas of Niger and Nigeria to northern Sudan, Eritrea, Ethiopia and Uganda and has antimicrobial activity. It is very attractive to ants. It is locally known as dugdugna [16].

Extraction and filtration methods

The leaves of the three medicinal plants were thoroughly washed with running tap water and shade dried. The dried plant leaves were disintegrated by feeding it into a hammer mill. The objective for powdering the plant material was to rupture its organ, tissue and cell structures so that its medicinal ingredients are exposed to the extraction solvent. Furthermore, sizes of the leaves were reduced to maximize the surface area, which in turn enhances the mass transfer of active principle from plant material to the solvent. Then 100 grams from each powdered plants were extracted separately with ethanol-aqueous, chloroform and water. It was left out on an electronic shaker for 48 hrs at 100 rpm. The extract so obtained was separated out from the marc (exhausted plant material) by allowing it to trickle into a holding tank through the built-in false bottom of the extractor, which is covered with a filter cloth. The marc is retained at the false bottom, and the extract was received in the holding tank. From the holding tank, the extract was further filtered using Whatman filter paper no.1 to remove fine or colloidal particles from the extract. The filtered extract was then concentrated under vacuum using the rotary evaporator to produce a thick concentrated extract. Finally, the extracts were dried by placing it in the water bath at 40°C. The final residue was kept in tightly closed clean glass bottles and stored at 4°C for further use.

Antibiotics susceptibility profile of clinical isolates

Each isolated gram negative and gram positive organism was tested against twelve and five standard antibiotic discs respectively. The standard antibiotic discs used in the current study were
Amikacin (30 µg), Ampicillin (10 µg), Cephalexin (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Co-trimoxaz (25 µg), Gentamycin (10 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Rifampin (5 µg) and vancomycin (10 µg). An antibiotic sensitivity test was carried out using the modified Kirby Bauer's agar discs diffusion technique. After the inoculation of test organisms in the Muller Hinton agar media which was prepared in various agar plates, the antibiotic discs were placed using disc dispenser. The plates with the antibiotic discs were incubated at 37°C for 24 hrs to observe the zones of inhibition produced by the standard antibiotics. Then the sensitivity status was given based on the National Health Laboratory's standard table.

Multiple antibiotic resistance index study

The MAR index of each of the isolates was determined using the formula [17].

MAR index=a/b

Where a: number of antibiotics to which the isolate was resistant to

b: number of antibiotics to which the isolate was subjected to

Antibacterial activity of plant extracts against clinical bacterial isolates

The selected multidrug-resistant organisms which were previously subjected to the standard antibiotics were then tested against the medicinal plant extracts. First, a sterile swab was used to make a suspension from each prepared bacteria isolate in which they were adjusted to 0.5 McFarland standards. The antimicrobial bioassay was performed by using both disc diffusion and agar well diffusion methods. The dried extracts were dissolved in 5% DMSO to prepare one concentration of 500 mg/ml. Suspensions of the bacterial strains were inoculated on Muller Hinton agar plates using a sterile swab. The swab was streaked evenly over the surface of the medium to ensure confluent growth. One hundred µl of the concentration was introduced into the respective holes using micropipette. An equal amount of DMSO was used as a negative control and finally, all the plates were incubated at 37°C for 24 hrs to observe the zone of inhibition produced by the extract.

MIC and MBC

Following antimicrobial bioassay, the determination of Minimum Inhibitory Concentration (MIC) and Maximum Bactericidal Concentration (MBC) was conducted. Dilution tests were used to determine the MIC directly by using serial dilutions of the plant extracts with the highest antibacterial activity against clinical isolates in broth with the range of 1.9531 mg/ml-250 mg/ml) concentrations. The bacterial inoculums were adjusted to 0.5 McFarland standards and added to the broth. After incubation overnight, the tubes were examined for turbidity produced by bacterial growth. The MIC was defined as the lowest concentration at which there was no visible growth of the test organisms [17]. Afterward, MBC (minimum bactericidal concentration) was carried out by streaking the surface of Muller Hinton agar with non-turbid tubes from MIC. The plates were incubated overnight and investigated for bacterial growth. The MBC was defined as the lowest concentration at which no organisms were recovered [17].

Data analysis

All the experiments of antimicrobial susceptibility testing were performed in triplicate. The results were expressed as the mean ± standard deviation (SD). Data were statistically analyzed by using one-way analysis of variance (ANOVA) followed by Tukey's HSD test using SPSS version 20. P-values were calculated and $P<0.05$ was considered as statistically significant.

Result

Percentage yield of the plant extracts

The percentage yield was calculated from all the individual leave extracts of the selected plants. The crude extracts of *Lannea fruticosa* showed the highest yield percentage within all solvent-solvent comparisons with aqueous extract (22.6%), chloroform extract (7.6%) and ethanol-aqueous extract (19.04%). Higher yield was also seen in aqueous extracts of *Chenopodium murale* and *Malva parviflora* which were 20.2% and 19.5% respectively as it can be seen in Figure 1.

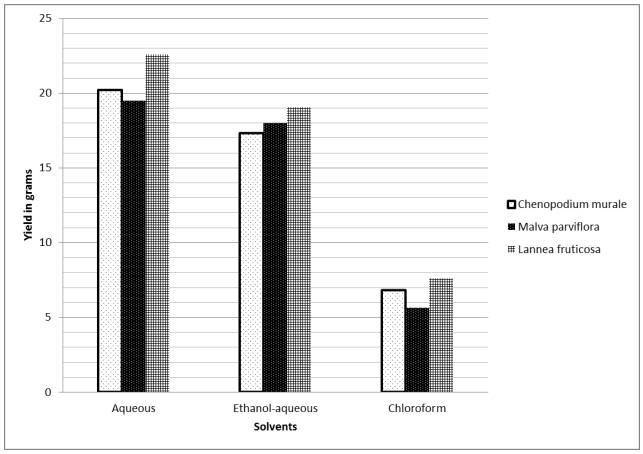

Figure 1: Percentage yield of the different extracts.

Demographic data of catheterized patients

During the study period, a total of 24 catheterized patients were randomly selected in which 20 were males with the remaining 4 being females and 79% of the patients were over the age of 50 years. After all the samples streaked and incubated for 24 hrs on nutrient agar medium they were assessed for bacterial growth. Bacterial growth was observed on 20 of the 24 plates used with some of the plates having more than one colony (Table 1). Then subsequent sub-culturing was carried out for those plates with more than one colonial growth for the sake of separating the multiple colonies forming units. The entire gram staining procedure was carried out and a total of 27 organisms were isolated in which 23 organisms were found out to be gram-negative and the remaining four-gram positive organisms. After that, all the organisms were differentiated by using their respective selective and differential Media mentioned in the methodology part. Final identification (speciation) of bacteria was done on the basis of biochemical testing. This step was used as a confirmatory step to know the exact identity of the organisms and some of them were repeatedly found. Then the identified organisms were tested against standard antibiotics and the profile is recorded (Table 2).

Background information		Number of cases	Percent
Sex	Male	20	83.3%
	Female	4	16.7%
Age group	<50	5	21%
	50+	19	79%
Bacterial Growth	Growth	20	83.3%
	Non Growth	4	16.7%
Gram staining	Gram negative	23	85.1%
	Gram positive	4	14.9%
	Total number of organisms isolated	27	100%

Table 1: Demographic data of catheterized patients.

Gram-Negative														
Sample no	**Isolated Organisms**	**Amikacin**	**Ampicillin**	**Cephalexin**	**Ceftazidime**	**Ceftrixone**	**Ciproflxacin**	**Co-trimoxaz**	**Gentamycin**	**Nitrofurnto**	**Nalidixic acid**	**Tetracycline**	**Chloromphenicol**	**MAR INDEX**
N=1	*E. coli*	S	R	S	S	S	R	R	R	S	R	R	S	0.5
N=2	*E. coli*	S	R	S	S	S	S	R	S	S	S	S	S	0.17
	P. aeruginosa	S	R	S	R	S	R	S	S	S	R	R	R	0.5
N=3	*P. mirabilis**	S	R	R	I	R	S	R	R	R	R	R	R	0.83
N=4	*E. coli**	S	R	R	R	R	R	R	R	I	R	R	S	0.83
N= 5	*Margarella margais*	S	R	R	R	S	R	R	R	R	R	R	S	0.75
N=6	*E. coli*	S	R	R	R	R	R	S	I	S	R	R	S	0.67
N=7	*Providencio sturati*	S	R	S	S	R	R	R	S	R	R	R	S	0.58
N=8	*E. coli*	S	R	S	S	S	R	R	R	S	R	S	S	0.41
N=9	*Marganella morgani*	S	R	R	S	S	R	S	R	R	R	R	S	0.58
N=10	*Marganella morgani*	S	R	R	R	S	R	R	R	R	R	R	S	0.75
N=11	*E. coli*	S	R	R	R	R	R	S	R	S	R	R	S	0.67

N=12	*E. coli*	S	I	S	S	S	S	S	S	S	S	S	S	0.08
N=13	*Pseudomonas copacia*	S	R	R	R	R	R	S	R	R	R	R	S	0.75
N=14	*Klebsilla oxytoca*	S	R	R	R	R	R	R	S	S	R	R	S	0.67
	Proteus mirabillis	S	R	S	S	S	S	R	S	R	R	R	R	0.5
N=15	*Hafnia aluci*	S	R	S	I	S	R	S	S	S	R	R	R	0.5
	*Pseudomonas aeruginosa**	S	R	R	S	I	R	R	S	R	R	R	R	0.75
N=16	*P. mirabilis*	S	R	S	S	S	S	R	S	R	R	R	S	0.41
N=17	*Serratia liquefacts*	S	R	R	R	R	S	S	S	R	S	R	I	0.58
N=18	*Citrobacter fecalis*	S	R	R	R	R	S	R	S	R	R	R	R	0.75
N=19	*E. coli*	S	R	S	S	S	S	S	S	S	S	S	S	0.08
	Citrobacter diuersus	S	R	R	S	I	I	R	S	R	R	R	R	0.75

Gram Postive

Patient no	Isolated Organisms	Ampicillin	Ciprofloxacin	Tetracycline	Rifampin	Vancomycin	MAR INDEX
N=4	*Streptococcus* group D	S	R	R	R	S	0.6
N=9	*Streptococcus* group D	R	S	R	R	S	0.4
N=10	*Streptococcus* group D	S	S	R	R	S	0.4
N=20	*Streptococcus* group D	R	R	R	R	S	0.8

Table 2: Drug sensitivity status of the isolated microorganisms.

Percentage contribution of bacterial isolates

From the 27 isolates, twelve different organisms were identified namely *E. coli*, *P. aeruginosa*, *P. mirabilis*, *Streptococcus* group D, *Margarella morgani*, *Providencio sturati*, *Klebsiella oxytoca*, *Hafnia aluci*, *Pseudomonas copacia*, *Serratia liquefacts*, *Citrobacter fecalis* and *Citrobacter diuersus*. Among the isolated bacteria three different Gram negative enterobactriacea *E. coli*, *P. aeruginosa* and *P. mirabilis* with percentage contribution of 33%, 8.3%, 12.5% respectively were exclusively selected (Table 3) and again from these each species the organisms with the highest MAR INDEX were selected for the study which were *E. coli* (0.83), *P. aeruginosa* (0.75), *P. mirabilis* (0.83) (Table 4).

S. No	Pathogens	No. of Positive cases	Percentage
1	*Escherichia coli*	8	33%
2	*Proteus mirabilis*	3	12.5%
3	*Pseudomonas aeruginosa*	2	8.3%

Table 3: Percentage contribution of tested bacterial isolates.

Isolated Organisms	Resistant To	Mar Index Result
E. coli	10 standard antibiotics	0.83
P. mirabilis	10 standard antibiotics	0.83

P. aeruginosa	9 standard antibiotics	0.75

Table 4: Multiple antibiotic resistance index of the selected organisms.

Antibacterial activity of Malva parviflora extracts against the test isolates

The activity of ethanol extract of *Malva parviflora* against the MDR *P. aeruginosa* was found to be significant with an inhibition zone of 17.5 mm which is greater than almost all the twelve drugs used. The plant extracted using the aqueous had also a considerable effect against the MDR *P. mirabilis* with a zone of inhibition of 12.5 mm. However, all of *Malva parviflora's* extracts have shown no activity against the MDR *E. coli* (Table 5).

Solvent	**Malva parviflora**		
	E. coli	*P. mirabilis*	*P. aeruginosa*
	500 mg/ml M ± SD	500 mg/ml M ± SD	500 mg/ml M ± SD
Aqueous	0.00 ± 0.00	12.5 ± 0.7	0.00 ± 0.00
Ethanol	0.00 ± 0.00	0.00 ± 0.00	17.5 ± 0.7
Chloroform	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Positive control	17.5 ± 0.7	15.5 ± 0.7	16.5 ± 0.7
Negative control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P-value	0.000	0.000	0.000
Post hoc (Tukey HSD)	P>E=C=A=N	P>E=C=A=N	P=E>C=A=N

Table 5: Antibacterial activity of *Malva parviflora*.

Antibacterial activity of Chenopodium murale extracts against the test isolates: There was no inhibition zone seen with all extracts of *Chenopodium murale* except using the aqueous extract against the MDR *P. aeruginosa* with 9.5 mm inhibition zone (Table 6).

Solvent	**Chenopodium murale**		
	E. coli	*P. mirabilis*	*P. aeruginosa*
	500 mg/ml M ± SD	500 mg/ml M ± SD	500 mg/ml M ± SD
Aqueous	0.00 ± 0.00	0.00 ± 0.00	9.5 ± 0.00
Ethanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Chloroform	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Positive control	17.0 ± 0.4	15.5 ± 0.7	16.5 ± 0.7
Negative control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P-value	0.000	0.000	0.000
Post hoc (Tukey HSD)	P>E=C=A=N	P>E=C=A=N	P>E=C=A=N

Table 6: Antibacterial activity of *Chenopodium murale*.

Antibacterial activity of Lannea fruticosa extracts against the test isolates: Aqueous extracts of *Lannea fruticosa* against both the MDR *P. mirabilis* and *P. aeruginosa* showed a significant result of 20 mm and 19.5 mm inhibition zones respectively. Also, the chloroform extracts of this plant against these above isolates were found to be promising with 16.5 mm and 18 mm zone of inhibitions respectively. The maximum result from all extracts was displayed by the Aqueous extract of *Lannea fruticosa* against the MDR *P. mirabilis* with an inhibition zone of 20 mm which is also higher than all the drugs used for antimicrobial susceptibility testing under the study (Table 7).

Solvent	**Lannea fruticosa**		
	E. coli	*P. mirabilis*	*P. aeruginosa*
	500 mg/ml M ± SD	500 mg/ml M ± SD	500 mg/ml M ± SD
Aqueous	0.00 ± 0.00	20.00 ± 0.7	19.5 ± 0.7
Ethanol	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 1.4
Chloroform	0.00 ± 0.00	16.5 ± 0.7	18.00 ± 0.7
Positive control	17.5 ± 0.7	17.5 ± 0.7	17.5 ± 0.7
Negative control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P-value	0.000	0.000	0.000
Post hoc (Tukey HSD)	P>E=C=A=N	P=A=C>E=N	P=C=A>E=N

Table 7: Antibacterial activity of *Lannea fruticosa*.

Minimum inhibitory concentration and maximum bactericidal concentration of the selected plant extracts

The MIC values of aqueous extracts of *Lannea fruticosa* against the MDR *P. mirabilis* and *P. aeruginosa* were seen at 1.953 mg/ml. The lowest MIC value was seen at 3.90 mg/ml in the ethanol-aqueous extract of *Malva parviflora* against the MDR *P. aeruginosa*. The MBC values of aqueous extracts of *Lannea fruticosa* against the MDR *P. mirabilis* and *P. aeruginosa* were seen at 3.90 mg/ml. The lowest MBC value was recorded at 15.86 mg/ml in the ethanol-aqueous extract of *Malva parviflora* against the MDR *P. aeruginosa*. The range of concentrations for MIC was 1.953 mg/ml to 3.90 mg/ml and for MBC was 3.90 mg/ml to 15.6 mg/ml (Table 8).

Plant extract	**Solvent used**	**MICs and MBCs of the plant extracts with the highest antimicrobial activities on the isolated organisms (mg/ml)**			
		P. mirabilis		*P. aeruginosa*	
		MIC	MBC	MIC	MBC
Malva parviflora	Ethanol-Aqueous	-	-	3.90 mg/ml	15.86 mg/ml
Lannea fructicosa	Aqueous	1.953 mg/ml	3.90 mg/ml	1.953 mg/ml	3.90 mg/ml

Table 8: MIC and MBC values.

DISCUSSION

Infectious diseases are becoming the main cause of mortality and morbidity worldwide. Multi drug-resistant bacteria are the major contributing factors to hugely raised bars of UTIs. Such increase has been attributed to the empirical drug usage, availability of few drug classes and undermining the usage of culture and DST for patients with UTIs. Lately, the use of herbal medicines to control pathogenic microorganism has been a source of hope for human health and wellbeing [18].

The present study constitutes of three medicinal plants, from which aqueous extracts showed the highest yield, whereas the percentage yield of chloroform extracts was relatively low. The aqueous extract of *Lannea fruticosa* showed the highest yield with 22.6% followed by aqueous extracts of *Chenopodium murale* and *Malva parviflora* which were 20.2% and 19.5% respectively. As reported in a similar study, aqueous extract of *L. indica* and *A. reticulate* expressed the highest percentage yield [19]. Moreover, in another study chloroform extract of *Morinda citrofolia* showed the lowest percentage yield compared to ethanol and methanol extracts [20]. In this study, catheterized patients were selected for carrying out DST as well as AST against the resistant pathogens. The study population includes twenty-four urine samples collected from catheterized UTI patients with the majority of them above the age fifty and in which twenty were males and four were females. The result revealed that all cases but four showed no growth and this may be due to sample collection techniques, or insufficient media constituents required for growth. From the total plates that showed bacterial growth, twelve different organisms were isolated and identified. Of these one was a Streptococcus group D whereas the remaining eleven isolates were Gram-negative bacterias namely *E. coli*, *P. aeruginosa*, *P. mirabilis*, *Margarella margais*, *Providencio sturati*, *Klebsiella oxytoca*, *Hafnia aluci*, *Pseudomonas copacia*, *Serratia liquefacts*, *Citrobacter fecalis,* and *Citrobacter diuersus*. From the twelve isolates only Gram negative enterobacteriacea were taken into consideration and organisms such as *Serratia liquefacts*, *Hafnia aluci*, *Margarella margais* were very uncommon and not selected for the purpose. And from the considered organisms only the most prevalent UTI causing bacteria; *E. coli*, *P. aeruginosa*, and *P. mirabilis* were conveniently selected. This was done purposefully as these three organisms,(a) contributed more than 50% in which each of them

contributed 33%, 8.3% and 12.5% respectively, (b) are most common causes of UTIs worldwide, (c) and these species had high MAR INDEX , *E. coli* (0.83), *P. aeruginosa* (0.75), *P. mirabilis* (0.83) [21].

During the current study, the bacterial isolates were mostly resistant to the tested antibiotics. This phenomenon may be due to genetic changes since antimicrobial resistance occurs naturally over time [22]. However, the irrational use of antibiotics is also playing its role in accelerating this process. A recent finding which strongly supports the idea is that 80%-90% of antibiotic prescriptions were found to be written by general practitioners, of that 30% are considered to be completely unnecessary [23]. Also, inappropriate use of antibiotics, such as taking them for viral conditions like flu, or for mild infections that may clear-up without treatment is known to fuel resistance. A recent study in England, reported that One in three (34%) of the samples analyzed were found to be resistant to antibiotic trimethoprim which was once the first choice treatment for UTIs [24].

In this study almost all the isolates were found to be multi drug-resistant pathogens with the majority of them having MAR INDEX greater than 0.5. In another similar study majority of the clinical isolates were found to have MAR INDEX greater than 0.2 [17] and this indicates that the isolates in this study were more resistant than those [17]. This can be attributed to the fact that the patients in our case were using catheters for many years. Moreover, the undesirable finding in our study is that the majority of the Gram-negative pathogens were found to be resistant to Ciprofloxacin and Gentamycin which were some of the highly effective drugs recently. In a similar one study, *E. coli* was highly resistant to ampicillin, amoxicillin, tetracycline, and trimethoprim and sulfamethoxazole [25]. In our study, *E. coli* and *P. mirabilis* isolates which were tested against the extracts were resistant to 10 out of the 12 standard antibiotics and *P. aeruginosa* was resistant to 9 of standard drugs. *P. mirabilis* was only sensitive to Amikacin and ciprofloxacin whereas *E. coli* isolate was only sensitive to Amikacin and chloramphenicol. In another study, amikacin was found to be the most effective anti-bacterial agent against *E. coli* isolates which is in agreement with the finding in our case [26].

The antibacterial activity of these extracts was performed against highly drug-resistant clinical bacterial isolates. In general, a promising result was found from these plant extracts against these highly drug-resistant bacteria. *Lannea fruticosa* showed the highest inhibition zone among the selected medicinal plants which is 20 mm and 19.5 mm against poly drug-resistant *P. mirabilis* (which was sensitive to Amikacin 21 mm and Ciprofloxacin only) and *P. aeruginosa* respectively in its aqueous form at 500 mg/ml concentration. Not only this but also 18 mm and 16.5 mm inhibition zones was seen by chloroform extract of this plant against *P. aeruginosa* and *P. mirabilis* respectively at the same concentration. A considerable 10 mm inhibition zone was also recorded by the ethanol extract of this medicinal plant at 500 mg/ml concentration. From the isolates tested *P. aeruginosa* was found to be highly sensitive to the plant extracts of the current study which is similar to a study by in which a multidrug resistant *P. aeruginosa* was found to be highly sensitive from other bacteria to the crude extracts of that study [27]. But it can be noted that the results of comprises a lower inhibition zone compared to the result of the current study which is 20 mm inhibition zone by the extracts of *Lannea fruticosa* against *P. mirabilis*. Ethanolic extract of *Malva parviflora* showed an inhibition zone of 17.5 mm against *P. aeruginosa*, while its aqueous extract showed 12.5 mm inhibition zone against *p. mirabilis*. A study showed a maximum inhibition zone of 10 mm against *P. aeruginosa* by the ethanolic extracts of the medicinal plants, so it can easily be noticed that the antibiotic power of the ethanolic extract of *Malva parviflora* is also really promising when comparing with such findings [28]. In another similar study conducted the aqueous extract of *Tribulus terrestris*, *Cinnamom verum* and *Punica granatum* were used to test the antimicrobial activity on five different bacteria (*E. coli, K. pneumonia, P. aeruginosa, P. vulgaris and S. aureus*) isolated from urine samples of UTI infected patients and when comparing to our study *Lannea fruticosa* showed a higher inhibition zone than *Tribulus terrestris* and lower inhibition zone compared to *Cinnamom verum* and *Punica granatum* against *E. coli* and *P. aeruginosa* isolates [21]. A 9.5 mm inhibition zone by aqueous extract of *Chenopodium murale* against *P. aeruginosa* also adds the reliability of the selected medicinal plants as an antibiotic source.

MIC provides a precise concentration of drug to guide the choice of both the drug and the dose. The MIC and MBC of extracts with the highest antimicrobial activities against the isolated organisms were evaluated which were the ethanol extract of *Malva parviflora* against *P. aeruginosa* and aqueous extracts of *Lannea fruticosa* against both *P. mirabilis* and *P. aeruginosa* isolates. The range of concentrations for MIC was (1.953 mg/ml to 3.90 mg/ml) and for MBC was (3.90 mg/ml to 15.86 mg/ml). As all the extracts used against *E. coli* did not show any activity, MIC and MBC were not calculated.

The MIC values of aqueous extracts of *Lannea fruticosa* against *P. mirabilis* and *P. aeruginosa* was both at 1.953 mg/ml. The lowest MIC value was 3.90 mg/ml in ethanol-aqueous extract of *Malva parviflora* against *P. aeruginosa*. In another study done in Iran on selected plants which were *Marribum vulgari*, *Saturja montana*, *Myrtus comminus L*, *Amaranthus retraflexus*, the seed of *Cumminum cyminum L* and *Peganum harmal* showed activity against MDR *P. aeruginosa* and *E. coli* isolates [28]. The MIC values were calculated to be in a range of 2.5-20 mg/ml.

The MBC values of aqueous extracts of *Lannea fruticosa* against *P. mirabilis* and *P. aeruginosa* were both 3.90 mg/ml. The highest MBC value was recorded 15.86 mg/ml in the ethanol-aqueous extract of *Malva parviflora* against *P. aeruginosa*. In a similar study the MBC values of crude methanolic extracts of *Syzygium aromaticum*, *Glycerrhiza glabra, Laurus nobilis,* and *Brassica rapa* against all tested MDR UTI causing bacterial strains ranged between 15->15 mg/ml [29].

Conclusion

As indicated in our previous published paper, the same crude extracts from these three medicinal plants used in this study were tested for antibacterial activity against three standard bacteria such as *E. coli*, *S. aureus*, and *P. aeruginosa* in which they revealed astonishing antibacterial activity towards those microorganisms except to *E. coli*. Such result may be attributed to the presence of active phytocompounds present in these plants namely tannins, flavonoids, and glycosides which are known to have antibacterial property.

Generally, all plant extracts revealed a good antibacterial effect with a very remarkable inhibition zone against the isolated organisms even better than some antibiotics supplemented to the patients. Therefore, evaluating the activities of these medicinal plants on *in vivo* activities and further toxicological studies will be beneficial as it will help in formulating effective antibiotics against infectious organisms.

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