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 emphasises 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 μL, injector temperature 260.0 °C and ion-source temperature 230.0 °C was employed. The oven temperature was operated according to the following mode: 100 °C held for 1 min, rising at the rate of 10 °C per min up to 250 °C with 6 min hold, rising at the rate of 15 °C per min upto 300 °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 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 (Stary, 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

| Plant extract | Carbohydrates | Phenols | Tannins | Flavonoids | Saponins | Glycosides | Steroids | Terpenoids | Alkaloids |
|---------------|---------------|---------|---------|------------|----------|------------|----------|------------|----------|
| 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*.

![GC-MS chromatogram of the methanolic extract of Elettaria cardamomum leaves](image)

Figure 1: GC-MS chromatogram of the methanolic extract of Elettaria cardamomum leaves
| Peak | R.Time | Peak Area | Area %  | Name |
|------|--------|-----------|---------|------|
| 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   | 0.57    | 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-butanol |
| 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-Oxatri cyclo [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-Naphthenemethanol |
| 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-, (1alpha) |
| 36   | 17.327 | 982860    | 0.26    | 2(4H)-Benzo furanone, 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) dehydro-2-methylene-5, 5,8A-T |
| 39   | 17.755 | 4119627   | 1.10    | 2,6,10-Trimethyl, 14-ethylen-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- |
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-cyclocadiene
48 19.613 709077 0.19 3-Hydroxy-7-methoxy-3-phenyl-4-chromane
49 20.003 1001904 0.27 Heptadecanoic acid
50 20.134 675976 0.18 Phenol, 2-[(4-methoxyphenyl) ethenyl]-, (e)-
51 20.201 673485 0.18 N-Nonadecan-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-3-trans-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 (Albanicol) 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 (Albanicol) 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
| Retention Time | Name of the compound | Structure | Nature | Activity |
|---------------|----------------------|-----------|--------|----------|
| 11.323        | 2-Propenoic acid, 3-phenyl-, methyl ester | ![Structure](image) | Organic compound | Antiarthritic, Antioxidant, Cancer Preventive, Additive |
| 11.497        | 2-methoxy-4 Vinylphenol | ![Structure](image) | Phenolic compound | Antibacterial, Antioxidant, Antiseptic, Antiviral, Fungicide, Cancer preventive |
| 15.690        | Beta-D- Glucopyranose, 1,6- Anhydro- | ![Structure](image) | Sugar moiety | Preservative |
| 19.064        | Pentadecanoic acid | ![Structure](image) | Fatty acid | Rare Fatty acid in nature, flavoring agent |
| 19.306        | Hexadecanoic acid, Ethyl ester | ![Structure](image) | Palmitic acid ester | Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Flavor, Lubricant, Antiandrogenic, Hemolytic 5-Alpha reductase Inhibitor |
| 20.498        | Phytol | ![Structure](image) | Diterpene | Antimicrobial, Anticancer, Anti-inflammatory, Diuretic |
| 20.797        | 9, 12,15-Octadecatrienoic acid, (Z, Z, Z)-  | ![Structure](image) | Linolenic acid | Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, |
| Compound                        | Activity                                                                 |
|--------------------------------|--------------------------------------------------------------------------|
| Octadecanoic acid              | Antihistaminic, Antieczemic, 5-Alpha Reductase Inhibitor, Antiandrogenic, Antiarticular, Anticoronary, Insectifuge |
| Flavonoid fraction             | Hypercholesterolemic, Antiarthritic, Anti-inflammatory, Hepatoprotective, Nematicide, Antimicrobial |
| Vitamin E                      | Antioxidant, anticancer, antitumor, antibronchitic, anti-inflammatory activities |

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, stigmaster-5-en-3-ol, 4-hydroxy-1, 7-diphenyl-1-Penten-3-one, 4-methyl-1-phenyl-1-Butanone, 3-Heptanone, 5-hydroxy-1, 7-dihydroxy-2-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, 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).

![FTIR analysis of leaves of Elettaria cardamomum methanolic extract](image)

**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⁻¹) | 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 (-CH₂ 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 as certain 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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