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Research Article

Comprehensive Investigation of *Juniperus virginiana* Essential Oil: GC/MS Analysis, Thermoanalytical Characterization, and Evaluation of Bioactive Potential

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Abstract

Due to the ever-increasing unregulated antibiotic use, there have been increased risks of antibiotic-resistant bacterial infections. Natural agents, their products, and derivatives have been used for centuries to cure a variety of ailments. In the present study, an attempt was made to explore the physiochemical properties of the CEO. GC-MS analysis was used to identify the volatile components present in the oil. Additionally, TGA and DSC analysis were carried out to understand the thermal properties of the oil. The oil was characterized by higher amounts of terpenoids. The essential oil has potent anticancer, cytotoxic, antimalarial, and anti-inflammatory activity. This study concludes that the *Juniperus virginiana* essential oil could be used as a therapeutic agent.

Keywords: Anticancer, Antioxidant, Cedarwood, Cytotoxicity, Essential oil

INTRODUCTION

Infectious disease has afflicted mankind since the dawn of time. When Alexander Fleming developed penicillin in 1923, he provided a ray of hope for the eradication of infectious diseases [1]. The contemporary struggle against bacterial infections began with this discovery, but since then, unregulated antibiotic use and over-prescription have increased the frequency of antibiotic-resistant bacteria [2]. Diseases caused by antimicrobial-resistant microorganisms have become more common and diverse in both hospitals and communities over the last few years. Drug resistance is becoming more common due to a combination of microbial features, antimicrobial use selection pressures, cultural and technological changes that facilitate the spread of drug-resistant organisms [3]. Antimicrobial resistance increases morbidity, death, and healthcare costs. To control these issues, new immunizations, cautious drug use, novel antimicrobial agents, and enhanced public health measures are necessary. Common bacteria with multidrug resistance cause community and nosocomial infections. (MDR) are *Pseudomonas aeruginosa, Staphylococcus aureus*, *Salmonella spp.*, coagulase-negative *Staphylococcus*, *Shigella*, *Enterococcus sp.* and *Escherichia coli* [4,5].

Since then, the current antimicrobial crisis is fueled by a decline in antibiotic discovery and the rise of drug-resistant organisms in human infections.[6]. This has led to an urgent need for new antibiotics [7] and recognizing this, the scientific community has shown interest in herbal medicines with antimicrobial properties. Herbal therapy supports natural healing processes, aiming to address imbalances gently. Globally, plant extracts and essential oils are explored for antimicrobial properties and alternative treatments, given their diverse activities[8,9,10]. Therefore, essential oils can be exploited as a reservoir of active biological compounds for reducing bacterial resistances.

Essential oils are complex, aromatic, volatile, oily liquids obtained and exploited from different parts of plants. Historically, medicinal plants have always been a part of pharmaceutical and dietary therapy [11,12]. Essential oils have been used since Ancient Egypt, when they were produced by

steeping plant parts in animal fats or vegetable oils [13]. The Great Plague, caused by *Yersinia pestis*, began in 1347 and spread across Europe, killing one-third of the population [14]. People who were exposed to essential oils were reported to have better immunity as compared to their other counterparts [15]. Rene Gattefosse, a French chemist, invented the term "Aromatherapy" in 1937 and conducted research into essential oils that proved their therapeutic capabilities [16]. Today, essential oils are being utilised to treat a variety of illnesses, including cancer, pain, stress, and infectious disease. Essential oils are made up of a variety of volatile substances and thus represent multi-component systems, whereas their main components are single-component systems [17]. These are mixtures of over 22 chemical compounds produced by aromatic plants as secondary metabolites, including sesquiterpenes (terpenes, aliphatic aldehydes, alcohols, and esters), as well as non-volatile components (hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins, and flavonoids) [18]. The composition varies based on factors like plant species, region, and extraction methods [19]. These oils exhibit a range of biological activities, such as antiviral, analgesic, antimicrobial, and anti-inflammatory effects.[20].

Cedarwood essential oil (CEO) can be extracted from the needles, leaves, bark, and berries of cedar trees and is a yellow-colored sticky oil. The most frequent species of these trees in nature are *Cedrus atlantica, Cedrus deodara, Juniperus virginiana,* and *Juniperus mexicana*, also known as Atlas, Himalayan, Virginian and Texan Cedarwood, respectively [21]. *Juniperus virginiana L.* (Eastern Red Cedar) is a dioecious, aromatic conifer from the *Cupressaceae* family. The tree has a history of medicinal use for colds, measles, skin issues, and rheumatic pains[22]. Cedarwood essential oil exhibits antibacterial, antifungal, anti-inflammatory, antispasmodic, diuretic, and insecticidal properties attributed to bioactive compounds including alpha-cedrene, beta-cedrene, thujopsene, cedrol, widdrol, and sesquiterpenes [23]. Additionally, Cedarwood essential oil, once part of the 'mithridat' poison antidote, has historical use in religious rites and spiritual practices, including collective prayer and solo meditation[24]. Cedarwood essential oil is recognized by scientists and herbalists for its efficacy in addressing skin issues, arthritis, lung and kidney problems, anxiety, and stress through aromatherapy. Despite its commercial significance, the essential oil from Juniperus virginiana deserves further study.

This study explores Juniperus virginiana essential oil (CEO) for its effectiveness against microbial infections. Utilizing advanced techniques like DSC, Thermogravimetry, GC-MS, FT-IR, and HPTLC, it analyzes the oil's thermal properties. The research delves into its potential applications as an antimicrobial, antioxidant, anticancer, and antimalarial agent.

MATERIALS AND METHODS

Extraction of oil using Hydro-distillation method

Cedarwood essential oil was produced in the laboratory using fresh wood sample of *Juniperus virginiana* plant. 200 g of sample was weighed and placed in a 2L Erlenmeyer conical flask. The flask was then connected to the Clevenger apparatus. Further, 1L of double distilled water was added to the flask and heated up to 100°C. Subsequently, the vapour phase was collected in a graduated cylinder. After a duration of 4h, the crudely extracted EO was separated from the aqueous layer as per the procedure mentioned by Want *et al* [25].

GCMS Analysis

GC-MS analysis utilized a Clarus 600GCMS system with a 30 m GsBP®5MS capillary column (0.25 mm internal diameter, 0.25 µm film). Helium (99.999%) served as the mobile phase at 1.20 mL/min. The injector, held at 250°C, injected a 1 µL sample (split ratio 150:1). The initial oven temperature was 40°C for 3 min, then increased to 230°C at 10°C/min, holding for 3min. Total run time was 25 min, reaching a maximum temperature of 350°C with a 2 min equilibration time. Mass spectra (40 to 1000 m/z at 70 eV) were compared with NIST & Wiley libraries, and retention indices with literature for component identification. [26].

Thermogravimetric analysis (TGA)

STA 250 was used to analyse the TGA measurements. The tests were carried out in a nitrogen gas atmosphere at a flow rate of 300 mL/min. The samples weighed 20 mg and were placed in aluminium crucibles. The readings began at 30°C and increased at a pace of 20°C per minute.

Differential scanning calorimetry (DSC) analysis

A differential scanning calorimeter, model DSCQ20, was used to obtain the DSC essential oil profile. In aluminium crucibles, 4 mg sample was introduced. A nitrogen gas flow of 40 mL/min was used to analyse the sample. Over a temperature range of 150 to 300°C, a dynamic scan was executed at a rate of 20°C/min.

Fourier Transform Infrared Spectrometry (FTIR) analysis

Initially, IRPRESTIGE 21 Shimadzu Fourier transform infrared spectrometer was preheated and stabilized. A sample was placed in a NaCl pellet, with another pressed onto it, creating a uniform oil membrane. This setup was then positioned in the infrared spectrometer sample holders. Infrared absorption spectra were collected in the range of 4000–650 cm^{-1} under designed conditions, with a resolution of 8 cm^{-1} and 32 accumulations for analysis.

In-vitro Antimalarial screening

In-vitro antimalarial assays in 96 well microtiter plates were used to assess the essential oil's effectiveness against *Plasmodium falciparum*. In an enhanced RPMI-1640 growth media, *Plasmodium falciparum* and its drug-resistant variant were maintained. Parasites were treated with 5% D-sorbitol to obtain ring stage cells, followed by synchronization of *P. falciparum* and the drug-resistant variant. To evaluate the percentile parasitemia (rings), the JSB staining method [27] was utilized to measure an early ring phase parasitemia of 0.8 to 1.5% at 3% hematocrit in 200 µL of RPMI-1640 medium and sustained with 50 % RBCs (O+). The essential oil stock solution was diluted in DMSO at a concentration of 5 mg/mL, with further dilutions ranging from 0.1 µg/mL to 2 µg/mL. 20 µL of the diluted samples were placed in test wells and duplicate wells, each containing the cell preparations. In a candle jar, the culture plates were incubated at 37°C for about 36 to 40 h. After incubation, thin blood smears from each well were produced and stained with JSB stain. Chloroquine and Quinine served as reference drugs. Microscopic examination revealed the transformation of ring-stage parasites into schizonts and trophozoites at various test substance concentrations. Minimum inhibitory concentrations (MIC) were documented, limiting overall maturation, with recorded IC50 values for each *P. falciparum* strain.

Assessment of Antioxidant activity

DPPH assay

The radical scavenging potential of CEO was studied through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [28] with slight variations. Oil concentrations from 1 mg/mL to 10 mg/mL were prepared from a 100 mg/mL stock in dimethyl sulfoxide (DMSO). The total reaction volume was 4 mL, including sample, methanol as a diluent, and 2 mL of DPPH in each tube. After a dark incubation period of 30-45

minutes, absorbance was measured at 515 nm using a UV-Visible spectrophotometer.

The percentage inhibition of the DPPH radical for each concentration was determined by making use of the following formula:

$$\text{Percentage DPPH radical scavenging activity} = \left[\frac{\{OD(control) - OD(sample)\}}{OD(control)}\right] * 100$$

Phosphomolybdate Assay

This assay was used to determine the total antioxidant capacity of CEO. 0.1 mL sample solution aliquot was added to 1 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were covered and placed in a 95 °C water bath for 90 min. The absorbance of the reaction mixtures was measured at 765 nm once the samples cooled down. Ascorbic acid was utilized as a control.

The antioxidant capacity was calculated using the formula:

$$\text{Percent Total antioxidant capacity} = \left[\frac{\{OD(sample) - OD(control)\}}{OD(sample)}\right] * 100$$

Examination of Anticancer activity by MTT assay

HeLa cells were provided by the National Centre for Cell Science (NCCS) in Pune, were cultured in Dulbecco's Modified Eagle Medium DMEM with 10% fetal bovine serum FBS and antibiotics (Penicillin and Streptomycin). Cultures were passaged weekly, and the culture medium was replaced twice a week; the cells were maintained at 37°C, 5% CO2, 95% air, and 100% relative humidity. Trypsin-ethylenediaminetetraacetic acid was used to produce single-cell suspensions, which were then counted and diluted to 1 × 10^5 cells/mL in 5% FBS medium. After 24 hours, 96-well plates containing 10,000 cells per well were treated with oil samples dissolved in dimethyl sulfoxide (DMSO). To obtain final concentrations of 5, 10, 20, 40, 60, 80, and 100 mg/mL, 100 µL of each concentration was administered in the plates and were incubated for 48 hours. Nothing was in the control wells. Following 48 hours, 5 mg/mL of MTT was added, the mixture was incubated for 4 hours, and formazan crystals were measured at 595 nm using a microplate reader after dissolution in DMSO.

The percentage of cells inhibited was calculated using the formula:

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

Evaluation of Cytotoxicity of CEO

The essential oil's cytotoxicity was tested on Chinese Hamster Ovary (CHO) cells maintained at 37°C, 5% CO2, 95% air, and 100% relative humidity. Cells were passaged weekly, and single-cell suspensions were created using trypsin-ethylenediaminetetraacetic acid. Viable cells were counted and diluted with 5% FBS medium to achieve a final density of 1 × 105 cells/mL. Cytotoxic activity was assessed using the tetrazolium salt MTT(3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide), based on the reagent cleavage by dehydrogenases in live cells. Percentage cell inhibition was evaluated at final doses of 5, 10, 20, 40, 60, 80, and 100 mg/mL, and the concentration inhibiting cell growth by 50% (IC50 value) was determined.

HPTLC fingerprint profiling of CEO

Concentrated extracts of the CEO were homogenized in 20 mL of methanol and stored at 4 ºC. High-Performance Thin Layer Chromatography (HPTLC) analysis was carried out to establish the chromatographic profile referring to an optimized methodology deduced by Moein *et. al* [29]. Approximately 2 µL of the essential oil sample on an aluminium pre-coated silica gel plate. CAMAG TLC system with VisionCats Software processed data. The solvent system Toluene: Ethyl acetate (9.7:0.3 v/v) in a Twin trough Glass Chamber (TTC, 10*10 chamber) was used for TLC plate development. After saturation, plates were derivatized with vanillin-sulphuric acid and heated at 120 ºC for 3 min. Examination under visible light and UV light at 254 and 366 nm followed.

Determination of Anti-inflammatory activity

The anti-inflammatory assay, i.e., the albumin denaturation assay, was carried out with minor changes as described by Foe *et al.* [30]. A range of diluted CEO concentrations prepared in dimethyl sulfoxide and 3 % bovine serum albumin (BSA) fraction in sterile distilled water were added in separate reaction mixture tubes. The test tubes were heated for 20 minutes at 60 ℃ after 20 minutes of incubation at 37 ℃. The absorbance of these solutions was measured using a spectrophotometer at 660 nm. The following formula was used to compute the percentage inhibition of precipitation:

$$\text{Percentage inhibition of denaturation} = 100 - \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) * 100$$

RESULTS AND DISCUSSION

GC-MS

The GC-MS chromatogram (Figure 1) of the essential oil of *Juniperus virginiana* revealed the presence of 22 distinct compounds. Comprising mostly sesquiterpenes, along with terpenes, diesters, and organic compounds, the essential oil makes up 99.9% of the total composition. (Table 1) summarizes the retention time (RT), compound names, and percentage area for all the identified compounds of CEO in this study. The highest percentage peak shown is 19.5 while the lowest is 0.29. The most predominant compound of CEO was alpha-Cuprenene (19.5%) followed by alpha-trans Atlantone (14.32%); alpha-Himachalene (13.62%); gamma-E-Atlantone (9.6%); Diethyl Phthalate (9%); gamma-Himachalene (5.82%); Allohimachalol (4.67%); beta-Himachalene oxide (4.22%); alpha-Z-Atlantone (3.66%); Limona Ketone (1.92%); Calarene Epoxide (1.74%). The retention time in minutes for each of the components were 15.59, 18.65, 14.87, 17.83, 15.09, 15.22, 17.4, 16.84, 17.92, 10.29, 17.25, respectively. Compared to previous studies on *Juniperus virginiana* essential oil, the mentioned composition exhibits significant variations [28]. The presence of Longiborneol and Allohimachalol in CEO serves as antimicrobial [31, 32], and Longiborneol also has antiseptic properties, while gamma-E-

Atlantone (Atlantone) functions as a potent anticancer component[32,33]. Alpha-E-Bisabolene and gamma-E-Atlantone are potential food additives, while components like gamma-Himachalene and alpha-Cuprenene serve as organoleptics, contributing aromatic sensations. The diverse composition of CEO positions it prominently in pharmaceutical and cosmetic applications.

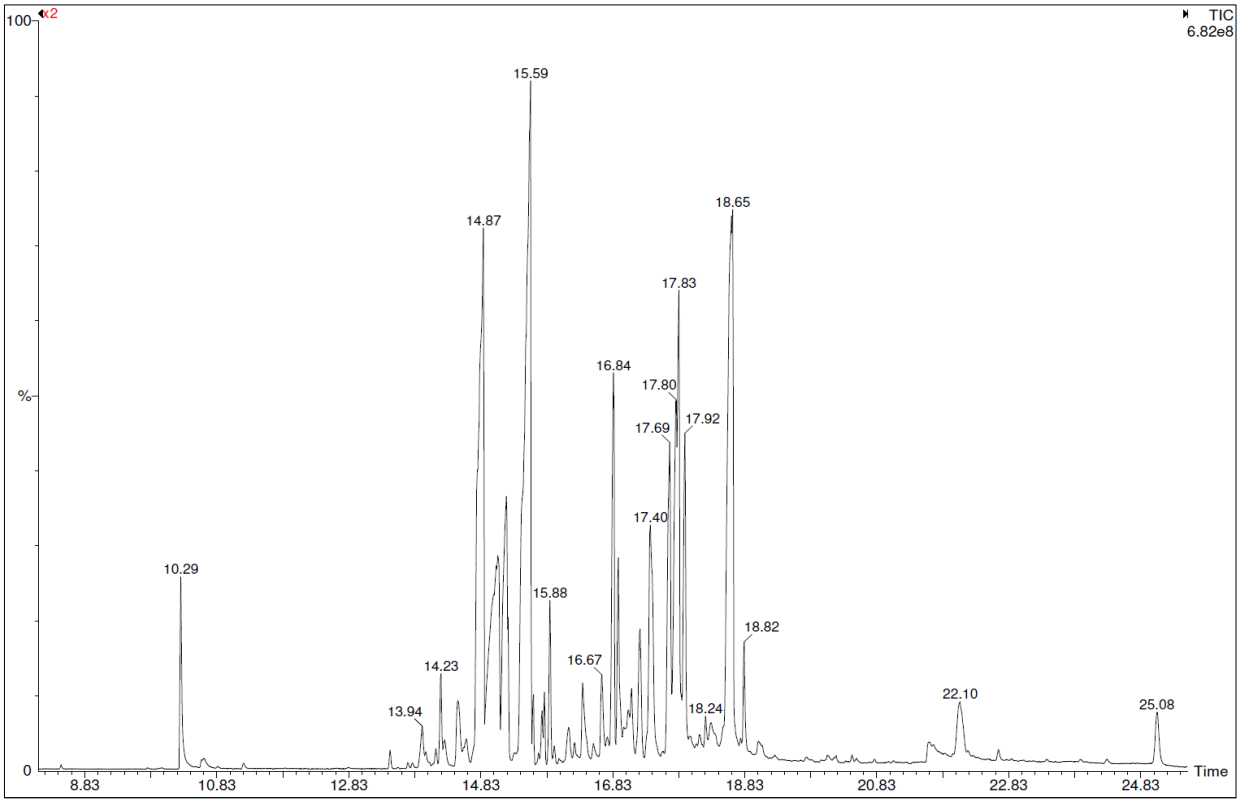

Figure 1: Representative GC-MS chromatogram of CEO

Table 1: Chemical composition of *Juniperus virginiana* essential oil

Retention Time (min)	% Area	Name
15.59	19.5	Alpha-Cuprenene
18.65	14.32	Alpha-trans Atlantone
14.87	13.62	Alpha-Himachalene
17.83	9.6	Gamma-E- Atlantone
15.09	9	Diethyl Phthalate
15.22	5.82	Gamma-Himachalene
17.4	4.67	Allohimachalol
16.84	4.22	Beta-Himachalene oxide
17.92	3.66	Alpha-Z-Atlantone
10.29	1.92	Limona Ketone
16.92	1.74	Calarene Epoxide
17.25	1.74	Beta-Himachalene
14.49	1.61	Himachala-2,4-Diene
15.88	1.3	Alpha-E-Bisabolene
14.23	1.17	Longifolene
18.82	1.12	10,11-E-Dihydroatlantone
16.37	1.11	Himachalene epoxide
25.08	1.01	Bis(2-ethylhexyl)adipate
16.67	0.94	Longiborneol
17.12	0.87	Thujopsan-2-alpha-ol
13.94	0.76	2,2-Dimethyl, 2,3-Dihydro-1H-Indene
15.8	0.29	Gamma-dehydro-ar-Himachalene

Thermogravimetric profile

Cedarwood essential oil (CEO) exhibited high thermal stability, as seen in the thermogram (Figure 2). A single decomposition event occurred between 105°C and 310°C, resulting in a 99.83% mass loss due to the evaporation of volatile components. The TGA curve stabilized with increased temperature, indicating the oil's resistance to mass loss at elevated temperatures, consistent with its thermal stability observed in previous studies. Previous studies suggest that(34), the presence of various constituents in the essential oil is one of the factors that could be correlated with its thermal tolerance or susceptibility when exposed to varying temperatures.

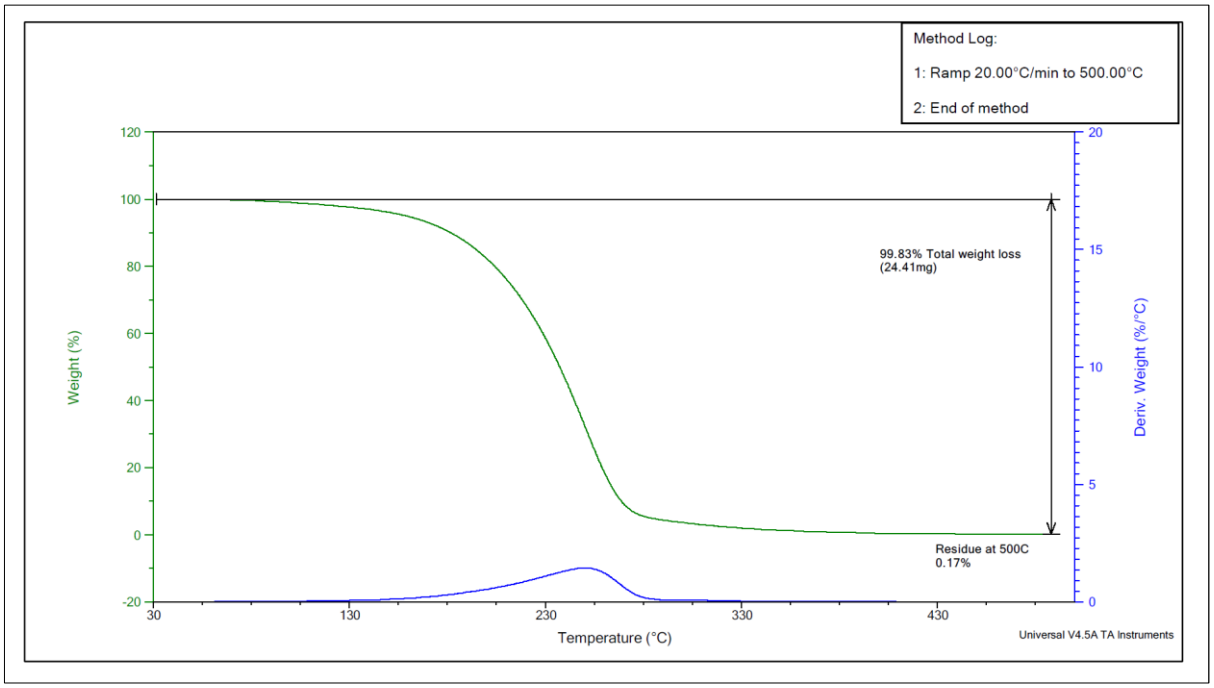

Figure 2: Thermogravimetric profile of *Juniperus virginiana* essential oil

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) was undertaken to explore how heat moves within the sample under controlled circumstances, with the aim of evaluating its thermal resilience. The DSC plot of cedarwood essential oil (CEO) displayed in Figure (3) shows that with rising temperatures, the heat flow within the oil initially declines, indicating an endothermic process between 50°C and 75°C. Following this, there is a modest increase in heat flow, peaking at 109.8°C, accompanied by an exothermic change suggesting absorption of heat by the sample. A swift endothermic decrease is observed at 170.98°C, marking a shift in heat flow. As the temperature climbs further, there's an abrupt endothermic drop at 281.83°C, succeeded by a gradual rise in heat flow up to 375°C. This analysis of thermal behavior provides insights into the stability of the essential oil as temperature varies.

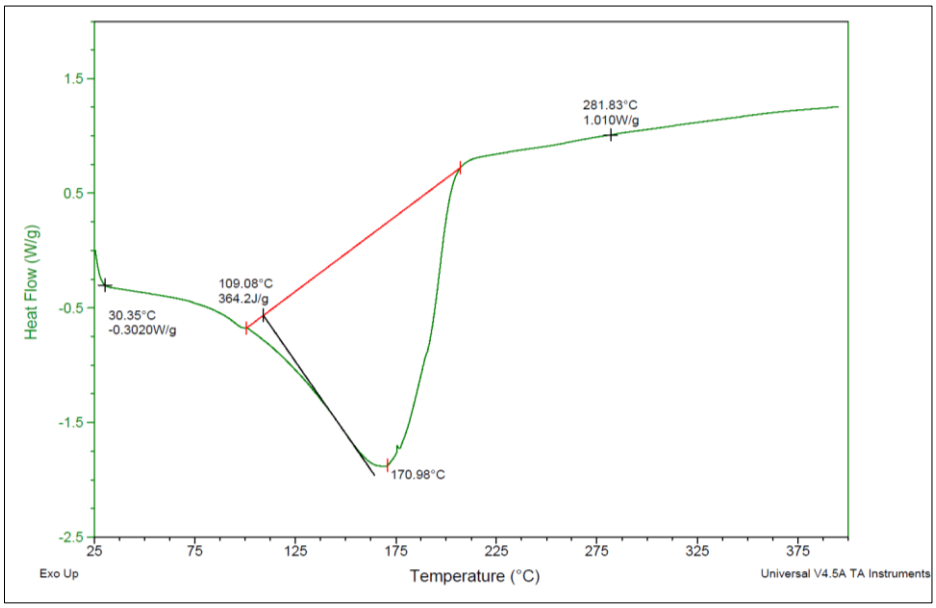

Figure 3: Differential scanning calorimetry profile of *Juniperus virginiana* essential oil

FTIR analysis

FTIR Analysis, or Fourier Transform Infrared Spectroscopy, scans samples with infrared light (Figure 4). Peaks in the FTIR spectrum pinpoint functional groups, like intramolecular hydrogen at 3317.3 cm-1 and aldehydic bonds at 2944.6 cm-1 and 2832.8 cm-1. Conjugated aldehyde, weak bonds, and aromatic rings appear at 1662.4 cm-1, 1449.9 cm-1, and 1412.7 cm-1. Absorption at 1021.3 cm-1 and 1114.5 cm-1 suggests the presence of the alcoholic group (C-O), aiding compound identification.

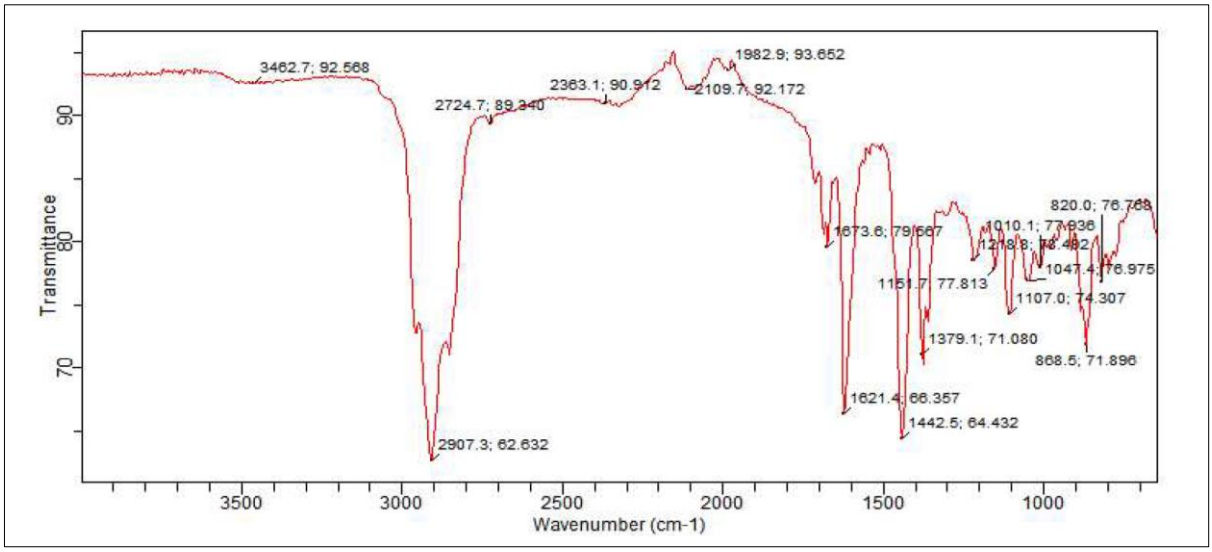

Figure 1: FTIR analysis of *Juniperus virginiana* essential oil

Antimalarial activity

The anti-plasmodial activity of CEO was assessed by determining the minimum inhibitor concentration needed to inhibit 50% of cells in drug-sensitive Plasmodium falciparum and quinine-resistant Plasmodium falciparum. The IC50 values were higher than standard drugs, with 1.10 µg/mL against drug-sensitive and 2.04 µg/mL (Figure 5) against quinine-resistant strains. This outcome is attributed to terpenes like α-himachalane, α-cuprenene, γ-atlantone, α-atlantone, and longifolene, indicating Cedarwood essential oil as a valuable source for potential anti-malarial drugs. [35]. There are also studies carried out where terpenes such as longifolene have biological activity against larvae [36]. To the best of our knowledge, this is the first study to show that CEO has antimalarial efficacy against *Plasmodium falciparum*.

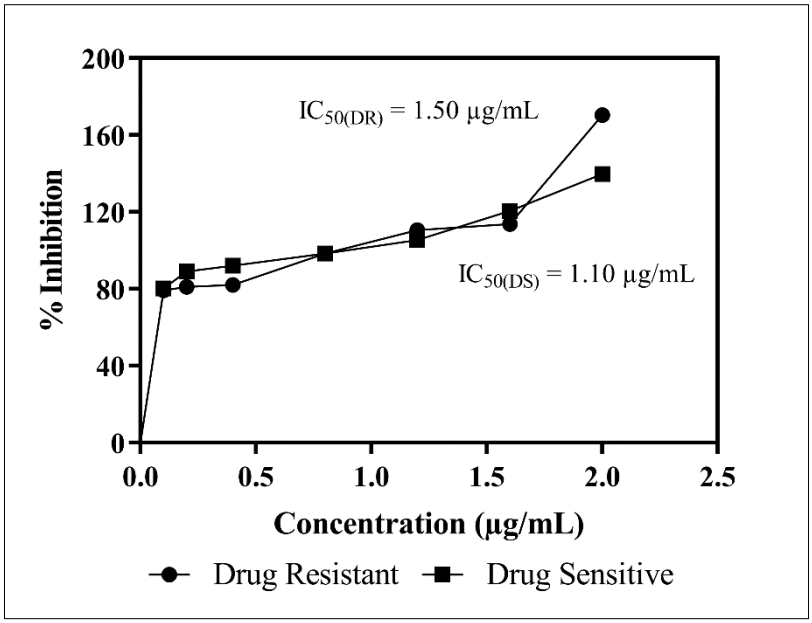

Figure 5: Anti-malarial Activity of *Juniperus virginiana* against drug resistant and drug sensitive strains of *Plasmodium falciparum*

Antioxidant Activity

DPPH assay

In order to understand the antioxidant capacity of CEO, an in vitro antioxidant test was performed. To investigate the free radical scavenging activity of naturally occurring chemicals, different quantities of essential oil were treated to the DPPH (2,2′ - diphenyl-1-picrylhydrazyl) free radical scavenging technique in this work. the lowest at 1 mg/mL (33.86 ± 0.04%) and the highest at 10 mg/mL (66.54 ± 0.08%), yielding an IC50 value of 3.28 mg/mL. (Figure 6). The existence of Gamma-E-Atlantone & Alpha-Z-Atlantone, Alpha-trans Atlantone [37], and similar compounds, which were validated by our GC-MS investigation and have previously been described as effective anti-oxidant agents, may be related to the compound's ability to scavenge free radicals. As a result, the presence of such components contributes to the antioxidant effects of cedarwood essential oil extracted from *Juniperus virginiana.*

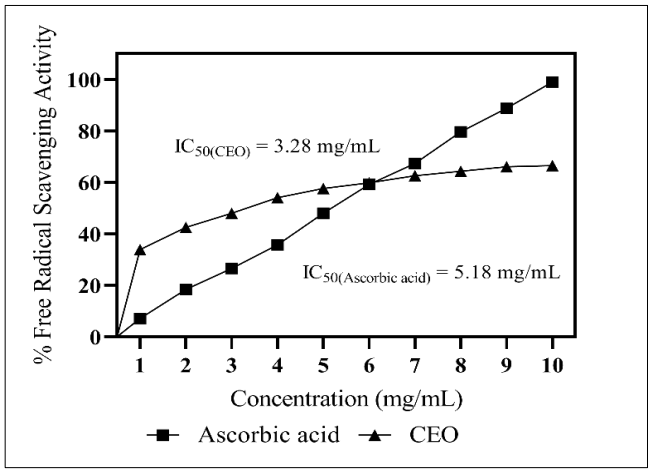

Figure 6: Percentage Free Radical Scavenging Activity of CEO using Ascorbic acid as control

Phosphomolybdate Assay

The assay, based on phosphomolybdate ion reduction in the presence of an antioxidant, creates a green phosphate complex which is quantified spectrophotometrically [38]. Using the calibration curve (Figure 7), the total antioxidant capacity of *Juniperus virginiana* essential oil was quantified at doses from 1 to 10 mg/mL. The graphical representation showed *Juniperus virginiana* essential oil and ascorbic acid with an IC_{50} value of 1.99 mg/mL and 2.01 mg/mL respectively. This suggests that cedarwood essential oil, with potent antioxidant activity, can be utilized similarly to ascorbic acid, a well-known antioxidant.

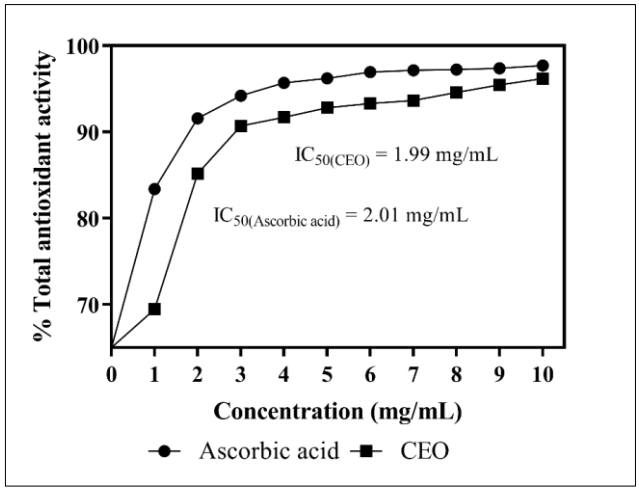

Figure 7: Percentage Total antioxidant activity of CEO using Ascorbic acid as control

Anticancer activity

Earlier research demonstrates essential oils' significant anticancer properties against various cell lines[39]. EOs induce programmed cell death, involving changes in membrane fluidity, decreased ATP synthesis, altered pH gradient, and loss of mitochondrial potential, crucial for cell death [40]. Certain essential oils have been identified as promising anticancer medicines and are currently being studied for their cytotoxic and antiproliferative properties in cancer cell lines or experimental animals [41]. The results exhibited that the EO of *Juniperus virginiana* have a significant inhibition of the HeLa cell line with IC_{50} of 42.16 mg/mL (Figure 8). Sesquiterpenes, in both oxygenated and hydrocarbon forms, were particularly potent antitumor leads [42,43]. Several studies concluded that increasing sesquiterpene content in EOs resulted in increased anticancer activity [44]. The presence of bioactive chemicals in CEO was supported by dose-dependent toxicity in an in vitro assay conducted in cancer cell lines.

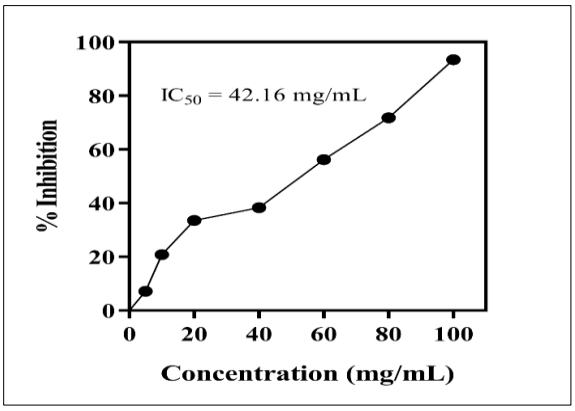

Figure 8: Anticancer activity of *Juniperus virginiana* essential oil against HeLa cancer cell line

Cytotoxic activity

The cytotoxicity of, *Juniperus virginiana*, against the Chinese hamster ovary (CHO) are shown in Figure. The results exhibited that the EO of *Juniperus virginiana* have a significant inhibition of the CHO cell line with IC_{50} of 45.9 mg/mL. According to the findings, *Juniperus virginiana* essential oil improved the inhibition rates of CHO cell lines in a dose-dependent manner (Figure 9). It is likely that the essential oil's anticancer properties are related to the presence of the sesquiterpene molecule Atlantone [33] and the synergistic effect of all the terpenes present in the essential oil, which was detected as a prominent component in our GC-MS analysis and can be explored in the future as a potential source of cytotoxic agents [45]. Cedarwood essential oil was found to have anticancer and cytotoxic activities against the particular cell line in this investigation for the first time.

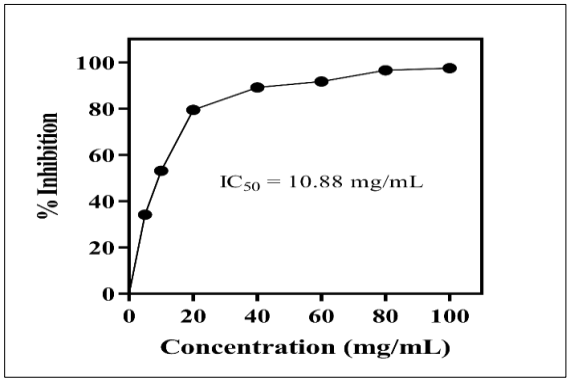

Figure 9: Cytotoxic activity of *Juniperus virginiana* essential oil against CHO cell line

HPTLC

Essential oils are complex mixtures with diverse constituents due to the numerous processes and multiple parameters involved in their extraction procedure [46]. High-Performance Thin Layer Chromatography (HPTLC), plays a crucial role in identifying bioactivities[47]. HPTLC is widely acknowledged by regulatory bodies for identifying essential oils and detecting adulteration. [46,48].The present study determines the chromatographic profile of the *Juniperus Virginia* essential oil in Toluene: Ethyl acetate (9.7:0.3 v/v) solvent system. For visualisation, the universal reagent for natural products as well as the most often used reagent in TLC analysis of essential oils, vanillin-sulfuric acid, was employed. It appears specific for the visualisation of monoterpenes, steroids and triterpenes [49]. Under UV light at 254 nm, compounds with two or more conjugated double bonds appeared as dark zones against the light-green-fluorescent TLC plate. Monoterpenes, triterpenes, and steroids exhibited characteristic colours aiding identification. In the TLC analysis, vanillin-sulfuric acid was used for visualizing natural products in the essential oil. Plates were heated for 5–10 minutes at 100–105°C, enhancing colour development. Monoterpenes had a mild grey tint, sterol steroids appeared in greyish blue, and triterpenes emitted purple-violet or reddish/blue colours under different lights. Flavonoids fluoresced under UV-366 nm. TLC plates displayed distinct blue, violet, and brown bands, and the densitogram revealed four unique peaks in the essential oil.(Figure 10). Additionally, the essential oil also presented four distinctive peaks on the densitogram (Figure11).

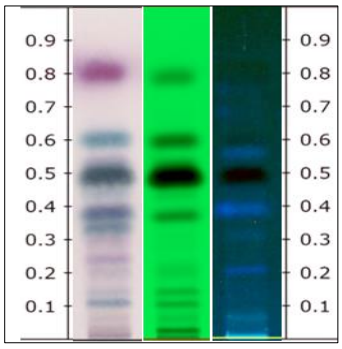

Figure 10: TLC analysis of *Juniperus virginiana* essential oil

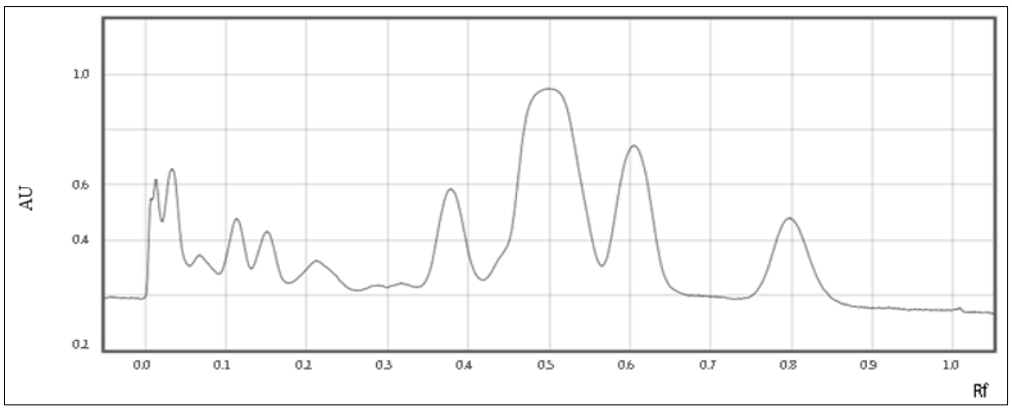

Figure 11: Densitogram of *Juniperus virginiana* essential oil

Anti-inflammatory assay

Inflammation is a protective response to tissue injury, characterized by heat, redness, pain, swelling, and compromised physiological functions[50]. The anti-inflammatory activity of the essential oil *Juniperus Virginia* was evaluated against denaturation of the bovine serum albumin method. The inhibitory concentration of cedarwood essential oil was determined using different concentrations of albumin serum ranging from 1-10 mg/mL. The highest inhibition rate occurred at a CEO concentration of 10 mg/mL, with a percent inhibition of 88.97 ± 0.01. Inhibiting protein denaturation is crucial in mitigating inflammatory disorders like rheumatoid arthritis, cancer, and diabetes. [51]. Cedarwood essential oil (Figure 12) exhibits anti-inflammatory potential, possibly due to compounds like Longiborneol, supported by historical antibacterial use for inflammation.

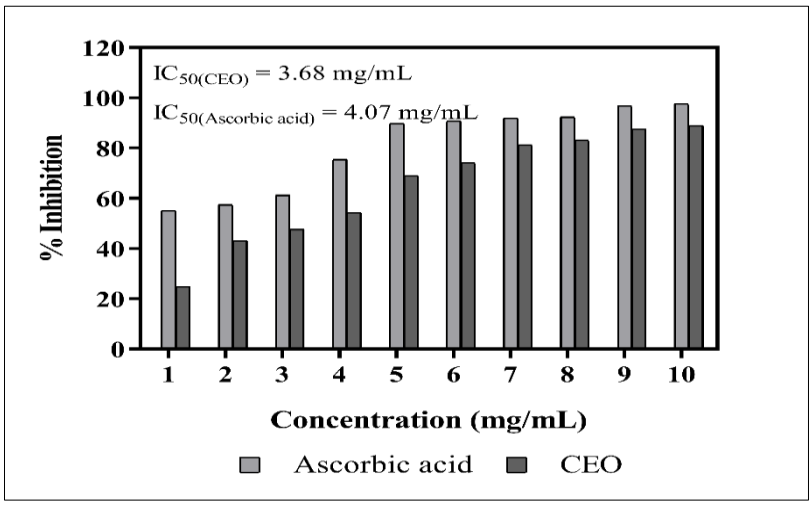

Figure 12: Anti-inflammatory activity of *Juniperus virginiana* essential oil using Ascorbic acid as positive control

CONCLUSION

The prevalence of antibiotic resistance has escalated due to the unrestrained utilization of antibiotics, thus making drug resistance a significant apprehension in disease management. Essential oils and other plant extracts have piqued curiosity as sources of natural agents with medicinal use throughout history. They are being tested to see if they could be used to treat a variety of infections and several disorders. Considering the increasing interest in natural alternatives, we endeavored to conduct this study on cedarwood essential oil. The GC-MS analysis revealed a list of bioactive components found in the essential oil of *Juniperus virginiana*. Thermogravimetric Analysis and Differential Scanning Calorimetry were used to analyze the stability and heat resistance of the CEO. Not only does the essential oil have antimalarial action against drug-resistant *Plasmodium*, but it also has substantial antioxidant, anti-inflammatory, and anticancer properties. The encouraging attributes of the cedarwood essential oil (CEO) have prompted us to draw various conclusions, including its potential application in liposomal form, nanoparticles, essential oils derived from alternative plants, essential oil constituents, as well as antibiotics. This will allow us to better understand its synergistic activity in the presence of other compounds as well as its medical use in therapy.

Abbreviations

MDR, Multidrug Resistance; CHO, Chinese hamster ovary; CEO, Cedarwood essential oil; DMSO, Dimethyl sulfoxide; DPPH, 2,2′-diphenyl-1-picrylhydrazyl; DSC, Differential Scanning Calorimetry; FBS, Foetal bovine serum; GC-MS, Gas chromatography mass spectrometry; HeLa cell line, Henrietta Lacks cell line; HPTLC, High Performance Thin Layer Chromatography; TGA, Thermogravimetry Analysis; MTT Assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay.

Ethics declarations

Ethics approval and consent to participate: Not applicable.

Data Availability Statement: All data generated or analysed during this study are included in this published article

Author Contributions: All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ethical statement: No animals were harmed during this study.

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