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Abstract

As medicinal plants known for their importance in different uses especially pharmaceutical usage due to presence of the active compounds represented by terpens, phenols, alkaloids. . . etc. and one of these important medicinal plants is our plant under study which is *Cymbopogon citratus* locally known as lemon grass that belongs to a Poaceae family. In this study lemon grass was collected from Anbar province desert then extracted by Clevenger apparatus so we got oil and identify the chemical compounds found in it by using general tests which shown the presence of alkaloids, phenols, tannins and flavonoids while cardiac glycosides and saponins were absent and the sample was identified using GC-Mass which revealed the presence of the following compounds (2-Methyl-Z, 13-octadecadienol) with percentage 39.86%. We can separate some pure phytopharmaceutical, which in turn can be used as lead molecule for producing the novel agent having good therapeutic activity. In order to combat pathogenic bacterial and zone of inhibition of growth with diameters of 22mm, the antibacterial activity of lemongrass oil on micro-organisms has been examined using of well diffusion method. This study was conducted to determine the phytoconstituents presents in *Cymbopogon citratus* plant.

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

INTRODUCTION

Natural products, primarily from plants, were explored by people in the treatment of different diseases for thousands of years now [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 (Figure 1), spontaneously emerging worldwide, mostly in tropical and savannan areas. Lemongras, barbed wire grass, silky heads, lemongrasses, chadedartigalongue, tanglad or 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].**Figure (1)***Cymbopogon citratus***2. MATERIALS AND METHODS2.1. Collection and preparation**

Dry *Cymbopogon citratus* leaves were purchased from a local market in Anbar and were classified received from Ministry of Agriculture/ State Board for Seeds Testing and Certification in Baghdad.

2.2 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 (Figure 2). 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 [12]. The sample weight of a plant.

2.3. Culture preparation:

Streptococcu ssp. obtained from Al-Ramadi Hospital and use centrifuge at 3000rpm, twice washed and resuspended in 0.1 per cent pepton water, loop of 24-hour surface development on a 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 37C. The turbidity was changed to meet that of the Mcfarland tube (108 CFU/ml). Then 1:10 dilution of the cell suspension was done to have an inoculum concentration of 107 CFU/ml.

2.4. Antibacterial screening test of extracts using disk diffusion method:

Jorgensen et al. [20] conducted the disk diffusion check by normal protocol. On the entire surface of Muller-Hinton agar (MHA), the inoculum suspension of bacterial insulates was swabbed (pH7.3). 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 20ml 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 that grass oil in diameters of the inhibition zone. 22 mm against *Streptococcu ssp.*was found to be of good antibacterial activity [21].

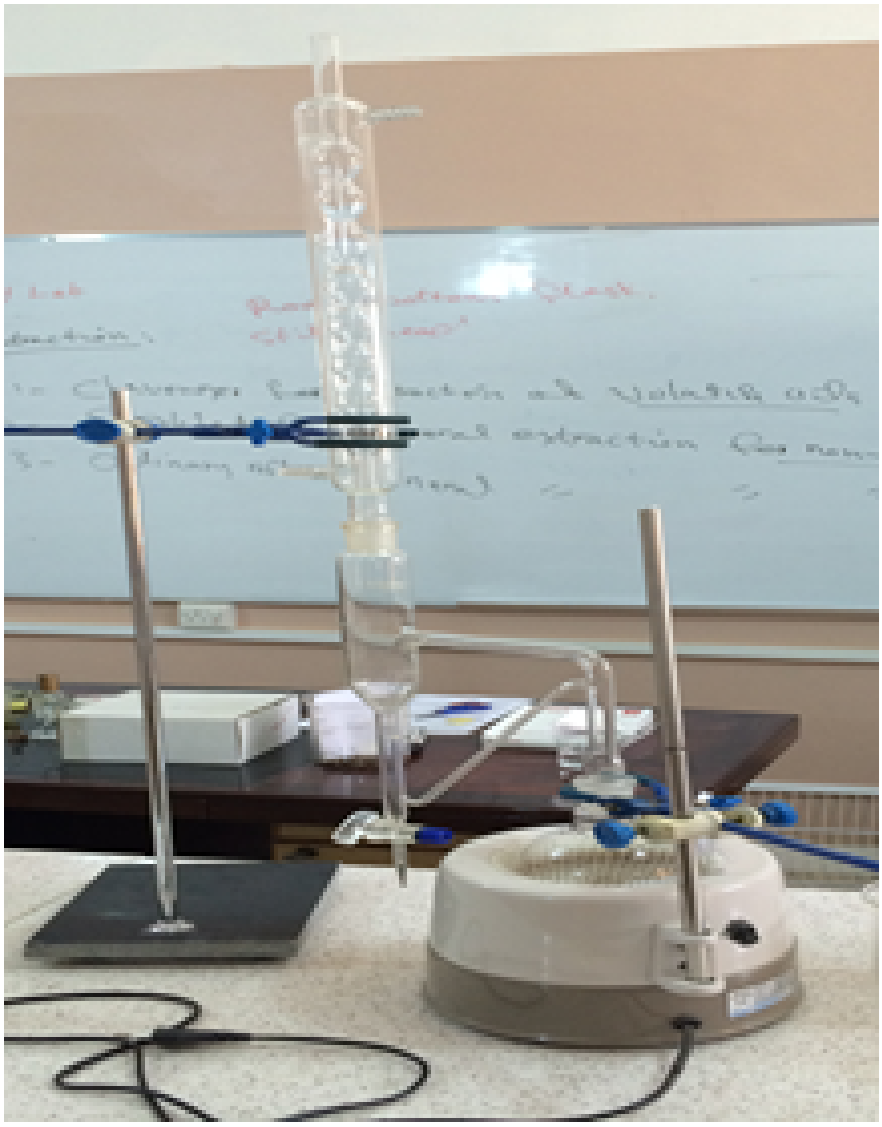
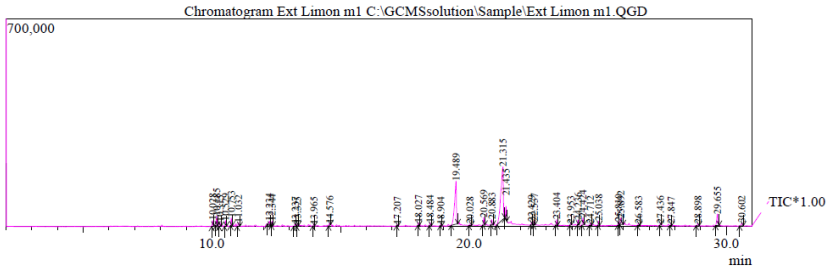


Figure (2): Explain Clevenger apparatus

Antibacterial activity of *Cymbopogon citratus* oil was seen against *Streptococcus ssp* , [23]. The oil showed maximum activity against bacteria as shown in the Table 2.

3.Result and Dissection

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 [9]. 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 [10].

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As shown from the GC/MS results, we can identify a number of extractions in the leaves of our Iraqi plant (*C. citratus*) which n-Hexadecanoic acid, Palmitic acid, Pentadecanecarboxylic acid, with a good quantity of extract in these leaves. 2-Methyl-Z, 13-octadecadienol, and it is used for several chemical syntheses [11].

Table (1) 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

The key substance in the extract with n-hexadecanoic acid is 22.49% and this analysis results in similar results [13] and classified many extracts with 13-octadecadienol as the main compound with 39.86% in lemon. The result of this research was Lemon consistency depends on the content of octadienol [12]. 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 [14].

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 [15, 16]. Terpenoids can be synthesized as Isoprene Unit [17], 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 (18). 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 [19]. Antibacterial activity of lemongrass oil on bacterial isolates showed that oil had the broadest spectrum of activity on the test bacteria. The oil was a good source of various phytochemicals like alkaloids, flavonoids, glycosides, saponins, and tannins. The antibacterial activity *Cymbopogon citratus* oil was clearly shown by the present study against Streptococcus ssp. (22)

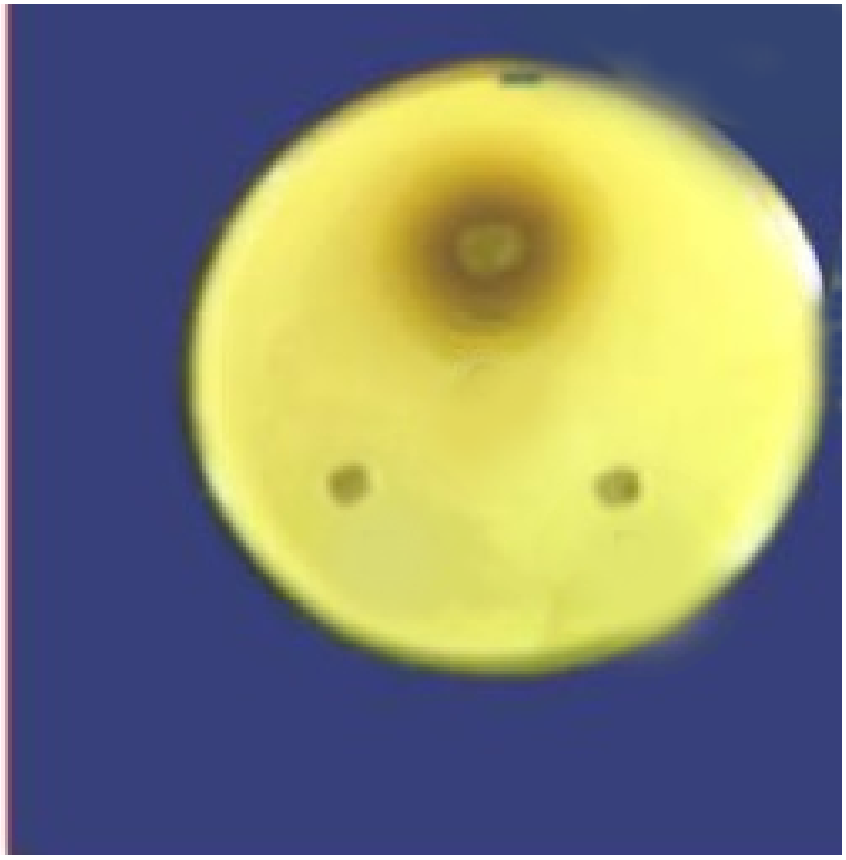

Fig.3. Inhibitory zone of different concentration of Lemongrass oil against bacteria.

Table (2): Antibacterial activity of essential oil against pathogenic bacteria.

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

4.CONCLUSION:

Therefore, we can isolate certain pure phytopharmaceutical products, which in turn can serve as lead molecules to allow the inexperienced agents providing good therapeutic activity and which are responsible for the changed biological activities of this plant. *Cymbopogon citratus* produces 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.

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

5.REFERENCES

1. Ebunlomo A.O., A.O. Odetola O. Bamidele J.N. Egwurugwu S.Maduka, J. Anopue. Effects of Emilia praetermissa leaf extract on the haematological and biochemical parameters of stress induced

ulcerated Wistar rats. Afr. J. Biochem. Res., 2012; 6: 185-189.

2. Aja P.M., Nwachukwu N., Ibiam U.A., Igwenyi I.O., Offor C. E.,Orji U.O.. Chemical constituents of Moringaoleifera leaves and seeds from Abakaliki, Nigeria. Am. J. Phytomed. Clin.Therapeut., 2014; 2: 310-321.

3. Uraku A.J., Okaka A.N.C., Ibiam, U.A., Agbafor, K.N., Obasi N.A., Ajah P.M., Obai, O.U., Nwalo, F. Antiplasmodial activityof ethanolic leaf extracts of Spilanthes uliginosa, Ocimum

basilicum (Sweet Basil), Hyptis spicigera and Cymbopogon citratus on mice exposed to Plasmodium berghei Nk 65. Int. J.Biochem. Res. Rev., 2015; 6(1).

4. Achi N.K., Ohaeri O.C. GC-MS determination of bioactive constituents of the methanolic reactions of Cnidoscolusaconitifolius. Br. J. Pharm. Res., 2015; 5: 163-172.

5. Vazquez-Briones M.C., Hernandez L.R., Guerrero-Beltran, J.A.Physicochemical and antioxidant properties of Cymbopogoncitratusessential oil. J. Food Res., 2015; 4: 36-45.

6. Nambiar V.S., Matela, H. Potential functions of lemon grass(Cymbopogon citratus) in health and disease. Int. J. Pharmaceut.Biol. Arch., 2012; 3: 1035-1043.

7. Shah G., Shri R., Panchal V., Sharma N., Singh B., Mann, A. S.Scientific basis for the therapeutic use of Cymbopogon citratus,stapf (Lemon grass). J. Adv. Pharm. Technol. Res., 2011; 2: 3-8.

8. Harborne, T.B. (1973). Phytochemical methods. Halasted press. Johnwiely & Sons, New York.. 178.

9. Skoog D.A., Holler F.J., Crouch S.R. Principles of Instrumental Analysis. 6th Edition. Brooks/Cole Cengage Learning, Chapters 11, 20, 26, 27, 2007.

10. Oregon State University. GC-MS: How does it Work?Environmental Health Sciences Center Corvallis OR 97331 http://www.unsolvedmysteries.oregonstate.edu/MS_ 05, 2012.

11. Pengelly A. The Constituents of medicinal plants (Eds): An Introduction to the chemistry and therapeutics of herbal medicine.United Kingdom: CABI Publishing. pp. 85-103, 2004.

12. Negrelle, R.R.B., Gomes, E.C. *Cymbopogon citratus* (DC.) Stapf:chemical composition and biological activities. Revista Brasileira de Plantas Medicinais, 2007; 9: 80-92.

13. Shah G., Shri R., Panchal V., Sharma N., Singh B., Mann, A.S.Scientific basis for the therapeutic use of Cymbopogon citratus,stapf (Lemon grass). J. Adv. Pharmac. Technol. Res., 2011; 2(1):3-8.

14. Breitmaier E.In: Terpenes: Flavors, Fragrances, Pharmaca,Pheromones, Wiley-VCH Verlag GmbH & Co. KGaA, Germany,1 st Edition, 2008.

15. Connolly J.D., Hill R.A. Triterpenoids, Natural Product Reports,27, 2010: 79-132.

16. http://greenhouseseeds.nl/shop/terpens-in-resin.html#11

17. Hanson J.R. Diterpenoids, Natural Product Reports, 26, 2009,1156-1171.

18. http://berkeleypatientscare.com/2010/10/08/terpenesterpenoidsand-cannabis/

19. Quintans-Júnior L. J., Guimarães A. G., Santana M. T., Araújo B.E. S., Moreira F. V., onjardim L. R., Araújo A. A. S., Siqueira J.S., Antoniolli A. R., Botelho M. A., Almeida J. R. G. S., Santos

M. R. V. Citral reduces nociceptive and inflammatory response in rodents. Braz. J. Pharmacogn., 2011; 21: 497-502.View publication.

20-Jorgensen, J. H . , Turidge. J.D. And Whashington. J. A. 1999. Antibacterial Susceptibility Tests:Dilution and Disk Diffusion methods.In:Murray, P. R. , Barron. E. J. , Praller. M. A. , Twnover. E. C. and Yollken. R. H. , Eds. Manual of Clinical Microbiology. A J M press. , Washington, DC. , PP. 1526-1562.

21-Indue, M. N. , Hatha. A. A. M. , Abirosh. C. , Harsha. U. and Vivekanandan. G. 2006. Antimicrobial activity of some of the south –indian spices against serotyper of Escherichia coli, Salmonella, Listeria monocytogenes and Aeromonas hydrophila. Brazillian Jornal of Microbiology. 37(2):147-158

22-1. Abalaka ME, Daniyan SY, Dyeleke SB, Adeyemo SO (2012) The antibacterial evaluation of moringa oleifera leaf extracts on selected bacterial pathogen. Journal of microbiology research 2(2): 1-4.

23- Napolean P, Anitha J, Emilin RR (2009) Isolation, analysis and identification of phytochemicals of antimicrobial activity of Moringa oleifera Lam. Current Biotica 3(1): 33-37.