Research Article

Chemical Composition, Antimicrobial and Brine Shrimp Lethality of the Essential Oil of *Cuminum cyminum* L.

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ABSTRACT

Cuminum cyminum is a traditional herbal remedy intensively used in Sudanese traditional medicine as diuretic, carminative, stimulant, astringent, emmenagogic and antispasmodic. In this study the cytotoxicity (LC_{50}) of the essential oil of *Cumunum cyminum* was carried out using brine shrimp lethality test. It showed high toxicity (LC_{50}= 30.404 µg/ml) .The effect of the *C. cyminum* essential oil on bacterial and fungal growth was also investigated using agar well diffusion method in Nutrient Agar and Potato Dextrose Agar. The antimicrobial effect of *C. cyminum* essential oil (1:100 v/v) was significant against *S. aureus*, *E. coli*, *K. pneumonia, A. niger, C, albicans* and *F. oxysporum*. The chemical composition of the essential oil of *C. cyminum* was qualitatively and quantitatively identified by Gas Chromatography Mass Spectrometer (GC-MS). The main chemical constituents were 2-Caren-10-Al (29.64%), Benzaldehyde,4-1-methyethyl (28.55 %), c-Terpinene (16.58%) and 2-J-Pinene (12.06%). The biological activity of the essential oil of *Cumunum cyminum* may be due to the presence of these compounds. The results of this study indicate the potential use of the essential oil of *Cumunum cyminum* as an antimicrobial and an antitumor agent (LC_{50}= 30.404 µg/ml).

Keywords: *Cuminum cyminum*- essential oil- antimicrobial- Cytotoxicity- GCMS.

INTRODUCTION

The use of medicinal plants for the treatment of human ailments based on hearsay, folklore, or tradition, without scientific proof, is a potentially harmful and dangerous practice. Information in the literature regarding proper usage of medicinal herbs (dosage, dose frequency, physical condition, sensitivity of user, and possible interaction with prescribed drugs) is still limited[1].

Essential oils diluted from several plants have been used for a long time in perfumery, aromatherapy, food and flavors. Many essential oils are known to be potent antibacterial and antifungal agents. Certain essential oil components have been reported to have anticancer activity[2-5]. The advantage of these components in anticancer therapy is their low or negligible toxicity. Therefore, such components can highly be useful in the development of anti-cancer therapeutics, and used for the long-term treatment of chemoprevention and chemotherapy of cancer [6,7].

Cytotoxicity of herbs should be studied in order to evaluate their safe usage. Brine Shrimp Lethality Bioassay is a rapid and inexpensive general bioassay which has been developed for screening, fractionation and monitoring of biologically active natural products[8,9]. The lethality is recorded as the concentration when 50% of the larvae are killed within 24h of contact with the extract. LC_{50} values were below 249 µg/ml are considered as highly toxic, 250–499 µg/ml as median toxicity and 500–1000 µg/ml as light toxicity. Values above 1000 µg/ml are regarded as non-toxic[10.] From a pharmacological point of view, a good relationship has been found with Brine Shrimp Lethality Test (BLT) to detect antitumor compounds in terrestrial plant extracts[11.]

According to unique position and varied climate, terrain, and flora and fauna, the Sudanese have developed a unique traditional culture. Their unique indigenous knowledge of herbs led to the development of numerous general health and preventative remedies. Some are useful and some, although widely accepted due to custom and tradition, proved harmful[12.]

Cuminum cyminum is stomachic, diuretic, carminative, stimulant, astringent, emmenagogic and antispasmodic. It is valuable in dyspepsia diarrhea and hoarseness, and may relieve flatulence and colic. In the West, it is now used mainly in veterinary medicine, as a carminative, but it remains a traditional herbal remedy in the East. It is supposed to increase lactation and reduce nausea in pregnancy. It has been shown to be effective in treating carpal tunnel syndrome, as well as diarrhea, indigestion, and morning sickness. Cumin also shows

potential as a natural way to increase breast size. Used in a poultice, it relieves swelling of the breast or the testicles. Cumin stimulates the appetite[13].

In this study we are investigating the antibacterial and antifungal activity of the essential oil of *Cuminum cyminum* using Agar Well Diffusion Assay. The cytotoxic properties of the essential oil are also studied using the brine shrimp lethality bioassay technique. The phytochemical analysis of the essential oil was also performed using GCMS technique. This technique is very useful tool for the isolation of bioactive compounds from plant extracts.

MATERIALS AND METHODS

Plant Material

Cuminim cyminum L, Fruits, Vernacular name (Shamar), Family Apiaceae, collected from Northern Sudan (Shamilia), identified and authenticated by Dr. Haider Abdelgadir Herbarium Curator. Herbarium material was deposited at The Medicinal & Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan.

Extraction of Oil

Plants Essential oils were extracted by Hydro distillation using Clevenger Apparatus [14]. Essential oil yield 15%. Plant extract was dissolved in sterile Dimethyl sulfoxide (DMSO) and kept as a stock solution. The Plant extract was filtered using Chromafil CA-45/ 25 S (MACHEREY-NAGEL postfach 10 13 52 D-52313 Duren). The essential oil content was measured on the basis of volume / weight × 100.

1. Brine Shrimp Lethality Test

Test sample, *Artemia salina* (shrimp eggs) was placed in natural sea water, and eggs hatched within 48 hrs, providing a large number of larvae (nauplii). The tested essential oil (20 mg) was dissolved in 0.02 ml DMSO, and completed to 2ml with seawater. From this solution 5, 50 and 500µl were transferred to vials (triplicate for each concentration), forming concentrations of 10, 100 and 1000 µ/ml respectively. Volume was made to 5 ml with seawater. 10 larvae were placed in each vial using a Pasteur pipette. Etoposide (7.4625 µg/ml) was used as positive control. Vials were left for 24 hrs and numbers of survived larvae were counted. Data was analyzed by Finney Probit Analysis computer program to determine LC_{50} values with 95% confidence intervals[8].

2. Antimicrobial Activity

2.1. Tested organisms are shown in table (1)

2.2. Preparation of media and inoculation of test organism

Antibacterial and Antifungal Activity of *C. cyminum* essential oil was performed using Nutrient Agar and Potato Dextrose Agar (PDA) respectively. Nutrient Agar was prepared (20 gm in distilled water 1 liter), (PDA) Sterilized molten media (45 ml) at 45-50 ˚C each were placed into 100 ml conical flasks. 0.1 ml of bacterial or fungal suspension was added to the flasks, shaken, and then poured into 19cm Petri dishes. They were left to solidify and two wells for each extract concentration were made using a sterile cork borer[15].

2.3. Essential oil dilutions

As adopted by Belboukhari *et al*[15], essential oil extract in propanol concentrations were prepared as follows; 1:100 (0.1 ml oil + 4.45 ml 2- propyl alcohol + 4.45ml water). 1:250 (2.8 ml solution 1+ 3.6 ml alcohol + 3.6 ml water). 1:500(1.6 ml solution 1 + 4.2 ml alcohol + 4.2 ml water). 1:1000 (1ml solution 1 + 4.5ml alcohol + 4.5 ml water).

2.4. Tests and measurements

Using micropipette and sterilized tips, 0.1 ml of the extract concentrations was added, each to its respective well. Control plates were also prepared where 0.1 ml of alcohol and water (1:1) was pipetted into the well. Petri dishes were placed in the refrigerator to ensure good diffusion of extract, and incubated at 37˚C. Inhibition zones were measured to the nearest (mm) and recorded after 48 hrs of incubation.

3. Gas Chromatography Mass Spectrometry

The quantitative and qualitative analysis of the oil was carried out using GC-MS apparatus at The National Research Center in Dokki, Cairo, Egypt under the following conditions:

Instrument: Hewlett Packered 5989 A system equipped with Wiley 138, NBS Library software. Column: DB-5, Film Thickness: capillary GC, Injection volume: 1.0 µl, Oven temperature program: 40˚C/5 min., 40-160 ˚C/min., 160- 300˚C/min.Split ratio: 1:50, Scan mass range: 40-400, MS ionization voltage: 70.

The essential oil was identified by matching its mass spectra with those recorded in the Mass Spectrometry Library and compared with those of reference compounds.

RESULTS AND DISCUSSION

Following the procedure of McLaughlin *et al*. [8], the lethality of *Cuminum cyminum* essential oil was evaluated by BSLT and showed relatively high cytotoxicity with LC_{50} value of 30.404.

This result is supported by Bajracharya *et al.* [16], a study constructed to show the potential anticarcinogenecity of essential oils from some spices investigated by Brine Shrimp bioassay. It verified the high toxicity of cuminum oil (LC_{50}= 0.53 ig/ml). This method is a rapid, inexpensive and simple procedure[17]. The cytoxicity of *Cuminum cyminum* essential oil indicates its potentiality for containing bioactive compounds that might have antitumor or pesticidal activity[18]. Furthermore, the folklore use of this plant as a medicine must be monitored closely by the health authorities based on the oil's lethality.

This study showed that the antibacterial activity of *C. cyminum* essential oil with 1:100 dilutions was high against *S. aureus*, *E. coli* and *K. pneumoniae,* with inhibition zones of 16 mm, 16 mm and 15 mm respectively (Figure 1). The oil dilution 1:250 was active against the first two tested organisms, with inhibition zones 13 mm and 12 mm. The results suggest that the oil is relatively effective and contains active inhibitory compounds for some pathogenic bacteria. The activity against *E. coli* is supported by Shetty *et al.*[19]. On the other hand, the antibacterial activity against *S. aureus* is verified by Leopold *et al.*[20]. In a study constructed by Singh *et al*.[21], the essential oils extracted from the seeds of seven spices, *Anethum graveolens, Carum capticum, Coriandrum sativum, Cuminum cyminum, Foeniculum vulgare, Pimpinella anisum* and *Seseli indicum* have been studied for antibacterial activity against eight pathogenic bacteria, causing infections in the human body. It has been found that the oil of *C. cyminum* is very effective against all tested bacteria. These oils were equally or more effective when compared with standard antibiotics, at a very low concentration. In another study investigated by Derakhshan *et al.*[22], the antibacterial activity of essential oil and alcoholic extract of cumin against *Klebsiella pneumoniae* ATCC13883 and clinical *Klebsiella pneumoniae* (ceftazidime-resistant strain) were evaluated on the minimum inhibitory concentration by the broth-dilution method. Synergistic or antagonistic effect with antibiotic disks was tested in agar media involving sub-MIC concentration of oil and alcoholic extract. The results suggested that the essential oil and alcoholic extract of cumin seed could be used in medicinal industries.

The current study revealed the high fungal growth inhibition by the essential oil of *Cuminum cyminum*. Measurements of inhibition zones revealed that the essential oil of *Cuminum cyminum* has a relatively high inhibitory effect against some disease-causing fungi (Figure 2). The antifungal effect of *C. cyminum* essential oil with dilution (1:100 v/v) was high against *A. niger* and *C. albicans* with inhibition zones 18 mm for each, followed by *F. oxysporum* with inhibition zone of 14 mm. These results suggest the possible usage of *C. cyminum* essential oil as an industrial antifungal agent. Shetty *et al.* and Leopold *et al.* [19, 20] work supports the attained results. In a study of the antimicrobial activity of cumin essential oil against fungi namely (*Aspergillus* and *Penicillium* spp.) and yeasts (*Saccharomyces* and *Candida spp*.) the cultures were found to be more sensitive to cumin volatile oil and cumin aldehyde than bacteria and fungi had MIC values 10 to 20 times lower than those of bacteria.

This essential oil has been chemically and biologically investigated[19,20,23]. The seed essential oil of *Cuminum cyminum* was analyzed by GC-MS. The main constituents identified as shown in (Table 2) were; 2-Caren-10-Al (29.64%), Benzaldehyde, 4-(1-methylethyl) (28.55 %), c-Terpinene (16.58%), and 2-J- Pinene (12.06%). The results agree to a great extent with those stated in the literature[19, 24].

CONCLUSIONS

The results from this study showed a high toxicity of the essential oil of *Cumunum cyminum* (LC_{50}= 30.404 µg/ml). This indicates its potential use as an antimicrobial, antitumor agent.

The essential oil of *C. cyminum* showed a relatively good inhibitory effect against *E. coli* and *S. aureus*, and a fair growth inhibititin against *K.pnemonea* in addition to the two mentioned organisms.Furthermore, it showed a relatively high inhibitory effect against *A. niger* and *C. albicans*, and a fair growth inhibition against *F.oxysporum*. These results support the use of this herb in traditional medicine in Sudan.

More *in vitro* and *in vivo* tests are required to ascertain its medicinal properties with special consideration to the high toxicity of the essential oil.

The main chemical compounds identified in the essential oil of *C. cyminum*, analyzed by GCMS were 2-Caren-10-Al (29.64%), Benzaldehyde, 4-1-methyethyl (16.58%) and 2-J-Pinene (12.06%). The bioactivity of the essential oil may be attributed to its chemical constituents. More fractionation, isolation and chemical analysis must be performed to specify single chemical compounds responsible for the bioactivity.

Table 1: Tested organisms

Organisms	Source
Aspergillus flavus	Egypt local strain isolated and identified at the natural and microbial chemistry research products department NRC By Prof. Mohamed Mabrook Atallah. Supplemented under the auspices of the author, Dr. Atallah.
Aspergillus niger	Serial number ATCC9763 (Filamentous Fungi)
Aspergilus sp.	Species unidentified
Bacillus subtilis	Serial number NRRL- 543
Candida albicans	Serial number ATCC9763 (Yeast Fungi)Personal
Escherishia coli	Serial number NRRL 210
Fusarium oxysporum	Contact, Professor Mohamed Fareed, Egypt, NRC
Klebseilla pnemonia	Serial number NRRL- B-117
Staphyloccocus aureus	NRC- Laboratory-1101, Dr. Ahmed Aldewani

Table 2: The chemical composition of *Cuminum cyminum*

Compound	R.T.(min.)	Composition (%)	Chemical Formula	Molecular Weight	Base Peak
Ethane,1,1`-oxybis-	3.50	0.03	$C_4H_{10}O$	74	59.11
Ā-Pinene.(-)	8.98	1.18	$C_{10}H_{16}$	136	93.09
2-J-Pinene	10.87	12.06	$C_{10}H_{16}$	136	93.07
J-Myrcene	11.06	0.33	$C_{10}H_{16}$	136	93.09
1-Phellandrene	11.61	0.41	$C_{10}C_{16}$	136	93.09
Benzene, 1-methyl-4(1-methyl ethyl)	12.50	6.43	$C_{10}H_{14}$	134	119.05
c-Terpinene	13.98	16.58	$C_{10}H_{16}$	136	93.09
a-Terpinolene	14.52	0.07	$C_{10}H_{16}$	136	93.10
Trans-Sabinene hydrate	15.25	0.08	$C_{10}H_{18}O$	154	71.22
Cis-P-2-Menthen-1-ol	16.69	0.06	$C_{10}H_{18}O$	154	92.12
Ethanone,1-(1,4 dimethyl-3-cyclohexen-1-yl)	16.95	0.08	$C_{10}H_{16}O$	152	81.10
Trans-2-caren-4-ol	17.33	0.02	$C_{10}H_{16}O$	152	81.10
Limonene oxide	17.49	0.06	$C_{10}H_{16}O$	152	71.16
3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	18.00	0.43	$C_9H_{18}O$	154	71.11
1,3-Cyclohexadiene-1-methanol,4-(1-methylethyl)	18.52	0.94	$C_{10}H_{16}O$	152	109.05
P-mentha-trans-2,8,dien-1-ol	19.78	0.03	$C_{10}H_{16}O$	152	135.07
Benzaldehyde,4-(1-methylethyl)	21.12	28.55	$C_{10}H_{12}O$	148	133.04
5-Isopropenyl-1,2-Dimethyl-cyclohex-2-ENOL	21.68	0.03	$C_{11}H_{18}O$	166	109.00
2-Caren-10-Al	23.15	29.64	$C_{10}H_{14}O$	150	107.05
1,4,Cyclohexadiene-1-methanol, 4-(1-methyl ethyl)	23.64	0.26	$C_{10}H_{16}O$	152	79.11
2-Caren-10-Al	23.86	0.16	$C_{10}H_{14}O$	150	79.11
2(E)-(4-Methyl-3 penten-ylidiene)-butadienal	24.53	0.07	$C_{10}C_{14}O2$	166	69.11
c- Cadinene	24.68	0.10	$C_{15}H_{24}$	204	161.11
2,4 (10)- thujadien	24.96	0.16	$C_{10}H_{14}$	134	91.10
Isocaryophyllen	25.98	0.20	$C_{15}H_{24}$	204	79.11
3,4-Dimethyl-2-oxocyclopent-3-enylacetic acid	26.42	0.62	$C_9H_{12}O3$	168	122.11
Trans-d-Farnesene	26.92	0.08	$C_{15}H_{24}$	204	69.15
Unidentified	27.67	0.58	-	-	119.06
ā- Chamigrene	28.67	0.03	$C_{15}H_{24}$	204	121.06
Caryophyllene oxide	30.90	0.06	$C_{15}H_{24}O$	220	79.12
3a(1H)- Azulenol, 2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methyl ethyl)-,[3R-(3a,3aā,8aā)	31.63	0.31	$C_{15}H_{26}O$	222	161.09
J-Eudesmol	34.21	0.02	$C_{15}H_{26}O$	222	95.13
(5RS,6SR,7SR)-2,2,6,7-	36.39	0.02	$C_{13}H_{22}O$	210	119.11

tetramethylbicyclo[4.3.0.]non-9-ene-5,7-diol					
2-Pentadecanone,6,10,14-trimethyl	37.68	0.04	$C_{18}H_{36}O2$	268	146.10
Ester-4-en-3-one,17-hydroxy-,(17j)	40.21	0.06	$C_{18}H_{26}O$	274	231.12
Androst-5-en-3d-ol	40.47	0.08	$C_{19}H_{30}O$	274	274.19
6-Fluro-1-hydroxy-4-methoxy-2-methylanth-racene-9,10-dione	40.82	0.03	$C_{16}H_{11}FO4$	286	122.14
2-(2-Hydroxyphenyl)-2,4,4-Trimethylchroman	42.86	0.03	$C_{18}H_{20}O2$	268	92.12

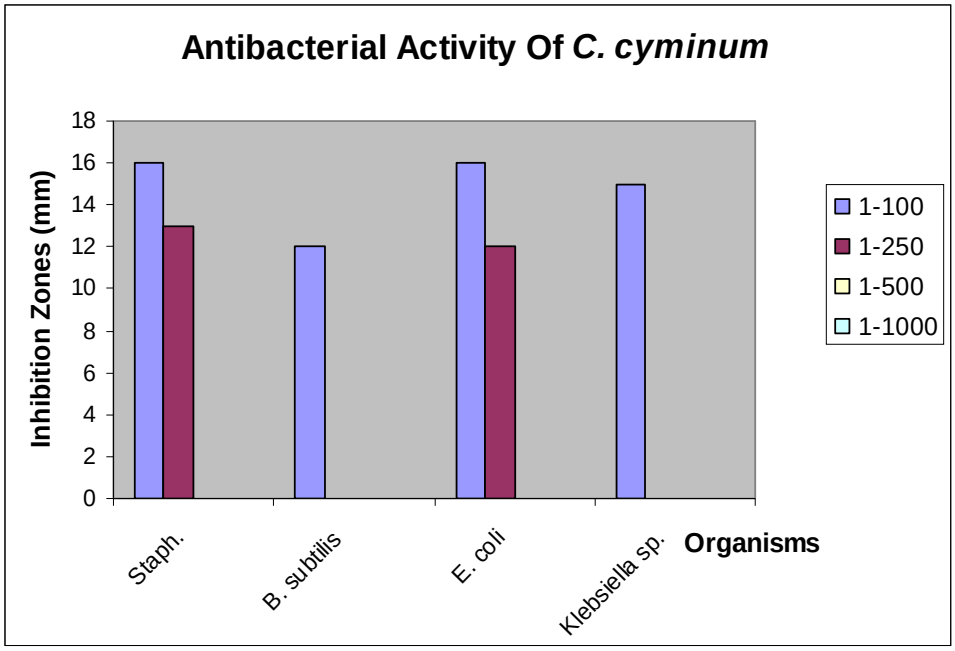

Fig. 1: A histogram showing the antibacterial activity of *C. cyminum*

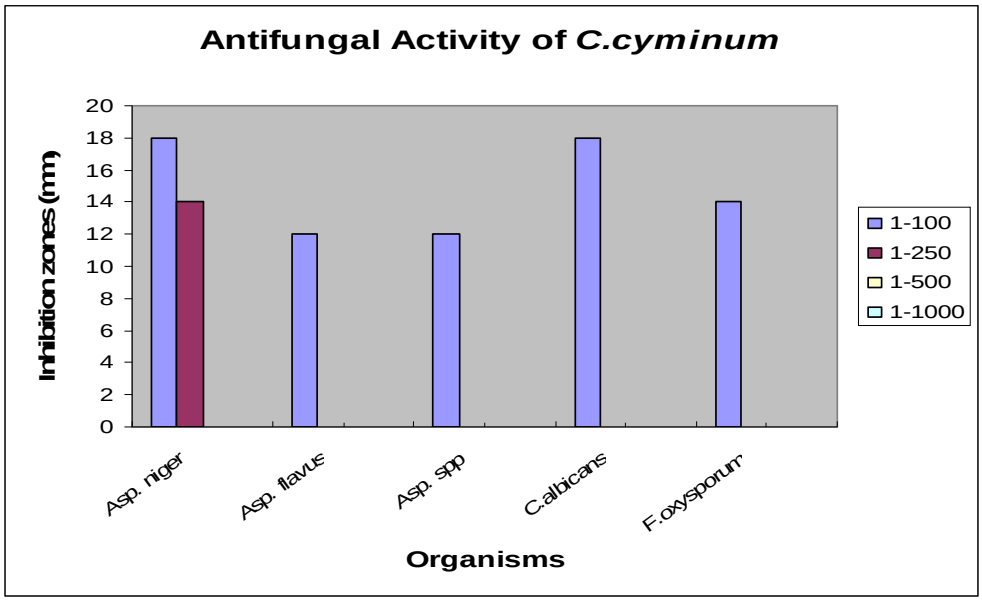

Fig. 2: A histogram showing the antifungal activity of *C.cyminum*

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