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In-vitro Antimicrobial, Anti-oxidant Activities and Cytotoxicty of *Carum carvi* L

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Abstract: *Carum carvi* L. was used traditionally in different populations for many medical complains. The study was aimed to investigate antimicrobial, anti-oxidant activities and cytotoxicty of fixed oil of *Carum carvi* L. (seeds)**.** The oil was extraction by petroleum ether (60-80°C) using a Soxhlet apparatus. The oil of *Carum carvi* L. seeds were tested against four standard bacterial species: two Gram-positive bacteria viz, *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and fungal strains viz, *Candida albicans* (ATCC 7596) using the disc diffusion method. The antioxidant activities were conducted via DPPH radical scavenging assay and cytotoxicty using brine shrimp assay. Antimicrobial activity of fixed oil of *C.carvi* L. dissolved in methanol (1:10), showed high activity against the Gram-negative bacteria (*P. aeruginosa* & *E. coli*) (18 & 14 mm). It also showed against Gram positive bacteria (*S. aureus* & *B.subtilis*) (14 & 13 mm) and against (*C. albicans*) (14 mm). The tested anti-oxidant activity gave (18±0.06 RSA %) in comparison to the control of propylgalate (92±0.01 RSA %). In addition cytotoxicity (brine shrimp lethality Bioassay) verified the safety of the examined extract with an IC_{50} less than 1000µg/ml. This study conducted for essential oil of *C. carvi* L. seeds proved to have potent activities against antimicrobial activity *In-vitro* with verified safety evidence for use.

Keywords: *Carum carvi* L., Antimicrobial Activity, Anti-Oxidant Activity, Cytoxicity (Brine Shrimp)

1. Introduction

Medicinal plants represent a rich source of antimicrobialagents. Plants are used medicinally in different countries andare a source of many potent and powerful drugs [1]. Therehas been an increasing incidence of multiple resistances inhuman pathogenic microorganisms in-recent years, largelydue to in discriminate use of commercial antimicrobial drugscommonly employed in the treatment of infectious diseases [2]. The drug resistant bacteria and fugal pathogens have further complicated the treatment of infectious diseases [3]. The increasing antibiotic resistance of some pathogens thatare associated with foodborne illness is another concern [4] [5].

Carum carvi L., belonging to the family: Apiaceae, is one of the earliest cultivated herbs in Asia, Africa and Europe. In folk medicine, this plantis used as a carminative for stomach disorders, diarrhea, and colic, as well as particularly in veterinary medicine [6].

Caraway has a long history of use as ahousehold remedy especially in the treatment of digestive complaints where its antispasmodic action soothes the digestivetract and its carminative action relieves bloating caused by wind and improves the appetite [7] [8] [9]. It is often added to laxative medicines to prevent griping [8]. The seed is antiseptic, aromatic, anesthetic, anodyne, antianxiety, diuretic, mildly expectorant, fungicidal, muscle relaxant, soporific, tonic, emmenagogue, expectorant, galactogogue and stimulant [7]

[10]. It can be chewedraw for the almost immediate relief of indigestion and can also bemade into infusions. The seed is also used in the treatment ofbronchitis and are an ingredient of cough remedies, especiallyuseful for children and for mothers for increasing breast milk. Atea made from the seeds is a pleasant stomachic and carminative, it has been used to treat flatulent colic [10] [11]. The seed is used inTibetan medicine where it is considered to have an acrid tasteand a heating potency. It is used to treat failing vision and loss of appetite [12]. An essential oil from the seed is used in perfumery, forscenting soap, as a parasiticide etc. [13]. Also *C.carvi* L. are used in traditional Sudanese medicine and other folk medicines as a carminative, since it is effective against spasmodic gastrointestinal complaints, flatulence, irritable stomach, indigestion, lack of appetite, and dyspepsia in adults [14]. Therefore, the study was aimed to investigate antimicrobial, anti-oxidant activities and cytotoxicty of fixed oil of *Carum carvi* L. (seeds).

2. Materials and Methods

2.1. Plant Materials

The Caraway (*Carum carvi* L.), was collected from Khartoum central Sudan during September to October 2016, and the plant was kindly identified and authenticated by Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) in Khartoum, Sudan. Seeds were air-dried, under the shade, pulverized and stored prior to extraction. Shade with good ventilation and then ground finely in a mill and kept in the herbarium until oil extract preparation Figure (1).

Figure 1. *Carum carvi L. (Seeds).*

2.2. Extraction of Oil

Air-dried seeds of Caraway (*Carum carvi* L.), was separately powdered and extracted with 1 L ofpetroleum ether (60-80°C) using a Soxhlet apparatus. This process of extraction was repeated for 6h, thepetroleum ether distilled out by distillation assembly, then concentrated by hot plate drying and air-drying at temperature of 40±2°C.

2.3. Test Microorganisms

The oil solution of *Carum carvi* L. was tested against four standard bacteria species: two Gram-positive bacteria viz., *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and one standard fungal strains viz, *Candida albicans* (ATCC 7596) using the Disc diffusion method. The standard bacterial and fungal strains used in the study were obtained from the Department of Microbiology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

Testing of Antibacterial and Antifungi Susceptibility

Disc diffusion method:

The paper disc diffusion method was used to screen the antibacterial and fungi activity of plant oil and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [15]. Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of plant oil. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

2.4. Anti-Oxidant

DPPH radical scavenging assay:

The DPPH radical scavenging was determined according to the method of Shimada [16], with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

2.5. Toxicity Testing Against the Brine Shrimp

2.5.1. Storage of Artemiasalina Eggs

Eggs of *Artemiasalina* were stored at low temperatures (4°C), they will remain viable for many years.

2.5.2. Hatching Shrimp

Brine shrimp eggs, *Artemiasalina* were hatched in artificial seawater prepared by dissolving 38g of sea salt in one litre of distilled water. After 24-72h incubation at room temperature (37°C), the larvae were attracted to one side of the vessel with a light source and then collected with pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing artificial seawater.

2.5.3. Brine Shrimp Assay

Bioactivity of the oil was monitored by the brine shrimp lethality test [17]. Brine shrimp lethality bioassay was carried out to investigate the cytoxicity of plant extracts. 50 mg of *Artemiasalina* (Leach) eggs were added to a hatching chamber containing artificial Sea water (75 ml). The hatching chamber was kept under an inflorescent bulb for 48h for the eggs to hatch into shrimp larvae. 20 mg of test extracts of the various plant species were separately dissolved in 2 ml of methanol, then 500, 50, and 5µl of each solution was transferred into vials corresponding to 1000, 100, and 10 µg/ml, respectively. Each dosage was tested in triplicate. 10 larvae of *A. salina* Leach (taken 48 – 72h after the initiation of hatching) were added to each vial (Figure 4). The final volume of solution in each vial was adjusted to 5ml with Sea water immediately after adding the shrimps. One drop of dimethyl sulphoxide (DMSO) was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. LD_{50} values were determined at 95% confidence intervals by analysing the data on a computer loaded with a "Finney Programme." The concentration at which it could kill 50% larvae (LD_{50}) was determined. LD_{50} values below 200ppm are generally considered as significant according to [18] [19] [20].

2.6. Statistical Analysis

All data were presented as means ± S.D. Statisticalanalysis for all the assays results were done usingMicrosoft Excel program (2010).

3. Results and Discussion

The oil of *Carum carvi* L. seeds were tested against four standard bacterial species: two Gram-positive bacteria viz, *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and fungal strains viz, *Candida albicans* (ATCC 7596) using the disc diffusion method. The antioxidant activities were conducted via DPPH radical scavenging assay and cytotoxicty using brine shrimp assay.

The oil of *Carum carvi* L. seeds family (Apiaceae) was screened for antimicrobial activity against four bacterial species: two Gram-positive bacteria viz., *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and fungal strains viz, *Candida albicans* (ATCC 7596)using the disc diffusion method.

The oil of *Carum carvi* L. dissolved in methanol (1:10) showed high activity (18 & 14 mm) against Gram negative bacteria (*P. aeruginosa* & *E. coli*) and (14 mm) against (*S. aureus* & *C. albicans*). It also showed (13 mm) againstGram positive bacteria (*B. subtilis*) Table (1) and Figure (2).

Therefore this result showed that the oil tested inhibited the growth of all microorganisms though the sensitivities of microorganisms varied.

This result was similar to that reported by [21]. who found that the plant oil showed high activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, *Mycobacterium tuberculosis.*

*C. carvi*essential oils inhibited thegrowth of *Aspergillusparasiticus*and yeast and Gram-positiveand Gram-negative bacteria [22]. Thus, according to ourinvestigation caraway can be used as a potent antimicrobialagent for human pathogens.

As shown in Table 2, the results of cytotoxicity of the *Carum carvi* L. (seeds) was determined by using brine shrimp lethality Bioassay were shown in Table (2). The tested have IC_{50}<1000µg/ml <1000 µg/ml.

As shown in Table 3, the results of antioxidant activity *Carum carvi* L. (seeds) showed antioxidant activity againstthe DPPH free radical (18 ± 0.06 RSA%). Table 3 indicates the anti DPPH of ethanol extract of *Carum carvi* L. (seeds) the reading and propyl gallate was used as standard drug level. The tested antioxidant activity gave (18 ± 0.06 RSA %) in comparison to the control of propyl galate levels gave (92 ± 0.01 RSA %).

Table 1. *The antimicrobial activity oil of Carum carvi L. and reference antibiotics against the standard bacteria and fungi.*

Standard microorganisms	Concentration (mg/ml) Mean Diameter of Growth Inhibition Zone (mm)				Gentamicin 30 µg/ml
	100	50	25	12.5	
Bacillus subtilis	13	-	-	-	20
Escherichia coli	14	-	-	-	29
Staphyococcus aureus	14	13	-	-	30
Pseudomonas aeruginosa	18	-	-	-	25
Tested fungi used					Nystatin
Candida albicans	14	-	-	-	15

Table 2. *The Cytotoxicity oil of Carum carvi L. according to the brine shrimp assay.*

Name of plants	Concentrations (µg/ml)	Total Number of shrimps	dead	survivors	Mortality (%)	IC_{50} (µg/ml)
Carum carvi L.	1000	10	04	06	40	2371
	100		00	10	00	
	10		00	10	00	

Table 3. *Antioxidant activity of the oil of Carum carvi L. using DPPH assay.*

No.	Name of Samples	%RSA+SD (DPPH)
1.	*Carum carvi* L.	18±0.06
2.	Propyl galate	92±0.01

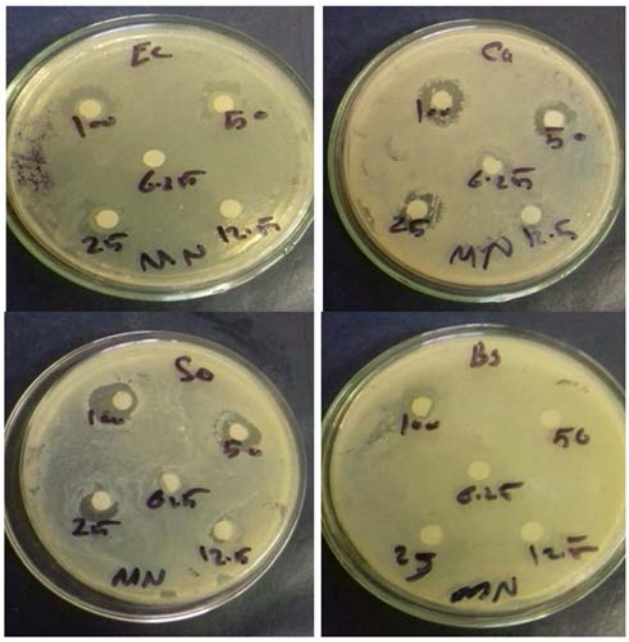

Figure 2. *Antimicrobial activity of fixed oil of Carum carvi L. seeds. Zone of inhibition of oil against standard bacteria andfungi.*

4. Conclusion

The oil of *C.carvi* L. showed the various degree of inhibitory activity against the microorganisms tested. The obtained results may justify the use of the Sudanese whole plant of *C.carvi* L. as antimicrobial therapy in traditional medicine in Sudan and the neighboring countries. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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