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In vitro Antimicrobial Evaluation and Chemical Profiling of Essential Oils Obtained from the Selected Medicinal Plant Species

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Abstract

Acne vulgaris is one of the most prevalent dermatological conditions, associated with the proliferation of *Cutibacterium acnes* and *Staphylococcus epidermis.* The emergence of antibiotic-resistant strains and the adverse effects associated with conventional therapies have created a need for new, novel, safer, and more effective alternative treatments. This study aimed to evaluate the antimicrobial potential of essential oils extracted from selected medicinal plants traditionally used in herbal medicine for the treatment of acne. A comprehensive ethnobotanical survey was conducted, including a field survey; collection, identification, and authentication of medicinal plant specimens traditionally used for skin ailments; extraction and isolation of essential oils; phytochemical screening; *in vitro* antimicrobial screening; and determination of bioactive compounds by GC-MS. Five medicinal plants were selected to carry out antimicrobial activity against acne. The plant specimens were successfully identified as *Cyperus rotundus, Eucalyptus tereticornis, Pogostemon cablin*, *Cymbopogon nardus*, and *Cymbopogon flexuosus*. Phytochemical screening revealed the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, phenols, saponins, and glycosides. Among the tested essential oils, CNEO and CFEO exhibited the strongest antimicrobial activity with an MIC value of 1024 µg/ml. Based on the findings, CFEO was characterised by GC-MS, which revealed more than 100 bioactive compounds present in it, with multiple peaks. Overall, from this study, the essential oils showed potential antimicrobial activity against the bacterial strains. This may be due to the presence of multiple bioactive phytoconstituents in the essential oil, which may be the reason for its antimicrobial activity.

Major Finding: The essential oils of *Cymbopogon nardus* and *Cymbopogon flexuosus* show good antimicrobial activity against acne-causing bacterial strains.

Keywords: Agar Diffusion Assay, Essential Oils, *In vitro* Antimicrobial Activity, Minimum Inhibitory Concentrations (MIC), Phytochemicals

1. Introduction

Acne is the most prevalent type of dermatological condition, affecting a significant number of individuals worldwide. Acne affects approximately 50% of adolescents, and these individuals have been affected by acne for more than thirty years[1]. The pathogenesis of acne vulgaris is multifactorial and involves increased sebum production, follicular hyperkeratinisation, bacterial proliferation, and inflammation[2]. The pilosebaceous unit, where microorganisms like *C. acnes* and *S. epidermidis* are implicated, *C. acnes* and *S. epidermidis* are among the principal microorganisms implicated in acne development, contributing to inflammation and lesion formation within the pilosebaceous unit[3]. A chronic inflammatory illness of the pilosebaceous unit, acne vulgaris is characterised by the presence of comedones (blackheads and whiteheads), papules, pustules, nodules, cysts, and scars in severe instances[4]. Acne vulgaris may be differentiated from other types of acne by its appearance. It is one of the most prevalent skin disorders in the world, and it primarily affects teens. However, it can also

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continue into maturity or even begin at that age. Acne vulgaris is a condition that not only affects a person's appearance but also has substantial psychological effects, such as lowering one's expectations of themselves, anxiety, and sadness[5]. With substantial clinical and psychological repercussions, acne vulgaris is a disorder that manifests itself in a variety of ways. Hereditary alterations, sebum production, follicular hyperkeratinisation, bacterial colonisation, and inflammation are all involved in its aetiology, which is characterised by a complex interplay between these factors[6]. To establish effective treatment techniques, it is essential to have a solid understanding of these processes. Current management of acne vulgaris includes topical agents, systemic antibiotics, retinoids, hormonal therapy, and procedural interventions, with treatment tailored according to disease severity and patient characteristics[7]. These therapies primarily aim to reduce sebum production, inhibit bacterial proliferation, normalise follicular keratinisation, and suppress inflammation; prolonged use of antibiotics has also contributed to the emergence of antibiotic-resistant strains of *C. acnes* and *S. epidermidis*, thereby reducing treatment efficacy and highlighting the need for safer and more effective alternative therapies. Research that is now being conducted into the pathogenesis and treatment of acne continues to improve the outcomes for patients, which offers promise for the development of medicines that are more focused and successful in the future. Reducing the production of sebum, controlling the multiplication of bacteria, reducing inflammation, and normalising keratinisation are the goals of the treatment for acne vulgaris[8]. Since ancient times, medicinal plants have been used by traditional healers in traditional medicinal systems like Ayurveda and the Chinese medicinal system[9]. For the entirety of human history, a wide range of communicable diseases have been successfully treated with medicinal plants and herbal remedies. There is a progressive increase in the utilisation of folk medicine by traditional practitioners for the treatment of skin problems, and the use of plant medications constitutes a significant component of this practice[10]. The primary polar compounds have a propensity to cross natural membranes, which ultimately results in poor bioavailability. This is due to their large molecular size, which causes them to be poorly absorbed. Additionally, these compounds do not get drawn in by passive diffusion because of their lower lipid solubility[11]. Antibiotics are chosen to treat acne vulgaris; due to this, *C. acnes* and *S. epidermidis*, two of the main acne-causative organisms, developed resistance against antibiotics. Essential Oil (EO), Alcoholic Extract (AE), and hydro-alcoholic extracts (HE) may be used as a safe, natural, efficient, and multifunctional alternative herbal treatment. This study aimed to evaluate the anti-acne potential of EOs, AEs, and HAEs obtained from the selected medicinal plants commonly used in traditional Indian medicine. The proposed work aims to make a systematic data and screen the extracts to identify and determine the most potent essential oil against acne-causing bacteria, and characterise its bioactive constituents. We hypothesised that the selected medicinal plant extracts, particularly the essential oils, would exhibit significant antimicrobial activity against *C. acnes* and *S. epidermidis* owing to their rich phytochemical composition.

2. Materials and Methods

The materials and methods followed in this research work included a field survey, collection, identification, and authentication of plant specimens, extraction and isolation of essential oils, phytochemical screening, antimicrobial activity of the essential oils, and determination of bioactive compounds by GC-MS.

3. Collection, Herbaria, Identification, and Authentication of Plant Specimens

Five medicinal plants traditionally used by traditional medicinal practitioners of the region were collected from the College of Horticulture, Pasighat, East Siang District, Arunachal Pradesh, India, during February–April. Herbarium specimens were prepared and submitted to Prof. Gireesh Chandra, College of Agriculture, Pasighat, Central Agricultural University, Imphal, for identification and authentication. The specimens were authenticated as *Cyperus rotundus, Eucalyptus tereticornis, Pogostemon cablin, Cymbopogon nardus*, and *Cymbopogon flexuosus* (F. No. COA/CAU/Plant Path./2024/1282 (A), dated October 12, 2025).

3.1 Extraction and Isolation of Essential Oil

The collected fresh plant raw materials were thoroughly rinsed with tap water to remove adhering debris. They were shade-dried at room temperature and then ground

into a powder using a mechanical grinder. The ground powder was subjected to extraction using ethanol and hydroalcoholic solvents by the cold maceration method. Briefly, the plant powder was soaked in 95% ethanol and hydroalcoholic solvent (70:30, ethanol) at a 1:10 (w/v) ratio and kept at room temperature for 72 h with intermittent vigorous shaking to facilitate extraction. The extracts were filtered using Whatman No. 1 filter paper, and the solvents were recovered using a rotary evaporator[12]. The filtered extracts were lyophilised into powder using a lyophiliser and then stored in a closed airtight container at 4°C for further studies.

The collected fresh leaves of the selected medicinal plants for isolating essential oils were rinsed with tap water and then shade-dried for two to three hours to remove excess water. After drying, the fresh leaves were chopped into small pieces, and then the essential oils were extracted via the hydro-distillation method using a Clevenger apparatus, where fresh plant material and distilled water were mixed in the ratio of 1:3 (w/v) and distilled for 3 hours. From the condensed mixture, the essential oils were carefully pipetted out, collected and stored in amber vials at 4°C for further use[13]. The photo image of the extraction and isolation is shown in Figure 4.2. The essential oil yield of all the plants was calculated using the formula:

$$\text{Essential Oil Yield} = \frac{\text{Volume of oil obtained}}{\text{Weight of the Fresh Leaves}} \times 100$$

4. Phytochemical Screening

Qualitative Phytochemical screening was performed using reference phytochemical procedures to detect the presence or absence of various secondary metabolites, such as alkaloids, flavonoids, tannins, phenols, saponins, and glycosides, in the Alcohol and Hydroalcoholic extracts of all the plant species. Alkaloids were identified using Dragendorff's reagent, flavonoids by the alkaline reagent test, tannins by the ferric chloride test, saponins by the frothing test, phenolic compounds by the ferric chloride test, glycosides by Keller-Killiani's test, and terpenoids by the Salkowski test. The tests were performed in duplicate, and the results were recorded qualitatively as present (+) or absent (–) according to standard protocols[14,15].

5. *In vitro* Antimicrobial Assay

5.1 Bacterial Strains

In vitro antimicrobial screening was carried out using both the disc diffusion technique and the broth microdilution method for all essential oils obtained from the respective medicinal plants. To assess the antimicrobial activity of the essential oils, two bacterial strains, namely Propionibacterium acnes and Staphylococcus epidermidis, were procured from the Microbial Type Culture Collection and Genebank (MTCC), IMTECH, Chandigarh, India.

5.2 Preparation of Essential Oil Stocks

Primary stocks of Essential Oils were prepared by accurately weighing 20.5mg and dissolving it in DMSO (10 mL), resulting in a concentration of 2050 µg/mL. Two-fold serial dilutions in MHB were prepared. The groups were divided into control (growth control with MHB but no drug), test samples (Inoculum with MHB and essential Oil), and Standard (Inoculum with MHB and Linezolid).

5.3 Determination of Minimum Tested Inhibitory Concentration (MIC)

The Minimum Tested Inhibitory Concentration (MIC) of the selected essential oils was determined following the broth microdilution method with slight modifications[16]. Firstly, a primary stock solution of each essential oil was prepared by dissolving 20.5 mg of the oil in 10 mL of Dimethyl Sulfoxide (DMSO) to obtain a stock concentration of 2050 µg/mL. Two-fold serial dilutions were prepared in Mueller-Hinton Broth (MHB) to obtain final concentrations of 32, 64, 128, 256, 512, and 1024 µg/mL. The assay included a growth control (MHB with bacterial inoculum but without the test sample), a sterility control (MHB only), a negative control (MHB containing DMSO at the corresponding concentration), and a positive control consisting of Linezolid. Each well of a sterile 96-well microtiter plate received 50 µL of the test sample and 50 µL of the standardised bacterial inoculum (5×10^6 CFU/mL), giving a final volume of 100 µL per well. The plates were incubated at 35 ± 2°C for 20-24 h. Following incubation, bacterial growth was assessed by measuring the optical density (OD) using a microplate reader, and then the %growth (MIC) was determined using the equation:

$$\%Growth = (OD\ of\ test\ well \div OD\ of\ growth\ control) \times 100$$

The MIC was defined as the lowest concentration of the essential oil that inhibited visible bacterial growth, as confirmed by the corresponding reduction in optical density relative to the growth control. All experiments were performed in triplicate, and the mean values were used for analysis.

5.4 *In vitro* Antimicrobial Screening

A preliminary antimicrobial screening was conducted to evaluate the antibacterial activity of the essential oils extracted from the selected medicinal plants, namely, *Cyperus rotundus* (CREO), *Eucalyptus tereticornis* (ETEO), *Pogostemon cablin* (PCEO), *Cymbopogon flexuosus* (CFEO), and *Cymbopogon nardus* (CNEO), against acne-causing bacterial strains to screen out the most potent essential oil. The agar disc diffusion method, with slight modification, was employed by measuring the diameter of the zone of inhibition. Firstly, the solutions were prepared by diluting the Essential oils with analytical-grade Dimethyl Sulfoxide (DMSO) to obtain concentrations of 205, 410, 615, 820, and 1050 µg/ml. The Mueller-Hinton agar medium was prepared according to the manufacturer's instructions and sterilised before being poured into sterile Petri dishes. The bacterial inoculum was uniformly spread over the agar surface using a sterile cotton swab. Sterile filter paper discs (6 mm diameter) were impregnated with the respective essential oil solutions and placed on the inoculated agar plates at equal distances to prevent overlapping of the inhibition zones. DMSO served as the negative control, while Linezolid (1025 µg/mL) was used as the positive control. The culture plates were incubated in a BOD incubator at 37°C±2 for 24 hours. Following incubation, the antimicrobial activity was evaluated by measuring the diameter of the clear zone of inhibition (mm) surrounding each disc. All the experiments were performed in triplicate using both the Mueller-Hinton agar and blood agar media[17-19].

5.5 Gas Chromatograph-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of the most potent essential oil extracted from the fresh leaves of the selected medicinal plants was carried out to identify the volatile and semi-volatile constituents using a Thermo Scientific Triple Quadrupole GC-MS (trace 1300, TSQ8000 triple quadrupole MS) at the Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, India. The column temperature was first adjusted to 50°C for 4 min, and then it was increased to 320°C at a rate of 7°C per minute for 20 minutes. The temperature of the injector was adjusted to 280°C (split mode, 1:20, injection volume = 0.1 µL). The helium carrier gas flow rate was set at 1mL/min for a 60-min duration. Electron Ionisation at a potential of 70 eV was used to get mass spectra in the m/z 40-700 range. By comparing the GC retention time to predetermined standards and the mass spectra to library data, the chromatogram of the sample was identified[20].

6. Result and Discussion

6.1 Identification of Plant Specimens

The plant specimens were identified and authenticated by Prof. Gireesh Chandra, College of Agriculture, Pasighat, Arunachal Pradesh (Central Agricultural University, Imphal). They were identified as CREO, ETEO, PCEO, CNEO, and CFEO (COA/CAU/Plant Path./2024/1282(A) dated October 10, 2025). The prepared herbarium is provided in the supplementary file (S1).

6.2 Extraction and Isolation of Essential Oil

The percentage yield of the plant extracts and essential oils was calculated as shown in Table 1. The fresh chopped plant specimens were subjected to the extraction (isolation) of essential oils. After isolating the essential oils in a 1:1 ratio using the Clevenger apparatus, the percentage yield of all the essential oils was calculated, and the physicochemical parameters of the obtained essential oils were determined according to the Indian Pharmacopoeia and International Standards, as shown in Tables 1 and 2, respectively.

6.3 Phytochemical Screening

The preliminary phytochemical screening of the plant extracts was conducted, and various secondary metabolites were found to be present in the plant extracts as shown in Table 3.

6.4 *In vitro* Antimicrobial Screening

A preliminary antimicrobial screening using the agar disc diffusion method was conducted to evaluate the

Table 1. The % yield of essential oils for the plant species

S. No.	Plant Species	% Yield (EO)	% Yield (HAE)	% Yield (AE)	Appearance
1	*Cyperus rotundus*	0.98	8	6	Light yellow colour
2	*Eucalyptus tereticornis*	1.01	7	5	Whitish clear liquid
3	*Pogostemon cablin*	0.92	8	6	Pale light yellowish
4	*Cymbopogon nardus*	1.07	8	6	Light yellow colour
5	*Cymbopogon flexuosus*	1.02	7	5	Light yellow colour

Table 2. Physicochemical parameters of the essential oils.

S. No.	Plant Species	Relative density (g/cm^3)	Refractive index, λ (nD)
1	*Cyperus rotundus*	0.92	1.456±0.12
2	*Eucalyptus tereticornis*	0.91	1.458±0.07
3	*Pogostemon cablin*	0.99	1.479±0.09
4	*Cymbopogon nardus*	0.96	1.441±0.11
5	*Cymbopogon flexuosus*	0.98	1.497±0.07

most inhibitory potential of the essential oils and extracts obtained from the selected plant species against the test microorganism at a concentration of 200 µg/ml, as shown in Table 4. Essential oils exhibited a better zone of inhibition compared to those of the extracts (both AE and HAE). Based on this, the essential oils of all the plants were screened for their antimicrobial activity at different concentration ranges from 205 to 1025 µg/ml. The zone of inhibition was recorded at different concentrations (205-1025 µg/ml). Among

Table 3. Phytoconstituents of the plant extracts

S. No.	Plant name	Alkaloids	Flavonoids	Phenols	Tannins	Glycoside	Saponins
1	*Cyperus rotundus L.*	+	+	+	+	+	+
2	*Eucalyptus tereticornis*	-	+	+	+	+	-
3	*Pogostemon cablin*	-	+	+	+	+	+
4	*Cymbopogon nardus*	-	+	+	+	+	+
5	*Cymbopogon flexuosus*	-	+	+	+	+	+

Table 4. The Zone of inhibition diameter of all the essential oils and extracts.

S. No.	Sample (EOs)	Dose µg/ml	Zone of Inhibition Diameter (EOs)	Zone of Inhibition Diameter (AE)	Zone of Inhibition Diameter (HAE)
1	*Cyperus rotundus*	205	6	5	4
2	*Eucalyptus tereticornis*	205	6	4	5
3	*Pogostemon cablin*	205	6	4	6
4	*Cymbopogon nardus*	205	9	6	6
5	*Cymbopogon flexuosus*	205	12	7	8

Table 5. The Zone of inhibition diameter of all the essential oils

S. No.	Dose µg/ml	*Cyperus rotundus*	*Eucalyptus tereticornis*	*Pogostemon cablin*	*Cymbopogon nardus*	*Cymbopogon flexuosus*	*Linezolid*
1	205	6 mm	6 mm	6 mm	9 mm	12 mm	
2	410	7 mm	6 mm	7 mm	10 mm	14 mm	
3	615	10 mm	7 mm	9 mm	12 mm	18 mm	
4	820	12 mm	10 mm	11 mm	15 mm	17 mm	
5	1025	14 mm	11 mm	12 mm	16 mm	18 mm	26 mm

all the essential oils, both *Cymbopogon nardus* and *Cymbopogon flexuousus* exhibited good antimicrobial activity. Among all the essential oils, CFEO showed a better zone of inhibition, ranging from 15-20 mm and 16-21 mm, at concentrations of 815 and 1025 µg/ml, respectively. The zone of inhibition diameter of all the essential oils from the antimicrobial assay performed in triplicate is shown in Table 5.

Table 6. The percentage (%) growth inhibition of all the EOs

Sample name	Conc. µg/mL	Absorbance (OD)	% Growth inhibition
Control (-ve)	0	0.82±0.03	0.00
Linezolid	32	0.10±0.01	87.80
Linezolid	512	0.05±0.03	93.90
Linezolid	1024	0,04±0.01	95.12
CREO	32	0.38±0.02	53.66
CREO	512	0.24±0.01	70.73
CREO	1024	0.14±0.02	82.93
ETEO	32	0.33±0.02	59.76
ETEO	512	0.22±0.02	73.17
ETEO	1024	0.14±0.02	82.93
PCEO	32	0.31±0.02	62.20
PCEO	512	0.18±0.01	78.05
PCEO	1024	0.12±0.01	85.37
CNEO	32	0.20±0.01	75.61
CNEO	512	0.12±0.02	85.37
CNEO	1024	0.06±0.03	91.46
CFEO	32	0.19±0.03	76.83
CFEO	512	0.11±0.02	86.59
CFEO	1024	0.06±0.01	92.68

To determine and quantify the most antimicrobial potency of all the essential oils, the absorbance (optical density OD_{600}) of the broth microdilution was measured, and then the percentage (%) growth inhibition of all the essential oils (EOs) was calculated, as shown in Table 6. All the tested EOs at the selected concentrations of 32 µg/ml, 512 µg/ml, and 1024 µg/ml exhibited concentration-dependent growth inhibition against the acne-causing bacterial strains. A representative graph of Concentration against percentage growth. Linezolid (1000 µg/mL) was used as the positive control for comparison in the antimicrobial assays. Among all the EOs, *Cymbopogon flexuosus* essential oil (CFEO) exhibited the highest antimicrobial activity with an MIC value of 1024 µg/ml. Although this MIC indicates moderate antimicrobial activity compared with the reference drug (Linezolid), CFEO showed the best performance among the evaluated essential oils and was therefore selected for further phytochemical characterisation by GC-MS. A representative graph showing the relationship between concentration and percentage growth inhibition is shown in Figure 1.

6.5 Gas Chromatograph-Mass Spectrometry (GC-MS) Analysis

To understand the potential biological properties of essential oils from medicinal plants, we must know the various bioactive phytoconstituents or compounds present in them. As per the literature, even though it has been reported the presence of various phytoconstituents has been reported in the selected medicinal plant species,

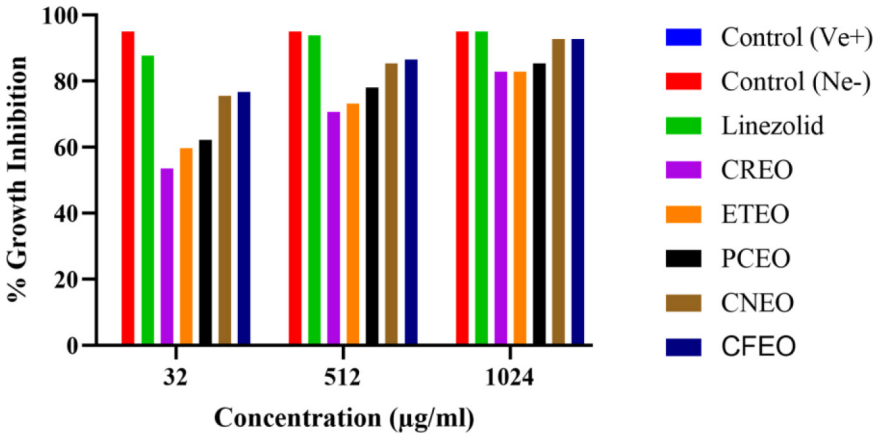


Figure 1. Graph of concentration against % growth inhibition.

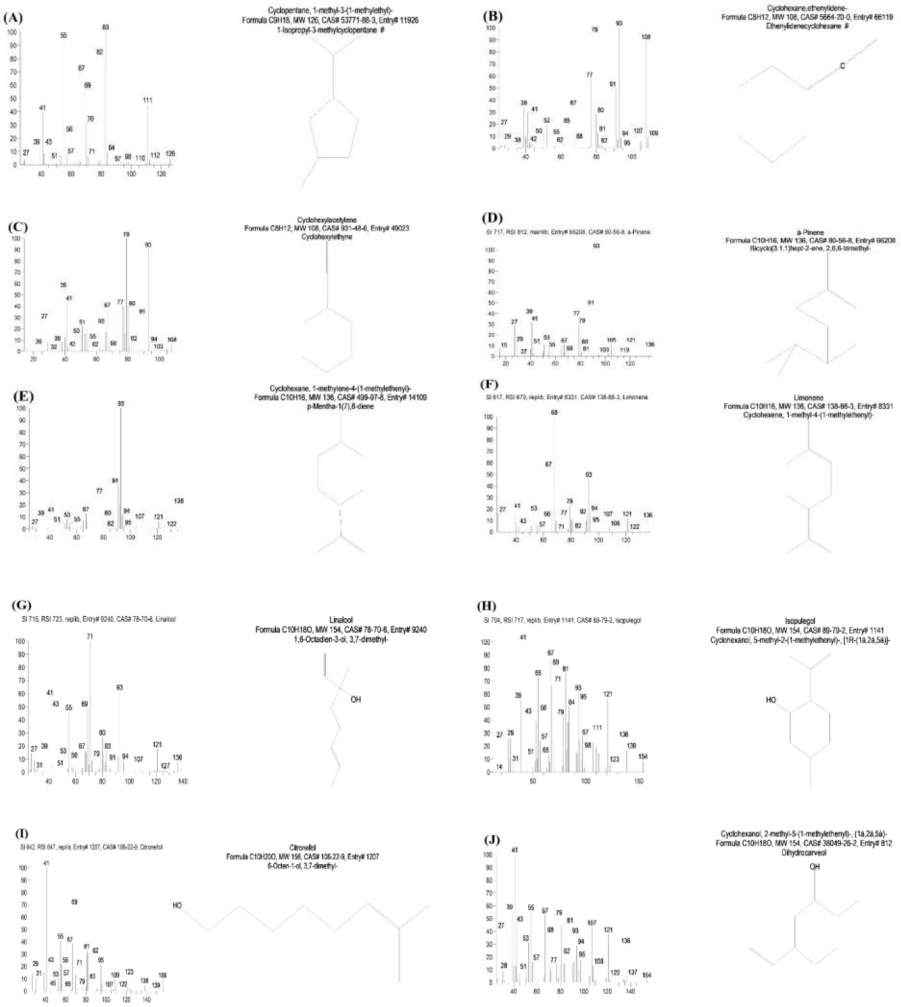


Figure 2. GC-MS chromatogram and structure of the bioactive compounds present in the CFEO.

GC-MS analysis was conducted to confirm the presence of various bioactive compounds that have been reported. From the *in vitro* antimicrobial study, it was found that the CFEO exhibited a better zone of inhibition, with % growth inhibition among all the essential oils; therefore, it was selected for GC-MS analysis. GC-MS chromatogram of the CFEO revealed the presence of many bioactive compounds present on it with multiple peaks. Putatively, 10 compounds with higher peak areas were identified and selected as peaks of the compounds of interest and were assigned to known phytocompounds listed in the NIST library by correlating their peak retention time, molecular weight, and molecular formula. These compounds that were putatively selected will be carried out for future studies. The identified bioactive compounds with compound name, molecular formula, and molecular weight are shown in Figure 2.

7. Conclusion

Acne is a preventable and curable skin disease. In current existing therapies, antibiotics are chosen to treat Acne vulgaris; due to this, *C. acnes* and *S. epidermidis*, two of the main causative organisms of acne, have developed resistance to antibiotics. So, there is a need to develop a novel therapy with fewer side effects and toxicity for the treatment of acne. Herbal medicines are gaining more acceptance because of better patient compliance, long history of use, fewer side effects, and also cost-effectiveness. In this proposed Research work, five medicinal plants were selected to carry out antimicrobial activity against acne. This proposed research study has scientifically validated that the essential oil of the selected plant species has moderate as well as promising antimicrobial activity. The plant specimens were successfully identified

as *Cyperus rotundus* (CREO), *Eucalyptus tereticornis* (ETEO), *Pogostemon cablin* (PCEO), *Cymbopogon nardus* (CNEO), and *Cymbopogon flexuosus* (CFEO). Preliminary phytochemical screening of the plant extracts confirmed the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, phenols, saponins, and glycosides. GC-MS profiling revealed the presence of several identified compounds that may contribute to the observed antimicrobial activity. Preliminary *in vitro* antimicrobial screening of all the essential oils showed good antimicrobial activity with a good zone of inhibition compared to the alcoholic and hydroalcoholic extracts at 205 to 1025 µg/ml concentrations. The essential oils of CNEO and CFEO evaluated in this study demonstrated good antimicrobial activity against the tested acne-causing bacterial strains. Among all the essential oils, CNEO and CFEO showed the highest antimicrobial activity with an MIC value of 1024 µg/ml. But in comparison to CNEO, CFEO exhibited better antimicrobial activity similar to the reference Linezolid.

However, the antimicrobial activity observed in this study was limited to *in vitro* investigations, and the findings should be interpreted with caution. The efficacy and safety of the selected essential oils require further validation through quantitative phytochemical analysis, isolation of the active constituents, computational studies like *in silico* molecular docking and formulation development, and *in vivo* and clinical investigations before their therapeutic potential can be established[21]. These findings support the traditional use of the selected medicinal plants and suggest that their essential oils may serve as promising natural sources for the development of anti-acne formulations. Studies have reported that the traditional practice of using polyherbal combinations rather than individual plants yields greater antimalarial activity[22]. The findings from this research study support the traditional use of these medicinal plants; further studies involving quantitative phytochemical analysis, detailed dose-response evaluation, statistical validation, and *in vivo* investigations are required to confirm their therapeutic potential as alternative anti-acne agents. Overall, the study provides preliminary scientific evidence supporting the antimicrobial activity of selected medicinal plant essential oils against acne-associated bacterial strains and establishes a foundation for future research on the development of safe and effective plant-based anti-acne therapies.

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Author Contributions

Conceptualisation: VKT and GS; Methodology: VKT and GC; Investigation: VKT; Data Curation: VKT and GS. Writing - Original Draft: VKT. Writing - Review and editing: VKT; Supervision: GS. All authors have read and approved the manuscript.

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