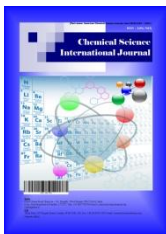

Chemical Science International Journal

31(2): 1-14, 2022; Article no.CSIJ.88917
ISSN: 2456-706X
(Past name: American Chemical Science Journal, Past ISSN: 2249-0205)

Chemical Composition, Bioactive Potential, and Thermal Behaviour of *Cyperus scariosus* Essential Oil

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Authors' contributions

This work was carried out in collaboration among all authors. Conception and design of study was done by authors VJ, DR, SW, NJ and MAS. Acquisition of data was by authors RP, SD, JK, and TJ. Analysis and interpretation of data was by authors VJ, DR, TJ and SW. Drafting the manuscript was by authors RS, SN, TJ, MAS and NJ. Revising the manuscript critically for important intellectual content was by authors SN, MAS, TJ, VJ and NJ. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CSJI/2022/v31i230276

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/88917

Original Research Article

Received 15 April 2022
Accepted 23 June 2022
Published 30 June 2022

ABSTRACT

Plant derived oils have recently been found to be more effective in pharmaceutical sectors than synthetic goods since these plants contain physiologically active chemicals with a wide range of therapeutic effects. Essential oil, also known as essence, volatile oil, etheric oil, or aetheroleum, is a complex mixture of volatile elements biosynthesised by living organisms, which is particularly abundant in aromatic plants. This research summarizes facts on the chemistry of essential oil isolated from *Cyperus scariosus* on the one hand and their most important biological activities on the other. The essential oil components were determined to be a complex mixture of volatile chemicals such as monoterpenes, sesquiterpene hydrocarbons, and their oxygenated derivatives

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using Gas Chromatography-Mass Spectrometry (GC-MS). Thermoanalytical techniques such as TGA and DSC were used to investigate the oil's stability. Infrared spectroscopy was used to provide further information on the existence of various functional groups. Furthermore, the extracted oil's antibacterial, antioxidant, and antimalarial activities were investigated. The antimalarial activity was tested against *Plasmodium falciparum*, and the antioxidant and free radical scavenging activity were calculated using the DPPH reagent. This comprehensive study will provide an overview of the chemicals extracted from *Cyperus scariosus* as well as the plant's convincing pharmacological qualities.

Keywords: GC-MS; DSC; TGA; FTIR; cyperus scariosus; antimicrobial; antioxidant; antimalarial; essential oil.

ABBREVIATIONS

GC-MS : Gas Chromatography-mass Spectrometry
FTIR : Fourier Transform Infrared Spectroscopy
TGA : Thermogravimetric Analysis
DSC : Differential Scanning Calorimetry
MIC : Minimum Inhibitory Concentrations
HPTLC : High-Performance Thin-Layer Chromatography
DPPH : 2,2'- diphenyl-1-picrylhydrazyl
LB : Luria-Bertani
RT : Room Temperature

1. INTRODUCTION

Due to rising consumer demand and interest in medicinal and aromatic plants for culinary, medicinal, and other anthropogenic purposes, their appeal continues to expand. Consumers are becoming more aware of the benefits and possible applications of medicinal and aromatic plants and their metabolites as they become more informed about food, health, and nutrition. Essential oils, being one of the many secondary metabolites produced by these plants, it would be worthwhile to have a deeper understanding of the chemistry and biological properties of these extracts, as well as their components, in order to find novel and useful applications in human health, agriculture, and the environment.

The Egyptians were likely the first to use essential oils for a variety of uses, including medical, cosmetic, religious ceremonies, and embalming the dead. The phrase essential oil comes from the medication Quinta essential, which was named by Paracelsus von Hohenheim of Switzerland in the sixteenth century [1,2,3]. Essential oils or “essences” owe their name to their flammability, and are produced by plants to protect them from pests and predators, to attract pollinators, or aid in seed dispersal [4,5]. They are aromatic, somewhat water-soluble, volatile, inflammable and soluble in alcohol liquids derived from various plant materials using acceptable processes [1]. Monoterpenes, sesquiterpenes, phenols, oxides, esters, aldehydes, and ketones are among the scent-producing chemicals found in extracted essential oils [2].

Although the term "aromatic" in modern usage refers to the attribute of emitting a fragrance that is either pleasant or offensive to the nose, an aromatic compound or moiety in chemistry has a chemical arrangement that results in electron delocalization, producing greater molecular stability [4]. As a result, essential oils can be a combination of aromatic and aliphatic (non-aromatic) components, all of which contribute to the smell [6]. They are volatile oils with a high refractive index and optimum rotation as a result of many asymmetrical components [7].

Plants' essential oils are made up of a variety of components, including chiral molecules with one or more asymmetric carbon atoms exhibiting optical activity [6]. These chiral substances of natural origin (mono- and sesquiterpenes) are frequently found in distinct enantiomeric distributions because they evolved through enzymatically regulated biosynthetic synthesis [8]. Therefore, they are also sources of fragrance compounds, notably enantiomers and valuable chiral building blocks in synthesis [9].

In recent years, there has been an increase in the biological and pharmacological properties of essential oils and their constituents [10,11]. At the moment, there is an increasing interest in EOs and their components, notably for their broad-spectrum antibacterial action, which can give, for example, alternative functional ingredients to extend the shelf life of food goods and assure microbiological safety for customers [12].

Cyperus scariosus is a member of the Cyperaceae family and is generally referred to as "Nagarmotha" in Hindi and "Nutgrass" in English [13]. It is also known as cyperus essential oil or cypriol essential oil. This essential oil is native to India and is collected from the roots of the Cyperus sedge plant. The oil is a mobile liquid that ranges in hue from dark yellow to brownish. It has a powerful, warm, and woody odour, akin to that of Vetiver and Oudh. It is extensively used as a significant element in the production of perfumes and incense sticks, but it has also been proven to have antibacterial, antifungal, and antioxidant characteristics, as well as some insecticidal and hypertensive medicine usage [14].

Apart from shedding light on biological activities such as anti-microbial, anti-oxidant, and anti-malarial, the goal of this extensive study of *Cyperus scariosus* essential oil is to identify aromatic compounds using GCMS, as well as determine chemical groups using FTIR combined with thermogravimetric (TG) and DSC for performing and determining effective thermochemical conversions. This objective will not only show the oil's multiple drug-like therapeutic activities, but will also assist researchers in approaching the utility, efficacy, and potency of essential oil isolated from *Cyperus scariosus*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extraction of Essential Oil

The *Cyperus scariosus* was bought from the regional market in Mumbai, Maharashtra. It was subsequently moved to a research facility. The essential oil was isolated from *Cyperus scariosus* using a Clevenger hydro-distillation technique. The oil was dried over magnesium sulphate after extraction and stored at 4°C in a dark brown bottle.

2.2 Gas Chromatography-mass Spectrometry (GC-MS)

A Clarus GC-MS 600C system equipped with a GsBP-5MS capillary column (Helium) with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 μm was used. To reveal sample components, the electron ionisation system (EI or "electron impact ionisation method to convert neutral molecules in the gas phase to ionised molecules appropriate for detection") was used. The GC-MS technique is utilised in electron impact mode, with an ionisation energy of 70 eV. In this approach, helium gas (99.999 percent purity) is used as a carrier gas. At a steady flow rate of 1.20 mL/min and an injection volume of 1 μL, this carrier gas exerts a consistent effect on the sample component (a split ratio of 150:1). The injector temperature was kept constant at 250°C, while the ion-source temperature was 220°C and the oven temperature was set from 40°C (isothermal for 3 minutes) to 230°C with a 10°C/min rise. The programme lasted 25 minutes in total. The sample components were determined by comparing them to the Mass Spectral Libraries of Wiley 6.0 and the National Institute of Standards and Technology (NIST), as well as retention indices literature data.

2.3 FTIR Analysis

A Carry 630 Fourier transform infrared spectrometer was used to analyse the functional groups of the sample oil. The system was initially pre-heated and stabilised. A drop of material was placed in a clean NaCl pellet. The salt pellet was then pushed inside the NaCl pellet, tiling the oil sample uniformly and vesicularly between two NaCl pellets. After gently rotating the NaCl pellets to generate a uniform liquid membrane and attaching and inserting them in the infrared spectrometer sample holders, the infrared spectrometer was calibrated to absorbance with a resolution of 8 cm^{-1}. Under the given conditions, the infrared absorption spectra of the oil sample were obtained in the spectral regions 3500–700 cm^{-1}.

2.4 Thermo Gravimetric Analysis

TGA analysis of the sample oil was carried out using Universal V4.5A TA Instruments. The experiment was conducted in a controlled nitrogen gas atmosphere with a flow rate of 300 mL/min. The samples were weighed at 26 mg and placed in crucibles made of aluminium.

Throughout the process, the sample was heated to 500 °C at a range of ambient temperatures at a constant flow rate of 20°C/min.

2.5 Differential Scanning Calorimetry

The DSC profile of an essential oil was created using a TA instrument type DSC Q20 V24.11. 8mg of sample was placed in aluminium crucibles to conduct the experiment. The samples were examined using a nitrogen gas flow rate of 40 mL/min. In addition, a dynamic scan was carried out over a temperature gradient of 25°C to 400°C at a continuous heating rate of 10°C/min.

2.6 Examination of Minimum inhibitory Concentration (MIC)

The potential of the essential oil was evaluated using the microdilution technique using 96-well microtiter plates in order to determine the least inhibitory concentration against the selected 9 resistant bacterial strains such as, Carbapenem-Resistant *Acinetobacter* (CRA), Carbapenem-Resistant *Pseudomonas aeruginosa* (CRP), Carbapenem-Resistant *E.coli* (CRE), Carbapenem-Resistant *Klebsiella pneumonie* (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) and 5 test fungal strains *Trichophyton rubrum, Microsporum gypseum, Aspergillus niger*, *Aspergillus clavatus* and *Candida auris*. The microbial 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 hours at 180 rpm. Following the incubation period, each well was given 5 µL of Resazurin dye (2mg/mL) to detect microbial proliferation by a pink coloration.[25]

2.7 Antioxidant Assay

The radical scavenging activity of *Cyperus scariosus* essential oil was investigated using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (NII-Electronic Library Service, n.d.), with minor changes. 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 set aside for 30 minutes at room temperature in the dark. At 515 nm, the absorbance was measured using a twin beam UV-VIS spectrophotometer. Methanol was used as a negative control. At concentrations ranging from 100 to 1000 µg/mL, a 3 mL aliquot of this solution was mixed with 100 µL of the sample. The reaction mixture was properly mixed before being incubated in the dark at room temperature for 15 minutes. At 515 nm, the absorbance was measured. The percent inhibition of the DPPH radical was calculated for each concentration using the following equation:

$$\text{Percentage inhibition (\%)} = \frac{\left(\text{A}_{\text{control}} - \text{A}_{\text{sample}}\right)}{\text{A}_{\text{control}}} \times 100$$

Plotting inhibition percentages against sample oil concentrations revealed the sample concentration that gave 50% inhibition (IC50). All experiments were done three times, and the IC50 values were determined by taking the average of the three repetitions.

2.8 Anti-Malarial Assay

According to Rieckmann et al, the invitro assay was performed in a microtiter plate following the microassay methodology with minor modifications. *P. falciparum* drug resistant and sensitive strains were cultivated and kept in Roswell Park Memorial Institute (RMPI) 1640 medium supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1% D-glucose, 0.23% sodium bicarbonate, and 10% heat inactivated human serum. *P. falciparum* non-synchronized parasites were synchronised after being treated with 5% D-sorbitol to get just the ring stage parasitized cells [15]. To begin, Jaswant Singh Bhattacharya (JSB) staining [16] was used to determine a ring stage parasitaemia of 0.8-1.5% at 3% haematocrit in a total volume of 200 µL of RPMI-1640 medium to estimate the percent parasitaemia (rings). Following that, dilutions of the oil (5 mg/mL) in culture media were made using DMSO as a solvent. The diluted material was then added to each test well, yielding a final concentration (at five-fold dilutions) ranging from 0.1 µg/mL to 2.0 µg/mL in triplicate wells containing parasitized cell preparation. The

plates were then incubated in a candle jar at 37 °C for 36 to 40 hours. Following the incubation period, thin blood smears were produced and stained with JSB stain. The slides were examined under a microscope to track the development of ring stage parasites into trophozoites and schizonts in the presence of various concentrations of the oil. The essential oil concentration that resulted in complete inhibition of schizont development was regarded as the lowest inhibitory concentration (MIC), while chloroquine and quinine were utilised as reference medicines in this investigation.

2.9 HPTLC

A solvent system consisting of Toluene: Ethyl acetate in a ratio of 9.7: 0.3, was utilized for identification and separation of the essential oil extracted from *Cyperus scariosus* using HPTLC. The essential oil was brought about on a 100.0x 100.0 mm silica gel 60 F 254 HPTLC plate (Merck). 50 µL of sample solution was delivered in 1 mL of methanol as 8 mm broad bands (delivery speed 150 nL/s). The plate was then developed at room temperature in a CAMAG twin-trough vertical development chamber that had been pre-saturated with the solvent solution indicated above for 20 minutes. The migration distance was maintained at 85 mm. Following this, the plate was subjected to densitometric scanning at a wavelength of 254 nm and 366 nm, utilizing a scanning speed of 20 mm/s and slit dimension of 5 mm x 0.2 mm, with deuterium and tungsten as the light sources.

3. RESULTS AND DISCUSSION

3.1 GC-MS Analysis

The essential oil was evaluated by using gas chromatography–mass spectrometry (GC–MS). The individual components were characterised, and twenty-four components were discovered, accounting for 99 percent of the essential oil [17]. The percentage chemical composition of the essential oil of *Cyperus scariosus* was determined and is shown in Table 1. It was discovered to be a mixture of several volatile molecules, including monoterpenes, sesquiterpene hydrocarbons, and their oxygenated derivatives, of which 72% were sesquiterpene, 12% ketone, 8% terpene, and 8% other compounds made up of ethers, esters, and organic compounds. The most common and prominent compounds were discovered to be alpha-Gurjunene (31.82%), rotundene (12.69%), epi-Cyclolorenone (8.03%), alpha-Copaene (6.38%), valencene (5.51%), beta-Selinene (3.82%), beta-Pinene (3.23%) and alpha-Pinene (2.01%) with retention times of 14.16, 14.91, 17.80, 13.79, 15.31, 15.24, 7.5 and 6.67 minutes respectively. The oil exhibited antibacterial, antimalarial, and antioxidant effects, which might be attributed to components such as beta & alpha-Pinene [18], alpha-Copaene [19], Spathulenol [20], and Valencene [21]. In addition, Myrtenal and 3-Cyclopentyl-6-Methyl-3,4-Heptadien-2-one have been shown to have anti-inflammatory, anti-aggregative, anticancer, antihyperglycemic, and biomarker properties [22]. The presence of aromatic components, which represents the quality of the oil, is ascribed to the medicinal benefits of essential oils derived from *Cyperus scariosus*.

3.2 FT-IR Analysis

Fourier Transform Infrared Spectroscopy, often known as FTIR Analysis or FT-IR Spectroscopy, is a widely used analytical method for detecting functional groups in samples by employing infrared light to detect diverse substances [15]. From the FTIR spectrum (Fig. 2) broad area of absorption between 3000 and 3500 cm^{-1} are allocated to the stretching vibrations due to intermolecular interactions through normal "polymeric" OH stretch. The characteristic peak at around 2922.2 & 2870.1 cm^{-1} represents methylene C-H symmetrical and asymmetrical stretch respectively, whereas a methylene C-H bend is observed at 1457.4 cm^{-1} in the fingerprint region. Aside from the aromatic tertiary amine C-N stretch and C-H out of plane bend, which were detected at 1317.7 cm^{-1} and 700.7 cm^{-1}, respectively, aliphatic fluro compounds (C-F) stretch, alkyl substitute ether (C-O) stretch, and alcohol (O-H) of plane bend were also observed at 1121.9 cm^{-1}, 1069.7 cm^{-1}, and 741.7 cm^{-1}. Furthermore, there are a few distinct peaks between 1900 cm^{-1} and 2100 cm^{-1}, indicating transition metal carbonyls such as Isothiocyanate (-NCS), in addition, the peak occurring between 1600 and 1800 cm^{-1}, alluding to alkenyl C=C stretch and ester groups like methyl ester. This FTIR results suggest that this study is a valuable technique for identifying the compounds in the selected essential oil sample.

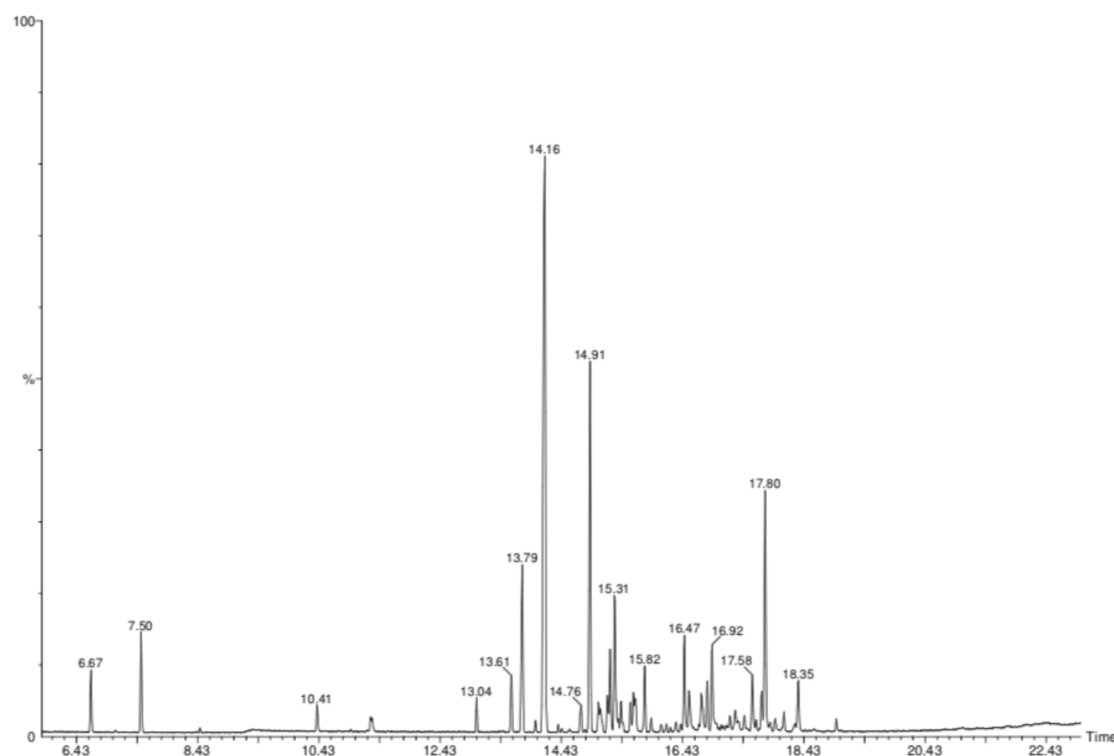

Fig. 1. Representative GC-MS chromatogram of *Cyperus scariosus* essential oil

Table 1. Representative GC-MS table of *cyperus scariosus* essential oil

Component name	Molecular formula	Molecular weight (g/mol)	Classification	Component composition (%)	Retention time (min)
Alpha-Pinene	C10H16	136.23	pinene (terpene)	2.01	6.67
Beta-Pinene	C10H16	136.23	pinene (terpene)	3.23	7.5
Myrtenal	C10H14O	150.22	monoterpene	-	11.29
3-Cyclopentyl-6-Methyl-3,4-Heptadien-2-one	C13H20O	192.3	Ketone	1.00	13.04
Alpha-Copaene	C15H24	204.35	sesquiterpene	6.38	13.79
Alpha-Gurjunene	C15H24	204.35	sesquiterpene	31.82	14.16
Spirolepechinene	C15H24	204.35	sesquiterpene	1.15	14.76
Rotundene	C15H24	204.35	sesquiterpene	12.69	14.91
Gamma-Gurjunene	C15H24	204.35	sesquiterpene	2.21	15.04
Beta-Selinene	C15H24	204.35	sesquiterpenes	3.82	15.24
Valencene	C15H24	204.35	sesquiterpene	5.51	15.31
Alpha-Bulnesene	C15H24	204.35	sesquiterpenoids	1.13	15.42
Eremophila-1(10),8,11-triene	C15H22	202.33	Organic compound	1.00	15.58
7-epi-Alpha-Selinene	C15H24	204.35	Terpenes	2.75	15.62
Spathulenol	C15H24O	220.35	tricyclic sesquiterpenoid	2.39	15.82
Alpha-Calacorene	C15H20	200.32	Organic compound	-	15.92
Caryophyllene oxide	C15H24O	220.35	Ether	3.11	16.47

Component name	Molecular formula	Molecular weight (g/mol)	Classification	Component composition (%)	Retention time (min)
diethyl Phthalate	C12H14O4	222.24	diethyl ester	1.94	16.54
Muurola-4,10(14)-dien-1-beta-ol	C15H24O	220.35	Organic compound	1.86	16.74
epi-Cyclolorenone	-	-	Ketone	3.49	16.92
Mustakone	C15H22O	218.33	sesquiterpenoid	1.88	17.58
epi-Cyclolorenone	-	-	Ketone	8.03	17.80
Cyclolorenone	-	-	Ketone	2.58	18.35
Nootkatone	C15H22O	218.33	sesquiterpenoid	-	18.97

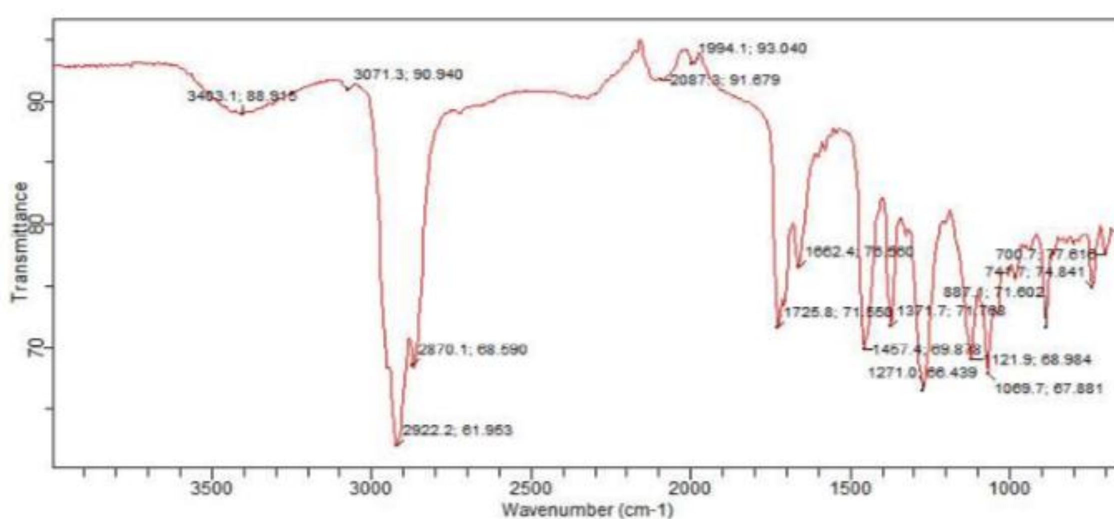

Fig. 2. FT-IR analysis of *Cyperus scariosus* essential oil

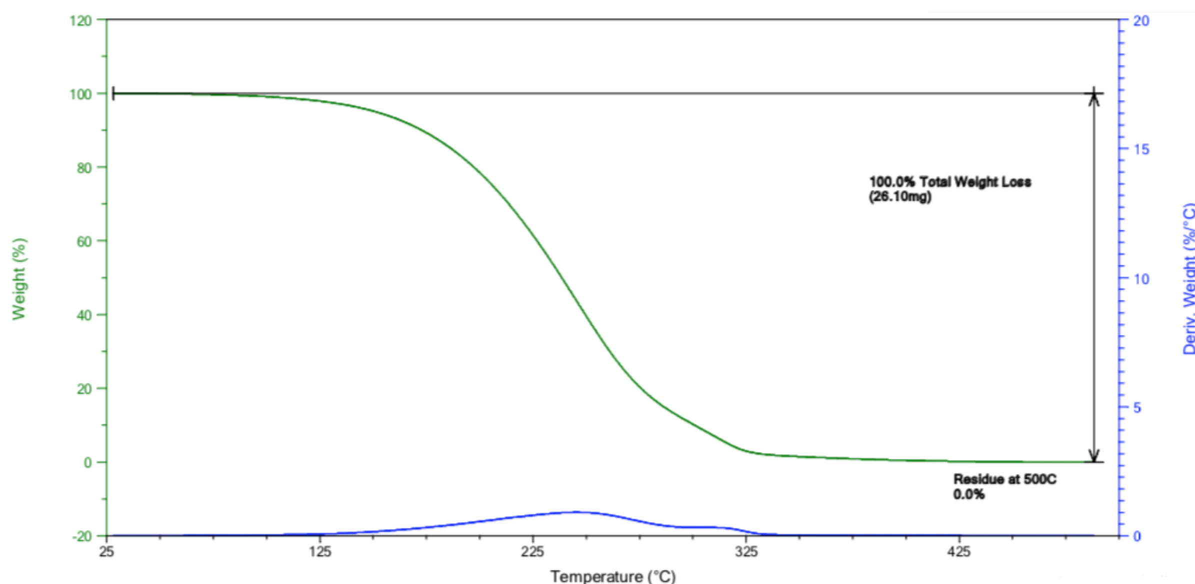

Fig. 3. Thermo-gravimetric analysis of *Cyperus scariosus* essential oil

3.3 TGA Analysis

Thermogravimetric analysis (TGA), is a type of thermal analysis that evaluates changes in physical and chemical properties of materials as temperature rises [23]. Thermogravimetric analysis is critical for determining the stability and mass loss of essential oils as temperatures rise, it also aids in identifying the nature of the reaction that occurs as a result of high temperatures, resulting in sample disintegration or mass loss [24]. The thermogram derived from the thermogravimetric investigation is depicted in Fig. (3). It is noted that the essential oil displayed a single thermal degradation event, with $_T$onset and $_T$max of 125°C and 330°C, respectively, and thereafter the curve stabilizes beyond this temperature. This demonstrates that the selected essential oil has a greater temperature for mass loss, which corresponds to its high stability.

3.4 DSC Analysis

Differential scanning calorimetry (DSC) is one of the thermal techniques that can provide significant information about tested materials [25]. The information collected from DSC curves can be useful in understanding thermal transitions when the material is subjected to particular treatments [26,27]. Exothermic and endothermic behaviors were observed (Fig. 4), with the endothermic pattern being strongly associated to double bonds and a sign of decomposition, whilst the exothermic event might be defined by oxidation processes [28]. The DSC curve of *Cyperus scariosus* essential oil revealed an endothermic event until the temperature reached 170.97 °C, corresponding to its volatilization, immediately following the Glass transition phase at 32.48 °C, with a decrease in heat flow of -1.069W/g. At 99.19°C and 240.12°C, the melting enthalpies of essential oil were 487.3 J/g and 20.36 J/g, respectively. However, the smooth peak seen beyond 260.62°C, may be related to the auto-oxidation on process of the sample. This thermogram depicts the heat profile of the essential oil when subjected to escalating temperatures, providing proof for the oil's stability.

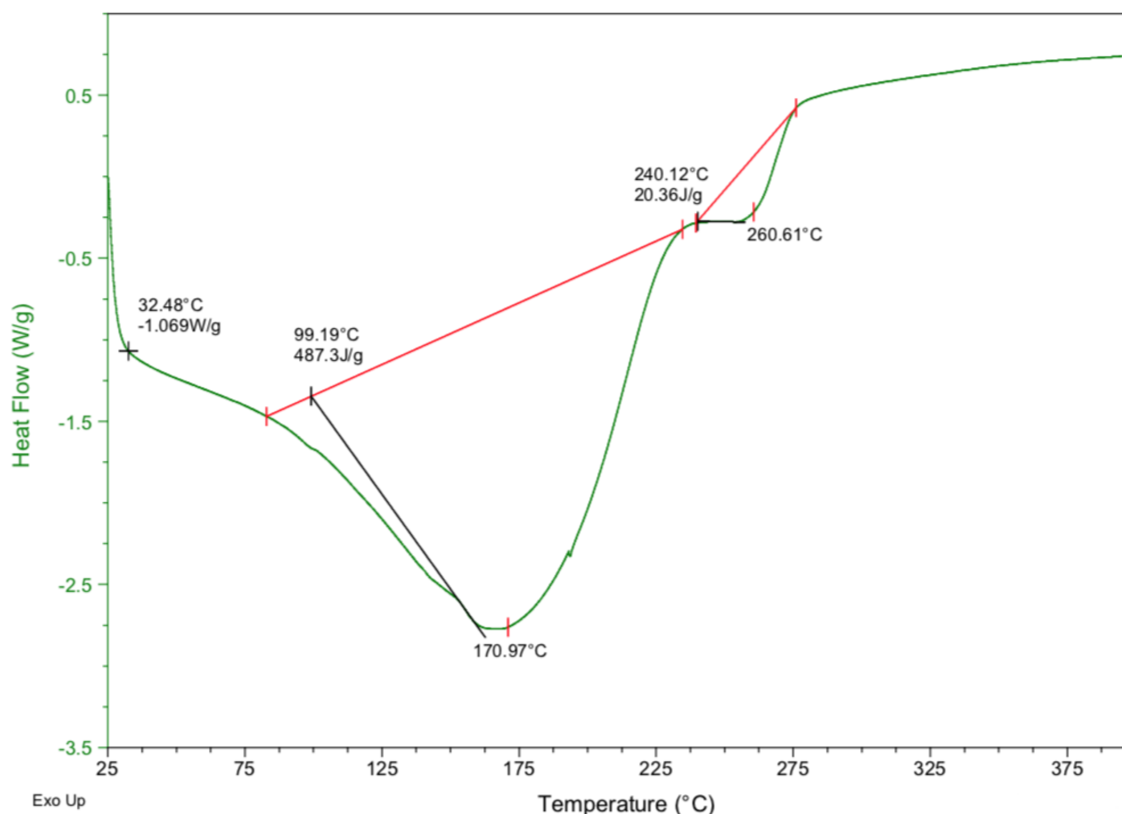

Fig. 4. DSC analysis of *Cyperus scariosus* essential oil

3.5 HPTLC

The use of chromatographic fingerprints is a consistent and reasonable way for assessing the quality and confirming the presence of active chemotypes in traditional medicines [29]. HPTLC is still one of the most adaptable, dependable, and cost-effective separation techniques for the investigation of botanicals and herbal medications. It ensures reliable findings when used with standardized processes, which is critical in the routine identification of complex fingerprints of plant extracts and medicinal compounds [30,31]. An accurate, easy, precise, specific, and quick HPTLC-densitometric approach for concurrently detecting components present in *Cyperus scariosus* essential oil was developed. HPTLC profile of *Cyperus scariosus* essential oil was subjected to the solvent system Toluene: Ethyl acetate (9.3:0.7 v/v), which revealed many blue to violet-colored bands after derivatizing with vanillin sulphuric acid under UV 366 nm, indicating the presence of various essential oil compounds, with Rf values ranging between 0.0 to 0.90. Sulfuric acid, as a universal reagent for natural products, is effective for detecting terpenoids or steroids since it shows unique colours for monoterpenes, triterpenes, and steroids. Under white light, monoterpenes, triterpenes, and steroids appear as dim grey, blue or red-violet (purple), and grey dots, respectively (Fig. 6). Under UV illumination at 254 nm, compounds with at least two conjugated double bonds would quench fluorescence and appear as black zones against the light-green fluorescent backdrop of the TLC plate. Monoterpenes often have a mild grey tint, while sterol steroids have a greyish blue colour. Triterpenes, on the other hand, emit purple-violet under white light and reddish or blue under 366 nm, whereas flavonoids emit fluorescence under UV-366 nm [32,33]. The present study's HPTLC profile of essential oil indicate the diversity present at the biochemical level in *Cyperus scariosus* essential oil, which may also be used as a reference for proper identification or authentication.

3.6 Minimum Inhibitory Concentration Determination

In the present study, the higher percentage of sesquiterpenes is detected in the essential oil of *Cyperus scariosus* and thus it has shown notable antibacterial activity. The results of the MIC for the essential oil against drug resistant organisms, which seemed susceptible to a degree as it was inhibited between 95 and 250 µg/mL, are shown in Table 2. However, the overall antimicrobial activity screening results indicated that these herbal medications have the potential to be effective therapies for bacterial infections. Furthermore, monoterpenes like Myrtenal and other terpenes like alpha and beta-pinene, alpha-gurjunene, and Valencene have been connected to the antibacterial activity of many essential oils [18,21]. As previously stated, the antibacterial action of *Cyperus scariosus* essential oil is mostly due to its composition, particularly the presence of sesquiterpenes in high concentrations, validated by GC-MS. They work by dissolving the microbial cytoplasmic membrane, increasing its permeability, and allowing large protons and ions to pass through however, attributing the antibacterial activity to a specific molecule is difficult. Spathulenol (2.39%) and other tricyclic sesquiterpenoid molecules contain one hydrogen bond donor and one acceptor on their surface, allowing them to interact and pass through the cellular membrane [34]. The antibacterial action of sesquiterpenes such as spathulenol is described as a cell membrane-disrupting mechanism that results in the release of K+ ions from bacterial cells [35]. *Cyperus scariosus* essential oil was tested for its antifungal activity against *Candida auris, Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323), *Trichophyton rubrum*, and *Microsporum gypseum* (Table 2). The present study revealed low antifungal activity ranging around 1000 µg/mL, except against *Microsporum gypseum,* which was found out to be 500 µg/mL. Apart from alpha-pinene and beta-pinene, no additional antifungal compound was detected and verified by GC-MS in the oil, explaining its limited action.

3.7 Antimalarial

Malaria and other tropical diseases caused by single-celled parasites affect millions of people globally, with a high frequency in tropical countries [16]. Quality pharmacological treatment for these disorders is severely limited to a few types of medications, many of which are associated with high toxicity, variable efficacy, and resistance, posing a public health dilemma in developing countries [36]. As a result, new medicines for the treatment of parasitic infections are required. *Cyperus scariosus* essential oil inhibited the growth of drug sensitive *Plasmodium falciparum* and Q-resistant strain of *Plasmodium falciparum* in a dose-dependent manner, as seen in Table 3. The antimalarial

activity (IC50 values) of the essential oil against the aforementioned strains ranged from 0.39 µg/mL to 0.77 µg/mL, indicating that it might be considered a valuable source of antimalarial agent due to the inclusion of chemicals that bestow the activity, such as α- and β-Pinene [18], mainly affecting the ring stage of the parasite and Spathulenol [20], being a powerful anti-malarial agent found in the oil and verified by GC-MS analysis. Furthermore, oxygenated sesquiterpenes are known to act and hinder the production of the isoprene side chain of the benzoquinone ring of ubiquinones linked to coenzyme Q at the trophozoite and schizont phases [37]. Fig. 7 illustrates percent inhibition vs oil concentration ranging from 0.1 to 2 µg/mL.

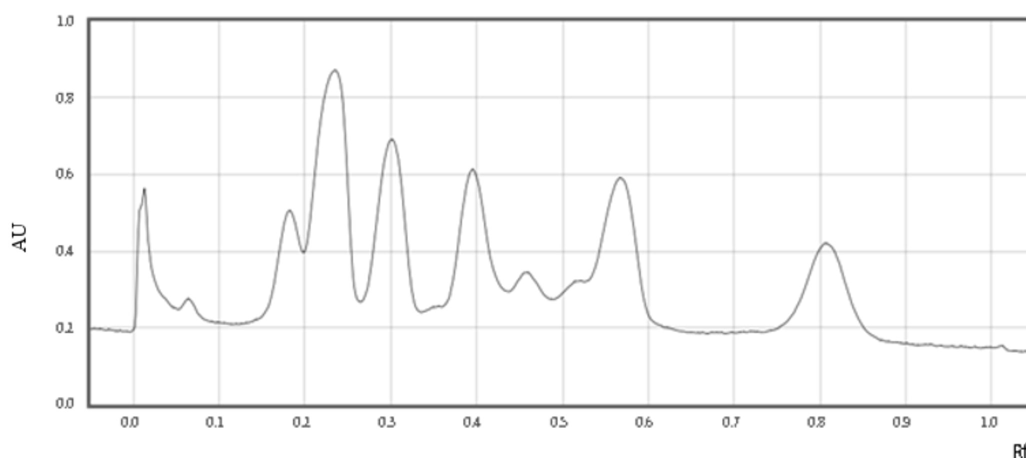

Fig. 5. HPTLC peak densitogram profile of *Cyperus scariosus* essential oil

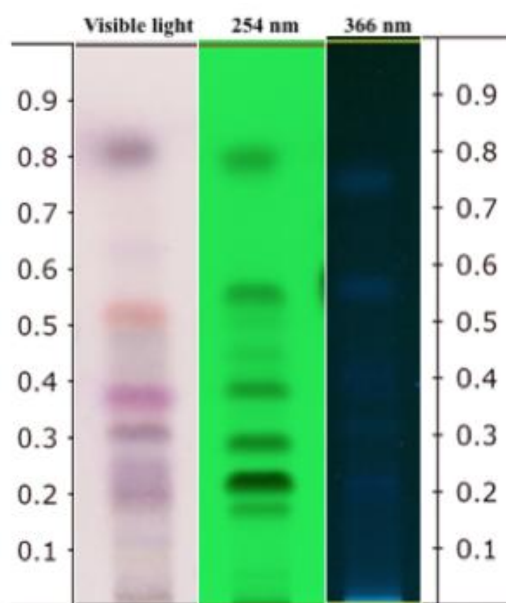

Fig. 6. HPTLC fingerprint of *Cyperus scariosus* essential oil at UV 254, UV 366 and white light after derivatization with vanillin sulfuric acid

Table 2. Minimum Inhibitory concentration (µg/mL) against test strains

Serial No.	Strain Name	MIC (µg/mL)
1	Carbapenem Resistant *Acinetobacter species*	250 ± 0.05
2	Carbapenem Resistant *Pseudomonas aeruginosa*	250 ± 0.05
3	Carbapenem Resistant *E. coli*	125 ± 0.05

Serial No.	Strain Name	MIC (μg/mL)
4	Carbapenem Resistant *Klebsiella pneumonie*	125 ± 0.02
5	Extended Spectrum beta lactamase *E. coli*	100 ± 0.06
6	Vancomycin resistant *Enterococci*	250 ± 0.04
7	Quinolone resistant *Salmonella*	100 ± 0.06
8	Methicillin resistant *Staphylococcus aureus*	125 ± 0.05
9	Methicillin resistant *Staphylococcus aureus*	125 ± 0.05
10	*Candida auris*	1000 ± 0.05
11	*Aspergillus niger*	1000 ± 0.02
12	*Aspergillus clavatus*	1000 ± 0.05
13	*Trichophyton rubrum*	1000 ± 0.04
14	*Microsporum gypseum*	1000 ± 0.06

Table 3. Anti-malarial activity of *Cyperus scariosus* essential oil

***Cyperus scariosus* essential oil**	**Drug sensitive *Plasmodium falciparum***		**Drug resistant Q.Resistant**		**Standard drugs**	**IC_{50} Value**
Concentration (μg/mL)	**Inhibition %**	**IC_{50}Value**	**Inhibition %**	**IC_{50}Value**		
0.1	7.89		16.78		Chloroquine (control)	0.020 μg/mL
0.2	15.63		21.27		Quinine (control)	0.268 μg/mL
0.4	17.89		34.75			
0.8	24.56	0.39 μg/mL	36.54	0.77 μg/mL		
1.2	33.30		38.65			
1.6	91.89		57.89			
2.0	92.40		58.94			

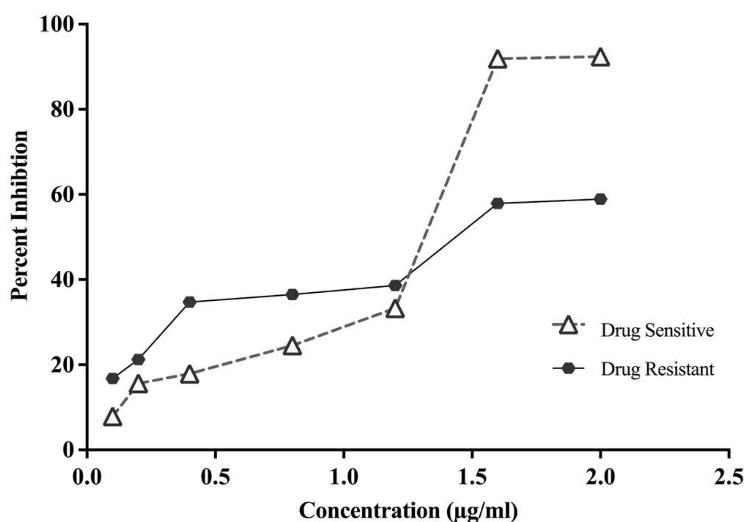

Fig. 7. Anti-malarial activity of *Cyperus scariosus* essential oil

Table 4. Antioxidant activity of *Cyperus scariosus* essential oil

Concentration (μg/mL)	% Inhibition
100	33.07 ± 0.02
200	37.85 ± 0.04

Concentration (µg/mL)	% Inhibition
300	41.82 ± 0.03
400	47.76 ± 0.03
500	49.57 ± 0.05
600	53.21 ± 0.05
700	53.43 ± 0.06
800	56.06 ± 0.04
900	56.26 ± 0.02
1000	57.17 ± 0.04

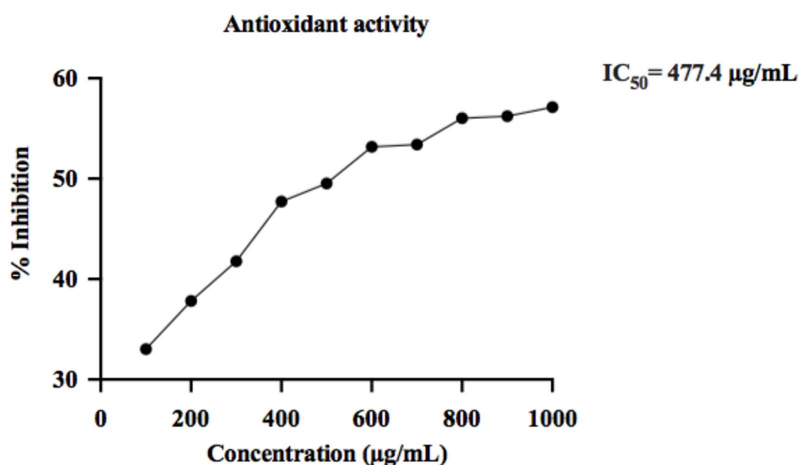

Fig. 8. Anti-oxidant activity of *Cyperus scariosus* essential oil

3.8 Antioxidant

The potential antioxidant activity of the essential oil isolated from *Cyperus scariosus* was determined according to the basis of scavenging activity of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a stable free radical with a characteristic absorbance at 517 nm when dissolved in alcohol. Antioxidant molecules scavenge free radicals by changing the color of the DPPH assay solution from dark purple to light yellow, resulting in a decrease in absorbance value [38]. In this study, the essential oil was diluted at 100 µg intervals from 100 to 1000 µg. The lowest free radical scavenging activity was measured at 0.1 mg/mL with a value of 33.07%, while the greatest activity was 57.17% measured at 1 mg/mL with an IC50 value of 477.4 µg/mL (Table 4). These high antioxidant results were obtained due to the presence of chromatographically determined components such as Myrtenal [22], Alpha-Copaene [19], Beta-Selinene [39], and Valencene [21], which have previously been reported as powerful anti-oxidant agents. As a result, the presence of such components contributes to the antioxidant effects of nargarmotha essential oil extracted from *Cyperus scariosus*.

4. CONCLUSION

Essential oils have seen a surge in popularity in recent years. There has been an increase in the biological and pharmacological properties of essential oils and their constituents. As a result, essential oils will remain indispensable natural element. The evidences presented above on the biological extracts and pharmacological activities of *Cyperus scariosus* demonstrated that it is a natural and potentially effective technique to seek for novel chemicals for the treatment of various ailments. More research on this plant's therapeutic potential is being conducted.

ACKNOWLEDGEMENTS

We would like to acknowledge Diya Laboratory, and Microcare Pvt ltd for helping us with analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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https://www.sdiarticle5.com/review-history/88917