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Chemical Composition, Essential Oil Characterization and Antimicrobial Activity of *Carum copticum*

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Abstract

Medicinal plants are an important source of bioactive compounds which can be used for the treatment of various diseases. The aim of the present study was to explore the phytochemical content and biological evaluation of *Carum copticum* essential oil and crude extracts. Antimicrobial activity of *Carum copticum* against six gram negative bacteria, three gram positive bacteria and one fungal strain were estimated. Furthermore, metallic content (Cr, Fe, Mn, Ni, Mg Zn, Cu and Ca), nutrient content and identification of the biologically active constituents were also evaluated. GC-MS characterization showed that p-cyme-3-ol (38.00%), o-cymene (37.44%), gamma-terpines (21.07%) and beta-pinene (1.42%) were present as major constituents. Antimicrobial activity of essentials oil was significant as compared with crude extract and exhibited maximum activity against *B. atrophaeus* (43 mm). The phytochemical screening confirmed the presence of terpenoids, steroids, flavonoids, alkaloids, glycosides and reducing sugar. Metallic screening displayed the highest Ca (191.67 mg/l) concentration followed by Mg (52.275 mg/l), Fe (1.610 mg/l) and Mn (0.941 mg/l). The results obtained exposed that *Carum copticum* may be a significant source with interesting antimicrobial action and health protective potential.

Keywords: *Carum copticum*; GC-MS analysis; Metallic content

Introduction

Medicinal plants have been used for centuries as remedies for human health and diseases because they contain secondary metabolites of medicinal value. Spices and herbs are essential part of human diets as they have been used since ancient times for flavoring, aromatic and therapeutics values. Currently, new strains of drug resistant pathogens is a very serious problem for the effective treatment of microbial diseases [1], as well as many antibiotics are associated with adverse effects on host like hypersensitivity and allergic reactions [2]. This situation forced researchers to start the quest for new and alternative antimicrobial drugs for the treatment of infectious diseases [3]. Hence natural medicine is considered as safe alternatives of synthetic drugs. Approximately 20 percent of the plants spices in the world have been found to have therapeutic value and a sustainable number of new pharmaceutical drug introduced in the market are obtained from natural products or semi-synthetic resources [4].

Carum copticum (syn: *Trachyspermum ammi*, commonly called ajwain) is an aromatic, annual plant, belonging to the Apiaceae family which is commonly grows in Iran, India, Pakistan and Egypt. *Carum copticum* is a traditional herb, extensively used as flavoring agent and for treatment of various infectious diseases in human and animals. In Persian traditional medicine, the seeds of *Carum copticum* were used for its therapeutic effects such as diuretic, anti-vomit, carminative, anti-helmentic, expectorant, analgesic, anti-asthma, anti-dyspnea and anti-spasm [5]. Data from the literature has confirmed the wide spectrum of biological activity of *Carum coptcium* like antiviral [6], anti-inflammatory [7], antifungal [8], antipyretic [9] and antioxidant activities [10]. *Carum coptium* fruits are important industrial product for the food and/ orflavoring industry and contains up to 5% essential oils [11]. Some important biological activities of essential oils (EOs) of *Carum copticum* like antioxidant [12], anti-cholinergic [13] and analgesic effects [14] have been reported earlier. Traditionally, the aqueous extract of *Carum copticum* is extensively used to alleviate the symptoms of flu in children [15].

Keeping in view the long term pharmacological and day to day use, the objective of the current study is to evaluate the nutritional value of *Carum copticum* and to analyze the chemical constituents of essential oils by GCMS technique. In addition the inhibitory activity of the crude extracts and essential oils were investigated against pathogenic microbes by disc diffusion assay.

Materials and Methods

Chemicals

All the chemicals used were of analytical grade and were obtained from standard commercial supplier, while the water was glass distilled.

Sample collection and identification

Carum copticum was purchased from local market of Peshawar in February 2012. The plant species were identified by experts in PCSIR Laboratories Complex, Peshawar, Pakistan.

Crude extraction: The dry plant material was grinded to fine powder. Crude ethanolic, aqueous and ethyl acetate extracts were prepared by soaking 250 g of plant material in 1 L of solvents at room temperature for 48 hours then were filtered using Whatman No. 1 filter paper. The diluted extracts were concentrated by rotary evaporator and stored at 4°C.

Proximate analysis: The proximate analysis (carbohydrate, fats, protein, moisture and ash) of *Carum copticum* was determined by the method of the Association of Official Analytical Chemists (AOAC) methods. Crude carbohydrate value was determined by the following difference formula (100–(moisture+ash+protein+fat)). The nitrogen content was determined by kjeldahl method and multiplied to factor 6.25 to find the protein content. The weight difference method was used to find moisture and ash content. Nutrient contents are reported in percentage (%) [16].

Phytochemical screening: The method of Sofowora and Trease and Evans were employed to evaluate the presence of secondary active metabolites like flavonoids, glycosides, steroids and terpenoids in the selected plant [17,18].

Analysis of the essential oils

GCMS analysis of the oils was carried out on a 6890 series instrument equipped with FID and a HP-5 fusedsilica capillary column (30 m × 0.25 mm (internal diameter, film thickness 0.25 mm). The temperature was programmed from 50-240°C at 4°C/min; from 240°C to 270°C at 15°C/min; held isothermal at 50°C for 1 min and at 270°C for 15 min. The temperatures of both auto-injector and detector were kept at 280°C; sample injection volume was 1 µL, while 100:1 split ratio was maintained throughout the analytical procedure. Nitrogen was used as carrier gas at a flow rate of 1.2 mL/min. Compounds were identified by comparison of their retention indices and mass spectra with the data given in the literature [19,20].

Metallic screening by Atomic Absorption Spectrophotometer (AAS)

Metallic content i.e., Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Zinc (Zn), Chromium (Cr), Nickel (Ni) and Manganese (Mn) were determined by atomic absorption spectrophotometry. Sample was prepared by washing the *Carum copticum* with double distilled water to remove air born pollutants. For complete removal of moisture the sample was oven dried in a hot air oven at 70-80°C for 24 hours. 0.5 g sample was accurately weighed and placed in crucibles. The ash was digested with perchloric acid and nitric acid (1:4) solution. The sample was left to cool and contents were filtered through whatman filter paper No. 42. Sample solution was made up to a final volume of 25 mL with distilled water and was spectrally analyzed using atomic absorption spectrophotometer (Hitachi Model Z-8000 Japan). Standard solutions of heavy metals (1000 mg/L), namely Ca, Mg, Fe, Cu, Zn, Cr, Ni and Mn were procured from Merck. Solutions of varying concentrations were prepared for all the metals by diluting the standards [21].

Analysis of antimicrobial activity

Test microorganisms: Six Gram negative bacteria namely *Eschericia coli* (*E. coli*), *Klebsiella pneumonia* (*K. pneumonias*), *Salmonella typhi* (*S. typhi*), *Pneumococcus auroginosa* (*P. aeruginosa*), *Erwinia carotovora* (*E. carotovora*), *Agrobacterium tumefaciens* (*A. tumefaciens*), three Gram positive bacteria namely *Bacillus subtilis* (*B. subtilis*), *Bacillus atrophoeus* (*B. atrophaeus*), *Staphylococcus aureus* (*S. aureus*) and one fungal strain i.e., *Candida albicans* (*C. albican*) were used to determine the antimicrobial activity of the *Carum copticum* extract.

Determination of Antimicrobial Activity: Antibacterial activity of solvent extracts i.e., aqueous, ethanolic, ethyl acetate and essential oil were determined by Disc-diffusion method on nutrient agar medium in terms of diameters of inhibition zone against selected microbes. Nutrient agar media (2.8 g 100 ml^{-1}) and nutrient broth (1.3 g 100 ml^{-1}) were prepared by dissolving in distilled water. Agar media was decanted into petri plates and incubated for 16 hours at 37°C to check any contamination. The stock cultures were freshened by streaking on fresh agar plates in a laminar flow hood and incubated at 37°C for 24 hours. Next day streaked cultures were inoculated into the nutrient broth in flasks and kept in the shaking water bath (Model; GLSC-SBR-04-28) for 16 hours, at 200 rpm at 37°C. The microbial cultures were standardized in test tubes by comparing with 0.5 McFarland (turbidity) Standard. 100 µl of standardized microbial cultures were spread on each nutrient agar plate. The stock solutions of all the extracts and oil in 1-2 mg disc in 12 µl volume were applied on these discs in triplicates. Antimicrobial potential was recorded for each extract in terms of mm of zones of inhibition around each disc [22,23].

Results

The yields of extracts in different solvents were calculated and recorded as percentage of plant material. The extractive value was highest for aqueous extract (26.28%), followed by ethanolic (15.4%) and ethyl acetate (8.23%) extract. Proximate analysis of *Carum copticum* demonstrated highest carbohydrates (30%) value followed by crude fat (25.3%) and protein (17.5%). While moisture, ash and crude fiber content were 11.43%, 13.14% and 9.65% respectively (Table 1).

Moisture	Ash	Crude Fat	Crude Fiber	Carbohydrate	Protein
11.43	13.14	25.3	9.65	30	17.5

Table 1: Proximate (%) analysis of *Carum copticum*.

The results of phytochemical analysis in different solvents extracts are shown in Table 2 which revealed the presence of terpenoids, alkaloids, glycosides, flavonoids, steroids and reducing sugar while tannins were not detected in any solvent extracts. The ash content of *Carum copticum* seeds was analyzed by AAS for elemental estimation as presented in Table 3.

S No	Phytochemical	Aqueous	Ethyl Acetate	Ethanol
1.	Steroids and terpenoids	+	++	+++
2.	Glycosides	+	++	++
3.	Tannins	_	_	_
4.	Flavonoids	_	++	+++
5.	Reducing sugar	+++	++	++
6.	Alkaloids	+	++	++

Table 2: Phytochemical screening of *Carum copticum.*

The results exposed that Ca (191.67 mg/l) was present in highest concentration followed by Mg (52.275 mg/l), Fe (1.610 mg/l), Mn (0.941 mg/l) and Zn (0.807 mg/l). Eleven chemical constituents representing 99.9% were identified by GC-MS analysis as shown in Table 4. The predominant constituents were p-cymen-3-ol (38.00%), o-cymene (37.44%), gamma.-terpinen (21.07%) and beta.-pinene (1.42%), thymol (p-cymen-3-ol) and o- cymene (75%) of the total oil.

S No	Analyte	Wavelength	Mean (mg/l)	Std Dev	RSD (%)
1	Mn	279.5	0.941	0.039	4.15
2	Cr	357.9	0.179	0.0086	4.83
3	Fe	248	1.61	0.0182	1.13
4	Ni	232	0.427	0.0181	4.23
5	Zn	213.9	0.807	0.0017	0.21
6	Cu	324.8	0.119	0.0096	8.1
7	Ca	422.7	191.67	0.0309	0.4
8	Mg	285.2	52.275	0.0114	0.55

Table 3: Elemental investigation of *Carum copticum* by using atomic absorption spectroscopy (AAS).

All crude extracts (ethanolic, aqueous and ethyl acetate) and essential oil of *Carum copticum* showed varying degree of growth inhibition against the selected microbial strains by disc diffusion assay (Table 5). The zone of inhibition of essential oil ranged from 18 to 43 mm against the selected microorganisms. The diameter of zone of inhibition for ethanol, ethyl acetate and aqueous extract ranged from 15.5 to 32.5 mm, 13.5 to 29 mm and 11 to 25 mm respectively. Essential oil exhibited strong activity against some gram positive and gram negative microbes than the crude extracts and standards antibiotics i.e., ciprofloxacin and azithromycin were used as standard drugs. Essential oil exhibited highest activities against *B. atrophaeus* (43 mm) followed by *K. pneumonias* (41 mm) *B. subtilis* (40 mm) and *S. aureus* (36.5 mm). However, no activity was detected against *P. aruginosa* and *A. tumfacines*. Among the crude extracts ethanolic and ethyl acetate extracts were more effective than aqueous extracts against the selected microbes. Crude ethanolic and ethyl acetate extracts showed significance activity against all the tested microbes. The ethanolic extract of *Carum copticum* was found to be more sensitive against *E. corotovora* (32.5 mm), *K. pneumonias* (22 mm) and *S. typhi* (20.5 mm), while ethyl acetate extract showed wider zone of inhibition against *E. coli* (29 mm) followed by *B. subtilis* (20.5 mm) and *C. albican* (18 mm). Although aqueous extract was least active but it showed promising activity against *E. coli* (25 mm).

ID#	Name	R. Time	Area	Conc (%)
1	alpha.-Phellandrene	9.610	40185	0.52
2	alpha.- Pinene	9.937	13439	0.20
4	Bicylo [3.1.0]hexane, 4-methylene-1-(1-methylethyl)	12.131	3744	0.05
5	beta.-Pinene	12.339	96720	1.42
6	beta.-Myrcene	13.239	41139	0.60
9	alpha.-Terpine	14.783	24237	0.36
10	o-Cymene	15.404	2551693	37.44
15	gamma-Terpinen	17.578	1436270	21.07
36	p-menth-1-en-8-ol	26.763	8277	0.12
42	Propanal, 2-methyl-3-phenyl	29.763	9979	0.15
49	p-Cymene-3-ol	32.688	2589957	38.00

Table 4: Chemical composition of *Carum coptium* essential oil by GC/MS analysis.

Organisms	Zone of inhibition in mm					
	Aqueous	Ethyl Acetate	Ethanol	Essential Oil	Positive Control	Negative Control
	2 mg/12 µl	2 mg/12 µl	2 mg/12 µl	2 mg/12 µl	Ciprofloxacin 30 µg/6 µl	DMSO
Gram negative bacterial strains						
Escherichia coli	25	29	15.6	21	37	0
Klebsiella pneumonia	11	16	22	41	29	0
Salmonella typhimurium	12.5	14.5	20.5	32	23	0
Pseudomonas aeruginosa	11.5	15.5	16.5	N.D	32	0
Erwinia carotovora	13	14.5	32.5	32.5	17	0
Agro bacterium tumefaciens	15	16	18	N.D	25	0
Gram positive bacterial strains					Azithromycin 50 µg/6 µl	
Bacillus subtilis	13	20.5	16.5	40	23	0
Bacillus atrophaeus	11.5	13.5	17	43	27	0
Staphylococcu aureus	N.D	17.5	15.5	36.5	21	0
Fungal strains					Clotrimazole 50 µg/6 µl	

Candida albicans	11.5	18	21.5	18	32	0

Table 5: Antimicrobial activity of *Carum copticum* essential oil and extracts against pathogenic microbes.

Discussion

The result of the proximate analysis showed that *Carum copticum* is rich in nutrients and has high carbohydrate (30%) value followed by crude fat (25.3%) and protein (17.5%). The current study revealed optimum moisture content (10.5%), as the acceptable range is from 6 to 15% for most vegetable drugs [24]. Previously a contrasting result has been reported for the proximate analysis of *Carum copticum* where the percentage concentration of carbohydrate, protein, crude fat, moisture, fiber and ash were 47.54%, 20.23%, 4.83%, 11.6% and 11.5% respectively [25]. The results of phytochemical analysis (Table 2) indicated that *Carum copticum* is rich in phytochemicals such as alkaloids, flavonoids, terpenoids, glycosides, steroids and reducing sugar. Presences of these bioactive compounds may contribute to its medicinal value. For instance, flavonoids and saponins have been reported to have anti-oxidants, anti-microbial, anti-cancer and anti-allergic activities and cardiac glycosides have also been reported as antifungal as well as cardiotonics [26]. Furthermore, quantitative estimation of ash verified *Carum copticum* as good source of minerals. Ca was present in highest concentration followed by Mg and Fe while the concentrations of Mn, Cr, Ni, Zn and Cu were low. Minerals elements are extremely important for various metabolic processes as they act as cofactors and help in transmission of nerve impulses and water balance [27]. Ca is necessary for the structure and metabolism of bones and for blood clotting and muscle contraction, while Mg act as activator of many enzymes involved in carbohydrate metabolism and synthesis of nucleic acids [28]. Moreover, Cr is proved to be toxic at 5 mg/L for different plants species [29]. Our study recorded very low concentration of Cr than its recommended level for toxicity of plants.

Similarly, chemical composition of *Carum copticum* oil showed the presence of p-Cymen-3-ol (thymol), O-cymene and gammam-terpinen as the main constituents of oil. Kahjeha et al. reported that the main constituent of essential oil were thymol (48%), p-Cymene (15%) and gamma-Terpinen (30%) [30]. While in another report the major constituent were characterized as p-cymene (39.1%), oleic acid (10.4%), linoleic acid (9.9%) and gamma terpinen (28.6%). We identified thymoland γ-terpinene as the two main compounds in ajwain oil which are in strong agreement with the report of Khajeh et al. and Mohagheghzadeh et al. O-cymene was the third most abundant compound which is one of p-cymene isomers with ortho substituted alkyl group [30,31].

Essential oil and crude extract (ethanol, ethyl acetate and aqueous) of *Carum copticum* exhibited different degree of anti-microbial activities against selected microbial strains. Our result demonstrated that essential oil was highly effective against *B. atrophaeus*, *K. pneumonias, B. subtilis* and *S. aureus* than ciprofloxacin and azithromycin. No antimicrobial activity of oil was observed against *A. tumefacines* and *P. aeruginosa*. It may be due to the multidrug resistance-characteristics of *P. aeruginosa*. Results of antimicrobial study also revealed that ethyl acetate and ethanol extract of plant exhibited broad spectrum activity against the tested microorganisms. Ethanolic extract of *Carum copticum* was active against all tested bacterial and fungus strains and showed highly significant activity against *K. pneumonias* followed by *E. carotovora*. Ethanolic extract was found be more active against *K. pneumonias* when compared to ciprofloxacin. Ethyl acetate extract was equally effective against both gram positive and gram negative bacteria and displayed highest activity against *E. coli* and *B. subtilis*, while its activity against *E. coli* was greater as compared to the standard antibiotic i.e., ciprofloxacin. Although aqueous extract was least active against all the tested microorganisms but its antimicrobial potential against *E. coli* (25 mm) was similar to the standard drug. Aqueous extract depicted least activity this may be due to the lack of solubility of the active components in water. The traditional healers use water as the solvent for extraction but organic solvent extracts exhibited more consistent antimicrobial potential [32,33].

The strong antimicrobial potential of the studied plant can be attributed to thymol and its precursors, cymene and terpinene [34], have strong antimicrobial activities. The antimicrobial activity of thymol may induce through the modification of the cell membrane permeability and leakage of intracellular material. P-cymene a major compound detected in *Carum copticum* oil is a hydrophobic molecule and causes swelling of the cytoplasmic membrane [35]. The antimicrobial potential of thymol, p-cymene, caracole, and γ-terpinene against *E. coli* and *S. aureus* has been reported in literature [36]. Furthermore, studies have been reported that the use of whole essential oil has more antibacterial activity than the mixed major compounds. It also revealed the vital synergistic effect of minor components of essential oil [37,38]. The results of the current study are in accordance with Goudarzi et al. [39] which showed antimicrobial potential of *Carum copticum* oil against *S. aureus, E. coli* and *S. typhimurium* while no activity was recorded against *P. aeuroginosa*. Similarly Singh et al. [40] reported that *Carum copticum* oil was very effective against *C. diphtheriae*, *S. aureus*, *S. haemolyticus*, *E. coli*, *Klebsiella* spp. and *P. vulgaris*. Previously published literature has shown that gram positive bacteria are usually more susceptible to essential oil and extracts than gram negative bacteria because of the outer membrane protein of gram negative bacteria which restricts diffusion of compounds through it [41], while our data did not demonstrate any selectivity toward gram positive and gram negative bacteria.

Conclusion

Carum copticum is a good source of minerals, nutrients and can be used in Ayurvedic system to cure various ailments. Furthermore the essential oil and crude extracts of the plant exhibited highest activity against certain microbes than standard antibiotics used, indicating that this plant may act as potential agent for drug developments and may provide a lead for modern drug design. However in order to develop a strong basis for appreciating the curative effect of this spice, future research is needed to explore the unexploited potential of this plant.

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