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Short Comminication

IN VITRO ANTIBACTERIAL ACTIVITY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF ETHANOLIC EXTRACT OF LEAVES OF *ELETTARIA CARDAMOMUM* L. MATON

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ABSTRACT

Objective: The objective of the present study is to determine the possible phytoconstituents identified by gas chromatography and mass spectrometry (GC-MS) analysis of an ethanolic leaves extract of *Elettaria cardamomum* L. Maton.

Methods: The extraction of *E. cardamomum* was done by cold solvent extraction system at room temperature. GC-MS analysis of lyophilized ethanolic leaves extract of plant samples was carried out by GC-MS-GC Clarus 500 Perkin Elmer**.**

Results: In *E. cardamomum*, 21 phytochemicals were identified among which retinal, 9-cis-showed the highest area (44.86%) and benzeneethanamine, α-methyl- showed the lowest area (0.12%). The major compounds identified were retinal, 9-cis-, 1-heptatriacotanol, phytol, n-hexadecanoic acid, naphthalene,decahydro-1,1,4atrimethyl-6-methylene-5-(3-methyl-2-4-pentadienyl)-[4aS-(4aα,5α,8aα)]-, β-pinene, 2H-pytan-3-ol,6-ethenyltetrahydro-2,2,6-trimethyl-and cyclopropane, trimethanol, (2-methyl-1-propanylidene).

Conclusion: GC-MS analysis revealed the presence of hydrocarbon alkane, ester, terpenes, phenolic compounds, steroids, and fatty acid**s** in *E. cardamomum*. These active phytoconstituents contribute to the medicinal efficacy of the plant, and the plant can be used for the sourcing of these compounds.

Keywords: *Elettaria cardamomum*, Gas chromatography-mass spectrometry, Phytoconstituents, Antibacterial.

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INTRODUCTION

Recently, medicinal plants are increasingly gaining importance in pharmaceutical and scientific societies, as they are the richest biological resources of traditional medicines, food supplements, and nutraceuticals [1-3]. Plants are the traditional sources for many chemicals and used as pharmaceutical biochemicals, fragrances, food colors, and flavors in different countries especially in India [4-7]. Most herbal medicines were prepared from crude plant extracts, which comprise a complex mixture of different secondary metabolites [8-10]. Gas chromatography and mass spectroscopy (GC-MS) is the methods used for testing the amount of some active principles in herbs used in cosmetics, drugs, pharmaceutical, and food industry. The chemical features of these constituents considerably differ among different species. The GC-MS analysis of the obtained plant extracts revealed the presence of various active principles in herbs which are used in cosmetics, drugs, pharmaceutical, and food industry [11,12].

Elettaria cardamomum L. Maton (green cardamom) is a perennial shrub of the family Zingiberaceae. Its seeds are used as a spice, so it is called the queen of spices [13]. It stands third in the list of expensive spices throughout the world preceded by saffron and vanilla. The medicinal importance of cardamom is also mentioned in Ayurveda and Unani systems [14]. Apart from its usage in cooking, cardamom also possesses a wide range of therapeutic features such as antifungal, antibacterial, antiviral, and diuretic and carminative properties [15]. It is also applied to fight against cardiac diseases, renal problems, anorexia, asthma, and bronchitis. Furthermore, it demonstrates as an antioxidant, antiplatelet aggregation, anti-hypersensitive, and anticancerous attributes [16]. Hence, the present study was carried out to assess the phytochemical nature of medicinal plants along with their antimicrobial activities from the leaf ethanol extract of *E. cardamomum* by GC-MS analysis.

METHODS

Collection and identification of plant material

E. cardamomum L. Maton used for the investigation was obtained in Idukki District, near Munnar, Kerala, South India. The taxonomic identification of the plant was confirmed at the Rapinat Herbarium and Centre for Molecular Systematic, Tiruchirappalli, with the voucher number: SM001. A voucher specimen of the plant was deposited to that the Rapinat Herbarium for future reference. Fresh leaves of *E. cardamomum* were washed under running tap water and air dried at room temperature.

Sample extraction

The dried leaves are pulverized well in a Udy Cyclone Mill, and a 100 g sample of dried plant powder was extracted in 500 ml of ethanol in a round-bottomed flask for 72 h. The ethanolic extract was filtered using Whatman 40 filter paper, and the residue was rejected. The obtained extract was concentrated and evaporated to dryness, stored at 4°C in an airtight container for further use.

GC-MS analysis of *E. cardamomum*

The GC-MS analysis of *E. cardamomum* was carried out on a GC clarus 500 perkin elmer, Carrier gas: 1 ml/min, Split: 10:1, Detector: Mass detector: Turbomass gold-perkin elmer, Software: Turbomass 5.2, sample injected: 2 µl, column: Elite-5MS (5% diphenyl/95% Dimethyl polysiloxane), L × I.D. 30 m × 0.25 mm, df 0.25 µm, oven temperature Program: 110°C with 2 min hold, up to 200° C at the rate of 10°C per min without hold, up to 280°C at the rate of 5 °C/ min with 9 min hold, injector temperature 250 °C, total GC running time 36 min, inlet line temperature 200 °C, source temperature 200 °C Electron energy:70eV, Mass scan (m/z): 45–450, solvent Delay: 0-2 min, and total MS running time: 45 min. The spectrum obtained in GC-MS compounds was

Table 1: Phytocomponents identified in the alcoholic extract of leaves - *E. cardamomum*

No.	RT	Name of the component	MF	MW	Peak area %
1	4.65	β-Pinene	$C_{10}H_{16}$	136	3.09
2	5.32	Benzeneethanamine, α methyl-	$C_9H_{13}N$	135	0.21
3	7.14	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethylene-(α-compholenal)	$C_{10}H_{16}O$	152	0.16
4	7.67	Bicyclo [3.1.1]heptan-3-ol, 6,6-dimethyl-2,23-trimethylene-[1s-(1α,3α,5α)]-	$C_{10}H_{16}O$	152	1.08
5	8.00	2 (10)-pinen-3-one,(±)-[pinocarvone]	$C_{10}H_{14}O$	150	0.071
6	8.71	Bicyclo [3.1.1]hept-2-ene-2-corboxaldehyde, 6,6-dimethyl-(Myrtenal)	$C_{10}H_{14}O$	150	0.69
7	8.87	Bicyclo [3.1.1]hept-2-ene-2-methanal, 6,6-dimethyl-(Myrtenal)	$C_{10}H_{16}O$	152	1.13
8	11.02	Cyclopropane, trimethanol,(2-methyl-1-propanylidene)	$C_{10}H_{16}$	136	1.68
9	11.16	1-Cyclohexene-1-methanol, 4-(1-methylethenyl)-{p-mentha-1,8-dien-7-ol}	$C_{10}H_{16}O$	152	0.58
10	12.94	Tran-3 (10)-caren-2-ol	$C_{10}H_{16}O$	152	0.59
11	13.23	Bicyclo 2.25.1]heptan-2-ol, 1,3,3-trimethyl-	$C_{10}H_{18}O$	154	0.98
12	15.14	2H-pytan-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	$C_{10}H_{18}O_2$	170	1.41
13	18.31	Asarone	$C_{10}H_{16}O_3$	208	0.39
14	19.68	Methyl jasmonate	$C_{13}H_{20}O_3$	224	0.35
15	2031	Longifolenaldehyde	$C_{15}H_{24}O$	220	0.38
16	22.99	Aroma decanoic acid	$C_{15}H_{24}O$	220	1.35
17	25.74	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.87
18	26.66	Naphthalene, decahydro-1,1,4atrimethyl-6-methylene-5-(3-methyl-2-4-pentadienyl)-[4aS-(4aα,5α,8aα)]-	$C_{20}H_{32}$	272	2.98
19	28.62	Phytol	$C_{20}H_{40}O$	296	7.54
20	29.34	Retinal, 9-cis-	$C_{20}H_{28}O$	284	44.86
21	33.52	1-Heptatriacotanol	$C_{37}H_{76}O$	536	26.07

E. cardamomum: *Elettaria cardamomum,* MF: Molecular formula, MW: Molecular weight, RT: Retention time

Table 2: Antibacterial activity of *E. cardamomum* leaves

S. No	Conc. of extract (µg/ml)	Zone of inhibition (mm) (the mean±SD)			
		A. niger	***A. flavus***	***E. coli***	***K. pneumonia***
1	25	7±0.15	8±0.75	11±0.55	10±0.72
2	50	13±0.40	15±0.35	18±0.58	14±0.60
3	100	18±0.25	19±0.25	23±0.24	16±0.22
4	Penicillin (10)	20±0.25	24±0.30	26±0.65	18±0.25

Ethanolic extract of *E. cardamomum* observed the inhibition zone of diameter and shows antibacterial activity against microorganisms. *E. cardamomum, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, E. cardamomum: Elettaria cardamomum*

compared with the spectrum of known components using the NIST library.

RESULTS AND DISCUSSION

The GC-MS analysis of the ethanol extract of *E. cardamomum* clearly shows 21 peaks and indicates the presence of 21 phytochemical compounds. The identification of the phytochemical compounds was based on the retention time, molecular formula, and molecular weight. The percentage of peak area for each compound was calculated assuming total eluted compounds as percent. The details of eluted compounds were given in Table 1.

In this analysis, Retinal, 9-cis- (44.86%) showed the highest area %, followed by 1-Heptatriacotanol (26.07%), Phytol (7.54%), n-Hexadecanoic acid (3.87%) as major phytoconstituents.

Antibacterial activity

The above-obtained plant extract was evaluated for their antibacterial activity in comparison with standard antibiotic penicillin (10 µg/ml) *in-vitro* by disc diffusion method [17] using *Aspergillus niger*, *Aspergillus flavus*, *Escherichia coli,* and *Klebsiella pneumonia* as test organisms. Each extract was individually loaded on the 3 mm sterile disc at the concentration of 25 µg/ml, 50 µg/ml and 100 µg/ml and subjected to antibacterial activity. The results were recorded by measuring the zone of growth inhibition surrounding the disc. The experiments were done in triplicate. The ethanolic extract of *E. cardamomum* showed a maximum zone of inhibition against *A. niger* (18 mm at 100 µg/ml) and *K. pneumonia* (16 mm at 100 µg/ml) when compared to that of standard (Penicillin). The antibacterial activities are mentioned in Table 2. It shows that the antibacterial activity is due to the presence of major phytochemicals like retinal, 9-cis present in leaves of the plant.

CONCLUSION

The ethanolic leaf extract of *E. cardamomum* has shown the presence of diverse classes of compounds possessing pharmacological and industrial importance. The extract exhibits the importance of antibacterial against bacterial strains. GC-MS analysis has been found useful in the identification of several constituents such as retinal, 1-Heptatriacotanol, Phytol, and n- hexadecanoic acid present in the ethanolic leaves extract of *E. cardamomum*. Further studies are needed to explore the potential of phenolic compounds from the leaves of *E. cardamomum* for application in drug delivery, nutritional, or pharmaceutical fields.

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AUTHOR'S CONTRIBUTIONS

PP has carried out the research. NE and SA have provided study conception, the design of work, drafting of the manuscript, and critical revision.

CONFLICTS OF INTEREST

We have no conflicts of interest to declare.

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