

COMPOSITION AND ANTIMICROBIAL SCREENING OF THE ESSENTIAL OIL OF LEMON GRASS *Cymbopogon citratus* (DC.) FROM EGYPT

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ABSTRACT: *Lemon grass, Cymbopogon citratus* (DC.) is a perennial grass that grows spontaneously around the world, mainly in the tropical regions. The present work reports the antimicrobial activities of the essential oil from *C. citratus* as well as the content of its essential oil. Two Gram-positive bacteria (*Bacillus subtilis* and *Streptomyces* spp.); one Gram-negative bacterium, *Escherichia coli* O157; five fungal strains (*Fusarium oxysporum*, *Rhizopus stolonifer*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Aspergillus niger*) and three yeasts (*Candida albicans*, *Candida tropicalis* and *Saccharomyces cerevisiae*) were used for evaluation of antimicrobial properties of *C. citratus* essential oil. The disc diffusion method was used to evaluate the zone of growth inhibition at various concentrations of the oil. It can be concluded that essential oil of *C. citratus* has activities against Gram-positive bacterium (*B. subtilis*), two fungal strains (*M. phaseolina* and *A. niger*) and a yeast (*S. cerevisiae*) more than against Gram-negative bacterium, *E. coli* O157, and Candidal strains. The chemical composition of the essential oil obtained by hydrodistillation was analysed by GC/MS and the resulting oil contained geraniol (43.08%), citral (38.96%) and myrcene (11.22%) being the main constituents, which may provide the antimicrobial properties of the essential oil against tested organisms.

Keywords: *Cymbopogon citratus*, Essential oil, Antimicrobial activity, Bacteria, Fungi, Yeast, Chemical composition, GC/MS analysis

INTRODUCTION

Aromatic and medicinal plants produce a wide variety of volatile terpene hydrocarbons (aliphatic and cyclic) and their corresponding oxygenated isoprenoid derivatives and analogues. Mixtures of these substances, which are known as essential oils, can be isolated from diverse parts of plants by steam distillation. The antimicrobial properties of essential oils are well recognized for many years (Hammer *et al.*, 1999; Cosentino *et al.*, 1999; Daferera *et al.*, 2000). Several plants and herb species used traditionally have potential antimicrobial and antiviral properties (Sumathi *et al.*, 2011) and this has raised the optimism of scientists about the future of phyto-antimicrobial

agents (Das *et al.*, 1999). In the last decades, application of synthetic toxins for control of weeds, pests and plant diseases caused serious environmental problems (Razavi, 2011). Resistance of some bacteria has created an urgent need to develop alternative antimicrobial drugs from herbs that are safe, cheap and may overcome the resistance of the pathogens (Geidam *et al.*, 2007; Bonjar *et al.*, 2004). The investigation of plants for bioactive secondary metabolites is an area which most plant scientists have recently focused with an aim of discovering new clinically useful and commercially important plant products (Dewick, 1997). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests (Suffredini *et al.*, 2004).

Lemon grass, *Cymbopogon citratus* (DC.) is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Carlin *et al.*, 1986). The plant is a native herb from India and is cultivated in other tropical and subtropical countries. The plant is widely used in tropical countries as a source of ethnomedicines (Di Stasi *et al.*, 1989; Duke, 1989; Tortoriello and Romero, 1992). As a medicinal plant, lemon grass has been considered a carminative and insect repellent. It is popularly used in Brazil mainly due to its central nervous system action, with few controlled studies about this type of activity (Blanco *et al.*, 2009). In developing countries where medicine are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed (Cowan, 1999). It is probable that a large number of plants with biological activities remain untested.

The principle aim of the present work was to study the antimicrobial activity of commercial essential oil (EO) obtained from lemon grass. Also, chemical composition of essential oil extracted which are may be responsible for antimicrobial activity, were identified by using GC/MS analysis from the lemon grass.

MATERIALS AND METHODS

1. Plant material

Lemon grass, *C. citratus* was collected from the Sekem company plantation in the city of Bilbeis, Sharkeiah governorate during the flowering period. The plantation is certified for organic biodynamic agriculture by COAE (Center of Organic Agriculture in Egypt).

2. Isolation of essential oil (EO):

The EO of lemon grass was extracted from the entire plant (stems, leaves, and flowers) by hydro-distillation using a Clevenger-type apparatus for 3 h as described by Negahban *et al.*, (2006). The oily layer which formed on top of the aqueous distillate was separated and dried with anhydrous sodium

sulfate (0.5 g). The extracted EO was kept in sealed airtight glass vials and covered with aluminum foil at 5° C until further analysis.

3. Antimicrobial activity

3.1. Microbial strains

For the determination of antibacterial activity of *C. citrates* essential oil, two Gram-positive bacteria; *Bacillus subtilis* and *Streptomyces* spp.; one Gram-negative bacterium *Escherichia coli* O157; five fungi strains (*Fusarium oxysporum*, *Rhizopus stolonifer*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Aspergillus niger*) and three yeasts (*Candida albicans*, *Candida tropicalis* and *Saccharomyces cerevisiae*) were used. The selected microorganisms were obtained from culture collection of the Department of Agriculture Botany, Menoufiya University, Egypt.

3.2. Screening for antibacterial and anti-yeast activities

Antimicrobial activity was tested by disc-diffusion inhibition on agar method as described by Musyimi *et al.*, (2008). Culture suspension (200 µl) of the tested bacteria (2×10^6 CFU/ml) was spread on the media. Circular paper discs of 6.0 mm in diameter were cut out from Whatman No. 1 filter paper using a paper punch. Each sterile filter disc dipped in a known concentration of the essential oil for about 2 min, then were gently transferred to the inoculated agar media. Antimicrobial activity was determined by measuring the zone of growth inhibition around the discs after 48 h of incubation at 30°C.

3.3. Screening for antifungal activity

For screening the antifungal activity of EO, the agar-disc diffusion method was used. The discs of mycelial felt (6 mm diameter) of the pathogenic fungi were transferred aseptically to the centre of Petri dishes. Tolcofos-methyl was used as a reference fungicide. The treatments were incubated at 25° C for 72 h. The antimicrobial activity was determined by measuring the diameter (mm) of the growth inhibition zones including the 6 mm disk. The measurements of inhibition zones were carried out for three sample replications and values were the average of three replicates.

3.4. Antibiotic and minimum inhibitory concentrations (MIC) assay

The standard discs (6 mm diameter) of some antibiotics were served as positive antibacterial control and compared to the control disc to nullify the effect of the solvent on the growth of the tested organisms. The antibiotics used were chloramphenicol (C) (30µg), clarithromycin (CLR) (15 µg), gentamicin (CN) (120µg), erythromycin (E) (15µg), norfloxacin (NOR) (10µg). The antibiotic disks were placed on the surface of the media; incubation time was 48 or 72 hours at 30 or 25° C according to the microbial organisms used.

The inhibition zones were measured with a millimeter ruler including the diameter of the disc. Minimum inhibitory concentrations (MIC) were determined after 48 h for the bacteria and yeasts and after 72 h for fungal strains. Serial dilutions of the EO were made in a concentration range from 1.25 to 40.00 mg/ml. Each concentration was tested in triplicate. The MIC was determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate.

4. GC/MS analysis

The oil compounds were isolated, identified and quantified on a Shimadzu GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (GC-MS QP-5050A). The GC-MS system was equipped with a TRACSIL Meta X5 column (Teknokroma S. Coop. C. Ltd., Barcelona, Spain; 30 m × 0.25 mm i.d., 0.25 µm film thickness). Analyses was carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 40°C for 5 min; rising at 3.0°C/min to 200°C and held for 1min; rising at 15°C/min to 280°C and held for 10 min. The injector and detector were held at 250 and 300°C, respectively. Diluted samples (1:10 pentane, v/v) of 0.2 µL of the extracts were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 45-450. The identification of individual compounds of essential oil was accomplished using two different analytical methods: (a) KI, Kovats indices in reference to *n*-alkanes (C₈-C₃₂) by National Institute of Standards and Technology (NIST) 2009; and (b) mass spectra (authentic chemicals and Wiley spectral library collection). Identification was considered to be tentative when it was based on mass spectral data only. The relative concentration of each compound in the essential oil was quantified according to the peak area integrated by the analysis program.

5. Statistical analysis

Data of all measurements were then subjected to one-way analysis of variance (ANOVA) appropriate for a randomized complete block design (CoStat system for Windows, version 6.311, CoHort Software (2006), Berkeley, CA, USA).

Results

1. Antimicrobial activity

The antimicrobial activity of lemon grass EO was evaluated against three bacterial genera, three yeasts and five fungal genera. According to the results given in Table 1, the EO had variable antimicrobial activity against all tested microorganisms. Lemon grass EO showed the highest antibacterial activity against *B. subtilis* with an inhibition zone of 15 mm. Gram-positive

Table 1. Antimicrobial activity of *Cymbopogon citratus* essential oil (EO) against some micro-organisms tested by disc-diffusion inhibition on agar method

Microbial strains		Diameter of inhibition zone (mm)										MIC**	MIC for Tolcofos-methyl (µg/ml)
		Essential oil (mg/ml)				Antibiotics (µg)					Control		
		5	10	20	40	C 30	CLR 15	CN 120	E 15	NOR 10			
Bact.	<i>Streptomyces</i>	NE	NE	7.0±0.0 ^c	9.3±0.6 ^b	18.0±1.0 ^a	NE	n.d.	17.6±1.2 ^a	NE	NE	20	n.d.
	<i>B. subtilis</i>	7.3±0.6 ^d	8.3±0.6 ^d	11.0±0.0 ^c	15.3±0.6 ^b	22.0±1.0 ^a	n.d.	14.3±0.6 ^b	19±1.0 ^a	14.0±0.0 ^b	NE	5	n.d.
	<i>E. coli</i> O157	NE	NE	NE	NE	20.7±1.0 ^a	NE	8.0±0.0 ^b	9.3±0.6 ^b	NE	NE	NE	n.d.
Yeasts	<i>C. albicans</i>	NE	NE	NE	NE	22.0±1.0 ^b	26.0±1.0 ^a	n.d.	18.7±1.5 ^b	8.7±0.6 ^c	NE	NE	n.d.
	<i>C. tropicalis</i>	NE	NE	NE	NE	20.0±0.0 ^b	22.0±1.0 ^a	n.d.	17.7±1.2 ^c	9.3±0.6 ^d	NE	NE	n.d.
	<i>S. cerevisiae</i>	7.0±0.0 ^d	8.3±0.6 ^{cd}	10.6±0.6 ^b	13.0±1.0 ^b	9.3±0.6 ^{bc}	9.6±0.6 ^{bc}	n.d.	9.6±0.6 ^b	NE	NE	5	n.d.
Fungi	<i>F. oxysporum</i>	NE	NE	NE	NE	NE	NE	NE	n.d.	NE	NE	NE	64
	<i>R. stolonifer</i>	NE	NE	NE	7.0±0.0	NE	NE	NE	n.d.	n.d.	NE	40	40
	<i>R. solani</i>	NE	NE	NE	NE	NE	NE	NE	n.d.	NE	NE	NE	8
	<i>M. phaseolina</i>	7.3±0.6 ^c	8.0±0.0 ^c	9.3±0.6 ^b	13.3±0.6 ^a	NE	NE	NE	n.d.	NE	NE	5	96
	<i>A. niger</i>	NE	NE	7.0±0.0 ^b	8.3±0.6 ^a	NE	NE	NE	n.d.	NE	NE	20	120

Means followed by the same letter(s) within each vertical column are not significantly different at P = 0.05.

* Diameter of inhibition zone including disc diameter of 6 (mm). Values are means of three replication ±SD.

**MIC, minimum inhibitory concentration as (mg/ml).

NE, no inhibition zone observed.

n.d., not determined.

bacteria were shown to be more sensitive to the lemon grass EO than the Gram-negative ones. The growth of Gram-negative bacteria *E. coli* O157 was not inhibited. The growth inhibitions of tested microorganisms ranged from 5 mg/ml (w/v) to 40 mg/ml (w/v) with the lower MIC value against *B. subtilis* at 10 mg/ml. It means that the EO had most activity against Gram-negative ones. Furthermore, the most susceptible yeasts for *C. citratus* EO was *S. cerevisiae* (13 mm), it had great activity against *S. cerevisiae* but the EO was ineffective against *C. albicans* and *C. tropicalis*. The concentration effect on *F. oxysporum* and *R. solani* inactivation can be seen in Table 1. Under the same experimental conditions, 40 mg/ml of *C. citratus* EO had a low inhibitory effect on the growth of *R. stolonifer* and had no effect on the growth at other concentrations of oil. *M. phaseolina* was the most sensitive fungal strain (7 mm) at 5 mg/ml of EO and it showed inhibitory effects at all added concentration. The disks impregnated with 5, 10, 20, and 40 mg/ml of lemon grass EO were not active against *F. oxysporum* and *R. solani*, whereas had no inhibitory effects at any of the four concentration tested.

3.2. GC/MS analysis

The hydrodistillation of the dried aerial parts of *C. citratus* gave light yellowish oil with yield of 1.2 % (w/w). Thirteen components were identified in the oil, representing 98.97% of the total composition (Table 2).

Table 2. Chemical composition of essential oil isolated by hydrodistillation from aerial parts of *C. citratus* analyzed by GC-MS

Number	RI ^a	Compound ^b	Peak Area (%) ^c
1	10.725	myrcene	11.22
2	12.293	trans-beta-ocimene	0.61
3	14.894	alpha-terpinolene	0.91
4	16.793	gamma-isogeraniol	0.33
5	17.188	citronellal	0.17
6	17.572	trans-limonene oxide	0.60
7	18.420	limonene oxide	2.18
8	19.264	6-nonenal,	0.29
9	21.372	citral	38.96
10	22.750	geranial	43.08
11	23.623	2-heptadecanone (CAS)	0.15
12	26.062	Geranic acid	0.20
13	27.543	Geranyl acetate	0.27
Total			98.97

^a RI, retention index on a TRACSIL Meta X5 column

^b Compounds are listed into order of their elution from a TRACSIL Meta X5 column

^c Compound percentage

The major components of the essential oil were geranial (43.08%), citral (38.96%) and myrcene (11.22%). Given the composition of the lemon grass EO, marked inhibitory effects would be expected, as observed in Table 2.

Discussion

The use of natural products can be considered as an important antimicrobial agent. The results from this study indicated that the EO of *C. citratus* exhibited antimicrobial activity. Therefore, antimicrobial activity of *C. citratus* may be related to these components. The chemical composition of the EO from *C. citratus* could be changed according to geographical distribution and might be an effective factor on its antimicrobial activity. Moreover, most of the studies investigating the action of essential oils against food spoilage organisms agreed that, essential oils are slightly more active against Gram-positive than Gram-negative bacteria (Cosentino *et al.*, 1999; Lambert *et al.*, 2001; Karaman *et al.*, 2003). Burt (2004) reported that citral and geraniol had inhibitory effect on *L. monocytogenes*. It is established that these compounds are the main components of lemon grass EO (Adeleke *et al.*, 2001). Bakkali *et al.* (2005) reported that pro-oxidant activities of volatile terpenic and phenolic components of EOs may damage eukaryotic cell membrane, in particular those of mitochondria, and thus promote the release of Ca^{2+} and cytochrome c. This may lead to late apoptosis and/or necrosis including damage to proteins and DNA and overall cytotoxic effects (Bakkali *et al.*, 2006). The antimicrobial activity of *C. citratus* EO would be related to its monoterpene components. Indeed, in essential oils, it was shown that monoterpenes in essential oils are able to destroy cellular integrity resulting in respiration inhibition and permeability alteration (Cox *et al.*, 2000). Some oils of basil types showed strong antibacterial activity against *E. coli* (Nour *et al.*, 2009). In general, the toxic activity of essential oils is mostly due to the presence of phenols, aldehydes and alcohols (Sacchetti *et al.*, 2005). However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the antimicrobial activity exhibited. In the oil, the possible compounds synergistic and antagonistic effects would play an important role in microbial inhibition and should also be taken into consideration. It is also possible that various minor components may be involved in some type of synergism with other active components (Yu *et al.*, 2004). The results of the present study suggest the possible use of *C. citratus* essential oil as natural compounds, of potential antimicrobial activity.

Conclusion

Antimicrobial properties of the essential oils and various extracts from many plants have recently been of great interest, because their possible use as natural additives emerged from a growing tendency to replace synthetic

compounds with natural ones. Extracted volatile oils from *C. citratus* with steam distillation were found to have antibacterial and antifungal activity. The essential oil of *C. citratus* has activities against Gram-positive bacterium (*B. subtilis*), two fungi strains (*M. phaseolina* and *A. niger*) and yeast (*S. cerevisiae*) than against Gram-negative bacterium *E. coli* O157 and *Candidal* strains. All these results indicated that EO is a source of biologically active compounds. However, further studies are needed to be done to obtain more information regarding the practical effectiveness of the extracts in animal models.

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التركيب ودراسة النشاط المضاد للميكروبات للزيت الأساسي المستخلص من حشيشه الليمون المصرية

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الملخص العربي :

حشيشه الليمون عبارة عن عشب ينمو دائما بصورة تلقائية (برية) في جميع أنحاء العالم وخاصة في المناطق الاستوائية وفي مصر يوجد في جبل عنبه والصحراء الشرقية وصحراء التبين بندرة. وتهدف الدراسة الحالية إلى دراسة النشاط المضاد للميكروبات للزيت الأساسي لحشيشه الليمون بالإضافة إلى مكونات الزيت الأساسي فتم استعمال نوعين من البكتيريا الموجبة لجرام وهما: باسيلس سبتيلس ، ستريتوميسيس وكذلك نوع من البكتيريا السالبة لجرام وهي: اشيرشيا كولاي O157 وأيضاً خمس أنواع من الفطريات وهم: فيوزاريوم اوكسيسبوريم ، ماكروفومينا فاصولينا ، اسبرجلس نيجر ، رايزوكتونيا سولاني ، رايزوبيس ستولونيغيرا وثلاثة أنواع من الخمائر وهم: نوعين من الكانديدا ، السكرومييسيس ، وذلك لتقييم الخصائص الإيجابية على الميكروبات للزيت الأساسي لحشيشه الليمون عن طريق قياس قطر منطقة التثبيط في طبقة الآجار باستخدام تركيزات مختلفة من الزيت الأساسي. أوضحت النتائج أن الزيت الأساسي لحشيشه الليمون له تأثير مهلك على كل من البكتيريا الموجبة لجرام (باسيلس سبتيلس) ، و جنسين من الفطريات (ماكروفومينا فاصولينا ، اسبرجلس نيجر) وخميرة (السكرومييسيس) أكثر من نشاطه ضد البكتيريا السالبة لجرام (اشيرشيا كولاي O157) وسلالات الكانديدا المستخدمة تحت الاختبار. وعن طريق تحليل التركيب الكيميائي للزيت الأساسي بواسطة إستعمال جهاز الكروماتوجرافي الغازي المرتبط بمطياف الكتلة GC/MS للتعرف على مكونات الزيت اتضح أن geranial (٤٣,٠٨ %) ، citral (٣٨,٩٦ %) ، myrcene (١١,٢٢ %) هي المكونات الرئيسية ، والتي قد تبرهن امتلاك هذا الزيت الأساسي لخصائص إيجابية على الميكروبات تحت الاختبار.