

Chemical Composition and Anti-scabies Activity of Essential Oil of *Elettaria Cardamomum* Maton. Leaves

Bharat Sharma¹, Neeru Vasudeva^{1*}, Sunil Sharma¹

¹ Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, Haryana, India

ABSTRACT

The objective of this study was to investigate the chemical constituents of essential oil of *Elettaria cardamomum* leaves and assess its anti-scabies potential.

Essential oil obtained by hydrodistillation of fresh leaves of *E. cardamomum*, subjected to gas chromatography, gas chromatography-mass spectrometry for identification and quantification of components. Anti-scabies potential of essential oil of *E. cardamomum* leaves against *S. scabiei* was investigated by contact bioassay method.

GC and GC-MS analysis results revealed the presence of 44 compounds, representing 96.42% of the oil. The volatile components in leaves of *E. cardamomum* were made up of largely of oxygenated monoterpenes including terpinen-4-ol, eucalyptol, p-cymene, trans-phytol and cis-sabinene. The anti-scabies study revealed that 10% *E. cardamomum* oil showed 100% mortality within 60 min.

This study demonstrated the potential of *E. cardamomum* leaves essential oil as a scabidical agent, therefore can be used as an alternative for the cure and effective control of *S. scabiei*.

Keywords: *Elettaria cardamomum*, Essential oil, GC, GC-MS, Anti-scabies

INTRODUCTION

Sarcoptes scabiei var *hominis* “itch mite”, family Sarcoptidae, causes a contagious pruritic skin infestation in animals and humans (Scabies). According to WHO, it affects more than 130 million people ubiquitously at any time and prevalence varies from 0.35 to 46%¹. In tropical regions, it is epidemiologi-

*Corresponding Author: Neeru Vasudeva, e-mail: neeruvaseva@gmail.com

Bharat Sharma ORCID Number: 0000-0002-8498-3950

Neeru Vasudeva ORCID Number: 0000-0001-5656-6562

Sunil Sharma ORCID Number: 0000-0002-0768-4367

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cally evidenced that, scabies generally cause pyoderma and eventually serious illness due to invasion by opportunistic bacteria. Such infections can lead to cellulitis, bacteraemia and sepsis, kidney and heart disease that will increase health burden in resource-poor communities ². Due to suboptimal efficacy of few available therapies, the treatment of *Sarcoptes scabiei* infection is getting hindered in human being ¹.

Elettaria cardamomum Maton., cardamom, universally known as “queen of spices”, is dried fruit of a herbaceous perennial shrub belonging to the family Zingiberaceae. Cardamom is endemic to South Asia but is mercantily cultivated in Southern India on the shady slopes of Ghats (mostly in Tamilnadu, Kerala and Karnataka), Nepal, Sri Lanka, Guatemala, Thailand, Mexico, Tanzania and Central America ³. In Indian Ayurvedic system of medicine, it is used for alleviating skin and urinary problems ⁴. Cardamom is commonly used as antiseptic, carminative, expectorant, diuretic, breath freshener, desiccant, stomachic, anti-emetic and as an aphrodisiac ⁵⁻⁷. *E. cardamomum* have been reported to have various biological potential such as antimicrobial ⁸⁻⁹, anti-inflammatory ¹⁰, bronchodilator ¹¹, blood pressure lowering ¹², gastroprotective¹³⁻¹⁷, sedative and anticonvulsant ¹⁸, anticancer, antihypertensive, antioxidant and anti platelet aggregation ¹⁹.

Due to biological and medicinal importance, the present study is carried out to analyse and characterise the bioactive constituents present in essential oil of leaves of *E. cardamomum* by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) technique and further to assess the *in vitro* anti-scabies potential of essential oil against *Sarcoptes scabiei*.

METHODOLOGY

Plant material

The leaves of *E. cardamomum* were collected from Botanical Garden, Malah, Morni Hills, India. The leaf samples were identified and authenticated by Dr. Satish Kumar, Taxonomist at Department of Botany, Government College of Girls, Bhodia Khera, Fatehabad, Haryana. A voucher specimen (GJUP-COG160015 I) was preserved in the Herbarium of Department of Pharmaceutical sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India. The fresh leaves (100g) of were distilled for 6 h to obtain oil by hydro-distillation method using Clevenger’s apparatus. The oil was collected over anhydrous sodium sulphate in a glass vial to obtain pure oil, without any traces of moisture and stored at 40 °C until used ²⁰.

Analysis of the essential oil

The gas chromatographic (GC) analysis of essential oil was carried out using a Shimadzu GC-2010 Gas chromatography equipped with flame ionization detector using Rtx 5 MS capillary column (RESTEK Company: crossbond 5% diphenyl/ 95% dimethyl polysiloxane) having dimensions 30m (Length) x 0.25mm (diameter) x 0.25 μ m df (film thickness). The sample (0.2 μ L) was injected into the column with a split ratio of 1:100. The analytical conditions were: carrier gas (N 1.21 mL/min, 69.0 kPa), injector temperature 260 °C, detector (FID) temperature 280 °C, oven temperature 50 °C (2 min hold) to 280 °C (9 min hold) at 3 °C/min. The retention indices (RIs) were in relation to homologous series of n-alkane (C₉ to C₃₃) on the Rtx 5 MS capillary column under the same chromatographic condition.

GC-MS analysis was performed using GCMS-QP2010 Plus, Shimadzu, system equipped with mass selective detector, having ion source temperature 230 °C, Interface Temp. – 270 °C, Solvent Cut Time – 2.50 min threshold of 1000ev and mass range was 40-650 m/z, Rtx 5 MS capillary column and aforementioned chromatographic conditions, with He used as a carrier gas.

Compounds were identified using two methods, one of the methods was based on comparison of mass spectra with the data in NIST or Wiley library. The other one was by comparison of their retention indices (RIs) with those which reported in literature for Rtx 5 MS capillary column.

***In vitro* anti-scabies activity**

Collection of mites

The *Sarcoptes scabiei* mites were isolated from scabies and ear cerumen of infested legs and ears of rabbits under clinical examination by Dr. Snahil Gupta, Assistant Professor, Department of Veterinarian Parasitology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. The morphologically characterized mites were placed in petri dishes and motile adult mites were collected for testing.

Contact bioassay

The essential oil was diluted with paraffin oil to get concentrations of 1%, 5% and 10%. Ten mites were placed in each petri dish and then in petri dishes 1 mL of diluted solution was added in direct contact with adult mites. Three replicates were performed for each concentration of oil. Permethrin 5% was used as a positive control and liquid paraffin was used as a negative control. The mites were inspected under stereomicroscope (Olympus) 20, 40, 60, 80 min after

inoculation. Mites were considered dead when no movement was seen even after touching it with needle and no gut movement was observed over 2 min¹.

Statistical analysis

% Mortality was calculated and expressed as mean±SEM and significance of difference was determined by two-way ANOVA test using Graph Pad Prism.

RESULTS AND DISCUSSION

Essential oil analysis

Hydrodistillation of leaves of *E. cardamomum* generated yellowish green liquid with a yield of 2%. The GC and GC-MS analysis of essential oil of leaves of *E. cardamomum* allowed the identification and quantification of 44 components which accounts for 96.42% of the total oil, as presented in Figure 1. The identified components of the essential oil of *E. cardamomum* as well as their percentage area and retention indices are reported in Table 1. The oxygenated monoterpenes (65.62%) dominated in essential oil, with terpinen-4-ol (32.99%), eucalyptol (19.82%), p-cymene (10.17%), trans-phytol (5.26%), cis-sabinene (2.53%), linalool (2.41%), as the most abundant constituents, followed by oxygenated sesquiterpenes (9.45%) with caryophyllene oxide (6.59%), β -eudesmol (0.99%), trans-nerodilol (0.59%). Sesquiterpenes hydrocarbons (6.04%) slightly prevailed over monoterpenes hydrocarbons (4.30%). The monoterpenes hydrocarbons were mainly represented by β -pinene (1.54%), β -ocimene (0.85%), α -thujene (0.75%), α -pinene (0.53%). β -Farnesene (3.45%) was the major constituent among sesquiterpene hydrocarbons in essential oil of *E. cardamomum* leaves. The findings obtained were compared with those reported earlier on *E. cardamomum* seeds, fruits or seed coat essential oil analysed by GC-MS.

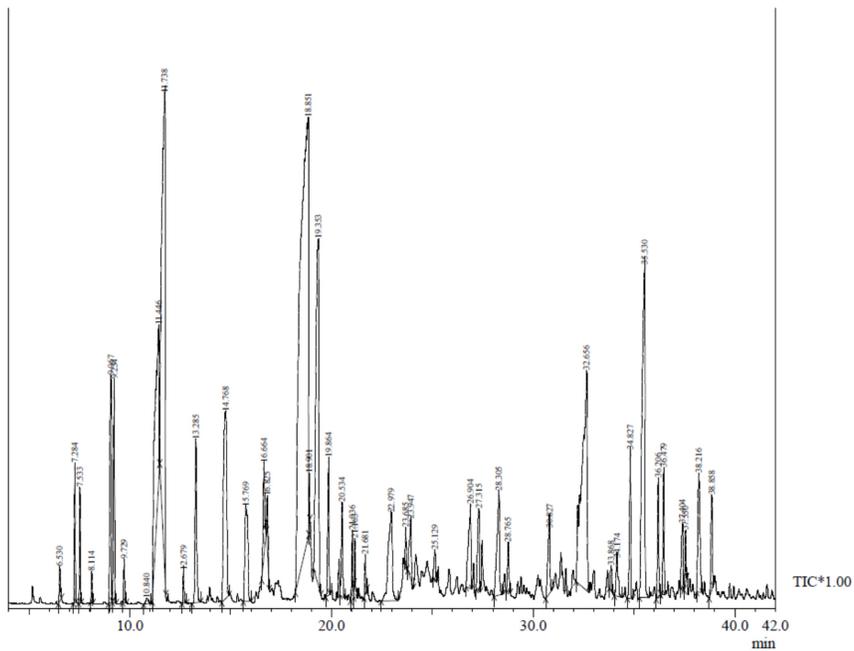


Figure 1. GC-MS chromatogram for essential oil of *Elettaria cardamomum* leaves

Table 1. Volatile components in essential oil of leaves of *Elettaria cardamomum*

Peak No.	Name	Area%	RI ^a (Lit.)	RI ^b (Exp.)	R. Time ^c
1	Heptan-2-ol	0.21	896	903	6.530
2	α -Thujene	0.76	928	928	7.284
3	α -Pinene	0.53	941	930	7.533
4	Camphene	0.12	946	945	8.114
5	cis-Sabinene	2.53	972	970	9.067
6	β -Pinene	1.54	976	975	9.234
7	Myrcene	0.22	993	988	9.729
8	δ -3-Carene	0.11	1011	1015	10.840
9	p-Cymene	10.17	1025	1029	11.446
10	Eucalyptol	19.83	1031	1035	11.738
11	γ -Terpinene	0.19	1063	1057	12.679
12	β -Ocimene	0.85	1050	1070	13.285
13	Linalool	2.41	1102	1104	14.768
14	cis-Menth-2-en-1-ol	0.91	1122	1126	15.769
15	Camphor	0.34	1143	1145	16.664
16	Pinocarvone	1.43	1146	1148	16.825
17	Borneol	1.56	1165	1170	17.703
18	Terpinen-4-ol	32.99	1177	1174	18.851
19	Cryptone	0.76	1192	1189	18.901
20	α -Terpineol	2.37	1193	1203	19.353
21	trans-Piperitol	0.61	1208	1214	19.864
22	Nerol	0.55	1226	1229	20.534
23	Ascaridole	0.22	1237	1240	21.036
24	4-Phenyl-2-butanone	0.21	1251	1243	21.163
25	trans-2-Decenal	0.20	1265	1254	21.681
26	Bornyl acetate	0.59	1286	1283	22.979
27	Carvenone	0.29	1252	1298	23.685
28	Isolatedene	0.38	1377	1304	23.947
29	cis-Methyl-cinnamate	0.78	1380	1372	26.904
30	Methyl cinnamylate	0.37	1394	1381	27.315
31	Caryophyllene	0.86	1417	1415	28.765
32	α -Bergamotene	0.43	1433	1465	30.827
33	β -Farnesene	3.45	1455	1510	32.656
34	β -Bisabolene	0.16	1506	1540	33.868

35	γ -Cadinene	0.34	1513	1543	33.927
36	trans-Nerolidol	0.59	1569	1548	34.174
37	Caryophyllene epoxide	0.20	1580	1565	34.827
38	Caryophyllene oxide	6.06	1582	1583	35.530
39	Carotol	0.45	1587	1600	36.206
40	Humulene epoxide	0.51	1594	1607	36.479
41	Caryophylladienol II	0.27	1631	1632	37.404
42	β -Eudesmol	0.99	1641	1636	37.550
43	Guaiyl acetate	0.36	1712	1654	38.216
44	trans-Phytol	5.26	2099	1988	38.858

^aRI, programmed temperature retention index as determined on Rtx 5 MS capillary column using a homologous series of n-alkanes (C₉ to C₃₃);

^bRI, Identification was based on the compound of retention indices with those of published data (NIST);
^cRetention Time.

Ashokkumar et al.²¹ characterised essential oil content of four varieties of *E. cardamomum* capsules, of which 1,8-cineole (28.94%–34.91%), sabinene (11.17%–13.50%), α -terpineol (12.47%–14.89%) and α -terpinyl acetate (26.68%–29.60%) constituents were detected as major constituents and also reported their use in aroma, food, pharmaceutical and cosmetic domains. Han and Parker²² showed the presence of α -terpinyl acetate (38.00%), linalyl acetate, 1,8-cineole/eucalyptol (36.00%) in *E. cardamomum* essential oil and demonstrated anti-inflammatory and immune modulatory potential due to presence of eucalyptol. Iranian *E. cardamomum* essential oil showed the presence of α -terpineol acetate (11.78%), nerolidol (8.82%), linalool (10.15%), α -pinene (8.11%), 1,8-cineole (4.25%), geranyl acetate (3.47%), γ -terpinene (3.88%), according to Asadollahi-Baboli and Mani-Varnosfaderani²³. Kaskoos et al.²⁴ characterised essential oil of *E. cardamomum* fruits and recorded monoterpenes (87.60%) of total volatiles such as 1,8-cineole (35.60%), α -terpineol (4.90%), α -terpinylacetate (27.10%), thujyl alcohol, linalool (4.10%) and sesquiterpenes as valencene (1.00%), t-caryophyllene (0.80%).

Gradinaru et al.²⁵ analysed *E. cardamomum* fruit essential oil and α -terpinyl acetate (39.59%), 1,8-cineol (31.27%) were found as major constituents. Study also revealed that oxygenated monoterpenes (84.54%) were dominant over monoterpenes (8.27%) and also investigated combination effect of *E. cardamomum* fruit essential oil with amoxicillin or ciprofloxacin against methicillin-resistant clinical isolates. The volatile oil of seed of *E. cardamomum* was reported to have pinene (2.80%), sabinene (1.60%), myrcene (36.00%), 1,8-cineole, graninol and terpinyl acetate as the major constituents²⁶⁻²⁷. These

differences in chemical composition could be due to different distillation technique used, climate or growing conditions of plant.

***In vitro* anti-scabies activity**

The *E. cardamomum* essential oil was evaluated for their *in vitro* anti-scabies potential against *S. scabiei* mites. % Mean mortality for the mites treated with three concentrations of oil is presented in Table 2. *E. cardamomum* essential oil demonstrated scabicial potential as its 10% concentration caused 100% mortality within 60 min whereas 5% diluted solution took 80 min to kill all the mites. Based on % mean mortalities study, it was found that Permethrin (reference) killed all the mites within 60 min but in negative control group, mortality was only 1.58% and most of mites remained alive after 80 min of treatment. The scabicial effects produced by *E. cardamomum* essential oil were significant ($p < 0.0001$) as compared with the respective control groups as demonstrated in Figure 2.

Table 2. *In vitro* anti-scabies activity of *E. cardamomum* essential oil against *S. scabiei*

Test agent	Conc.	% Mortality (mean± SEM)			
		20 min	40 min	60 min	80 min
E. cardamomum essential oil	1%	30.00± 0.57***	42.66± 0.56***	57.33± 1.00***	80.34± 0.59***
	5%	39.44± 1.53***	48.14± 1.15***	86.14± 0.57***	100.00± 0.00***
	10%	47.81± 1.15***	85.33± 0.99***	100.00± 0.00***	100.00± 0.00***
Positive control		68.00±0.20	89.00± 0.40	100.00± 0.00	100.00± 0.00
Negative control		00.00 ± 0.00	00.00 ± 0.00	01.58±0.08	01.58± 0.08

Data are expressed as mean±SEM; n=3

***, $p < 0.0001$ indicates highly significant results

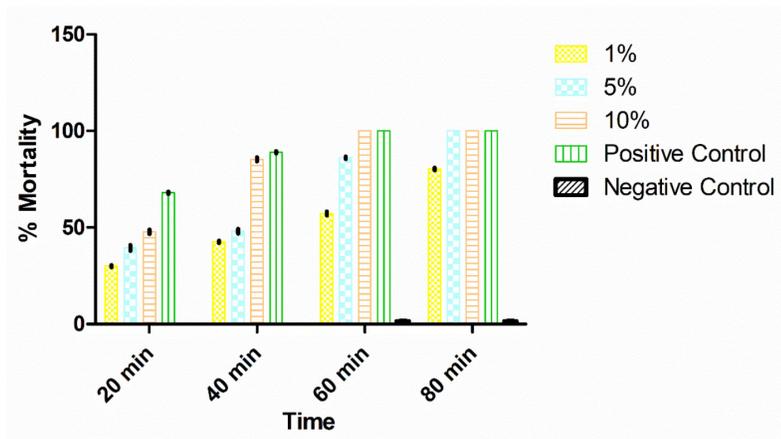


Figure 2. Anti-scabies activity of *E. cardamomum* leaves essential oil against adult *S. scabiei*. Data are analysed by two-way ANOVA and expressed as mean±SEM, n=3.

Adupa et al.²⁸ reported the toxicant, fumigant and repellent potential of eucalyptol against the maize weevils. Abbassy et al.²⁹ study inferred the pronounced insecticidal activity of terpinen-4-ol and γ -terpinene against tested insects *Spodoptera littoralis* and *Aphis fabae* L.

It was also found that eucalyptol enhances the superoxide dismutase and glutathione-s-transferase enzymatic activity, which play a role in protection mechanism of *S. scabiei* mites³⁰. The natural components i.e. terpinen-4-ol, γ -terpinene and eucalyptol have been reported to exhibit insecticidal activity may be responsible for anti-scabies potential of *E. cardamomum* oil. Fang et al.¹ studied ten essential oils and reported that 1% clove and palmarosa oil killed all the motile mites within 20 and 50 min, respectively. Using contact bioassay, clove oil (1.56%) killed all the mites after exposure of 15 min while nutmeg oil showed moderate toxicity against scabies mites³¹. Aboelhadid et al.³² stated that 20% lemon oil caused 100% mortality of mites after 24 h and also investigated the elevation in hydrogen peroxide level that leads to considerable cellular damage. *Elsholtzia densa* also found to possess acaricidal potential against *S. scabiei* and at 16 mg/mL concentration killed all the mites within 16 h period³³.

A number of previous studies have been performed on fruits and seed of *E. cardamomum*. The present study is an attempt made to identify the constituents of volatile oil of *E. cardamomum* leaves and find its utilization for anti-scabies activity. The chemical composition of the leaves is more or less similar to fruits and seeds though the concentrations of the constituents vary. Furthermore, in

view of its action against *S. scabiei* mites, it may prove to be beneficial to the patients with scabies disease and could be explore as alternative to current medicines.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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