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Research Article

Exploring the Therapeutic Potential of *Chrysopogon zizanioides* Essential Oil: A Comprehensive Study on Its Chemical Composition, Thermal Analysis, and Biological Activities

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Abstract

Natural bioactive compounds sourced from plants and plant parts have become integral to drug discovery as natural products. The overreliance on synthetic drugs has adversely affected human health. This study focuses on the essential oil from *Chrysopogon zizanioides*, commonly known as "Vetiver" to explore its medicinal value. The oil underwent various analytical and biological assessments. Extraction involved hydro-distillation of the roots, and GC-MS analysis unveiled its composition. FAME analysis provided insights into fatty acid composition. Infrared spectroscopy identified different functional groups. Thermal stability was gauged using TG-DTA and DSC methods. The essential oil's antibacterial potency was tested against bacterial strains. Antimalarial and antituberculosis potential were evaluated against *Plasmodium falciparum* and *Mycobacterium tuberculosis*, respectively. Vetiver essential oil's ability to counter free radicals was determined via the DPPH reagent. Its impact on biofilm produced by 5 cultures was studied. The essential oil's effects on cancerous (HeLa) and normal (CHO) cell lines were assessed using the MTT assay. In conclusion, *Chrysopogon zizanioides* essential oil exhibits considerable potential with diverse activities. It could serve as a non-toxic alternative for infection prevention and find utility in the food industry

Keyword: *Chrysopogon zizanioides*, Essential oil, Thermal Characterization, GCMS, Antimicrobial, Biofilm.

INTRODUCTION:

In these pressing times of antibiotic resistance and side effects from synthetic drugs, antimicrobial medications are proving to be not effective in treating new infections [1]. Moreover, 700,000 people die annually from antimicrobial resistance (AMR), and another 10 million are doomed to die by 2050 [2]. As a result, to address the issue of AMR, it is critical to search for innovative alternative antimicrobial agents [3]. Novel substitutes include secondary metabolites of plants, that have been a part of Indian Ayurveda since time immemorial and have great potential, for biological activity, most notably antibacterial, antifungal, and antioxidant characteristics. [4]

Essential oils (EOs) are fragrant, slightly water-soluble, inflammable, volatile, alcohol-soluble liquids extracted from various plant sources using various procedures such as distillation and solvent extraction [5]. The essential oils are recognized by their unique compositions of bioactive substances, primarily terpenoids, particularly monoterpenes, and sesquiterpenes. The presence of these bioactive substances is in charge of giving EOs their biological significance [6]. They find their application in diverse avenues like pharmaceutical alternative medicines, natural food preservatives, and also as constituents in fragrances, cosmetics, aromatherapy, phytotherapy, spices, and nutrition [7] which has led to their increased research.

'*Chrysopogon zizanioides*' is a perennial plant native to India, the thick fibrous adventitious roots produce Vetiver oil, widely employed in the fragrance sector as a significant odour contributor and as a flavour enhancers component in the food sector [8]. Despite its peculiar scent, Vetiver oil has a variety of biological properties, namely antifungal, anti-bacterial, anti-parasitic, anti-convulsant, anti-depressant, antioxidant, anti-inflammation, analgesic, skin anti-ageing, and cytotoxic against cancer [9] . In addition, the yearly market demand for Vetiver oil was anticipated to be up to 250 tons, worth roughly $200 million per year [10]. This surge can be attributed to the plant's adaptability, which is linked to its chemical makeup. Terpenoids, sesquiterpenoids, hydrocarbons, alcohols, ketones, acids, phenols, and nitrogenous chemicals have been

recognized as significant constituents in Indian Vetiver oil using a gas chromatography-mass spectroscopy (GC-MS) study[11].VEO has limitless benefits, thus exploration into the biological characteristics of *V. zizanioides* is crucial for various applications.

The present research's principal goal was to examine the various therapeutic effects of Vetiver essential oil, in order to understand the potential for its use in the pharmaceutical sector, as an alternative to currently available drugs in the market. Therefore, the investigation included various assays to determine its biological activities, namely anti-microbial, anti-oxidant, anti-malarial, anti-cancer, and cytotoxicity. Furthermore, the EO was studied using a variety of high-throughput analytical methods such as FT-IR, GC-MS, FAME, TGA, and DSC, in order to reveal the biologically active compounds making the essential oil extracted from *Chrysopogon zizanioides*' potential substitute to synthetic medications, and could also be employed in the food industry.

MATERIALS AND METHODS:

Sample collection and extraction of essential oil:

The Vetiver plant was obtained from a garden (Latitude: 25°27'56.4"N Longitude:78°35'49.4"E) located in Jhansi, Uttar Pradesh, India. Initially, in order to validate our plant sample, it was authenticated at Agharkar Research Institute, Department of Science and Technology, India by Dr. R. K. Choudhary. With the help of organoleptic and taxonomic characteristics, the sample submitted was successfully identified as *Chrysopogon zizanioides* (L.) Roberty belonging to *Poaceae* Family with authentication number (22-104). Furthermore, the Vetiver roots were used to extract the essential oil, and the extraction process was carried out in the research facility in Matunga, Mumbai, India according to [12]. The extraction procedure involved subjecting the 200 g of shade-dried roots to hydrodistillation using a Clevenger apparatus for a span of 24 hr.

GC-MS analysis to determine the composition of essential oil:

To comprehend the composition of the essential oil under study, GC-MS analysis was employed, and the Shimadzu GCMS-QP2010 system was used. In this system, a Gas Chromatograph is interfaced with a Mass Spectrometer and a J & W DB-1 column was used. A capillary column with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 µm, as well as for GC-MS detection, an electron ionisation device in electron impact mode with 70-eV ionization energy was utilized. Helium gas of (99.999%) purity was used as the carrier gas, with a constant flow rate of 1.8 mL/min and an injection volume of 1 µL, with a split ratio of 100:1. The injector temperature and ion-source temperature were fixed at 280.00°C and 240.00°C, respectively, while the oven temperature was set at 50.00°C for 1.00 min with the isothermal condition, then 180.00°C/min rise to 260.00°C with 2.00 min isothermal condition. The program lasted 30.00 min in total. The components were identified by comparing their mass spectra to those present in the Wiley and NIST libraries.

Analysis of functional groups present in VEO by FTIR technique:

FTIR spectra of the oil samples were studied using IRPRESTIGE 21 (SHIMADZU) FTIR spectrometer. The oil sample was divided equally between the two NaCl pellets afterward the second pellet was pressed onto the other [13]. The infrared spectrometer was adjusted to absorbance with a resolution of 1 cm^{-1} and a 45-time repetition scan after gently rotating the NaCl pellets to make the oil form a homogeneous liquid membrane before attaching and inserting them into the infrared spectrometer sample holders. Spectra were collected using NaCl pellets in the spectral range 4000-400 cm^{-1} and were recorded as average curves from 45 acquisitions, with a resolution of 4 cm^{-1} [14].

TGA analysis to understand the thermal stability of the essential oil:

The STA 2500 equipment NETZSCH, Germany was employed to carry out the TGA analysis. The tests were conducted in a nitrogen gas atmosphere with a 300 mL/min flow rate. The sample, weighing 21.40 mg, was introduced into the aluminum crucible. The temperature of the study ranged from room temperature to 500° C, the sample was heated at a rate of 5.5°C/min.

Differential scanning calorimetry (DSC) analysis was undertaken to establish a calorimetric profile:

The DSC essential oil profile was created using a TA Instruments DSCQ20 differential scanning calorimeter. In the metal crucible, the essential oil sample weighing 8.00 mg was placed. A nitrogen gas flow rate of 40 mL/min was used to analyse the sample under study. A dynamic scan was performed at a heating rate of 10°C/min over a temperature range of 25 to 375° C.

Antimicrobial action of essential oil extracted from *Chrysopogon zizanioides*:

Bacterial strains employed for the study:

The antibacterial capability of the test materials was evaluated using 9 resistant bacterial strains. *Carbapenem-Resistant Acinetobacter spp* (*CRA*), *Carbapenem-Resistant Pseudomonas aeruginosa (CRP), Carbapenem-Resistant E.coli (CRE), Carbapenem-Resistant Klebsiella pneumoniae (CRK), Extended Spectrum beta-lactamase E.coli (ESBL), Quinolone resistant Salmonella (QRS),* and *Vancomycin-resistant Enterococci (VRE), Methicillin-resistant Staphylococcus aureus (MRSA), Erythromycin resistant Streptococci* (ERS). Gram-negative bacteria make up the first 6 strains, on the other hand, Gram-positive bacteria make up the last 3. These resistant bacterial strains were grown for 24 hrs at 37°C on MH agar and then were used for the investigation.

Determination of Minimum Inhibitory Concentration (MIC) of essential oil:

The potential of the VEO was assessed by the microdilution method adopting 96-well microtiter plates in order to ascertain the minimal inhibitory concentration against the selected bacterial strains according to [15]. Employing sterile saline solution, the bacterial suspensions were regulated until they attained a concentration of 1.0×10^5 CFU/mL. Later, the essential oil was dissolved in a combination of 5% DMSO, 0.1% of polysorbate-80 (1 mg/mL), and then in order to reach the desired concentrations, it was added to a Luria-Bertani medium (100 µL) possessing a bacterial inoculum of 1.0×10^4 CFU/mL. Then the inoculated plates were incubated at a temperature of 37°C for about 24 hrs at 180 rpm.

Determination of capacity of essential oil to capture free radicals:

The radical scavenging activity of Vetiver essential oil was tested using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay[16] , with minor variations. 1 mL methanol solution of 0.5 mM DPPH radical, as well as 2.0 mL of a 0.1 M sodium acetate buffer at pH 5.5, were added to a 2.0 mL sample. The solutions were thoroughly mixed and set aside for 30 min at room temperature in the dark. The absorbance was measured using a twin beam UV-VIS spectrophotometer 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

ranging from 100 to 1000 µg/mL. The reaction mixture was thoroughly mixed before being incubated in the dark for 15 min at room temperature. The absorbance was measured at 515 nm.

For each concentration, the % inhibition of the DPPH radical was computed using the following equation:

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

Plotting inhibition %s versus sample oil concentrations yielded the sample concentration that provided 50% inhibition (IC_{50}). All of the experiments were repeated three times, and the IC_{50} values were calculated using the average of the three repeats.

Antituberculosis Strain Collection:

M. tuberculosis sensitive strain (H37Rv) was obtained from the National Institute for Research in Tuberculosis in Chennai, Tamil Nadu.

Evaluation of potential action of essential oil against *Mycobacterium tuberculosis* H37Rv strain:

In vitro testing of Vetiver essential oil against a susceptible strain of *Mycobacterium tuberculosis* (H37Rv) was performed. The oil was assessed using a classic growth-based method, which incorporated the L.J. MIC method (Lowenstein and Jensen). During the experiment, 2% Malachite green solution, homogenized eggs solution, and mineral salt solutions were added to Lowenstein Jensen (LJ) medium. Inoculated with a mycobacterium suspension strain whose concentration was equivalent to the McFarland standard, the medium containing various concentrations of essential oils (100, 50, 12.5, 6.25, 3.125, 10, 5, 2.5, 1.25, 8, 4, 2, 1, 0.5, 0.25 µg/mL) was then incubated at 37°C with regular monitoring. The reference *Mycobacterium tuberculosis* H37Rv strain was tested with isoniazid, a well-known drug with a concentration of 0.2 mg/l [17].

Cell lines maintenance under regulated conditions:

The National Centre for Cell Science in Pune provided a Human cervical cancer cell line (HeLa), and a normal cell line, Chinese Hamster Ovary (CHO) cells. The cell lines were maintained at regulated conditions of 37°C, 5% CO_2, 95% air, and 100% relative humidity in Dulbecco's Modified Eagle Medium (DMEM) supplemented with antibiotics (Pen-strep) and 10% foetal bovine serum (FBS). Later, once the trypsin-EDTA treatment was given at 37 °C, then the cells were extracted for subculturing.

Cytotoxic Screening of essential oil extracted from *Chrysopogon zizanioides*:

The MTT assay was adopted to determine the cytotoxic action of the VEO as mentioned in [18] by introducing small variations. In 96-well microplates the cells were seeded at a density of 5×10^4 cells/well and then were incubated in a CO_2 incubator with regulated environmental conditions at 37° C, 5% CO_2 overnight. Subsequently, the entire confluent cells were subjected to 6 varying essential oil concentrations after observing them under a microscope, which were then kept for incubation overnight in the CO_2 incubator at 37° C, 5% CO_2. Once incubation was completed, then the cells were observed under a microscope and transferred to the wells with 10 µL of 5 mg/mL, afterwards, an MTT reagent was added, followed by incubation for 4 hrs. The medium with MTT was then removed followed by the introduction of formazan crystals which were then dissolved by adding 100 µL of DMSO. Ultimately, the absorbance was measured at 595 nm in the 96-well ELISA plate reader. The assay was carried out in triplicate.

The cell % inhibition is calculated using the following form;

$$\%cell\ inhibition = \left[100 - \left(\frac{OD\ at\ sample - OD\ at\ blank}{OD\ at\ control - OD\ at\ blank}\right)\right] \times 100$$

Anti-Malarial assay to understand the essential oil's capability against *Plasmodium falciparum:*

An in vitro anti-malarial test was performed in a sterile 96 well microtiter plate in accordance with Kotecka B and Rieckmann's approach, with slight modifications [19]. Drug-sensitive and drug-resistant strains of *Plasmodium falciparum* were cultured in RPMI-1640 medium, which contained 25 mM HEPES, 0.23 % sodium bicarbonate, 1% D-glucose, and 10% heat-inactivated human serum. Only ring-stage parasitized cells were obtained after treatment with 5% D-sorbitol, resulting in the synchronisation of both the *Plasmodium falciparum* strains. To conduct the study, Jaswant Singh Bhattacharya (JSB) staining was used to establish an initial ring stage parasitaemia of 0.8 to 1.5% at 3 % haematocrit in a total volume of 200 µL of medium RPMI-1640, which was then maintained with 50 % RBCs (O^+) [20]. The essential oil was prepared as a 5 mg/mL stock solution in DMSO, with dilutions ranging from 0.4 to 100 mg/mL. The test and duplicate wells received 20 µL of diluted liquid containing parasitized cell preparations. In a candle jar, the culture plates were incubated at 37°C for 36 to 40 hrs. After incubation, thin blood smears were taken from each well and stained with JSB stain. Chloroquine and Quinine were used as reference drugs in the study. When varied dosages of the test chemicals were employed, microscopic analysis of the slides revealed the evolution of ring-stage parasites into schizonts and trophozoites. The MICs that inhibited both *Plasmodium falciparum* strains from growing into schizonts were recorded, and the estimated IC_{50} values for each were compared to the standard values.

Evaluation of Biofilm inhibition capability of essential oil:

The biofilm growth test used in this investigation was based on a technique previously published with slight alterations [21]. Standard MTCC Chandigarh cultures of *Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Proteus mirabilis, and Pseudomonas aeruginosa*, as well as other laboratory isolates, were used for the biofilm inhibition experiment. On Nutrient agar medium, the test organisms were isolated, and a selected isolated colony were chosen and inoculated in Luria Bertani Broth. The culture suspension was adjusted to 0.5 O.D. according to McFarland Standards. The crystal violet test was performed in 96-well microtiter plates, with 100 µL sterile LB and an equal amount of distilled water in each well, further 100 µL sample of Vetiver essential oil was added. Instead of placing the samples in the positive and negative control wells, each was given 30 µL of chloramphenicol and 100 µL of sterile distilled water with LB. Bacterial suspension of 20 µL was prepared by diluting an overnight culture cultured in LB broth and added to 96-well plates to enable bacterial growth and biofilm development, the plates were sealed and incubated overnight at 37°C in a static atmosphere for 24 hrs. Following incubation, the cells and inadequate medium were removed, and the adherent biomass was washed three times with distilled water. Each plate was air-dried inverted at room temperature for 30 min. After that, 400 µL of 1% Crystal violet dye was used to color the biomass. After that, the plates were incubated for 30-40 min at room temperature (RT). The plates were drained and cleaned three times with distilled water to remove any remaining color After cleaning, the dishes were dried for 10-20 min in a hot-air oven at 40°C. Following the drying of the wells, 400 µL of methanol was added. The absorbance was measured in an ELISA plate reader at 570 nm, and the % biofilm inhibition was calculated using the equation below.

$$\% \, Inhibition \, = \frac{(A_{control} - A_{test})}{A_{control}} \times 100$$

RESULT AND DISCUSSION:

GC-MS technique for the Analysis of essential oil's potential therapeutic molecules:

GC-MS is primarily used to identify a large number of volatile components and their concentrations. It describes the chemistry of essential oil. GC-MS was used to examine the chemical constitution of *Chrysopogon zizanioides* essential oil, which is represented in **Figure (1)**. This resulted in the identification of 27 distinct compounds of the total oil composition, which are listed in **Table (1)** [22]. Sesquiterpene (37.15 %) which consists of cyclic and oxygenated sesquiterpenes, the organic compound (29.62 %), fatty acid ester (11.11 %), terpene (14.81 %) which includes cyclic and monoterpene which are the most prominent classes in Vetiver essential oil. The abundant components identified were trans--p-tert butyl cyclohexyl acetate (21.47%); Benzyl benzoate (11.36%); Alpha Acoradiene (10.49%) and trans-Isolongifolanone (10.078%) with the retention time of 10.54, 15.97, 16.26 and 14.25. The rest of the oil corresponds to minor parts of hydrocarbon and some other identified compounds [23]. Due to the different composition of elements, the Vetiver essential oil has certain therapeutic properties owing to molecules such as Fenchyl Alcohol, Cuparene, Alpha Copaene; Aromadendrene, and several more compounds which depicts several potential biological properties, which makes the essential oil used for pharmaceutical purpose.

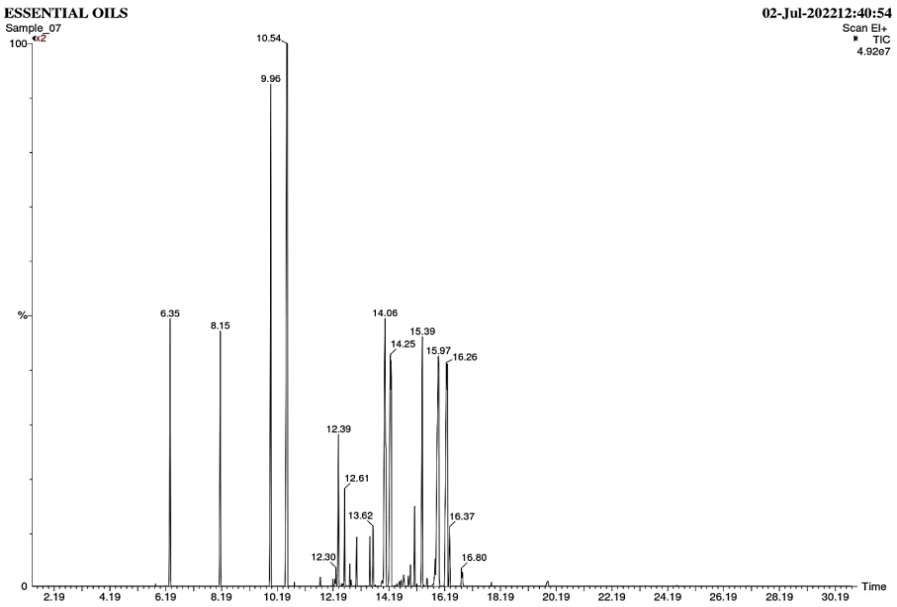

Figure 1: GC-MS analysis shows various compounds present in the essential oil of *Chrysopogon zizanioides* with their respective retention time.

Table 1: Pharmacological relevant compounds present essential oil extracted *Chrysopogon zizanioides*

Component name	Molecular formula	Molecular weight	Classification	Composition %	Retention time
Fenchyl alcohol	$C_{10}H_{18}O$	154	Monoterpenoid	4.623	6.35
Fenchyl alcohol	$C_{10}H_{18}O$	154	Monoterpenoid	4.147	8.15
cis-p-tert butyl cyclohexyl acetate	$C_{12}H_{22}O_2$	198	Carboxylic ester	9.585	9.96
trans--p-tert butyl cyclohexyl acetate	$C_{12}H_{22}O_2$	198	Carboxylic ester	21.471	10.54
alpha Copaene	$C_{15}H_{24}$	204	Sesquiterpene	0.062	10.8
Seychellene	$C_{15}H_{24}$	204	Sesquiterpene	0.202	11.74
Non-3-en-2-one	$C_9H_{16}O$	140	Enones	0.133	12.19
alpha-Curcumene	$C_{15}H_{22}$	202	Sesquiterpene	0.392	12.3
Kephalis	$C_{14}H_{24}O_2$	224	Organic Compound	9.585	12.39
Cuparene	$C_{15}H_{22}$	202	Sesquiterpenoid	1.673	12.61
alpha Alaskene	$C_{15}H_{24}$	204	Sesquiterpene	0.335	12.79
beta (E)- Ionone	$C_{13}H_{20}O$	192	sesquiterpenoids	0.847	13.04
Sandal mysore core	$C_{13}H_{22}O$	194	Organic Compound	0.772	13.51
Diethyl Phthalate	$C_{12}H_{14}O_4$	222	Organic Compound	1.11	13.62

trans-Isolongifolanone	$C_{15}H_{24}O$	220	Organic Compound	10.078	14.25
trans-Arbusculone	$C_9H_{14}O_2$	154	Organic Compound	0.267	14.72
alpha-Sinensal	$C_{15}H_{22}O$	218	Sesquiterpenoids	0.194	14.89
alpha hydroxy terpenyl acetate	$C_{12}H_{20}O_3$	212	Monoterpenoid	0.487	14.97
4 butyl-5-3-methyl butyl - 6- 1- methyl ethenyl -2H- pyran-2one	$C_{12}H_{18}O_2$	262	Organic Compound	1.443	15.12
Aromadendrene	$C_{15}H_{24}$	204	Sesquiterpenoid	5.443	15.39
cis- Floral super	$C_{13}H_{22}O_3$	180	Tetrahydrofurans	0.191	15.56
Benzyl benzoate	$C_{14}H_{12}O_2$	212	Benzoate Ester	11.362	15.97
alpha Acoradiene	$C_{15}H_{24}$	204	Sesquiterpene	10.493	16.26
Germacrene D	$C_{15}H_{24}$	204	Sesquiterpenes	1.144	16.37
cis- Carvyl tiglate	$C_{12}H_{18}O_2$	234	Organic Compound	0.664	16.8
3,7,11-Trimethyl 2,6,10-Dodecatrien-1ol	$C_{15}H_{26}O$	222	Monoterpenoid	0.1	17.87
2,6-dimethyl-Pyrazine	$C_6H_8N_2$	108.14	Pyrazines	0.36	19.9

FTIR characterization of essential oil to detect its functional groups:

Fourier transform infrared spectroscopy (FTIR) is a technology that has been utilised for structural and compositional study for many years [24]. FTIR spectroscopy is strong optical spectroscopy that determines the vibration properties of chemical functional groups in a sample by measuring the vibration of an excited molecule by IR light at a specified wavelength range [25].

The FTIR spectra of VEO are shown in **Figure (2)**. It is obvious that it has many absorption peaks corresponding to various oxygen functional groups. The peak at 3500.80 cm^{-1} is the stretching vibration of –OH; the peak at 3388.93 cm^{-1} can be associated with overlapping of -OH and N-H stretching vibrations; the absorption peak at 1444.68 cm^{-1} and 1689.64 cm^{-1} indicate C=C and C=O stretching vibrations; the absorption peak at 1367.53 cm^{-1} suggests it is C–H bending, NO_2, C=S and C-N stretching vibrations; the absorption peaks at 1124.5 cm^{-1} and 1172.72 cm^{-1} reveals Si-O-Si and C-O stretching from the cellulose structure of CF stretching vibrations; the absorption peaks at 744.52 cm^{-1} and 779.24 cm−1 demonstrate =C–H bending vibration. The peaks at 1020.34 cm^{-1}, 1041.56 cm^{-1} and 1074.35 cm^{-1} indicate =C-O-C stretch ethers, C—O (H) cycloalkane 3-hydroxy-ladamantan and C-F stretching respectively. The peak at 1284.59 cm^{-1} and 2929.87 cm^{-1} shows C-H_2 and C-H_2 asymmetric stretching vibrations. CH_3 asymmetric stretching is observed at 1454.33 cm^{-1}. The peaks at 2870.08 cm^{-1} and 2954.95 cm^{-1} indicate –C-H stretching of alkane and the peak at 1728.22 cm^{-1} indicate ester (C-O-C) groups.

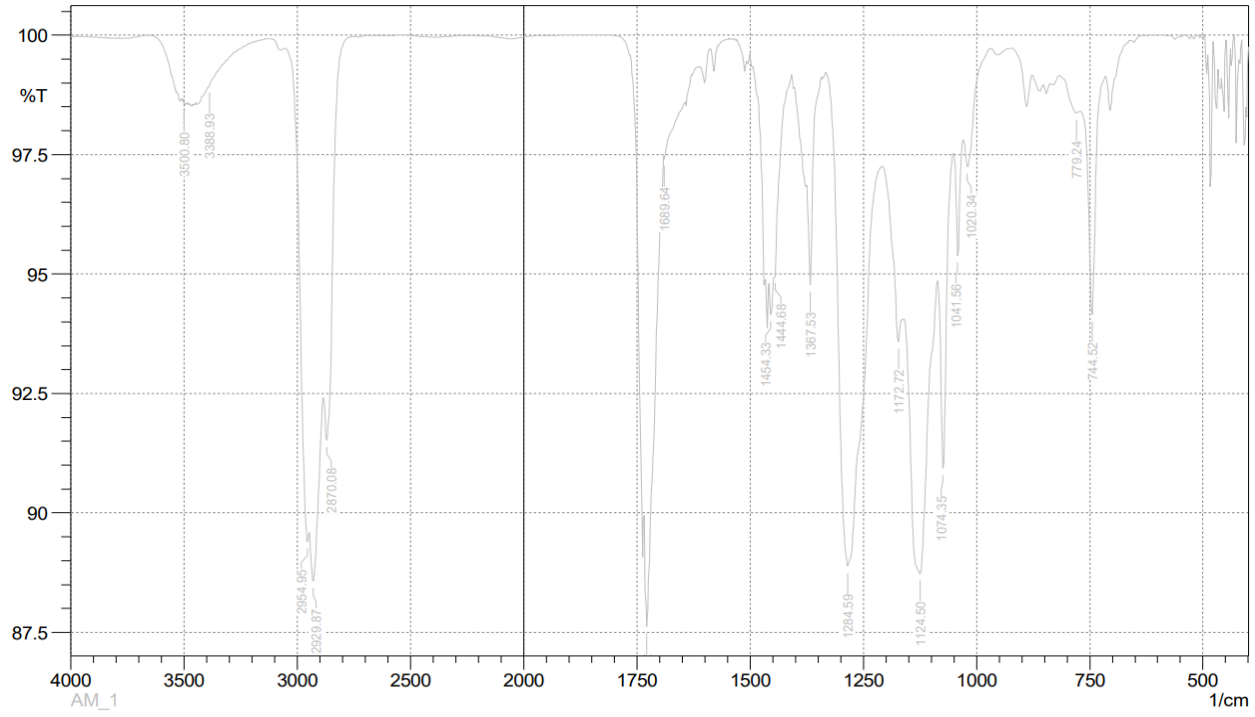

Figure 2: Functional group analysis of essential oil derived from *Chrysopogon zizanioides* by FTIR Profile

TGA – DTG analysis to determine the thermal stability of essential oil:

Concurrent Thermogravimetric and Differential Thermal Analysis (TGA-DTA) of VEO was performed to evaluate its thermal endurance. The thermogram of VEO is depicted in **Figure (3)**. The Vetiver oil curve demonstrates stability to a temperature of 200⁰ C, indicating an exothermic process. The onset peak was observed at 229.9°C until the end at 234.6°C within the complex peak. Temperatures ranging between 229.9°C and 234.6°C indicate an endothermic process. After 260°C, it stabilizes, suggesting an exothermic process. The high temperature in the DTA testing suggests that the sample has a high-temperature tolerance and great stability. The Vetiver oil curve denotes exothermic from 50°C to 150°C.

The descending TGA thermal curve implies a weight decrease. The onset temperature is the temperature at which the weight loss begins. The onset temperature point for the mass loss was observed at 203.1°C until 220°C. The curve continues to indicate an endothermic reaction until 300°C. Furthermore, when the temperature rises, the slope of the curve decreases.

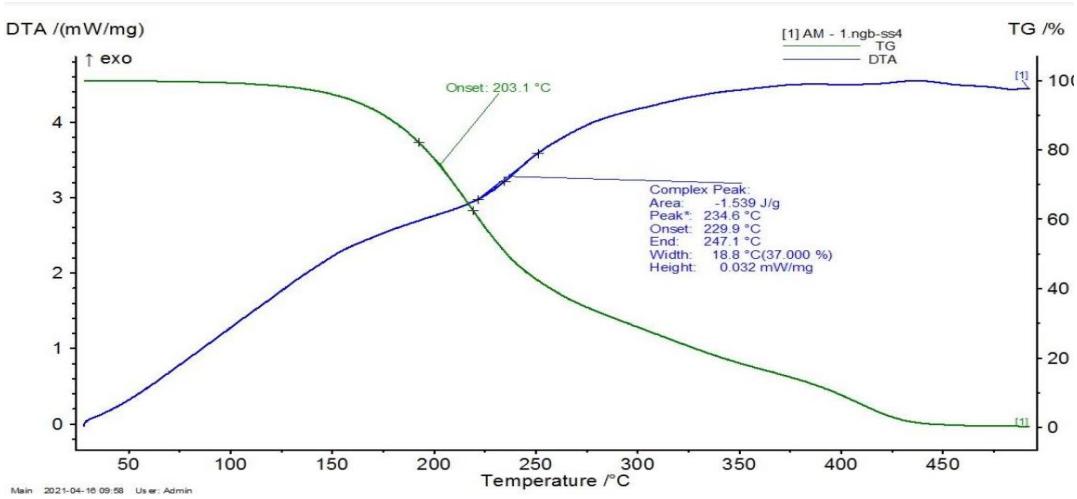

Figure 3: TG-DTA analysis of *Chrysopogon zizanioides's* essential oil indicating its thermal tolerance at wide temperature range

Calorimetric profiling of essential oil by DSC analysis:

Differential Scanning Calorimetry (DSC) analysis was carried out to understand the heat flow through the sample under regulated conditions, in order to understand its thermal stability[26]

The DSC curve of Vetiver oil shown in **Figure (4)**, indicates that as the temperature rises, the heat flow inside the oil sample drops, as evidenced by endothermic drop, in the temperature range of 50°C to 75°C, then slightly rises, peaking at 134°C with an exothermic shift suggesting heat absorption by the sample. At 201.30°C, a fast endothermic drop demonstrating heat flow is detected. When the temperature is pushed even higher, there is a sudden endothermic decline at 273.23 °C, followed by a linear rise in heat flow to 375°C as the temperature rises. This thermogram depicts the heat profile of the essential oil when subjected to escalating temperatures, providing proof of the oil's stability.

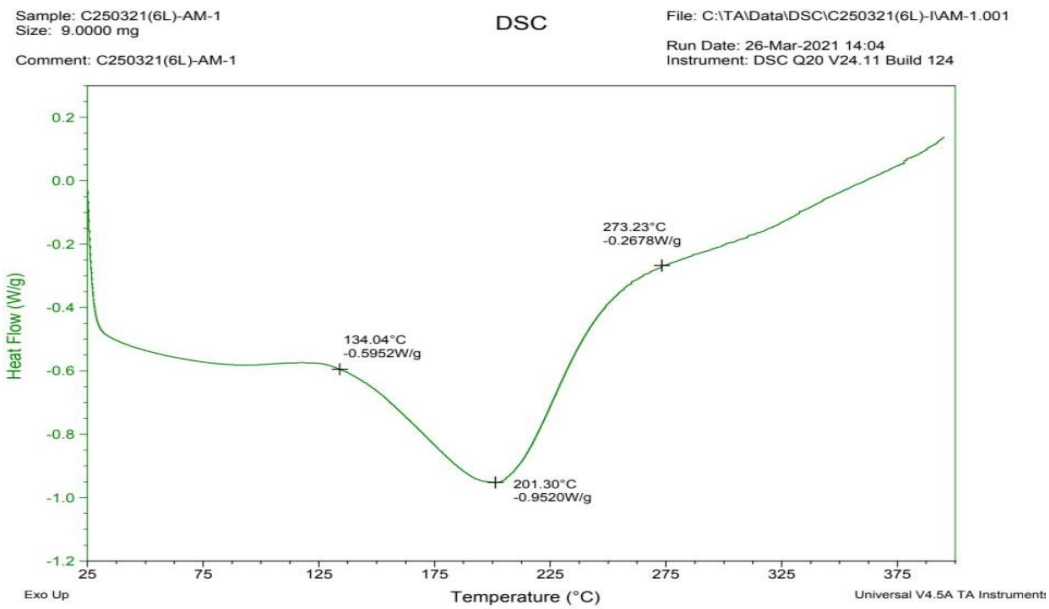

Figure 4: DSC profile of *Chrysopogon zizanioides* derived essential oil to comprehend heat exchange

Biological assays:

Determination of Bactericidal capacity of essential oil:

The current investigation evaluated the potential of the VEO against 9 resistant bacterial strains, using a Minimal Inhibitory concentration method. The action of the essential oil is illustrated in **Table (2) & Figure (5)**. The experimental findings demonstrated that the *Carbapenem-Resistant Acinetobacter spp* was highly susceptible to the essential oil with a MIC value of 62.5 µg/mL, however, the other 4 strains, namely *Carbapenem-Resistant E.coli, Carbapenem-Resistant Klebsiella pneumoniae, Vancomycin-resistant Enterococci, and Methicillin-resistant Staphylococcus aureus* were more tolerant to the VEO with the MIC of 200 µg/mL, as compared to others.

The above data communicates that the VEO was competent against the resistant bacterial strains employed for the test. Previous studies have mentioned that the possible target of the essential in order to trigger bactericidal effect could be the cell membrane. The components of the essential oil alter the integrity of the cell membrane, thereby disrupting it, which results in the release or loss of the cellular content and ultimately cell death [27]. Previous studies have also mentioned that due to the presence of certain components, like Monoterpenes and Sesquiterpenes and their oxygenated derivatives have shown to exhibit potential anti-microbial activity [28]. These components have been validated by our GCMS analysis in the VEO, which could be responsible for its anti-bacterial action. The VEO was also evaluated against *Mycobacterium tuberculosis (H37Rv)*, where it gave a MIC value of 100 µg/mL.

Table 2: Anti-bacterial action of *Chrysopogon zizanioides* derived essential oil against antibiotic-resistant bacterial strains

Sr No.	Bacterial strain		MIC value
1	CRA	*Carbapenem Resistant Acinetobacter spp*	62.5 ± 0.07
2	CRP	*Carbapenem Resistant Pseudomonas aeruginosa*	125 ± 0.05
3	CRE	*Carbapenem Resistant E. coli*	200 ± 0.08
4	CRK	*Carbapenem Resistant Klebsiella pneumoniae*	200 ± 0.09
5	ESBL	*Extended Spectrum beta lactamase E. coli*	125 ± 0.07
6	VRE	*Vancomycin-resistant Enterococci*	200 ± 0.08
7	QRS	*Quinolone resistant Salmonella*	100 ± 0.05
8	MRSA	*Methicillin-resistant Staphylococcus aureus*	200 ± 0.04
9	ERS	*Erythromycin resistant Streptococci*	100 ± 0.06

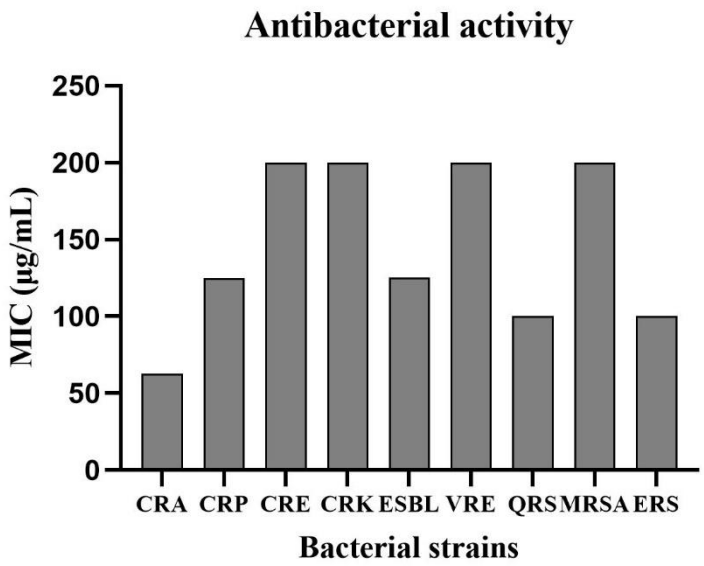

Figure 5: Action of *Chrysopogon zizanioides* derived essential oil against antibiotic-resistant bacterial strains

Free radical scavenging potential of essential oil by DPPH assay:

According to the DPPH method, antioxidants react with the stable free radical 1,1-diphenyl-2-picrylhydrazyl and convert it to 1,1-diphenyl-2-picrylhydrazine which results in discoloration of the deep violet color. The degree of discoloration indicates the free antioxidant's radical scavenging activities, and it has been discovered that known antioxidants like Cysteine, Glutathione, Ascorbic Acid, Tocopherol, and Polyhydroxy Aromatic Compounds reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability [29]. In this study, the essential oil was diluted from a range of 100 to1000 µg at intervals of 100 µg, which is given in **Table (3)**. Results of the assay suggest that the highest % inhibition of free radical DPPH was recorded at a concentration of 1000 µg with a value of 85.38%, whereas the lowest % inhibition activity was seen at 100 µg which was 39.24%, which is graphically represented in **Figure (6)**. Correlation of the antioxidant activity can be done with different components of the oil having various vital activities which were identified using GC-MS. Components such as alpha Copaene [30], Non-3-en-2-one [31], and Aromadendrene [32] were identified by the GC-MS profile, previous studies have proved that this compound has promising antioxidant properties.

Table 3: Free radical scavenging action of essential oil by DPPH assay

Sr No.	Concentration (µg)	% Free Radical Scavenging Activity
1	100	39.24 ± 0.06
2	200	42.55 ± 0.02
3	300	48.48 ± 0.09
4	400	52.82 ± 0.05
5	500	54.81 ± 0.01
6	600	59.86 ± 0.03
7	700	66.17 ± 0.07
8	800	70.37 ± 0.07
9	900	77.79 ± 0.08
10	1000	85.37 ± 0.07

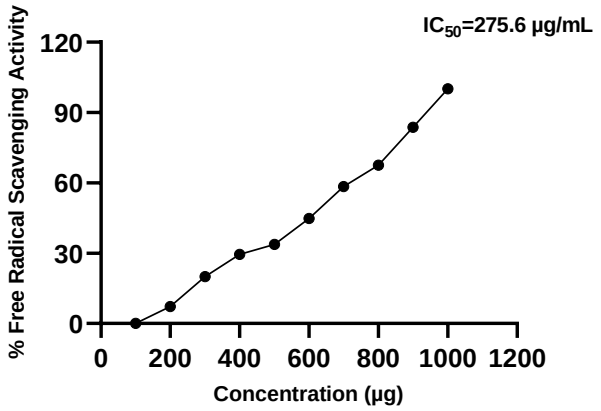

Figure 6: Anti-oxidant action of *Chrysopogon zizanioides* extracted essential oil at different concentrations with an IC_{50} value of 275.6 µg/mL

Cytotoxic action evaluation of essential oil towards cell lines:

MTT reduction is commonly used as a cytotoxic test for a variety of chemical substances to investigate mitochondrial/nonmitochondrial dehydrogenase activity. In this case, this assay was employed to observe the cytotoxic effect exerted by Vetiver essential oil on the selected HeLa and CHO cell lines, which is represented through **Figure (7)**. According to the results it was observed that CHO cells were more sensitive to essential oil as they exhibited an IC_{50} value of 15.81 µL/mL representing that at this concentration the oil will successfully inhibit 50% of the total cell population. Meanwhile, HeLa cells were comparatively more tolerant to the oil since they had an IC_{50} value of 37.11 µL/mL, present in **Table (4)**. This cytotoxic activity can be correlated with the presence of compounds identified by GC-MS analysis such as Aromadendrene [32] and alpha Copaene [30], these compounds have previously also exhibited cytotoxic effects. Therefore, this activity represents that the volatile oil derived from Vetiver grass has the potential to alter mitochondrial enzymatic activity and trigger a pre-injury that leads to cell death. Essential oils have also been linked to mitochondrial membrane damage, as they produce depolarization of mitochondrial membranes by lowering membrane potential and modifying the fluidity of membranes, causing them to become abnormally permeable thus leading to cytotoxic effects [33].

Table 4: Inhibitory action of essential oil on HeLa and CHO Cell lines

concentration (µg/mL)	Hela		CHO	
	% Inhibition	IC_{50}	% Inhibition	IC_{50}
5	2.13 ± 0.09		4.06 ± 0.04	
10	8.42 ± 0.08		44.13 ± 0.05	
20	45.71 ± 0.05		69.68 ± 0.06	
40	62.08 ± 0.06	37.11	97.6 ± 0.09	15.81
60	96.18 ± 0.04		97.45 ± 0.04	
80	96.08 ± 0.08		97.6 ± 0.09	
100	96.59 ± 0.07		98.59 ± 0.07	

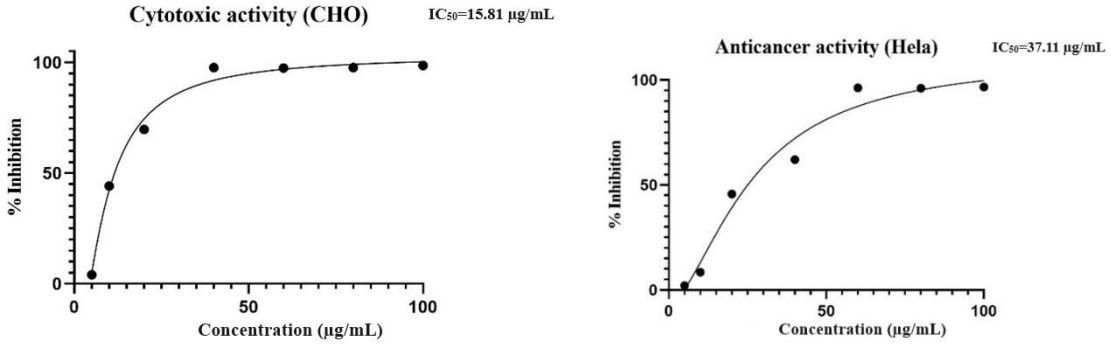

Figure 7: Anti-cancer and cytotoxic potential of *Chrysopogon zizanioides* derived essential oil at different concentrations with given IC_{50} value of both the cell lines.

Determination of Antimalarial potential against *Plasmodium falciparum*:

Malaria is an acute febrile illness caused by several strains of the parasitic protozoan microorganism called *Plasmodium*. It is the world's most serious tropical disease, according to WHO's recent report of malarial death in 2020 stood at 627,000[34]. In recent years, the widespread use of chloroquine (CQ) to prevent and cure falciparum malaria has resulted in the emergence of CQ-resistant *Plasmodium falciparum* strains throughout the afflicted region [35]. The widely accepted theory is that quinoline-containing medications affect haem disposal, which is the mechanism by which intra-erythrocytic-stage malaria parasites detoxify haem in the feeding vacuole[26]. The global rise of drug resistance to the most economical antimalarial treatment has compelled us to develop novel antimalarial approaches to combat the disease. Therefore, the adoption of natural remedies derived from plant products may usher in a new phase of malaria treatment. The anti-plasmodial properties **Table (5)** of *Chrysopogon zizanioides* essential oil against drug-resistant and drug-sensitive *Plasmodium falciparum* strains were assessed using MIC, which is depicted in **Figure (8)**. VEO with a concentration of 3.97 µg/mL suppresses 50% of cells against a drug-sensitive strain of *Plasmodium falciparum*, whereas VEO with a concentration of 5.04 µg/mL suppresses 50% of cells against a drug-resistant strain of *Plasmodium falciparum*, given in **Table (5)**. A graphical representation of survival vs essential oil concentration can be seen here.

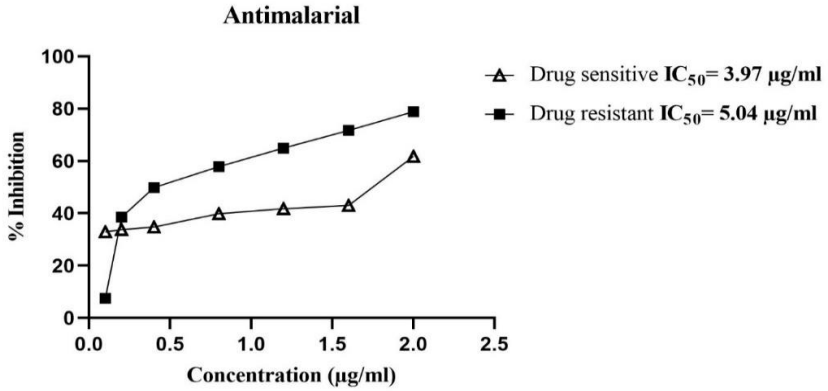

Figure 8: Action of VEO against Drug-sensitive and resistant strains of *Plasmodium falciparum*

Table 5: Anti-malarial potential of *essential oil toward Plasmodium falciparum* strains

VEO	**Drug-sensitive**		**Drug-resistant**	
Concentration (µg/mL)	**Inhibition %**	**IC_{50}Value**	**Inhibition %**	**IC_{50}Value**
0.1	32.95	3.97 µg/mL	7.56	5.04 µg/mL
0.2	33.75		38.56	
0.4	34.75		49.89	
0.8	39.85		57.89	
1.2	41.75		64.98	
1.6	43.00		71.68	
2.0	61.75		78.90	
Standard drugs	Chloroquine (control)	Quinine (control)		
IC_{50} value	0.020 µg/mL	0.268 µg/mL		

Determination of capacity of essential oil to inhibit biofilm formation by bacterial strains:

The ability of the essential oil to inhibit biofilm formation was determined by the indirect biomass-quantification technique using crystal violet. Five test cultures namely *Staphylococcus aureus, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa,* and *Escherichia coli* were employed for testing the biofilm inhibition activity of the Vetiver essential oil, given in **Table (6)**. According to the results, the essential oil showed promising activity against all organisms at a concentration ranging from 10 to 100 µg/mL. The most prominent results were shown against *Pseudomonas aeruginosa* with the highest biofilm inhibition activity of 83.23% at a concentration of 100 µg/mL. *Staphylococcus aureus* biofilm was inhibited up to 67.89% at a concentration of 100 µg/mL which was the lowest compared to all other organisms. The remaining test cultures *Proteus vulgaris, Proteus mirabilis*, and *Escherichia coli* also showed satisfactory results with inhibition of 74.56, 71.36, and 76.85 % respectively at the highest concentration. The lowest % inhibition (4.6%) was shown for *Proteus mirabilis* and the highest (14.65%) was observed for *Pseudomonas aeruginosa* at 10 µg/mL. Thus, indicating that the Vetiver essential oil has the highest inhibitory activity against *Pseudomonas aeruginosa*, which could be understood through **Figure (9)**. Vetiver has shown reliable results as an anti-biofilm agent, previous studies have concluded that root extract of *Vetiveria zizanioides* has shown Antibiofilm activity against methicillin-resistant *Staphylococcus aureus* [36].

Table 6: Anti-biofilm action of *Chrysopogon zizanioides* derived essential oil against common bacterial strains

Organisms	Concentration (µg/mL)	% Inhibition
Staphylococcus aureus	10	8.2 ± 0.05
	20	15.7 ± 0.08
	40	34.33 ± 0.09
	60	46.17 ± 0.02
	80	52.33 ± 0.06
	100	67.89 ± 0.03
Proteus vulgaris	10	12.34 ± 0.06
	20	26.78 ± 0.03
	40	42.14 ± 0.06
	60	56.78 ± 0.02
	80	67.45 ± 0.08
	100	74.56 ± 0.04
Proteus mirabilis	10	4.6 ± 0.04
	20	12.34 ± 0.06
	40	21.36 ± 0.05
	60	34.65 ± 0.03
	80	58.54 ± 0.07
	100	71.36 ± 0.05
Pseudomonas aeruginosa	10	14.65 ± 0.04
	20	24.65 ± 0.07
	40	39.89 ± 0.08
	60	51.63 ± 0.04
	80	68.97 ± 0.02
	100	83.23 ± 0.05
Escherichia coli	10	12.36 ± 0.04
	20	21.34 ± 0.02
	40	34.65 ± 0.09
	60	46.78 ± 0.05
	80	62.45 ± 0.03
	100	76.85 ± 0.08

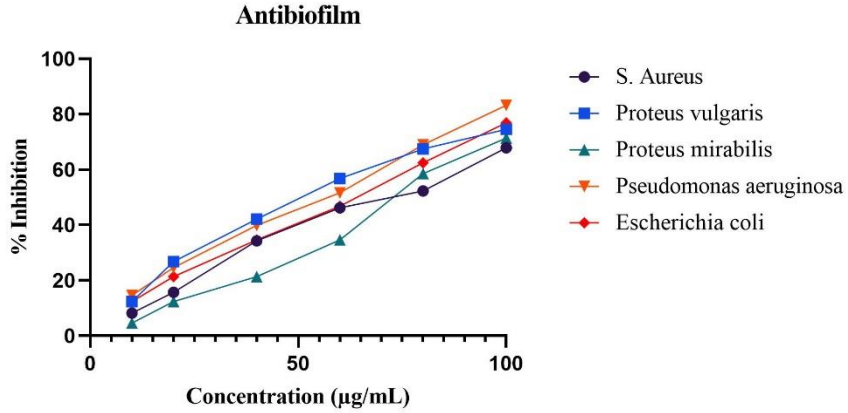

Figure 9: Biofilm suppression of *Chrysopogon zizanioides* derived essential oil against 5 bacterial cultures.

CONCLUSION:

As the world is shifting toward the use of natural products for various purposes, specifically for pharmaceuticals in order to deal with emerging ailments. Our study is a piece of evidence that explores the physio-chemical and biological potential of essential oil extracted from *Chrysopogon zizanioides* (Vetiver). The findings in our work detail the composition of VEO and indicates its promising use as, an anti-bacterial, anti-oxidant, anti-malarial, and anti-biofilm agent. The essential oil was also found to be effective against the HeLa cell line, demonstrating its anti-cancer potential, as well as showing its potential action against *Mycobacterium tuberculosis.* The above-presented activities demonstrate that VEO is a mixture of various potential compounds which was validated in our study through GC-MS analysis. Therefore, VEO can be employed as an alternative to synthetic medication, and could also be explored further in other sectors as well.

Author Contributions

All the authors mentioned in the study made enormous practical and cognitive contributions to the research and have provided their approval for it to be published.

Conflict of Interest

The authors declare that there were no commercial or financial links that existed during the research that may be regarded as a potential conflict of interest.

Abbreviations

VEO; Vetiver Essential Oil, GC-MS; Gas chromatography-mass spectrometry, FTIR; Fourier Transform Infrared Spectroscopy, TG-DTA; Thermogravimetric and Differential Thermal Analysis, DSC; Differential Scanning Calorimetry, FAME; Fatty Acid Methyl esters, MIC; Minimum Inhibitory Concentrations, DPPH; Assay, 2,2′- diphenyl-1-picrylhydrazyl Assay, RT; Room temperature, HeLa; Human cervical cancer cell line, CHO; Chinese hamster ovary, MTT Assay; 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay, FBS; Foetal bovine serum.

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