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Antimicrobial Activity of Aqueous and Alcoholic Lemongrass Extract on *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* Isolated from Burns in Al-Ramadi Teaching Hospital

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ABSTRACT

The present work aimed at exploring the antimicrobial activity of aqueous and alcoholic lemongrass extract alongside three specific human pathogens (*Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*), which were well-known to cause several diseases. The inhibitory effect of aqueous and alcohol extract was studied using the agar-well diffusion method. The study showed that 10% aqueous lemongrass extract indicated maximum antimicrobial activity of 30, 28 and 25 mm alongside *E. coli, C. albicans* and *P. aeruginosa*, respectively. The ethanolic lemongrass extract displayed the highest inhibitory result at 26, 23 and 20 mm, respectively. In contrast, the methanolic lemongrass extract showed the highest inhibitory result at 26, 25 and 22 mm, respectively. This study resolved that both aqueous and alcoholic lemongrass extract had unlimited potential as a microbicide and could be used for numerous human pathogens.

Key words: Microbicide, antimicrobial activity, lemongrass, burns, aqueous, alcoholic extract

INTRODUCTION

In the last era, there was a notable increase in the use of medicinal plants and herbs as main sources for the production of medicinal drugs or as a source of the active constituents that enter into the composition of the drug. It is also used as a raw material to produce some chemical materials and pharmaceuticals. Consequently, it could be a suitable cure for some disorders resulting from various bacterial infections. In Iraq, several studies have been accomplished on the effect of plant extracts on microbes (Ali *et al.,* 2020). For instance: the extracts of mint, mandarin, bitter melon, amber and pomegranate peel were studied on the growth of different types of bacteria, molds and yeasts. These plants were selected for the abundance of these plants in the local environment and the scarcity of studies on their effectiveness in inhibiting microbial growth. Lemongrass is a special type of herb characterized by long green leaves with a white pulp end (Pylak *et al.,* 2019). Its taste is the same as the fresh lemon, with a stronger flavorin white-pulp. It is commonly used in the manufacturing of various types of tea, oils, sauces and many food products (Ghazali *et al.,* 2020).
Fresh lemongrass can be directly used or its derived products such as lemongrass oil and lemongrass tea. Lemon tea has been found to boost the body's production of red blood cells, often because lemongrass contains a relatively high number of antioxidants. It also has a unique aroma. In addition to some essential nutrients, the body needs to produce new and healthy blood cells, such as folic acid, zinc, iron and copper (Kaur *et al.,*2021). Lemongrass contains natural diuretic compounds, so regular use of this herb may improve the body's ability to eliminate accumulated toxins. Lemongrass has antiseptic and antibacterial properties, which may make it effective in fighting and treating some types of infections that may affect the body, such as ringworm, urinary tract infections and scabies (Valduga *et al.,* 2019).
It may help in the treatment of some skin diseases (high content of vitamin A) such as vaginal candidiasis, acne, eczema, psoriasis and internal or external wounds, with effects that may make it superior to some other herbs (thyme) commonly used as a treatment for similar diseases. A mixture of a few drops of its oil with coconut oil can be applied straight away to the infected part (Shahrajabian *et al.,* 2019).

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Escherichia coli a member of the Enterobacteriaceae family is commonly known as normal florae in the intestine of humans and animals. Most *E. coli* strains do not cause disease or may lead to diarrhea for a few days. In comparison, *E. coli* O157: H7 may cause severe stomach spasms, diarrhea and nausea (Karimi *et al.,* 2018). It might be from water or food contaminated (vegetables and meat) with these bacteria. Adults often recover in seven days. In contrast, elderlies and children may develop a deadly infections, such as kidney failure (Ye *et al.,* 2019). Signs and symptoms usually emerge after 3-4 days of contact with this bacterium, identified by diarrhea (watery or bloody), stomach cramps, soreness to the touch and nausea. This strain has the ability to secrete a potent toxin that destroys the lining of the small intestine. This can lead to bloody diarrhea (Yang *et al.,* 2020). *E. coli* disease occurs mainly after eating or drinking a small amount of precooked burger or contaminated pool water.

Pseudomonas spp. is another gram-negative rod, facultative anaerobic belonging to the Enterobacteriaceae family. This bacterium can be isolated from different environments, such as damp areas, wash basins, toilets, non-chloride swimming pools and some antiseptic liquids. *Candida albicans* is a fungal microbe normally found in the human gut. It has the ability to cause UTI, oral thrush, and my enter into the bloodstream, which leads to Candidemia and consider a model for the study of fungi.

P. aeruginosa and *C. albicans* can be found in the armpits and genitals of healthy people. Its illness ranges from a simple exterior to a severe mortal condition, which is more powerful in immune-compromised patients. Contaminated medical utensils, such as drains and gasmasks, may lead to infection with *P. aeruginosa*. These infections with this type of bacteria usually occur in hospitals due to direct contact with contaminated wash basins, disinfection solutions and containers used to collect urine from bladder catheters with this bacterium (Nas *et al.,* 2019).

MATERIALS AND METHODS

The present study comprised of 76 (51 males and 25 females) specimens. These specimens were collected from patients who came to the AL-Ramadi teaching hospital with different types of burn injuries, under surgeon supervision, through the period from December 2021 to March 2022. The ages ranged from 18 to 65 years old. These strains were isolated using morphological examination on special culture media and biochemical tests supported by VITEK-2 system version 08.01. The agar-well diffusion technique evaluated the antimicrobic effect of lemongrass extracts. Specimens were taken using single sterile swaps with sterile normal saline. These specimens were placed into BHI broth and transported straight to the laboratory. After that, those specimens were cultured into different culture media for microbial examination.

Several microscopical and cultural methods were used for the characterization of these bacteria. The methods included: Gram stain and culture on a special medium (NA, MA, SDA and CBA; Figs. 1 and 2) for 24 h at 37°C. Biochemical tests, such as IMVC, Urease, Coagulase, Oxidase, and Catalase, were performed for diagnosis confirmation. This was confirmed by VITEK-2 system version 08.01. The ability of *P. aeruginosa* to produce pyocyanin and protease was examined by culturing on cetrimide agar and skim milk agar, respectively (Raghavendra *et al.,* 2019).

Ten g lemongrass powder was dissolved into 100 ml of dd D.W. (100 mg/ml) on an orbital shaker for seven days. Centrifugation of the mixture was done at 5000 rpm for 5 min before evaporation of supernatant using a freeze-dryer. The concentrations 2.5, 5, 7.5 and 10% of each extract were prepared by taking an

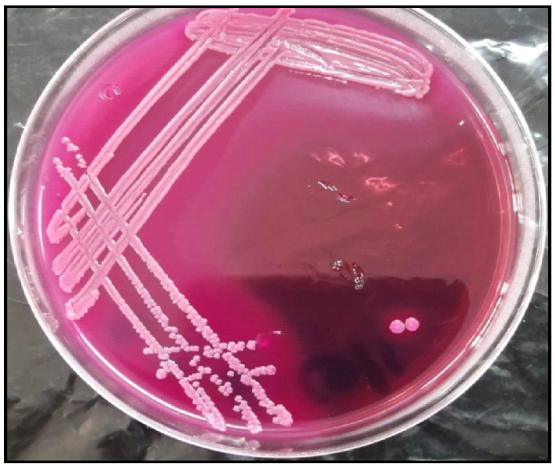

Fig. 1. Colony morphology of *Escherichia coli* on MacConkey agar.

Fig. 2. Colony morphology of *Pseudomonas aeruginosa* on Chocolate agar.

appropriate amount of the filtrate and placing it in a specific volume of sterile distilled water (Dzimitrowicz *et al.,* 2019).

Ten g lemongrass powder was dissolved into 100 ml (70%) of absolute ethanol or methanol on an orbital shaker for seven days. Afterward, a similar procedure in aqueous extraction was recurrent, excluding using 10% dimethyl sulfoxide (DMSO) as a dilutant in place of D.W., in which 100 mg and 50 mg of the extract were suspended separately into 1 ml DW containing 10% dimethyl sulfoxide (DMSO) to obtain a final-concentration of 100 mg/ml. Later mixed well using a vortex, the supernatant was taken out and kept in flasks at 4°C until use.

The inhibitory effect of the examined lemongrass extracts was evaluated using the agar-well diffusion technique. Each isolated bacteria (*E. coli*, *C. albicans* and *P. aeruginosa*) were cultivated for 18 h in broth (BHI) at 37°C. The turbidness of the broth was qualified to McFarland tube no.0.5 (1.5×108CFU). Afterward, 100 µl of 18 h of each broth was mixed with freshly prepared MHA at 42°C and poured into Petri dishes. Four boreholes (7 mm length) were pressed aseptically using sterile cork-piercing after solidifying. Three wells were filled with 30, 50 and 100% (separately) of the test material suspended in 10% DMSO. The four bore holes were fill-in with 10% (DMSO) as a control. These dishes were cultivated at 37°C for 24 h, then the area of inhibition was measured in mm (Gummuluri *et al.,* 2019).

The micro-broth dilution method tested all active plant extracts for their minimum inhibitory concentration. The prepared extract was serially twice diluted into a nutrient broth medium. This procedure was performed in replicates for each dilution (50.0, 25.0, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195 and 0.098 mg/ml). One µl of each bacterial isolate (1 × 107 CFU/ml) was cultivated with the above dilution. The last two replicate wells were not cultivated. Then the inoculated microtiter plates were incubated at 37°C for 18 h. The lowest concentration of the extract (the highest dilution) which inhibited the growth of the tested microorganism was considered as a MIC, and the antibiotic tetracycline was used as a positive control. Sterile distilled water and 10% DMSO were used as a negative control (Bhuyar *et al.*, 2020; Parvekar *et al.*, 2020).

The contents of the resulting MIC wells were cleaved using sterile cotton swabs on an agar plate free of antibacterial agents and incubated at 37°C for 18 h. The lowest concentration of the extract that did not show any bacterial growth was considered MBC.

In testing the sensitivity of bacteria to aqueous lemongrass extract, the container dishes were inoculated at 37°C for 24 h, followed by spreading a volume of 0.1 ml of the bacterial suspension on the center sterile agar surface. These plates were left at room temperature for 15 min for inoculum adsorption. After solidification, wells (7 mm length) were pressed aseptically with the use of sterile cork-piercing. A micropipette was used for transferring 50 µl from each concentration of aqueous lemongrass extract under study and placed inside the hole. Meanwhile, 50 µl of sterile distilled water was placed into one hole as a control. Later the plates were incubated at 37°C for 18 h. Finally, the inhibitory zone was measured as it represented the area of no bacterial growth nearby the well (El-Ishaq *et al.,* 2019).

RESULTS AND DISCUSSION

The highest inhibition rate was recorded at a 100 mg/ml concentration of the aqueous extract against *E. coli*. In contrast, for *P. aeruginosa*, the concentration was 250 mg/ml highest inhibition rate. A high inhibition rate was recorded for *C. albicans* at 150 mg/ml (Table 1). The highest rate of inhibition for the ethanolic extract was recorded at a

Table 1. The minimum inhibitory concentration of ethanol, methanol and aqueous extracts

Bacterial isolates	Extract type	MIC (mg/ml)
Escherichia coli	Ethanol	250
	Methanol	350
	Aqueous	100
Pseudomonas aeruginosa	Ethanol	300
	Methanol	300
	Aqueous	250
Candida albicans	Ethanol	400
	Methanol	300
	Aqueous	150

concentration of 250 in *E. coli*. For *P. aeruginosa*, the highest inhibition was recorded at a concentration of 300 mg/ml of ethanol. A higher inhibition rate was also recorded in *C. albicans* at the level of lemongrass extract, reaching 400 mg/ml.

The highest rate of inhibition for the methanolic extract was recorded at a concentration of 350 in *E. coli*. In *P. aeruginosa*, the highest inhibition was documented at a concentration of 300 mg/ml. A higher inhibition rate was also recorded in *C. albicans* at the level of lemongrass extract reaching 300 mg/ml (Table 1). The obvious effect of lemongrass extract at different concentrations may be attributed to the bacteria's ability to resist some of the active substances in the lemongrass plant.

A significant effect of aqueous lemongrass extract on the length of the inhibitory zone (mm), was 30 mm at a concentration of 10% with a significant difference from other concentrations of *E. coli* (Table 2). As for *P. aeruginosa* and *C. albicans*, the inhibition diameter was 25 and 28 mm, respectively, at 10% concentration of aqueous lemongrass extract.

Table 2. The inhibitory zone (mm) of aqueous lemongrass extract

Concentration / Bacterial isolates	2.5%	5%	7.5%	10%	LSD (0.05)
Escherichia coli	11	21	26	30	3.43
Pseudomonas aeruginosa	10	15	20	25	2.17
Candida albicans	15	17	23	28	4.54

The outcomes of arithmetical evaluation, as shown in Table 3, specified a significant effect of aqueous lemongrass extract on the length of the inhibitory zone (mm), in which its highest inhibition rate was 26 mm at a concentration of 10% with a significant difference from other concentrations of *E. coli*.

Table 3. The inhibitory zone (mm) of the ethanolic lemongrass extract

Concentration / Bacterial isolates	2.5%	5%	7.5%	10%	LSD (0.05)
Escherichia coli	9	15	23	26	11.3
Pseudomonas aeruginosa	9	16	12	28	23.3
Candida albicans	10	15	26	29	31.3

As for *P. aeruginosa* and *C. albicans*, the inhibition diameter was 20 and 23 mm, respectively, at a 10% concentration of the ethanolic lemongrass extract.

Similarly, the outcomes of arithmetical evaluation, as shown in Table 4, specified a significant effect of aqueous lemongrass extract on the length of the inhibitory zone (mm), in which its highest inhibition rate was 26 mm at a concentration of 10% with a significant difference from other concentrations of *E. coli*. As for *P. aeruginosa* and *C. albicans*, the inhibition diameter was 22 and 25 mm, respectively, at a 10% concentration of the methanolic lemongrass extract.

Table 4. The inhibitory zone (mm) of the methanolic lemongrass extract

Concentration / Bacterial isolates	2.5%	5%	7.5%	10%	LSD (0.05)
Escherichia coli	8	17	22	27	38.2
Pseudomonas aeruginosa	11	14	18	32	56.3
Candida albicans	12	19	25	30	22.3

CONCLUSION

It is clear from the results that aqueous, ethanolic and methanolic lemongrass extract had a significant effect on the growth and activity of *E. coli, C. albicans* and *P. aeruginosa* isolated from burns and at different concentrations on these types of bacteria. A clear indication that deserves attention and focuses was on obtaining anti-microbial sources from safe and low-chemical plant sources.

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