Anti-Bacterial Activity, Level of Cytotoxicity and Chemical Constituents of Essential Oil of Lemongrass Under Three Different Artificial Growth Conditions

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Objective: To evaluate the antibacterial activity of Essential Oils (EO) extracted from Lemongrass (LG) (Cymbopogon citratus, stapf.) against a broad range of food pathogens.

Design: Experimental Study In Vitro.

Setting: University of Bahrain, Bahrain.

Method: EO from LG were extracted by hydrodistillation and their chemical composition were determined by gas chromatography-mass spectrometry (GC-MS) analysis. These EO were tested for antibacterial activity against gram-negative and positive bacteria in three different agricultural growth media. The cytotoxicity of the EO extracts of LG was tested using the Brine Shrimp Test (BST) method.

Result: The EO pooled mixture exhibited significant antibacterial activity against all the bacterial test strains ($p>0.05$); the most significant was against P. Vulgaris with 17–24 mm inhibition zone in the diffusion test ($p>0.001$). EO yield for sodium and phosphorus was detected in compost, 1.9% and 0.6%, respectively. For potassium, the highest level was detected for hydroponic (3.2%) and sodium the highest level for sand (1.8%).

Conclusion: The LG oil revealed significant antibacterial activity against Gram-positive and negative bacterial strains due to the presence of diverse chemical components.

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The emergence of frequent multidrug-resistant pathogenic bacterial strains has increased the urgency to seek better and safer antibacterial options with minimal side-effects. Many drugs of plant origins are showing promising results due to their anti-inflammatory and antimicrobial health benefits[1]. One of the most commonly used plants is the lemongrass (Cymbopogon citratus stapf.) (LG), due to its commercially valuable essential oils (EO) and wide use in food technologies as well as in traditional medicine[1]. Cymbopogon species are indigenous in tropical and semi-tropical areas. The plant is usually harvested for its rich chemical composition and citral content, which varies according to the maturity stage of the plant[2].

Tajidin et al found as many as 65 different compounds, some of them only in trace amounts in different maturity stages of the plant; however, the main components were citral, juniper camphor, neral, nerol, 3-undecyne, geranyl acetate, geranial and myrcene.

Balakrishnan et al found anti-oxidative protective abilities of LG against free radicals when DNA is subjected to hydrogen peroxide and treated with LG extracts. The LG extracts showed notable anti-pathogenic abilities against dangerous animal and plant bacterial strains[3]. LG EO had a positive effect against the fungal species of Candida[4]. LG aqueous extracts have a substantial effect on the cardiac rate in mice study[5].

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This indicates that LG can have major uses in the medicinal industry. The citral content of LG has anti-tumor and anti-mutagenic effect[6]. A neurobehavioral study of beta-myrcene on rodents was investigated by de Silva[7]. Myrcene and LG EO analgesic property was studied by Lorenzetti et al[8]. The effect of myrcene on nociception in mice was studied by Rao et al[9]. Myrcene blocks the pain receptors and has a long-lasting effect than analgesic drugs. The anthelmintic property of LG was investigated by Sherwani et al[10]. Aqueous extracts of LG against earthworms resulted in paralysis and death with increased concentrations[10,11].

The use of EO extracted from aromatic plants as antimicrobial agents is gaining attention. EO are rich in biologically active compounds causing antimicrobial effects[11]. The prevention of food spoilage can be achieved by the use of EO.

The aim of this study is to evaluate the antibacterial activity of EO extracted from LG. Furthermore, to assess the productivity of LG under green-house growth conditions.

METHOD

Microbial strains of human pathogens were used in the antimicrobial bioassay, American Type Culture Collection (ATCC), which included the Gram-negative bacteria: Klebsiella pneumonia, Proteus vulgaris, Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa, Salmonella typhi and Gram-positive bacteria: Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis and Staphylococcus epidermidis.

Agar well diffusion method by Perez et al was performed to evaluate the antimicrobial activity[12]. Overnight culture of each bacterial strain (1 ml) was placed in a sterile Petri plate, followed by 20 ml of molten nutrient agar (NA, 45°C) into the Petri plate, then rotated to mix the culture and medium thoroughly. Six wells (4-5 mm diameter) were made in each of these plates using a sterile cork borer. Approximately 50 µl of LG solvent extracts were added using sterilized micropipettes into the wells and allowed to diffuse at room temperature. The plates were incubated for 24 hours at 37°C. Control experiments comprising inoculums without plant extract were set up. The diameter of the inhibition zones (average of three regions) were recorded.

The Brine Shrimp Bioassay (BST) experiment was performed according to Meyer et al[13]. Brine shrimp eggs were imported from North American Brine Shrimp (NABS) company and were hatched in a shallow rectangular box (22x32 inches) under the same general conditions described in the literature except that natural instead of artificial seawater was used.

The EO were analyzed by GC-MS using a Perkin Elmer Claus 600 C mass selective detector coupled with PE Claus 600 gas chromatograph, PE Elite-5MS capillary column (30m×0.2mm (ID), film thickness 0.25µm). Operating conditions were as follows: carrier gas, helium with a flow rate of 1 mL/min; column temperature, zero min at 80ºC,80–280ºC at 10ºC/min and finally held for 20 min at 280ºC; injector temperature, 250ºC; source temperature, 200ºC; volume injected, 1 µL of the oil in dichloromethane (0.1%); split ratio, 1:20. The MS measurements were as follows: ionization potential, 70 eV; ion source temperature, 200ºC; quadrupole 100ºC, solvent delay 3.0 min, mass range 25–600 amu, EI mode.

Data obtained were analyzed with the statistical software JMP8 (SAS Corporation, Wisconsin, USA, 2006), and means were separated at P ≤0.05 level of significance. The normality test with Shapiro Wilk was assessed to determine if the data were normally distributed. Data were analyzed by one-way ANOVA to assess the variance with different means and one independent variable. Results were expressed as the mean and SD. Value of P <0.05 was considered statistically significant.

Descriptive analyses were performed using the total mean and the independent sample analysis for the comparison between the two baseline treated groups. Frequency analysis assessed the number of subjects within each sample.

RESULT

Antibacterial activity of LG (Cymbopogon citratus) EO was tested against Gram-negative bacterial strains of K. pneumonia, P. Vulgaris, E. coli, S. marcescens, P. aeruginosa, S. Typhi and Gram-positive bacterial strains of S. aureus, M. luteus, B. subtilis and S. epidermidis by agar diffusion method.

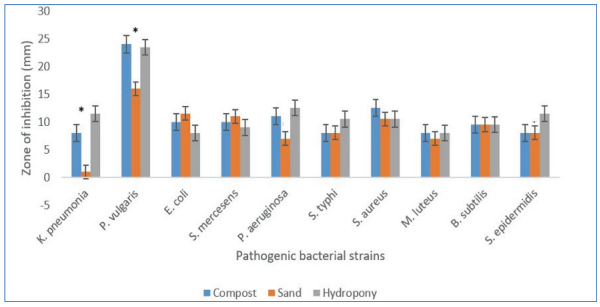

Figure 1: Effect of Different Growing Methods on Microbial Activity of Lemongrass

LG extract showed a significant antimicrobial effect against all tested strains (Gram-negative bacteria: K. pneumonia, E. coli, S. marcescens, P. vulgaris, P. aeruginosa, S. typhi and Gram-positive bacteria: B. subtilis, S. epidermidis, M. luteus and S. pneumonia), see figure 1. The highest antimicrobial activity was against P. vulgaris from 17 to 24 mm zone with a statistically significant change compared to compost and hydropany (P<0.05). In all other Gram-negative bacteria, the level was between 5 to 10 mm. No statistical differences were detected among the different substrates (compost versus sand and hydropony), except for K. pneumonia (P=0.543) and P. vulgaris (P=0.564).

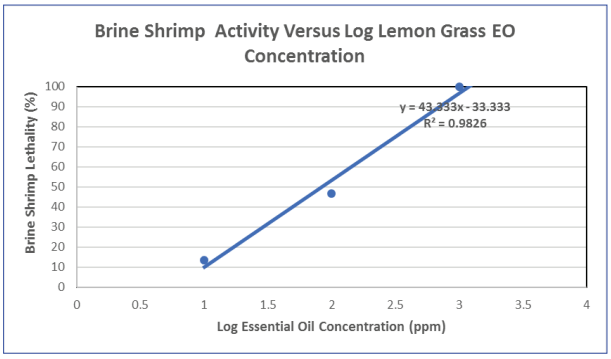

Figure 2: Graph Plot of Brine Shrimp Bioassay for Determination of Cytotoxicity of Lemongrass Essential Oil. LC50 Value Obtained When Brine Shrimp Activity Was Plotted Against Log of Essential Oils Concentration (Ppm) Through the Slope Which Was Found To Be 83.18 Ppm and R^2 Was 0.982

The degree of lethality was directly proportional to the concentration of the extract, ranging from slightly toxic with the lowest concentration (10 µg/mL) to highly toxic with the highest concentration (1000 µg/mL) compared to methanol. When considering the same concentration across different plant species EO (LGES, SOES, and ROES), shrimp mortalities were almost similar in going from one plant to another. Lethal concentration LC50 of each plant investigated at 24 hours was obtained. A plot of percentage of the shrimps killed against plant extract concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. LC50 was obtained from the best-fit line of the slope and were recorded, see figure 2.

The percentage of EO content (w/w) of LG was 0.14% in plants grown in sand, 0.30% in hydroponic, and 0.45% in compost, see table 1. EO components, analyzed by GC-MS, consisted of myrcene (RT=4.68), limonene (5.30), eucalyptol (5.38), geranyl tiglate (6.11), neral (8.18), geraniol (8.20), geranial (8.50), geranyl acetate (8.75), neryl acetate (9.45), and geranyl propanoate (9.95) (numbers in parenthesis indicate retention time). Myrcene was highly affected by growth media; it increased from 6.22% in plants grown in the sand to 19.34 and 24.31% in hydroponic and compost, respectively. Neral decreased from 29.53% in plants grown in the sand to 12.63% and 5.43% in hydroponics and compost, respectively, while geraniol increased from 3.08% in plants grown in the sand to 3.15% in hydroponic and decreased to 1.59% in those grown in hydroponic and compost. Geranial decreased from 36.66% in plants grown in the sand to 14.58% and 5.54% in hydroponically and compost grown plants, respectively. A high percentage of geranyl acetate was only found in plants grown in compost (66.88%), while geranyl propanoate content was higher in plants grown hydroponically (31.70%).

Table 1: Chemical Concentration of Lemon Grass Essential Oil in Different Growth Media

Retention time (RT)	Compound	Concentration %			
		Sand	Hydroponic	Compost	Significance
4.68	Myrcene	6.22 b	19.34 b	24.31 a	*p>0.01
5.30	Limonene	0.71	Trace	Trace	*p>0.01
8.18	Neral	29.53 a	12.63 b	5.43 c	*p>0.05
8.20	Geraniol	3.08 a	3.15 a	59 b	*p>0.001
8.50	Geranial	36.66 a	14.58 b	5.45 c	*p>0.05
8.75	Geranyl acetate	1.33	Trace	66.41	*p>0.001
9.95	Geranyl propanoate	21.84 b	31.70 a	25.48 b	*p>0.267

Note: Means followed by the same letter are not significantly different at p≤ 0.05 according to DMRT.

Shoot height increased progressively at different rates, in all growth media. However, the rate of increase was more pronounced in hydroponic and compost than in sand. Significant differences (p≤ 0.05) were found in shoot height between plants grown in compost and both those grown in hydroponic and sand starting five weeks after transplanting. Significant differences (P≤ 0.05) in shoot height between plants grown in sand and hydroponic started to show 11 weeks after transplanting. Thirteen weeks after transplanting, there were no significant differences between compost and hydroponically grown plants, and in week 15 and 16, hydroponic exceeded compost. Plant height was increased by 93% in plants grown in sand; those grown in hydroponic and compost increased by 180 and 273%, respectively, in 11 weeks period. In 16 weeks, it increased by 357 and 333% in hydroponic and compost plants, respectively, see figure 3.

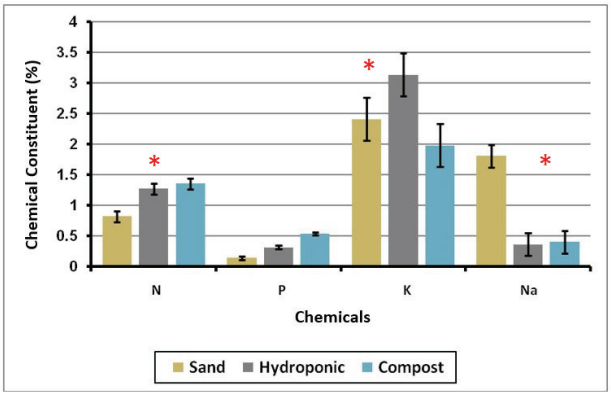

Figure 3: Effect of Growth Media on Lemongrass Shoot Chemical Constituents Essential Oil Yield (%)

Significant differences (p≤ 0.05) in nitrogen, sodium and potassium content of plant shoot were found between plants grown in sand and those in hydroponic and compost. Overall, for sodium and phosphorus, the highest level was detected in compost, (1.9%) and (0.6%). For potassium, the highest level was detected for hydroponic (3.2%) and for sodium, the highest level for sand (1.8%).

DISCUSSION

This study reveals the effect of the different growth methods on the antimicrobial effect of LG except in K. pneumonia and P. vulgaris. The inhibition zone was 1 mm and 17 mm for K. pneumonia and P. vulgaris, respectively, when the LG was grown in the sand; inhibition zone was 8 mm and 24 mm, respectively, when LG was grown on the compost. This preference for compost indicates that LG needs precursors found in the substrate or irrigating water to produce the antimicrobial agent needed to control the growth of some bacteria. These results are supported by Naik et al who confirmed the toxicity effect of LG oil against S. aureus, B. cereus, B. subtilis, E. coli, K. pneumonia[14]. Naik et al found that LG did not show antimicrobial effect against P. aeruginosa. Another study confirmed the antimicrobial effect of LG EO against the food-borne pathogens: S. aureus, E. coli, B. cereus and S. Typhimurium in cream-filled cakes[15].

Finding new therapeutic options for Gram-negative bacteria is a challenge because their infection is hard to treat compared to Gram-positive bacteria[16]. A study found the antimicrobial property of LG (Cymbopogon citratus) oil against gram-positive and negative pathogenic bacteria isolated from pets including turtles[17].

Lethality values of LG EO to brine shrimp revealed significant lethality to Artemia salina with exposure to different doses of the plant EO.

Our study revealed that plants grown in compost or hydroponic had stunted growth, nutrient deficiency, and salt injury most

likely caused by the high Na accumulation in plant shoot tissue. Mazhar et al and Brady et al described the symptoms of plants affected by salinity show as 1) leaves necrosis, scorching, or mottling, leaves shedding and tip burn and death, 2) fragmentation of cuticle and injury to cell membrane due to increased solute leakage, 3) inhibited vegetative growth with reduction of shoot growth, and 4) loss of their leaves prematurely[18,19]. Some of these symptoms were observed in plants grown in sand. LG and sage suffered from nitrogen deficiency (optimum percentage in plant tissue is 2-5%[20].

The concentration of sodium in palmarosa and LG tissue was increased by increasing salinity; increasing soil fertility plays an important role in increasing herb and oil yield in both plant species[21]. Hydroponic and compost had a positive effect on the growth and yield of plants. This is due to the availability of all nutrients needed for optimum growth. The compost act as a slow-release fertilizer, which increases the supply of nitrogen and phosphorus to crops, and improves physical, chemical, and biological conditions of the soil, which, in turn, enhances plant growth and yield[22]. Hydroponics also provides the optimum nutrient needs by plants; this also plays an important role in increasing plant growth and yield[22-25].

Our study revealed that the EO yield was low in all plant species grown in sandy soil compared with those grown in hydroponic and compost. This may be due to low nutrient availability in the soil as well as the high salinity levels (up to 18dS/m) that caused an adverse effect on plant growth. Increasing soil salinity level strongly influences the EO biosynthesis and this may affect the synthesis and composition of oil and cause a yield reduction in Lamiaceae species[26,27].

The reduction in EO biosynthesis in thyme (Thymus vulgaris) is attributed to the increase in soluble salts in the soil solution, which in turn increased its osmotic pressure, which leads to decrease in plant water and nutrient uptake[24]. The suppression of plant growth under saline conditions is due to the toxicity of NaCl, and reduction in plant dry weight, which was due to the inhibition of hydrolysis of reserved foods and their translocation to growing shoots and the subsequent reduction in EO yield[28]. The reduction in EO content was associated with the reduction in leaf number and thickness as a result of salt stress[27].

The increasing soil salinity level (up to 4.2dS/m) resulted in a gradual increase in sage (Salvia officinalis) EO concentration[29]. The same result was obtained by Ozturk et al in Lemon balm[30]. Our results contrast the previous studies, possibly due to different plant cultivars and high salt levels in our experiment. Increasing salinity has no effect on the EO percentage of peppermint, but it increases EO percentage in lemon balm[31]. Dracocephalum moldavica planted in compost has a high EO percentage due to the effect of compost in accelerating metabolism reaction as well as stimulating enzymes[32]. This increment may be due to the effect of compost on mass production and oil content.

EO content of LG grown in sand was 0.14% compared to the results obtained by Pandey et al where the EO yield of LG (Cymbopogon flexuosus) wildly grown in India, was 0.75%[33]. All previous authors attributed the differences in the EO percentage to reasons such as season or harvest time, geographic origin, climatic conditions, or the site where plants were grown.

CONCLUSION

The LG oil revealed significant antibacterial activity against Gram positive and negative bacterial strains due to the presence of diverse chemical components.

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