BIODIVERSITAS
Volume 23, Number 10, October 2022
Pages: 5298-5301

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231039

Short Communication: Antimicrobial of lemongrass (*Cymbopogon citratus* L.) volatile oil and cytotoxic effects against L20B and MCF-7cell lines

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Manuscript received: 10 August 2022. Revision accepted: 14 October 2022.

Abstract. *Hasan ZYM, Al-Halbosiy MMF, Al-Lihaibi RK, Al-Nauimi EH. 2022. Short Communication: Antimicrobial of lemongrass (Cymbopogon citratus L.) volatile oil and cytotoxic effects against L20B and MCF-7cell lines. Biodiversitas 23: 5298-5301.* Lemongrass (*Cymbopogon citratus* L.) has been used in different countries in folk remedies for coughs, malaria, pneumonia, and others for many years. The development of bacterial resistance to available antibiotics has obligated finding for new agents to serve as potent antibacterial drugs. The present investigation deals with the effect of volatile lemongrass oil cultivated in Iraq, on different bacterial species and evaluates the cytotoxic activity of the extracted oil on L20B and MCF-7cell cancer cell lines. The plant samples were collected from the college of science /Baghdad University /scientific garden and classified as *C. citratus* L. by the plant herbarium at the same college. The volatile plant oil was extracted from fresh leaves at the laboratory of the plant biotechnology department of Biotechnology Research Center at Al-Nahrain university/Iraq. With the aid of the Clevenger apparatus, a hydro-distillation method was employed to quantify the lemon grass's volatile oil. The extracted essential oil and the plant crud maceration were screened for their antibacterial activity against two Gram-negative bacteria (*Escherichia coli*, and *Vibrio cholera*) and two Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) using the well-diffusion method and disc diffusion method. The biological survey also included the cytotoxic effect of oil subjected to the anticancer activity in vitro on two cancer cell lines; L20B mouse cell line that expresses the genes for human cellular receptors for polioviruses, and the second line was the MCF-7 breast cancer cell line. As a result of this screening study, it was shown that the plant seemed to be rich in essential oil content. The Iraqi cultivated plant produced 1.5%v/w essential oil. The volatile oil affected both Gram-negative and Gram-positive strains in comparison to the crud plant extract among the selected bacterial cultures, the highest antibacterial activity was recorded against the Gram-positive strain *S. aureus* by well diffusion method. Besides, the plant oil showed an inhibitory effect on L20B cell line with a percent inhibitory growth rate reaching 47.1% at 1.125 µl/mL of the oil concentration. While for the other cell line, MCF-7 cell line, the inhibitory growth rate percentage appeared for almost all concentrations in comparison with control after 24 hours, and even at a concentration of 0.3125µl/mL, the inhibitory growth rate percentage reached up to 86%. This study was conducted to high lightened the benefits of this plant as little study had been done for an Iraqi cultivated plant and the results showed the potent biological effects of the plant especially the volatile oil as an antimicrobial and as a potent cancer inhibitory agent.

Keywords: Antibacterial activity**,** *Cymbopogon citratus*, cytotoxicity, L20B cell line, MCF-7 cell line, volatile oil

INTRODUCTION

Plants produce chemical compounds in special pathways, these compounds are known as secondary metabolites. These natural products were regarded as more than 12,000 different structural compounds only about 10% of the total had been estimated and isolated from plants for their medicinal purposes were estimated (Zhou et al. 2015). In almost all plant production, these substances serve as plant rules of defense mechanisms against predation by insects and herbivores. Moreover, to overcome any bad environmental stress and various fungal, microorganisms, and even viral infections (Denaro et al. 2020). All these products utilized an alternative agent to overcome the risks of antibiotics resistance, and the huge side effects, normally intended in synthetic medicines nowadays (Mishra 2010), and since the tiny microorganisms evoke genetic ability to acquire virulence elements, causing well-known general antibiotics responded, as well as these natural compounds might be useful in the new infectious pathogen strains (Farooq and Ngaini 2021). Some of these secondary metabolites possessed the ability to inhibit or kill different microorganism strains in unique mechanisms than the traditional antibiotics currently used that eluted a significant clinical value in the treatment of resistant microbial strains, even in coronavirus (Luo et al. 2020). In a study investigated the effects of phytochemical component extracted from an Iraqi herb *"Lepidium draba"* against pathogenic bacteria isolated from skin infections, the extracted compounds exerted potent inhibiting growth against these microorganisms, even in very low concentrations (Najim et al. 2020). One the famous medicinal plant rich with many active constituents is *Cymbopogon citratus* belongs to Gramineae family, rich in the presence of cyclic mono-terpene, besides other active constituents such as phenols, flavonoids, tannins and even

alkaloids. A study at *Viet Nam* Institute of medicinal plants under the Ministry of Health concluded that lemon oil had been shown to have potent antimicrobial activity, making the oil to be used as a disincentive and preservative agent beside, possessing powerful pesticide and anti-fungal properties (Viktorová et al. 2020).

Lemongrass oil showed antimicrobial, insecticidal, and insect-repellant activity. Lemongrass oil also had cytotoxic properties or presented a protective role on some cancer cell lines for humans, thus the major component (citral) acted to induce the apoptosis in tumor cell lines by activating the procaspase-3 in several cancer cell lines in a dose /time-depending manner. As glutathione S-transferase (GST) plays a key role in the detoxification of cellular oxidative damage caused by carcinogenic and toxic chemicals, the use of citral for cancer prevention has been reported (Oladeji et al. 2019). Estimation of lemon grass cultivated in Iraq and study of the biological activity of volatile lemongrass oil on pathogenic microorganisms and even the anticancer activity are considered to be the main project aim in the present work.

MATERIALS AND METHODS

Plant collection and extract preparation

The fresh leaves of the plant were collected from the college of science /Baghdad University /scientific garden and classified as lemongrass (*Cymbopogon citratus* L.) at the plant herbarium at the same college. The crud extract was prepared from small cut pieces of fresh leaves, digested with distilled water for 2 hrs at 60°C then concentrated and dried by rotary evaporator to be kept in dry cool containers till use. The volatile oil was extracted in the lab from fresh leaves with the aid of Clevenger apparatus as a water distillation method for about 8 hrs (Dangkulwanich and Charaslertrangsi 2020).

Antibacterial activity of lemongrass crud extract and volatile oil

The microorganisms used for the present study were *Escherichia coli, Staphylococcus aureus, Bacillus cereus,* and *Vibrio cholera* (received from Biotechnology Research Center /Al-Nahrain University). The Muller Hinton agar (MHA) in the concentration of 3.1%w/v was used in the current study to be sterilized and poured into a sterile Petri plate, then swabbed with each culture of microorganisms separately. A well puncher was made to be filled with 50 µL of oil in a concentration of 50%v/v and 25% w/v from the crud extract after sterilization with 0.22mm millipore filter. The plates were incubated overnight at 37°C. The antibacterial activity was determined by measuring the diameters of inhibition zones for each strain. The same protocol proceeded after disc saturation with the same concentration of both plant extract; the crud and the essential oil in each experiment were repeated twice (Mohammed et al. 2020).

Anticancer cells activity of lemongrass volatile oil

Cancer anti-proliferation assay was held through the colorimetric test using the vital cell consuming dye (3- [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT) dye, Sigma-Aldrich). Both cancerous cells (about 100 µL) were cultured using two tissue culture microtiter plates of 96-well seeded with 2.4×10^4 suspended with cancerous cells into each plate separately, then incubated for 24 hrs at 37°C. Aliquot of 100 µL extracted plant oil were added in each well that diluted with 0.1% v/v dimethylsulfoxide (DMSO) with concentrations of 0.0781, 0.156, 0.312, 0.625, 1.125, 2.5, 5, 10 µl/mL. The time of exposure for both plates was 24 hrs incubated in CO_2 incubator at 37°C. The control group is represented by the untreated cancer cells. All treatments were done in triplicate. The medium was discarded at the end of incubation time and 150 µL from the yellow MTT dye solution (2 mg/mL) was added to all wells, to be then re-incubated for another 4 hrs. In the end, a purple formazan crystal was formed, which was read at 490nm after dissolving the precipitating crystals with the aid of 100 µL DMSO reagent. The percentage of growth inhibition for each cell line (L20B, MCF-7) was calculated as follows (Trang et al. 2020).

$$\text{Growth inhibition \%} = \frac{\text{Control} - \text{Treatment cell}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

Plant volatile oil content

The *C. citratus* fresh leaves cultivated in Iraq produced 1.5% v/w volatile oil. This result indicated that the Iraqi cultivated plant is rich in essential oil content. A study by Viktorová et al. 2020, showed in their results about the lemongrass essential oil content prepared by the vacuum distillation of fresh *Cymbopogon* leaves had yielded 0.56% (v/w) in native plants. This difference could be explained by several factors, including genetic factors, the age of the plant, the season of harvest, or the plant environment, besides, as the differences in the chemical composition of the oil extracted (Viktorová et al. 2020).

Antibacterial activity of lemongrass volatile oil and crud extract

The lemongrass oil and crud extract caused an inhibition on the growth of bacterial species. The highest inhibition effect was shown in Gram-positive bacteria (*S. aureus* and *B. cereus*) by well diffusion method to reach maximum inhibition 23 mm and 16 mm respectively for the plant essential oil (Table 1). The expected mechanism of lemongrass oil's antibacterial effects on tested Gram-positive bacteria, is due to citral, the main active constituent of the oil, that act as a potent antimicrobial component (Silva et al. 2008). However, more significant susceptibility was shown toward the Gram-positive bacteria than in Gram-negative bacteria, as the zones of inhibition were larger in the first species than the latent microorganism which could be due to the resistance mechanisms which might differ from each kind to result in alteration in *C. citratus* oil effects toward the different bacterial groups.

Table 1. Antibacterial effect of *Cymbopogon citratus* on some pathogenic bacteria

Type of plant extract and concentration		Method use	Average inhibition zone diameter (mm)			
			Gram-positive bacteria		Gram-negative bacteria	
			S. aureus	*B. cereus*	*E. coli*	*V. cholera*
Cymbopogon citratus	**Oil of *Cymbopogon citratus***	Disk diffusion	12 mm	12 mm	Negative	Negative
		Well diffusion	23 mm	16 mm	8 mm	12 mm
25%w/v	50%v/v	Disk diffusion	14 mm	8 mm	Negative	Negative
		Well diffusion	20 mm	18 mm	8 mm	10 mm

Table 2. Cytotoxic effect of lemongrass oil extract on the growth of L20B cancer cell line after 24 hrs exposure

Concentration (µl/mL)	Average cell absorbance at 490 nm Lemongrass oil O.D mean	% Inhibition rate (%IR) for L20B %IR
Negative control	0.155a	0.155
10.00	104.3b	32.69
5.00	0.122b	21.30
2.50	0.086b	44.00
1.125	0.082c	47.10
0.625	0.141d	9.00
0.3125	0.129d	16.00
0.15625	0.140e	9.70
0.078125	0.179e	0.00

Note: *Differences a, b, c, d, e are significant ($p<0.05$) to the compression column

Table 3. Cytotoxic effect of lemongrass oil extract on the growth of MCF-7 cell line after 24 h exposure

Concentration (µl/mL)	Average cell absorbance at 490 nm Lemongrass oil O.D mean	% Inhibition rate (%IR) for MCF-7 %IR
Negative control	0.349[a]	0.349
10.00	0.162[b]	53.60
5.00	0.090[b]	74.20
2.50	0.059[b]	83.00
1.125	0.054[c]	84.50
0.625	0.051[d]	85.30
0.3125	0.049[d]	86.00
0.15625	0.060[b]	83.00
0.07812	0.298[e]	14.00

Note: Differences (a, b, c, d and e) are significant ($p<0.05$) to compression column

According to a study done by Subramaniam, 2020, the terpenes found in lemongrass oil could inhibit the growth of Gram-positive bacteria but are inactive against Gram-negative bacteria (Subramaniam et al. 2020). Thus, because the essential oils could easily penetrate via Lipoteichoic acids content in Gram-positive bacterial membrane that facilitates penetration of these natural lipophilic substances toward cytoplasm causing changes in different organelles leading to suppress growth or microorganism cidal. Due to the presence of lipopolysaccharide in the case of Gram-negative bacteria made it difficult for volatile oil components to penetrate the bacterial cell membrane (Costa et al. 2019; Dawood et al. 2021).

Anticancer activity

Lemongrass oil significantly affected ($p<0.05$) the cancer cells of L20B line by decreasing their growth in almost all concentrations to get an inhibition rate (47.1%) at a concentration (1.125µl/mL) in comparison with the negative control. A weak cytotoxic effect (9%, 9.7%) was showed at the concentration (0.625, 0.07812) respectively (Table 2).

Cellular assays for screening medicinal plants for cancer can be in vitro and vivo cytotoxicity assays with other tests that may include different morphological parameter changes. Studies reports that demonstrating the anti-carcinogenic (or anti-tumor) properties of lemongrass evaluated the putative anti-tumor properties of the essential oil, and results showed that the oil inhibits the proliferation of both LNCaP and HeLa cancer cells used in these works potentially (Bayala et al. 2020; Khan 2020). The antiproliferative activity for both cancer cells of the plant-extracted essential oil appeared to be dependent on concentration.

On the other hand, the effect of Lemongrass oil in the growth of MCF-7 cell line investigated the cytotoxic effects of lemon grass in different concentrations on MCF-7 breast cancer cell line. The results showed significant differences ($P<0.05$) in the cell viability of MCF-7 that reached a maximum inhibition rate reach to (86%) at a concentration (0.3125 µl/mL) (Table 3).

Natural products are still a significant source of new drugs, especially in anticancer and antihypertensive therapy. Despite this importance, only a small percentage of plant species has been phytochemically and pharmacologically studied (Rachana and Manisha 2017).

In conclusion, Lemongrass volatile oil significantly affected both Gram-negative and Gram-positive bacteria in comparison to the crud plant extract, and among the selected bacterial cultures, the highest antibacterial activity was recorded against the Gram-positive strain *S. aureus* by well diffusion method. Besides; the plant oil showed an inhibitory effect on the breast cancer cell line MCF-7 cell line after 24 h exposure reached to 86% at a concentration of 0.3125µl/mL than with L20B cell line, where the highest inhibitory growth rate reached 47.1% at 1.125 µl/mL of the oil concentration after 24 hrs. These effects for cancerous cells cytotoxicity and antibacterial effects gave importance accept or the plant essential oil to be promised as a new drug discovery in the near future.

ACKNOWLEDGEMENTS

Authors would like to acknowledge the Biotechnology Research Center, Al-Nahrain University, Ministry of Higher Education, Iraq, for support in completing all work requirements.

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