

Synergistic Effect of the Methanolic Extract of Lemongrass and Some Antibiotics to Treat Urinary Tract Bacteria

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Abstract

Some medicinal plants are used traditionally in Saudi Arabia to treat many bacterial infections. Three plants, lemongrass (*Oymbopogon citrates*), lantana (*Lantana camara*), and wild olive leaves (*Olea europaea*) were collected, identified, extracted with either hot water or organic solvents (methanol, diethyl ether, ethyl acetate and n-butanol) to investigate their antibacterial activities against *E. coli*. The methanol extracts of lemongrass, lantana and olive showed the highest activities against *Escherichia coli* while aqueous extract exhibited the lowest activities. Thus, the antibacterial activities of the methanolic extract of the three tested plants were determined using agar well diffusion method against some bacterial pathogens, isolated from urine samples. The highest antibacterial activity was recorded for the methanolic extract of lemongrass against all tested bacteria, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *E. faecalis* and *S. aureus*. The tested bacteria differed with regard to their susceptibility to plant extracts. Lemongrass was the most active extract followed by lantana and wild olive extracts. Minimal inhibitory concentrations (MICs) of the methanolic extract of Lemongrass and some used antibiotics, Erythromycin, Tetracycline, Amoxicillin, Ciprofloxacin and Chloramphenicol were determined using fluorescein diacetate method. Synergistic effect of the methanolic extract of lemongrass with the previous antibiotics against the tested clinical bacterial isolates was determined and the Fractional inhibitory concentrations (FIC) of different combination of the extract and the antibiotics were determined. FIC index (FICI) was calculated and it was ranged from 0.08 - 0.98. The interaction between the tested plant extract and the tested antibiotics was either synergistic or additive effects and no antagonistic effect was recorded. In conclusion, methanolic extract of lemongrass singly or in combination with some antibiotics can be used to treat pathogenic bacteria that cause urinary tract infections.

Keywords

Antibacterial Activities, Fluorescein Diacetate, MIC, Methanol, Lemongrass, Antibiotics

1. Introduction

Klebsiella pneumoniae, *Proteus*, *Pseudomonas aeruginosa* and *Escherichia coli* are the most-frequent causative agents of acute urinary tract infections (UTI) that cause fatal infections if they are not treated properly or left untreated [1] [2] [3]. The urinary pathogens, *E. coli* found attached to the epithelium of the bladder and account to 75% - 95% of cases while other enterobacteria, *Klebsiella*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* and the Gram positive *Staphylococcus saprophyticus* isolates were less common. *Enterococcus faecalis* and *Streptococcus agalactiae* may be isolated from patients with complicated cystitis [4] [5]. Generally, all kinds of bacterial infections were treated with certain antibiotics but unfortunately and during recent years, bacteria are gradually becoming resistant due to the miss use of antibiotics. There is an accelerated search for novel antibacterial agents from plants which playing an important role in improving human health. For centuries in developed and undeveloped countries, plants or their extracts can be used as more effective and less toxic medical products or antibiotics to treat various infectious diseases [6]. Many plants produced many secondary products that can be used as antimicrobial agents which opened new avenues for novel natural antibiotics that can serve as substitutes for current antibiotics [7]. Many studied reported that plant extracts may have great action in treating multidrug resistant bacteria and fewer numbers of plant species have been studied for their secondary product productions while the rests of the plants still need studies [8] [9] [10]. Naik *et al.* (2010) [11] found that lemongrass strongly inhibited Gram positive bacteria compared to Gram negative bacteria while lantana exhibited antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *B. cereus* due to the presence of forty-one components including caryophyllene, humulene, sabinene, bicylogermacrene and davanone [12]. Themethanolic extract of wild olive showed the highest antibacterial activities compared to other organic solvents [13]. Phytochemical from plants can be used as antimicrobial agents [14], antiviral [15] and antioxidant [16]. They also stimulate enzyme activity [17] and immune system [18]. Plant extracts are rich in various components such as caffeine, tannins, amino acids, vitamins and saponins which are considered as hypocholesterolemic, neuroprotective, hypolipaemic, cardioprotective, anti infective, antimutagenic and antitumor activities as well as dental caries prevention [19]. The aim of this study was to evaluate the antibacterial activities of some plant extracts in vitro and the synergistic effect of the methanolic extract of lemongrass and some antibiotics to treat urinary tract bacteria was determined.

2. Material and Methods

2.1. Pathogenic Bacteria

The tested bacteria were *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus aureus*. All bacterial isolates were obtained from mid stream urine specimens collected from patients at King Fahad General Hospital, Saudi Arabia and identified and processed according to standard guidelines [20].

2.2. Medicinal Plant Materials

Pants, lemongrass stalk and leaves (*Cymbopogon citratus*), lantana leaves (*Lantana camara*) and wild olive leaves (*Olea europaea*) were collected during May 2015, from various areas of Al Baha region, Saudi Arabia. Identification of the previous plants was carried out at the Department of Biology, KAU, SA.

2.3. Preparation of Plant Extracts

After cleaning the collected plants, they were dried and extracted with either hot water or organic solvents (50 g/200ml of the solvent) for 24 h. The organic solvents used were methanol, n-butanol, ethyl acetate or diethyl ether and the resultant extract was filtered using a glass wool and dried at 40°C to dryness under reduced pressure using rotary evaporator. The water extract was dried using lyophilizer and all the obtained extracts were dissolved in 1 ml DMSO and stored -70°C until used [14].

2.4. Antibacterial Activities of the Plant Extracts and Antibiotics

Agar well diffusion assay was used to detect the antibacterial activities of the prepared extracts [21] and antibiotics. The antibiotics, Erythromycin, Tetracycline, Amoxicillin, Ciprofloxacin and Chloramphenicol (Sigma-Aldrich, St. Louis, Missouri, USA) were used.

Few microbial colonies were collected in sterile nutrient broth and adjusted to the turbidity of the standard of 0.5 McFarland solutions [22] which has 4×10^6 CFU/ml. Each Mueller Hinton agar (Oxoid) plate was inoculated with 1.0 ml of the bacterial suspension. Agar wells were done using sterile cork borer (0.6 mm) and each well was filled with 100 μ l of the tested plant extract in DMSO. All inoculated plates were incubated at 37°C for 24 h. Dimethyl sulfoxide (DMSO) was used as negative control and Ampicillin was used as a positive control. Mean diameter of inhibition zone of three replicated plates was determined. Minimal inhibitory concentrations (MICs) of plant extract, antibiotics or the mixture of both for all tested bacteria were determined in 96 well ELISA trays by the methods described by Chand *et al.* (1994) [23] and was modified by Aly and GumgumJee (2011) [9]. Each well contained 175 μ l of the bacterial suspension (4×10^6 CFU/ml), 20 μ l the plant extract, or the solvent as control. After 40 minutes of incubation, fluorescein diacetate (FDA, 5 μ l of a 0.2% w/v in acetone) was added and the plates were incubated for 90 minutes. The obtained Green color from FDA hydrolysis was determined at 490 nm using an ELISA tray reader.

2.5. Fractional Inhibitory Concentration

Fractional inhibitory concentration (FIC) is the lowest concentrations of the extract and the antibiotics in combination giving no detectable bacterial growth after incubation. FIC index values were calculated using the formula:

FIC index = MIC of extract in combination/MIC of extract alone + MIC of antibiotics in combination/MIC of antibiotics alone.

The combination defined synergy if $\Sigma\text{FIC} \leq 0.5$, additive if $0.5 < \Sigma\text{FIC} \leq 1$, indifference if $1 < \Sigma\text{FIC} \leq 4$ and antagonism as $\Sigma\text{FIC} > 4$ [24] [25] [26].

2.6. Statistical Analysis

Each experiment was carried out three times replicates and the means \pm standard deviation was recorded. Analysis of variance, ANOVA was used to determine the significant at $P \leq 0.5$.

3. Results

Lemongrass (*Oymbopogon citrates*), lantana (*Lantana camara*), and wild olive (*Olea europaea*) were belonging to families Gramineae, Verbenaceae and Oleaceae, respectively (Table 1). The leaves of lantana and wild olive and shoot system of lemongrass were collected, identified, extracted with either hot water or organic solvents (methanol, diethyl ether, ethyl acetate and n-butanol) to investigate their antibacterial activities against *E. coli*. The methanolic extracts of lantana, lemongrass and wild olive showed the highest activities against *E. coli* with diameter of inhibition zones 28, 22 and 19 mm, respectively while their aqueous extract exhibited the lowest activities, 7.7, 8.1 and 7.8 mm as shown in Table 2. Thus, the antibacterial activities of the methanolic extract of the three tested plants were determined against the tested bacteria, (Table 3)

Table 1. The studied medicinal plants to detect their antibacterial activities and the used parts.

Common name	Scientific name	Family	Used part
Lantana	<i>Lantana camara</i>	Verbenaceae	Leaves
Lemon grass	<i>Oymbopogon citrates</i>	Gramineae	Leaves
Wild olive	<i>Olea europaea</i>	Oleaceae	Leaves

Table 2. Antibacterial activities of the aqueous and organic extracts of three tested plant against *E. coli* as test organism.

Used plant	Type of the extract					F value
	Aqueous extract (hot)	Methanol extract	Diethyl ether	Ethyl acetate	n-Butanol	
Lantana	7.7 \pm 1.0	28 \pm 1.5	12 \pm 0.6	19 \pm 2.0	15 \pm 1.4	1.12*
Lemon grass	8.1 \pm 1.7	22 \pm 2.0	10 \pm 0.6	17 \pm 2.6	13 \pm 2.5b	32.4*
Olive	7.4 \pm 0.4	19 \pm 2.0	10 \pm 0.4	18 \pm 2.5	11 \pm 1.5	3.46*

*: significant results where the calculated F value > F Tabulated.

Table 3. The antibacterial activities (diameter of the inhibition zone, mm) of the methanolic extract of Lantana, lemongrass and olive against different pathogenic bacteria and compared to Ampicillin (positive control).

Pathogenic bacteria	Diameter of the inhibition zone (mm)			
	Lantana	Lemon-grass	Olive	Ampicillin (control, 5 µg/ml)
<i>E. coli</i>	22 ± 2.0	28 ± 1.5	10 ± 2.0	30 ± 2.5
<i>K. pneumoniae</i>	20 ± 2.1	29 ± 0.5	14 ± 1.5	29 ± 1.5
<i>P. mirabilis</i>	20 ± 2.2	24 ± 1.4	10 ± 0.4	33 ± 0.9
<i>P. aeruginosa</i>	20 ± 1.0	25 ± 1.6	8 ± 0.5	34 ± 0.9
<i>E. faecalis</i>	18 ± 1.7	20 ± 1.4	19 ± 0.4	26 ± 0.5
<i>S. aureus</i>	14 ± 1.1	20 ± 1.0	20 ± 1.5	27 ± 1.0
Activity index [†]	19*	24.3	13.1*	29.8

[†]Activity index was calculated as the mean value of net zones of inhibition (mm) against the pathogenic bacteria, * Significant difference compared to Ampicillin at $p \leq 0.05$.

obtained from urine samples using agar well diffusion method. The tested bacteria were *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *E. faecalis* and *S. aureus*. The highest antibacterial activity was recorded for the methanolic extract of lemongrass, followed by lantana and finally olive leaf extract, against all tested bacteria, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *E. faecalis* and *S. aureus*, with inhibition zone diameter ranged from 20 - 28 mm, 14 - 22 mm and 8 - 20 mm. The tested bacteria differed with regard to their susceptibility to the plant extracts. Lemon grass methanolic extract was the most active extract for inhibition of the Gram negative *E. coli* and *K. pneumoniae* followed by *P. mirabilis* and *P. aeruginosa*. The effect of lantana leaf extract was also on Gram negative bacteria especially *E. coli* and *K. pneumoniae* while olive leaf extract inhibit mainly Gram positive bacteria, *S. aureus* and *E. faecalis*. Minimal inhibitory concentrations (MICs) of the methanolic extract of lemongrass and some used antibiotics, Erythromycin, Tetracycline, Amoxicillin, Ciprofloxacin and Chloramphenicol were determined using fluorescein diacetate method (Table 4). Synergistic effect of the methanolic extract of lemongrass with the previous antibiotics against the tested clinical bacterial isolates was determined (Table 5) and the Fractional inhibitory concentrations (FIC) of different combinations of lemongrass methanolic extract and some selected antibiotics were determined (Table 6). FIC index (FICI) was calculated and it was ranged from 0.08 - 0.98. The interaction between plant extract and the tested antibiotics was either synergistic effect (ΣFIC or $FICI \leq 0.5$) or additive effect ($0.5 < \Sigma FIC \leq 1$) and no indifference ($1 < \Sigma FIC \leq 4$) or antagonistic ($\Sigma FIC > 4$). The interaction between the tested plant extract and the tested antibiotics was either synergistic or additive effects and no antagonistic effect was recorded. In conclusion, methanolic extract of lemongrass singly or in combination with some antibiotics can be used to treat urinary tract infections with different pathogenic bacteria.

Table 4. Minimal inhibitory concentration (MIC) $\mu\text{g/ml}$ of methanolic extract of lemon grass and different antibiotics using Fluorescein diacetate method.

Tested bacteria	Minimal inhibitory concentration (MIC)					
	Plant extract (mg/ml)	Antibiotic ($\mu\text{g/ml}$)				
		Amx	Chl	Cip	Ery	Tet
<i>E. coli</i>	1.5 \pm 0.3	3.7 \pm 0.6	1.9 \pm 0.1	0.07 \pm 0.0	1.8 \pm 0.4	1.96 \pm 0.1
<i>K. pneumoniae</i>	1.5 \pm 0.1	0.9 \pm 0.4	3.7 \pm 0.4	0.07 \pm 0.0	3.9 \pm 0.1	1.96 \pm 0.1
<i>P. mirabilis</i>	2.2 \pm 0.5	3.7 \pm 0.9	0.9 \pm 0.0	0.03 \pm 0.0	1.9 \pm 0.3	1.96 \pm 0.3
<i>P. aeuroginosa</i>	2.2 \pm 0.7	7.5 \pm 0.6	3.7 \pm 0.4	0.03 \pm 0.0	1.9 \pm 0.1	3.96 \pm 0.4
<i>E. faecalis</i>	2.5 \pm 0.7	15.6 \pm 0.1	1.9 \pm 0.2	0.03 \pm 0.0	0.9 \pm 0.1	3.96 \pm 0.5
<i>S. aureus</i>	2.7 \pm 0.7	31.2 \pm 0.1	1.9 \pm 0.0	0.036 \pm 0.0	3.9 \pm 0.2	7.86 \pm 0.7

Ery: Erythromycin, Tet: Tetracycline, Amx: Amoxicillin, Cip: Ciprofloxacin, Chl: Chloramphenicol.

Table 5. Minimal inhibitory concentration (MIC) $\mu\text{g/ml}$ of the mixture of methanolic extract of lemon grass and different antibiotics using Fluorescein diacetate method.

Tested bacteria	Minimal inhibitory concentration (MIC) $\mu\text{g/ml}$				
	PE + Amx	PE + Chl	PE + Cip	PE + Ery	PE + Tet
<i>E. coli</i>	1.7 \pm 0.6	0.9 \pm 0.1	0.03 \pm 0.0	1.4 \pm 0.2	1.4 \pm 0.2
<i>K. pneumoniae</i>	0.5 \pm 0.4	0.4 \pm 0.4	0.05 \pm 0.0	3.0 \pm 0.5	1.7 \pm 0.2
<i>P. mirabilis</i>	1.7 \pm 0.9	0.4 \pm 0.03	0.02 \pm 0.0	1.0 \pm 0.4	1.5 \pm 0.2
<i>P. aeuroginosa</i>	3.5 \pm 0.1	1.7 \pm 0.4	0.02 \pm 0.0	1.0 \pm 0.1	3.5 \pm 0.5
<i>E. faecalis</i>	2.3 \pm 0.2	0.9 \pm 0.2	0.02 \pm 0.0	0.5 \pm 0.0	3.5 \pm 0.4
<i>S. aureus</i>	2.1 \pm 0.3	0.9 \pm 0.04	0.02 \pm 0.0	0.9 \pm 0.2	7.0 \pm 0.3

PF: Plant extract, Ery: Erythromycin, Tet: Tetracycline, Amx: Amoxicillin, Cip: Ciprofloxacin, Chl: Chloramphenicol

Table 6. Fractional inhibitory concentrations index of combination of the methanol extract of lemon grass and some antibiotics.

Tested bacteria	PE + Amx		PE + Chl		PE + Cip		PE + Ery		Tet + PE	
	FIC index	Eff.	FIC index	Eff.	FIC index	Eff.	FIC index	Eff.	FIC index	Eff.
<i>E. coli</i>	0.45	S	0.20	S	0.42	S	0.70	A	0.7	A
<i>K. pneumoniae</i>	0.55	A	0.46	G	0.71	A	0.08	S	0.9	A
<i>P. mirabilis</i>	0.45	S	0.10	S	0.66	A	0.52	A	0.78	A
<i>P. aeuroginosa</i>	0.46	S	0.20	S	0.66	A	0.52	A	0.89	A
<i>E. faecalis</i>	0.92	A	0.05	S	0.66	A	0.51	A	0.98	A
<i>S. aureus</i>	0.81	A	0.02	S	0.66	A	0.23	S	0.89	A

S: synergistic effect, A: additive, Eff.: Effect, PF: Plant extract Ery: Erythromycin, Tet: Tetracycline, Amx: Amoxicillin, Cip: Ciprofloxacin, Chl: Chloramphenicol.

4. Discussion

The resistance of bacterial pathogens to ampicillin and trimethoprim sulfamethoxazole was increased and became major therapeutic problems. Multidrug-resistant bacteria isolates are widely distributed in the hospitals through patient contacts and are increasingly being isolated from community-acquired infections [2] [27] [28] [29]. In normal genitourinary tracts, the Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and occasionally *Pseudomonas aeruginosa* and the Gram-positive bacteria, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. saprophyticus* were recorded. In hospitalized patients, *E. coli* accounts for about 50% of cases, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas* and *Serratia* account for about 40% and the Gram-positive bacterial cocci account for the remainder [30]. Many studies were carried out to extract and characterize plant products that inhibit the most pathogenic bacteria which are difficult to be effectively treated due to antibiotics limitation and availability [14] [31]. Inhibition of bacterial growth without harming the host cells represent the qualities required for safe and potent therapeutic agent. The plants lemongrass, lantana and wild olive were used traditionally in folkloric medicine in Saudi Arabia for their therapeutic activity in treating different pathogens and diseases [32] [33]. Lemongrass is a perennial aromatic medicinal grass mainly found in Najd, in the northern and eastern parts and commonly used as an infusion or tea for kidney remedy and fevers [32]. Aqueous and organic solvents of lemongrass, lantana and wild olive were compared. Methanol significantly extracts the active ingredient of the plants and was the most active in inhibition of *E. coli* growth. Similarly, the methanolic extract of *Olae* showed antibacterial activity similar to that of the antibiotic amoxicillin against *P. aeruginosa* [34]. In addition, the methanol extract of *Spondias mombin* showed excellent antibacterial activity with a mean zone of inhibition ≥ 11.1 and activity index of 0.8 - 1.1 [35]. The antimicrobial effect of any extract depends on the used method for extraction, the used solvents and the used plant. The methanolic extract of lemongrass showed excellent antibacterial activities against all the tested bacteria with bacterial index of 24.3 and no significant difference was found when compared to Ampicillin (positive control). Lower activities were obvious in case of lantana extract and olive extract where the difference was significant at $p \leq 0.05$ compared to Ampicillin. It was reported that the extract of lemongrass was effective against some bacteria including *Acinetobacter baumannii*, *Aeromonas veronii*, *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae* [36] [37]. The differences in the antibacterial effects of the three tested plant extracts may be due to the qualities and quantities of active compounds in the extract. The extract of lemongrass was among the most significant active and nontoxic natural extracts due to free and bound flavonoid fractions and considered an important antimicrobial agent for many kinds of infections [11] [38]. The presence of the major volatile compounds nerol, geranial and geraniol in lemongrass extract was confirmed using Gas chromatography-mass spectrometry and all the tested pathogens are variably susceptible to these compounds [39]. The results of Al Yousef (2013) [40] showed that Lemongrass has a fungitoxic effect and cause a reduction in fungal viability and spore germination, loss of pigmentation

and disrupted conidiospore structure, supporting a possible role of infection control and the use as antifungal agents. Promising antifungal activity of olive leaves against some plant pathogens was due to the presence of phenolic compounds [41]. Broth micro-dilution technique was used to determine the antibacterial activities and the same method was used for the antibiotic griseofulvin [42]. The calculated minimal inhibitory concentrations (MICs) for lemongrass were greater than that obtained for Erythromycin, Tetracycline, Amoxicillin, Ciprofloxacin and Chloramphenicol. FIC index (FICI) was ranged from 0.08 - 0.98. The most prominent and synergistic interaction was observed between lemongrass and Amoxicillin (Amx) against *E. coli*, *P. mirabilis* and *P. aeruginosa* with $\Sigma\text{FIC} \leq 0.5$. Ahmad A and Viljoen (2015) [39] calculated the MICs of lemongrass extract and Ag⁺ for all the tested pathogens and they ranged from 0.032 - 1 mg/ml and 0.004 - 0.064 mg/ml, respectively, while the MIC of their combination (FICI values) were ranged from 0.258 - 2.186 which indicating synergy, additive and indifferent interactions. The combination of Ampicillin and fresh garlic extract showed synergistic interaction for different *S. aureus* isolates [43]. Further studies are needed for isolation, separation and identification of the active materials to be applied as alternative drug for treatment of urinary system infections. The phenolics and polyphenols, essential oils; alkaloids; lectins and polypeptides are the most useful phytochemicals act as antimicrobial compounds. Their mechanisms of action vary according to their structure (Aly *et al.*, 2013) [10] which may include enzyme inhibition, disruption of cell membranes, inactivation of the protein, complex formation with soluble proteins, DNA and bacterial cell walls [44] [45].

5. Conclusion

Very few studies confirm the viability of some plant extracts as antibiotics on urinary tract infections bacteria. In this study we supported the promising use of plant extract especially crude extracts of lemongrass, singly or in combination with some commonly used antibiotics. Similar combinations are usually used by the local people to treat bacterial infections associated with many diseases. Moreover, from our results, we suggest that lemon grass could be useful in the development of new antimicrobial drugs, especially against urinary tract infections caused by bacteria. In addition, more investigations are needed to identify potential medical uses of more traditional and herbal plants against different kinds of infections.

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