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Chemical analysis and antimicrobial activity of Cumin seeds extracted oil against some bacterial and fungal isolates

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Abstract

Cuminum cyminum seeds oil was extracted by using Soxhelt extraction ,the chemical analysis is carried out by means of GC-MS ,1H-indene derivatives (59.77%) and Cuminic aldehyde(13.77%) are respectively the major compound in the extracted oil . The Cumin oil exhibits a strong antibacterial activity against four clinical bacterial isolates (*Escherichia coli, Staphylococcus aureus, Klebsiella sp.* and *Pseudomonas aeruginosa*) and strong to moderate antifungal activity against three fungal isolates (*Aspergillus flavus* ,*Candida albicans and Cryptococcus sp*.). The minimum inhibitory concentration (MIC) of the Cumin extracted oil was applied against the clinical isolates of bacteria and *E.coli* was the most sensitive isolate, with the lowest MIC value.

Keywords*:* *Cuminum cyminum*; Cumin oil; Antibacterial, Antifungal activity

التحليل الكيميائي و الفعالية المضادة للميكروبات لزيت بذور الكمون المستخلص

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الخلاصة

تم استخلاص زيت بذور الكمون (*Cuminum cyminum*) Cuminبأستخدام جهاز الاستخلاص السوكسليه Soxhelt . وقد تم التحليل الكيميائي للزيت المستخلص بأستخدام كروماتوجرافيا الغاز–طيف الكتلةGC –MS ووجد ان حوالي59,77% من محتوى الزيت كان مشتق الأندين1H–Indene derivative و13.77% هو الديهايد الكمون Cumin aldehyde وهذا بالتتابع حسب اعلى النسب . وقد أظهر زيت الكمون المستخلص فعالية بيولوجية عالية في تثبيط اربع عزلات جرثومية سريرية (*Escherichia coli ,Staphylococcus aureus, و Pseudomonas aeruginosa*).كما اظهر فعالية معتدلة تجاه ثلاث عزلات فطرية(*Aspergillus flavus Candida albicans , و Cryptococcus sp.*).التركيز المثبط الأدنى من زيت الكمون المستخلص للعزلات الجرثومية السريرية تم تحديده ولوحظ أن جرثومة *E. coli* هي اكثر الانواع الجرثومية تحسساً لزيت الكمون حتى مع التراكيز الواطئة منه.

Introduction

Medicinal plants have always been considered a healthy source of life for all people [1]. Nowadays people are being bombarded with a thousand of unhealthy products, and also the problem of antibiotic resistance that is a worldwide public health problem that continues to grow [2]. All that makes the use of medicinal plants is the best choice for the treatment of different diseases and infections [1]. One of these medicinal plants is Cumin (*Cuminum cyminum)* its an herbaceous annual plant of the Umbellifera family and is used as a condiment and as an ingredient in many food industries [3,4]. From the most important medical uses of Cumin are to reduce inflammation increase ,urination , prevent gas and suppress muscle spasms and it is also used as an aid for ingestion , jaundice , diarrhea and flatulence [5]. Cumin cultivate in Western Asia, South Mediterranean and the main producer countries today are India, Iran and Indonesia [3].

Generally the medicinal importance and the spicy flavor of Cumin belong to the Cumin seeds [5]. Cumin seeds are oblong in shape longitudinally ridged and yellow-brown color [3]. Also Cumin seeds contain essential oil (up to 5%) that imparts the generally the medicinal importance and the spicy flavor of Cumin belong to the Cumin seeds [5]. Cumin seeds are oblong in shape longitudinally ridged and yellow-brown color [3]. Also Cumin seeds contain essential oil (up to 5%) that imparts the characteristic aroma to the seeds [4]. Essential oils extracted by hydro distillation from fruits of Cuminum cyminum the main components of Cuminum cyminum oil were p-mentha-1, 4-dien-7-al, cumin aldehyde, Gama-terpinene, and α-pinene [12] , Cumin essential oil has a broad antibiotic spectrum against Gram-positive and Gram- negative bacteria [15], and more research showed that Cuminum cyminum essential oil is active in general against all fungi [20] .

Cumin seeds are the most popular spice all over the world and they are very popular in western to the central Asia[6] they are found in a large amount in our Iraqi market and sold in a low price , the aim of this study is to analyse the chemical composition and testing the ability of Cumin oil from a seeds collected from Basra markets and extracted in our laboratories by soxhelt and Ether as a solvent, to detect its antibacterial action on the growth of clinical isolates of some bacterial and fungal isolates.

Materials and methods

Materials

Cuminium cyminum seeds were obtained from the local market of Basra city in Iraq. Four types of media were used [Nutrient agar, Nutrient broth, Sabouraud-Dextrose agar (Himedia company) and Muller-Hinton agar (CDH) .Four clinical isolates of bacteria (*Escherichia coli, Staphylococcus aureus, Klebsiella sp.* and *Pseudomonas aeruginosa*) and three fungal isolates (*Aspergillus flavus , Candida albicans and Cryptococcus sp*.) are used, all of these isolates obtained from Pharmacology department in the college of pharmacy .

Methods

Oil extraction and chemical analysis

Cumin seeds oil was extracted using Soxhelt extraction[4] , which carried out in standard apparatus for 12 hours using (20)gm of Cumin seeds with (200)ml of Diethyl ether as a solvent. Then the extracted oil was collected in a dark glass container and stored in a freezer until use.

The chemical analysis for the extracted oil was examined using GC-MS analysis which is performed by using Shimadzu GC-2010 system coupled with a Shimadzu GCMS-QP2010 Ultra network mass selective detector and equipped with DB-1MS capillary fused silica column(30m ,0.25mm I.D. ;0.25µm film thickness). The Cumin oil solution (1µ) in Diethyl ether (HPLC grade) was injected and analyzed with the column held initially at 40 C° for 5 min and then increased to 250 C° with a 4 C° /min heating ramp and subsequently kept at 250 C° for 1 min. other

operating conditions were as follows: carrier gas, He (99.999%); with a flow rate of 1.82 ml/min: injector temperature, 250 C° ; split ratio,1:50. Mass spectra were taken at 70ev. Mass range was from m/z 35-50 amu. the relative percentage amount of the separated compounds were calculated from total ion chromatograms by a computerized tegrator literature data and MS data by MS data obtained from NIST 08 library [7].

Antimicrobial activity

The antibacterial activity of the extracted oil was assayed by using four clinical bacterial isolates [*E. coli* isolated from stool specimen, *Klebsiella sp*., *Psudomonas aeruginosa* and *Staphylococcus aureus* all isolated from blood specimen] with a microbial concentration 10^6 CFU/ml and by Well-diffusion method on Muller-Hinton agar [8,9] and the inhibition zone was measured in millimeter (mm). Four Petri dishes were used as an experimental unit and the trial was repeated twice. Cultures were incubate at 37 C° for 24 hours. Similarly, the antifungal activity of the extracted oil was tested against three fungal isolates [*Aspergillus flavus, Candida albicans, Cryptococcus sp.*] and by using Sabouraud Dextrose agar medium, three Petri dishes were used as an experimental unit and the trial is repeated twice. The fungal cultures were incubate at 27 C° for three days [11].

Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) of the Cumin extracted oil against the four clinical isolates of bacteria is carried out using Well diffusion method[10,25], different concentration of the extracted oil (0.95 ,0.85 ,0.75 ,0.65 ,0.6 ,0.55 , 0.45 ,0.35 ,0.1 ,0.04 ml/ml) were prepared using Diethyl ether as diluent [15]. The MIC of the Cumin oil for each bacterial isolates was regarding as the lowest concentration that inhibited visible growth of the bacterial isolates on the agar plate after 24 hours incubation at 37 C°. And the Diethyl ether used in this test as a negative control [10].

Results and Discussion

Result of GC-MS analysis of *Cuminum cyminum* seed extracted oil in table(1) and figure (1)show the chemical composition of the extracted oil , in which the main components of the extracted oil were 1Hindene derivatives (59.77%), Cuminic aldehyde (13.77%) and Cuminol (3.08%). The antibacterial activity of the extracted oil in table(2) and figures(2) show a good antibacterial activity when compared with the standard antibiotic with a nearly concentration [24], that belongs to the chemical composition of Cumin extracted oil which contain Cuminic aldehyde ,P-menth-2-en-7-ol ,Gamma-Terpine , β-Pinene and else all of these compound despite of their little concentration but they are contribute in the antibacterial activity because they are known bactericidal compounds[12,13] , especially the Cumin aldehyde which has a high concentration (13.77%) in the extracted oil and this compound exhibited a considerable inhibitory effect against different Gram positive and Gram-negative bacteria isolates [14,15,16,17]. Also it was supported by (MIC) results of the extracted oil against the bacterial isolates table (3).

Isolation and extraction of each compounds and testing its antimicrobial activity are processes required long time that's it can be done as a further step for this study, there are no reports about the antimicrobial activity of 1H-indene derivative that is found in the extracted Cumin oil, but some of the 1H-indene derivatives have antimicrobial activity and use as antibiotic intermediate [22]. The results of MIC in table (3) show that the Cumin extracted oil is more active against *E. coli* (4% ml/ml) than the other used clinical isolates, in this action it resembles the antimicrobial activity of the Cumin essential oil against *E. coli* which records in some research were lowest MBC value (1µl/ml) [23]. That's because of the some similarity between the research extracted oil and the Cumin essential oil in the chemical structure which have nearly the same bactericidal compounds [12]. Also the present study which reported antibacterial potential of a different types of spices including Cumin

found a potent antibacterial potential against *E. coli* and *Bacillus subtilis* [13].The mechanism of action of Cumin oil in some bacterial cells can be represented by cell elongation , repression of capsule expression in some bacteria and inhibition of Urease activity [18,19]. The high MIC value against the other clinical isolates of bacteria can attributed to the fact that some of Cumin extracted oil compounds especially the aromatics that most of which have an antimicrobial activity[18] may lost or volatile after a period of time even when stored in the freezer by repeated use of oil and put its container for hours in room temperature during the tests period , that explain the high MIC value because the accounting of MIC value is the latest test in this study and carried out after a few months of Cumin oil extraction while detecting of antimicrobial activity is carried out under the same conditions of MIC test but shortly after oil extraction. Susceptibility of E. coli in the study, suggests that other compounds of the oils have exerted a antimicrobial effect [23].

The Cumin extracted oil has antifungal activity against the fungal isolates table(2) and figure(3) that also belongs to the presence of some compounds in its chemical composition like Cumin aldehyde , P-cymen and Pinene [12,20]because these compounds and other phenolic compounds of Cumin oil are able to inhibit some important fungal enzymes like Pectinase which is used by some fungi to hydrolyze the fruit cell wall and invade the host cell and form fruit decay[21]and some research found that the Cumin oil blocked the growth of fungi and can completely stop fungal growth in modest concentration [5], the Cumin essential oil has an antifungal activity against *Aspergillus sp*. and *Candida albicans* [12,20] and that resembles the research Cumin extracted oil, that mean the both oils have the same action because they have the same active compounds.

Conclusion

In this study we observed that the Cumin (*Cuminum cyminum*) plant is used from most of the people as a kind of spicy in their food and its seeds are present in our markets and sold in a low price and the seeds extracted oil have an antibacterial and antifungal activity all that can enable us to use this extracted oil in the treatment of human, animals and plant microbial disease. And also about (59.77%) of Cumin extracted oil is 1H-indene derivative and because Cumin extracted oil have an antibacterial and antifungal activity there for this compound may have an antimicrobial activity that lead us to further extraction for this compound and testing its antibacterial and antifungal activity . Finally the Cumin extracted oil must be kept in a tightly closed container in the freezer and avoid put it for a long time in a room temperature to keep its volatile compounds.

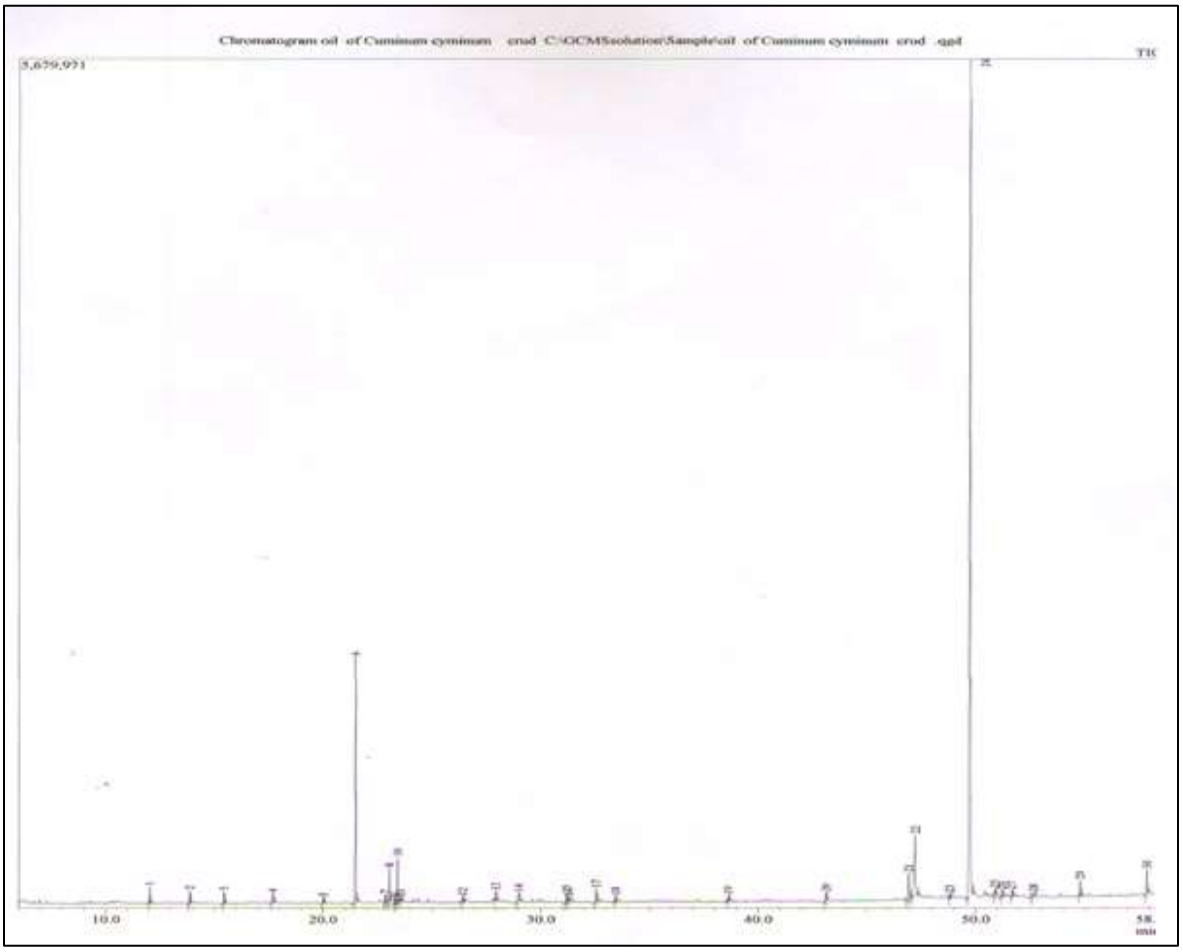

Fig-1- GC-MS analysis of *Cuminum cyminum* extracted oil

Table-1-The chemical composition of *Cuminum cyminum* extracted oil.

NO	Compounds	RT	%
1	Beta-Pinene	12.031	0.85
2	Beta-Cymene	13.885	0.67
3	Gamma-Terpine	15.455	0.64
4	Undecane	17.687	0.69
5	Phellandral	20.021	0.32
6	Cumic aldehyde	21.492	13.77
7	P-Menth-2-en-7-ol,trans.	22.878	0.44
8	2-Caren-10-al	23.058	1.87
9	1-Phenyl propane-1,3-diol	23.318	0.31
10	Cuminol	23.430	2.61
11	2-Methyl-1,3-cyclopentadione	23.580	0.23
12	Trifluoroacetyl- camphenilol	26.447	0.31
13	Cuminic acid	27.954	0.66
14	Spiro[4,4]non-3-en-2-one,4 methyl-3-(1H-tetrazol-5-yl)oxa	29.011	0.77
15	P-isopropylphenetole	31.213	0.52
16	Admantane-1-carbohydrazide,N2-(4-methylbenzylideno)	31.306	0.40
17	14-Methyl-8-Hexadecenal	32.566	0.85
18	Carotol	33.458	0.35
19	5H-3,5a-Epoxynaphth[2,1-c]oxepin.	38.651	0.35
20	1-(+)-Ascorbic acid 2,6-dihexadecanoate	43.144	0.54
21	Linoleic acid	46.936	2.19
22	6-Octadecenoic acid	47.220	6.26
23	2-(1,3-Benzodioxol-5-yl)-1-(5-ethyl-2-hydroxy-4-methox)	48.828	0.23
24	1H-indene derivative*	49.731	59.77
25	1,3-Cyclohexadiene,1,2,3,4,5,6-hexamethyl	50.907	0.44
26	Eicosane	51.222	0.51
27	Oxalic acid monoamide, N-(4-ethylphenyl)-,propyl ester	51.681	0.38
28	2,3-Dihydroxypropyl elaidate	52.672	0.37
29	3-Methyl-2-butenoic acid, cyclobutyl ester	54.810	1.01
30	2-Butenoic acid,2-methyl-,dodecahydro-8-hydroxy-8a-methyl-3,5-bis(methylene).	57.870	1.69

RT: retention time , %:Area% ,*1H-Indene,2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl.

Table-2- Antibacterial and antifungal activity of *Cuminum cyminum* extracted oil against the clinical isolates of bacteria and fungi.

Clinical isolates of bacteria	Inhibition zones (mm)
Staph. aureus	37
E.coli	40
P.aeruginosa	40
Klebsiella sp.	45
Fungal isolates	
Aspergillus flavus	19
Candida albicans	20
Cryptococcus sp.	22

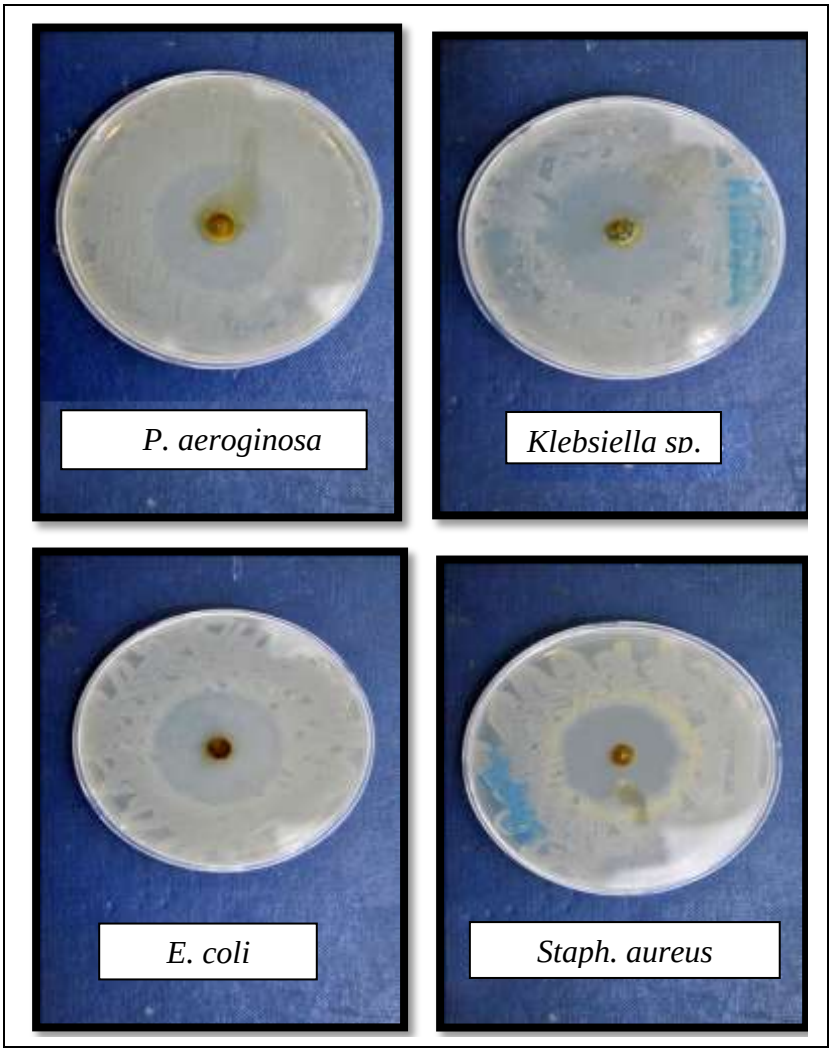

Fig-2- Antibacterial activity of *Cuminum cyminum* extracted oil.

Fig-3-Antifungal activity of *Cuminum cyminum* extracted oil.

Table-3- Minimal inhibitory concentration of *Cuminum cyminum* extracted oil against the clinical isolates of bacteria.

Clinical isolates of bacteria	MIC (ml/ml)
Staph. aureus	0.6
E. coli	0.04
P. aeruginosa	0.65
Klebsiella sp.	0.35

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