Advances in Research

23(5): 50-69, 2022; Article no.AIR.91810
ISSN: 2348-0394, NLM ID: 101666096

GC-MS Analysis, Thermal Characterization and Biomedical Applications of Essential Oil from *Cymbopogon martinii*: *In vitro* Approach

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Authors' contributions

This work was carried out in collaboration among all authors. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2022/v23i5912

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/91810

Original Research Article

Received 09 July 2022
Accepted 18 September 2022
Published 22 September 2022

ABSTRACT

Background: Plant derived metabolites have served as the foundation of sophisticated systems in traditional medicine that have existed for hundreds of years and continue to supply mankind with novel treatments. Owing to its antibacterial characteristics, Gingergrass (*Cymbopogon martinii*) has been utilized in aromatherapy as a skin tonic. It's also been employed in Ayurvedic medicine to treat skin conditions and nerve discomfort. It helps with neuralgia, epilepsy, and anorexia, among other central nervous system disorders. Geraniol, the main constituent of *Cymbopogon martinii* essential oil, has numerous biological properties, including antibacterial, antioxidant, and anti-inflammatory activities, and may constitute a new class of therapeutic medicines against pancreatic

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and colon malignancies. In this study, an attempt was made to explore the physiochemical and bioactive properties of *Cymbopogon martinii* Essential oil to understand the chemical composition and thermal stability of the oil along with its efficacy against biological agents. The hydro-distillation method was used to extract the Gingergrass (*Cymbopogon martinii*) Essential oil and its chemical composition as well as Thermal characteristics was determined. The essential oil's potential to scavenge free radicals was evaluated, as well as its anti-inflammatory effect was determined. The essential oil's antibacterial potential was tested against a variety of drug-resistant microorganisms, while its antimalarial efficacy was investigated against *Plasmodium falciparum*. In addition, the EO was tested against Hela and CHO cell lines for anticancer and cytotoxic activities, respectively.

Results: The GC-MS Analysis of the essential oil characterized 10 major compounds which included different classes of alcohols, esters, ketones, monoterpenes and sesquiterpenes. Thermogravimetric and differential calorimetric analysis revealed that the oil showed a stable thermograph when exposed to constant increasing temperatures. The essential oil exhibited promising antimicrobial and antimalarial activity against the drug-resistant pathogens. Further, moderate free radical scavenging activity was observed during DPPH Assay. The essential oil also showed reliable anti-inflammatory, anticancer and cytotoxic activity.

Conclusion: According to the research, *Cymbopogon martinii* essential oil has a number of medicinal benefits and can be used as a therapeutic agent to address a range of health issues.

Keywords: Cymbopogon martini; essential oil; GC-MS; FTIR; TG-DTA; DSC; antimicrobial; antimalarial; antioxidant; anti-inflammatory.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectroscopy; FT-IR: Fourier Transform Infrared Spectroscopy; DSC: Differential Scanning Calorimetry; TGA: Thermogravimetric Analysis; MIC: Minimum Inhibitory Concentrations; DPPH: 2,2'- diphenyl-1-picrylhydrazyl; LB: Luria-Bertani.

1. INTRODUCTION

Due to high consumer demand in culinary, medicinal, and other anthropogenic purposes, interest in medicinal and aromatic plants and their appeal continues to expand. Scientists are becoming increasingly aware of the advantages and possible uses of medicinal and aromatic plants and their metabolites [1]. Essential oils are one of the many secondary metabolites produced by these plants. Essential oils have been used for medicinal purposes from ancient times since they are fascinating and strong natural plant products. They have been used for thousands of years as fragrances, flavours for food and beverages, and to treat both the body and the mind, hence being of paramount importance [2]. Since millennia, essential oils have been utilised medically to treat skin infections as well as respiratory, digestive, endocrine, cardiovascular, and nervous system disorders. In the cosmetic and perfume industries, they are employed as aromatic agents. Alcohols, ethers, oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles and primarily terpenes are some of the volatile organic molecules found in essential oils [1]. EOs (essential oils) are volatile liquids or semi-liquids extracted from plants using steam distillation [3].

EOs are of scientific and popular interest because (a) they may act synergistically with other preservation techniques, (b) they are generally recognised as safe and (c) they show antioxidant, antibacterial, antidiabetic, antimutagenic, non-toxigenic and antimycotic properties that are promising for their use as bioactive compounds in various foods [4]. Plants synthesize essential oils for a variety of reasons, including protection against fungi and bacteria, allelopathic activity, insect defence (terpenoids are known to repel animals and insects), and pollinator and dispersal agent attraction to aid seed and pollen dispersal (the aromas and scents of the plants are responsible for the attraction of insects by the essential oils, characterised by volatility and a strong odour). Plants can be protected by essential oils from viruses, bacteria, fungus, insects and herbivores. Antimicrobial, sedative, anti-inflammatory, antibacterial, antiviral, antifungal (fungicidal) and preservation for foods are some of the key properties of essential oils [5]. The chemical characteristics and bioactivity of essential oils have piqued people's curiosity in

recent years. Essential oils have been demonstrated in studies to have antibacterial, antioxidant, cytotoxic, and antimalarial properties, suggesting that they might be employed as a substitute for synthetic substances in the health, environment, and agriculture sectors [1].

It would be beneficial to have a deeper knowledge of the chemistry and biological characteristics of these extracts, as well as their individual components, to find novel and useful applications in human health, agriculture, and the environment. Essential oils might be used as efficient substitutes or complements to synthetic chemical molecules without causing the same negative side effects. Even though many plants have been studied for their essential oils, there are still a great number of oils that are yet to be explored, one such important essential oil belongs to the plant *Cymbopogon martinii* (commonly known as "Gingergrass oil"). *Cymbopogon martinii* belongs to the grasses species of the genus *Cymbopogon* (lemongrasses) native to India and Indochina, but widely grown for its fragrant oil in many countries. The essential oil is known to have high demand in perfumery industries, tobacco products, and cosmetics. *Cymbopogon martinii* is very important member of Gramineae family which is very famous for its high oil content [6]. The main chemical elements of its oil are geraniol and geranyl acetate, which have a rose-like aroma and are in high demand in the perfumery, food flavouring, and pharmaceutical sectors. It is also one among the world's top ten essential oils [7]. The oil's antibacterial, insect repellent, and pain-relieving characteristics make it important in the pharmaceutical industry [8]. According to Millezi et al. [9], the essential oil of *Cymbopogon martinii* reduces biofilm by 60 to 93 percent (2020).

Over time, researchers have paid close attention to the chemical makeup of the plant, which has aided in the development of *Cymbopogon martini* essential oils for aromatherapy and other medicinal applications. There has been a significant amount of research done on the medicinal potential of extracts from *Cymbopogon martinii* plant species, wherein its essential oil and its components are known for their antimicrobial [10,11] anti-helmintic [12], antiparasitic [13], anti-inflammatory [14], anticonvulsant [15] and antioxidant activities [16]. The objective of this research study was to identify the chemical composition, fatty acid profile, and thermal characteristics of the essential oil extracted from *Cymbopogon martinii*, as well as to evaluate the oil's biological potential. The essential oil of *Cymbopogon martinii* was first analysed using GC-MS to identify its volatile content and fatty acid profile. Thermogravimetry (TGA) and Differential Scanning Calorimetry (DSC) were used to investigate the oil's thermal characteristics. The oil was also tested to see whether it might be used as an antibacterial, antioxidant, anticancer, and antimalarial agent.

2. METHODS

Sample collection and extraction of essential oil: *Cymbopogon martinii* was purchased from a local market in Mumbai, Maharashtra. After which, it was taken to the research facility. Clevenger hydro-distillation method was used to extract the essential oil from the grass. [17] 100 g of the plant sample and 800 ml of distilled water were combined in a 2-liter flask. The flask was kept in a balloon warmer that was attached to a refrigerator for three hours to ensure the condensation of the essential oil. After distillation, two phases were produced: an aqueous phase (aromatic water) and an organic phase (essential oil), which is less dense than water. The oil was dried over magnesium sulphate after extraction and stored at 4°C in an amber container. The yield of essential oil was expressed in g relative to 100 g of dry vegetable matter. Following extraction, a yield of 12% was obtained and estimated using the formula below:

$$\text{Yield (\%)} = \frac{\text{Amount of extracted oil (g)}}{\text{Amount of dry vegetal matter mass (g)}} \times 100$$

Gas chromatography-mass spectrometry (GC-MS) analysis: The extracted essential oil was tested both qualitatively and quantitatively using the Shimadzu GCMS-QP2010 system along with the RTX-5MS capillary column (5% diphenyl/ 95% dimethyl polysiloxane) which was 30 m long with an internal diameter of 0.25 mm and the film of the stationary phase was 0.25 µm thick. In the beginning, electron impact mode produced 70 eV of ionization energy. In this process, Helium gas (99.999%) was utilized as a carrier gas at a constant flow rate of 1.00 mL/min, and an injection volume of 1 µL was used, whereas the carrier gas saver split ratio was set at 5.0. The injector temperature was kept at 250°C, whereas the ion-source temperature was 220°C and the oven temperature was set from 70°C (isothermal for 1 min), with an increase up to 260°C at the fifth interval. The

total span of the program was 42 min. The mass spectra were recorded across the range of 50 to 650 m/z. The *Cymbopogon martinii* essential oil components were determined on a comparative basis with the Mass Spectral Libraries of Wiley 6.0 and the National Institute of Standards and Technology (NIST) as well as the literature data of retention indices collected from [18].

Fatty acid methyl ester (FAME) analysis: FAME analysis was carried out for *Cymbopogon martinii* essential oil by using the method specified by R.K Lall [19] as a reference with slight modifications. A sample of 100 mg was placed in centrifuge tubes, while 1 mL of toluene, and 0.5 M sodium methoxide were added to this sample, and it was further purged with nitrogen gas. In a water bath, the mixture was heated to 60°C for 50 minutes before cooling for 5 minutes. To halt the reaction, 0.2 mL glacial acetic acid was added to the mixture. After adding 5 mL of distilled water and 1 mL of hexane to the mixture, the tubes were vortexed for 2 minutes. In a glass vial, the hexane layer was extracted and dried for 15-20 seconds over anhydrous sodium sulphate [19]. The resulting FAME were analyzed with the Shimadzu GCMS-QP2010 system, which consists of a Gas Chromatograph connected to a Mass Spectrophotometer and a 30 m long capillary column (FAMEWAX) with a 0.32 mm internal diameter and 0.25 µm film coating. In the beginning, electron impact mode produced 70 eV of ionization energy. Helium (99.999 percent) was utilized as the mobile phase, with a flow rate of 3 ml/min, to separate the volatile components. The injector temperature was kept at 250°C, and the sample volume injected was 1 µl (split ratio of 1:50). The starting oven temperature was kept at 120°C for 1 minute before being raised to 170°C at a rate of 20°C/min, then raised to 210°C at 3°C/min, and lastly raised to 250°C at 20°C/min and held for 10 minutes. At 70 eV, mass spectra were logged spanning the range of 40 to 1000 m/z. At 70 eV, mass spectra were logged spanning the range of 40 to 500 m/z. A comparison of their mass spectra with those of Wiley and NIST libraries [20] and those described by Adams 2007 [18] as well as a comparison of their retention indices with literature was carried out to identify the components.

Fourier transform infrared (FTIR) analysis: The functional group was analysed with a stabilised IRPRESTIGE 21 Shimadzu Fourier transform infrared spectrometer. After preheating, a drop of essential oil was placed in a clean KCl pellet and subjected to the stabilised apparatus to perform the functional group analysis. The other salt pellet was pressed against the KCl pellet to create a vesicular tiling of the oil sample between the two KCl pellets. The KCl pellets were rotated to generate a homogenous liquid membrane before being placed in sample holders of infrared spectrometer. The infrared spectrometer was calibrated to absorbance with a 1 cm-1 resolution and a 45-time repetition scan before fastening and inserting the mixture onto the sample holder. Under the operative conditions, the infrared absorption spectra of essential oil were obtained across the spectral range of 4000–400 cm^{-1}. The resolution was 4 cm^{-1} and the number of spectral accumulations was 45.

Thermogravimetric and differential thermal analysis (TGA): Thermogravimetric and Differential Thermal analysis analysis were conducted using NETZSCH STA 2500. The instrument was turned on the night prior to the study to stabilize cryostat and the balance properly overnight. A syringe or a disposable pipette was used for handling the sample and was placed in an aluminium crucible. The crucibles are cleaned with a mixture of 0.1M hydrochloric acid (HCl) in water, put in a muffle oven and kept at high temperatures for some time, to ensure that all the residues have been removed prior its use. An empty crucible was placed in the balance and a measurement launched with the same exact conditions (heating rate, gas flow, kind of crucible, etc.) as the ones expected to be used in the measurement of the sample for blank measurement. Initially, measurements were recorded from 30℃ while the heating rate was 20℃/min [21]. Prior to beginning the analysis, the sample's TGA curve was subtracted from the most stable baseline from the executed blank measurements. The DTG of the mass loss curve's first derivative was calculated in order to accurately take into account any thermal event. By drawing two horizontal lines across the TGA curve, one before and one after the event, and taking the difference between them, one can calculate the mass loss caused by that particular event. The start and end of the event, or the locations where the curve's value is zero, were found using the curve [22].

Differential scanning calorimetry: To generate the DSC profile of the essential oil under research, a TA Instruments with DSCQ20 model was used. The instrument was switched on

approximately an hour before the measurement for the furnace to reach thermal equilibrium. A suitable sample crucible has been chosen considering compatibility with the measured sample. Here, an aluminum crucible was used where sample was introduced using a syringe or a disposable pipette. 3 mg sample was placed in an aluminium crucible. While preparing the sample, a specific portion from the bulk material is selected and if necessary mixed or stirred prior preparing the sample. If the sample was liquid, (made of glass or plastic) was used to pour the sample into the crucible. Purge gas flow was set to 40 mL/min where Nitrogen was used as a purge gas. Further, dynamic scan was performed at a heating rate of 10°C/min throughout a temperature range of 25°C to 375°C. To prevent inaccurate results, any heat alteration or contamination of the material was avoided during sample preparation. The temperature programme consists of a number of cycles with two different rates of heating and cooling. The first cycle's goal is to eliminate the sample's prior thermal history before the measurement. The phase change enthalpy and temperature will be ascertained using the remaining cycles [22].

Antimicrobial activity: To assess the antimicrobial potential of *Cymbopogon martini* EO, a microdilution test was carried out according to CLSI standard methods with the following modifications [23]. The least inhibitory concentration against the selected 9 resistant bacterial strains was determined using a microdilution approach employing 96-well microtiter plates. The 9 resistant bacterial test strains included Carbapenem-Resistant *Acinetobacter* (CRA), Carbapenem-Resistant *Pseudomonas aeruginosa* (CRP), Carbapenem-Resistant *Escherichia coli* (CRE), Carbapenem-Resistant *Klebsiella pneumonie* (CRK), Extended Spectrum beta-lactamase *Escherichia coli* (ESBL), Quinolone resistant Salmonella (QRS), Vancomycin-resistant Enterococci (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA) and Erythromycin resistant *Streptococci* (ERS). The microbial suspensions were adjusted until a concentration of 1.0×10^6 CFU/mL was reached. Furthermore, the essential oil was dissolved in a mixture of 5 % DMSO and 0.1 % polysorbate-80 (1 mg/mL), and then added to a Luria- Bertani medium (100 µL) with a bacterial inoculum of 1.0×10^5 CFU/mL to attain the necessary concentrations. The plates were inoculated and incubated for 24 hours at 180 rpm at 37°C. After the incubation period, 5 µl of Resazurin dye (2 mg/mL) was added to each well to identify microbial growth by a development of pink coloration.

Anti-tuberculosis activity: The in vitro Anti Tuberculosis Activity of *Cymbopogon martini* essential oil was assessed against a susceptible strain of Mycobacterium tuberculosis (H37Rv) obtained from the National Institute for Research in Tuberculosis, Chennai, Tamil Nadu. Minimum Inhibitory Concentration of the essential oil was determined using Lowenstein and Jensen Method. The Lowenstein and Jensen (LJ) medium employed 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 [24]. Two-fold serial dilutions of the same were prepared in the supplemented L.J medium to attain 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 test culture suspension whose concentration was equivalent to the McFarland standard and incubated at 37°C with regular monitoring for growth. The reference *M. tuberculosis* H37Rv strain was tested against isoniazid as a standard drug with a concentration of 0.2 µg /L [25].

***In-vitro* antimalarial screening:** Using a 96-well microtiter plate, an *In-vitro* antimalarial experiment was performed to determine the efficiency of the essential oil against *Plasmodium falciparum*. In enriched RPMI-1640 growth medium, *Plasmodium falciparum* and its Quinine resistant strain were maintained. [26] Both parasites were treated with 5% D-sorbitol to achieve ring stage cells, followed by synchronisation of *P. falciparum* and Quinine resistant *P. falciparum*, as described by Lambros and Vanderberg [27]. In this assay, a parasite suspension comprising primarily of ring stage parasites was adjusted to 0.8 to 1.5% parasitaemia and 3% haematocrit before being added to 200 µl of RPMI-1640 medium. [28] The percentage parasitaemia (rings) was measured using the JSB (Jaswant Singh Bhattacharya) staining method, and the suspension was uniformly maintained with 50% RBCs (O^+). [29] A stock solution of the essential oil was prepared in 5mg/ml DMSO with subsequent dilutions ranging from 0.4 µg/ml to 100 µg/ml. 20 µl of the diluted samples were pipetted in test wells and duplicate wells containing both the parasitized cell suspensions respectively. In a candle jar, the

plates containing the preparation were incubated at 37°C for 36 to 40 hours [28]. After incubation, thin blood smears were taken from each well and stained with JSB staining technique. Chloroquine and Quinine were utilised as the experiment's control drugs. When varied concentrations of the test samples were employed, the development of ring stage parasites into schizonts and trophozoites was observed in a microscopic analysis of the slides. The concentration which demonstrated the complete maturation of both the *P. falciparum* strains into schizonts was noted as the Minimum Inhibitory Concentrations (MIC). The recorded IC_{50} value for each was later compared with the standard values.

Antioxidant activity: Measurement of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was carried out according to Karamać et al. [30]. According to which a 2.0 mL sample was mixed with 1 mL of a 0.5 mM DPPH radical methanol solution and 2.0 mL of a 0.1 M sodium acetate buffer at pH 5.5. The solutions were properly mixed and left at room temperature in the dark for 30 minutes. At 515 nm, the absorbance was measured with a twin beam UV-VIS spectrophotometer. Methanol was used as a negative control. At concentrations ranging from 100 to 1000 g/ml, a 3 ml aliquot of this solution was mixed with 100 µl of the sample. Before being incubated in the dark for 15 minutes at room temperature, the reaction mixture was thoroughly mixed and at 515 nm, the absorbance was measured. For each concentration, the % inhibition of the DPPH radical was computed using the following equation:

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

The sample concentration that gave 50% inhibition was determined by plotting inhibition percentages against sample oil concentrations (IC50).

Anti-inflammatory activity: The anti-inflammatory activity of the *Cymbopogon martini* essential oil was evaluated by employing Inhibition of protein denaturation assay. Inflammation is caused because of protein denaturation which in turn is observed because of loss of tertiary and quaternary structure of proteins. In this study, the potential of the Essential oil to inhibit inflammation was determined. A reaction mixture comprising of 1ml of essential oil sample of concentrations ranging from 10 to 100 µg/mL and 1% aqueous solution of egg albumin except for the control tube which included standard (Diclofenac) and saline was taken. The test tubes were incubated at 37°C for 20 minutes and further heated to 51°C for 20 minutes. Followed by the incubation, the test tubes were cooled and absorbance of the test solutions was determined at 660 nm using a spectrophotometer [31].

The percentage inhibition was calculated using the following formula:

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

Anticancer screening: The Anticancer potential of *Cymbopogon martini* essential oil was evaluated against human cervical cancer HeLa cell line which was obtained from National Centre for Cell Science (NCCS), Pune. Dulbecco's Modified Eagle Medium (DMEM) with 10% foetal bovine serum (FBS) and antibiotics (Penstrep) was used to culture the Hela cell line. Cells were passaged weekly, and the culture medium was replaced twice a week. The cell line was maintained at 37℃, 5% CO_2, 95% air and 100% relative humidity. Single cell suspensions were made by detaching monolayer cells with trypsin-ethylenediaminetetraacetic acid, and viable cells were counted using a haemocytometer. The cells were diluted in FBS medium with 5% FBS to obtain a final density of 1×10^5 cells/ml. Further, 100 µl of cell suspension was seeded in each well of 96-well plates at plating density of 10,000 cells/well and incubated at 37℃, 5% CO_2, 95% air and 100% relative humidity for cell adherence. [32] Followed by 24 h of incubation, the cells were treated with serial concentrations of essential oil sample. They were first dissolved in dimethyl sulfoxide (DMSO) and then diluted in serum free media to obtain final concentrations of 5, 10, 20, 40, 60, 80 and 100 µg/ml, 100 µl of each concentration was added to each well in 96-well plate. Each well had a final volume of 200 µl and were further incubated for 48 hours at 37℃, 5% CO_2, 95% air and 100% relative humidity. As a control, a medium with no sample was used. After incubation period, 15 µl of MTT (5 mg/ml) in phosphate buffered saline was added in each well and again incubated at 37°C for 4 hours. The medium containing MTT was then decanted, and the formed formazan crystals were solubilized in 100 µl of DMSO. The optical density was measured at 595 nm using micro plate reader. The percentage cell inhibition was calculated using the following formula:

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

Cytotoxicity: "The cytotoxicity of the *Cymbopogon martini* essential oil was assessed against Chinese Hamster Ovary (CHO) cells obtained from National Centre for Cell Science (NCCS), Pune. The cells were grown in Dulbecco's Modified Eagle Medium (DMEM) comprising of 10% foetal bovine serum (FBS) and antibiotics (Penstrep). The cells were maintained and passaged once a week as per the protocol mentioned above. Cytotoxicity activity was examined by employing the tetrazolium salt, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) colorimetric technique. 100 µl of cell suspension was seeded into each well on a 96-well plate and incubated at 37°C, 5% CO2, 95% air, and 100% relative humidity for cell adhesion" [33]. Followed by 24 h incubation, the cells were introduced with serial concentrations of oil sample which were prior dissolved in neat dimethyl sulfoxide (DMSO) and further diluted in serum free medium. Final concentrations of 5, 10, 20, 40, 60, 80 and 100 µg/ml was obtained by adding 100 µl of each concentration in the wells and obtaining a final volume of 200 µl. Later, the plates were incubated at 37℃, 5% CO_2, 95% air and 100% relative humidity for 48 h. Medium without sample was maintained as a control. Post 48 h of incubation, 15 µl of MTT (5 mg/ml) in phosphate buffered saline was added to each well and incubated at 37℃ for 4 h. The MTT-containing media was discarded, and the formazan crystals formed were solubilized in 100 µl of DMSO. The absorbance was determined at 595 nm by employing micro plate reader. The cytotoxicity was estimated as the concentration of sample that inhibited 50% of cell growth (IC_{50}). The percentage cell inhibition was determined using the following formula:

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

3. RESULTS

3.1 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

A total of 52 components of *Cymbopogon martini* essential oil were identified (Table 1). The essential oil was characterized by the presence of Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- (13.81%); .alpha. -Terpineol (12.98%); Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1S)- (10.74 %); Caryophyllene (6.96%); Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alp (5.6%); Cyclohexene, 4-isopropenyl-1-methoxymethoxymet (4.79%); Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4dimethyl-7-(4.62%); Cyclohexanol, 5-methyl-2-(1 methylethenyl)- (3.77%); Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylet (3.5%); Isobornyl thiocyanoacetate (3.37%). These major peaks were present at a retention time of 15.924; 8.275; 6.657; 12.71; 5.738; 4.374; 14.991; 9.335; 12.184; and 8.683 minutes respectively.

3.2 Fatty Acid Methyl Ester (FAME) Analysis

The essential oil was characterized by the presence of component such as Erucic acid followed by, 4,7-Methano-1H-indene, octahydro-5-(2-octyldecyl); 2-methyltetracosane; 2,6,6,9,2', 6',6',9'-Octamethyl [8,8']bi[tricyclo[5.4.0.0]], 4,7-Methano-1H-indene, octahydro-5-(2-octyldecyl); Hexadecanoic acid, methyl ester; Methyl stearate; Hexacosane; Eicosane; Heneicosane; Decane, 1-iodo-. The major peaks were present at the retention time of – 3.414, 5.982, 11.790, 2.635, 3.493 and 3.220 which corresponds to the presence of 4,7-Methano-1H-indene, octahydro-5-(2-octyldecyl), 2-methyltetracosane, Erucic acid, Hexadecanoic acid, methyl ester, Methyl stearate and 2,6,6,9,2',6',6',9'-Octamethyl [8,8'] bi [tricycle[5.4.0.0]]. The essential oil was predominantly associated with the presence of Erucic acid which depicted 40.60% percentage area followed by the presence of 4,7-Methano-1H-indene, octahydro-5-(2-octyldecyl) and 2-methyltetracosane with percentage area of 16.62% and 11.03%.

3.3 Fourier Transform Infrared (FTIR) Analysis

FTIR spectrum of *Cymbopogon martinii* essential oil represented 10 diverse peaks at different corresponding wavenumbers (Fig. 1). Firstly, a strong peak at 426.27 is seen which represents a C-I group. A C=O & C-O stretch is seen at wavenumbers 1018.41 & 1818.87. The peak at 1056.99 corresponds to C-N stretching. The presence of SiO stretch can be correlated with the presence of a peak at 1109.07. The 1130.29 peak reflects CN. The presence of N-H bend may be seen in the peaks 1591.27. Peak 2289.5 indicates an C≡N stretch, while peaks 2953.02 and 3442.94 indicate a C-H & O-H stretch.

Table 1. Table representing the major components identified from the gas chromatogram of *Cymbopogon martini*

Sr. no	Component name	Molecular formula	Molecular weight	Classification	Component composition (%)	Retention time (mins)
1	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	C12H16O	208.2536	sesquiterpene	13.81	15.924
2	.alpha.-Terpineol	C10H18O	154.25	monoterpenoid	12.98	8.275
3	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	C10H16O	152.2334	monoterpenoid	10.74	6.657
4	Caryophyllene	C15H	203.3511	terpenoid	6.96	12.71
5	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alp	C10H18O	154.2493	terpenoid	5.6	5.738
6	Cyclohexene, 4-isopropenyl-1-methoxymethoxymet	C12H20O2	152.24	monoterpene hydrocarbon	4.79	4.374
7	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(	C10H8	128.17	sesquiterpene	4.62	14.991
8	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	C10H18O	154.25	terpenoid	3.77	9.335
9	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylet	C15H24	204.3511	terpenoid	3.5	12.184
10	Isobornyl thiocyanoacetate	C13H19NO2S	253.360	monoterpenoid	3.37	8.683

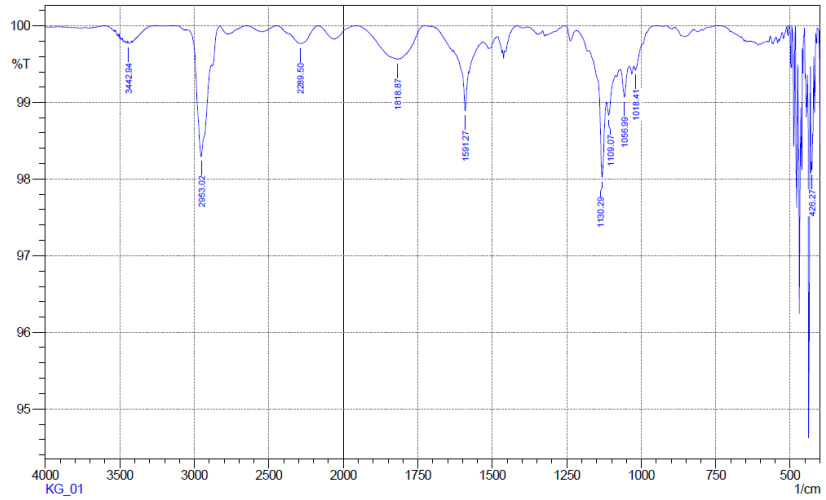

Fig. 1. FTIR spectra representing different functional components present in the *Cymbopogon martinii* essential oil

3.4 Thermogravimetric and Differential Thermal Analysis (TGA)

Thermogravimetric and differential thermal analysis curve provides a convenient way of obtaining kinetic parameters for a decomposition reaction and thus helps to determine the thermal stability during a simple pyrolysis reaction [34]. The TG-DTA analysis of *Cymbopogon martinii* essential oil was carried out to determine the thermal stability and mass loss when subjected to constant increasing temperatures (Fig. 2). The thermogravimetric curve represents a single-stage decomposition reaction. Initially, the oil shows stability at temperatures ranging from 50 to 100°C. At 155.8°C there is an onset of thermal degradation thus showing a sharp decrease in the curve up to 200°C after which the curve continues to gradually decrease when exposed to temperatures between 200 to 450°C. This curve represents complete mass loss from 100% to 0% thus, depicting decent thermal stability. The differential thermal analysis curve initially shows a small endothermic dip at around 50°C from 0.5 to less than 0.2 mW/mg of sample, further as temperature increases the curve shows an exothermic behavior with a complex peak at an onset temperature of 190.6°C, the curve later stabilizes at around 250°C ranging from 0.5 to 1.4 mW/mg of sample.

3.5 Differential Scanning Calorimetry

The differential scanning calorimetry curve of *Cymbopogon martinii* essential oil shows an endothermic and exothermic behavioral pattern (Fig. 3). Which helps to elucidate the structural and functional changes in the oil at increasing temperatures. Initially, the DSC curve revealed an endothermic behavior starting from 88.08 °C up to 143.03°C with a decrease in heat flow from -0.9207W/g to -1.389 W/g. This corresponds to the volatilization of components and sample phase transition. Further, the curve shows an exothermic pattern until the temperature of 201.64°C is reached with an increase in heat flow to -0.09691 W/g. The curve later adopts a stable trend at a temperature of 307.20°C with an increased heat flow of 0.1254 W/g, this represents functional changes and auto-oxidative reactions of the sample oil.

Antimicrobial activity: The susceptibility of the test organisms towards the oil was in the range of 100 to 500 µg/mL (Table 2). Carbapenem-Resistant Acinetobacter (CRA)and Quinolone resistant Salmonella (QRS) were most susceptible to the essential oil as both showed a MIC of 100 µg/mL. Methicillin-resistant *Staphylococcus aureus* (MRSA) was most resistant as it was inhibited at a minimum concentration of 500 mg/mL. The other organisms showed MIC range between 125 to 250 µg/mL (Fig. 4). Anti-Tubercle activity of *Cymbopogon martini* essential oil was evaluated using MIC method. The MIC of essential against Mycobacterium tuberculosis (H37Rv) was observed to be 25 µg/mL which was relatively higher as compared to the standard.

Antimalarial activity: The concentration of the essential oil required to inhibit 50% of the cells

against against the *Plasmodium falciparum* strain and Quinine Resistant Strain was estimated to be 1.56 µg/ml and 2.26 µg/ml, respectively (Table 3). Both the IC_{50} values were higher as compared to the standard drugs Chloroquine and Quinine which exhibited IC_{50} values 0.020 µg/ml and 0.268µg/ml, respectively (Fig. 5). This could be due to the presence of additional components in the essential oil.

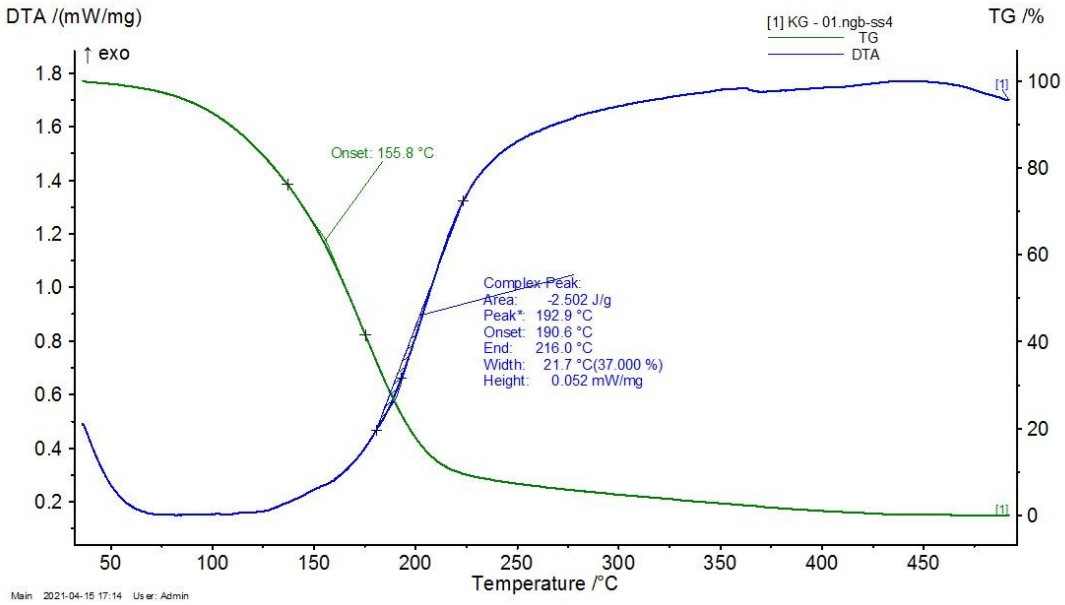

Fig. 2. Thermogram representing the thermogravimetric and differential thermal analysis of *Cymbopogon martinii* essential oil at constantly increasing temperatures

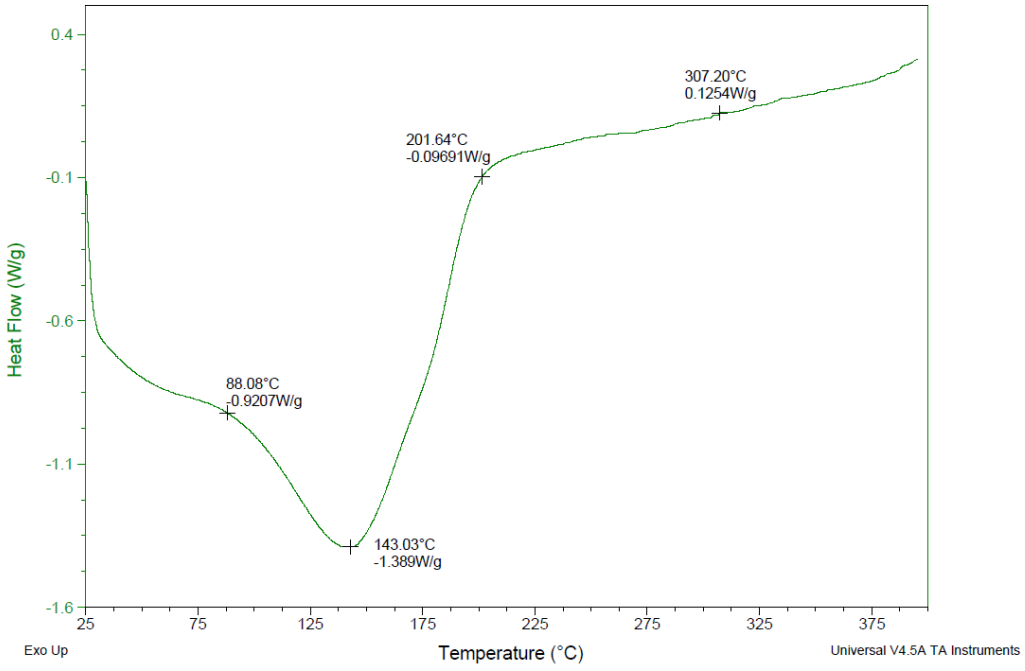

Fig. 3. Thermogram representing differential scanning calorimetric analysis of *Cymbopogon martinii* essential oil at constantly increasing temperatures

Table 2. Examination of antimicrobial properties of essential oil isolated from *Cymbopogon martini*

Serial no.	Strain name	MIC (µg/mL)
1	Carbapenem Resistant *Acinetobacter species*	99.93 ± 0.09
2	Carbapenem Resistant *Pseudomonas aeruginosa*	200.05 ± 0.04
3	Carbapenem Resistant *E. coli*	250.06 ± 0.05
4	Carbapenem Resistant *Klebsiella pneumonie*	249.95 ± 0.07
5	Extended Spectrum beta lactamase *E. coli*	199.93 ± 0.09
6	Vancomycin resistant *Enterococci*	250.05 ± 0.04
7	Quinolone resistant *Salmonella*	99.93 ± 0.09
8	Methicillin resistant *Staphylococcus aureus*	499.93 ± 0.09
9	Erythromycin resistant *Streptococci*	124.93 ± 0.09

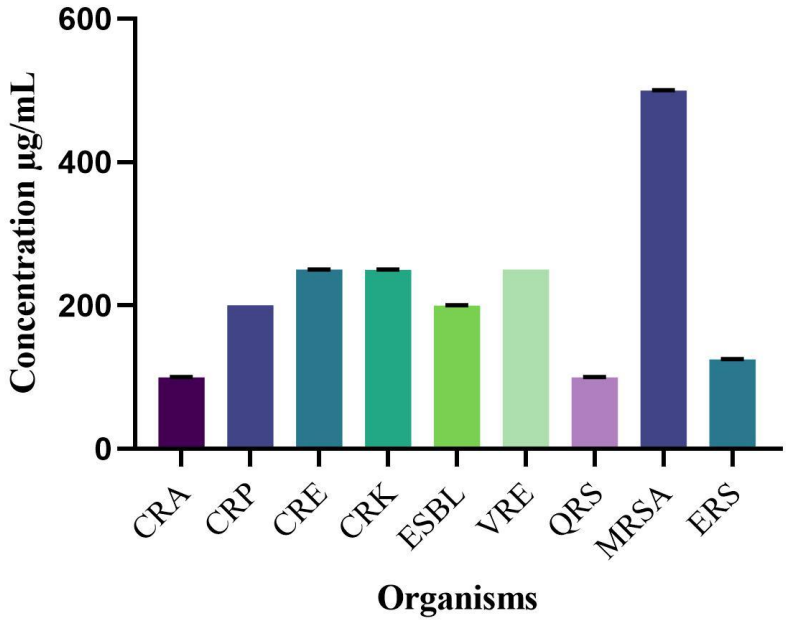

Fig. 4. Graph representing the minimum inhibitory concentration of *Cymbopogon martinii* essential oil against selected drug-resistant strains

CRA- Carbapenem Resistant Acinetobacter species, CRP- Carbapenem Resistant Pseudomonas aeruginosa, CRE- Carbapenem Resistant E. coli, CRK- Carbapenem Resistant Klebsiella pneumonie, ESBL- Extended Spectrum beta lactamase E. coli, VRE- Vancomycin resistant Enterococci, QRS- Quinolone resistant Salmonella, MRSA- Methicillin resistant Staphylococcus aureus, ERS- Erythromycin resistant Streptococci.

Table 3. Evaluation of anti-malarial activity of *Cymbopogon martini* essential oil

Concentration (µg/ml)	Drug sensitive plasmodium falciparum		Drug resistant plasmodium falciparum		Standard drug (Control)	
	% Inhibition	IC50	% Inhibition	IC50	IC50 (µg/ml)	
0.1	12.8	1.56	7.56	2.26	chloroquine	0.02
0.2	16.7		10.24		quinine	0.268
0.4	26.7		12.34			
0.8	34.5		18.54			
1.2	40.2		24.3			
1.6	52.3		36.2			
2.0	59.05		41.2			

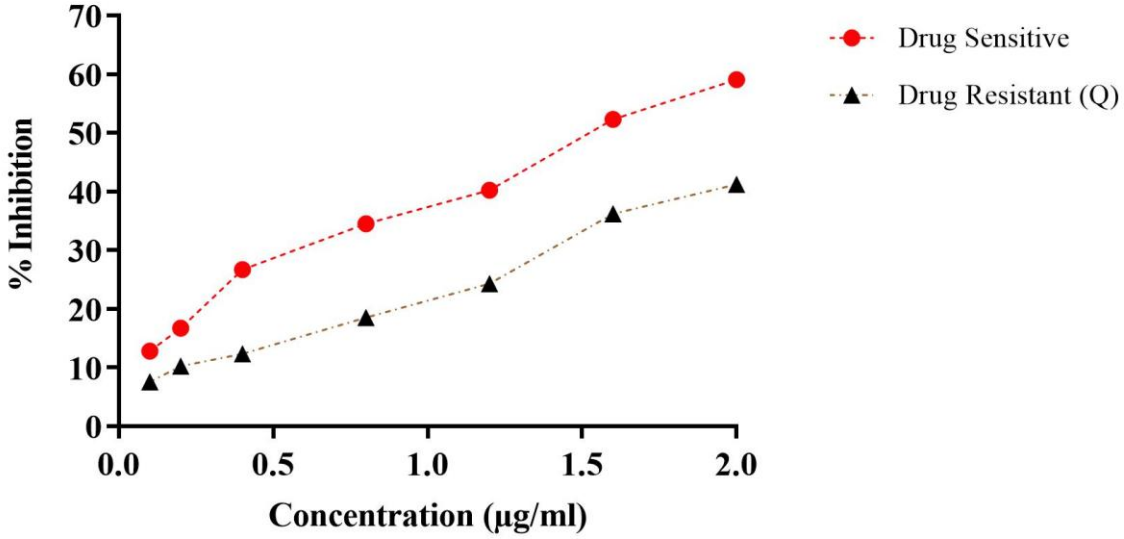

Fig. 5. Graphical representation of antimalarial activity imparted by *Cymbopogon martini* essential oil on drug-resistant as well as drug-sensitive strains of *plasmodium falciparum*

Antioxidant activity: The highest radical scavenging activity was recorded at the oil concentration of 1000 µg/ml with a percentage inhibition activity of about 88.27%, whereas the lowest percentage activity was seen at 100 µg/ml which was 70.71% (Table 4). Compared to standard ascorbic acid which showed the highest activity at 1000 µg/ml with a percentage inhibition activity of 98.98% whereas the lowest percentage inhibition activity was seen at 100 µg/ml which was 7.14% (Fig. 6). This shows that the essential oil has promising antioxidant potential, however when compared to the standard the sample showed a lesser percentage of inhibition at the highest concentration.

Anti-inflammatory activity: There was a minimal increase in the range of percentage inhibition of inflammation (Table 5). At lowest concentration of 10 µg/ml of essential oil the percentage inhibition was found to be 92.76 % and at highest concentration of 100 µg/ml it was found to be 96.21 %. The initial percentage inhibition for standard was 89.81% and for highest concentration it was 97.68 % (Fig. 7). It can be observed that as the concentration of essential oil increases the optical density decreases which corresponds to good anti-inflammatory behavior. The overall percentage inhibition of inflammation was found to be like that of the selected standard i.e., Diclofenac.

Anticancer: After spectrophotometric analysis of the essential oil, a decrease in the optical density with an increase in the concentration of the essential oil was observed. Initial concentration exhibited a darker shade of formazan crystals whose intensity decreased with the concentration giving a colourless product. As a result, it can be concluded that at concentrations 5, 10, 20, 40, 60, 80, and 100 µg/ml of the sample, inhibition in proliferation of the Hela cells was observed (Table 6). The formazan production was inhibited resulting in a colourless product. The IC_{50} value was found to be 55 µg/ml, indicating the concentration of the oil required to inhibit 50 % of the Hela cells (Fig. 8).

Cytotoxicity: According to spectrophotometric analysis, the optical density decreased as the concentration of the essential oil increased. The initial concentration resulted in a deeper shade of formazan product, which diminished in intensity as the concentration was increased, eventually leaving a colourless product. Hence, it can be concluded that concentrations of 5, 10, 20, 40, 60, 80, and 100 µg/ml inhibited CHO cell proliferation (Fig. 9) by halting formazan formation and leaving behind a colourless product. The IC50 value was reported to be 24.23 µg/ml, which indicates the oil concentration required to inhibit 50% of the CHO cells (Table 7).

Table 4. Evaluation of DPPH assay of *Cymbopogon martini* essential oil

Concentration (ug/mL)	Sample (Ginger grass essential oil)	
	OD (515 nm)	% Inhibition
100	0.287	70.71
200	0.26	73.47
300	0.241	75.41
400	0.199	79.69
500	0.189	80.71
600	0.178	81.84
700	0.163	83.37
800	0.136	86.12
900	0.126	87.14
1000	0.115	88.27

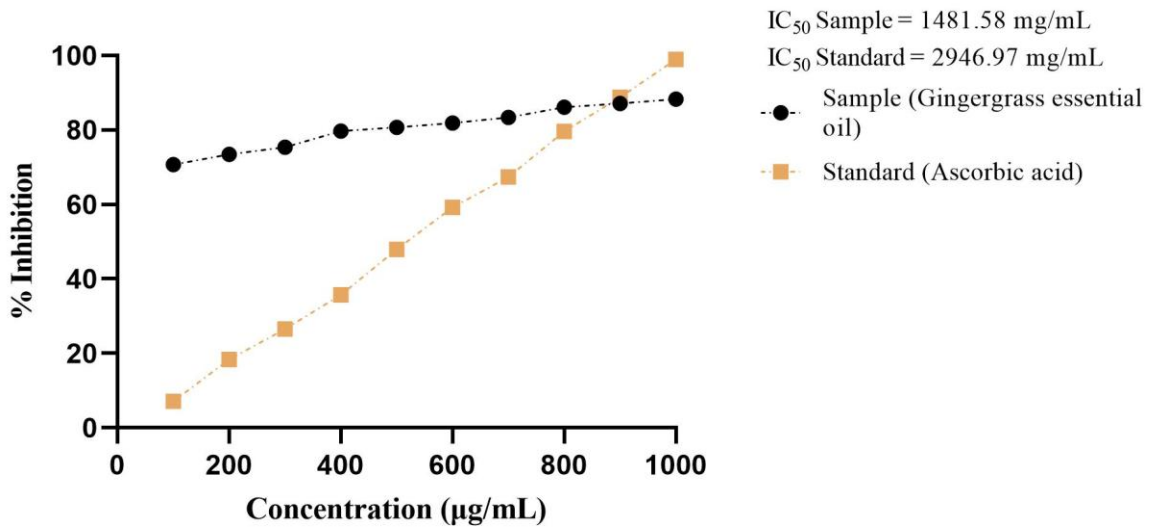

Fig. 6. Graphical representation of the antioxidant activity shown by *Cymbopogon martinii* essential oil using DPPH assay

Table 5. Evaluation of anti-inflammatory activity of *Cymbopogon martini* essential oil

Concentration (µg/mL)	Sample (Ginger grass essential oil)	
	OD (515 nm)	% Inhibition
10	0.128	92.76
20	0.117	93.38
30	0.108	93.89
40	0.096	94.57
50	0.096	94.57
60	0.095	94.62
70	0.094	94.68
80	0.09	94.91
90	0.068	96.15
100	0.067	96.21

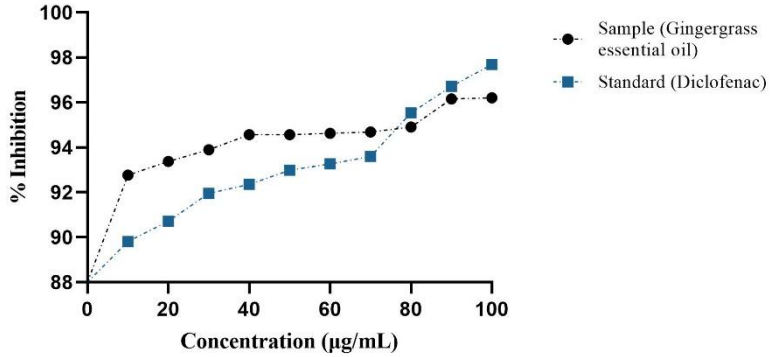

Fig. 7. Graphical representation of anti-inflammatory activity shown by *Cymbopogon martini* essential oil using protein denaturation assay

Table 6. Evaluation of anticancer activity of essential oil extracted from *Cymbopogon martini*

Anticancer activity			
concentration (µg/mL)	**OD (595 nm)**	**% Inhibition**	**IC50**
5	1.12	7.63	55.0
10	1.04	14.48	
20	0.952	22.02	
40	0.742	40.02	
60	0.578	54.07	
80	0.349	73.69	
100	0.25	82.18	

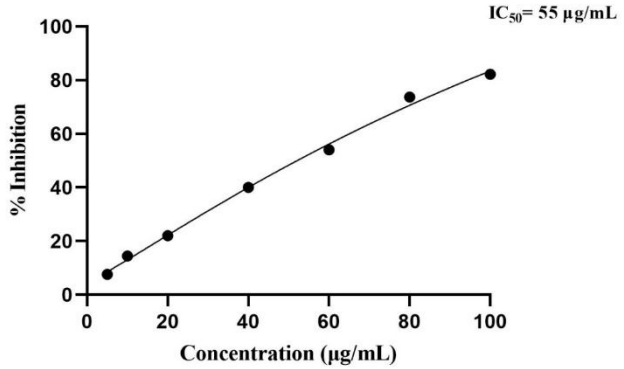

Fig. 8. Graphical representation of anticancer activity depicted by *Cymbopogon martini* essential oil on HeLa cell line

Table 7. Evaluation of cytotoxicity of essential oil extracted from *Cymbopogon martini*

Cytotoxic activity			
Concentration (µg/mL)	**OD (595 nm)**	**% Inhibition**	**IC50**
5	0.704	31.19	24.235
10	0.634	38.46	
20	0.568	45.32	
40	0.36	66.94	
60	0.182	85.45	
80	0.137	90.12	
100	0.123	91.58	

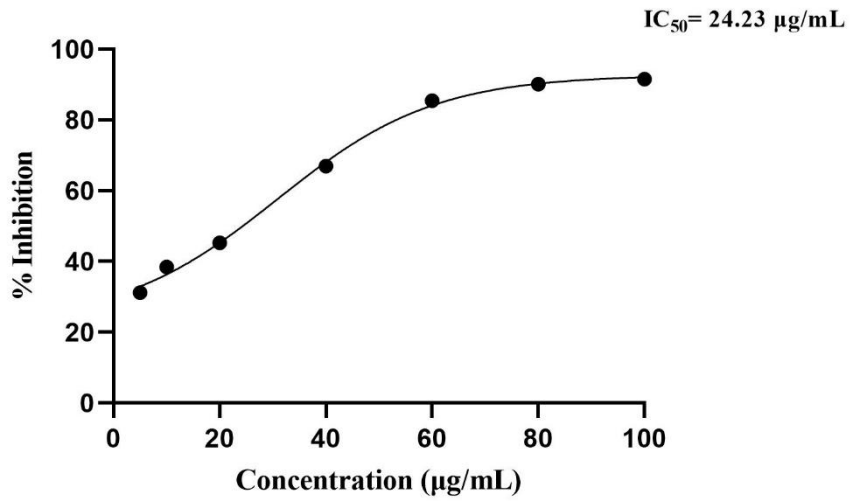

Fig. 9. Graphical representation of the cytotoxic activity depicted by *Cymbopogon martinii* essential oil on CHO cell line

4. DISCUSSION

Various components present in the sample were identified and separated using Gas chromatography-mass spectrometry. These compounds majorly involved different classes of alcohols, esters, ketones, monoterpenes and sesquiterpenes. However, the chemical composition reported was found to be quite different when compared to other published reports. The essential oil of *Dendranthema indicum Var. Aromaticum* when analysed by GC-MS showed higher amounts of oxygenated monoterpenes and fatty hydrocarbons. The main volatile components of *Dendranthema indicum Var. Aromaticum* were α-Thujone (21.63%); Neointermedeol (12.6%); β-Thujone (9.53%); cis-Sabinol (5.13%) Sabinyl acetate (5.83%) Isothujol (3.31%); Sabinyl acetate (5.13%) (Z)-Tibetinspiroether (3.31%) β-Caryophyllene (3.09%). [35] Many other reports also showed a difference in their reports when compared. FAME (Fatty acid methyl esters) analysis was carried out for the presence of fatty acids in *Cymbopogon martinii* essential oil and were detected efficiently using GC-MS. After the analysis of the chromatogram, 13 different fatty acid methyl esters peaks were found within the sample. Erucic acid, which represented a percentage area of 40.60%, was the main component of the essential oil. It was followed by 4,7-Methano-1H-indene, octahydro-5-(2-octyldecyl), and 2-methyltetracosane, which represented percentage areas of 16.62% and 11.03%, respectively. FTIR analysis is a technique for identifying organic, inorganic, and polymeric materials by scanning them with infrared light. The infrared spectrum of absorption, emission, and photoconductivity of the sample is obtained using this method [36]. The study's FTIR results indicate that it is a useful technique for identifying various structural and functional components in the *Cymbopogon martinii* essential oil.

Thermogravimetric and differential thermal analysis curve provides a convenient way of obtaining kinetic parameters for a decomposition reaction and thus helps to determine the thermal stability during a simple pyrolysis reaction [34]. The TG-DTA analysis of *Cymbopogon martinii* essential oil was carried out to determine the thermal stability and mass loss when subjected to constant increasing temperatures. It represents reliable thermal stability of the essential oil and thus contributes to its potential activity. The differential scanning calorimeter (DSC) is a crucial tool in thermal analysis as it provides information on a material's heat capacity and heat enthalpy, which may be used to assess the thermal stability of a compound [37]. An endothermic and exothermic behavioural pattern can be seen in the differential scanning calorimetry curve of *Cymbopogon martinii* essential oil which helps to elucidate the structural and functional changes in the oil at increasing temperatures. The differential scanning calorimeter profile proves the stability of *Cymbopogon martinii* essential oil at highly elevated temperatures.

The minimum inhibitory concentration of *Cymbopogon martini* essential oil was determined against drug-resistant organisms which were Carbapenem-Resistant Acinetobacter (CRA), Carbapenem-Resistant *Pseudomonas aeruginosa* (CRP), Carbapenem-Resistant *Escherichia coli* (CRE), Carbapenem-Resistant *Klebsiella pneumonie* (CRK), Extended Spectrum beta-lactamase *Escherichia coli* (ESBL), Quinolone resistant Salmonella (QRS), Vancomycin-resistant Enterococci (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA) and Erythromycin resistant Streptococci (ERS). The antimicrobial activity detected can be characterized by the presence of compounds such as gamma elemene and azulene which were confirmed by GC-MS analysis. Studies have shown that azulene and its derivatives have proven potential applications that concern various areas of medicine, including anti-inflammatory with peptic ulcers, antineoplastic with leukemia, antidiabetes, antiretroviral with HIV-1, antimicrobial with antimicrobial photodynamic therapy, and antifungal activities [38]. Gamma elemene has also shown presence in essential oils such as Curcuma wenyujin with promising antimicrobial activity [39]. The essential oil's antibacterial mechanism could include simultaneous cytomembrane rupture, allowing intracellular molecules like protein and K+ to flow out [40]. The antimalarial activity of the *Cymbopogon martini* essential oil was determined as Minimum inhibitory concentration against the parasite, *Plasmodium falciparum and* Quinine Resistant Strain. The GCMS analysis did not confirm the presence of an antimalarial component, but it did confirm the presence of 1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-xyclodecadi which has exhibited modest inhibitory activity against the malarial parasites. From a study conducted by Srinivasan et. al, larvicidal activity of *Elaeagnus indica* plant leaf extracts against dengue and malarial vectors it can be seen that Cyclodecadiene possess antimalarial potential [41].

One of the most important biological aspects of essential oils for dealing with oxidative stress is their antioxidant activity. In eukaryotes, reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and hydrogen peroxide cause mitochondrial DNA damage, which slows the electron transport chain, resulting in ROS accumulation [42]. The antioxidant activity of the essential oil was assessed using the 2,2′ -diphenyl-1-picrylhydrazyl (DPPH) test in this study. It is a quick and low-cost method for evaluating antioxidants' radical scavenging activity, in which DPPH is reduced by obtaining a hydrogen atom from antioxidants, resulting in a colour change from violet to pale yellow [43]. The essential oil exhibited promising antioxidant potential, however when compared to the standard the sample showed a lesser percentage of inhibition at the highest concentration. This antioxidant activity of *Cymbopogon martini* essential oil can be correlated to the presence of component such as alpha-terpineol which was confirmed by GC-MS analysis. Previous studies have shown that alpha-terpineol has presented good antioxidant and anti-proliferative activity in vitro and also has encouraging results against chronic myeloid leukemia and breast carcinoma [44].

Inflammation is a protective response triggered by tissue injury or infection, and it protects human body against invaders by removing dead or damaged host cells [45]. In inflammatory diseases such as rheumatoid arthritis, cancer and diabetes, protein denaturation results in the generation of autoantigens. Inflammatory activity can thus be reduced by inhibiting protein denaturation. In this study, anti-inflammatory activity of *Cymbopogon martini* essential oil was evaluated in the study by incorporating Inhibition of protein denaturation assay using egg albumin method. The anti-inflammatory activity exhibited by the *Cymbopogon martini* essential oil can be attributed to the presence of a terpenoid, Caryophyllene which was confirmed by GC-MS analysis. Essential oil extracts were investigated for anti-inflammatory action in the TPA-induced mouse ear edema model (TPA), with a topical application of 2 mg/ear, and in an in vitro quantitative lipoxygenase inhibition assay, at a dosage of 100 µg/mL, in a study conducted by Salleh et al. Results with an inhibitory effect value of more than 50% are deemed to have considerable activity. In both the TPA and the lipoxygenase inhibitory assays, the essential oil inhibited 73.6 % and 76.0 %, respectively. The main component, caryophyllene oxide, was evaluated for lipoxygenase inhibition and found to be 82.4 % effective [46]. Some essential oils have been shown to have anti-inflammatory properties in addition to their ability to scavenge free radicals. Essential oils like chamomile eucalyptus, rosemary, lavender, millefolia and other plants (pine, clove, and myrrh) have been utilised in mixed formulations as anti-inflammatory agents in the past [47]. Essential oils' anti-inflammatory properties can be linked to

their antioxidant properties, as well as their interactions with signalling pathways including cytokines and regulatory transcription factors, as well as their effects on the expression of pro-inflammatory genes.

Essential oils 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 affected cell's membrane fluidity, a decrease in adenosine triphosphate (ATP) production, a change in pH gradient, and the loss of mitochondrial potential, all of which are key precursors to cell death. [42] MTT assay was carried out to determine anticancer activity of *Cymbopogon martini* Essential oil on Hela cells. Metabolic activity can be assessed by determining the activity of a mitochondrial enzyme succinate dehydrogenase using MTT assay. MTT is employed for the quantification of viable cells in cell population using 96 well plate format. It is extensively used on the *in vitro* evaluation of the anticancer and cytotoxic potency of the test sample. The MTT-based assay relies upon the cellular reduction of reduction of MTT which is a yellow water-soluble tetrazolium dye by the mitochondrial dehydrogenases to purple coloured formazan crystals as an end product [48]. According to the GC-MS results, there is presence of Ledene oxide-(II), Caryophyllene and 2-Methyl-Z,Z-3,13-octadecadienol in the essential oil and previous studies have shown that they possess good anticancer potential. In a study based on Anticancer effects of *Myric rubra* Essential Oil, various concentrations (100-400 mug/mL) were tested against A549 lung cancer cells. According to the GC-MS analysis, Ledene oxide-(II) and Caryophyllene were major constituents of the essential oil. The survival rates documented post 48h exposure of the essential oil resulted in inhibition of cell line proliferation in a concentration-dependent manner [49].

The cytotoxicity of *Cymbopogon martini* essential oil was evaluated using MTT cell proliferation assay by employing Chinese hamster ovary (CHO) cells. The MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) colorimetric assay determines the functional condition of mitochondria, which indicates cell viability or metabolic activity. The assay is based on the ability a mitochondrial dehydrogenase [nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase enzymes] enzyme present in living cells to converts yellow tetrazolium MTT salt to purple MTT formazan, which is precipitated in healthy cells [50]. MTT determines cell respiration, and the amount of formazan produced is proportional to the number of live cells in the culture. A change in the amount of formazan produced in response to an increase or decrease in cell number indicates the degree of cytotoxicity caused by the test sample. The IC50 value of the essential oil is the concentration at which 50% of the cells die when treated with it and can be used to estimate the degree of cytotoxicity [48]. The cytotoxicity of the essential oil can be corresponded to the presence of n-Hexadecanoic acid present in *Cymbopogon martini* essential oil which was confirmed by GCMS analysis. Previous cytotoxicity studies performed on the n-Hexadecanoic acid extracts of *from Kigelia pinnata Leaves* against HCT-116 cell lines, using MTT assay demonstrated an IC50 value of 0.8 µg/mL [51]. Owing to the presence of n-Hexadecanoic acid in the sample, it can be concluded that the *Cymbopogon martini* essential oil exhibits a significant Cytotoxic activity.

5. CONCLUSION

Understanding the extent and mode of inhibition of certain compounds present in the essential oils may contribute to the successful application of the essential oils in food and cosmetic industry as well as for therapeutic use. For this very reason, physiochemical properties of the *Cymbopogon martini* essential oil were evaluated by performing various analysis which included GS-MS, FTIR, FAME, TG-DTA, DSC which helped to identify the crucial components present in the essential oil having therapeutic benefits. The thermal stability represented the potency and robustness of the oil at high temperatures. Furthermore, the study also confirmed antimicrobial activity of the oil against the selected drug-resistant pathogens as well as exhibited inhibitory activity against drug – resistant *Plasmodium falciparum* strain. In addition to this, the DPPH and egg albumin protein denaturation assay revealed free radical scavenging and anti-inflammatory potential of the essential oil, respectively. The results obtained during MTT assay showed moderate anticancer and significant cytotoxic activity against the selected Hela and CHO cell lines, respectively. We can thus conclude that the *Cymbopogon martini* essential oil possess various beneficial properties and can be a good candidate for medicinal applications. Nevertheless, in addition to the significance of the study certain limitations

were also observed with respect to the plant extracts. They have considerably low bioavailability and appropriate toxicological analysis are required. Further, investigations should be carried out on the mode of action as well as on the toxicological effects of the essential oil to optimize their potential therapeutic applications.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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