

Research Article

Phytochemical Investigation And Antibacterial Activity Of Dried Lime *Citrus Aurantifolia*

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Article History:

Received: June 9, 2024

Accepted: June 30, 2024

Published: July 21, 2024

Abstract: To continue the efforts of screening natural products to obtain more active drugs and safer to use, this study established to screening the phytochemical compounds on dried lime *Citrus aurantifolia* and evaluate the antibacterial activity of its water extract on several bacteria species. Via GC-MS analysis, the most phytochemicals were found in water extract of *C. aurantifolia*, dl-Limonene (34.05%), alpha. -Terpineol (13.45%), Terpinene gamma (4.56%), Alpha.-terpinolene (4.56%), Pinene beta (3.88%), endo-Borneol (3.26%), beta.-Bisabolene (3.31%). The Kirby-Bauer disk diffusion susceptibility test was utilized to determine the antibacterial activity of the extract at various concentration (200, 100, 50, 25, 12.5, and 6.25 mg/ml), it's found to inhibited the bacterial growth as dose depending manner, where the inhibition zone were (25, 25, 19, 25, 26 and 23mm) against *E. faecalis*, *St. aureus*, *B. cereus*, *E.coli*, *K. pneumoniae* and *P. aeruginosa*, respectively. In conclusion, the dried lime has a high potential to be used as antibacterial drug in many fields.

Keywords: Dried lime, phytochemicals, GC-MS analysis, antibacterial activity

How to Cite:

Al Kateeb, A.I., Shamran, D.J., & Abed, E.H. (2024). "Phytochemical investigation and antibacterial activity of dried lime *Citrus aurantifolia*". *Interdisciplinary Studies on Applied Science* 1(1): 12 – 19. <https://doi.org/10.58613/isas112>



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INTRODUCTION

Presently, scientists have been searching for alternative treatment or drugs to fighting pathogens in response to the rise in antibiotic resistance. Furthermore, in recent years there has occurred a decrease in the detection of novel antibacterial drugs (Tüfekci et al., 2020). Various traditional medicinal plants would be the finest alternative source of quality and crucial herbal formulations for the treatment and protection from a variety of diseases in addition, plant-derived treatments are considered the safest and most acceptable among people (Satabathy et al., 2020). Plants are extraordinarily rich in bioactive natural compounds (Al Kateeb, 2022). A large number of secondary metabolites (SMs) are formed in higher plants generated by primary metabolites support plant defense against pests, phytopathogens, insects, herbivores, as well as other challenges (Jain et al., 2019). The physiological and developmental condition of the plant influences the formation of all these pharmaceutically important chemicals, which produce in trace degrees (Twaij and Hasan 2022).

Citrus peels, which provide almost all of citrus "residue," contain an abundance of potential purposes and contain numerous important compounds, includes flavonoids, limonoids, alkaloids, essential oils, and pectin. Citrus fruits provide an enormous variety of industrial byproducts. (Kazeem et al., 2020; Xiao et al., 2021). *C. aurantifolia* is member the Rutaceae family, which involves 900 species and 150 genera (Mohammed, 2016). *C. aurantifolia* is a popular raw material for cosmetics, food flavouring, beverage flavor enhancers, and traditional medicine (Swandiny et al., 2021). A number of studies have shown the *C. aurantifolia* includes biological activities such as an insecticidal, larvicidal, and repellent properties (Galovičová et al., 2022), antimicrobial, antioxidant, and anticancer activities (Julaeha et al., 2022) antimicrobial, analgesic, astringent, antiviral, antifungal, diuretic, anti-cholesterol, appetite stimulant, and treatment of constipation. The wide range of biological functions shown via *C. aurantifolia* is clarified by the presence of secondary metabolites (Shchérzade et al., 2021).

Lime Basra, noomi Basra, or dry black lime are the names given to this commodity in Iraq. These are also known as Black Lemons, Omani Lemons in Iran (originating in Oman), and Loomi in various Arab nations throughout the region. The fruit is used in almost every tropical home, primarily to flavour food, but also to make drinks and for a range of therapeutic purposes. *C. aurantifolia* is recognized for its aroma and taste and for the health advantages resulting with the bioactive compounds contained in the plant. (Boulos, 1998). Due to each section in plant contains unique bioactive ingredients and biochemical processes, further research into these chemicals is required. Detailed knowledge of the constituents of the dry black lime fruit will lead to a better and specifically diverted application and also to know its purity only be obtained by means of carefully performed GC/MS experiments. Our study aims to analyze phytochemical compounds in the dried lime with GC-MS analysis and investigation their antibacterial activity against several pathogenic bacteria that show high resistance to the antibiotics that are found in the pharmacies.

MATERIAL AND METHODS

Plant Extraction

The dried lime was obtained from the market in Al Muthanna city, powdered by electric blender then 50 g was taken and mixed with distilled water in a ratio 1:6 (plant: water) and boiled until $\frac{1}{4}$ the solution stay. After that, the solution was filtered by filter paper then dried under room temperature until full dryness (Handa et al., 2007).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis All chemical constants in dried lime were determined via GC-MS. The condition used for the analysis shown in the following Table 1.

Table 1. The conditions of GC-MS used to analysis of plant extract

Device	Shimadzu GCMS QP 2010 ULTRA
Column	RXI-5MS Capillary column (30m; 0.25 mm; 0.25 µm).
Carrier gas	Helium (1 ml/min flow)
Column furnace temperature	40°C
Injection temperature	2
Pressure:	100 kPa
Injection mode	Split
Split ratio	25
Injection volume	1µl
Oven temperature program	3 min at 40°C, 40°C to 240°C in 4°C/min increment, 240 °C, 240 °C 10min/Total 63 min.
Interface temperature	250°C
Ion source temperature	200°C

Antibacterial activity method

The bacterial isolates used in this study were obtained from the Department of Life Sciences at the College of Science, Al-Muthanna University. Bacterial species were purified using specialized *Enterococcus faecalis*, *Staph. aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and *P. aeruginosa*. Kirby-Bauer disk diffusion susceptibility test was used to evaluate the antibacterial activity of dried lime *C. aurantifolia* extracts in different concentrations (200, 100, 50, 25, 12.5 and 6.25 mg/ml). The overnight bacterial culture adjusts to 0.5 McFarland standard in 0.9% sterilized normal saline. By sterile cotton swab the inoculum spread on Muller Hinton agar medium. By using A small filter disk, at 6mm diameter saturated with 100 µl of each concentration of plant extracts, also place on that plate. Then normal saline filled on disc as a negative control and using for control. After 24 h. of incubation of all plates at 37°C, the microbial growth inhibition zones (I.Z.) were measured. All experiments were carried out in duplicate (Humphries et al., 2021).

RESULTS AND DISCUSSION

The current study investigated the phytochemical compounds of dried lime water extract and its antibacterial activity against six of bacterial species. Table 2 and Figure 1 showed the compounds that were found in lime extraction. The yield of hot water extraction of dried lime was (1 gm). GC-MS analyse compound according to retention time and the result of GC-MS illustrated the presence of 49 compound occurred in many groups like monoterpenes and sesquiterpenoids but the largest amount was dl-Limonene (34.05%), alpha. -Terpineol (13.45%), Terpinene gamma (4.56%), Alpha. terpinolene (4.56%), Pinene beta (3.88%), endo-Borneol (3.26%), beta. -Bisabolene (3.31%). These compounds known to give the special flavor of the lime also using in perfumes industry and medicine as local antiseptic according to (Ranganna et al., 1983; Boulos, 1998). Our results agree with (Mohammed et al., 2024) work, where they study the essential oils of lime and found the above mention compounds. Also, (Olatunya & Akintayo., 2017) found limonene, Terpineol and Alpha and beta pinene in the dried lime.

Table 2. Phytochemical compounds of *Citrus aurantifolia* water extract

Peak	R. time	Area%	Name
1	9.532	2.63	Pinene alpha
3	10.073	1.17	Camphene
4	11.193	3.88	Pinene beta
5.	11.871	0.80	beta.-Myrcene
6	12.321	0.27	Phellandrene alpha
7.	12.822	2.25	alpha.-Terpinene
8	13.162	4.98	Benzene, 1-methyl-2-(1-methylethyl)- (CAS)
9	13.424	34.05	dl-Limonene
10	14.153	0.18	1,3,6-Octatriene, 3,7-dimethyl-, (E)- (CAS)
11	14.546	4.56	Terpinene gamma
12 .	15.714	4.56	Alpha.-terpinolene
13	16.204	0.75	Linalool
14	16.696	2.59	Fenchol alpha
15	17.031	0.20	Bornyl bromide
16	17.519	0.91	Terpin-3-en-1-ol
17 -	17.916	0.98	Cyclohexanol, 1-methyl-4-(1-methylethenyl)
18	18.366	0.34	BICYCLO[2.2.1]HEPTAN-2-OL, 1,7,7-TRIMETHYL
19	18.568	0.59	Bicyclo[2.2.1]heptane, 2-chloro-1,7,7-trimethyl-, (1R-endo
20	18.738	3.26	endo-Borneol
21	19.175	1.39	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)
22.	19.778	13.45	alpha.-Terpineol
23	19.975	1.64	Terpineol gamma
24	20.261	0.38	Decanal (CAS)
25	20.762	0.20	Octanal, 7-methoxy-3,7-dimethyl-
26	21.128	0.29	Citronellol
27	21.660	0.20	Carvone
28	22.081	0.29	Geraniol
29	23.827	0.23	Carvacrol
30	25.960	0.19	NERYL ACETATE
31	26.614	0.39	Geranyl acetate
32.	26.942	0.35	BETA. ELEMENE
33	27.448	0.17	Lauric aldehyde
34	27.879	1.01	TRANS(.BETA.)-CARYOPHYLLENE
35	28.394	1.45	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-
36	29.008	0.22	alpha.-Humulene (CAS)
37	30.549	0.25	TRANS-.ALPHA.-BISABOLENE
38.	30.757	3.31	beta.-Bisabolene
39	30.987	0.17	Spathulenol
40	32.721	0.28	Caryophyllene alcohol
41	32.888	0.50	Sabinene
42	33.344	1.90	ALPHA.-PINENE, (-)-
43	34.482	0.36	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,
44	34.580	0.19	Eudesmol
45	35.658	0.41	Neoisolongifolane, hydroxy-
46.	36.116	0.74	alpha.-Bisabolol
47	40.731	0.28	1,3,4,6,7,8-HEXAHYDRO-4,6,6,7,8,8-HEXAMETHYLCYCLOPENTA(G)-2-BENZOPYRAN OR GALAXOLI
48	42.527	0.27	Hexadecanoic acid, methyl ester (CAS)
49	51.312	0.19	Pentacosane
		100.00	

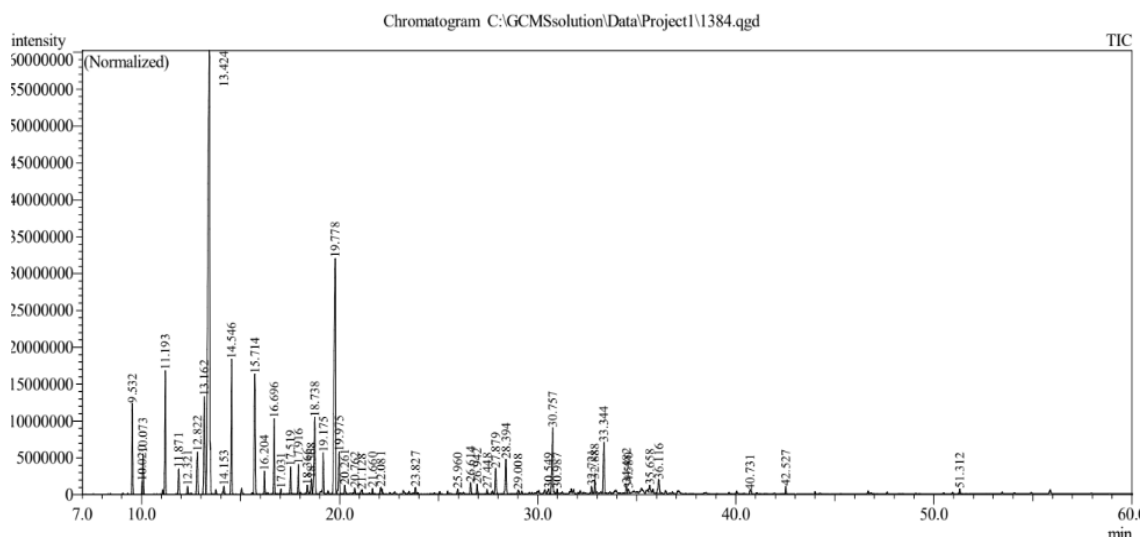


Figure 1. GC-MS chromatograms of *Citrus aurantifolia* extract

Table 3. The antibacterial activity of dried lime water extract against several bacteria (by mm)

Bacteria	Extract concentration mg/ml					
	200	100	50	25	12.5	6.25
<i>E. faecalis</i>	25	21	19	19	16	12
<i>S. aureus</i>	25	23	18	16	12	8
<i>B. cereus</i>	19	19	14	13	9	9
<i>E. coli</i>	25	23	23	20	16	11
<i>K. pneumoniae</i>	26	25	19	18	12	7
<i>P. aeruginosa</i>	23	18	15	10	0	0

The results of antibacterial activity of dried lime shown in Table 3, the inhibition zone against *K. pneumoniae* was between 26 and 7 mm depending on concentrations. Also, the inhibition zones were 25 mm against each of *E. faecalis*, *St. aureus*, *E. coli* and it was 23 mm against *P. aeruginosa* while it was 19 mm against *B. cereus*. The antibacterial activity of the lime extract return to its different phytochemicals, these compounds have ability to disrupting the bacterial cell membranes, inhibiting the essential enzyme and destroying the resistance mechanisms of the bacterial cells (Guo et al., 2021). Our results agreed with (Al Farraj et al., 2018), where they found high antibacterial activity of different extractions of dried lime including water extract against several bacteria. Also, in other study by (Olatunya et al., 2024) they work on fresh lime and dried lime and found the same high antibacterial activity against different bacterial species. The highly antibacterial activity of lime extract against these pathogenic bacteria return to the high amount of phytochemicals in it like limonene, alpha and beta pinene, also the presence of alpha, beta and gamma terpineol as illustrated in Figure 1 and Table 2 (Al-Jabri and Hossain., 2014). This antibacterial activity gives way to the possibility of treating the diseases caused by infection with these pathogenic bacteria. Where *S. aureus* has been found to be resistant to many of the available antibiotics via many mechanisms (Guo et al., 2020). While by using lime extract all the concentrations possess antibacterial effect on this bacterium. The same applies to the other bacteria used in this study. As most types of bacteria have started to resist the conventional antibiotics used to eliminate them, which requires finding new agents to get rid of these pathogenic and deadly bacteria (Bayode et al., 2021; Murray et al., 2019). Limonene is a monoterpenoid compound

make the major compound in the essential oils of citrus family, it's found to destruct the bacterial cell integrity by effecting the cell membrane permeability leading to cell death (Han et al., 2019). Furthermore, Limonene is found to significantly inhibit both gram positive and negative bacteria as well as fungi (Hsouana et al., 2011). Alpha pinene also showed high antibacterial activity and one of the suggested mechanisms was generating promoters for heat shock in the targeted bacteria (Melkina et al., 2021). Additionally, in further study terpineol and its isomeric showed antibacterial activity via destroying cell membrane and cell wall of the bacterial cells (Huang et al., 2021).

CONCLUSION

In the present study several phytochemical compounds were be determined in the water extract of dried lime. Also, the extract showed high antibacterial activity on many bacterial species. this activity thought to be related to the presence of many phytochemicals' compounds in the dried lime water extract. Further studies will be established to screen the antioxidant, anti-inflammatory and anticancer activity of dried lime.

Acknowledgments

The authors acknowledge all members of the Coordinated Collections for supporting this study.

Personal Interest

The authors proclaim no conflicts of interest

Funding Source

Current study did not receive a special funding.

Ethical Approval

Non-applicable.

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