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Chemical Composition and Antimicrobial Activity of Essential Oil Obtained from *Jasminum Sambac*

Vikas Jha[1]*; Aneesh Risbud[3]; Darpan Kaur Matharoo[2]; Geetika Preman[4]; Shreya Thube[2]; Anjali Bhosale[1]; Fatimabi Aslam Shaikh[2]; Farheen Khan[2]; Krishnandu Manna[1]; Nikita Tendulkar[2]

[1]National Facility for Biopharmaceuticals, G. N. Khalsa College, Mumbai, Maharashtra, India.

[2]Department of Five Years Integrated Course in Bioanalytical Sciences, GNIRD, G.N. Khalsa College, Matunga-19, Mumbai, Maharashtra, India.

[3]Department of Biomedical Engineering, The Pennsylvania State University, University Park, PA 16802-4400, USA.

[4]Faculty of Nutrition and Biomedicine, TUM School of Life sciences, 85354 Freising, Germany.

***Corresponding Author(s): Vikas Jha**

National Facility for Biopharmaceuticals, Guru Nanak Khalsa college, Mumbai 400019 India.

Email: vikasjjha7@gmail.com

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Abstract

The need for efficient plant-based therapies is being acknowledged all across the world as the prevalence of infectious diseases is growing. Essential oils have been intensively investigated in the hunt for plant-derived biomolecules that can substitute synthetic medications and, to some extent, remove or lessen their adverse effects. In this study, an attempt has been made to give a thorough view on the investigation of essential oil obtained from the *Jasminum sambac* plant as a suitable option for synthetic analogues. The various components present in the essential oil were identified using GC-MS analysis. The oil's antibacterial activity was determined using the Agar Disc Diffusion assay and Microbroth dilution assay, while its antioxidant activity was determined using the DPPH (2,2' - diphenyl-1-picrylhydrazyl) reagent by Free Radical Scavenging assay where the IC_{50} value was observed to be 79.71 µg/mL. The oil exhibited a variable degree of antimicrobial nature against 9 bacterial and 3 fungal strains with the highest activity seen against *Klebsiella pneumoniae*. Furthermore, the antimalarial properties of *Jasminum sambac* essential oil were investigated, and the results revealed it to be a viable therapeutic agent in the future. In conclusion, the role of *Jasminum sambac* essential oil in biological interactions and its potential as therapeutic agent was determined.

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Introduction

The negative environmental consequences of population increase, industrialization, urbanisation, and other variables have resulted in health safety issues owing to the emergence of fast developing infectious diseases as human civilization has progressed towards an advanced technological period [1]. These concerns have prompted a surge in scientific research efforts to investigate the uses of different undiscovered plant-derived antimicrobials in order to determine their antimicrobial capacity [2]. Essential (volatile) oils being one such component derived from aromatic and therapeutic plants have been recognised to have biological activities since antiquity such as antimicrobial, antimalarial and antioxidant characteristics among them [3]. A thorough evaluation of plant extracts for these qualities has become more relevant as interest in the use of essential oils in pharmaceutical industries is growing [4]. Essential oils (EOs) are recognised to have antibacterial effects to different degrees, which are mostly owing to the presence of bioactive components. Antiviral, nematicidal, antifungal, insecticidal, and antioxidant activities are among them [5]. Furthermore, infectious diseases caused by pathogenic microorganisms pose a significant risk to health, particularly in immunocompromised individuals, necessitating the development of low-cost, highly-effective antimicrobial medicines.

Jasmine (*Jasminum*) is a genus of small trees and vines belonging to the Oleaceae family with over 600 species [6]. Their flowers and leaves have long been used for a variety of purposes. For instance, throughout Asia, these flowers have been used to cure a range of diseases such as diarrhoea, fever, conjunctivitis, stomach discomfort, dermatitis, asthma, abscess, breast cancer, uterine haemorrhage, and toothache. *Jasminum sambac* commonly known as "Mogra" is one of the most widely grown plants in Asia, particularly Thailand, due to its great medicinal potential [7]. This plant has traditionally been used for analgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorant, and anti-spasmodic purposes [8].

Essential oil is prevalent in *Jasminum sambac* flowers and obtained in abundance [9]. Essential oils of Jasmine are utilised in skin care products as scent, whereas the plant roots are utilised as analgesic, flowers as lactifuge, and flower extract as deodorant; leaves and flowers as antipyretic and decongestant [10]. Only a few pharmacological investigations of *J. sambac* have been published, despite the fact that the entire plant is used and recommended in traditional medicine. The flower was found to be effective in preventing puerperal lactation [11] and the essential oil was shown to have antimicrobial properties [12]. Another study was conducted in which two complimentary test techniques, DPPH free radical scavenging and beta-carotene-linoleic acid assays, were used to screen the essential oil of *Jasminum sambac* from Djibouti for potential antioxidant activity. In conclusion, the study found that *J. sambac* essential oil and methanol extract exhibit antibacterial and antioxidant properties in vitro, indicating that the plant might be used by traditional healers to treat a variety of infections [13].

Plants and their secondary metabolites have benefitted humans immensely since ages. They provide (and continue to provide) a practically limitless supply of food, fodder for domesticated animals, fibres for clothing, and, not least, medicaments during the plant-human co-evolution. Essential oils are among the many plant products that require special attention [14]. Even with its immense advantages, a limited amount of research has been carried out with respect to *Jasminum sambac* and hence the objective of our study is to test the biological activities of the essential oil and their components; including anti-malarial, antimicrobial and antifungal. The results of our research demonstrate that JEO has the potential to operate as a natural therapeutic agent for a variety of infectious diseases.

Materials and methods

Collection and preparation of plant material for extraction of oil

Fresh flowers present in fully bloomed state were randomly collected from a local botanical garden in Mumbai, Maharashtra, India (Latitude 19.0097° N and Longitude 72.8488° E) for oil extraction. The flowers were washed to get rid of any dirt particles settled on the flowers. Post washing, the petals were separated from the sepals, weighed and air-dried at room temperature before proceeding ahead with the extraction process. The essential oil was extracted as performed by Mahanta *et al* [15] by hydro distillation method using a Clevenger-type apparatus. Any traces of residual moisture present in the extracted oil were eliminated by using anhydrous sodium sulphate. The oil was then stored at 4°C in dark until further experimental analysis.

GC-MS analysis

The extracted essential oil was tested both qualitatively and quantitatively using the Shimadzu GCMS-QP2010 system along with the Rtx-5MS capillary column (5% diphenyl/ 95% dimethyl polysiloxane) which was 30m long with an internal diameter of 0.25 mm and the film was 0.25 µm thick.

In this process, Helium gas (99.999%) was utilised as a carrier gas at a constant flow rate of 1.00 mL/min, and an injection volume of 1 µL was used, whereas the carrier gas saver split ratio was set at 5.0. The injector temperature was kept at 250°C, whereas, the ion-source temperature was 220°C and the oven temperature was set from 40°C (isothermal for 3 min), with an increase up to 230°C at the fourth interval. The total span of the program was 64 min. The mass spectra were recorded across the range of 40 to 1000 m/z. The JEO components were determined on a comparative basis with the Mass Spectral Libraries of Wiley 6.0 and National Institute of Standards and Technology (NIST) as well as the literature data of retention indices obtained from [16].

Microbial strains used for experimental studies

A range of Gram positive bacterial strains which comprised of *Enterococcus hirae* MTCC 2728, *Propionibacterium acnes* MTCC 1951, *Staphylococcus epidermidis* MTCC 3615, *Streptococcus pneumoniae* MTCC 655, MRSA 13 and four Gram-negative micro such as organisms *Proteus mirabilis* MTCC 1429 *Escherichia coli* MCC 2412, *Klebsiella pneumoniae* MCC 2451, *Pseudomonas aeruginosa* MCC 2080, and *Mycobacterium tuberculosis* were used for assessing the bioactivity of the oil. Additionally, three fungal strains namely *Candida albicans* MCC 1151, *strain number MTCC 2196* and *Botrytis* were also used for experimental analysis. Out of all the above-mentioned strains, some were procured from the National Centre for Cell Science (NCCS), Pune, whereas, some were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh and the rest of the bacterial and fungal cultures were obtained from existing laboratory test isolates. Active bacterial cultures were prepared by inoculating loopful of cells from their respective glycerol stock cultures into flasks containing sterile Luria-

Bertani (LB) broth, whereas fungal cultures were inoculated in Sabouraud Dextrose Broth (SDB) and incubated for 24 h at 37°C.

Antimicrobial analysis

Determination of antibacterial activity by disc diffusion test

Bacterial cultures that had been cultivated overnight at 37°C in sterile Luria-Bertani (LB) broth were adjusted to 1.0×10^5 CFU/mL. Then under aseptic conditions, on top of the agar layer in each petri plate containing about 20 mL Luria-Bertani Agar (LA), 50 µL suspension culture was spread plated with a sterile glass spreader. Further, one sterile filter disc (6 mm) was inserted on each plate and 10 µL of test oil sample was added to each disc, and another disc with Kanamycin (1 mg/mL) served as a positive control. After a 24 h incubation period at 37°C, the plates were examined to determine the zone of clearance.

Determination of antifungal activity by agar disc diffusion assay

Initially, each of the 24 h fungal culture (adjusted to 1.0×10^6 CFU/mL) was spread out onto Sabouraud Dextrose Agar petri plates (SDA). On the agar plates, two sterile filter discs with a diameter of 6 mm were placed, impregnated with 10 µL each of JEO and Ketoconazole (1 mg/mL) respectively, wherein Ketoconazole served as a positive control. For a duration of 3-7 days, the plates were incubated at 30°C. Later the plates were observed for clearance areas and their diameters were noted. Later the plates were observed for zone clearance areas and their diameters were noted.

Evaluation of Minimal inhibitory concentration (MIC) against Bacterial strains

The Resazurin Microtiter Assay (REMA) was used to assess the essential oil's Minimum Inhibitory Concentration (MIC) against the test bacterial strains. A 96-well transparent microtiter plate with a flat bottom was used for this test. In sterile 1X Mueller Hinton broth, a two-fold serial dilution of the oil was done from 1000 µg/mL to 1.953 µg/mL. To each well 100 µL of bacterial culture (adjusted to 1.0×10^6 CFU/mL) was added. Finally, the mixture in each well was evenly mixed. The sealed and cultured microplates were incubated at 37°C for 24 h. After the incubation period, 5 µL of Resazurin (2 mg/mL) was added to each well and the plate was incubated at 37°C for 30 min, after which the MIC of oil was determined. The colour shift of Resazurin in the microplate wells was used to determine microbial growth. The minimum inhibitory concentration (MIC) was defined as the lowest oil sample concentration that caused no colour change (clear) thereby completely inhibiting the microorganisms.

Evaluation of minimal inhibitory concentration (MIC) against fungal strains

The fungi strains were put to the test against JEO to obtain its Minimum Inhibitory Concentration (MIC) value against the test fungal strains. Ketoconazole (1 mg/mL) was utilised as a Positive Control in the experiment, which was carried out on a 96-well microtiter plate. The test was carried out using a two-fold serial dilution procedure in 1X Mueller Hinton broth, with a highest oil concentration of 1000 µg/mL up to a lowest concentration of 1.953 µg/mL. Each well consisted a volume of 100 µL of overnight grown fungal culture adjusted at 1.0×10^6 CFU/mL, and all wells were properly mixed. Later, the test microtiter plate was sealed and incubated at 37°C for 24 h. Following the incubation time, 5 µL of Resazurin dye (2 mg/mL) was added to each well to identify microbial growth by the emergence of pink hue. The well with the lowest inhibitory concentration had the minimum oil concentration, exhibiting no colour change.

Assessment of anti-mycobacterial activity by Minimum Inhibitory Concentration (MIC) assay

In DMSO, a working stock of oil was made with a concentration of 100 mg/mL. The *Mycobacterium* culture was cultivated for 20 days in Middlebrook 7H9 broth base medium supplemented with Middlebrook ADC (Bovine Albumin fraction V, Dextrose, Catalase) Growth Supplement and 0.2 percent glycerol were added in a sterile McCartney glass tube. After observing adequate growth, the culture was adjusted to 1.0×10^5 CFU/mL, and the required volume of culture was introduced to each McCartney glass tube. Then, in each tube, JEO was introduced at various concentrations ranging from 100 µg/mL to 1000 µg/mL. The tubes were kept in a CO_2 incubator at 37°C for 28 days. Following the incubation time, 100 µL of the incubated mixture was added to each microtiter well, along with 25 µL of 0.4 mg/mL Resazurin, in order to evaluate microbial growth by colour change to pink. The lowest inhibitory concentration against *Mycobacterium tuberculosis* was recorded with no colour change.

In-vitro antimalarial analysis

The extracted JEO was tested for antimalarial activity at the Microcare laboratory and TRC in Surat, Gujarat. The *in vitro* antimalarial assay was performed in 96-well microtiter plate according to Rieckmann and coworkers' [17] micro assay technique with minimal changes. The *P. falciparum* strain was cultured in RPMI 1640 medium supplemented with 25 mM HEPES, 1% D-glucose, 0.23 % sodium bicarbonate, and 10% heat inactivated human serum.

P. falciparum asynchronous parasites were synchronized using 5% D-sorbitol to obtain only the ring stage parasitized cells. An initial ring stage parasitaemia of 0.8 to 1.5 % at 3% haematocrit in a total volume of 200 µL of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O+) for carrying out the assay. Each of the test samples had a stock solution of 5 mg/ml produced in DMSO, and further dilutions were made with culture media. In duplicate wells containing parasitized cell preparation, the diluted samples in 20 µL volume were added to the test wells to produce final concentrations (at fivefold dilutions) ranging from 0.4 µg/ml to 100 µg/ml. In a candle jar, the culture plates were incubated at 37°C. Thin blood smears from each well were produced and stained with JSB stain after 36 to 40 h of incubation. The lowest inhibitory concentrations were defined as the test concentrations that prevented complete maturation into schizonts (MIC). Reference drugs used in this study were Chloroquine and Quinine.

Antioxidant analysis via DPPH free radical scavenging assay

Oil stock with a concentration of 10 mg/mL was prepared in dimethyl sulfoxide at various concentrations ranging from 0.1 mg/mL to 1 mg/mL. (DMSO). Each tube had a total reaction volume of 4 mL, which included the sample, methanol as a diluent, and 2 mL of DPPH. For around 30 - 45 min, the reaction mixture was incubated in the dark. The absorbance was measured at 515 nm using a UV Visible spectrophotometer after the incubation time.

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

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

Results and discussion

Gas Chromatography- Mass pectrometry (GC-MS) analysis

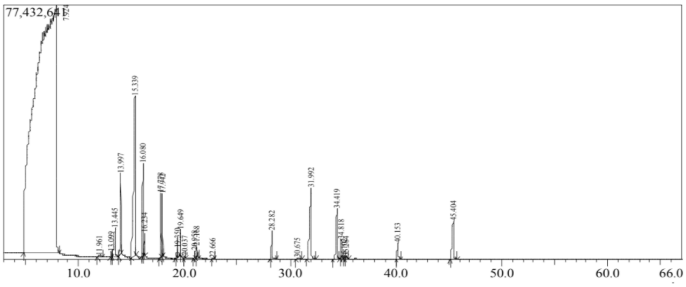

Figure 1: Resentative GC-MS chromatogram of *J.sambac* essential oil

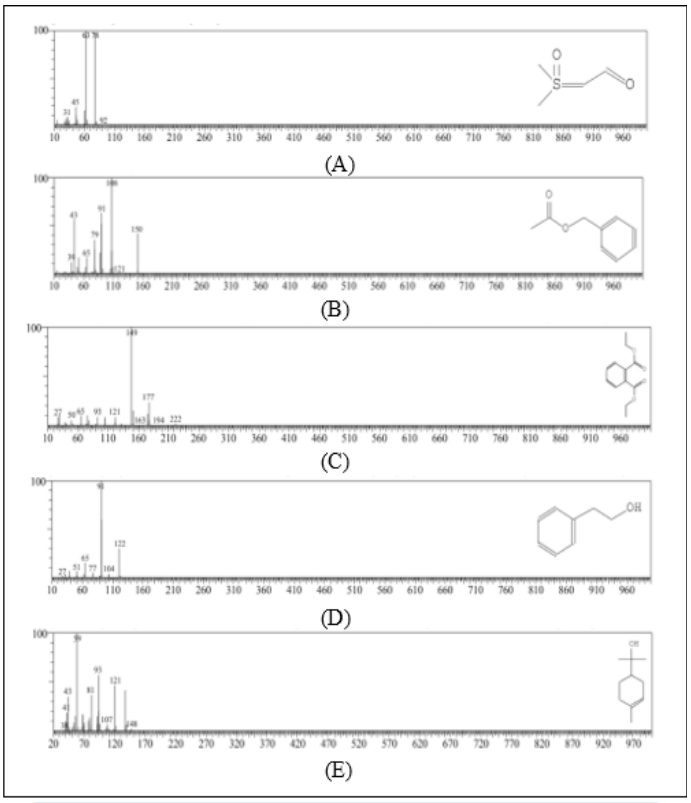

Figure 2: GC-MS mass fragments of five major components of J.sambac **(A)** Dimethylsulfoxonium formylmethylide; **(B)** Acetic acid, phenylmethyl ester; **(C)** Diethyl Phthalate **(D)** Phenylethyl Alcohol; **(E)** L-.alpha.-Terpineol

The chromatographic profiles as shown in Figure 1 and Figure 2 was obtained by GC-MS technique wherein 41 distinct compounds were identified (Table 1). The main components in the essential oils were monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes. Results of the analysis are summarized in the below tables. In the case of *J. sambac* we observed ten peaks. In the peaks, it shows the highest peak percentage is 85.33 and the least is peak number three at 0.91 percent. The analysis reported Dimethylsulfoxonium formylmethylide (85.33%) as the predominant component of *J.sambac* essential oil followed by Acetic acid, phenylmethyl ester (4.09%); Diethyl Phthalate (2.17%); Phenylethyl Alcohol (1.33%); L-.alpha.-Terpineol (1.08%); Cinnamaldehyde, .alpha.-pentyl- (1%); c, 2-hydroxy-, phenylmethyl ester (0.91%); 1,6-Octadien-3-ol, 3,7-dimethyl- (0.81%); 1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate (0.73%); Propanoic acid, phenylmethyl ester (0.54%) (Figure 2). The respective retention time for each of the components were 7.92, 15.33, 31.99, 13.99, 16.08, 34.41, 45.40, 13.44, 17.77, 17.94 min respectively. However, the above mentioned composition varies significantly as compared to the GC-MS analysis for *Jasminum sambac* flowers by S. Elumalai et al [18].

Table 1: Chemical composition of *J.sambac* essential oil

Retention time (min)	% Area	Name
7.924	85.33	Dimethylsulfoxonium formylmethylide
15.339	4.09	Acetic acid, phenylmethyl ester
31.992	2.17	Diethyl Phthalate
13.997	1.33	Phenylethyl Alcohol
16.08	1.08	L-.alpha.-Terpineol
34.419	1	Cinnamaldehyde, .alpha.-pentyl-
45.404	0.91	Benzoic acid, 2-hydroxy-, phenylmethyl ester
13.445	0.81	1,6-Octadien-3-ol, 3,7-dimethyl-
17.778	0.73	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate
17.942	0.54	Propanoic acid, phenylmethyl ester
28.282	0.43	Lilial
40.153	0.28	Benzyl Benzoate
34.818	0.27	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester
19.649	0.21	Benzeneethanol, .alpha.,.alpha.-dimethyl-, acetate
21.168	0.2	Undecanal, 2-methyl-
16.234	0.18	Cyclohexanol, 1-methyl-4-(1-methylethylidene)-
19.35	0.09	(1Alpha,3beta,4beta)-p-menthane-3,8-diol
20.958	0.09	3-Allyl-6-methoxyphenol
13.099	0.07	p-Cresol
30.675	0.06	2(3H)-Furanone, 5-heptyldihydro-
11.961	0.05	Benzyl alcohol
35.284	0.04	Cinnamaldehyde, .alpha.-pentyl-
20.037	0.03	4-tert-Butylcyclohexyl acetate
22.666	0.01	Longifolene
35.102	0	2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl-

Antimicrobial analysis

Antibacterial activity analysis

The antibacterial activity of JEO (Figure 3) was determined against four strains of Gram-positive and four strains of Gram-negative bacteria by paper disc diffusion test as shown in Table 2. The largest zone of inhibition was observed in case of *Klebsiella pneumoniae* (13.95 ± 0.07 mm) followed by *Pseudomonas aeruginosa* (11.03 ± 0.05 mm) and *Streptococcus pneumoniae* (10.99 ± 0.03 mm). The antibacterial activity might have resulted due to the presence of antibacterial compounds such as Dimethylsulfoxonium formylmethylide [19], p-Cresol [20], Undecanal, 2-methyl- [21] and Linalool [22] in the essential oil which was revealed by GC-MS analysis. These compounds have previously been reported as antibacterial in nature on an array of various microorganisms. The results obtained against some of the bacteria were in accordance with the previously published

report which also carried an analysis on JEO [23]. Due to the notable results and the presence of multiple antibacterial components, JEO can be considered as a highly potent antibacterial agent against pathogenic bacterial strains in focus.

Table 2: Antibacterial activity of JEO. Kanamycin acid was used as Positive control

Sr. No.	Strain Name	Inhibition zone* (mm)	
		JEO	Positive control
1	*Enterococcus hirae*	10.02 ± 0.06	21.04 ± 0.04
2	*Propionibacterium acnes*	9.00 ± 0.08	29.10 ± 0.06
3	*Proteus mirabilis*	10.02 ± 0.07	20.99 ± 0.08
4	*Staphylococcus epidermidis*	10.03 ± 0.05	18 ± 0.06
5	*Streptococcus pneumoniae*	10.99 ± 0.03	13 ± 0.06
6	*Escherichia coli*	0	30.08 ± 0.05
7	*Klebsiella pneumoniae*	13.95 ± 0.07	29.97 ± 0.04
8	*Pseudomonas aeruginosa*	11.03 ± 0.05	11.05 ± 0.06

*Above mentioned values are given as Mean ± Standard Deviation (n=3)

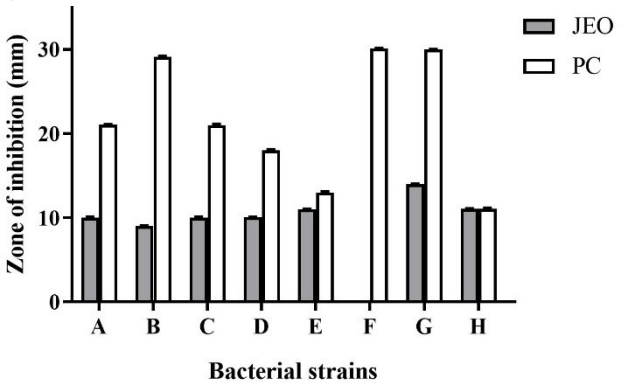

Figure 3: Antibacterial activity of JEO tested against **(A)** *Enterococcus hirae*, **(B)** *Propionibacterium acnes*, **(C)** *Proteus mirabilis*, **(D)** *Staphylococcus epidermis*, **(E)** *Streptococcus pneumoniae*, **(F)** *Escherichia coli*, **(G)** *Klebsiella pneumoniae*, **(H)** *Pseudomonas aeruginosa*

Antifungal activity analysis

The antifungal activity of JEO was evaluated against three fungi as shown in Figure 4. The highest and lowest zones of inhibition were observed against *Candida Albicans* (18 ± 0.08 mm) and *Botrytis* (15.02 ± 0.07mm) respectively (Table 3). The antifungal activity might have been caused due to the presence of various antifungal components such as Dimethylsulfoxonium formylmethylide [19], 1,6-Octadien-3-ol, 3,7-dimethyl- [24], and Longifolene [25] observed in GC-MS analysis. The results obtained for Candida Albicans [26] and *Aspergillus niger* [27] are in accordance with the data obtained in recent published reports, wherein, different essential oils consisting of the same oil component were studied for their anti-fungal property.

Table 3: Antifungal activity of JEO. Ketoconazole was used as Positive Control

Sr. No	Strain Name	Inhibition zone* (mm)	
		JEO	Positive control
1	*Aspergillus niger*	16.03 ± 0.06	18.97 ± 0.04
2	*Botrytis*	15.02 ± 0.07	18.00 ± 0.06
3	*Candida albicans*	18.00 ± 0.08	16.03 ± 0.04

*Above mentioned values are given as Mean ± Standard Deviation (n=3)

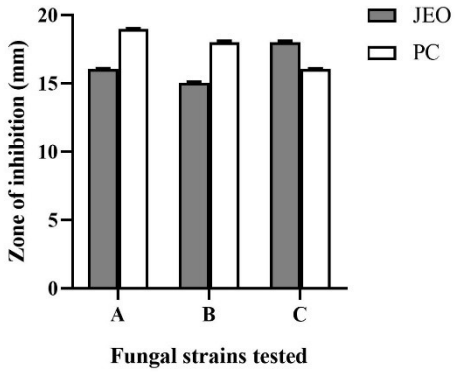

Figure 4: Antifungal activity of JEO tested against **(A)** *Aspergillus niger*, **(B)** *Botrytis*, **(C)** *Candida albicans*

Minimum inhibitory concentration for bacteria

The MIC values of JEO against each bacterium is mentioned in Table 4. The results obtained, determined that, JEO successfully inhibited the growth of all the test microbial strains as shown in Figure 5. The concentration range for minimum inhibitory concentration started from 18.23 µg/mL for *Klebsiella pneumoniae* and extended up to 291.67 µg/mL for *Propionibacterium acnes* and *Staphylococcus epidermidis.* The highest MIC values were obtained against the gram-positive bacteria *Propionibacterium acnes* (291.67 ± 155.9 µg/mL), *Staphylococcus epidermidis* (291.67 ± 155.9 µg/mL) and followed by *Enterococcus hirae* (166.67 ± 28.93 µg/mL) followed by the gram-negative bacteria *Proteus Mirabilis* (145.83 ± 77.95 µg/mL). The results were found to be remarkable for these before-mentioned bacteria. These values indicate the resistance potential of the microorganisms against JEO at higher concentrations.

The MIC for anti-mycobacterial activity was also carried out and the resulted value was 6.25 ± 0.03 µg/mL. This activity might have been observed due to the presence of one of the Anti-tuberculosis components for e.g. Undecanal, 2-methyl- [21] observed via GC-MS analysis.

Table 4: Minimum Inhibitory Concentration (µg/mL) of JEO against test bacterial strains.

Sr. No.	Bacterium	Minimum Inhibitory Concentration[a] (MIC) (µg/mL)
1	*Enterococcus hirae*	166.67 ± 28.93
2	*Propionibacterium acnes*	291.67 ± 155.9
3	*Proteus mirabilis*	145.83 ± 77.95
4	*Staphylococcus epidermidis*	291.67 ± 155.9
5	*Streptococcus pneumoniae*	104.17 ± 29.46
6	*Escherichia coli*	0
7	*Klebsiella pneumoniae*	18.23 ± 9.74
8	*Pseudomonas aeruginosa*	36.46 ± 19.49
9	*Mycobacterium tuberculosis*	6.25 ± 0.03

[a]Minimum Inhibitory Concentration values are expressed as Mean ± Standard Deviation (n=3)

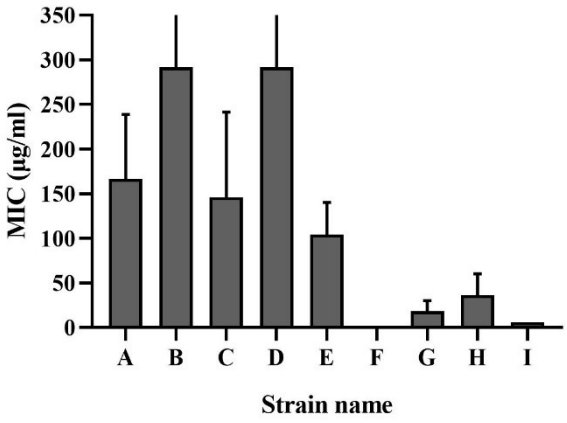

Figure 5: MIC values (µg/mL) of JEO obtained against **(A)** *Enterococcus hirae*, **(B)** *Propionibacterium acnes*, **(C)** *Proteus mirabilis*, **(D)** *Staphylococcus epidermis*, **(E)** *Streptococcus pneumoniae*, **(E)** *Escherichia coli*, **(F)** *Klebsiella pneumoniae*, **(H)** *Pseudomonas aeruginosa* **(I)** *Mycobacterium tuberculosis*

Minimum inhibitory concentration for fungi

The values obtained for the MIC of JEO against the various fungal strains ranged from 26.04 µg/mL to 72.92 µg/mL (Table 5). The lowest MIC value was observed against *Candida albicans* (26.04 ± 7.37 µg/mL) and the highest was observed against *Botrytis* (72.92 ± 38.98 µg/mL). The difference between each MIC value of the fungi was seen to be significant as shown in Figure 6. These results indicate that JEO showed comparatively significant antimicrobial results against bacterial strains as compared to the fungal strains used in the current study.

Table 5: Minimum Inhibitory Concentration (µg/mL) of JEO against test fungal strains.

Sr. No	Fungi	Minimum Inhibitory Concentration* (MIC) (µg/mL)
1	*Aspergillus niger*	36.46 ± 19.49
2	*Botrytis*	72.92 ± 38.98
3	*Candida albicans*	26.04 ± 7.37

*Minimum Inhibitory Concentration values are expressed as Mean ± Standard Deviation (n=3).

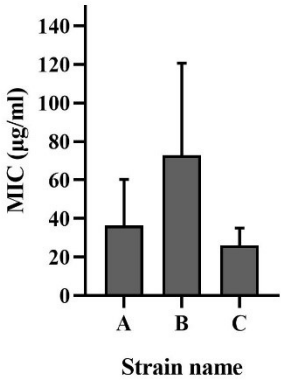

Figure 6: MIC values (µg/mL) of JEO obtained against **(A)** *Aspergillus niger*, **(B)** *Botrytis*, **(C)** *Candida albicans*

Invitro antimalarial analysis

The antimalarial activity of *J.sambac* essential oil (Figure 7) was analyzed by Minimum Inhibitory Concentration (MIC) method against drug sensitive and drug resistant strains of *Plasmodium falciparum* (Table 6). The IC_{50} values of JEO against drug sensitive and drug resistant *P.falciparum* strains were observed at 0.4 µg/mL to 1.04 µg/mL respectively, it can be considered as a vital source of anti-malarial properties due to the presence of compounds capable of conferring anti- malarial activity such as Cinnamaldehyde, alpha.-pentyl- and Benzoic acid as validated previously from GC-MS analysis. Previous studies have reported Cinnamaldehyde [28] as an effective larvicidal and mosquito repellent compound, whereas Benzoic acid [29] has shown significant results when tested against *P. falciparum.* The consequent IC_{50} values obtained against both the strains *P. falciparum* were observed to be relatively low as compared to the previous antimalarial studies [9,30] conducted against *P. falciparum* strains due to the abundance of Cinnamaldehyde which is a major component of *J.sambac.*

Table 6: Anti-malarial Activity of JEO against *Plasmodium falciparum*

JEO	[DS]*Plasmodium falciparum*		[DR]*Plasmodium falciparum*	
Concentration (µg/ml)	% Inhibition	IC_{50} value (µg/mL)	% Inhibition	IC_{50} value (µg/mL)
0.1	25.19	0.40	34.75	1.04
0.2	26.5		34.85	
0.4	30.85		35.7	
0.8	31.25		37.4	
1.2	32.44		37.65	
1.6	32.8		38.45	
2	34.05		40.95	
Chloroquine (Control)		0.020		
Quinine (Control)		0.268		

[DS]Chloroquine and Quinine sensitive *Plasmodium falciparum* strain and Chloroquine and Quinine resistant *Plasmodium falciparum* strain.

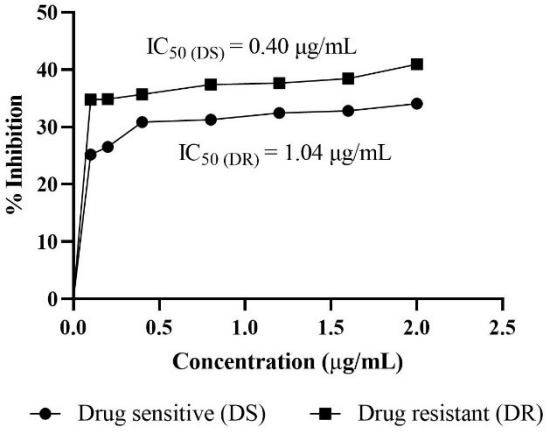

Figure 7: Anti-malarial activity of JEO against drug resistant and drug sensitive strains of *Plasmodium falciparum*

Antioxidant analysis via DPPH free radical scavenging assay

In this study, different concentrations of JEO were subjected to the DPPH (2,2' - diphenyl-1-picrylhydrazyl) free radical scavenging technique to determine the free radical scavenging activity of naturally occurring compounds (Figure 8). The lowest free radical scavenging activity was reported at 700 mg/mL whereas the highest activity was recorded at an oil concentration of 200 µg/mL with an IC_{50} value of 79.71 µg/mL (Table 7). These antioxidant results may have been obtained due to the presence of a few components observed by GC-MS analysis such as Benzyl alcohol [31], p-Cresol [32], L-.alpha.-Terpineol [33], and 3-Allyl-6-methoxyphenol [34] which have previously been reported to exhibit anti-oxidant properties.

Table 7: Percentage Free Radical Scavenging Activity of JEO from 200 mg/mL to 1000 mg/mL.

Concentration (µg/mL)	Absorbance measured at 515 nm	Free Radical Scavenging Activity* (%)
Control (DPPH)	0.725	
200	0.218	69.94 ± 0.03
300	0.15	79.31 ± 0.02
400	0.161	77.79 ± 0.01
500	0.164	77.38 ± 0.04
600	0.121	83.31 ± 0.03
700	0.093	87.17 ± 0.02
800	0.066	90.90 ± 0.02
900	0.076	89.52 ± 0.03
1000	0.04	94.48 ± 0.01

*% Free Radical Scavenging Activity is expressed as Mean ± Standard Deviation (n=3).

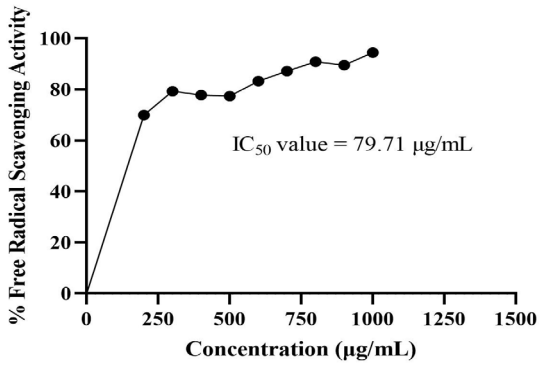

Figure 8: Percentage Free Radical Scavenging Activity of JEO

Conclusion

The current study delves into the chemical composition of JEO as well as its antibacterial, anti-malarial, antioxidant, and anti-mycobacterial activities. The GC-MS analysis of the oil reported the presence of Dimethylsulfoxonium formylmethylide (85.33%) as the major component followed by Acetic acid, phenylmethyl ester (4.09%); Diethyl Phthalate (2.17%); and Phenylethyl Alcohol (1.33%). Because of the presence of a number of antibacterial and antifungal chemicals in the essential oil, it successfully demonstrated anti-microbial action against all of the test pathogens. The anti-malarial effect of JEO at lower concentrations was investigated because to its strong action against drug-resistant and drug-sensitive stains of Plasmodium falciparum. Furthermore, the oil's antioxidant ability was assessed using the DPPH free radical scavenging activity test. Till date, to the best of our knowledge, very few studies have been conducted to check the chemical and antimicrobial properties of JEO. Further research is needed to separate these different components using the HP-TLC based Bioautography technology. MS-MS and NMR analytical methods can be used to confirm the separated oil constituents. In conclusion, JEO is a very less researched source of numerous plant metabolites that should be further studied in order to get natural alternatives to synthetic industrial by-products, therefore substituting them in a variety of medicinal, food, cosmeceutical, and nutraceutical applications.

Ethics declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Data availability statement: All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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.

Abbreviations: EO: Essential Oil; DPPH: 2:2-diphenyl-1-picrylhydrazyl ;JEO: *Jasminum* Essential Oil; GC-MS: Gas Chromatography-Mass Spectrometry; DMSO: Dimethyl Sulfoxide; NIST: National Institute of Standards and Technology; MTCC: Microbial Type Culture Collection and Gene Bank; NCCS: National Centre for Cell Science; LB: Luria-Bertani; LA: Luria-Bertani Agar; SDB: Sabouraud Dextrose Broth; SDA: Sabouraud Dextrose Agar; MIC: Minimum Inhibitory Concentration; DS: Drug Sensitive; DR: Drug Resistant; DPPH: 2:2'-diphenyl-1-picrylhydrazyl; DMSO: Dimethyl Sulfoxide; HP-TLC: High-Performance Thin-Layer Chromatography; MS-MS: Mass-Spectroscopy; NMR: Nuclear Magnetic Resonance; REMA: Resazurin Microtiter Assay; MIC: Minimum Inhibitory Concentration; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; RPMI: Roswell Park Memorial Institute.

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