

Antimicrobial and Cytotoxic Effect of *Cymbopogon Citratus* (Lemongrass) On Some Cell Lines

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Abstract: This study was about the effect of *Cymbopogon citratus* (lemongrass) volatile oil that cultivated in Iraq, on different bacterial species and evaluates the cytotoxic activity of oil on some cancer cell lines. The essential oil was screened against two gram negative bacteria (*Escherichia coli*, *Vibrio cholera*) and two gram positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*). The cytotoxic effect of oil was carried on two cancer cell lines (L20B and MCF-7).

The oil affected both gram negative and gram positive strains. Among the selected bacterial cultures, the highest antibacterial activity was recorded against *Stap. aureus*. The plant oil showed an inhibitory effect on L20B cell line growth rate and MCF-7 cell line growth rate after 24 hr. for almost all concentrations in comparison with control.

Key words: *Cymbopogon citratus*, volatile oil, cytotoxicity, antibacterial activity, L20B cell line, MCF-7 cell line

I. 1-INTRODUCTION:

Plants had produced a chemical compounds in a special pathways, known as secondary metabolites. These natural products were regarded as 12,000 different structural compounds only about 10% of total had been estimated and isolated from plants for their medicinal purposes(1). In almost of plant production for these substances had been served as plant rule of defense mechanisms against predation by insects, herbivores, more over; to overcome any bad and environmental stress and various fungal, microorganisms, even viral infections(2). These products utilized an alternative agents to overcome risks of the antibiotics resistance, and the huge side effects, normally intended in synthetic medicines now-a-days (3), and science the tiny microorganisms evoke genetic ability to acquire virulence elements, make general well known antibiotics unresponded, as well as might be

useful in the new infectious pathogen strains(4). Some of these secondary metabolites possessed the ability to inhibit or kill different micro-organism strains in a unique mechanisms than the traditionally antibiotics currently used that eluted a significant clinical value in treatment of resistant microbial strains even in corona virus(5,6). One the famous medicinal plant rich with many active constituents is *Cymbopogon citratus* (lemongrass); this genus contains about 55 species of grasses, which mainly had been native to warm temperate and tropical regions of the Old World and Oceania. The plant grows natively in India and mainly in Latin American and African counties, consumed as teas, soups, and curries(7). Lemongrass oil has disincentive and preservative activity as well as a powerful pesticide and anti-fungal properties, it had been used in Brazilian folk medicine as tea for anxiety internally and insect repellants externally, but the recurrence used may cause of contact dermatitis. The major active phytochemical in lemongrass, is the volatile oil which composed mainly from geraniol, citral and citronellol, each possessed antiseptics, preservative activity, as well as flavoring agent(8,9).

This study is aimed to high lighten the biological activity of lemongrass volatile oil that had been cultivated locally in Iraq, specially the antimicrobial and anticancer activity.

II. MATERIAL AND METHODS:

2.1-Plant collection and extract preparation:

The plant was collected from college of science /Baghdad University /scientific garden and classified as *Cymbopogon citratus* (lemongrass) by the plant herbarium at the same collage. The volatile oil was in

the lab with aid of Clevenger apparatus as a water distillation method (10).

2.2-Antibacterial activity of lemongrass volatile oil:

The microorganisms used for the present study were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio cholera* (received from Biotechnology Research Center /Al-Nahrain University).

The Muller Hinton Agar (MHA) in concentration of (3.1% w/v) was used in current study to be sterilized and poured into a sterile Petri plates, then swabbed with each culture of microorganisms separately. A well puncher was made to be filled with 50 µl of oil. The plates were incubated overnight at 37°C. The antibacterial activity was determined by measuring the diameters of inhibition zones for each strain (11).

2.3-Anticancer Cells Activity of *Cymbopogon citratus*(lemongrass) volatile oil(12):

This work was held at Biotechnology Research Center, using two kinds of cancer cell lines; L20B cell line: which is a mouse cell line that expresses the genes for human cellular receptor for *polio viruses*, and the passage number was (20).

The second line was the: MCF-7 cell line which represent cancer breast cell of a 69-year-old Caucasian woman isolated in 1970.

Cancer anti-proliferation assay was held through the colorimetric test using the vital cell consuming dye (MTT dye, Sigma-Aldrich). Both cancerous cells (about 100 µL) had been cultured using two tissue culture microtiter plates of 96-well seeded with 2.4×10^4 suspended with cancerous cell into each plate separately, then incubated for 24 hours at 37°C. Aliquot of, 100 µL extracted plant oil were added in each well that diluted with 0.1% v/v DMSO with concentrations of (0.0781, 0.156, 0.312, 0.625, 1.125, 2.5, 5, 10) µl/ml. Time of exposure for both plates was 72 hours incubated in CO₂ incubator at 37°C. Control group represented by the untreated cancer cells. All treatments were done in triplicate. Medium was discarded at the end of incubation time and 150 µL from the yellow MTT dye solution (2 mg/mL) had been added to all wells, to be then re- incubated for another 4 hours. Endly formazan crystal was formed, that was read at 490nm after dissolving the precipitating crystals with aid of 100 µL DMSO reagent. The percentage of growth inhibition for each cell line (L20B , MCF-7) was calculated as follow(13) :

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

III. 3-RESULT AND DISCUSSION:

3.1- Plant volatile oil content:

Each 100 gm *Cymbopogon citratus* (lemongrass) fresh leaves yielded about 1.5ml volatile oil.

3.2-Antibacterial Activity:

The anti-bacterial activity of *Cymbopogon citratus* (lemongrass) against bacterial species is summarized in table (1). Also the lemongrass oil cause an inhibition on the growth of bacterial species ,the highest inhibition effect was show in gram positive bacteria (*S. aureus* and *B. Cereus*) by well diffusion method to reach to maximum inhibition 23 mm and 16 mm respectively . The expected mechanism of lemongrass oil antibacterial effects on tested G+ve microorganisms, is due to citral, the main active constituent of oil, that act as a potent antimicrobial component(7). However, more significant susceptibility was shown toward the G+ve bacteria than in G-ve bacteria, as the zones of inhibition were larger in first specie than the latent microorganism which could be due to the resistance mechanisms which might be differ from each kind to result in alteration in *Cymbopogon citratus* oil effects toward the different bacterial groups. The antifungal activity presented by lemongrass oil and citral were similar, which indicated significant association between the effect and the presence of citral in lemongrass oil because citral is able to form a charge transfer complex with an electron donor of fungal cells, resulting in fungal death(14). According to a study done by *Geetha and co.*,2020, who concluded that the terpenes found in the lemongrass oil could be the phytochemicals that inhibit the growth of gram positive bacteria but are inactive against gram negative bacteria (15). Thus because the essential oils could easily penetrate via lipoteichoic acids content in G+Ve bacterial membrane that facilitate 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 case of G-Ve bacteria this made difficulties for volatile oil components to penetrate the bacterial cell membrane(16,17)

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

Type of plant		Method used	Inhibition Zone diameter (mm)			
			Gram positive bacteria		Gram negative bacteria	
			<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>V. cholera</i>
<i>Cymbopogon citratus</i>	Oil of <i>Cymbopogon citratus</i>	Disk diffusion	12 mm	12 mm	Negative	Negative
		Well diffusion	23 mm	16 mm	8 mm	12 mm
		Disk diffusion	14 mm	8 mm	Negative	Negative
		Well diffusion	20 mm	18 mm	8 mm	10 mm

3.2- Anticancer activity:

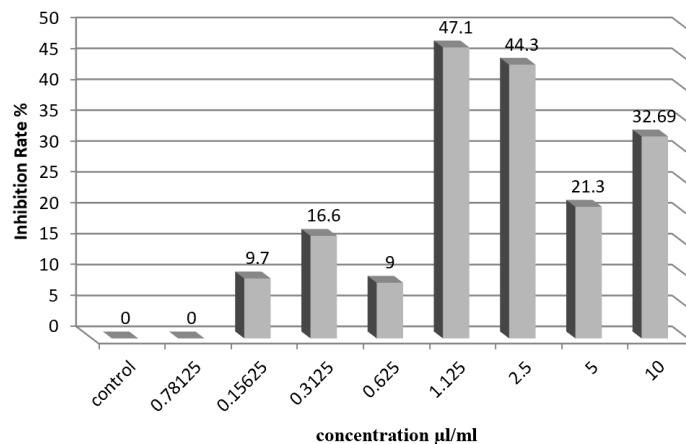
Table 2 showed that lemongrass oil significantly affected ($p < 0.05$) the cancer cells of L20B line by decreasing their growth in almost all concentrations to get inhibition rate (47.1%) at concentration (1.125 $\mu\text{l/ml}$) in comparison with the negative control. A weak cytotoxic effect (9%, 9.7 %) was showed at the concentration (0.625, 0.07812) respectively (Figure 1).

Table(2) Cytotoxic effect of Lemongrass oil extract on the growth of cancer cell line(L20B)*

Concentration $\mu\text{l/ml}$	Cell viability (Absorbance) % (m) L20B	
	Lemongrass oil O.D mean	Inhibition rate %
Negative control	0.155A	0.155
10	104.3B	32.69%
5	0.122B	21.3%
2.5	0.086B	44.3 %
1.125	0.082C	47.1%
0.625	0.141D	9%
0.3125	0.129D	16.6%
0.15625	0.140E	9.7%
0.078125	0.179E	0%

*Differences A,B,C,D,E are significant ($p < 0.05$) to compression column

A numerous reports demonstrating the anti-carcinogenic (or anti-tumor) properties of lemongrass (18). Cellular assays for screening medicinal plants for cancer can be divided into cytotoxicity assays and other assay types that include morphological assays. The major advantage of cytotoxicity assay is that all potential mechanisms concerning cellular proliferation are monitored simultaneously (19).

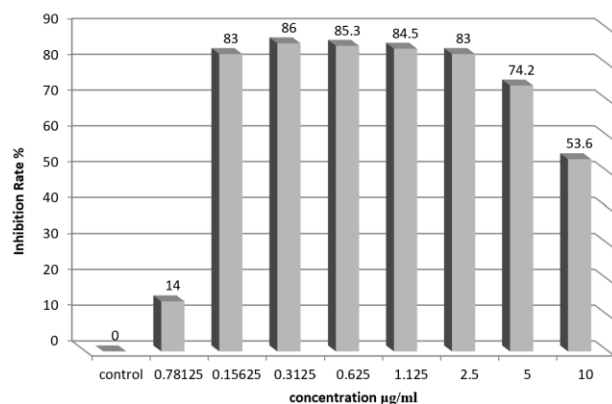
Figure(1) Growth inhibition percentage of *Cymbopogon citratus* on L20B after 72hr

On the other hand the effect of Lemongrass oil in the growth of MCF-7 cell line was show in the Table 3 and Figure(2) which investigated the cytotoxic effects of lemon grass different concentrations on MCF-7 breast cancer cell line. The results showed a significant differences ($P < 0.05$) in cell viability of MCF-7 that reach maximum inhibition rate reach to (86%) at concentration (0.3125 $\mu\text{l/ml}$).

Table (3) Cytotoxic effect of Lemongrass oil extract on the growth of (MCF-7) cell line*

Concentration(μ l/ml)	Cell viability (Absorbance) % (m) MCF-7	
	Lemongrass oil O.D mean	Inhibition rate %
Negative control	0.349A	0.349
10	0.162B	53.6%
5	0.090B	74.2%
2.5	0.059B	83 %
1.125	0.054C	84.5%
0.625	0.051D	85.3%
0.3125	0.049D	86%
0.15625	0.060B	83%
0.07812	0.298E	14%

*Differences A,B,C,D,E are significant ($p < 0.05$) to compression column



Figure(2) Growth inhibition percentage of Cymbopogon citratus on MCF-7 after 72 hr

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 pharmacology studied (20).

IV. CONCLUSION

Lemongrass (*Cymbopogon citratus*) volatile oil showed significantly effects in cancerous cells cytotoxicity and anti-bacterial effects and it can be promise as a new drug discovery in the near future.

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