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RESEARCH ARTICLE

PHYTOCHEMICAL CHARACTERIZATION USING VARIOUS SOLVENT EXTRACT AND GC-MS, FT-IR ANALYSIS OF ETHANOL EXTRACT OF *JASMINUM SAMBAC* LINN.

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ABSTRACT

The aim of the present study was to investigate the Ethanol extract leaf from *Jasminum sambac* Linn. Qualitative analysis of showed the presence of alkaloids, carbohydrates, flavonoids, tannins, phenol, steroids, terpenoids and glycosides. Absence of leaves extracts protein, mucilage, saponins, fats and fixed oils. This work deals with the phytochemical screening and GC-MS, FT-IR studies of the Ethanol extract. The highest peak area of (40.87%) was obtained by 7-Tetradecenal, (Z) - ($C_{14}H_{26}O$) at retention time of (18.959) and the lowest peak area of (0.16%) was obtained by Phenol, 3, 5-bis (1,1-dimethylethyl)- ($C_{14}H_{22}O$) at retention time of (12.294). The FT-IR spectroscope studies shows different characteristic peak values of many functional groups in the extract. FT-IR analysis of leaf extract confirmed the presence of amide, alcohol, phenol, alkane, carboxylic acid, aldehyde, ketone, alkene, primary amine, aromatic esters, alkyl halide, and aliphatic amine compounds. This study summarizing the information about the phytochemical constituent's presence in ethanolic leaf extracts and these constituents may be responsible for pharmacological activites.

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INTRODUCTION

Herbal medicines are promising choices of modern synthetic herbal drugs. Herbal drugs are no side effects are considered to be safe. Generally herbal formulations involve use fresh and dried plant materials. The use of plants for prevention and treatment of various health ailments has been in practice from time immemorial and it is estimated that about 25% of drugs prescribed are derived from plants, moreover, WHO essential medicine list contains 252 % drugs out of which 11% is exclusively of plant origin (Rates, 2001). *Jasminum sambac*. Linn (Oleaceae) is commonly known as motia or lily Jasmine is a scandent or sub-erect shrub with young pubescent branches, broadly ovate or elliptic, opposite leaves, white, very fragrant flowers cultivated nearly throughout the tropical and sub-tropical parts of the world. It is a well known glabrous twinning shrub widely grown in gardens throughout India. It is useful in treating diseases of the mouth and teeth, especially for toothache (Kirtikar *et al*., 1993). The genus *Jasminum* is reported to include about 64 species (Anonymous, 2011) out of which 40 are indigenous to the Indian subcontinent (Irulappan, 1994). Jasmine plants are of great economic value of a field crop for the florist, landscape, medicinal and pharmaceutical industries (Green and Miller, 2009).

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The *Jasminum sambac* flowers and leaf is largely used in folk medicine to prevent and treat breast cancer. The flowers are used by the women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding (Joshi, 2000). *Jasminum sambac* are widely used in Ayurveda for anti-ulcerative, anti-cancer and antileprotic, skin diseases and wound healing activity. *Jasminum sambac* leaf ethyl acetate extract analysed anti-diabetic activity (Upaganlawar *et al*., 2009) and anti tumour –activity (Radu *et al*., 2002). Ethanol extract of *Jasminum sambac* leaf are used as anti-cancer agent (Talib *et al*., 2010) Cytotoxic activity (Rahman *et al*., 2011) and antimicrobial activity (Hussaini *et al*., 2009). Methanol extract of leaf *Jasminm sambac* is used as anti oxidant activity (Abdoul Latif *et al*., 2010). Analgesic activity and anti-stress activity (Baby and Aimy, 2010).

GC-MS is a powerful technique used for many applications which are highly sensitive and specific in nature. Generally its application is oriented towards the specific detection and potential identification of compounds based on the molecular mass in a complex mixture. The combination of a principle separation technique Gas- Chromatography (GC) with the best identification technique Mass- Spectrometry (MS) made GC–MS is an ideal of qualitative and quantitative analysis for volatile and semi-volatile compounds (Karthishwaran *et al*., 2012). Therefore an attempt was made to screen the bioactive

compounds, to evaluate the bioactive potential and characterize them by GC-MS analysis.

The objective of the study is to identify the possible functional groups and organic compounds present in the active fraction of ethanol extract of *Jasminum sambac* leaves using spectroscopic (FT-IR and GC-MS) studies. This may provide an insight in its use for traditional medicine. The Fourier Transform Infrared (FTIR) spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants. Moreover, FTIR spectroscopy is an established time-saving method to characterize and identify the functional groups (Grube *et al*., 2008).

MATERIALS AND METHODS

Collection and preparation of plant materials

The fresh leaf of *Jasminum sambac* was collected from the natural habitats of Villupuram District, Tamilnadu, India. The leaf was washed thoroughly for 3 times with running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The leaf was cut and dried in the shadow ground into fine powder and stored in air tight polythene bags for further study.

Chemical and reagents

The chemicals like Petroleum ether, Chloroform, Ethyl acetate, Ethanol and reagents used and analysed for phytochemical screening to test the aerial parts and leaf part of *Jasminum sambac* Linn. For Mayer's reagent, Ninhydrin's reagent Felhing solutions A and B and Borntrager's reagent, Ferric chloride solution, Gelatin, lead acetate, Sodium hydroxide, Hydrochloric acid, Sulphuric acid which were purchased from SD Fine chemical, Mumbai.

Phytochemical Screening of the *Jasminum sambac* leaf Extract

Phytochemical test was conducted to identify the presence or absence of secondary metabolites namely alkaloids, flavanoids, glycosides, saponins, tannins, terpenoids, carbohydrates, resins, sterols and lipid/fat according to the method outlined by (Evans *et al*., 1999).

GC-MS Analysis

Preparation of extracts

About 25 g of the powdered leaf was soaked in 95% methanol for 12hrs. The extracts were filtered through Whatman 41 filter paper along with 2g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was made wet with 95% ethanol along with sodium sulphate. The filtrate was concentrated on bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phyto-components in the plant material, 2µl of this solution was employed for GC-MS analysis (Merlin *et al.,* 2009). GC-MS analysis of these extracts were performed used a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary columns (30mmX0.25mm 1D X 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1) Injector temperature 250°C Ionsource temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectrum was taken at 70 eV a scan interval of 0.5sec and fragment from 45 to 450 Da. Total GC running time was 36 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass.

Identification of components

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation of mass spectrum GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage, amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2. This was done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further, it helps to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their putative biological or therapeutic relevance.

Fourier Transform Infrared Spectroscopic Analysis (FT-IR)

Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond between spectrum. By interpreting the infrared absorption spectrum, the chemical bonds between molecules can be determined. The leaf of *Jasminum sambac* was oven dried at 60°C and ground into fine powder using a mortar and pestle. Two milligrams of the sample was mixed with 100mg K Br (FT-IR grade) and then compressed to prepare a salt disc (3mm diameter). The disc was immediately kept in the sample holder and FT-IR spectrum was recorded in the absorption range of 4000 and 400cm^{-1}. All investigations were carried out with a Shimadzu FT-IR spectrometer.

RESULTS AND DISCUSSION

Preliminary phytochemical screening was done in petroleum ether, chloroform, ethyl acetate and ethanol extracts of *Jaminum sambac* of the four solvent extracts tested, steroids

showed their presence in all the four extracts, terpenoids and proteins in three extracts, carbohydrates, phenolic compounds, saponins and phytosterols in two extracts, flavonoids and quinones were noticed in only one extract. Glycosides and alkaloids were completely absent in all the extracts (Table 1).

The FT-IR spectrum was used to identify the characteristic peaks and functional groups of the active components based on the peak value in the region of infrared radiation (Table 4; Figure 2). The results revealed the presence of different phytochemicals which are formed the plants normal metabolic processes. The leaf extract from *Jasminum sambac* was subjected to FT-IR analysis and the functional groups of the components were separated based on their peak ratios. The result confirmed the presence of normal polymeric (OH stretch) alcohol, phenol aromatic rings(C-H stretch), alkenes, (C-O stretch) alcohol, ether, (C=S stretch) group thio, (C-N stretch) amines, NO_2 nitro compound, (C-H stretch) alkanes, (C=C stretch) aromatic rings, (C=C stretch) alkenes, (C=O stretch) ketones, (C=N stretch) nitriles, alkynes, (O-H stretch) monomeric alcohol, phenol, which showed major peaks at 667.37, 1039.63, 1321.24, 1456.26, 1539.20, 1645.28, 1732.08, 2355.08, 2924.04, 3354.21, 3585.37, cm^{-1} respectively (Figure 2; Table 4). Hence, the leaf extracts subjected to FT-IR analysis are used for the identification of chemical constituents present in *Jasminum sambac.* In addition FT-IR spectroscopy is proved to be a reliable and sensitive method for detection of bio-molecular composition by (Komal Kumar *et al*., 2011).

GC-MS analysis of the extracts

The Gas- Chromatography and Mass- Spectroscopy analysis of *Jasminum sambac* 20 compounds was identified with the ethanol extract. The GC-MS identification of phytochemical compounds is based on the peak area molecular weight and molecular formula. The chromatogram (Figure 1) of ethanol leaves extracts shows 6 prominent peaks of 7-Tetradecenal, (Z)- ($C_{14}H_{26}O$) with retention time of 18.959 and peak area percentage of 40.87. n-Hexadecenoic acid ($C_{16}H_{32}O_2$) with retention time of 17.274 has the peak area percentage of 35.93, Di-n-octyl phthalate ($C_{24}H_{38}O_4$)with retention time of 23.076 has the peak area percentage of 6.04, Phytol ($C_{20}H_{40}O$) with retention time of 18.596 has the peak area percentage of 3.45 and 9- Octadecenoic acid (Z)-($C_{18}H_{34}O_2$) with retention time of 19.099 has the peak area percentage of 2.86 and 1-Octadecyne ($C_{18}H_{34}$) with retention time of 15.865 has the peak area percentage of 1.87. The other less prominent peaks at other retention times molecular formula is shown in (Table 2). The total ion chromatograph (TIC) showing the peak identities of the various compounds are shown in (Figure 1). The structure of compounds and molecular structure and biological activity of leaf extract are presented in (Table 3).

It also proved the presence of functional groups and secondary metabolites present in the leaf of *Jasminum sambac* leaf extract provide different compounds presents in the many biological activities.

Table 1. Preliminary phytochemical screening of different extracts of *Jasminum sambac* leaves

S.No	Chemical constituents	Test	Leaf extract			
			Petroleum ether	chloroform	Ethyl acetate	Ethanol
1	Alkaloids	Mayer's Test	+	-	+	+
2	Carbohydrates	Fehling's Test	-	-	-	+
3	Flavanoids	Chinasoda Test	-	-	+	+
4	Tannins	$Fecl_3$ test	-	-	+	+
5	Phenol	Gelatin test	-	-	-	+
6	Protein	Ninhydrin test	-	-	-	-
7	Gum/Mucilage	Molish Test	-	-	-	-
8	Steroids	Sulphuric acid Test	-	+	+	+
9	Terpenoids	Salkowaski test	-	-	+	+
10	Glycoside	Borntrager Test	-	+	-	+
11	Saponins	Foam Test	-	-	+	-
12	Fats and fixed oils	Spot Test	+	-	-	-

Table 2. Phytocompounds identified in the Ethanolic extract of the *Jasminum sambac* Linn by GC-MS analysis

Peak number	R.Time	Area %	Name of the compounds	Molecular weight	Molecular formula
1	11.377	0.25	Bicyclo(2.2.1)heptane-2,5-diol,1,7,7-trimethyl-,(2-endo,5-ex	170	$C_{10}H_{18}O_2$
2	12.294	0.16	Phenol,3,5-bis(1,1-dimethylethyl)-	206	$C_{14}H_{22}O$
3	15.170	0.45	Hexadecanoic acid	256	$C_{16}H_{32}O_2$
4	15.553	0.58	2(4H)-Benzofuranone,5,6,7,7A-Tetrahydro-6-Hy	196	$C_{11}H_{16}O_3$
5	15.701	0.79	R-Limonene	184	$C_{10}H_{16}O_3$
6	15.865	1.87	1-Octadecyne	250	$C_{18}H_{34}$
7	16.122	0.27	1-Octadecyne	250	$C_{18}H_{34}$
8	16.313	0.56	1-Octadecyne	250	$C_{18}H_{34}$
9	16.769	0.51	Eicosanoic acid,methyl ester	326	$C_{21}H_{42}O_2$
10	17.051	0.42	9- Octadecenoic acid (Z)-	282	$C_{18}H_{34}O_2$
11	17.274	35.93	n-Hexadecenoic acid	256	$C_{16}H_{32}O_2$
12	17.428	0.96	Hexadecenoic acid,Ethyl ester	284	$C_{18}H_{36}O_2$
13	18.167	0.16	Octadecenoic acid	284	$C_{18}H_{36}O_2$
14	18.467	0.96	9- Octadecenoic acid, Methyl ester,(E)-	296	$C_{19}H_{36}O_2$
15	18.596	3.45	Phytol	296	$C_{20}H_{40}$
16	18.959	40.87	7-Tetradecenal,(Z)-	210	$C_{14}H_{26}O$
17	19.099	2.86	9- Octadecenoic acid (Z)-	282	$C_{18}H_{34}O_2$
18	23.076	6.04	Di-n-octyl phthalate	390	$C_{24}H_{38}O_4$
19	27.052	1.04	Squalene	410	$C_{30}H_{50}$
20	31.368	1.87	2,5,7,8-Tetramethyl-2-(4,8,12=Trimethylridecy	430	$C_{19}H_{50}O_2$

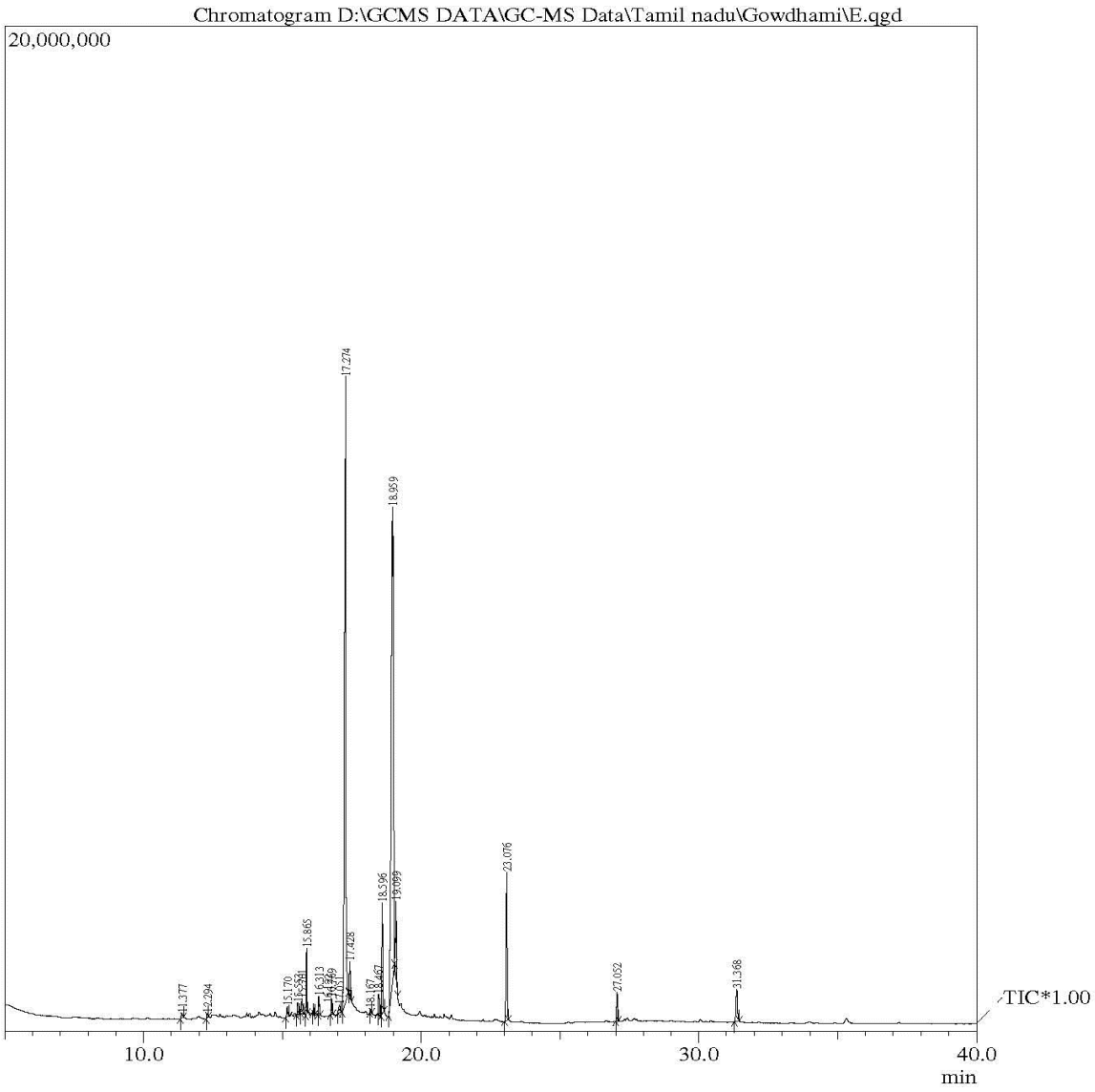


Figure 1. GC-MS Chromatogram of Ethanolic extract of *Jasminum sambac* Linn.

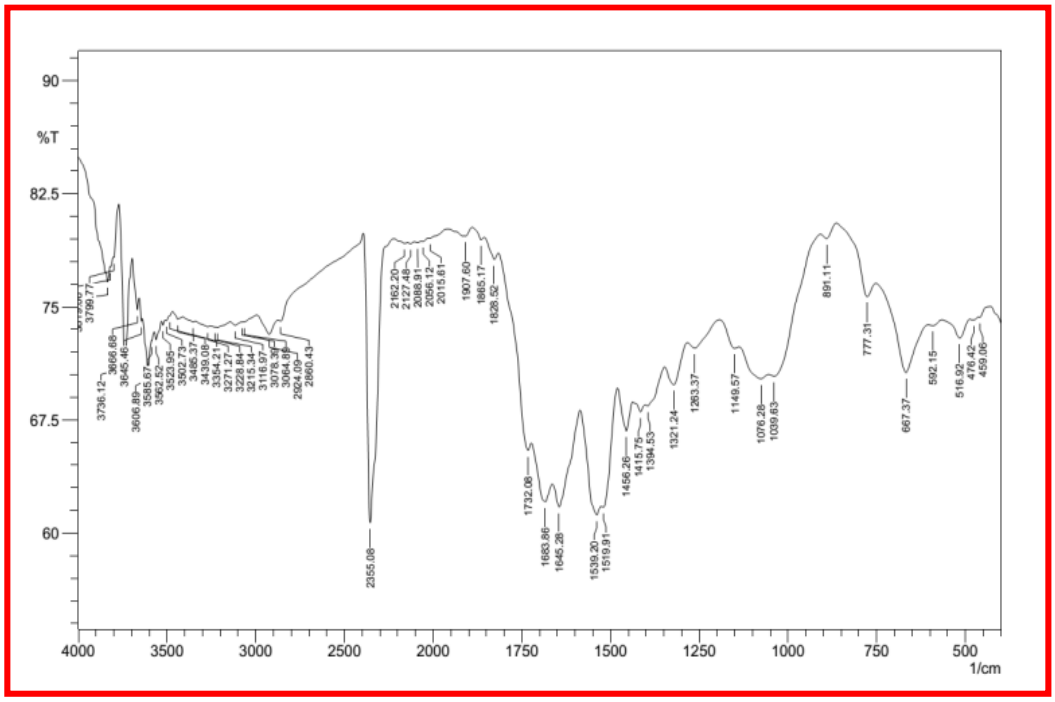


Figure 2. FT-IR Spectrum of Ethanol extract of *Jasminum sambac* Linn.

Table 3. Chemical structures of the most prevailing compounds of the Ethanol extract of *Jasminum sambac* Linn

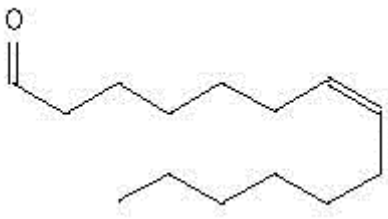
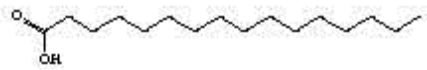
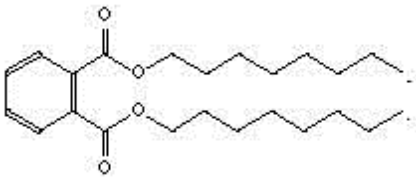
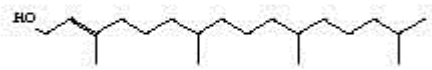
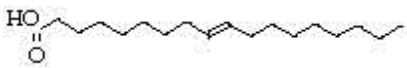
S.No	Name of the compounds	Chemical structure of the compounds	Nature compounds	Biological Activity
1	7-Tetradecenal,(Z)-		Flavors fragrances, Sesquiterpenoids	Cytotoxity, immuno medulatory activity, anti-malarial activity, anti-cancer activity, anti-oxidant, anti-prurity, antinociceptic
2	n-Hexadecenoic acid		Palmitic acid	Anti-inflammatory, antioxidant, Hypochloesterolemic, nematicide, pesticide, anti-androgenic
3	Di-n-octyl phthalate		Plasticizer compounds	Liver hitopathology enzyme activity β-glucuronidase activity
4	Phytol		Diterpene	Antimicrobial,anticancer,cancer preventive,diuretic anti-inflammatory
5	9- Octadecenoic acid (Z)-		Fatty acid	Antibacterial, antioxidant
6	1-Octadecyne		Ester compound	Anti-inflammatory agent, Antibacterial agent, Fragrance

Table 4. FTIR spectral peak and functional groups obtained for the leaf extract (Ethanol) of *Jasminum sambac* Linn.

Extracts	Peak value	Types of stretching	Functional group	Corr. Area
Ethanol	667	C-H bending	Aromatic rings (Alkenes)	1.48
	1039.63	C-O bending	Alcohol, Ether, Carboxylic acid, Ester	0.454
	1321.24	NO2 bending	Nitro compound	0.349
	1456.26	C-H bending	Alkanes	0.321
	1539.2	C=C bending	Aromatic rings	0.5
	1645.28	C=C	Alkenes	0.791
	1732.08	C=O bending	Aldehydes, Ketones, Carboxylic acid	0.288
	2355.08	C=N bending	Nit riles, Alkynes	6.95
	3585.67	O-H bending	Monomeric alcohols, phenol	0.002
	3645.46	O-H bending	Monomeric alcohols, phenol	0.023

The FTIR spectrum confirmed the presence of alcohol, phenol, alkane, alkyne, alkyl halides, aldehyde, carboxylic acids, aromatics, nitro compounds and amines in leaf extracts. The GC-MS analysis reveals the presence of various types of compounds of *Jasminum sambac* leaves are given below. There are 7-Tetradecenal,(Z)-($C_{14}H_{26}O$) having Cytotoxity, anticancer activity, antioxidant, antiprurity, antinociceptic, 9-Octadecenoic acid (Z)-methyl ester ($C_{19}H_{36}O_2$) having Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic (Dr. Duke's and Yeong *et al*., 1989). Phytol ($C_{20}H_{40}O$) is biological activity of cancer prevention autoimmune response, n-hexadecanoic acid ($C_{16}H_{32}O_2$) biological activity of antioxidant, Hypochloesterolemic and nematicide activities.

Conclusion

The ethanolic leaf extracts of *Jasminum sambac* contain many important phytochemical components such as alkaloids,

carbohydrates, Steroids, terpenoids. The GC-MS analysis showed a number of medicinal active components, so the present study of GC-MS work establishes the presence of many constituents in these species. Further studies were needed to isolate the bioactive compounds that could be used to formulate new and more potent drugs of natural origin. Hence, this study may be useful to explore the pharmacological and biosynthetic activity of *Jasminum sambac*.

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