



Science

## PHYTOCHEMICAL SCREENING, GC-MS AND FT-IR ANALYSIS OF METHANOLIC EXTRACT LEAVES OF *ELETTARIA CARDAMOMUM*

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### Abstract

In this modern era, medicinal plants are at great attention to the researchers as most of the drug industries depend on medicinal plants for the production of therapeutic compounds. In many countries especially in India, plants are the conventional source of pharmaceutical biochemical, food colours, flavours and fragrances. Hence, the main aim of the study was the identification of bioactive compounds from the leaves of *Elettaria cardamomum* by Gas Chromatography and Mass spectroscopy (GC-MS). The GC-MS study revealed the presence of various compounds like Vitamin E, Squalene, Eucalyptol, Stigmast-5-en-3-ol, 4H-1-Benjopyran-4-one, 2,3-dihydro-5, 7-dihydroxy-2-pheny, Octadecanoic acid, Phytol, Hexadecanoic acid in the methanolic extract of *Elettaria cardamomum*. Henceforth, the *Elettaria cardamomum* may have chemo preventive, antidiabetic, anti- microbial and anticancer activity due to the presence of secondary metabolites in the methanolic extract. The results of FTIR analysis confirmed the presence of alcohol, phenols, alkanes, alkyl halides and alkynes. In the present study, leaf sample of this plant was analyzed for the first time. This work will help to identify the active components, which may be used for therapeutic purposes. This study offers a platform of using *Elettaria cardamomum* leaves as herbal alternative for many diseases.

**Keywords:** Methanol Extract; Phytochemical; *Elettaria Cardamomum*; GC MS And FTIR.

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### 1. Introduction

Traditional medicine is the entirety of knowledge, skills and study based on the beliefs, theories and experiences indigenous to different cultures that are used to prevent and diagnose physical and mental illness. For millions of years, herbal remedies have healed the sick and passed on to next generation (WHO). The World Health Organization has been encouraging countries to

identify and exploit traditional medicine since 1980. The Indian traditional system of medicine namely Ayurveda and Siddha emphasizes the use of plant based medicines and treatments (Kirtikar et al., 1918). Everyday new diseases are being identified due to our disruptive life style, but the fact is that our nature contains cure for all diseases and potentially worthy treasures in medicinal plants are still unknown. It is estimated that almost 25% of prescribed medicines contain plant extracts or active compounds produced from plants. For examples – aspirin (analgesic), vinblastine and paclitaxel (anticancer agents) exclusively derived from plant sources (Pankaj et al., 2011). By keeping in mind the scope of medicinal plants we should spend some more time and resources in developing new medicines.

*Elettaria cardamomum* (Zingiberaceae) or cardamom is commonly known as queen of spices for the versatile use in cooking practice. Cardamom is a perennial shrub with fleshy, thick and lateral roots and the plant grows to a height of eight feet (Kapoor, 2000). It is native to South Asia but it is commercially cultivated in Sri Lanka, Tanzania, Morocco, Guatemala and Southern India (El-Malti et al., 2007).

Cardamom has antifungal, antibacterial (Agaoglu et al., 2005; Bansod and Rai, 2008; Singh et al., 2009), antioxidant (Singh et al., 2009; Lin et al., 2009; Sultana et al., 2010), gastro protective effect (Jamal et al., 2006) and anticancer properties (Sengupta et al., 2005).

Cardamom oil is used in perfumery, food and in medicine which is used as a powerful antiseptic, stimulant, expectorant, aromatic, carminative, stomachic, diuretic and anti-spasmodic (Baytop, 1984; Korikontimath et al., 1999). In Saudi Arabia and the Near East, Cardamom is used largely in the preparation of “Gahwa” a strong cardamom coffee concoction (Baytop, 1984).

Within an era, there are a number of advances in analytic techniques including Gas Chromatography-Mass Spectroscopy (GC- MS) and Fourier Transform Infrared spectroscopy (FTIR) that are used for identification and determination of phytochemical compounds (Roberts and Xia, 1995). GC- MS is a very compatible and the most commonly used technique for the identification and quantification purpose. FTIR is the most powerful tool for identifying the functional groups present in compounds (Ronald, 1997). The presented study is carried out on the bioactive compounds present in the *Elettaria cardamomum* leaves by the use of GC-MS and FT-IR techniques.

## **2. Materials and Methods**

### **2.1.Plant Material**

The medicinal plant used for the study were collected from Ch. Devi Lal Herbal Nature Park - Chuharpur, Yamunanagar (Haryana) and maintained in the University nursery.

### **2.2.Preparation of the Extract**

The fresh and healthy cardamom leaves were washed 2-3 times with running water and then air dried under shade. Afterwards, the dried leaves were grinded with mechanical grinder and the powder was kept in small-labeled plastic bags. 100 g of leaves of cardamom were subjected to

successive extraction with methanol solvent using Soxhlet apparatus. The solvent were evaporated under reduced pressure and stored in desiccators at 4 °C. The methanol extract was used for GC-MS and FTIR analysis.

### 2.3. Phytochemical Screening

Phytochemical analysis was carried out for identification of terpenoids, flavonoid, tannins, phenols, phytosterols, alkaloids and saponins according to standard methods (Kumar et al., 2007).

#### 2.3.1. Test for Saponins

- a) **Foam test-** 1 mL solution of extract was diluted with distilled water to 20 mL and shaken for 15 min. Development of stable foam confirms the presence of saponins.
- b) 1 mL extract was treated with 1% lead acetate solution and formation of white precipitates suggests the presence of saponins.

#### 2.3.2. Test for Tannins and Phenols

The test extract was taken in water, warmed and filtered. 5 mL of filtrate was allowed to react with 1mL of 5% ferric chloride solution. Dark green or deep blue color shows the presence of tannins and phenols.

#### 2.3.3. Test for Amino acids and Proteins

Small quantity of the extract was dissolved in minimum quantity of water and filtered. Filtrate was subjected to Millons test and Biuret test.

#### 2.3.4. Test for Sugars

Small quantity of extract was dissolved in 4 mL of distilled water and filtered and the filtrate was subjected to Molisch's test and Iodine Test.

#### 2.3.5. Test for Glycosides and Sterols

**Salkowaski test-** 10 mg of extract was dissolved in 2 mL of chloroform and 2mL of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for few minutes and the development of red color in chloroform layer indicated the presence of glycosides and sterols.

#### 2.3.6. Test for Alkaloids

**Mayer's test-** 2-3 mL filtrate when mixed with a few drops of Mayer's reagent results in formation of precipitate (Shankar et al., 2014).

### 2.3.7. Test for flavonoids

**Shinoda test-** The extracts were dissolved in alcohol. One piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color confirms the presence of flavonoids (Shankar et al., 2014).

### 2.3.8. Test for Terpenoids

About 0.5 g plant extract in separate test tubes was taken with 2 mL of chloroform and concentrated sulphuric acid was added carefully to form a layer. Observation for presence of reddish brown color at interface was recorded to show positive results for the presence of terpenoids (Venkatesan et al., 2009).

### 2.4.GC-MS Analysis

Methanol extract of *Elettaria cardamomum* leaves was analyzed with the help of GC-MS analyzer (GCMS-QP2010 Plus). Helium was used as carrier gas at a constant flow of 1.2 mL/min, an injection volume of 2.0  $\mu$ L, injector temperature 260.0  $^{\circ}$ C and ion- source temperature 230.0  $^{\circ}$ C was employed. The oven temperature was operated according to the following mode: 100  $^{\circ}$ C held for 1 min, rising at the rate of 10  $^{\circ}$ C per min up to 250  $^{\circ}$ C with 6 min hold, rising at the rate of 15  $^{\circ}$ C per min upto 300  $^{\circ}$ C with 20 min hold up. The total GC-MS running time was about 46 min.

### 2.5.Identification of Components

Identification was based on molecular mass, molecular structure and calculated fragments. Interpretation of mass spectrum GC-MS was done using the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the WILEY library. Compound name, molecular weight, retention time, percentage and structure of various components of the test materials were ascertained.

### 2.6.FTIR Spectroscopic Analysis

FTIR analysis was performed using Perkin Elmer spectrophotometer system, which was used to detect the characteristic peaks and their functional groups using ATR (Attenuated Total Reflectance) accessory. The IR scan was performed in the wave number region of 4000-550  $\text{cm}^{-1}$  (mid- infrared range).

## 3. Results and Discussion

Phytochemical compounds such as flavonoids, tannins, aromatic compounds or secondary metabolites act as defense mechanism against many microorganisms. The therapeutic properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as flavonoids, alkaloids, tannins, saponins, phytosterols and phenolic compounds (Britto and Sebastian, 2012). The presence of flavonoids, alkaloids, saponins, alkaloids, phenolic

compounds, phytosterols, and terpenoids are used in antiplasmodic, analgesic and bactericidal activities (Sary, 1998). Results for preliminary phytochemical screening of the *Elettaria cardamomum* methanol extract is given in the Table 1.

Table 1: Preliminary phytochemical screening of *Elettaria cardamomum* methanol extract

<u>Plant extract</u>	<u>Carbohydrates</u>	<u>Phenols</u>	<u>Tannins</u>	<u>Flavonoids</u>	<u>Saponins</u>	<u>Glycosides</u>	<u>Steroids</u>	<u>Terpenoids</u>	<u>Alkaloids</u>
Methanol extract	+	+	-	+	+	+	+	-	+

(+) = Positive (present); (-) = Negative (absent)

The components present in the methanol extract of *Elettaria cardamomum* were identified by GC-MS analysis (Figure 1). The active compounds with their retention time (RT), molecular formula and molecular weight (MW) in the methanol extract of leaves of *Elettaria cardamomum* re-presented in Table 2. Twenty-two compounds were identified in methanol extract of leaves of *Elettaria cardamomum*.

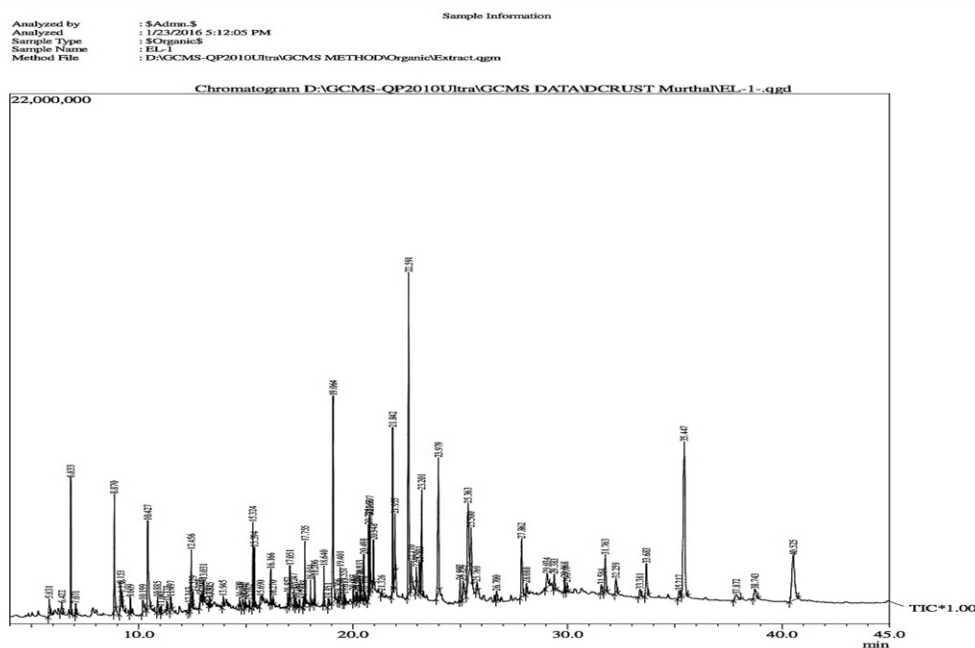


Figure 1: GC-MS chromatogram of the methanolic extract of *Elettaria cardamomum* leaves

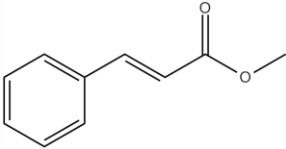
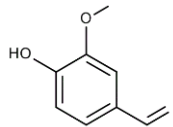
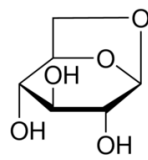
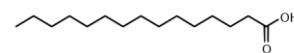
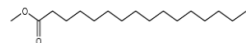
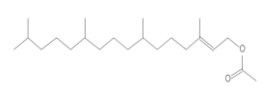
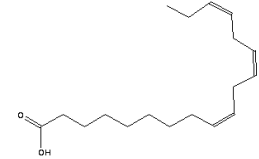
Table 2: GC-MS analysis of phytochemicals identified in the methanolic leaves extract of  
*Elettaria cardamomum*

<u>Peak</u>	<u>R.Time</u>	<u>Peak Area</u>	<u>Area%</u>	<u>Name</u>
1	5.831	2245644	0.60	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methyl
2	6.422	1293890	0.35	3-Hexenoic acid, (E)-
3	6.833	11227585	3.01	Eucalyptol
4	7.071	1015506	0.27	Benzene acetaldehyde
5	8.870	9882210	2.65	Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1s)-
6	9.153	3215177	0.86	Benzenepropanal
7	9.238	681789	0.18	Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl-
8	9.609	1254867	0.34	3-Cyclohexene-1-methanol, Alpha.4-
9	10.199	2045877	0.55	2,3-Dihydro-benzofuran
10	10.427	12864652	3.45	4-Phenyl-2-butanone
11	10.885	1894235	0.51	Cinnamaldehyde, (E)-
12	11.041	366311	0.10	Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl-
13	11.323	628908	0.17	2-Propenoic acid, 3-phenyl-, methyl ester
14	11.497	1547664	0.42	2-Methoxy-4-vinylphenol
15	12.343	607787	0.16	4-Epi-cubedol
16	12.456	5758227	1.54	2-Propenoic acid, 3-phenyl-, methyl ester, (z)
17	12.529	947072	0.25	2-Hepten-3-ol, 4,5-dimethyl-
18	12.873	1270547	0.34	2-(1,3-Dithian-2-yl)-1,5,5-trimethyl-3-methyl
19	12.963	233296	0.06	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-
20	13.031	1738968	0.47	1-Hydroxymethyl-2-methyl-1-cyclohexene
21	13.280	172140	0.05	2-Furanmethanethiol, 5-methyl-
22	13.325	283642	0.08	Cis-.beta. -farnesene
23	13.945	846456	0.23	Cyclopropane carboxylic acid, 2,2-dimethyl-
24	14.709	514301	0.14	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-
25	14.861	1618070	0.43	Cis-Z-.alpha. -bisabolene epoxide
26	14.972	621474	0.17	9-Eicosene, (E)-
27	15.162	455187	0.12	(-)-5-Oxatricyclo [8.2.0.0(4,6)] dodecane
28	15.324	5863438	1.57	3A(1H)-azulenol, 2,3,4,5,8,8a-hexahydro-6, 8A-D
29	15.394	3973843	1.07	Benzene, 1,2,4-trimethoxy-5-(1-propenyl)-, (z)-
30	15.690	1617751	0.43	Beta. -D-glucopyranose, 1,6-Anhydro-
31	16.166	2322736	0.62	2-Naphthalenemethanol
32	16.270	608336	0.16	Spiro [4.5] dec-8-en-7-one
33	16.957	1046848	0.28	Tetradecanoic acid
34	17.051	2473341	0.66	Tetracyclo [5.3,1,0e2, 6-0e8, 11] undecan-4-ol, 6-
35	17.247	1207147	0.32	Cyclohexane, 1,2,3,4,5,6-hexachloro-, (1.alpha)
36	17.327	982860	0.26	2(4h)-Benzofuranone, 5,6,7,7a-tetrahydro-6-h
37	17.491	566590	0.15	4-Hydroxy-3, 5,5-trimethyl-4-[(1e)-3-oxo-1-bute
38	17.688	746785	0.20	(Albicanol) decahydro-2-methylene-5, 5,8A-T
39	17.755	4119627	1.10	2,6,10-Trimethyl, 14-ethylene-14-pentadecne
40	18.014	1672465	0.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
41	18.206	1897125	0.51	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R

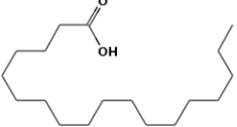
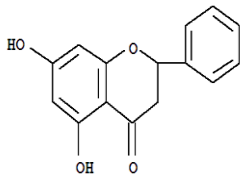
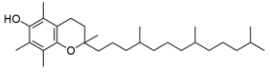
42	18.640	2645523	0.71	Hexadecanoic acid, methyl ester
43	18.851	825470	0.22	Cis-9-Hexadecenoic acid
44	19.064	21965987	5.89	Pentadecanoic acid
45	19.306	508895	0.14	Hexadecanoic acid, ethyl ester
46	19.401	2348651	0.63	Benzene, 1,3-dimethoxy-5-[(1E)-2-phenylethenyl]-
47	19.551	1307399	0.35	1-Hydroxy-1, 7-dimethyl-4-isopropyl-2, 7-cyclodecadiene
48	19.613	709077	0.19	3-Hydroxy-7-methoxy-3-phenyl-4-chromanone
49	20.003	1001904	0.27	Heptadecanoic acid
50	20.134	675976	0.18	Phenol, 2-[2-(4-methoxyphenyl) ethenyl]-, (e)-
51	20.201	673485	0.18	N-Nonadecanol-1
52	20.313	1396296	0.37	9,12-Octadecadienoic acid (z, z)-, methyl ester
53	20.361	1672529	0.45	9-Octadecenoic acid (Z)-, methyl ester
54	20.498	3381083	0.91	Phytol
55	20.578	568527	0.15	Methyl stearate
56	20.725	6980248	1.87	9,12-Octadecadienoic acid (z, z)-
57	20.768	6548079	1.76	9-Octadecenoic acid
58	20.797	5362153	1.44	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-
59	20.948	3431484	0.92	Octadecanoic acid
60	21.326	247982	0.07	2,6-Dodecadien-1-ol, 3,7,11-trimethyl-, (E, E)-
61	21.842	14641181	3.93	Benzene, 1,3-dimethoxy-5-[(1E)-2-phenylethenyl]-
62	21.955	5366606	1.44	2-Methyl-3, 5-dinitrophenyl, Beta. -Phenyl propionate
63	22.591	36220037	9.71	7-Phenyl-4trans-heptenone-3
64	22.710	2963183	0.79	1,1'-Biphenyl, 2,2'-dimethyl-6, 6'-dinitro-
65	22.950	1796418	0.48	(1-Benzyl-2-O-tolyl-ethyl)-isonitrile
66	23.103	3063724	0.82	Benzene, (1-hexylheptyl)-
67	23.201	10538712	2.83	3-Heptanone, 5-hydroxy-1, 7-diphenyl-
68	23.979	18758286	5.03	3-Heptanone, 5-hydroxy-1, 7-diphenyl-
69	24.997	2419107	0.65	Cis-10-Nonadecenoic acid, methyl ester
70	25.129	1727900	0.46	(1-Benzyl-cyclopropyl)-methanol
71	25.363	15595779	4.18	4H-1-Benzopyran-4-one
72	25.500	5818625	1.56	4,6-Heptadien-3-one, 1,7-diphenyl-
73	25.769	1215487	0.33	1-Penten-3-one, 4-methyl-1-phenyl-
74	26.700	1305692	0.35	17-Ethynyl-17-hydroxyestr-5 (10)-en-3-one
75	27.862	9263463	2.48	1-Penten-3-one, 4-methyl-1-phenyl-
76	28.088	1341273	0.36	(Albicanol) decahydro-2-methylene-5, 5,8a-t
77	29.034	2961307	0.79	Coumaran-5, 6-diol-3-one, 2-[4-methoxybenzylidene]-
78	29.383	1692902	0.45	4-Pentenoic acid, 2,2-diethyl-3-oxo-5-phenyl-, ethyl ester
79	29.868	1573072	0.42	Squalene
80	29.977	1103730	0.30	(Albicanol) decahydro-2-methylene-5, 5,8A-T
81	31.584	1292488	0.35	Tetramethyl ether of catechin
82	31.763	5621658	1.51	2H-1-Benzopyran-6-ol
83	32.259	2737683	0.73	Tetramethyl ether of catechin
84	33.381	1420932	0.38	Beta. -Tocopherol
85	33.683	5784350	1.55	Gamma. -Tocopherol

86	35.217	1274033	0.34	Cholesterol
87	35.447	37247907	9.99	Vitamin E
88	37.872	1889897	0.51	Ergost-5-en-3-ol, (3.beta.24R)-
89	38.743	2735016	0.73	Stigmasterol
90	40.525	16941780	4.54	Stigmast-5-en-3-ol, (3.beta.)-

Table 3: Activity of phyto-components identified in *Elettaria cardamomum* leaf extract by GC-MS

Retention Time	Name of the compound	Structure	Nature	Activity
11.323	2-Propenoic acid, 3-phenyl-, methyl ester		Organic compound	Antiarthritic, Antioxidant, Cancer Preventive, Additive
11.497	2-methoxy-4-Vinylphenol		Phenolic compound	Antibacterial, Antioxidant, Antiseptic, Antiviral, Fungicide, Cancer preventive
15.690	Beta-D-Glucopyranose, 1,6- Anhydro-		Sugar moiety	Preservative
19.064	Pentadecanoic acid		Fatty acid	Rare Fatty acid in nature, flavoring agent
19.306	Hexadecanoic acid, Ethyl ester		Palmitic acid ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Flavor, Lubricant, Antiandrogenic, Hemolytic 5-Alpha reductase Inhibitor
20.498	Phytol		Diterpene	Antimicrobial, Anticancer, Anti-inflammatory, Diuretic
20.797	9, 12,15-Octadecatrienoic acid, (Z, Z, Z)-		Linolenic acid	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge,



				Antihistaminic, Antieczemic, Antiacne, 5-Alpha Reductase Inhibitor, Antiandrogenic, Antiart hritic, Anticoronary, Insectifuge
20.948	Octadecanoic acid		Fatty Acids	Hypercholesterolemic, Antiarthritic, Anti- inflammatory, Hepatoprotective, Nematicide, Antimicrobial.
25.363	4H-1- Benjopyran-4- one, 2,3- dihydro-5, 7- dihydroxy-2- phenyl		Flavonoid fraction	Antimicrobial, Antiinflammatory
35.447	Vitamin E		Vitamin	Antiageing, Analgesic, Antidiabetic, Antiinflammatory, Antioxidant, Antidermat itic, Antileukemic, Antitumor, Anticancer, Hepatoprote ctive, Hypocholesterolemic, Antiulcerogenic, Vasodi lator, Antispasmodic, Antibronchitic, Anticoronary

The GC-MS chromatogram of the major compounds detected was shown in (Fig. 1, Table 2 and Table 3). The results revealed that Vitamin E, Pentadecanoic acid, Eucalyptol, Octadecanoic acid, Squalene, stigmast-5-en-3-ol, (3.beta.), 4-phenyl-2-butanone, 3-Heptanone, 5-hydroxy-1, 7-diphenyl-, 1-Penten-3-one, 4-methyl-1-phenyl-, 4H-1-Benjopyran-4-one, 2, 3-dihydro- 5, 7-dihydroxy-2-phenyl are present as one of the major components in the methanol extract and has antioxidant, anticancer, antitumor, antibronchitic, anti-inflammatory activities. The structure and kinetics studies of n-Hexedeconic acid showed that it is an inhibitor of phospholipase, and hence it is an anti-inflammatory compound. Also, GC-MS studies have revealed antiarthritic, anticancerous, hypocholesterolemic, nematicide, pesticide, lubricant, and antiandrogenic activities that were also reported by (Kumar et al., 2010; Aparna et al., 2012).

The results of FT-IR analysis confirmed the presence of alcohols, phenols, alkanes, aromatic ring, alkyl halides, ether linkage and alkynes presented in Fig. 2 and Table 4 and in accordance with the results (Mohani et al., 2014; Das et al., 2011).

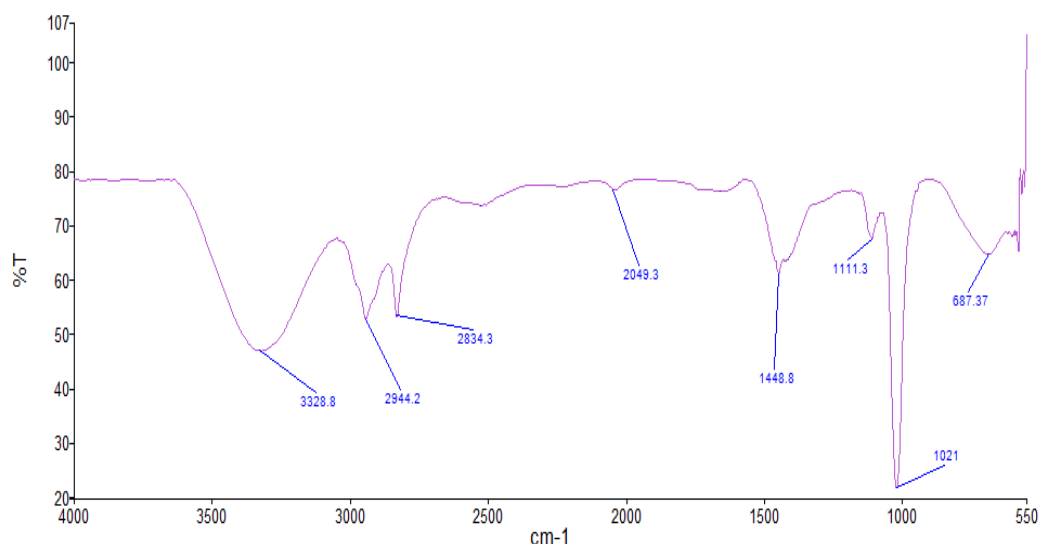


Figure 2: FTIR analysis of leaves of *Elettaria cardamomum* methanolic extract

Table 4: FTIR peak values of methanolic extract of *Elettaria cardamomum* leaves

Characteristic Absorption (cm <sup>-1</sup> )	Bond	Functional Group
3328.8	O-H stretch, H- bonded	Alcohols, Phenols
2944.2	C-H stretch	Alkanes
2834.3	C-H stretch	Alkanes
2049.3	C≡C stretch	Alkynes
1448.8	C=C stretch	Aromatic ring
1111.3	C-H wag (-CH <sub>2</sub> X)	Alkyl halides
1021	C-O-C stretch	Ether Linkage
687.37	C-H stretch	Alkanes

The results of the present study have given biochemical nature of biological and pharmacological properties of methanolic extracts and isolated phytoconstituents of *Elettaria cardamomum* to enrich our knowledge through GC-MS and FTIR analysis.

#### 4. Conclusion

In the present study, phytocomponents and their pharmacological activities have been identified from methanolic extract of *Elettaria cardamomum* (leaves) by GC-MS analysis. Hence, it is the hallmark to phytochemical, biomedical and pharmacognostical fields to carry out research

activities and drug formulations. It could be concluded that *Elettaria cardamomum* comprises of various bioactive compounds and acclaimed as a plant of phytopharmaceutical importance. Though, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the environment and agronomic products.

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### Conflict of Interest

The author's declare that they have no conflict of interest.

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