Study the antimicrobial activity of Lemon grass leaf extracts.

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Abstract

Antimicrobial properties of *Lemon grass* were investigated against both clinical and laboratory isolates of both bacteria and fungi using the disc diffusion method. Acetone extracts (15 mm zone diameter of inhibition, MIC 250 µg/mL and MBC 300 µg /mL) demonstrated the highest activity, followed by dichloromethane (7 mm zone diameter of inhibition, MIC 300 µg /mL and MBC 400 µg/mL), methane (7.5 mm diameter of inhibition, MIC 400 µg /mL and MBC 400 µg /mL) and hexane (5.8 mm zone zone diameter of inhibition, MIC 800 µg/mL and MBC 1000 µg/mL). Water extracts demonstrated the least activity against the test bacteria and fungi (4 mm zone diameter of inhibition, MIC 900 µg/mL and MBC 900 µg/mL). Phyto constituents present included Saponins, Tannins, Alkaloids and Flavonoids. *Lemon grass* can be used to source antibiotic substances for possible treatment of bacterial and fungal infections.

Key words :*Lemon grass*, antimicrobial property, extract antibiotic

INTRODUCTION

Lemon grass belongs to the section of *Andropogan* called *Cymbopogam* of the family Germineae. A very large genus of the family, including about 500 described species out of which eight species occur in Iraq. Due to the production of lemon grass oil as major component, two of the species i.e. *Cymbopogan citrates* and *C. flexuosus* are generally called Lemon grass (1).

Medicinal use of lemongrass is known to mankind since antiquity. Its oil has been used to cure various ailments like cough, cold, spitting of blood, rheumatism, lumbago, digestive problems, bladder problems, leprosy, and as mouth wash for the toothache and swollen gums. It is also been claimed to be stimulating, diuretic, anti purgative and sudorrific to reduce fever (5). To cure cholera, colic and obstinate vomiting only 3-6 drops of the oil are effective medicine of choice (5).

The oil has been found to posses bactericidal and anti fungal properties, which is comparable to penicillin in its effectiveness (12). The oil also contains male sex hormone agent (8). It is also reported to have strong activity against two dermatophytes, namely *Trichophyton rubrum* and *Microsporium gypsum* (11). Similarly pharmacological investigation on the essential oil of *C. citratus* revealed that it has a depressant effect on the CNS (2).

It has analgesic and antipyretic properties. The extract juice from the lemon grass contains inhibitor of the promotion stage of carcinogenesis induced by cotton oil. It is an oral anti tumor drug for the cancer and in combination with cyclodextrin lengthened the survival time (21,19).

Gallstone dissolving preparations have been made of oil (6). The lemon grass contains high percentage of Vitamin C, which is a characteristic of plants used as drug e.g., belladonna and jaborandi. Lemon grass oils show activity towards the phyto pathogenic fungi. A combination of lemon grass oil is given for use on human and domestic animal pathogens (10,24). This work was set out in order to investigate the antimicrobial activity of lemongrass extracts against some pathogenic bacteria and fungi and to ascertain the chemical constituents that may be present.

MATERIALS AND METHODS

Plant storage:

lemon grass were separated from stems, washed in clean water, and dried at room temperature (8). The dried plants were milled to a fine powder, and stored in the dark at room temperature in closed containers until required.

Extraction procedure:

Dried plant leaves were extracted by weighing samples of 1 g of finely ground plant material and extracting with 10 mL of acetone hexane, dichloromethane (DCM) or methanol (technical grade- Merck) and boiled water in polyester centrifuge tubes. Tubes were vigorously shaken for 3 to 5 min & shaking machine at high speed. After centrifuging at 3500 rpm for 10 min the supernatant was decanted into pre-weighed, labeled containers. The process was repeated three times to exhaustively extract the plant material and the extracts were combined. The solvent was removed under a stream of air in a fume cupboard at room temperature and the extraction efficiency was quantified by determining the weight of each of the extracts (9,16).

The antimicrobial activity of the crude extract was screened against four gram-negative bacteria; *Neisseria gonorrheae, Salmonella* sp.,*Pseudomonas aeruginosa*, *Proteus vulgaris* and two gram-positive bacteria; *Staphylococcus aureus* and *Streptococcus aerugenosa*. (clinical isolates) obtained from the Public health center laboratories isolates each of gram-negative bacteria *Escherichia coli* and *Salmonella typhi*); and gram-positive bacteria *S. aureus* and *Streptococcus pneumoniae*; and four fungi, *Aspergillus niger Aspergillus tamari* , *Candida albicans* and *Fusarium oxysporum* (standard laboratory isolates), all obtained from the Public health center laboratories.

Preliminary phytochemical studies:

The extracts were subjected to various phytochemical tests to determine the active constituents present in the crude aqueous and ethanolic extracts. The slightly modified method (20) was used.

The Antimicrobial activity screening:

The antimicrobial activity was determined by the paper disc diffusion method using Mueller-Hinton agar plates (MHA, oxoid) (for all bacteria) and potato dextrose agar plates (PDA, oxoid) (for the fungi) previously inoculated with 18 h old Nutrient broth (NB, oxoid) culture (0.5 Macfarland Standard) for the bacteria or spores (10^6 spores/mL for the fungi) suspension in Potato

Dextrose Broth (PDB, Oxoid) of the test organisms, respectively. Sterilized paper discs (6 mm), soaked in a known concentration of the crude extracts of *S. obtusifolia* (L.) (5000 µg/mL per disc) in DMSO were applied over each of the culture plates previously seeded with 0.5 McFarland (for bacteria) and 10^6spores/mL (for fungi). Antibiotic discs of ofloxacin (30 µg/m) was used as positive control for bacteria, clotrimazole (30 µg) was used for fungi and sterilized paper discs without extracts or antibiotics were used as negative controls for both the bacteria and fungi. The experiment was performed in triplicate. Incubations were at 37oC for 24 - 48 h for bacteria and *C. albicans* and at room temperature for 72 h for the other filamentous fungi. Following incubation the zones of inhibition formed were measured and the mean diameter obtained. Overall, cultured bacteria with halos equal to or greater than 7 mm and fungi with 10 mm halos were considered susceptible to the tested extract (18).

Determination of MIC and MBC:

The minimum inhibitory concentration (MIC) of the crude extracts was also determined using the same method except that the paper discs were soaked in different concentrations dispersed in water (10 - 2000 µL). After incubating at 24 h at 37°C, the MIC of each sample was determined by measuring the optical density in the spectrophotometer (620 nm), and comparing the result with those of the non inoculated NB and PDB (18). The minimum bactericidal concentration (MBC) of the plant extract on the clinical bacterial isolates was carried out according to (24) provision. Briefly, 1 ml was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and streaked on MHA (for bacteria) and PDA (for fungi) and incubated for 24 h (for bacteria) and 72 h (for fungi). The least concentration of the extract with no visible growth after incubation was taken as the minimum bactericidal concentration.

Evaluation of the synergistic effect of antibiotics and plant extracts or phytochemicals on the test organisms:

This evaluation was done according to (17). Aliquots of 100 µL of resistant bacterial cultures (0.5 MacFarland Standard) grown in 10 mL of nutrient broth for 6 h were inoculated in nutrient broth supplemented with the respective antibiotics (50 µg/mL) and 106 cells/mL fungal cultures grown in PDB supplemented with 100 µg/mL coltrimazole with different concentrations of plant extracts. The concentration for plant extracts ranged from 10 to 500

μg/mL, based on MIC values that had previously been evaluated. The growth conditions were the same as previously mentioned. After 48 h, the optical density of each sample was documented and compared to those of MIC to verify any synergistic effect among the tested compounds.

RESULTS AND DISCUSSION

All the extracts were acidic in nature (pH values ranging between 3-5). The acidity combined with bioactive components might enhance the antimicrobial activity of the extracts especially against the bacteria. Qualitative phytochemical investigation revealed that the extracts contained some phyto constituents. Saponins, tannins, alkaloids and flavonoids are present in the acetone extracts; tannins, alkaloids and flavonoids are found in the methanol extracts; alkaloids and flavonoids in water; and hexane extracts and saponins and tannins in dichloromethane extracts (Table 1). These bioactive components, beside other water soluble components which are naturally occurring in most plant materials, are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the anti-microbial property to plants (7,13,23).

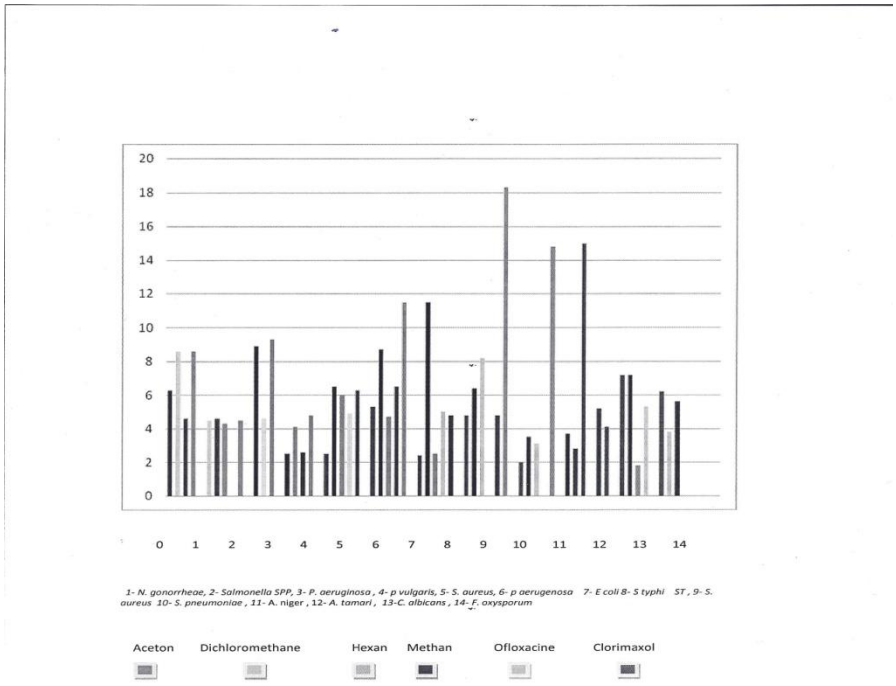
Table (1). Phytochemical constituents of root extracts of lemon grass.

Extract	pH	extraction	Saponins	Tannins	Alkaloids	Flavonoids	Balsams	Anthraquinone
Water	5.3	50	_	_	+	+	_	_
Acetone	5.1	40	+	+	+	+	_	_
Dichlorom ethane	5.0	28	+	+	_	_	_	_
Hexan	5.5	45	_	_	+	+	_	_
Methanol	5.2	38	_	+	+	+	_	_

Key: - - = absent,; + = present

All the extracts demonstrated antimicrobial activity against both the test bacteria and fungi with the acetone extracts demonstrating the highest activity (13 mm, followed by the dichloromethane extracts (8 mm, while the water extracts demonstrated the least activity (3 mm at 5000 µg/mL (Figure 1). The water extracts did not demonstrate any reasonable activity against all the clinical isolates; *N. gonorrheae*, *Salmonella* sp, *P. aeruginosa*, *P. vulgaris*, *S. aureus* and *S. aerugenosa*. The acetone extracts were active against three of the clinical isolates; N. gonorrheae (6 mm), S. aureus (2.3 mm) and *P.* zone diameter of aerugenosa (6.2 mm inhibition), and all of the laboratory isolates; *E. coli* (8.2 mm zone diameter of inhibition) *S. typhi* (11.9 mm), *S. aureus* (3.9 mm), *S. pneumoniae* (3.7 mm), A. niger (3.9 mm), *A. tamari* (7 mm), *C. albicans* (9 mm) and *F. oxysporum* (6 mm). The dichloromethane extracts had activity against both the clinical (N. gonorrheae – 8 mm zone diameter of inhibition, *Salmonella* sp – 5 mm zone diameter of inhibition, *P. vulgaris* – 8 mm zone diameter of inhibition and *S. aerugenosa* – 4 mm zone diameter of inhibition) and laboratory isolates [*E. coli* – 10 mm zone diameter of inhibition), *S. aureus* - 8 mm zone diameter of inhibition), *S. pneumoniae* (4 mmzone diameter of inhibition), A. niger (4 mm zone diameter of inhibition), A. tamari (6 mm zone diameter of inhibition) and *C. albicans* -(8 mm zone diameter of inhibition)] at 5000 µg/ml (Figure 1). Ofloxacin and coltrimazoledemonstrated the highest activities against both bacteria and fungi, respectively. The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds,

skin lesions and the lungs, especially in debilitate and immunodeficient patients (3) Infection caused by *Salmonella typhimurium* is a serious public health problem in developing countries and represents a constant concern for the food industry (14). Proteus mirabilis causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy, and it is a secondary invader of ulcers and pressure sores (4,22). The demonstration of activity against both gram-negative and gram-positive bacteria and fungi is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against both clinical and



(Figure 1) 1-N.gonorrheae,2-Salmonella spp,3-P.aeroginosa,4-P.vulgaris,5-S.aureus,6-P.aerogenosa,7-E.coli,8-S.typhi,9-S.aureus,10-S.pneumoniae,11-A.niger,12-A.tamari,13-C.albicans,14-F.oxysporum.

laboratory isolates is also an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms prevalent in hospital environments inhibition), *S. aureus* - 8 mm zone diameter of inhibition), *S. pneumoniae* (4 mm zone diameter of inhibition), *A. niger* (4 mm zone diameter of inhibition), *A. tamari* (6 mm zone diameter of inhibition)and *C. albicans* -(8 mm zone diameter of inhibition)] at 5000 µg/ml (Figure 1). Ofloxacin and coltrimazoledemonstrated the highest activities against both bacteria and fungi, respectively. The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immunodeficient patients (3). Infection caused by *Salmonella typhimurium* is a serious public health problem in developing countries and represents a constant concern for the food industry (18). Proteus mirabilis causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy, and it is a secondary invader of ulcers and pressure sores (3,21).

The demonstration of activity against both gram-negative and gram-positive bacteria and fungi is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against both clinical and laboratory isolates is also an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms prevalent in hospital environments. The MIC and MMC of the extracts ranged from 200- 2000 µg/mL, with the acetone extracts demonstrating the lowest values (MIC 200 µg/mL: MBC 300 µg/mL each) against *E. coli* and *S. typhi*, followed by the dichloromethane extracts against *S. typhi* (MIC 600 µg/mL, MBC 800 µg/mL) and *S. aureus* (MIC 300 µg/mL, MBC 400 µg/mL) (Table 2). Most of the MIC values were lower than the MBC values indicating that the extracts could be bactericidal in action. Low MIC and MBC values are also an indication of high efficacy. Lower MIC and MBC values (Table 2).

Most of the MIC values were lower than the MBC values indicating that the extracts could be bactericidal in action. Low MIC and MBC values are also an indication of high efficacy. Lower MIC and MBC values (Table 2) and higher zones of inhibition (Figure 1) for acetone extracts connotes higher solubility of phyto constituents in the acetone compared to the other solvents used. Different solvents have various degrees of solubility for different phyto constituents (15). Table 3 shows the effect of combination of extracts and antimicrobial agents on the test organisms. Results revealed an increased activity of both ofloxacin (30 µg/mL) and coltrimaxole (30 µg/mL) in the presence of the extracts (30 µg/mL). At 30 µg/mL, both ofloxacin and the extracts had no effect on *P. aerugenosa* (clinical isolate), but when combined, there was a remarkable activity (8 mm zone diameter of inhibition). At 30 µg/mL the activity of the extracts and ofloxacin against *E. coli* were 10 and 12 mm (zone diameter of inhibition), respectively but this increased to 18 mm when the extracts and the antibiotics were combined. A similar trend was observed with extract-coltrimaxole combination against the test fungi. At 30 µg/mL, the activity of the extracts alone against *A. niger* was 4 mm (zone diameter of inhibition) and that of coltrimaxole was 14 mm (zone diameter of inhibition), but this activity increased to 16 mm when the extracts and co-trimaxole were combined. Synergistic effect of some phyto-constituents on antibiotics against some resistant isolates had earlier been reported (18).

(Table 3). Synergistic activity of extracts of lemon grass (30 µg/ml) witj antibiotics (30 µg/ml).

Organisations	**Zone of inhibition (mm)**					
	E	**O**	**EO**	**E**	**C**	**EC**
Neisseria gonorrheae	6	8	14	X	X	X
Salmonella sp	-	4	10	X	X	X
Pseudomonas aeruginosa	-	-	8	X	X	X
Proteus vulgaris	-	10	12	X	X	X
Staphylloccocus aureus	2	4	6	X	X	X
Streptococcus aerugenosa	8	-	10	X	X	X
Escherichia coli (EC 002BFTY)	10	12	18	X	X	X
Salmonella typhi (ST 008BFTY)	12	-	14	X	X	X
Staphylloccocus aureus (SA012MBFTY)	6	20	20	X	X	X
Streptococcus Pneumoniae (SN006BFTY)	4	16	10	X	X	X
Aspergillus niger (AN082FFTY)	4	X	X	4	16	14
Aspergillus tamari (FT001FFTY)	6	X	X	6	-	-
Candida albicans (CA032FFTY)	8	X	X	8	8	8
Fusarium Oxysporum (FO004FFTY)	6	X	x	6	2	2

Key:E-Extract only;O-Ofloxcin alone;EO-Extract /Ofloxacin;C-Coltrimaxole alone;EC-Extract/Coltrimaxole;X-not detected

Conclusion

Extracts of *Lemon grass* in this study demonstrated a broad-spectrum of activity against both gram-positive and gram-negative bacteria and fungi. The broad-spectrum antibacterial activities of the plant extract, possibly due to the identified alkaloids, further confirm its use as a health remedy in popular medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea,pneumonia, eye infections and mycotic infections. Isolation, identification and purification of these phyto constituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemo therapeutic agents should be the future direction for investigation.

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دراسة الفعالية المضادة للمايكروبات لمستخلص أوراق حشيشة الليمون

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وزارة العلوم والتكنولوجيا

الخلاصة

درست فعالية مستخلصات حشيشه الليمون Lemon grass المضادة للإحياء المهجرية البكتيرية (عزلات سريرية ومختبرية) والفطرية باستخدام طريقة انتشار القرص، إذ أعطى مستخلص الأسيتون منطقة تثبيط بقطر 15 ملم وMIC 250 مايكرو غرام/ مل وMBC 300 مايكرو غرام/ مل، ويليه مستخلص الداي كلوروميثان، إذ أعطى منطقة تثبيط بقطر 7 ملم وMIC 300 مايكرو غرام/مل وMBC 400 مايكرو غرام/ مل، اما مستخلص الميثانول فأعطى منطقة تثبيط بقطر 7.5 ملم وMIC 400 مايكرو غرام/ مل و MBC 400 مايكرو غرام/ مل، في حين أعطى مستخلص الهكسان منطقة تثبيط بقطر 5.8 ملم وMIC 800 مايكرو غرام/ مل و MBC 1000 مايكرو غرام/ مل، وأظهر المستخلص المائي فعالية اقل تجاه الفطريات، إذ أعطى منطقة تثبيط بقطر 4 ملم وMIC 900 مايكرو غرام/مل و MBC 900 مايكرو غرام/مل، كما اظهرت النتائج احتواء حشيشه الليمون على الصابونيات والتانين والفلافونات والكلوريدات.