ISSN: 2637-7721

MEDDOCS Open Access Publisher

Journal of Plant Biology and Crop Research

Open Access | Review Article

Characterization of Physio-Chemical Properties and Evaluation of Bioactive Potentials of Essential Oils from *Elettaria Cardamomum*

Vikas Jha[1]*; Danish Shaikh[2]; Agraj Bhargava[1]; Arpita Marick[1]; Farzha Khan[1]; Vrushali Dhamapurkar[1]; Ashish Jhangiani[1]; Shruti Narvekar[2]; Rushiraj Shinde[1]; Mehul Nair[1]

[1]National Facility for Biopharmaceuticals, Guru Nanak Khalsa College of Arts, Science & Commerce, Mumbai-19, Maharashtra, India.

[2]Department of Biotechnology, Bhavan's College, Andheri, Mumbai-58, Maharashtra, India.

***Corresponding Author(s): Vikas Jha**

National Facility for Biopharmaceuticals, Guru Nanak Khalsa college, Mumbai 400019 India.

Email: vikasjjha7@gmail.com

Received: Sep 02, 2022

Accepted: Oct 04, 2022

Published Online: Oct 06, 2022

Journal: Journal of Plant Biology and Crop Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: *Elettaria cardamomum*; Antimicrobial; Antioxidant; Anticancer; Antimalarial; DSC; Essential oil; FAME; FT-IR; GC-MS; TG-DTA.

Abbreviations: CEO: *Elettaria cardamomum essential oil*; DSC: Differential Scanning Calorimetry; GCMS: Gas chromatography-mass spectrometry; FAME: Fatty Acid Methyl esters; FTIR: Fourier Transform Infrared Spectroscopy; DPPH Assay, 2,2′- diphenyl-1-picrylhydrazyl Assay; TG-DTA: Thermogravimetric and DSC: Differential Thermal Analysis; LB: Luria-Bertani; RT: Room temperature; MIC: Minimum Inhibitory Concentrations; HeLa: Human cervical cancer cell line; FBS: Foetal bovine serum; MTT Assay,3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay.

Abstract

Background: Due to their ability to kill disease-causing microorganisms and extend the shelf life of foods without the hazards associated with synthetic antimicrobial preservatives, essential oils derived from plants are quickly becoming popular as therapeutic agents and food additives. Similar reports on the exceptional biological and pharmacological activities of *Elettaria cardamomum* essential oil have been made. It is employed in Ayurveda and Unani medicine to treat migraines, gastrointestinal ailments, liver tonics, hypnotic and appetising effects, and gastric ulcers.

Objective: The objective of this study was to carry out the phytochemical profiling of *Elettaria cardamomum* essential oil and evaluate its bioactive potential in order to determine its therapeutic potential for application in food and pharmaceutical industries.

Methods: The *Elettaria cardamomum* essential oil was extracted via hydro-distillation of the cardamom fruit's crude capsules and was examined via GC-MS analysis. FAME and FT-IR analysis were further carried out for the detailed familiarity of the fatty acid and functional group composition in the essential oil. Furthermore, thermal characterization was carried out by employing TG-DTA and DSC techniques. The bioactive potential of the essential oil was also examined by determining the antibacterial activity against drug-resistant microorganisms. In addition to the Anti-tubercle and anti-malarial activity, Anti-biofilm activity was also examined. The free radical scavenging potential of the oil was evaluated using the DPPH assay. Further, the essential oil was subjected to MTT assay in order to determine its anticancer potency against HeLa cell line.

Cite this article: Jha V, Shaikh D, Bhargava A, Marick A, Khan F, et al. Characterization of Physio-Chemical Properties and Evaluation of Bioactive Potentials of Essential Oils from *Elettaria Cardamomum*. Plant Biol Crop Res. 2022; 5(2): 1068.

Results: The *Elettaria cardamomum* essential oil was characterized by the presence of terpenoids (49.21%); organic compounds (16.41%) and Fatty acids (7.46%). Moderate results were observed for antimicrobial and antibiofilm analyses against the selected test organisms as well as for *Mycobacterium tuberculosis* and *Plasmodium falciparum.* In addition to the moderate results for free radical scavenging activity, TG-DTA and DSC profiling of the essential oil accounted for its thermal stability and endurance against temperature variance. MTT assay evaluated the anticancer activity of the essential oil against the HeLa cell line exhibiting IC50 value of 38.02 µg/mL.

Conclusion: This study suggests a possible wide range of usage of the *Elettaria cardamomum* essential oil as a non-toxic substitute for the synthetic and chemical components employed in the food and pharmaceutical industries.

Introduction

Elettaria cardamomum (L.) Maton is a herbaceous perennial plant belonging to the Zingiberaceae family. It is also known as the "Queen of Spices" since, it is the third most valuable spice after vanilla and saffron [1,2]. The widespread usage of cardamom as a main ingredient in worldwide cuisines, as well as the age-old tradition of employing cardamom spices in medicine, demonstrates the spice's appeal. In South Indian Ayurvedic medicine, the cardamom mixture "Eladigana chooranm" is often used to treat arthritis, congestion, and itching. Also, crushed cardamom capsules are boiled with tea and water to give a pleasant scent to tea, which is known as "Elakkai tea" and has traditionally been used to treat fatigue and depression [2]. In traditional medicine, *Elettaria cardamomum* essential oil has been used as flavouring agent and as preservative in food items implying that the phytoconstituents isolated from it would also have similar bioactive potential [3].

Principal bioactive metabolites of *Elettaria cardamomum* essential oil are responsible for its distinctive strong aroma. These metabolites belong to various chemical classes including alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes [4]. Antioxidant, anti-inflammatory, antidiabetic, anticancerous, antibacterial, antiviral, and gastroprotective effects are all possible pharmacological and therapeutic capabilities of its major ingredients [5]. Recent studies indicate that such volatile compounds present in the *Elettaria cardamomum* essential oil play a significant role in the symptomatic management of oxidative stress that causes irreparable damage in neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease. It also plays a significant role in mood elevation by engaging the limbic system and the stimulation of the Dopaminergic, Cholinergic and Adrenergic neurones [6].

The essential oils extracted from *Elettaria cardamomum* are classified as GRAS (Generally Regarded as Safe). As pharmacological doses for diverse activities would range greatly from the minute amounts that are regarded organoleptically acceptable, it is critical that the bioactive compounds of *Elettaria cardamomum* be harnessed for their therapeutic potential [7].

Elettaria cardamomum essential oil can be used as food preservative and additive by the virtue of its antimicrobial activity. Microbial contaminants such as fungi and bacteria, have the tendency to deteriorate food quality by releasing metabolic toxins and increasing chances of human health hazards. Essential oils have also been reported to inhibit the mycotoxins released by pathogenic fungi and inhibit growth of organisms responsible for food spoilage. The use of synthetic and semi-synthetic preservatives carries the risk of bioaccumulation, endocrine disruption and also result in environmental challenges due to their non-biodegradable nature [8]. Synthetic chemicals employed as food preservatives pose a risk due to their bio-incompatibility and non-biodegradability. As a result of their eco-friendliness and biocompatibility, plant-based antimicrobials, such as essential oils, have sparked interest as a viable alternative to synthetic preservatives.

The *Elettaria cardamomum* essential oil has also been evaluated for its anti-microbial activity against human pathogens. Pathogenic microorganisms are constantly evolving into multidrug resistant strains, thus, posing a serious threat to human health and quality of life. as a result, research for new antimicrobials from natural sources has been encouraged [9]. *Elettaria cardamomum* essential oil has also been helpful in inhibiting the growth of foodborne pathogens like *Salmonella spp*., *E. coli O157:H7*, and *Listeria monocytogenes*.

Essential Oils successfully pass through the lipid bilayer of cell membranes due to their hydrophobic/lipophilic nature, interfering with ion transport, leakage of cellular components, alteration in proton motive force-mediated electron flow, and eventually lead to cell death [10]. Mechanisms of action for inhibiting the food spoilage and drug resistant pathogens by *Elettaria cardamomum* essential oil include deleterious effects on cellular membranes affecting their permeability and leakage of cytoplasmic materials (DNA, RNA, and Proteins).(8) The *Elettaria cardamomum* essential oil extract was tested against a wide range of pathogenic bacteria including several drug resistant strains including MRSA. It inhibited Methicillin Resistant Staphylococcus aureus biofilm by causing changes in cellular metabolism, extracellular polymer content and relative expression of MRSA biofilm formation related genes [11].

The objective of this study is to understand more about the phytochemical composition of *Elettaria cardamomum* essential oil, along with its therapeutic and food preservation properties. The essential oil was extracted from cardamom seeds using the hydro-distillation process. The phytochemical components were investigated using GC-MS. Thermal stability of the oil was examined using thermo analytical methods such as TG-DTA and DSC. The fatty acid content of the essential oil was determined using FAME analysis and GC-MS. Antimicrobial, antitubercle, antimalarial, antioxidant, anticancer, and antibiofilm activities of the essential oil were also evaluated.

Materials and methods

Sample collection and extraction

The *Elettaria cardamomum* fruit capsules obtained from a local market in Mumbai, India were used to extract the essential oil. The extraction procedure was carried out at a research facility using Clevenger hydro-distillation method [12]. Any residual moisture that might have been present in the generated oil was eliminated using anhydrous sodium sulphate. The oil was stored at 4 °C in the dark until an experiment was conducted.

GC-MS Analysis

A Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used to identify the components of the *Elettaria cardamomum* essential oil extracted from cardamom fruit capsules. The analysis was performed using, a Shimadzu GCMS-QP2010 system, which included a Gas Chromatogram coupled with a Mass Spectrometer, Rtx-5ms column with 5% diphenyl/ 95% dimethyl polysiloxane composition, 30 m long capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 µm. In the GC-MS analysis, an electron ionization device in electron impact mode with an ionisation energy of 70 eV was used. The operation employed helium (99.999 %) as a carrier gas, with a uniform flow rate of 14 mL/minute and an injection volume of 1 µL, including a split ratio of 10. Considering the temperature regulations, the injector temperature and ion-source temperature were held at 250°C and 220°C respectively. While the oven temperature was adjusted at 70°C for 1 minute with an isothermal condition. Later, an increase up to 260°C with 10 minutes of' isothermal condition was witnessed. The entire analytical procedure was of 42 minutes. Mass spectra in the range of 50 to 650 m/z were obtained at 70 eV. Several constituents of *Elettaria cardamomum* essential oil were determined by evaluating their mass spectra with those from the Wiley and NIST libraries, including those mentioned by R. P. Adams [13] and comparing their retention indices.

FAME Analysis

FAME Analysis was conducted in accordance with the method described by (Lall et al., 2009) with a few modifications. A weighing balance was used to accurately weigh 100 mg essential oil in 50mL centrifuge tubes using a class-A pipette. To this, 1mL of toluene and 5mL of 0.5 M sodium methoxide-were added and then purged with nitrogen gas. The centrifuge tube was heated in a water bath for 50 minutes prior to getting cooled for 5 minutes. Consequently, 0.2 mL glacial acetic acid was added to the centrifuge tube to restrict the production of sodium hydroxide as that could hydrolyse methyl esters to free fatty acids. Lastly, the centrifuge tubes were filled with 5mL distilled water, followed by 1mL of hexane, and vortexed for 2 minutes. In a 7mL glass ampoule, the hexane layer was extracted and dried over sodium sulphate for approximately 20 seconds. This approach required a total of 30 minutes for FAME preparation prior to GC-MS analysis.

The generated FAME was GC-MS analysed using a Shimadzu GCMS-QP2010 system, which included a Gas Chromatograph interfaced to a Mass Spectrometer, a capillary column with a length of 30 m, an internal diameter of 0.32 mm, and a film thickness of 0.25 m. An electron ionisation device in electron impact mode with an ionisation energy of 70 eV was used for GC-MS detection. Helium gas (99.999 %) was utilised as a carrier gas at a column flow rate of 3 mL/minute, the injector temperature was held at 250°C, and an injection volume of 1µL with a split ratio of 50 was used. The Gas chromatographic processing conditions began with the initial oven temperature of 120°C maintained for 1 minute before being heated at 170°C at a rate of 20°C/min, following another temperature increase to 210°C at a rate of 3°C/minute, and thereafter temperature raised to 250°C at a rate of 20°C/min and held for 10 minutes. Mass spectra were obtained at 70 eV ranging from 40 to 500 m/z. The components were determined by comparing their mass spectra to those of the Wiley and NIST libraries [14], as well as those described by R. Adams, 2007 [15], and by comparing their retention indices to those of the literature.

FTIR Analysis

Before adding a drop of sample to a clean KCl pellet, the Shimadzu IRPRESTIGE 21 Fourier transform infrared spectrometer was warmed and stabilized. The second salt pellet was then placed onto the KCl pellet for uniformly tiling the oil sample between two KCl pellets. After gently rotating the KCl pellets to generate a uniform liquid membrane and placing them in the infrared spectrometer sample holders, the infrared spectrometer was set to absorbance with a resolution of 1 cm^{-1} and a 45-time repetition scan. The extracted oil's infrared absorption spectra were obtained over the spectral range 4000–400 cm^{-1} in the design environment. The resolution of the measurement was 4 cm with 45 spectral accumulations.

Thermogravimetric and Differential Thermal Analysis (TG-DTA)

Thermogravimetric and differential thermal analysis was carried out for the acquired essential oil using STA 2500 instrument, NETZSCH, Germany. The analysis was performed in a nitrogen gas atmosphere with flow rate 300 mL/minute. The essential oil sample weighing 18.492 mg was placed in an aluminium crucible. Subsequently, the sample was heated till 500°C under a persistent flow rate of 5.5 °C/minute.

Differential Screening Calorimetric Analysis

Thermal analysis was carried out utilising differential scanning calorimetry (DSC) thermal analyzer (LINSEIS. DSC model P 10.). For the experiment, 5 mg of sample was placed in aluminium crucibles. The samples were analysed using a nitrogen gas flow rate of 40 mL/minute. Heating temperatures ranged from 25 °C to 375 °C, with a heating rate of 10°C/minute.

Antimicrobial activity

Preparation of microbial strains

The microbial strains employed in this study included 9 test organisms out of which Carbapenem-Resistant Acinetobacter spp, Carbapenem-Resistant *Pseudomonas aeruginosa*, Carbapenem-Resistant *Escherichia coli*, Carbapenem-Resistant *Klebsiella pneumonia*, Extended Spectrum beta-lactamase *Escherichia coli* and Quinolone-Resistant *Salmonella* were gram negative whereas Vancomycin-Resistant *Enterococci*, Methicillin-Resistant *Staphylococcus aureus*, Erythromycin-Resistant *Streptococci* were gram positive. These bacterial strains were cultivated on Mueller Hinton agar for 24 hours at 37°C.

Determination of Minimum Inhibitory Concentration (MIC) of microbial isolates

The micro broth dilution assay was used to obtain the minimal concentration of the *Elettaria cardamomum* essential Oil required to inhibit the test bacterial isolates and the process was carried out using 96-well microtiter plates [16]. According to the McFarland standard, the microbial suspensions were adjusted to get 1.0 x 10^5 CFU/ml. In order to achieve the necessary concentrations, the essential oil was dissolved in a 5 % DMSO and 0.1 % polysorbate-80 (1 mg/mL) solvent mixture before being introduced to 100 µL of Luria Bertani medium with a 1.0 x 10^4 bacterial inoculum. The inoculated plates were incubated for 24 hours at 37 °C. After incubation, 5 µL of Resazurin dye (2 mg/mL) was added to each well. The production of pink coloured resorufin, a product of resazurin reduction indicates microbial growth.

Determination of anti-tubercle potential

In vitro, Anti tubercle Activity of *Elettaria cardamomum* essential oil was evaluated against a susceptible strain of *Mycobacterium tuberculosis* (H37Rv) obtained from the National Institute for Research in Tuberculosis, Chennai, Tamil Nadu. The Minimum Inhibitory Concentration of the essential oil was determined using Lowenstein and Jensen Method. The Lowenstein and Jensen (LJ) medium used in the assay was supplemented with Potassium dihydrogen phosphate, Magnesium sulphate anhydrous, Magnesium citrate, Asparagine, Glycerol, 2% Malachite green solution, Distilled water, and Homogenized egg solution. The essential oil samples were prepared in DMSO as a stock solution [17]. Two-fold serial dilutions of the same were prepared in supplemented L.J medium to obtain final concentrations of 100, 50, 12.5, 6.25. 3.125, 10, 5, 2.5, 1.25, 8, 4, 2, 1, 0.5, 0.25 µg/mL. These dilutions were further inoculated with a strain of *Mycobacterium* suspension whose concentration was equivalent to the McFarland standard and incubated at 37°C with regular monitoring for growth. The reference *Mycobacterium tuberculosis* (H37Rv) strain was tested against Isoniazid as a standard drug with a concentration of 0.2 µg /L [18].

Evaluation of anti-biofilm activity

In vitro anti-biofilm activity of *Elettaria cardamomum* essential oil was determined against *Staphylococcus epidermidis, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis,* and *Staphylococcus aureus* by employing the crystal violet assay [19]. An isolated colony was picked from the test organisms' isolation on the Nutrient Agar medium and inoculated into the Luria Bertanii (LB) broth. The optical density of the culture suspension was calibrated to 0.5 O. D. in accordance with McFarland Standard. Furthermore, a sterile 96-well microtiter plate was filled with sterile distilled water, 100 µL of LB broth, and 100 µL of an essential oil sample. 200 µL of LB broth was maintained as a negative control and 50 µL of Chloramphenicol (30 mg/mL) with 150 µL of LB broth was used as a positive control. Each well contained 100 µL of the essential oil sample, and a 20 µL bacterial suspension with a final OD_{600} of 0.01 was added after it had been diluted with an overnight culture produced in LB broth. To allow bacterial growth and biofilm formation, the plates were incubated overnight at 37 °C for 24 hours under static conditions. Post incubation, the planktonic cells and the utilised medium were decanted, and the attached biomass was thrice cleaned with distilled water. Additionally, 400 µL of 1% Crystal violet dye was used to stain this biomass before the plates underwent a 30- to 40-minute incubation period at room temperature. After the incubation, the plates were drained and three times rinsed with distilled water to get rid of the unbound dye. Following 10-20 minute drying period in a hot air oven set to 40°C, 400 µl of methanol was poured into each well on the plate. In an ELISA plate reader, the optical density was measured at 570 nm, and the following formula was used to estimate the percentage inhibition:

$$\text{Percentage inhibition} = \frac{(\textit{Absorbance of negative control} - \textit{Absorbance of test})}{(\textit{Absorbance of negative control})} \times 100$$

Evaluation of antimalarial activity

An in vitro anti-malarial assay was performed in a sterile 96-well microtiter plate in accordance with the method illustrated by Kotecka B and Rieckmann [20]. *Plasmodium falciparum* drug-sensitive and resistant strains were developed in RPMI-1640 medium, which also included 25 mM HEPES, 0.23 % sodium bicarbonate, 1 % D-glucose, and 10% heat-inactivated human serum. Exposure with 5% D-sorbitol was used to obtain only ring-stage parasitized cells, which ended in the synchronization of drug-sensitive and drug-resistant *Plasmodium falciparum*. To carry out the experiment, Jaswant Singh Bhattacharya (JSB) staining was used to establish an initial ring stage parasitemia of 0.8 to 1.5 % at 3% haematocrit in a total volume of 200µL of medium RPMI-1640 and consistently maintained with 50 % RBCs (O^+) in a total volume of 200 µL of medium RPMI-1640 [21]. A 5 mg/mL stock solution of essential oil was made using DMSO, to which 20 µL of parasitized cell preparations was added, maintaining a range of dilutions from 0.4 µg/mL to 100 µg/mL in duplicate wells. The culture plates were then incubated at 37°C for 36 to 40 hours in a candle jar. Thin blood smears were made from each well after incubation and stained with the JSB stain. The reference drugs for the study were Chloroquine and Quinine. Microscopic observation of the slides confirmed the evolution of ring-stage parasites into schizonts and trophozoites when different concentrations of the test chemicals were used. The lowest inhibitory concentrations (MIC) that prevented both *Plasmodium falciparum* strains from developing into schizonts were recorded, and the estimated IC_{50} value for each was compared to the standard values.

Evaluation of antioxidant activity

The radical scavenging activity of *Elettaria cardamomum* essential oil was determined using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [22]. A mixture of 2.0 mL sample, 1 mL of methanol solution containing 0.5 mM DPPH radical, and 1 mL of 0.1 M sodium acetate buffer at pH 5.5 was added. Following adequate mixing, the solutions were kept at room temperature and placed in the dark for 30 minutes. A twin beam UV-VIS spectrophotometer was used to detect the absorbance at 515 nm. As a negative control, methanol was employed. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at concentrations of 100 to 1000 µg/ml. The reaction mixture was properly mixed and incubated for 15 minutes at room temperature in the dark. The absorbance was determined at 515 nm. For each concentration, the following formula was used to compute the percentage of DPPH radical inhibition:

$$percentage\ inhibition\ (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

The sample concentration that showed 50% inhibition was obtained by plotting inhibition percentages versus oil sample concentrations.

Evaluation of anticancer activity

In order to determine cell viability and cell proliferation the MTT assay was performed. This colorimetric assay relies on metabolically active cells to convert the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to purple formazan crystals [23]. In vitro anticancer activity of *Elettaria cardamomum* essential was evaluated against Human cervical cancer (HeLa) cell line. The cell line was obtained from the National Centre for Cell Science (NCCS), Pune and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS and antibiotic (Pen-strep). Cells were kept at 37°C, 5% CO2, 95% air, and 100% relative humidity, passaged weekly with the culture medium being replaced twice a week. Monolayer cells were detached using trypsin-ethylenediaminetetraacetic acid (Trypsin-EDTA) to create single cell suspensions, and viable cells were counted using a haemocytometer and diluted with FBS medium containing 5% FBS to achieve a final density of 1 x 10^5 cells/mL. 100 µL/well of cell suspension was seeded onto 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO2, 95% air, and 100% relative humidity. The cells were treated with increasing concentrations of oil sample after 24 hours. They were first dissolved in undiluted dimethyl sulfoxide (DMSO) and then diluted in serum free medium to provide the 7 concentrations. Final concentrations (5, 10, 20, 40, 60, 80, and 100 g/mL) were achieved by adding 100 µg/mL of each concentration to the plate. The final volume achieved in the wells was 200 µL, and the plates were incubated for 48 hours at 37°C, 5% CO2, 95% air, and 100% relative humidity. Medium with no sample was considered as the negative control. After 48 hours, 15 µL of MTT (5 mg/mL) in Phosphate buffered saline (PBS) was added to each well and incubated for 4 hours at 37°C. The medium containing MTT was then discarded, and the produced formazan crystals were dissolved in 100 µL of DMSO prior the quantification at 595 nm using an ELIZA plate reader. The percentage of cell inhibition was calculated using the following equation:

$$\text{Percentage cell inhibition} = \left\{100 - \left[\frac{(\text{Absorbance of sample})}{(\text{Absorbance of control})}\right]\right\} \times 100$$

Result and discussion

Chemical Composition of *Elettaria cardamomum* essential oil

The *Elettaria cardamomum* essential oil extracted through hydro-distillation was evaluated using Gas chromatography-mass spectroscopy (GC-MS) [24]. A total of 67 peaks were obtained through total retention time range of 3.38 to 35.22 minutes. The GC-MS profile of the essential oil revealed a diverse spectrum of compounds, including monoterpenes (23.88%); sesquiterpenes (22.38%); organic compounds (16.41%); terpenes (8.95%), and Fatty acid (7.46%). The major portion of the essential oil components consisted of (3E)-2,7-dimethyloct-3-en-5-yne (13.06%), Naphthalene, 1,2,3,5,6,8a- (7.41%), Caryophyllene oxide (6.94%), 3-Cyclopentyl-1-propyne (6.65%), Methyleugenol (6.25%), 1,3,6-Heptatriene, 2,5,6-trimethyl- (6.02%), Eucalyptol (5.19%), 1,2-Benzenediol, o-(4-butylbenzoyl)-o'-(2-methylbenzoyl)- (5.02%), trans-4-Methoxycinnamaldehyde (4.21%), Tetradecanoic acid (3.52%) and many other trace components. The present research outcomes on the quantification of *Elettaria cardamomum* as a bioactive ingredient were similar to previous studies that identified 1,8-cineole, -terpinyl acetate, and linalool as significant molecules [25]. *Elettaria cardamomum* essential oil is known to have therapeutic benefits owing to its antibacterial activity demonstrated by components such as 1,8-Cineole, 1,3,8-p-Menthatriene, Methyleugenol and δ-Cadinene. (3E)-2,7-dimethyloct-3-en-5-yne, 1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-, Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-, 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E) are some of the compounds which exhibited anticancer properties whereas the antioxidant activity observed can be attributed to presence of 4-(2,2,6-Trimethylbicyclo[4.1.0] hept-1-yl)-butan-2-one, 5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-, Salicylic acid, Cyclohexanone, 5-methyl-2-(1-methylethenyl)-, trans-, Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methyl and etc. Thus, demonstrating an extensive range of applications for *Elettaria cardamomum* essential oil in the food, cosmetic, and pharmaceutical industries.

FAME analysis of *Elettaria cardamomum* essential oil

Fatty acid analysis has gained significance as a result of the perceived growing understanding of the nutritional and health benefits of lipids [26]. Fatty acid methyl esters (FAME) are generated from the transesterification of lipids with methanol. The presence of these fatty acids in the essential oil sample was determined using GC-MS. The chromatogram (Table 2) on analysis of the sample presented 27 distinct fatty acid methyl esters. Characterization of the *Elettaria cardamomum* essential oil showed presence of 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-; Hexadecanoic acid,methyl ester; Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-; Octadecane, 1-bromo-; Methyl stearate; Tetradecanoic acid; Decane, 5-ethyl-5-methyl-; Tetracosane; N-(2-Aminoethyl)-3-aminopropyltrimethoxysilane; Pentadecanoic acid; Nonadecane, 9-methyl-; Isobutyl 2-(4-methylcyclohex-3-enyl)propan-2-yl carbonate; Isobutyl 2-(4-methylcyclohex-3-enyl)propan-2-yl carbonate; Heptacosane; 9-Hexadecenoic acid ; Hexacosane ; Hexatriacontane ; Scillarenin; Glycylglycine ethyl ester; Silane, dimethyl(docosyloxy) butoxy-; 2-Amino-4-dimethylaminomethylenepentanedinitrile; 4-Methoxymethoxy-hex-1-ene; 5-Hydroxy-2,2-dimethylhexan-3-one; 6 1-Ethyl-3-[2-(octadecylthio)ethyl]thiourea; 2-[3-Indolylmethyl]aminoethylthiophosphate; cis-2,3-Epoxyoctane and 1-(Methoxymethoxy)-3-methyl-3-hydroxybutane. The largest peak discovered was of 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-, covering a percentage area of 85.54, indicating its dominance in the essential oil.

Fourier transform infrared spectroscopy of *Elettaria cardamomum* essential oil

The infrared spectrum of absorption, emission, and photoconductivity of solids, liquids, and gases is obtained using the Fourier Transform Infrared Spectroscopy (FTIR) technique. It is employed in the detection of various functional groups in *Elettaria cardamomum* essential oil. Changes in chemical functional groups are visible in this spectrum [27]. At 2598.12cm^1 and 2926.01cm^{-1}, the S-H of thiol and the N-H stretching band, respectively can be seen. Aromatic C=C stretching and C=C of alkenes were detected at the peaks of 1591.27cm^{-1} and 1504.48 cm^{-1}. At 1132.21cm^{-1}, a C-O ester stretch is identified. C-H (alkane) stretching is indicated by the peaks at 2872.01 cm^{-1} and 2958.8 cm^{-1}, whereas CH_3 stretching is indicated by the peak at 1454.33 cm^{-1}. The presence of vinyl ether and amine groups in the essential oil is indicated by peaks at 1047.35 cm^{-1} and 781.17 cm^{-1}. Alcohol and ether are detected at a peak of 1093.64 cm^{-1}. At peak 1631.78 cm^{-1}, the N-H bending may be seen. S=O stretching and CH3 -Rock are indicated by the peaks at 1193.94 cm^{-1} and 1184.29 cm^{-1}. At peak 1238.3 cm^{-1}, C-N stretching is observed. The presence of amine groups is indicated by the peak at 3381.21 cm^{-1}.

TG-DTA Analysis to determine thermal stability of *Elettaria cardamomum* essential oil

Thermogravimetric Analysis (TGA) is a technique of monitoring the weight of a substance as a function of time or temperature under a regulated heating or cooling condition, according to international standards [28]. TGA examines decomposition/mass loss across a temperature range, whilst DTA monitors endothermic and exothermic event temperatures along with phase transitions. The thermogravimetric and differential analyses are critical for determining the essential oil's stability and mass loss as temperature rises, as well as identifying the nature of the reaction that leads to the sample's breakdown or mass loss [29]. Thermogravimetric curve (**Figure 1)** and mass loss of the essential oil, at varying temperatures, are observed. The onset temperature for mass loss was observed to be at 162.4°C continuing till 250-300°C, followed by the eventual stabilisation of the curve at a lower point. The analysis demonstrated higher temperature hold over mass loss denoting thermally stable and resistant capabilities. The DTA curve began with inconsistency but showed an onset of a complex peak at 215°C ending at 237°C further stabilising after 300°C. The DTA peak achieved at a higher temperature presented itself as an indicative of release of energy from the sample due to component breakdown via exothermic reaction. Hence showcasing and authenticating the essential oil's thermal stability and resistance.

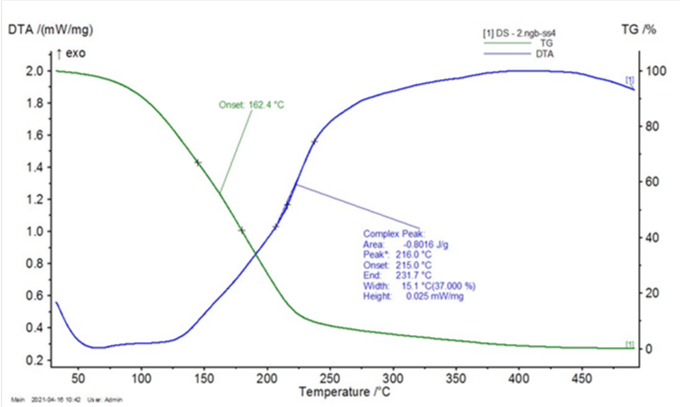

Figure 1: Thermogravimetric-DTA analysis curve of essential oil extracted from *Elettaria cardamomum.*

Differential Scanning Calorimetric analysis to establish Calorimetry profile of *Elettaria cardamomum* essential oil

Illustrated thermogram in **(Figure 2)** of *Elettaria cardamomum* essential oil developed through Differential Scanning Calorimetry (DSC) unveils its Calorimetric profile. It implies that as the temperature rises, the heat flow within the sample diminishes dramatically. This endothermic drop is reported at temperatures ranging from 25°C to 80°C. At 89°C heat flow of -0.792, W/g is noted. With the development in temperature the heat flow reduces substantially, this declines significantly between 85°C to 175°C. At 169°C, the observed heat flow is -0.9710 W/g. Heat flow accelerates exponentially with increasing temperature, with an exothermic shift of approximately 180°C and 225°C. At 210.29°C, a heat flow of -0.2291 W/g is measured. Following this, there is linear progression. This graph demonstrates the essential oil's endurance across a broad temperature range.

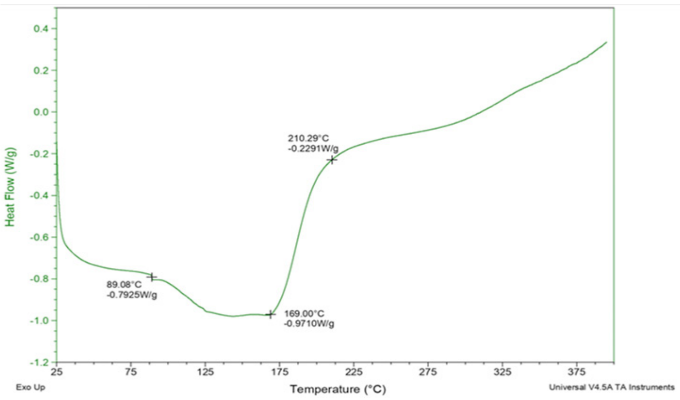

Figure 2: Calorimetric profiling of essential oil extracted from *Elettaria cardamomum.*

Evaluation of Antibacterial potential of *Elettaria cardamomum* essential oil against resistant bacterial strains

The micro broth dilution technique was used to examine the antibacterial activity of the essential oil against drug resistant strains. As seen in **(Table 1),** all of the tested strains indicated growth inhibition at varying MIC values. The most susceptible bacterial strain was *Carbapenem Resistant Pseudomonas aeruginosa* with a MIC value of 100 ± 0.02 µg/mL. Most resistant strain was *Carbapenem Resistant Klebsiella pneumoniae* with a MIC value of 250 ± 0.06 µg/mL. Potent activity of essential oil was seen against *Carbapenem Resistant Acinetobacter spps*, *Quinolone Resistant Salmonella*, and *Extended Spectrum Beta Lactamase* bacterial strains with MIC values of 125 ± 0.03 µg/mL, 125 ± 0.06 µg/mL, and 126 ± 0.07 µg/mL respectively whereas *Methicillin Resistant Staphylococcus aureus (MRSA)* exhibited a MIC value of 250 ± 0.02 µg/mL.

Elettaria cardamomum essential oil causes irreparable harm to MRSA by blurring the surface barrier of the cell wall altering the structure of the cells, which causes bacterial mortality [11]. Our GC-MS results show that *Elettaria cardamomum* essential oil contains a wide range of compounds. 1,8-cineole, one of the potential components of *Elettaria cardamomum* extracts is mono-terpenoid also known as Eucalyptol, which demonstrates antibacterial activity by promoting cell shrinkage and membrane damage [30]. Additonally, Sabinene, cymene, limonene, linalool, geraniol, and citral are also some of the components of Elettaria cardamomum essential oil with antibacterial activity. 2016). The MIC data imply that this oil can routinely aid in limiting the proliferation of foodborne pathogens. As a consequence of its substantial antibacterial capabilities, *Elettaria cardamomum* essential Oil plays an important role in the discovery of novel antibiotics. Moreover, Anti-Tubercle activity of essential oil was also evaluated using micro-broth dilution method via evaluating the MIC of the essential oil. The MIC against *Mycobacterium tuberculosis* (H37Rv) was observed to be 12.5 µg/mL which was relatively higher as compared to the standard.

Table 1: Determination of minimum inhibitory concentration of Elettaria cardamomum essential oil.

Sr. No.	Code	Bacterial Strains	MIC (µg/mL)
1	CRA	*Carbapenem Resistant Acinetobacter spp*	125 ± 0.03
2	CRP	*Carbapenem Resistant Pseudomonas aeruginosa*	100 ± 0.02
3	CRE	*Carbapenem Resistant E.coli*	250 ± 0.01
4	CRK	*Carbapenem Resistant Klebsiella pneumonie*	250 ± 0.06
5	ESBL	*Extended Spectrum beta lactamase E.coli*	126 ± 0.07
6	VRE	*Vancomycin resistant Enterococci*	200 ± 0.04
7	QRS	*Quinolone resistant Salmonella*	125 ± 0.06
8	MRSA	*Methicillin resistant Staphylococcus aureus*	250 ± 0.02
9	ERS	*Erythromycin resistant Streptococci*	2500.03

Anti-biofilm Activity of *Elettaria cardamomum* essential oil

The efficacy of the essential oil to suppress biofilm formation was evaluated using crystal violet assay. The biofilm inhibition potential of *Elettaria cardamomum* essential oil was assessed employing test cultures of *Staphylococcus epidermidis, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis,* and *Staphylococcus aureus* as shown in **(Figure 3)**. The essential oil exhibited promising potency against all species at concentrations ranging from 10 to 100 µg/mL. Our findings demonstrate that highest biofilm inhibition potential of 91.70 % at a concentration of 100 µg/mL was achieved against *Klebsiella pneumoniae*. Whereas the lowest capacity to inhibit the biofilm was 66.98 %, exhibited by *Enterococcus faecalis* at a concentration of 100 µg/mL. The remaining test cultures, *Staphylococcus epidermidis, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus* exhibited inhibition levels of 80.97 %, 75.04 %, 77.88 %, 78.12 %, and 70.94 % at the maximum dose, respectively [31]. At 10 µg/mL, *Escherichia coli* had the lowest percentage inhibition (1.04 %) while *Klebsiella pneumoniae* had the greatest (56.77 %). As a result, *Elettaria cardamomum* essential oil showed good inhibitory effect against *Klebsiella pneumoniae*. The propensity to hinder 50 % of biofilm growth was assessed through an IC_{50} value, and it was determined that *Klebsiella pneumoniae* had the lowest IC_{50} value of 29.30 µg/mL, indicating more effective suppression of biofilm development than compared to other test microorganisms. *Elettaria cardamomum* essential oil owes its efficacy to compounds like 4-Methoxycinnamaldehyde [32]. Essential oils regulate biofilm development by impeding peptidoglycan development and disrupting microbial membrane structures [33]. *Elettaria cardamomum* essential oil damages the biofilm barrier, causing the bacteria to lose metabolic activity, it negatively influences biofilm formation by increasing or decreasing the expression of biofilm-forming genes [11].

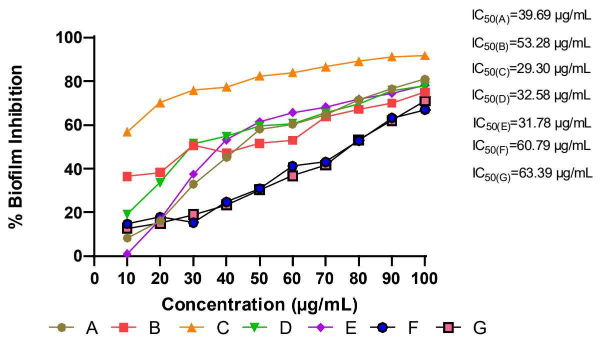

Figure 3: Biofilm inhibition of selected cultures by the essential oil of *Elettaria cardamomum*.
(A) *Staphylococcus epidermidis*, **(B)** *Proteus mirabilis*, **(C)** *Klebsiella pneumoniae*, **(D)** *Pseudomonas aeruginosa*, **(E)** *Escherichia coli*, **(F)** *Enterococcus faecalis*, **(G)** *Staphylococcus aureus.*

Antimalarial activity of *Elettaria cardamomum* essential oil

At relatively low concentrations, essential oils are reported to have antimalarial properties in vitro and in vivo [34]. IC_{50} values were determined for the anti-plasmodial activity of *Elettaria cardamomum* essential oil against drug-resistant and drug-sensitive strains of *Plasmodium falciparum.* **(Table 2)**, shows the IC_{50} value of *Elettaria cardamomum* essential oil. It was observed that 4.54 µg/mL of oil concentration suppressed 50% of the cells of Drug-sensitive strain of *Plasmodium falciparum* whereas the oil exhibited IC_{50} of 2.28 µg/mL against drug-resistant strain of *Plasmodium falciparum*. **(Figure 4)** The molecule β-Pinene-(1S) (-) shows antimalarial activity which is also confirmed by our GC-MS analysis. Previous research has shown that when two essential oil are combined, the overall interaction between them is synergistic, although the degree of synergism varies. The physical characteristics of essential oil constituents, such as their low density and lipophilicity, which permit quick diffusion across cell membranes, enable the anti-malarial formulation to deliver its active chemicals into the malaria parasite, enabling enhanced targeting. Although their interaction is dependent on their respective cellular targets, the final outcome depends on each essential oil [34].

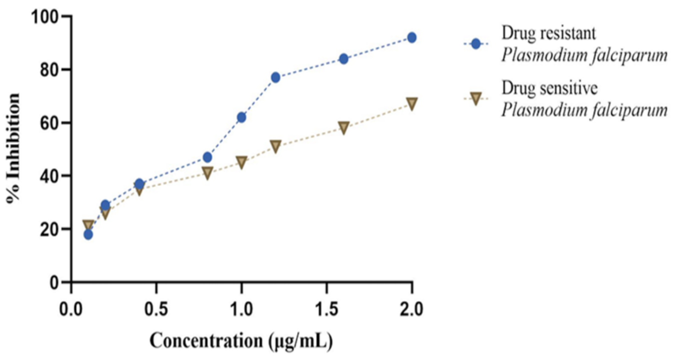

Figure 4: Evaluation of Antimalarial activity of *Elettaria cardamomum* essential oil.

Table 2: Anti-malarial evaluation of essential oils extracted from *Elettaria cardamomum.*

***Elettaria cardamomum* essential oil**			**Drug resistant *Plasmodium falciparum***		**Drug sensitive *Plasmodium falciparum***	
Standard Drugs	**IC_{50} value**	**Concentration (µg/mL)**	**% Inhibition**	**IC_{50}Value**	**% Inhibition**	**IC_{50}Value**
Chloroquine	0.020 µg/mL	0.1	18 ± 0.04	2.28 µg/mL	21 ± 0.04	4.54 µg/mL
		0.2	29 ± 0.05		26 ± 0.06	
Quinine	0.268 µg/mL	0.4	37 ± 0.02		35 ± 0.06	
		0.8	47 ± 0.04		41 ± 0.04	
		1.0	62 ± 0.03		45 ± 0.05	
		1.2	77 ± 0.06		51 ± 0.04	
		1.6	84 ± 0.05		58 ± 0.05	
		2.0	92 ± 0.06		67 ± 0.04	

Evaluation of Antioxidant potential of the *Elettaria cardamomum*

In this study, the ability of the *Elettaria cardamomum* essential oil to scavenge free radicals was assessed by a DPPH assay. The free radicals were captured by the essential oil in a dose-dependent manner, which is represented in **(Table 3)**. According, to the research findings, the oil sample demonstrated the highest potential at 1000 µg/mL with 86.41% activity, whereas the least capacity to neutralize free radicals was observed at 100 µg/mL with 11.20%. The graph depicts the antioxidant potential of *Elettaria cardamomum* essential oil at different concentrations with IC50 value as seen in (**Figure 5)**. The capability of the oil to scavenge the DPPH free radicals could be attributed to 1,8-Cineole [35], Eugenol [36], and β-caryophyllene epoxide [37], which were validated by our GC-MS analysis. Previous studies on *Elettaria cardamomum* essential oil reported 67.04 ± 0.04% antioxidant activity at 1000 µg/mL, which is considerably less compared to our results [38]. Furthermore, multiple studies have been carried out on essential oils and their constituents as a substituent food additive to synthetic antioxidants, like butylated hydroxytoluene, butylated hydroxyanisole, and propyl gallate, which have been linked to adverse health consequences in humans [39], and lead to the use of natural antioxidants in food and pharmaceutical industries.

Table 3: Anti-oxidant potential determination of *Elettaria cardamomum* essential oil by DPPH assay.

Sr.no.	Concentration (µg/mL)	% Inhibition
1.	100	11.2 ± 0.08
2.	200	15.1 ± 0.07
3.	300	19 ± 0.08
4.	400	27.4 ± 0.08
5.	500	35 ± 0.08
6.	600	42 ± 0.08
7.	700	61.3 ± 0.09
8.	800	64.4 ± 0.09
9.	900	78 ± 0.08
10.	1000	86.4 ± 0.08

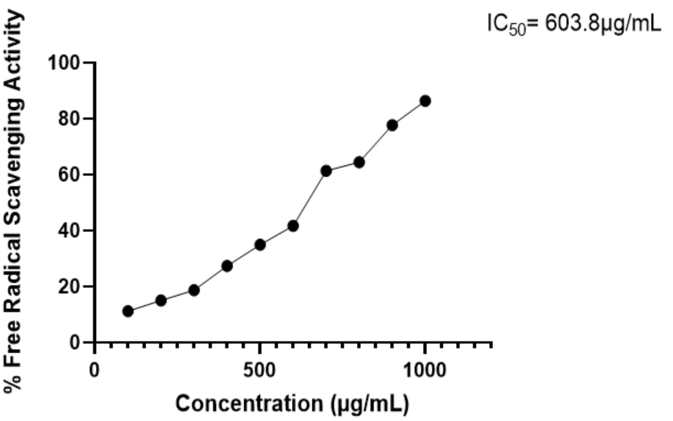

Figure 5: DPPH Free Radical Scavenging activity of essential oils obtained from *Elettaria cardamomum.*

Anticancer activity of *Elettaria cardamomum* essential oil

It was deduced that essential oils possess anticancer properties, as there is an association between the formation of reactive oxygen species and the origin of oxidation and inflammation, both of which can contribute to the disease [40]. In vitro studies have used essential oil extracts as they target cancer cells while inflicting minimal damage to healthy cells [41]. For the evaluation of the anticancer potential of the *Elettaria cardamomum* essential oil on the HeLa cell line, MTT assay was performed. The various concentrations made from the oil sample (5, 10, 20, 40, 60, 80, and 100 µg /mL) were tested against the HeLa cell line. MTT (3-(4,5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide) assay is one of the most widely utilized colorimetric assays to evaluate cell viability. This assay measures cell viability primarily by assessing mitochondrial function in cells by measuring the activity of mitochondrial enzymes like succinate dehydrogenase. MTT is converted to purple formazan crystals by NAD(P)H-dependent oxidoreductase enzymes in this assay, which is estimated spectrophotometrically at a specified wavelength of 550nm [42]. With the increasing concentration of the essential oil, the production of formazan crystals decreased resulting in a colourless product. IC_{50} value for the HeLa cell lines was found to be 28.71 mg/mL, signifying the required concentration of the essential oil for the 50 percent inhibition of the Hela cells. (**Table 4**) The HeLa cell lines present a dose-dependent susceptibility to the varied concentration of the essential oil. (**Figure 6**) As shown by the literature, essential oils (EOs) cause cancer cells to undergo programmed cell death by apoptosis, necrosis, cell cycle arrest, and organelle malfunction. This is accompanied by an increase in the impacted cell's membrane fluidity, decreased Adenosine Triphosphate (ATP) production, a shift in pH gradient, and complete depletion of mitochondrial potential, all of which are key precursors to cell death. This role can be attributed to chemical components found in *Elettaria cardamomum* essential oil namely artemisinin [43] and 1,8-Cineole [35].

Table 4: Evaluation of anticancer activity of essential oils obtained from Elettaria cardamomum.

Concentration of EO (µg /mL)	% Inhibition	IC_{50} (mg/mL)
5	4.1 ± 0.08	
10	6.7 ± 0.08	
20	30.5 ± 0.09	
40	63.1 ± 0.08	28.71
60	93.4 ± 0.09	
80	96.2 ± 0.08	
100	96.2 ± 0.08	

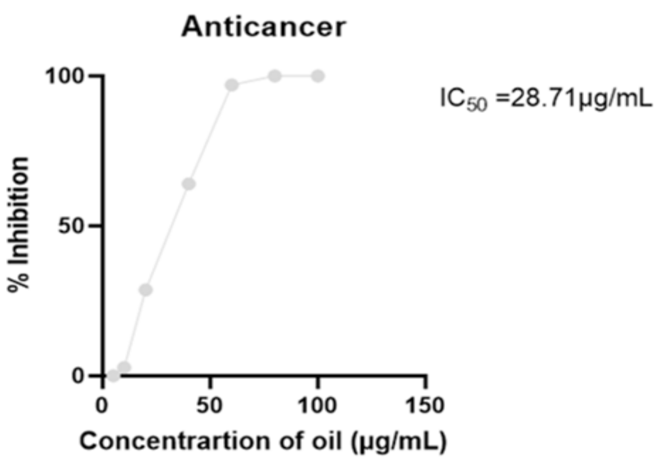

Figure 6: Evaluation of Anti-cancer potential of essential oil obtained from *Elettaria cardamomum.*

Conclusion

A paradigm shift has been witnessed towards the use of natural compounds in the pharmaceutical as well as food industries owing to their potential characteristics and negligible side effects. Our research explores the use of essential oil extracted from *Elettaria cardamomum* in order to comprehend its physio-chemical and biological potential. Therefore, the findings of our study evaluated the chemical composition of the oil through GC-MS analysis, which revealed major potential compounds. Furthermore, the thermal stability of the oil was investigated by adopting methods like TG-DTA and DSC. In addition, the anti-microbial potential was demonstrated against resistant bacterial strains and moderate antioxidant activity was also observed. The oil was found to be effective against malarial and tuberculosis pathogens with potential findings, along with this the oil also showed the anticancer potential towards the HeLa cell line. Further, the oil suppressed the biofilm formation by various microbial species. The capabilities shown above suggest that *Elettaria cardamomum* essential oil has a combination of numerous potential components, which was confirmed in our investigation using GC-MS analysis.

Author contributions

All of the authors mentioned have a significant, practical, and intellectual contribution to the work and have given their permission for it to be published.

Conflict of interest

The authors claim that there were no commercial or financial ties that might be interpreted as a possible conflict of interest during the research.

References

1. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Med (Basel, Switzerland). 2016; 22; 3: 25.
2. Ashokkumar K, Vellaikumar S, Murugan M, Dhanya MK, Ariharasutharsan G, et al. Essential Oil Profile Diversity in Cardamom Accessions From Southern India. Front Sustain Food Syst. 2021; 5: 1-8.
3. Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol. 2001; 91: 453-462.
4. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines. 2016; 3: 25.
5. Hamzaa R, Osman N. Using of Coffee and Cardamom Mixture to Ameliorate Oxidative Stress Induced in γ-irradiated Rats. Biochem Anal Biochem. 2012; 01: 1-6.
6. Auti ST, Kulkarni YA. Neuroprotective effect of cardamom oil against aluminum induced neurotoxicity in rats. Front Neurol. 2019; 10: 1-17.
7. Souza AG, Ferreira RR, de Oliveira ER, Kato MM, Mitra SK, et al. Chemical Stabilization behind Cardamom Pickering Emulsion Using Nanocellulose. Polysaccharides. 2022; 3: 200-216.
8. Maurya A, Prasad J, Das S, Dwivedy AK. Essential Oils and Their Application in Food Safety. Front Sustain Food Syst. 2021; 5.
9. Bhavaniramya S, Vishnupriya S, Al-Aboody MS, Vijayakumar R, Baskaran D. Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain Oil Sci Technol. 2019; 2: 49-55.
10. Reyes-Jurado F, Navarro-Cruz AR, Ochoa-Velasco CE, Palou E, López-Malo A, Ávila-Sosa R. Essential oils in vapor phase as alternative antimicrobials: A review. Crit Rev Food Sci Nutr. 2020; 60: 1641-1650.
11. Cui H, Zhang C, Li C, Lin L. Inhibition mechanism of cardamom essential oil on methicillin-resistant Staphylococcus aureus biofilm. Lwt. 2020; 122: 109057.
12. Elyemni M, Louaste B, Nechad I, Elkamli T, Bouia A, et al. Extraction of Essential Oils of Rosmarinus officinalis L. by Two Different Methods: Hydrodistillation and Microwave Assisted Hydrodistillation. Sci World J. 2019; 2019.
13. Ratnasingham S, Hebert PDN. bold: The Barcode of Life Data System. Mol Ecol Notes. 2007; 7: 355.
14. McPhillie MJ, Cain RM, Narramore S, Fishwick CWG, Simmons KJ. Computational methods to identify new antibacterial targets. Chem Biol Drug Des. 2015; 85: 22-29.
15. Adams RP. Identification of Essential Oil Components by Gas Chromatograpy/Mass Spectrometry, 4th Edition. Illinois USA Allured Publ Corp Carol Stream. 2007; 804-806.
16. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines. 2017; 4: 58.
17. Baldin VP, Scodro RB de L, Lopes-Ortiz MA, de Almeida AL, Gazim ZC, et al. Anti-Mycobacterium tuberculosis activity of essential oil and 6,7-dehydroroyleanone isolated from leaves of Tetradenia riparia (Hochst.) Codd (Lamiaceae). Phytomedicine. 2018; 47: 34-39.
18. Bueno J, Escobar P, Martínez JR, Leal SM, Stashenko EE. Composition of three essential oils, and their mammalian cell toxicity and antimycobacterial activity against drug resistant-tuberculosis and nontuberculous mycobacteria strains. Nat Prod Commun. 2011; 6: 1743-1748.
19. Somrani M, Debbabi H, Palop A. Antibacterial and antibiofilm activity of essential oil of clove against Listeria monocytogenes and Salmonella Enteritidis. Food Sci Technol Int. 2022; 28: 331-339.
20. Rieckmann KH, Campbell GH, Sax LJ, Ema JE. Drug sensitivity of plasmodium falciparum. An in-vitro microtechnique. Lancet (London, England). 1978; 1: 22-23.
21. Divatia SM, Rajani DP, Rajani SD, Patel HD. Novel thiosemicarbazone derivatives containing benzimidazole moiety: Green synthesis and anti-malarial activity. Arab J Chem. 2019; 12: 1641-1651.
22. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull (Tokyo). 1988; 36: 2090-2097.
23. Sarvmeili N, Jafarian-Dehkordi A, Zolfaghari B. Cytotoxic effects of Pinus eldarica essential oil and extracts on HeLa and MCF-7 cell lines. Res Pharm Sci. 2016;11: 476-483.
24. Krishnaveni M, Santhoshkumar J. Advances in Bioresearch FT-IR, GC-MS / MS Analysis of Essential Oil from Coriandrum. 2017.
25. Abdullah, Asghar A, Butt MS, Shahid M, Huang Q. Evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of Chromobacterium violaceum. J Food Sci Technol. 2017; 54: 2306-2315.

26. Lall RK, Proctor A, Jain VP. A rapid, micro-FAME preparation method for vegetable oil fatty acid analysis by gas chromatography. JAOCS, J Am Oil Chem Soc. 2009; 86: 309-314.

27. Singh AK, Srivastava ON. One-Step Green Synthesis of Gold Nanoparticles Using Black Cardamom and Effect of pH on Its Synthesis. Nanoscale Res Lett. 2015; 10.

28. Olatunji OO, Akinlabi SA, Mashinini MP, Fatoba SO, Ajayi OO. Thermo-gravimetric characterization of biomass properties: A review. IOP Conf Ser Mater Sci Eng. 2018; 423.

29. Olopade BK, Nwinyi OC, Adekoya JA, Lawal IA, Abiodun OA, et al. Thermogravimetric analysis of modified montmorillonite clay for mycotoxin decontamination in cereal grains. Sci World J. 2020; 2020.

30. Souissi M, Azelmat J, Chaieb K, Grenier D. Antibacterial and anti-inflammatory activities of cardamom (Elettaria cardamomum) extracts: Potential therapeutic benefits for periodontal infections. Anaerobe. 2020; 61: 102089.

31. Abdullah, Asghar A, Algburi A, Huang Q, Ahmad T, et al. Anti-biofilm Potential of Elletaria cardamomum Essential Oil Against Escherichia coli O157:H7 and Salmonella Typhimurium JSG 1748. Front Microbiol. 2021; 12.

32. Kerekes EB, Vidács A, Takó M, Petkovits T, Vágvölgyi C, Horváth G, et al. Anti-biofilm effect of selected essential oils and main components on mono- and polymicrobic bacterial cultures. Microorganisms. 2019;7.

33. Niu C, Gilbert ES. Colorimetric method for identifying plant essential oil components that affect biofilm formation and structure. Appl Environ Microbiol. 2004; 70: 6951-6956.

34. Van Zyl RL, Seatlholo ST, Viljoen AM. Pharmacological interactions of essential oil constituents on the in vitro growth of Plasmodium falciparum. Vol. 76, South African Journal of Botany. 2010: 662-667.

35. Cai ZM, Peng JQ, Chen Y, Tao L, Zhang YY, et al. 1,8-Cineole: a review of source, biological activities, and application. J Asian Nat Prod Res. 2021; 23: 938-954.

36. Nenadis N, Papapostolou M, Tsimidou MZ. Suggestions on the contribution of methyl eugenol and eugenol to bay laurel (Laurus nobilis l.) essential oil preservative activity through radical scavenging. Molecules. 2021; 26.

37. Dahham SS, Tabana YM, Iqbal MA, Ahamed MBK, Ezzat MO, Majid ASA, et al. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. Molecules. 2015; 20: 11808-11809.

38. Alam A, jawaid T, Alam P. In vitro antioxidant and anti-inflammatory activities of green cardamom essential oil and in silico molecular docking of its major bioactives. 2021; 15: 757-768.

39. Do Nascimento LD, de Moraes AAB, da Costa KS, Galúcio JMP, Taube PS, Costa CML, et al. Bioactive natural compounds and antioxidant activity of essential oils from spice plants: New findings and potential applications. Biomolecules. 2020; 10: 1-37.

40. Bayala B, Bassole IHN, Scifo R, Gnoula C, Morel L, et al. Anticancer activity of essential oils and their chemical components - A review. Am J Cancer Res. 2014; 4: 591-607.

41. Blowman K, Magalhães M, Lemos MFL, Cabral C, Pires IM. Anticancer Properties of Essential Oils and Other Natural Products. Evidence-based Complement Altern Med. 2018; 2018.

42. Aslantürk ÖS. In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages. Genotoxicity - A Predict Risk to Our Actual World. 2018; 1-18.

43. Anibogwu R, De Jesus K, Pradhan S, Pashikanti S, Mateen S, Sharma K. Extraction, isolation and characterization of bioactive compounds from artemisia and their biological significance: A review. Molecules. 2021; 26.