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STUDY OF BASIC CHEMICAL COMPONENTS AND ANTIMICROBIAL ACTIVITY OF LEMONGRASS LEAVES (*Cymbopogon citratus*)

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ABSTRACT

The study aimed to evaluate the antimicrobial activity using different concentrations of aqueous and alcoholic extracts of dried lemongrass leaves. Chemical phytochemical tests were performed for aqueous and alcoholic extracts of lemongrass. Antimicrobials activity was tested using agar disc diffusion method against *Escherichia coli* and *Staphylococcus aureus*. The results of the study showed that the aqueous extract of dried lemon leaves was highly effective (P≤0.05) against *S. aureus*, as the inhibition diameter was 22 mm for 50 dilution, while the inhibition diameter decreased to 15 mm for concentration 100. As for the alcoholic extract only, the diameter of inhibition decreased significantly (P≤0.05) as it was 16 mm for 50 dilution, and the diameter of inhibition decreased significantly (P≤0.05) to 8 mm for concentration 100 for *S. aureus* as well. The results of the study also showed that the effect of the alcoholic extract of lemongrass against *E. coli* was high, as the inhibition diameter was 20 mm for dilution 50, and a significant decrease (P≤0.05) fell to 12 mm for concentration 100. While the results of the study showed that the effect of alcoholic extract of lemongrass against *E. coli* the diameter of the inhibition was 14 mm for the dilution of 50, and it decreased significantly (P≤0.05) to zero for the concentration of 100.

Keywords: Lemongrass, antimicrobial activity, phytochemicals.

دراسة التركيب الكيميائي والفعالية المضادة للاحياء المجهرية لاوراق عشبة الليمون *Cymbopogon citratus*

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الخلاصة

هدفت الدراسة إلى تقييم النشاط المضاد للميكروبات باستخدام تراكيز مختلفة من المستخلصات المائية والكحولية لأوراق عشبة الليمون المجففة، اذ تم إجراء اختبارات المواد الكيميائية الفعالة للمستخلصات المائية والكحولية لعشبة الليمون، وتم فحص الفعالية المضادة للميكروبات باستخدام طريقة نشر قرص الوسط الصلب ضد بكتريا القولون والمكورات العنقودية الذهبية، وأظهرت النتائج أن المستخلص المائي لأوراق الليمون المجففة ذو فعالية معنوية عالية (P≤0.05) تجاه البكتريا العنقودية الذهبية، إذ كان قطر التثبيط 22ملم للتخفيف 50، بينما انخفض قطر التثبيط الى 15ملم للتركيز 100، اما بالنسبة للمستخلص الكحولي فقط انخفض قطر التثبيط معنويا (P≤0.05)، إذ كان 16ملم للتخفيف 50، وكذلك انخفض قطر التثبيط معنويا (P≤0.05) الى 8 ملم للتركيز 100 للبكتريا العنقودية ايضا، كذلك أظهرت النتائج أن تأثير المستخلص الكحولي لنبتة الليمون تجاه بكتريا القولون عالية، إذ كان قطر التثبيط 20 ملم للتخفيف 50، وانخفض معنويا (P≤0.05) الى 12 ملم للتركيز 100، بينما لوحظ أن تأثير المستخلص الكحولي لنبتة الليمون تجاه بكتريا القولون كان بقطر تثبيط 14 ملم للتخفيف 50، وانخفض معنويا (P≤0.05) الى الصفر للتركيز 100.

الكلمات المفتاحية: عشبة الليمون ، فعالية مضادة للميكروبات ، مواد كيمائية نباتية.

INTRODUCTION

Food safety from microbial contamination and spoilage is a concern for consumers of processed and canned food. So the save is performed and various techniques in the food system to extend the shelf life of food and control the growth of foodborne pathogens by heat treatment, decreasing water activity, and adding synthetic antimicrobial agents have many drawbacks including changes in the organoleptic properties of the food produced, the loss of many nutrients and the safety issues related to the use of chemical additives **(Negi, 2012; Francisco *et al.*, 2013)**. Recently, extensive studies have focused on the replacement of synthetic antimicrobials with natural antimicrobials from vital plant sources such as traditional medicinal plants, spices and herbs **(Manvitha & Bdya, 2014; Zulfa *et al.*, 2016)**. Lemongrass is native to Asia and in all Asian regions but now grows all over the world, for its subtle flavor in its fresh and dried leaves common to Asian cuisine and use in curries, tea, soups and milk, it is also suitable for cooking with poultry, seafood and fish **(Sanchez *et al.*, 2010; Wifek *et al.*, 2016)**. And it was also used in folk medicine **(Bhoj *et al.*, 2011)**, which was mixed with tea as sedatives, and to treat many fever diseases, and as an immunostimulant in India, Egypt and Iran **(Amirdivani & Baba, 2011; Tilaye *et al.*, 2018)**. Lemongrass has also been used in the problems of stomach disorders and was also used in mixing it with other herbal plants to effectively treat malaria and typhoid **(Depken, 2011)**. Aqueous or alcoholic extracts from dry leaves have been used as antimicrobial **(Danlami *et al.*, 2011)**, anti-inflammatory **(Arhoghro *et al.*, 2012)**, anti-hepatotoxic and anti-hypertonic agents **(Hasim *et al.*, 2015)**. It has also been used to treat fevers as a sleep aid. Several studies of its antioxidative efficacy and cancer chemopreventive properties were presented by **(Ramar *et al.*, 2014; Olorunnisola *et al.*, 2014)**. Studies have also demonstrated the possibility of using lemongrass as a therapeutic and preventive agent against coronary artery disease **(Jamuna *et al.*, 2017)**. The antimicrobial activity of lemongrass is due to its high content of phytochemicals and essential oils **(Calo *et al.*, 2015; Zulfa *et al.*, 2016)**. Therefore, this study aimed to evaluate FT-IR analysis to find the presence of functional groups in the alcoholic extract of lemongrass and to evaluate the antimicrobial activity of the aqueous and alcoholic extract of lemongrass, which has the borne pathogen.

MATERIAL AND METHODS

Lemongrass (*Cymbopogon citratus*) material

Lemongrass *(Cymbopogon citratus)* Mahmudiya/Baghdad, and then transferred to the laboratories of the market research and consumer protection center, university of Baghdad. Lemongrass leaves were washed well under running tap water with stirring and scrubbing in a large bowl to remove most of the dust and foreign weeds. Then they were dried first with a large stainless steel colander, then they were laid out in stainless steel trays also until they were dried in the room and under the ceiling fan, and then they were ground in the laboratory electric grinder to fine powder is and kept in airtight glass bottles and kept in the refrigerator until used according to **Sarin *et al.*, (2014**).

Aqueous Lemongrass extract preparation

Dried lemongrass leaves were extracted according to **Caroline *et al.* (2018**) by taking 30 g of dried leaves and placing them in a beaker with a capacity of 150 mL and adding distilled water to it up to 100 mL and placing it on a continuous shaker at a temperature of 60-70°C. Then it was cooled down and the extract was then filtered using Whatman No. 1 at 50^{o}C. The volume of the extract was reduced to 50 mL by placing it in the electric oven at 55°C, then it was packaged in a glass container and stored in the freezer (-18°C) until the required analyzes were performed.

Alcoholic Lemongrass extract preparation

Dried lemongrass leave were extracted according to **Caroline *et al.* (2018**) by taking 100 g of dried leaves and placing them in a beaker with a capacity of 150 mL and adding 400 mL of 100% (volume/volume) ethanol/overnight by placing it on a continuous shaker at a temperature of 50°C according to **Caroline *et al.* (2018)**. Then it was cooled down and the extract was then filtered using Whatman No.2 at 25^{o}C. The volume of the extract was concentrated using rotary vacuum evaporator with constant shaker agitation. Then, the extract was centrifuged at 220 g for 10 min at 50°C in order to evaporate or volatilize all the ethanol from the extract. The volume of the extract was reduced to 50 mL by placing it in the electric oven at 55°C, then it was packaged in a glass container and stored in the freezer (-18°C) until the required analyzes were performed.

Phytochemical analysis of lemongrass

Both the aqueous and alcoholic extracts of dried lemongrass leaves were used to detect and investigate the specific components of bio composites from different phytochemicals such as pH, tannin, carbohydrates, glycosides, phenols, resins, flavonoids, saponins, alkaloids, proteins, coumarins, terpenes and steroids using standard protocols. According to **Lalitha *et al.*, (2012); Aman & Dipak (2020); Zannou *et al.* (2016)**.

Antimicrobial activity

The standard disc diffusion method was used on human pathogenic bacteria *E. coli and S. aureus*, used Maconkey broth (HiMedia) to culture the bacteria and incubate at 37°C/ 24 h after which the fresh growing bacteria were taken and spread on McConkey agar plates to grow the bacteria. After that, sterile paper saturated with nanoscale plant extract with a diameter of 5 mm was placed, and the control sample was made with double distilled water, and another sample was made using only the blend extract. The dishes were placed together and simultaneously in an incubator and incubated at 37°C for 24 h. After that, the antibacterial activity was measured based on the inhibition of the area around the disc for all treatments.

Statistical analysis

The Statistical Analysis System- program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study **(SAS, 2012)**.

RESULT AND DISCUSSION

The results in (Table, 1) refer to the low moisture content of lemongrass 5.7%, which in turn has an antimicrobial activity and benefits during storage, and its high content of ash and crude fiber (6.50 and 75.60 % respectively) which leads to fast food digestion and well absorption by the body. And it contains good proportions of fats, raw ash, protein and carbohydrates 7.40, 6.50, 0.26 and 4.54 % respectively, thus leads to provide the body with energy and strengthens it which is necessary for the body (Table 1). Also, it was known that lemongrass essential oil has a good amount of high essential oils and its content of citral compounds, which was used as a source for the production of beta-carotene a vitamin A **(Wifek *et al.*, 2016; Biljana *et al.*, 2019; Wifek *et al.*, 2016)**.

Table (1): Chemical composition of lemongrass.

Components (N)	(%)
Moisture	5.70
Ash	6.50
Protein	0.26
Fat	7.40
Crude fiber	75.60
Charbohydrate	4.54

N= each unit was an average of triplicate

Phytochemical analysis

Results of this study presented the chemical detection of the active ingredients of lemongrass as in (Table 2). It was found that it contains many components such as resins, soaps, alkaloids, flavonoids, tannins, glycosides, total phenols, saponins and terpenes and lack of coumarins and steroids as presented in (Table 2) . Other study showed lemongrass leaves powder contained of saponins , phenols , resins, alkaloids, tannins, flavonoids, glycosides and terpenoids **(Thorat *et al.*, 2017)**. Also it was confirmed that lemongrass contained saponans, flavonoids and phenols in aqueous extract **(Ojo *et al.*, 2010; Arekemase *et al.*, 2015)**. The lack of steroids in this study was confirmed by **(Soares *et al.*, 2013; Manzoor *et al.*, 2013)**. It was also found that the aqueous extract is acidic in the reaction and that the pH = 5.5.

Table (2): Phytochemical scanning of aqueous and alcoholic lemongrass extract.

Phytochemical constituents	aqueous	alcoholic
pH	5.5	5.0
Alkaloids	+	+
Flavonoids	+++	++
Tannins	+	+
Glycosides	+	+
Total phenols	++	+
Coumarins	-	-
Saponins	+	+
steroids	-	+
Terpenes	+	+

+++ Strongly present; ++ Present; + Weakly present; - Absent **(Hamdia, *et al.*, 2019)**.

Antimicrobial activity

The results of studying the antibacterial activity of different concentrations of the aqueous and alcoholic extract of lemongrass for both bacteria E. coli and S. aureus was represented in (Table 3). The results showed that there was a significant inhibition of the aqueous extract more than the alcoholic extract for all the concentrations used, in addition, it was found that there was a clear and high significant inhibition of the concentrated extracts compared to the diluted ones. The results showed that there was a significant inhibition of the aqueous extract more than the alcoholic extract for all the concentrations used, in addition, it was found that there was a clear and high significant inhibition of the concentrated extracts compared to the diluted ones. The significant inhibition of both gram-positive and gram-negative bacteria of lemongrass aqueous extract can be attributed to the high content of total phenols and flavonoids available in lemongrass aqueous extract. This is what it has proven by previous studies have demonstrated the significant effect of phytochemicals, especially phenolic compounds and flavonoids, for their effective activity against Gram-positive and Gram-negative isolates **(Wany *et al.*, 2014; Singh *et al.*, 2011)**.

Table (3): Antimicrobial activity of Lemongrass extract of some type of bacteria.

Bacteria	Dilution	Inhibition zone (mm)	
		Aqueous extract	Alcoholic extract
E. coli	**100**	12	0
	75	11	10
	50	20	14
S. aureus	**100**	15	8
	75	12	12
	50	22	16
LSD value	zero	3.281 *	2.307 *
*** (P≤0.05).**			

= No inhibition-

CONCLUSION

The results of the current study showed that lemongrass extracts, due to its content of biological compounds and its antimicrobial activity, are highly effective against *E. coli* and *S. aureus* which cause some diseases.

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