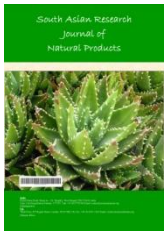

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Assessment of Thermal Behaviour, Physiochemical Characteristics and Bioactive Potentials of *Jasminum sambac* Essential Oil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Essential oils (EO) are intriguing natural compounds produced from aromatic plants, playing a significant role in the ancient pharmacopoeia. Recently, there has been a lot of interest in using several essential oils as alternative antibacterial and medicinal agents. One of such essential oils is derived from *Jasminum sambac* which is well known for its aroma and has various therapeutic benefits. It opens a door to alternative medicine for chronic conditions where commercially available treatments are of no benefit. This study is focused on determining the presence of various functional and bioactive components present in the essential oil and assessing its thermal stability, along with which behavior of biological agents was also monitored in presence of the oil. The *Jasminum sambac* essential oil was extracted using the hydro-distillation method, and its chemical makeup was ascertained using High performance thin layer liquid chromatography (HPTLC). The functional groups present in the essential oil were also identified using Fourier transform infrared spectroscopy (FTIR). The essential oil was thermally characterized using Thermogravimetric (TG) and Differential scanning calorimetric (DSC) analysis. The essential oil's anti-inflammatory potential was also evaluated. The antibacterial and antibiofilm ability of the essential oil was determined

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against various drug-resistant microbes and biofilm forming pathogens, respectively. Additionally, the percentage viability of CHO and HEK293 cell lines were evaluated in presence of the essential oil by employing the MTT assay. High performance thin layer liquid chromatography revealed 7 distinct bands which can be correlated to the presence of flavonoids, polyphenols, terpenes, quinones, steroids, and alkaloids. Thermal stability was achieved by the essential oil when exposed to high temperatures during Thermogravimetric and Differential scanning calorimetric analysis. Different functional components were identified using Fourier transform infrared spectroscopy. Reliable antimicrobial, antibiofilm and anti-inflammatory activities were also recorded. Further, the percentage viability in presence of the essential oil was also determined which can be directly correlated with its cytotoxicity. From the results of this study, it can be concluded that the *Jasminum sambac* essential oil has immense healing potential and can be employed as an alternative therapeutic agent.

Keywords: Jasminum sambac; HPTLC; FTIR; TGA; DSC; anti-inflammatory; antimicrobial; antibiofilm.

ABBREVIATIONS

HPTLC : High performance thin layer chromatography,
FT-IR : Fourier Transform Infrared Spectroscopy;
DSC : Differential Scanning Calorimetry;
TGA : Thermogravimetric Analysis;
MIC : Minimum Inhibitory Concentrations;
LB : Luria-Bertani.

1. INTRODUCTION

Current quality of life, drug-resistance, deteriorating food habits and adulteration has taken a toll on human health leading to severe chronic health conditions. There is an immediate need to find alternative therapeutic candidates to overcome and reduce such chronic ailments. Naturally occurring plant metabolites reported in medieval medical literatures have played a significant role in drug development and will likely continue to do so [1]. Plant metabolites such as essential oils have been employed since ages for treatment of infections as well as in aromatherapy. Such essential oils can be employed as aroma-therapeutics to combat such chronic health issues which synthetic drugs with major side effects fall short.

Essential oils are extensively used in cosmetics, pharmaceuticals, perfumery, disinfectants, confectionaries, and several other related products. They are composed of volatile compounds which give a characteristic odour and flavour to the plants and are obtained through hydro-distillation or extraction with solvents [2]. These volatile compounds include alcohols, ethers, aldehydes, ketones, esters, amines, amides, phenols and terpenes [3]. Essential oils have been widely employed for bactericidal, antiviral, fungicidal, anti-parasitical, insecticidal, medicinal and cosmetic applications [4]. Furthermore, essential oils are the most often used complementary and alternative medicine for treating fungal skin infections. Additionally, the literature on aromatherapy lists a variety of essential oils for use in dermatology, the majority of which are suggested for treating infections as they have the ability to suppress the bacteria that cause infections [5].

Jasminum sambac belongs to the *Oleaceae* family and comprises of a significant group of plants that are utilized all over the world for the extraction of their essential oils. About 200 species make up the genus, most of which are indigenous to Southeast Asia, the Pacific, Australia, and Africa. The plant's blooms are used to produce tea, while the roots, leaves, and flowers are all employed in traditional medicines to treat a wide range of ailments [4]. The primary constituents of *Jasminum sambac* are benzoic acid, benzaldehyde, benzyl acetate, benzyl alcohol, indole, benzyl benzoate, cis-3-hexenyl benzoate, cis-jasmone, ceosol, eugenol, farnesol, geraniol, linalool, methyl anthranilate, p-cresol The anti-bacterial, anti-depressant, anti-inflammatory, anti-septic, anti-spasmodic, anti-viral, aphrodisiac, astringent, calmative, cicatrisant, cooling, emenagogue, expectorant, galactogogue, hypotensive, nervine analgesic, parturient, sedative, and uterine properties of *Jasminum sambac* essential oil are responsible for its health benefits. It is effective in treating severe depression and calms the nerves while reviving and restoring energy [6]. Additionally, *Jasminum sambac* flowers are used for the production of perfumes, aromatizing agents, hair decorations and accessories, and employed as an ingredient in tea.

In terms of pharmacological activity, Jasminum sambac essential oil has been shown to possess antimicrobial properties against a wide range of bacteria, including *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Salmonella typhi, Salmonella typhimuri* and fungi such as *Candida albicans* and *Saccharomyces cerevisiae* [7]. One of the constituents of the EO, (R)-()-linalool, is responsible for the sedative effect on both autonomic nerve activity and mood states [8]. Non-diabetic rats with excision, incision, and dead space wounds showed improved wound healing when treated with an ethanolic extract of *J. grandiflorum Linn*. flowers from the *Jasminum* genus [9]. *Jasminum sambac* essential oilis used for labor since it reduces contractions and relieves pain and spasms. Jasmine, clary sage, rose, and lavender massages around the lower back have been said to have benefits during childbirth. Endorphins, which are produced naturally by the body and act as painkillers and mood enhancers, are stimulated to be released [10]. When the use of bromocriptine is constrained by cost or unavailability, jasmine blossoms can be utilized as an alternative that appears to be both efficient and affordable for reducing puerperal lactation [11].

Aromatherapy employs essential oils since centuries for their health benefits including relief from anxiety and depression, improved sleep cycle and mood alleviation. Aroma-therapeutic essential oils like rose, jasmine, tuberose and champa lead to improved physiological changes as mentioned above [4]. According to a study performed by Kubota *et al., Jasminum sambac* essential oil demonstrated a stimulating effect on brain activity and can be a good candidate for aroma-therapeutics [12].

In addition to aromatherapy, *Jasminum sambac* essential oil has also found its application in dentistry as it possesses antimicrobial activity against various oral pathogenic bacteria [7]. *Jasminum sambac* essential oil also exhibits anti-diabetic activity [13], anti-acne activity,[5] and improve blood cholesterol [14]. The essential oil from *Jasminum sambac* is highly used in perfume industry owing to its pleasant odor as well as for skincare products, in anti- irritation products, moisturizers, anti-aging, anti-hyperpigmentation lotions and in sunscreens [7,5].

The objective of the current study is to evaluate the therapeutic potential of *Jasminum sambac* essential oil as well as determine its thermal characteristics. The study also aims at determining the active components of the essential oil for its future applications in cosmetics, pharmaceutics, and aromatherapy by optimizing the concentrations of these components. To determine the therapeutic potential evaluation of its various biological activities like Anti-microbial, Cell viability, Anti-inflammatory and Anti-biofilm activity were conducted. Furthermore, Fourier transform infrared Spectroscopy (FTIR), Thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC) were carried out to determine the oil's functional components and thermal stability. High-performance thin-layer chromatography (HPTLC) was employed to determine a fingerprint representing various active components of the essential oil.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh flowers that were fully bloomed were randomly picked from a nearby botanical park in Mumbai, Maharashtra, India for oil extraction. To remove any dirt that had accumulated on the flowers, they were thoroughly cleaned. After being washed, the petals were separated from the sepals, weighed, and air dried at room temperature before the extraction procedure began. According to Mahanta *et al.* [15], the essential oil was extracted using the hydro distillation process with a Clevenger-style device. Anhydrous sodium sulphate was used to remove any lingering moisture that might have been in the produced oil. After which, the oil was stored at 4 ℃ in the dark until a subsequent experimental study.

2.2 FTIR Analysis

The functional groups of the sample oil were examined using a Carry 630 Fourier transform infrared spectrometer. Initially, the system was pre-heated and stabilized. In a clean KCl pellet, a drop of substance was inserted. After that, the salt pellet was placed into the KCl pellet, tiling the oil sample uniformly and vesicularly between two KCl pellets. The infrared spectrometer was calibrated to absorbance with a resolution of 8 cm^{-1} after gently rotating the KCl pellets to establish a homogenous liquid membrane and attaching and inserting them in the infrared spectrometer sample holders. The oil sample's

infrared absorption spectra were obtained in the spectral ranges 3500–650 cm^{-1} at the provided conditions.

2.3 Thermo Gravimetric Analysis

The STA 250 Instrument was used to perform TGA analysis on the oil sample. The experiment was carried out in a nitrogen gas atmosphere controlled by a flow rate of 300 mL/min. The samples were weighed at 20.45 mg and deposited in aluminium crucibles. The sample was heated to 500 ℃ at a variety of ambient temperatures at a constant flow rate of 20 ℃/min throughout the process.

2.4 Differential Scanning Calorimetry

A TA instrument type DSC Q20 V24.11 was used to develop a DSC profile of an essential oil. To conduct the experiment, 14mg of material was placed in aluminium crucibles. A nitrogen gas with a flow rate of 40 mL/min was used to evaluate the samples. A dynamic scan was also performed over a temperature gradient of 25℃ to 400℃ at a continuous heating rate of 20 ℃/min.

2.5 High Performance thin Layer Liquid Chromatography

For identification and isolation of the essential oil extracted from *Jasminum sambac* by HPTLC, a solvent solution consisting of Toluene: Ethyl acetate in a ratio of 9.7: 0.3 was used. The essential oil was extracted using a silica gel 60 F 254 HPTLC plate with dimensions of 100.0 × 100.0 mm (Merck). 50 µL of sample solution in 1 mL of solvent (methanol) were applied as 8 mm wide bands (delivery speed 150 nL/s). The plate was then developed for 20 minutes at room temperature in a CAMAG twin-trough vertical development chamber pre-saturated with the solvent solution mentioned above. The migration distance was kept constant at 85 mm. The plate was then subjected to densitometric scanning at a wavelength of 254 nm and 366 nm, scanning speed of 20 mm/s, slit dimension of 5 mm x 0.2 mm, light sources of deuterium and tungsten

2.6 Antimicrobial Activity

A microdilution test was conducted in accordance with CLSI standard procedures with the following modifications to evaluate the antibacterial properties of *Jasminum sambac* essential oil [16]. The test was carried out in a 96-well microtiter plate and nine antibiotic resistant bacterial strains were selected. The bacterial test strains included Carbapenem-Resistant Acinetobacter (CRA), Carbapenem-Resistant *Pseudomonas aeruginosa* (CRP), Carbapenem-Resistant *Escherichia coli* (CRE), Carbapenem-Resistant *Klebsiella pneumonie* (CRK), Extended Spectrum beta-lactamase *Escherichia coli* (ESBL), Quinolone resistant Salmonella (QRS), Vancomycin-resistant Enterococci (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA) and Erythromycin resistant Streptococci (ERS). To achieve a concentration of 1.0 x 10^5 CFU/mL, the microbial suspensions were adjusted. Additionally, the essential oil was dissolved in a 5 % DMSO and 0.1 % polysorbate-80 (1 mg/mL) mixture before being added to 100 µL of Luria-Bertani medium with a 1.0 x 10^4 CFU/mL bacterial inoculum to reach the required concentrations. Inoculated plates we incubated at 37°C for 24h at 180 rpm. In each well received 5 µl of Resazurin dye (2 mg/mL) was added after incubation. Microbial growth can be distinguished by the development of resorufin which is pink in colour and produced due to reduction of resazurin by viable cells.

2.7 Cell Viability Assay

The percentage cell viability of Chinese hamster ovary (CHO) and Human Embryonic Kidney (HEK293) cell lines was determined against *Jasminum sambac* essential oil employing the tetrazolium salt, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) colorimetric technique. The cell lines were obtained from National Centre for Cell Science (NCCS), Pune and cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% foetal bovine serum (FBS) and antibiotics (Penstrep). Every week, the culture media was changed, and every two weeks, the cells were passaged. The cell line was kept at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity. Trypsin-ethylenediamin-etetraacetic acid was used to separate monolayer cells into single cell suspensions, and viable cells were counted using a hemocytometer. The cells were diluted to a final density of 1 x 10^5 cells/ml in FBS medium containing 5% FBS. Additionally, 10,000 cells were plated in each well of 96-well plates using 100 µl of cell suspension, and the plates were incubated at 37°C, 5% CO_2, 95% air, and 100% relative humidity to test the adhesion of the cells. The cells were exposed to repeated quantities of the essential oil sample after 24 hours of incubation. They were first dissolved in dimethyl sulfoxide

(DMSO), and then their concentrations were diluted in serum-free media to reach final concentrations of 5, 10, 20, 40, 60, 80, and 100 µg/ml. 100 µl of each concentration was then added to each well in a 96-well plate. Each well had a final capacity of 200 µl and was incubated for an additional 48 hours at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity. A medium without any sample was utilised as a control. Following the initial incubation time, each well received 10 µl of MTT (5 mg/ml) in phosphate buffered saline, which was added and then re-incubated at 37°C for 4 hours. The generated formazan crystals were then solubilized in 100 µl of DMSO after the media containing the MTT was decanted. At 595 nm, the optical density was assessed using a microplate reader. The following formula was used to compute the percentage of cell inhibition:

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

2.8 Anti-inflammatory Activity

The *Jasminum sambac* essential oil's anti-inflammatory properties were evaluated using Inhibition of protein denaturation assay by employing egg albumin method. Protein denaturation, which is seen because of proteins losing their tertiary and quaternary structures, is what leads to inflammation. This study investigated the essential oil's potential to reduce inflammation. Except for the control tube, which contained the standard (Diclofenac) and saline, a reaction mixture containing 1 ml of essential oil sample with concentrations ranging from 10 to 100 µg/mL and 1 % aqueous solution of egg albumin was taken. The test tubes were incubated for 20 minutes at 37°C and then heated at 60 °C for 20 minutes. After the incubation, the test tubes were cooled and optical density of the test solutions was observed at 660 nm using a spectrophotometer [17]. The percentage inhibition was calculated using the following formula:

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

2.9 Anti-biofilm Activity

In vitro anti-biofilm activity of *Jasminum sambac* essential oil was determined against *Staphylococcus aureus*, *Proteus vulgaris, Proteus mirabilis* and *Pseudomonas aeruginosa* through the crystal violet assay [18]. The test organisms were isolated on the Nutrient agar medium from which an isolated colony was picked and inoculated in the Luria Bertanii (LB) broth. According to McFarland Standard, the optical density of the culture suspension was adjusted to 0.5 O. D. Further, sterile distilled water, 100 µl of LB broth and 100 µl of essential oil sample were added in sterile 96 well microtiter plate. The positive control comprised of 30 µg/ml Chloramphenicol and a solution of 200 µl of LB broth and 100 µl of sterile distilled water was used as a negative control. A bacterial suspension of 20 µl with a final OD_{600} of 0.01 was diluted with an overnight culture grown in LB broth and was added to each well containing 100 µl of the essential oil sample. The plates were kept for overnight incubation at 37 ℃ for 24 hours under static conditions to allow bacterial growth and biofilm maturation. Followed by incubation, the planktonic cells and the used medium were decanted, and the attached biomass was rinsed thrice using distilled water. Further, this biomass was stained with 400 µl of 1% Crystal violet dye and the plates were then incubated at Room Temperature for 30-40 min. Post incubation the plates were drained and rinsed thrice with distilled water for removal of the unbound dye. The plates were then dried in the hot-air oven at 40℃ for 10-20 min and 400 µl of methanol was added into the wells. The optical density was measured at 570 nm in ELISA plate reader and percentage inhibition was estimated by the formula:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of negative control - Absorbance of test})}{(\text{Absorbance of negative control})} \times 100$$

3. RESULTS AND DISCUSSION

3.1 FTIR Analysis

FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum [19]. For *Jasminum sambac* oil, the FTIR technique is utilised, which reveals a total of 21 peaks (Table 1 and Fig. 1). The band at 3436 cm^{-1} represents the asymmetric NH_2 group, while the band at 3063 cm^{-1} represents the triglyceride fraction. The peaks for the C-H, CH_3, and Azide groups are seen at 3030 cm^{-1}, 2929 cm^{-1}, and 2113 cm^{-1}, respectively. Aside from the peaks stated above, the FTIR spectra of *Jasminum sambac* essential

oil shows multiple low-intensity peaks at 697 cm^{-1}, indicating the C=O group. The C=C and hydroxyl stretches are displayed at 965 cm^{-1} and 916 cm^{-1} wavelengths, respectively. The C=C=C group is responsible for the bands formed at 1986 cm^{-1}. In addition, the CH_3 and carbonyl groups produce bands at 1494 cm^{-1} and 1677 cm^{-1}, respectively. Peaks for the CH_2 and CH3 bends are 1463 cm^{-1} and 1364 cm^{-1}. The CF_2 group propagates a strong peak at 1174 cm^{-1}. The second lowest band, seen at 834 cm^{-1}, forms the trans =C-H out of plane. Certain peaks, such as the C-O group with tertiary alcohol and polysaccharides at 1133 cm^{-1} and 1073 cm^{-1}, contain the same functional group. The CO-OH are visible at a wavelength of 1226 cm^{-1}. While the CO group recorded a peak at 1025 cm^{-1}, one of the lowest peaks for *Jasminum sambac* essential oil. The signal at 1722 cm^{-1} indicates the presence of the -CHO group. As a result, the presence of the group in the *Jasminum sambac* essential oil sample has been established. According to the FTIR data, this study is a helpful technique for identifying the components in the selected essential oil sample.

3.2 TGA Analysis

Thermogravimetry is a technique that analyses the change in weight of a sample over time or temperature in an inert or oxidative controlled environment [20]. Chemical changes in an oxidative environment provide useful information about the sample's properties. Fig. 2 depicts the thermogram produced from the thermogravimetric examination. The essential oil exhibited a single thermal deterioration event, with T_{onset} and T_{max} of 100°C and 250°C, respectively, and the curve then stabilized beyond these temperatures. The greatest apparent weight loss was recorded between 200 °C to 250 °C. This demonstrates that the chosen essential oil has a higher temperature for mass loss, indicating its stability.

3.3 DSC Analysis

Differential Scanning Calorimetry (DSC) is an analytical method in which the difference in temperature measurement is assessed as a utility of temperature by expanding the heat of a specimen and a reference [21]. A DSC experiment produces a heat flux vs. temperature or time curve. Exothermic reactions in the sample are represented by either a positive or negative peak, depending on the method used in the experiment. This curve can be used to estimate transition enthalpies [22]. The transition from amorphous to crystalline solid is an exothermic process that results in a peak in the DSC signal. As the temperature rises, the sample gradually approaches its melting point (Tm). As a result of the melting process, the DSC curve shows an endothermic peak. The DSC curve of *Jasminum sambac* essential oil exhibited an endothermic event until the temperature reached 160.12 °C, corresponding to its volatilization, immediately after the melting phase at 34.61 °C, with a -1.176W/g drop in heat flow (Fig. 3). The melting enthalpy of essential oil was 375.5 J/g at 102.30 °C. However, a smooth peak of 0.1575 W/g of heat flow was found beyond 250.93°C. This thermogram depicts the essential oil's heat profile when it is subjected to increasing temperatures, demonstrating the oil's stability.

3.4 High Performance thin Layer Liquid Chromatography Analysis

The current study aims to improve the concurrent HPTLC fingerprint characteristics of secondary metabolites present in the essential oil extract of *Jasminum sambac* [23]. High performance thin layer liquid chromatography is one of the comprehensive instrumental approaches that employs the full capabilities of thin layer chromatography to identify components, determine contaminants, and quantify active compounds [24]. HPTLC remains an efficient method for evaluating the quality and chromatographic fingerprint of medicinal plants, as well as distinguishing the chemotype variety of species [25]. Although HPTLC has been shown to be a linear, precise, and accurate method for detecting herbal components, the essential oil extracted from *Jasminum sambac* was exposed to the solvent system Toluene: Ethyl acetate (9.3:0.7 v/v). As indicated in the Fig. 4, distinct colours of bands were found after derivatizing with vanillin sulfuric acid under UV 366 nm, demonstrating the existence of diverse compounds of the essential oil. It represented seven bands of the *Jasminum sambac* essential oil, which has Rf value ranging from 0.1 to 0.9 as represented in Fig. 5. The phytochemical screening revealed the presence of significant secondary metabolites such as flavonoids, polyphenols, terpenes, quinones, steroids, and alkaloids, but tannins were not detected. The existence of these secondary metabolites indicate that the plant may have medicinal potential. According to the chromatogram, the highest essential oil peaks were 0.82 AU, 0.6

AU, and 0.79 AU. UV light at 245 nm and 366 nm was also used to scan the essential oil components. As a result, the HPLTC-based high-throughput analysis of *Jasminum sambac* essential oil is focused on the efficient detection of many components. It is notable that the increased use of analytical assistance for the evaluation of drug formulations and bulk medicines for various therapeutic targets generated from phytochemicals present in diverse plant essential oils has accelerated this research.

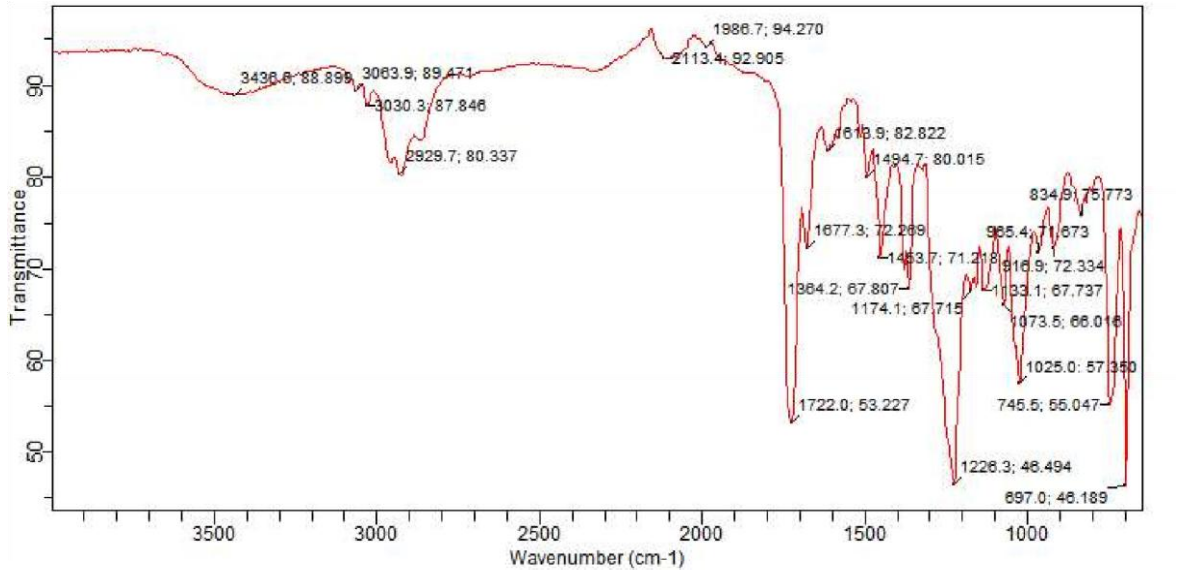

Fig. 1. Infrared spectrum representing the different functional components of *Jasminum sambac* essential oil

Table 1. Table representing different functional groups corresponding to the wave numbers obtained from different spectral peaks

Wave Number	Functional Group
3436	asymmetric NH_2 stretch
3063	originated from the vibrations of triglyceride fractions
3030	Aromatic C-H Stretch
2929	Symmetric CH_3 stretching and asymmetric CH_2 stretching
2113	azide stretching
1986	C=C=C stretching allene
1013	CCCR1, CCCR2, CCR1, CCR2, CCHR1, CCHR2
1494	vibrations of methyl and methylene groups
1677	carbonyl band
1463	CH2 bending vibration
1364	(CH_3, CH_2, bend)
1174	Ύas-CF_2
1722	carbonyl groups of aldehyde (-CHO) absorption
834	trans = C–H out-of-plane bending
965	C=C bending alkene
916	hydroxyl stretch
1133	C-O stretching tertiary alcohol
1073	stretching vibration of C–O, which showed the characteristic absorptions of polysaccharides and coumarin
1025	CO stretching
1226	CO-OH (org. acid), Amide III bands
697	C=O stretching, aromatic C-H

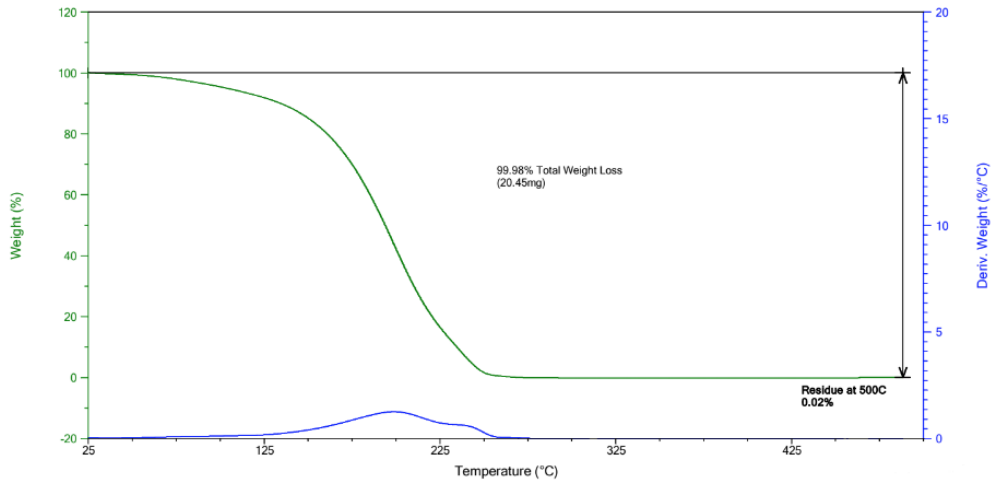

Fig. 2. Thermograph representing thermogravimetric analysis of *Jasminum sambac* essential oil

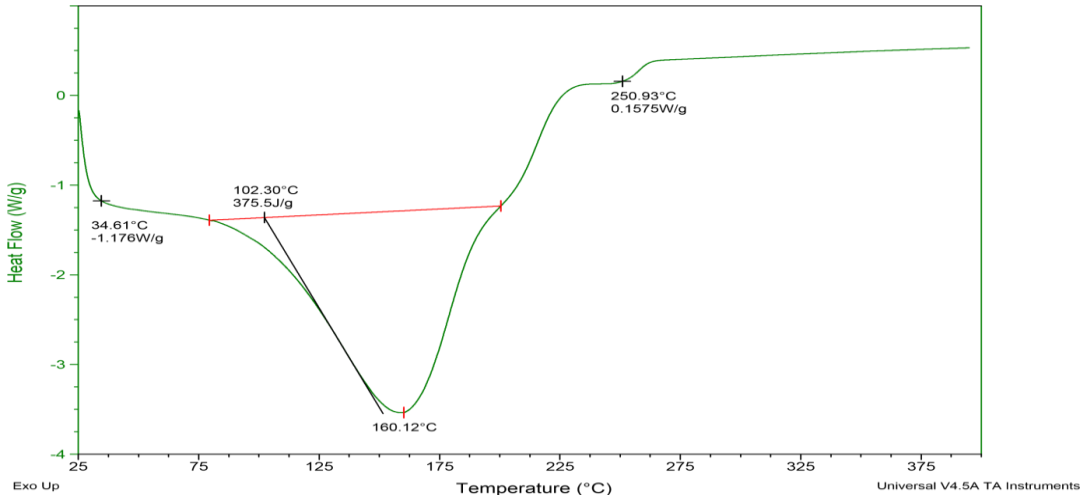

Fig. 3. Thermographic curve representing the differential calorimetric analysis of *Jasminum sambac* essential oil

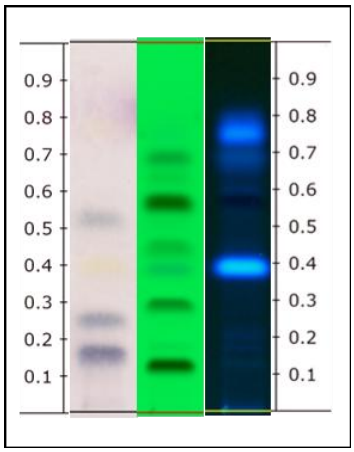

Fig. 4. HPTLC fingerprint of *Jasminum sambac* essential oil representing its chemical components

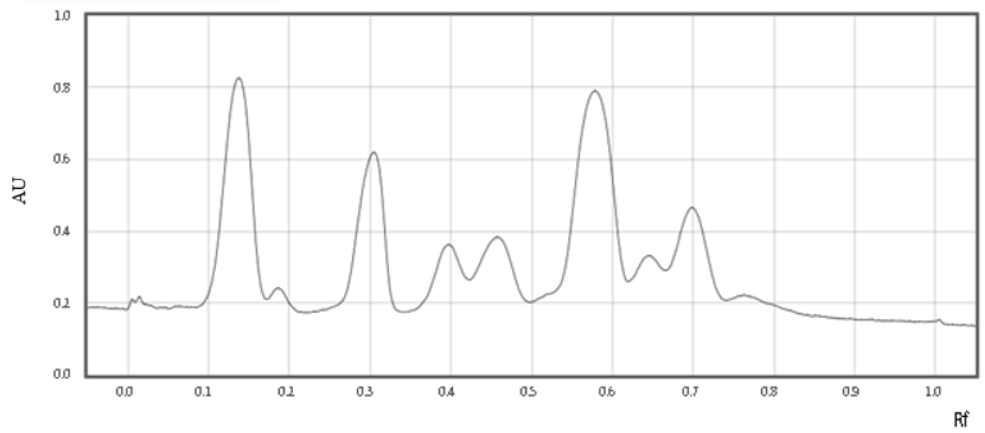

Fig. 5. Densitogram representing the peaks obtains from HPTLC analysis of *Jasminum sambac* essential oil

3.5 Antimicrobial Activity

Antimicrobial activity of *Jasminum sambac* essential oil was assessed by determining its Minimum inhibitory concentration (MIC) against antibiotic resistant organisms. The selected organisms were Carbapenem-Resistant Acinetobacter (CRA), Carbapenem-Resistant *Pseudomonas aeruginosa* (CRP), Carbapenem-Resistant *Escherichia coli* (CRE), Carbapenem-Resistant *Klebsiella pneumonie* (CRK), Extended Spectrum beta-lactamase *Escherichia coli* (ESBL), Quinolone resistant Salmonella (QRS), Vancomycin-resistant Enterococci (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA) and Erythromycin resistant Streptococci (ERS). According to the results it was observed that CRP showed the lowest MIC of 100 µg/ml suggesting that it was most susceptible to the essential oil. Whereas CRE, CRK, MRSA, ERS showed a MIC of 250 µg/ml which was the highest compared to the other organisms representing resistance. The remaining organisms which include CRA, ESBL and QRS showed moderate activity against the essential oil with an MIC of 125 µg/ml (Table 2). This mechanism of Jasminum sambac essential oil's antibacterial effect may include the disruption of cell membrane production, specifically membrane protein interference [26]. The oil's hydrophobic property allows it to separate from membranes, creating permeability that allows cell components to flow out and ultimately kills bacteria [27]. Previous studies have proven that there is presence of bioactive components such as linalool and benzyl acetate in the *Jasminum sambac* which have shown promising antimicrobial activity with an increased susceptibility exhibited by gram-negative bacteria [5]. This trend has also been fulfilled by our study (Fig. 6) as CRP a gram-negative organism was found to be most susceptible to the essential oil. Thus, it can be concluded that this antimicrobial activity of *Jasminum sambac* can be attributed to the presence of such bioactive compounds.

Table 2. Table representing the minimum inhibitory concentration of *Jasminum sambac* essential oil against antibiotic-resistant organisms

SR.NO	Organism	MIC (µg/mL)
1	CRA	125
2	CRP	100
3	CRE	250
4	CRK	250
5	ESBL	125
6	VRE	200
7	QRS	125
8	MRSA	250
9	ERS	250

3.6 Cell Viability Assay

The MTT assay is a standard method for assessing the cytotoxicity of chemical substances by measuring cell viability. The *Jasminum sambac* essential oil was diluted to obtain a concentration range of 5 to 100 µg/mL, which was used to detect the cytotoxic effect of the essential oil by assessing the percentage cell viability of CHO and HEK293 cell lines by employing the MTT tetrazolium dye assay. From the results it can be seen that for HEK293 cells, at 5 µg/mL a percentage viability of 98.14 % was observed whereas at the highest concentration of

the essential oil that is 100 µg/mL a percentage viability of 69.34 % was seen which represents approximately 30% reduction in viability. In case of CHO cells at 5 µg/mL a percentage viability of 97.57 % was observed whereas at 100 µg/mL a percentage viability of 68.32 % was seen which again depicts approximately 30% reduction in viability. However, the concentration of the essential oil required to inhibit 50% of the cellular population i.e the IC_{50} value was also calculated for both the cell lines which was found to be 71.47 and 107.73 µg/mL for HEK293 and CHO cell lines respectively. Based on the IC_{50} values it can be concluded that the essential oil was slightly more toxic to HEK293 cell when compared to CHO cells (Table 3 and Fig. 7). This cytotoxic activity of the essential oil can be correlated with the presence of compounds such as benzyl benzoate which has proven cytotoxic potential and is a major component of the *Jasminum sambac* essential oil [28].

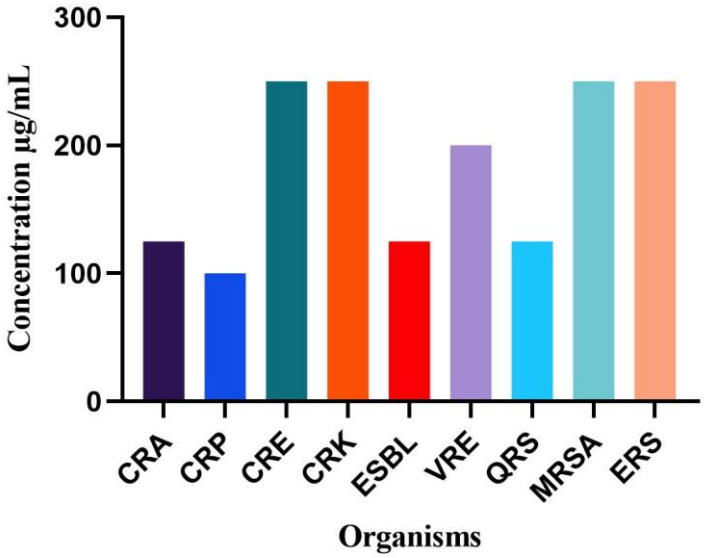

Fig. 6. Bar graph representing the minimum concentrations of *Jasminum sambac* essential oil required to inhibit antibiotic resistant organisms

Table 3. Table representing the percentage viability and IC_{50} value of *Jasminum sambac* essential oil on CHO and HEK293 cell lines

	Concentration (µg/mL)	% Viability	IC_{50} (µg/mL)
HEK293	5	98.14 ± 0.05	71.47
	10	97.60 ± 0.05	
	20	94.27 ± 0.07	
	40	89.16 ± 0.04	
	60	79.45 ± 0.05	
	80	74.45 ± 0.06	
	100	69.35 ± 0.05	
CHO	5	97.57 ± 0.07	107.73
	10	91.16 ± 0.05	
	20	85.63 ± 0.08	
	40	79.16 ± 0.04	
	60	73.33 ± 0.06	
	80	71.14 ± 0.05	
	100	68.32 ± 0.07	

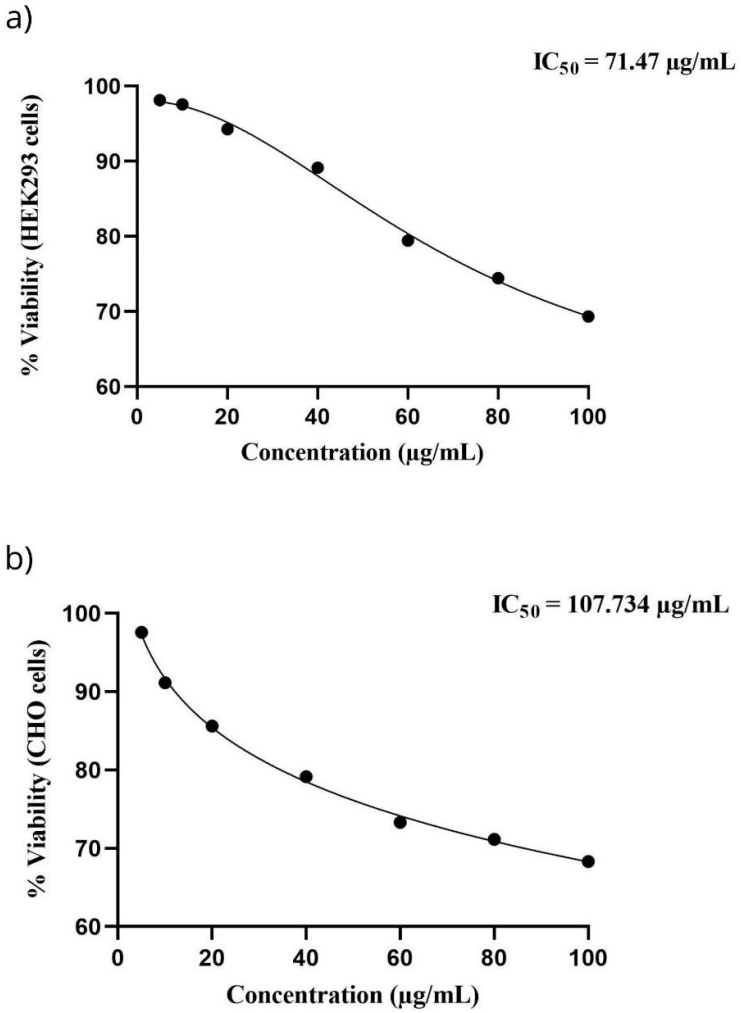

Fig. 7. Graphical representation of percentage viability of CHO and HEK293 cell lines at variable concentration of *Jasminum sambac* essential oil

3.7 Anti-inflammatory Activity

Inflammation is one of the body's defense mechanisms and is characterized by redness, swelling, pain, and a feeling of heat. Although the inflammatory response is crucial for the survival of the host, it can also result in chronic inflammatory disorders [29]. The Inhibition of Protein Denaturation Assay Using Egg Albumin Method was used in this study to assess the anti-inflammatory effect of *Jasminum sambac* essential oil. An increase in the concentration of essential oil is inversely proportion to the optical density recorded at 660nm which represents the trend of good anti-inflammatory activity. The highest percentage inhibition was observed at 100 µg/ml and was found to be 97.62 % whereas lowest percentage inhibition was seen at 10 µg/ml and was 92.93 % **(**Table 4). The initial percentage inhibition for standard was 89.81% and for highest concentration it was 97.68 % (Fig. 8). From this it can be concluded that *Jasminum sambac* essential oil exhibits better anti-inflammatory activity at lower concentration as compared to the standard Diclofenac. Linalool which is a major component of the *Jasminum sambac* essential oil possesses anti-inflammatory, antinociceptive, and other bioactive properties. Hence, the anti-inflammatory activity of the essential oil can be attributed to the presence of Linalool [29]. In a previous study conducted by Al-snafi *et al.,* a formulated topical gel which was comprised of *Jasminum sambac* extract was assessed against 1% diclofenac as positive control in rats for determining its anti-inflammatory activity. The results showed that the extract exhibited significant anti-inflammatory activity [30].

Table 4. Table showing the anti-inflammatory activity of *Jasminum sambac* essential oil and the standard diclofenac

Concentration (µg/mL)	Sample (*Jasminum sambac* essential oil)		Standard (Diclofenac)	
	OD (660 nm)	% Inhibition	OD (660 nm)	% Inhibition
10	0.125	92.93	0.18	89.81
20	0.114	93.55	0.164	90.72
30	0.105	94.06	0.142	91.96
40	0.093	94.74	0.135	92.36
50	0.09	94.91	0.124	92.98
60	0.087	95.08	0.119	93.27
70	0.065	96.32	0.113	93.60
80	0.051	97.11	0.079	95.53
90	0.048	97.28	0.058	96.72
100	0.042	97.62	0.041	97.68

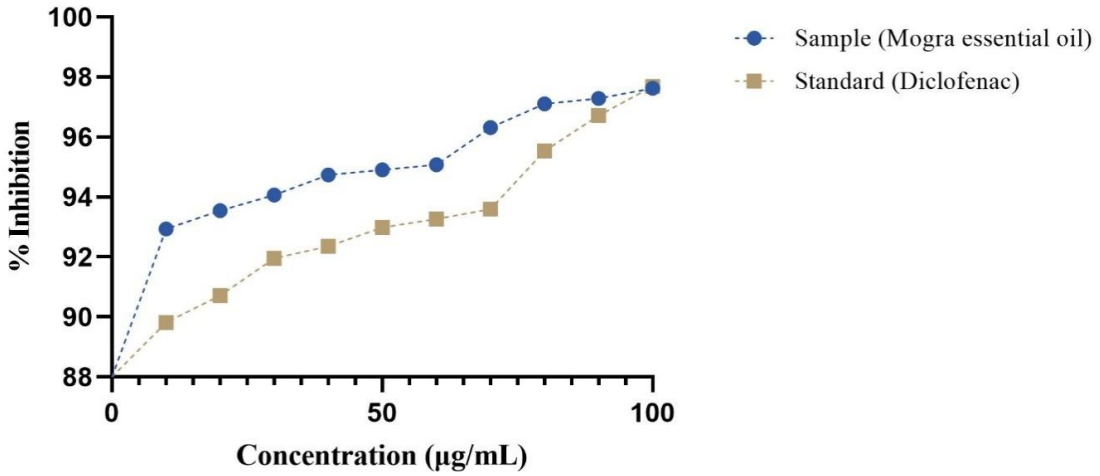

Fig. 8. Graph representing comparative curves of percentage inhibition of *Jasminum sambac* essential oil and standard diclofenac at variable concentrations

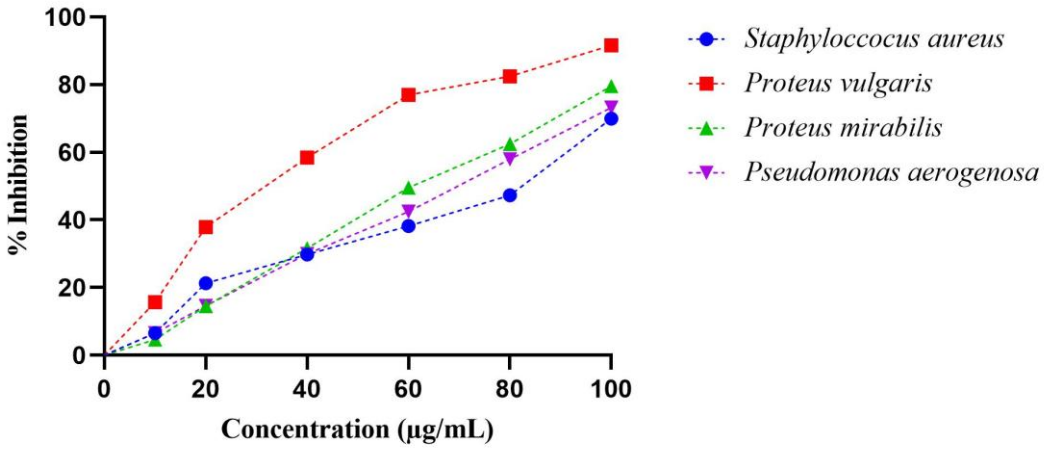

Fig. 9. Graphical representation of percentage biofilm inhibition by *Jasminum sambac* essential oil at various concentration against different biofilm forming organisms

Table 5. Table showing antibiofilm activity of *Jasminum sambac* essential oil against different biofilm forming organisms

organism	concentration (µg/mL)	% Inhibition
Staphylococcus aureus	10	6.52 ± 0.06
	20	21.31 ± 0.07
	40	29.81 ± 0.09
	60	38.21 ± 0.08
	80	47.29 ± 0.09
	100	69.97 ± 0.06
Proteus vulgaris	10	15.67 ± 0.03
	20	37.91 ± 0.07
	40	58.43 ± 0.08
	60	76.91 ± 0.08
	80	82.48 ± 0.08
	100	91.63 ± 0.06
Proteus mirabilis	10	4.6 ± 0.08
	20	14.57 ± 0.07
	40	31.63 ± 0.07
	60	49.57 ± 0.08
	80	62.49 ± 0.08
	100	79.55 ± 0.05
Pseudomonas aerogenosa	10	6.52 ± 0.07
	20	14.63 ± 0.06
	40	29.96 ± 0.06
	60	42.45 ± 0.07
	80	57.93 ± 0.09
	100	73.21 ± 0.08

3.8 Anti-biofilm Activity

The inhibitory activity of *Jasminum sambac* essential oil on biofilm formation against *Staphylococcus aureus*, *Proteus vulgaris, Proteus mirabilis* and *Pseudomonas aeruginosa* was evaluated using the Crystal Violet assay. *Proteus vulgaris* exhibited highest percentage inhibition of 15.67 % at lowest concentration of essential oil i.e. 10 µg/ml. It also shows highest percentage inhibition of 91.63 % at 100 µg/ml. Thus, results indicated that the essential oil shows promising anti-biofilm activity against *Proteus vulgaris* as compared to the other test organisms. The lowest percentage inhibition was exhibited by *Proteus mirabilis* which was 4.58 % at 10 µg/ml indicating its resistance against the essential oil as seen in (Table 5 and Fig. 9). The antibiofilm potential of the *Jasminum sambac* essential oil has not been explored much except for a study indicating its ability to inhibit *Streptococcus mutans* biofilm [31].

4. CONCLUSION

Although essential oils have been employed in traditional medicine since antiquity, research in this field is still in its early stages. A systematic and rigorous approach to the investigation of prospective biological activity and detections of vital components from phyto therapeutics is a development of recent decades. Therefore, in this research *Jasminum sambac* essential oil was thoroughly studied to understand its therapeutic potential with corelation to its functional components. Analytical methods such as HPTLC was employed to understand the chemical composition of the oil and understand its characteristics based on the component profile. TGA and DSC was carried out to unravel the thermal behaviour of the essential oil representing immense stability. Furthermore, the antimicrobial and antibiofilm activity was also assessed to determine the inhibitory potential of the therapeutic components present in the essential oil. Promising anti-inflammatory activity was also depicted by the essential oil by carrying out the egg albumin protein denaturation assay. In addition to this the CHO and HEK293 cell lines were treated with the essential oil which showed moderate cytotoxic activity. Thus, these results suggest that *Jasminum sambac* essential oil has great potential for uplifting the field of alternative therapeutics and thus more research on developing natural therapeutic formulations from *Jasminum sambac* should be adopted. However,

this study is also backed up with a limitation according to which the human toxicological profile of the oil should further be studied and based on which added invivo studies should be prioritised.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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