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GC-MS Analysis and Antimicrobial Activity of Essential Oil from (*Cymbopogon citratus*) against Pathogenic Bacteria

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ABSTRACT

Introduction: Our plant under research, Cymbopogon citratus, commonly referred to as lemon grass and a member of the family fabaceae, is one of those potential medicinal plants. It is known as a medicinal plant and is significant in various uses, particularly pharmaceutical usage due to the presence of the active compounds represented by terpens, phenols, alkaloids, etc. Our plant under study is one of these significant medicinal plants. **Objective:** In this work, lemongrass was harvested from the desert of the Anbar Region and then extracted using a Clevenger device to produce oil. To identify the phytoconstituents found in the Cymbopogon citratus plant, this study was done.

Materials and Methods: The sample was identified by GC-Mass, which indicated the presence of 2-Methyl-Z, 13-octadecadienol with percentage 39.86%. Alkaloids, phenols, tannins, and flavonoids were also present, however cardiac glycosides and saponins were not. **Results:** We isolated certain pure phytopharmaceuticals, which may then be employed as a lead molecule to create a new drug with potent therapeutic effects. The antibacterial activity of lemon grass oil on microorganisms has been investigated utilising the well diffusion method to battle pathogenic bacteria and zone of inhibition of growth with widths of 22 mm.

Conclusion: Using the well diffusion method, the antibacterial activity of lemongrass oil on microorganisms has been investigated in order to battle pathogenic bacteria and zones of inhibition of growth with diameters of 22 mm.

Keywords: Cymbopogon citrates, Clevenger apparatus, Antibacterial activity, GC-Mass.

INTRODUCTION

Natural products, primarily from plants, were explored by people in the treatment of different diseases for thousands of years [(1)]. Trado-medicine has been a global topic in the last decade and plays an important role both in healthcare and the global economy [(2)].

It is a matter of global significance. Medicinal plants have recently held their pivotal position in many people's universal health care schemes. In developed nations where commercial medication has a lengthy and continuous tradition of use [(3)]. Due to increased demand worldwide for medical products from medicinal plants, herbal plants manufacturers have been using the best extraction process to see and unlock their chemical compounds [(4)]. The main aims of this research were to evaluate the quality of active substances and to determine the chemical composition of essentials lemongrass oils grown in southern Delta conditions in the Nile region (Bilbeis, Sharqia, Egypt). *Cymbopogon citratus* is the scientific name of lemongrass. The name Cymbopogon derives from the Greek phrases 'kymbe' (boat) and 'pogon' (barley), which refer to a flower spike arrangement [(5)], a genus of approximately 55 grass species [(6)]. Whereas the term lemon-scented leaves derive in the ancient Latin [(7)].

Lemongrass is a genus of Poaceae grass. It is a herb with a fragrance that is known in Egypt, the Arab Peninsula, and tropical Africa of the North and West [(8)]. Tropical perennial herbs with green, long and slates leaves from 60-120 cm high with a good fragrance and flavor, spontaneously emerging worldwide, mostly in tropical and savannan areas. Lemongrass, barbed wire grass, silky heads, lemongrasses, chadedartigalongue, tanglad, hierbaluisa or gavatichaha, and many others are the common names of this grass. *Cymbopogon citratus* (Southwest Asia) is native to South India, but is grown in dense crops in many parts of the world [(9)].

PATIENTS AND METHODS

Collection: Dry *Cymbopogon citratus* leaves were purchased from a local market in Anbar.

Clevenger apparatus method (Hydrodistillation):

The Clevenger apparatus was named for its inventor, Joseph Franklin Clevenger, who had printed it in 1928. There are a few versions. The most famous is a part of a particular glassware, as can be seen above the rotund bottom flask. The flask, of an inconstant dimension, contains both the heated water and the plant to be harvested. Steam rises in the mixture of the condenser and the condensate fall into the narrow burette on the right. Oil floats on the bath, and is eventually extracted to the hot flask over the diagonal conduit. After a few hours of extraction, the amount of the oil can be determined directly together in the burette [(10)].

Culture preparation:

Streptococcus sp. was obtained from Al-Ramadi Hospital and centrifuged at 3000 rpm, twice washed and re-suspended in 0.1 per cent pepton water, loop of 24-hour surface development on NA slope of bacterial isolate, which is transported individually to 5 ml of brain heart infusion broth (PH 7.6) and incubated for 24 hours at 37 °C. The turbidity was changed to meet that of the Mcfarland tube (108 CFU/ml).

Antibacterial screening test of extracts using disk diffusion method:

The disk diffusion check was conducted by normal protocol. On the entire surface of Muller-Hinton agar (MHA), the inoculum suspension of bacterial insulates

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was swabbed (pH7.3) [11]. Aseptically mounted sterile 6-mm paper filter disk (Watman No.3) on MHA surface. Crude oil was applied immediately to 20 ml volume disks. A 10% DMSO and purified water aliquot of 20 ml was also applied as sample to a sterile paper disk while a disk screening was used as the successful check. The plates were left for 15 minutes at 37°C over 24 hr at ambient temperature. The experiment was conducted by grass oil and diameter of the inhibition zone was checked. 22 mm against *Streptococcus sp.* was found to be of good antibacterial activity [12]. Antibacterial activity of *Cymbopogon citratus* oil was reported against *Streptococcus sp* [13].

Ethical Consideration: The Scientific Research Ethics Committee at Anbar University granted approval in order to fulfil the requirements of the research.

RESULTS

GC-mass analysis

As shown from the GC/MS results, we identified a number of extractions in the leaves of our Iraqi plant (*C. citratus*), which included n-Hexadecanoic acid, Palmitic acid, Pentadecanecarboxylic acid, with a good quantity of extract in these leaves. 2-Methyl-Z, 13-octadecadienol, as in figure (1) and table (2). Furthermore, GC–MS analysis of essential oils of Cymbopogon citratus "lemon grass was shown in table (1). While GC–MS analysis of essential oils of *C. citratus* at average 19.492 were shown in figure (2), as well as, GC–MS analysis of essential oils of *C. citratus* at average of 21.315 were shown n figure (3). Lemongrass oil had antibacterial effect against bacteria after determination inhibitory zone with Diameter 22% as in figure (4) and table (3).

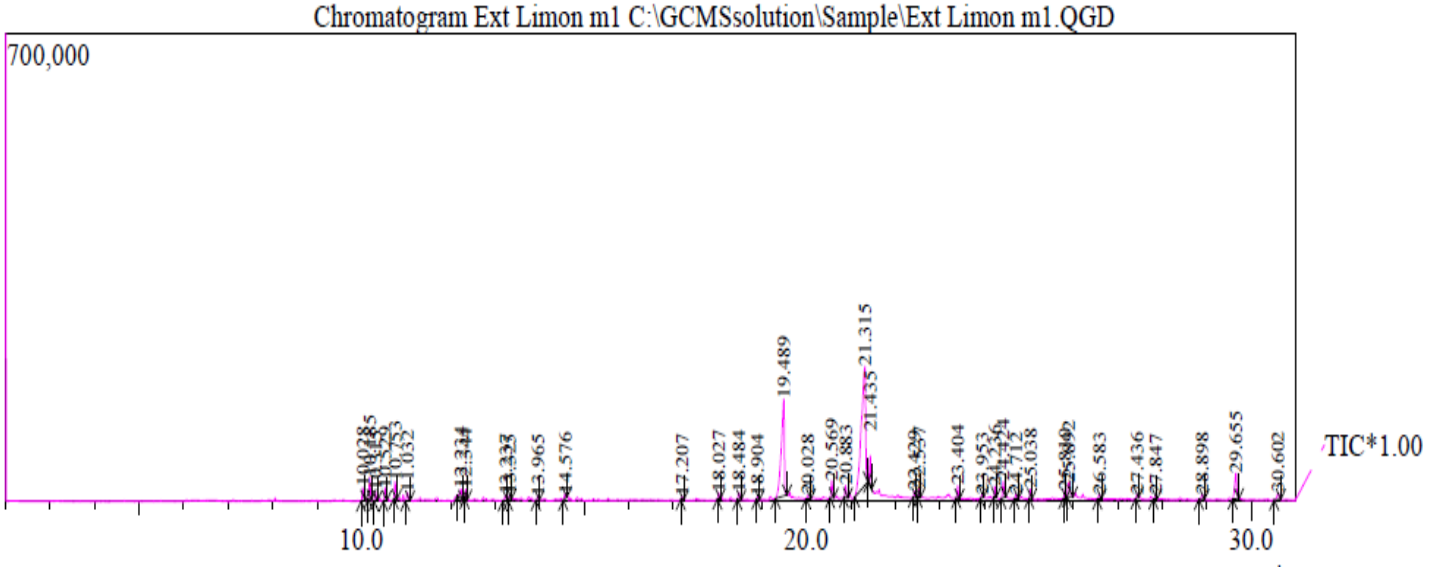
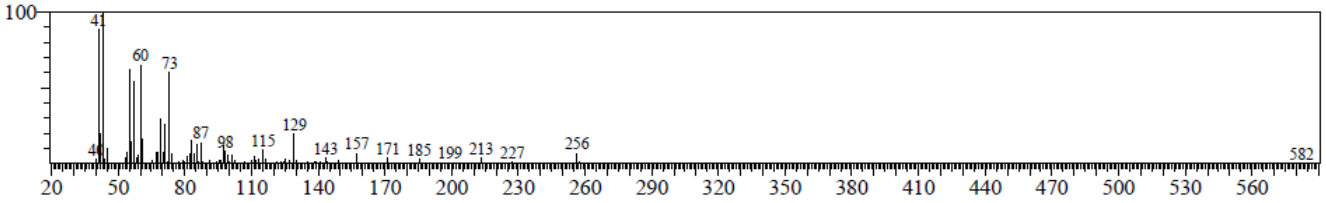


Figure (1): Chromatogram extraction (GCMS) for leaves of *C. citratus.*

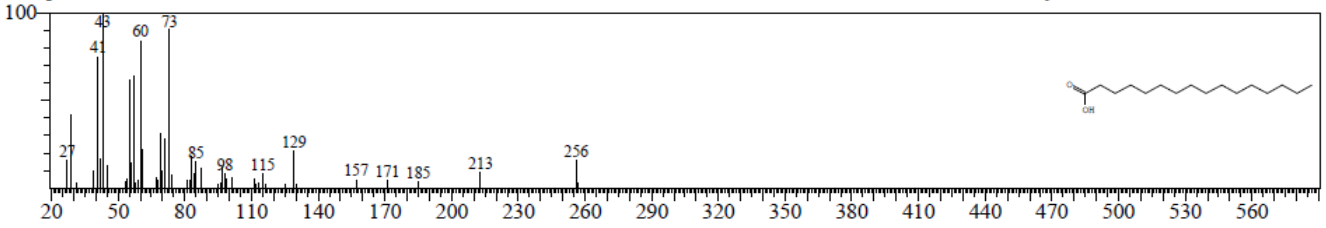
Table (1): GC–MS analysis of essential oils of *Cymbopogon citratus* "lemon grass

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark
1	10.028	9.992	10.083	38785	1.28	19712	2.32	1.95	MI
2	10.185	10.150	10.225	59429	1.96	33593	3.96	1.77	MI
3	10.315	10.275	10.358	24193	0.80	13713	1.62	1.77	MI
4	10.529	10.492	10.567	36828	1.22	19125	2.26	1.91	MI
5	10.753	10.752	10792	45129	1.49	25945	3.06	1.75	MI
6	11.032	10.983	11.092	28165	0.93	12731	1.50	2.21	MI
7	12.234	12.158	12.283	37652	1.24	14459	1.70	2.66	MI
8	12.344	12.317	12.375	24921	0.82	15112	1.78	1.67	MI
9	13.237	13.175	13.283	22192	0.73	7708	0.91	2.86	MI
10	13.325	13.292	13.375	16926	0.56	7130	0.84	2.34	MI
11	13.965	13.917	14.000	13407	0.44	6479	0.76	2.06	MI
12	14.576	14.533	14.617	17767	0.59	7256	0.86	2.36	MI
13	17.207	17.175	17.242	10232	0.34	5341	0.63	1.92	MI
14	18.027	18.000	18.067	17929	0.59	10364	1.22	1.72	MI
15	18.484	18.450	18.533	23721	0.78	10532	1.24	2.26	MI
16	18.904	18.875	18.950	11085	0.37	4961	0.58	2.25	MI
17	19.489	19.308	19.567	680096	22.49	145284	17.13	4.68	MI
18	20.028	19.992	20.100	12015	0.40	4842	0.57	2.48	MI
19	20.569	20.533	20.617	52771	1.74	26140	3.08	2.02	MI
20	20.883	20.842	20.933	34540	1.14	18370	2.17	1.88	MI
21	21.315	21.092	21.383	1205577	39.86	178523	21.05	6.75	MI

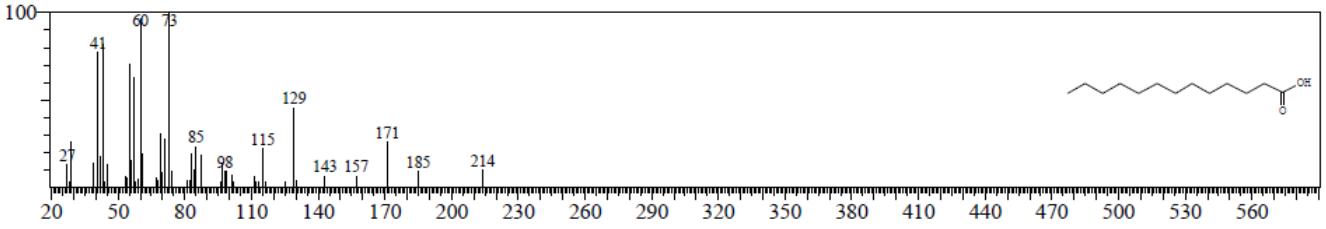
<< Target >>
Line#:17 R.Time:19.492(Scan#:2100) MassPeaks:96
RawMode:Averaged 19.267-19.583(2073-2111) BasePeak:43.05(4019)
BG Mode:19.583(2111) Group 1 - Event 1



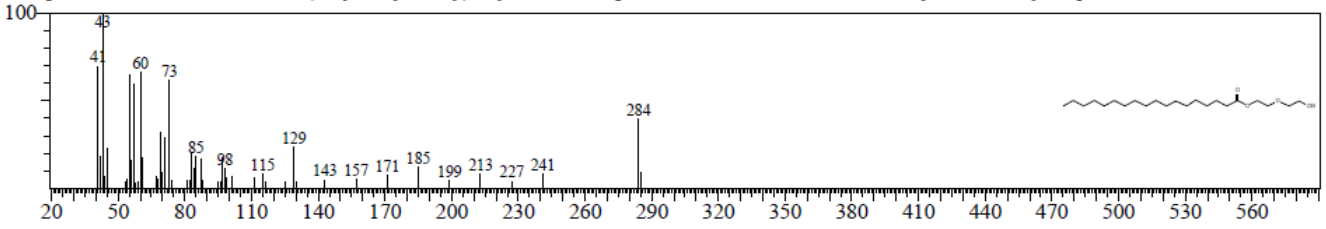
Hit#:1 Entry:80732 Library:NIST08.LIB
SI:95 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecanecarb



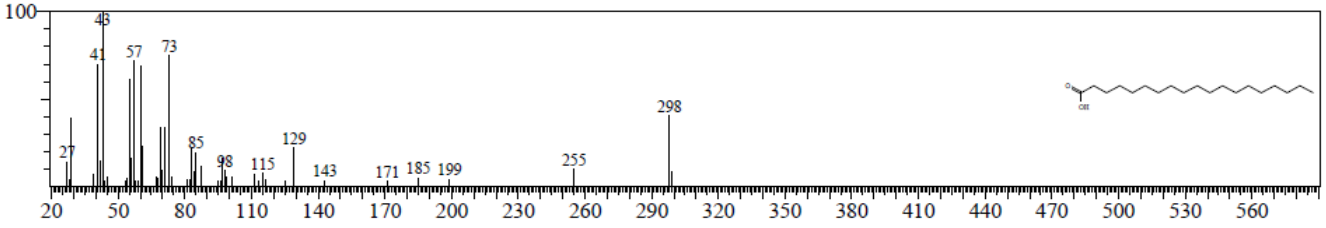
Hit#:2 Entry:52033 Library:NIST08.LIB
SI:91 Formula:C13H26O2 CAS:638-53-9 MolWeight:214 RetIndex:1670
CompName:Tridecanoic acid $$ n-Tridecanoic acid $$ n-Tridecoic acid $$ Tridecylic acid $$



Hit#:3 Entry:156739 Library:NIST08.LIB
SI:91 Formula:C22H44O4 CAS:106-11-6 MolWeight:372 RetIndex:2694
CompName:Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester $$ Aqua Cera $$ Atlas G 2146 $$ Cerasynt $$ Cerasynt Special $$ Clindrol SDG $$



Hit#:4 Entry:110794 Library:NIST08.LIB
SI:90 Formula:C19H38O2 CAS:646-30-0 MolWeight:298 RetIndex:2266
CompName:Nonadecanoic acid $$ n-Nonadecanoic acid $$



Hit#:5 Entry:120718 Library:NIST08.LIB
SI:90 Formula:C20H40O2 CAS:506-30-9 MolWeight:312 RetIndex:2366
CompName:Eicosanoic acid $$ Arachic acid $$ Arachidic acid $$ Icosanoic acid $$ n-Eicosanoic acid $$ Arachidic acid (synthetic) $$

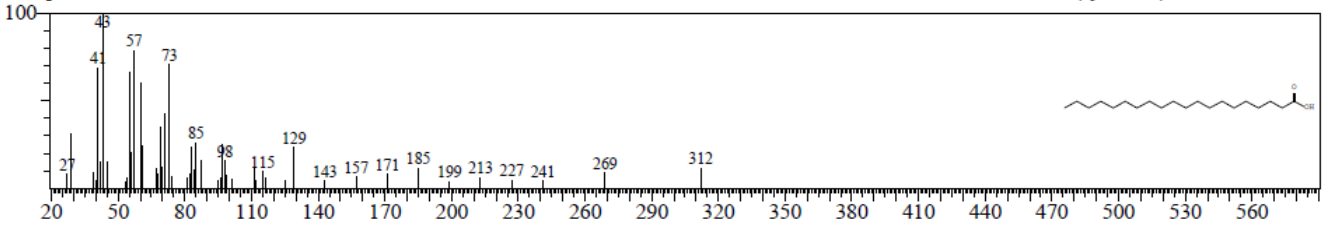
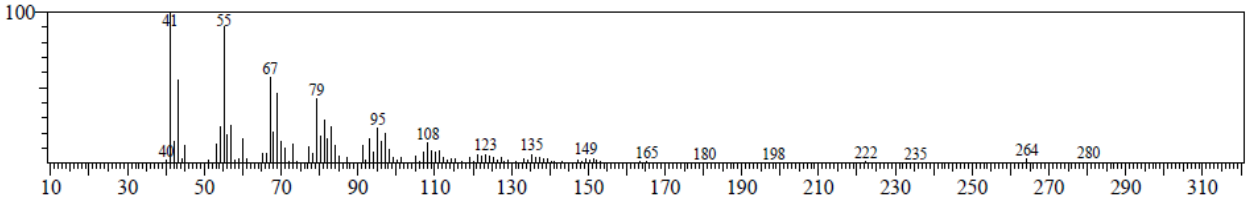
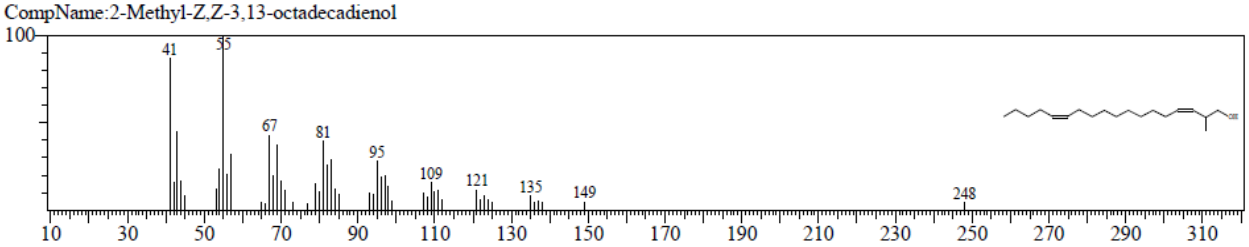


Figure (2): GC–MS analysis of essential oils of *C. citratus* (average of 19.492)

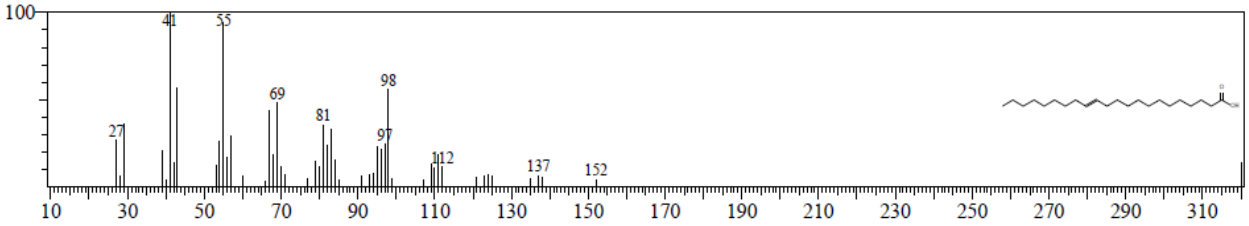
<< Target >>
Line#:21 R.Time:21.317(Scan#:2319) MassPeaks:122
RawMode:Averaged 21.150-21.392(2299-2328) BasePeak:41.05(7871)
BG Mode:21.133(2297) Group 1 - Event 1



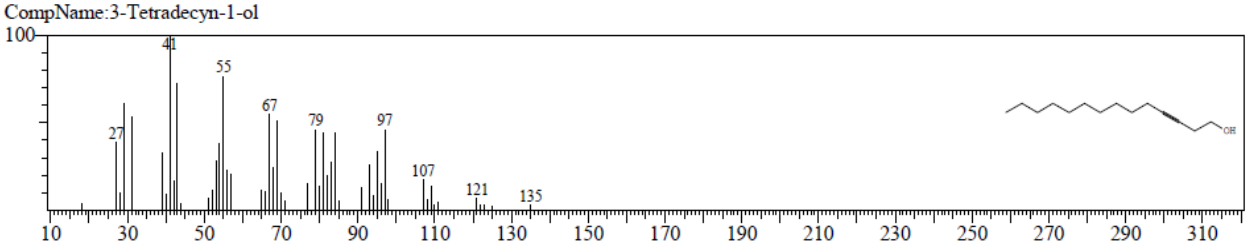
Hit#:1 Entry:97758 Library:NIST08.LIB
SI:89 Formula:C19H36O CAS:0-00-0 MolWeight:280 RetIndex:2104
CompName:2-Methyl-Z,Z-3,13-octadecadienol



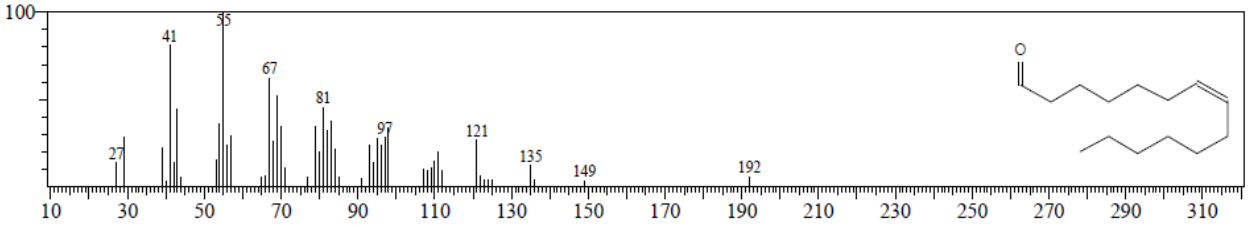
Hit#:2 Entry:137956 Library:NIST08.LIB
SI:88 Formula:C22H42O2 CAS:506-33-2 MolWeight:338 RetIndex:2572
CompName:(E)-13-Docosenoic acid $$ 13-Docosenoic acid, (E)- $$ trans-13-Docosenoic acid $$ Brassidic acid $$ (13E)-13-Docosenoic acid # $$



Hit#:3 Entry:49530 Library:NIST08.LIB
SI:88 Formula:C14H26O CAS:55182-74-6 MolWeight:210 RetIndex:1673
CompName:3-Tetradecyn-1-ol



Hit#:4 Entry:49536 Library:NIST08.LIB
SI:88 Formula:C14H26O CAS:65128-96-3 MolWeight:210 RetIndex:1609
CompName:7-Tetradecenal, (Z)- $$ Z-7-Tetradecenal $$ (7Z)-7-Tetradecenal # $$



Hit#:5 Entry:49534 Library:NIST08.LIB
SI:88 Formula:C14H26O CAS:0-00-0 MolWeight:210 RetIndex:1609
CompName:E-9-Tetradecenal

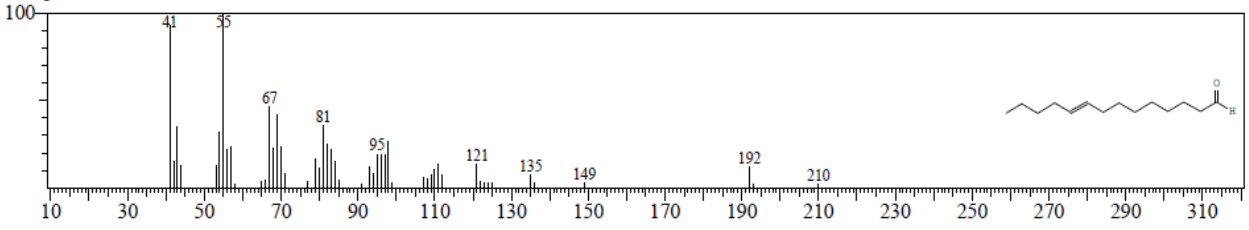


Figure (3): GC–MS analysis of essential oils of *C. citratus* (average of 21.315)

Table (2): Chemical Composition (%) of *Cymbopogon Citratus***.**

Peak	R.Time	Area %	Name
17	19.489	22.49	n-Hexadecanoic acid, Palmitic acid, Pentadecanecarboxylic acid
21	21.315	39.86	2-Methyl-Z, 13-octadecadienol

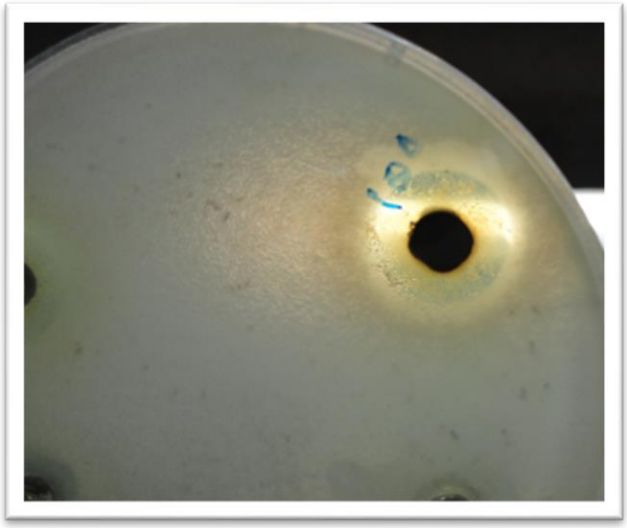


Figure (4): Inhibitory zone inhibition of Lemongrass oil against bacteria.

Table (3): Antibacterial activity of essential oil against pathogenic bacteria

Pathogenic bacteria	Diameter of inhibition zone (mm) of Essential oil (100%)
Streptococcu ssp.	**22**

DISCUSSION

GC-mass analysis:

In the Baghdad Environmental Testing Center, the sample has been analyzed. GC-MS Principle: GC/MS- a mixing of GC chromatography (GC) and Mass spectrometry (MS with two analytical techniques altered is used to study the dynamic mixes of organics and biochemical sources [(14)] .

Two key components are used in the GCMS. The section of gasses separates transformed molecules into vaporized bursts of pure chemicals in the sample. In a stationary phase stable in the column, an inert gas (mobile phase) is applied to transport the sample [(9)]. Compound spectra are joined by a mass-spectrometric column, which identifies and quantifies the chemicals based on a weight-to-weight (m/z) proportion. These spectrums can be stored and analyzed on the computer[(4)] .

The key substance in the extract with n-hexadecanoic acid is 22.49% showed in table (2)and this analysis results in similar results [(15)] and classified many extracts with 13-octadecadienol as the main compound with 39.86% in lemon as show in table (4). The result of this research was Lemon consistency depends on the content of octadienol [(10)]. Many modified groups of spontaneously founding compounds exist. Often, terpenoids comprise a category of natural base compounds, some of which are also derived from other sources, which are abundant in plants. Terpenoids are volatile substances which generate their fragrance for plants and flowers. They are popular to higher plants, oranges, conifers and eucalyptus in its leaves and fruits [(16)].

Plants, and trees, sap and tissue. But the word 'terpenoids,' containing hydrocarbons and their oxygenated derivatives, is more widely used. However, some scholars now use the term terpene to differentiate terpenoids [(17)]and [(6)]. Terpenoids can be synthesized as Isoprene Unit [(18)], Otto Wallach said. Isoprene law found that two or three units of isoprene are built into the terpenoid molecules. Distinctive law of isoprene suggests that the terpenoid molecule is synthesized in a head to tail form by two or more isoprene units [(19)]. Octadecadienol and other lemongrass are anti-inflammatory in vivo using edema prompted carrageenan and rat peritonitis. The use of Octadecadienol (100 and 200 mg/kg) was expected to minimize Paw edema and peritonitis, as leucocyte transformation to Peritoneal Cavity has been reduced [(20)]. When tested against bacterial isolates, lemongrass oil displayed the largest spectrum of antibacterial action, as shown in figure (5). Alkaloids, flavonoids, glycosides, saponins, and tannins were just a few of the many phytochemicals that the oil was an excellent source of. The current investigation against Streptococcus ssp. amply demonstrated the antibacterial activity of Cymbopogon citratus oil.[(21)].

CONCLUSION

Cymbopogon citratus produced several phytoconstituents such as flavonoids, alkaloids, phenolic and tannin compounds. C's extract. Citratus consists primarily of hydrocarbon monoterpenes, of which citrus is the primary constituent.

The main constituents are octadecadienol (39.86%), study from GC-MS (22.49 %).

In order for the quality adjustment of herbal medicinal products to be established, it is important to illuminate and reflect active phytopharmaceuticals, phytopharmaceutical modification of alternated extracts and the mode of action of isolated compounds and the clinical characteristics of the compounds.

In the changing environment situation, the importance in plants with therapeutic benefit is greatly applied to both the growth and development of the primary health sector. The nations. The countries. The

details would thus allow scientists and researchers to examine the bioactive compounds and illuminate the molecular mechanism of action.

Conflicts of Interest: The authors declare no conflict of interest.

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Author Contributions: Atheer A.Khashan , I have read and agreed to the manuscript and Authors contributed equally in the study.

REFERENCES

1. **Ebunlomo A, Odetola A, Bamidele O *et al.* (2012)**: Effects of Emilia praetermissa leaf extract on the haematological and biochemical parameters of stress induced ulcerated Wistar rats'. African J. Biochem. Res., 6 (14):185–189.
2. **Abdalla E, Elgharabawi G , Elsawy M (2011):** Structural and laboratory changes in the liver of female albino rats in cases of experimental high fat diet and curative role of some medicinal plants. The Egyptian Journal of Hospital Medicine , 44: 325 – 343
3. **Moustafa A, Helal E, Mohamed A(2009):** Structural changes in the pancreas of experimental diabetic rats under the effect of some hypoglycemic medicinal plants. The egyptian Journal of Hospital Medicine , 35 : 271 - 287.
4. **Achi N, Ohaeri O (2015):** 'GC-MS determination of bioactive constituents of the methanolic fractions of Cnidoscolus aconitifolius. J. Pharm. Res. Int., 44 (12): 163–172.
5. **Carmen M, Hernández L, Guerrero-Beltrán J (2012):** 'Physicochemical and antioxidant properties of Cymbopogon citratus essential oil. J. food Res., 4 (3): 36.
6. **Nambiar V, Matela H (2012):**Potential functions of lemon grass (Cymbopogon citratus) in health and disease. Int. J. Pharm. Biol. Arch., 3(5): 1035–1043.
7. **Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann A (2011):** Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). J. Adv. Pharm. Technol. Res., 2, (1): 3.
8. **Rhoades D (1979):** Evolution of plant chemical defense against herbivores. Herbiv. their Interact. with Second. plant Metab., 9(4): 3–54.
9. **Skoog D , FJ H (2007):** Crouch SR. Principle of instrumental analysis. Thomson Publ. India, 1(3): 145–147.
10. **Negrelle R and Gomes E (2007):** Cymbopogon citratus (DC.) Stapf: chemical composition and biological activities. Rev. Bras. Plantas Med., 9(1): 80–92.
11. **Jorgensen J (1999):** Antibacterial susceptibility tests: dilution and disk diffusion method. Man. Clin. Microbiol., 44(7):667.
12. **Indu M, Hatha A (2006):** Antimicrobial activity of some of the south-Indian spices against serotypes of Escherichia coli, Salmonella, Listeria monocytogenes and Aeromonas hydrophila. Brazilian J. Microbiol., 37(4): 153–158.
13. **Patel P, Patel N, Patel D *et al.* (2014):** Phytochemical analysis and antifungal activity of Moringa oleifera. Int. J. Pharm. Pharm. Sci., 6(5) : 144–147.
14. **Pengelly A , Bone K (2020):** The constituents of medicinal plants: an introduction to the chemistry and therapeutics of herbal medicine. Routledge, 55(8):5-9.
15. **Gilani A(1994)*:*** Pharmacological studies on hypotensive and spasmolytic activities of pure compounds from Moringa oleifera. Phyther. Res., 8(2): 87–91.
16. **Breitmaier E (2006):** Terpenes: flavors, fragrances, pharmaca, pheromones. Man. Clin. Microbiol., 45(7):687.
17. **Connolly J, a Hill R (2010):** Triterpenoids. Nat. Prod. Rep., 27(1): 79–132.
18. **Abdalla A, Alwasilah H, Mahjoub R, Mohammed H, Yagoub M (2016):** Evaluation of antimicrobial activity of Moringa oleifera leaf extracts against pathogenic bacteria isolated from urinary tract infected patients. J. Adv. Lab. Res. Biol., 7(2): 47–51.
19. **Al Naqqash Z, Al-Bazaz H (2019):** GC-Mass and Phytochemical Investigation of Cymbopogon citratus. Res. J. Pharm. Technol., 12(1): 67–73.
20. **Quintans-Júnior L (2011):** 'Citral reduces nociceptive and inflammatory response in rodents. *Rev. Bras. Farmacogn.*, 21(3): 497–502.
21. **Abalaka M, Daniyan S, SOyeleke S, Adeyemo S (2012):** The antibacterial evaluation of Moringa oleifera leaf extracts on selected bacterial pathogens. J. Microbiol. Res., 2(2): 1–4.