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Short communication

GC-MS INVESTIGATION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *CARUM COPTICUM* BENTH & HOOK

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The fruit oil of *Carum copticum* showed the presence of eleven components with carvacrol (45.20%) and δ–cymene (41.98%) as the major constituents by GC-MS analysis. The essential oil exhibited considerable in vitro antimicrobial activity with most of the gram-positive and gram-negative bacteria tested and the results are comparable with the standard bactericide. The pure oil inhibited the growth of *Phomopsis destructum*, *Aspergillus niger* and *A. flavus*.

Keywords: *Carum copticum*, agar overlay technique, zone of inhibition, microbicidal activity, essential oils

Carum copticum Benth & Hook (Syn. *Trachyspermum ammi* Linn.) is the Bishop's weed widely cultivated all over India (ASOLKAR et al., 1992). The seeds and its extractions are extensively administered in the Indian system of medicine as antiseptic, carminative and stimulant. MHASKAR and CAIUS (1931) suggested it as a remedy for snake bite. GEORGE and MARIAM (1957) investigated the plant for isolation of antibodies. QADRY and ATAL (1967) gave the pharamacognocy of its fruits. HANDA and co-workers (1986) formulated a liver protecting drug from the plant. SAXENA (1987) and BUCH and co-workers (1988) studied the effect of its volatile oil on ejaculated human spermatozoa.

RAMASASTRY (1983) reported Ca, Fe and its oxalate constituents. KARTA and KHAN (1969) isolated Δ^{5-6} and Δ^{7-8} octadecanoic acids from its seed fats. The effect of γ-rays on the thymol content of the oil was studied by THENGANA and DHYANSAGAR (1985) and GUPTA (1958) extracted demethylated azowan oil. MALLAIAH (1969) carried out the epoxidation studies of the oil and flavonoid pattern in its fruits was revealed by HARBORNE and WILLIAMS (1972).

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Hot water infusion of the fruit is a household remedy for a number of intestinal disorders. On account of the folklore medicinal use of the azowan, its essential oil was analysed by the capillary GC-MS method, and in order to rationalise its curative properties the oil was screened for its antimicrobial activity in the present studies.

1. Materials and methods

The fruits for the present study were collected from the plants specially grown in our botanical garden. A voucher specimen was preserved in the herbarium of the botany department. The essential oil was extracted using the volatile oil extraction apparatus (Paul Scientific, Calcutta). It consists of one round bottom flask, where weighed quantity of fruits were placed and distilled. The vapours were condensed and the volatile oil isolated was separated that gave a yield of 4.52% w/w. The characteristically smelling, colourless etherial oil was stored at 4 °C after drying over anhydrous sodium sulfate.

The isolated volatile oil was fed to a gas chromatograph (GC) where it was partitioned between the stationary liquid and the mobile gaseous phases. A peak was registered when its components emerged. Based on the number and areas of peaks recorded, its components were qualitatively and quantitatively estimated. The eluants were passed to the mass spectrograph (MS) which registered its mass fragmentation pattern indicating its molecular structure.

GC-MS analysis was carried out with the combined GC-MS system consisting of a Hitachi 163 GC and Hitachi M-80A mass spectrometer. A DB-1 fused silica column (60 m×0.28 mm i.d.) was used with helium as carrier gas. The temperature was held at 70 °C for 5 min then programmed at 240 °C at 3 °C min^{-1} and ionization voltage was 20 eV with scan rate of 0.0445 scans s^{-1}. Peak identification was confirmed by comparing GC retention index and mass spectrum with that of an authentic sample. Mass spectral data searching was carried out on Hitachi 0101 data processor.

The in vitro antimicrobial screening of the oil was carried out by the agar overlay technique (JASSEN et al., 1986) adopting the modified Bondi's paper disc method (LOUIS & ANDERSON, 1989). Bacteria were maintained and grown in Potato Dextrose Agar (PDA) and the fungi in chloramphenicol agar media. All the tests were conducted in triplicate and the average zones of inhibition were recorded. Blank and control tests were also performed. Activity of standard fungicides and bactericides was also simultaneously assessed for comparison.

2. Results and discussion

The percentage of the components present in the seed are shown in Table 1 and the zones of inhibition with different microorganisms at various dilutions are given in Tables 2–4. It showed good bactericidal activity with *Staphylococcus pyogenes* which causes rheumatic fever and infections in respiratory tract and *Corynebacterium diphtheriae* that causes diphtheria, though the activity dimmed with dilution. The oil showed considerable activity with gram-negative pathogenic bacteria, *Escherichia coli* causing diarrhoea and gastroenteritis and aerobic sporeformers (ASF) causing general infections. The activity of the pure oil was comparable with those of standard bactericides.

Table 1

Volatiles of Carum copticum

Compound	%
α-Pinene	0.38
β-Pinene	1.38
Myrcene	0.15
δ-Cymene	41.98
1,8-Cineole	0.23
Limonene	0.26
γ-Terpinene	1.79
Terpinene-4-ol	0.22
α-Terpineol	0.11
Thymol	0.48
Carvacrol	45.20
Unidentified	3.90

Table 2

Antimicrobial efficacy of the essential oil of Carum copticum *against gram-positive bacteria at different dilutions*

S. No.	Name of the bacterium	Average diameter of zone of inhibition in mm					
		Bactericide[a]	Pure oil	Dilutions			
				1:100	1:250	1:500	1:1000
1	*Staph. pyogenes*	25	22	20	10	10	10
2	*Strep. pyogenes*	26	16	R	R	R	R
3	*Strep. pneumoniae*	28	18	10	10	10	10
4	*Mycobacterium sp*	24	14	R	R	R	R
5	*Corynebacterium sp*	22	22	16	14	14	10
6	*Bacillus sp*	24	20	16	9	9	9
7	*A.S.F.*	18	16	14	10	10	10

[a] Bactericide is Streptomycin sulfate 10 mg ml^{-1}

R indicates resistance

Table 3

Antimicrobial efficacy of the essential oil of Carum capticum *against gram-negative bacteria at different dilutions*

S. No.	Name of the bacterium	Average diameter of zone of inhibition in mm					
		Bactericide[a]	Pure oil	Dilutions			
				1:100	1:250	1:500	1:1000
1	*Escherichia coli*	22	22	14	8	8	8
2	*Bordetella sp*	20	R	R	R	R	R
3	*Shigella sp*	18	R	R	R	R	R

[a] Bactericide is Streptomycin sulfate 10 mg ml^{-1}

R indicates resistance

Table 4

Antimicrobial efficacy of the essential oil of Carum capticum *against fungi at different dilutions*

S. No.	Name of the fungi	Average diameter of zone of inhibition in mm[a]					
		Fungicide[b]	Pure oil	Dilutions			
				1:100	1:250	1:500	1:1000
1	*Rhizopus sp*	17	10	R	R	R	R
2	*Sordaria sp*	19	11	9	9	9	9
3	*Phomopsis sp*	22	12	9	9	9	9
4	*Aspergillus niger*	16	14	R	R	R	R
5	*Aspergillus flavus*	18	14	R	R	R	R

[a] Includes diameter of the paper disc (6 mm)

R indicates resistance

[b] Fungicide is Nystatin 50 mg ml^{-1}

Terpinene 4-ol was reported to be the active component of juniper oil, which is administered in urinary bladder infections. Similarly the use of thymol as medicine is mainly because of its activity against dermatophytes. Likewise the therapeutic value of the present oil may be attributed to δ-cymene and carvacrol, the major constituents. The therapeutic use of Bishop's weed is further confirmed by the present study.

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