International Journal of Multidisciplinary Research and Development

Volume: 2, Issue: 8, 268-273
Aug 2015
www.allsubjectjournal.com
e-ISSN: 2349-4182
p-ISSN: 2349-5979
Impact Factor: 3.762

T Gowdhami
Department of Botany,
Annamalai University,
Annamalai Nagar- 608 002,
Chidambaram, India.

AK Rajalakshmi
Assistant professor,
Botany wing DDE,
Annamalai University,
Annamalai Nagar- 608 002,
Chidambaram, India.

Correspondence
T Gowdhami
Department of Botany,
Annamalai University,
Annamalai Nagar- 608 002,
Chidambaram, India.

Phytochemical Analysis of *Jasminum auriculatum* Vahl. Using FT-IR and GC-MS

T Gowdhami, AK Rajalakshmi

Abstract
The aim at the present study was to investigate the ethanol extract from *Jasminum auriculatum* Vahl. Qualitative analysis showed the presence of alkaloids, carbohydrates, flavonoids, tannins, phenol, steroids, terpenoids and glycosides in the different leaf extracts. The leaf extract did not contain proteins, mucilage, saponins, fats and fixed oil. This work deals with the phytochemical screening and GC-MS studies of the ethanol extract. The highest peak area (35.16%) was obtained by n-Hexadecanoic acid ($C_{16}H_{32}O_2$) at retention time of 17.275 and the lowest peak area (0.23%) was obtained by Octacosane ($C_{28}H_{58}$) at retention time of 30.441. The FT-IR spectroscope studies shows different characteristic peak values with many functional groups present like alkyl halides, aromatics, aliphatic amines, alkanes, amines, unsaturated esters, anhydride, alkene, alkynes, nitriles, carboxylic acids alcohol and phenol.The results important bioactive compounds leaf extracts and these constituents may be responsible for pharmacological activities.

Keywords: *Jasminum auriculatum,* Preliminary Phytochemical, GC-MS and FT-IR analysis.

Introduction
Medicinal plants have been used by human beings since ages in traditional medicine due to their therapeutic potential. The research on medicinal plants has led to the discovery of novel drug used against diverse diseases. According to the World Health Organization's report that nearly 65-80% of world's population in developing countries depends on the traditional medicine for their primary health care and treatment of ailments. The reasons for wide acceptance of herbal medicines are of their being comparatively less expensive, lesser side effects and being natural in origin and hence socially and culturally acceptable (Muniappan Ayyanar *et al*., 2011) [21]. Higher plants are sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on the green plants to represent a reservoir of effective chemotherapeutics, which are non-phytotoxic, more systemic and easily biodegradable (Kaushik *et al*., 2002; Chaman Lal *et al*., 2006) [16, 7]. Plants are rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are important source with a variety of structural arrangements and properties (De-Fatima *et al*., 2006) [9].

Jasminum auriculatum is a shrub used in traditional medicines, Ayurveda, Siddha and Unani (Vaidyaratanam, 2003; Bedi, 2008) [26, 6]. Studies conducted on this species showed that it possessed beneficial effects as suppurative, skin diseases, thermogenic, urolithiasis, ulcers and wounds. The present review highlights the various folk, ayurvedic uses, pharmacognostical, phytochemical and pharmacological studies conducted on *Jasminum auriculatum* and also the unexplored potential of the plant (Rajinder Raina *et al*., 2008) [23].

The aqueous and alcoholic extracts from flowers of *Jasminum auriculatum* were found to be effective against ethylene glycol induced lithiasis by reducing and preventing the growth of urinary stone (Bahuguna *et al*., 2009 a) [5]. It also exhibits diuretic effects at dose of 250 mg body weight by increasing the total volume of urine and concentrations of potassium and sodium salts in urine as compared to Frusemide. The alcoholic extract showed more effect (94.81%) (Bahuguna *et al*., 2009 b) [4] Then the aqueous extract (91.81%). More precise information about qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS) (Cong *et al*., 2007) [8]. For quantitative determination, gas chromatography with flame ionization detectors (GC-FID) and GC-MS are preferred (Lee *et al*., 2005; Lampronti *et al*., 2006; Haznagy Radnal *et al*., 2007) [19, 18, 15].

Traditional medicine is an important source of potentially useful compounds for the development of chemotherapeutic agents (Palombo *et al*., 2001) [22]. A wide range of medicinal

plant parts is used for extracting from raw drugs and they possess various medicinal properties (Uniyal *et al*., 2006) [25]. In developing countries the practice of medicine still relies heavily on plant and herbal extract for the treatment of human ailments. Dietary agents consists of a wide variety of biologically active compounds that are ubiquitous in plants and many of them have been used as traditional medicines (Aggarwal *et al*.,2003; Aggarwal *et al*., 2004; Aggarwal *et al*., 2006) [1, 2, 3]. The root is useful in skin diseases especially for ring-worm. The flowers are useful in burning sensation, hyperdesia, ulcers, odontalgia, stomatopathy, ophthalmopathy, cardiopathy, urolithiasis, nephrolithiasis, strangury and dermatopathy (Ghosh, 1984) [12]. *Jasminum auriculatum* leaves have been reported to contain lupeol and jasminol (Deshpande *et al*., 1967) [10]. The present work to investigate the phytochemicals present in the different leaf extract qualitatively by applying phytochemical test and quantitatively by Gas Chromatography- Mass Spectroscopy (GC-MS) and Fourier Tranform Infrared Spectroscopic Analysis (FT-IR) analysis.

2. Materials and Methods

2.1 Collection and preparation of plant materials

The fresh leafe of *Jasminum auriculatum* Vahl was collected from the natural habitats of Villupuram District, Tamilnadu, India. The specimen *Jasminum auriculatum* Vahl, was identified authentification using the specimen- BWH 78213 housed in the Botany wing, Annamalai University, Tamilnadu. The leaf was washed thoroughly 3 times with running tapwater 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 the air tight polythene bags for use.

2.2Extraction and processing

The shade dried plant material (200g) was crushed in Soxhlet extractor using petroleum ether, chloroform, ethanol, methanol successively (Green, 2004) [13]. The extracts were filtered using Whatman 41 filter paper and were concentrated. The concentrated methanol and ethanol extracts were subjected to qualitative phytochemical analysis and GC-MS analysis.

2.3 Chemical reagents

Chemicals and reagents like petroleum ether, chloroform, ethyy acetate, ethanol, sulphuric acid, hydrochloric acid, Mayer's reagents, Ninhydrin reagents, Felhing solutions A and B, Ferric chloride solution, gelatin, lead acetate, sodium hydroxide, were used to analyze phytochemicals present in the aerial parts of *Jasminum auriculatum* Vahl. Chemical purchased from Sd FiNe-Chem Limited, Mumbai.

3. Screening of phytochemical constituents

Phytochemical screening for the extracts was carried out as per standard methods prescribed by Harbone (1973) [14], Treas and Evans (1989) [24] to find out the presence of various constituents in different leaf extracts.

4. GC-MS Analysis

4.1 Preparation of extract:

About 25 g of the powdered leaf was soaked in 95% methanol for 12h. The extracts were then 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 then 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) [20]. GC-MS analysis of these extracts was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an 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.

4.2 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 database 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 is 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 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.

5. Fourier Transform Infrared Spectroscopic Analysis (FT-IR)

The fresh leaf of *Jasminum auriculatum* was oven dried at 60º C and ground into fine powder using a mortar and pestle. Two milligrams of the sample was mixed with 100mg KBr (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 spectra were recorded in the absorption range between 4000 and 400cm^{-1}. All investigations were carried out with a Shimadzu FT-IR spectrometer.

6. Results and Discussion

The phytochemical screening for crude extracts of from leaf *Jasminum auriculatum.* Table.1 represents the phytochemical *Jasminum auriculatum* Vahl and showed that the presence or absent of alkaloids, saponins, tannins, terpenoids, glycosides, carbohydrates, flavonoids, phenol and steroids.

The FT-IR spectrum was used to identify and detect the characteristic peaks and functional groups of the active components based on the peak value in the region of infrared radiation (Table 2; Figure 1). The results revealed the presence of different phytochemicals which are formed during the plants normal metabolic processes. The extract of

Jasminum auriculatum 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(C–Br stretch) normal alkyl halides, (C–H) aromatics, (C-N stretch) aliphatic amines, (C–N stretch) aromatic amines, (C–H rock) alkanes, (C–C stretch) aromatics, (N–H bend) 1° amines, (C=O stretch) $\alpha_{,\beta}$-unsaturated esters, (C=C stretch) anhydride (C=C stretch) alkene, (C≡C stretch) alkynes, (C≡C stretch) nitriles, (O–H stretch) carboxylic acids, (O–H stretch, H–bonded) alcohol and phenols. Which showed major peaks at 524.64, 775.38, 893.04, 1037.7, 1153.43, 1253.73, 1321.24, 1386.82, 1409.96, 1627.92, 1730.15, 1878.67, 1915.31, 2166.06, 2200.78, 2364.73, 2856.58, 2924.09, 3363.86, 3657.04, 3809.41, 3824.84, 3846.06, 3880.78, 3919.35 and 3959.86, $cm^{-}1$ respectively (Figure 2; Table 4).

Table 1: Preliminary Phytochemical screening of *Jasminum auriculatum* Vahl

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	-	-	-	-

GC-MS analysis of the extracts

During the GC-MS analysis of *Jasminum auriculatum* revealed the presence of 14 compounds identified in the ethanolic 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 leaf extract shows 6 prominent peaks as ethanol extract shows 6 prominent peaks as n-Hexadecanoic acid ($C_{16}H_{32}O_2$) with retention times of 17.275 and peak area of 35.16 and Cis cis,cis-7,10,13- Hexadecatrienal ($C_{16}H_{26}O$)with retention time of 18.985 and peak area 33.50 and Phytol ($C_{20}H_{40}O$)with retention time of 18.608 and peak area of 12.85 and 2,6,10,14,18,22-Tetracosahexaene,2,6,10,19,23, hexamethyl ($C_{30}H_{50}$)with retention time 27.060 and peak area of 5.28 and Di-n-octylphthalate ($C_{24}H_{38}O_4$) with retention time of 23.076 and peak area 4.75and 8, 11,14,Eicosatrienoic acid (Z,Z,Z) ($C_{20}H_{34}O_2$)with retention time of 19.099 and peak area 2.10. The other less prominent peaks at other retention times are shown in Table 2. The total ions chromatograph (TIC) showing the peak identities of the various compounds are identified as shown in Figure 1. The structure of compounds of ethanol leaf extract are presented in Table 3.

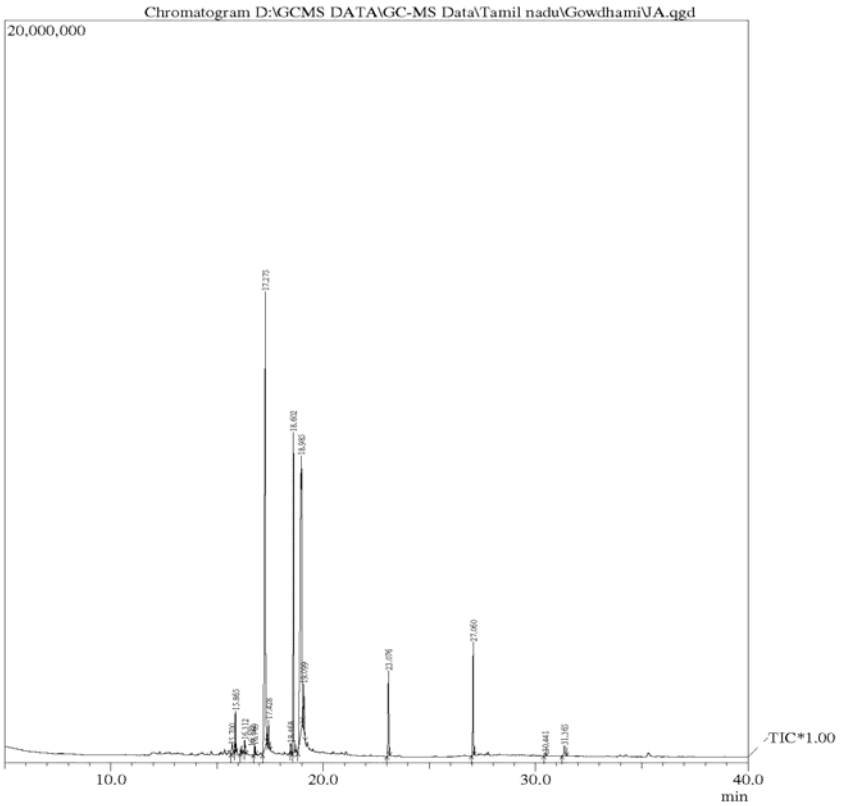


Fig 1: GC-MS analysis of *Jasminum auriculatum* leaves of ethanolic extract

Table 2: Chemical constituents present in the ethanolic extracts of *Jasminum auriculatum* Vahl.

Peak number	Retention Time	Name of the compound	Molecular formula	Molecular weight	Peak area %
		Ethanol leaf extract			
1	15.700	R-Limonene	$C_{10}H_{16}O_3$	184	0.78
2	15.865	1-Octadecyne	$C_{18}H_{34}$	250	1.53
3	16.312	1-Octadecyne	$C_{18}H_{34}$	250	0.44
4	16.769	Eicosanoic acid, Methyl ester	$C_{21}H_{42}O_2$	326	0.39
5	17.275	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	35.16
6	17.428	Ethyl nonadecanoate	$C_{21}H_{42}O_2$	326	1.18
7	18.468	Methyl 9-Octadecenoate	$C_{19}H_{36}O_2$	296	0.64
8	18.602	Phytol	$C_{20}H_{40}$	296	12.85
9	18.985	Cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234	33.50
10	19.099	8,11,14-Eicosatrienoic acid,(Z,Z,Z)-	$C_{20}H_{34}O_2$	306	2.10
11	23.076	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	4.75
12	27.060	2,6,10,14,18,22,-Tetracosahexaene,2,6,10,15,19,23-hexamethyl	$C_{30}H_{50}$	410	5.28
13	30.441	Octacosane	$C_{28}H_{58}$	394	0.23
14	31.365	2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecy	$C_{29}H_{50}O_2$	430	0.89

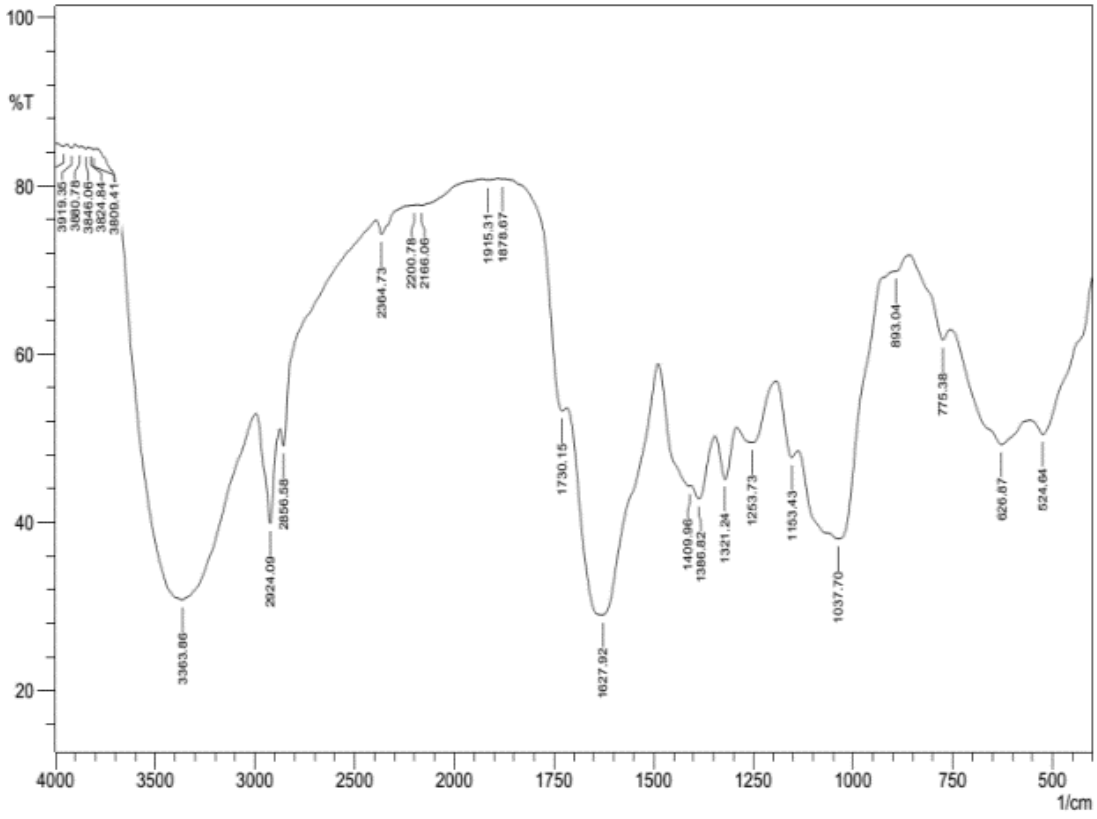


Fig 2: FT-IR Spectrum analysis of *Jasminum auriculatum* leaves of ethanolic extract

This study highlights the presence of many secondary metabolites in the leaves of *Jasminum auriculatum* Vahl, provide an overview of the different classes of molecules present that have led to their pharmacological activities. This study confirmed that the plant extract could be used for the treatment of various diseases. The GC-MS analysis of leaves extract showed the presence of various types of compounds in *Jasminum auriculatum.* Compounds like Phytol ($C_{20}H_{40}O$) having anti-cancerous activity (Yuenyongsawad *et al*.,2005) [28], n-Hexadecanoic acid ($C_{16}H_{32}O_2$) act as nematicide, pesticide, hemolytic, 5-alpha reductase inhibitor; 2,6,10,18,22-Tetracosahexaene,2,6,10,19,23, hexamethyl ($C_{30}H_{50}$) used to treat Cancer prevention, anticancer activity, Cis cis,cis-7,10,13- Hexadecatrienal ($C_{16}H_{26}O$) having Antioxidant activity, Di-n-octylphthalate ($C_{24}H_{38}O_4$) having liver hitopathology enzyme activity, β-glucuronidase activity and 8, 11,14,Eicosatrienoic acid (Z,Z,Z) ($C_{20}H_{34}O_2$) has been reported to have anti-inflammatory, Insectifuge, Hypatoprotective, Antihistaminic, Antieczemic, Antiacen, 5-Alpha reductase inhibitor, Antiarthritic, Anticoronary activities. (Lalitha Rani *et al.,* 2009) [17].

Table: 3. Chemical structure of the most prevailing compounds of the leaves extract of *Jasminum auriculatum* Vahl

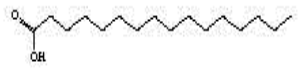
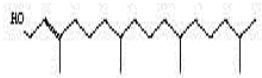
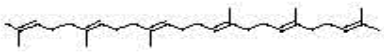
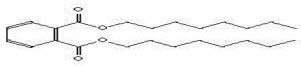
Name of the compounds	Chemical structure of the compounds	Nature of the compounds	Biological activity
Ethanol leaf extract			
n-Hexadecanoic acid		Fatty acid	Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavour, Hemolytic 5-Alpha reductase inhibitor
cis,cis,cis-7,10,13- Hexadecatrienal		Alcohol	Antioxidant activity
Phytol		Diterpene	Antimicrobial, Anti cancer, Anti-inflammatory Hypocholesterolemic, Nematicide, Anticoronary, Antiarthritic, Hepatoprotective, Anti -androgenic,
2,6,10,14,18,22-Tetracosahexaene,2,6,10,19,23, hexamethyl		Triterpene	Cancer prevention, anticancer activity
8, 11,14,Eicosatrienoic acid (Z,Z,Z)		Linolenic acid	anti-inflammatory, Insectifuge, Hypatoprotective, Antihistaminic, Antieczemic, Antiacen, Antiarthritic, Anticoronary
Di-n-octylphthalate		Plasticizer	liver histopathology enzyme activity, β-glucuronidase activity

Table 4. FT-IR Spectral peak and functional groups obtained for the leaf extract of *Jasminum auriculatum* Vahl

S.No	Peak value cm^{-1}	Types of stretching	Functional group	Corr. Area
1	524.64	C–H wag (–CH2X)	alkyl halides	4.008
2	775.38	C–H wag (–CH2X)	alkyl halides	0.455
3	893.04	C–H stretch	aromatics	0.024
4	1037.7	C–N stretch	aliphatic amines	19.109
5	1153.43	C–H wag (–CH2X)	alkyl halides	0.68
6	1253.73	C–H wag (–CH2X)	alkyl halides	1.649
7	1321.24	C–N stretch	aromatic amines	1.311
8	1386.82	C–H rock	alkanes	0.986
9	1409.96	C–C stretch (in–ring)	aromatics	1.69
10	1627.92	N–H bend	primary amines	33.656
11	1730.15	C=O stretch	α,β-unsaturated esters	0.359
12	1878.67	C=C stretch	Anhydride	0.002
13	1915.31	C=C stretch	Alkene	0.015
14	2166.06	–C≡C– stretch	alkynes	0.343
15	2200.78	–C≡C– stretch	alkynes	0.001
16	2364.73	C≡N stretch	nitriles	0.48
17	2856.58	C–H stretch	alkanes	0.429
18	2924.09	C–H stretch	alkanes	5.549
19	3363.86	O–H stretch	carboxylic acids	134.832
20	3657.04	O–H stretch, H–bonded	alcohol, phenol	6.107

7. Conclusion

The investigation concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

8. Acknowledgement

The authors are very much grateful to the authorities of Annamalai University for providing the facilities. We also thank Dr. Ajay Kumar, Advanced Instrumentation Research facility, Jawaharlal Nehru University, New Delhi, for the help of Gas Chromatography- Mass Spectrometry (GC-MS) analysis of *Jasminum auriculatum* ethanolic extract.

9. References

1. Aggarwal BB, Kumar A, and Bharti AC. Anticancer potential of curcumin preclinical and clinical studies. Anticancer Research 2003; 23:363-398.
2. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer, Biochemical pharmacology 2006; 71:1397-1421.
3. Aggarwal BB, Shishodia S. Suppression of the nuclear factor –kB activation pathway by spice-derived

phytochemicals; reasoning for seasoning, Annals of the New York. Academy of Science 2004; 1030:434-441.
4. Bahuguna Y, Singh M, Rawat M, Juyal V, Gupta V (2009b). Antilithiatic effect of flowers of *Jasminum auriculatum* Vahl. International Journal of Green Pharmacy2009b; 2: 155-158.
5. Bahuguna Y, Singh M, Rawat M, Juyal V, Gupta V. Diuretic activity of flowers of *Jasminum auriculatum* Vahl. Journal of Pharmacy Research 2009a; 2: 215-216.
6. Bedi S, Tanuja, Vyas SP. A hand book of Aromatic and Essential oil plants. Agrobios pvt.Ltd.Bombay 2008; pp.280-284.
7. Chaman Lal and Verma LR. Use of certain bio-products for insect-pest control. Indian Journal of Traditional Knowledge 2006; 5(1): 79- 82.
8. Cong Z, Meiling Q, Qinglong S, Shan Z, Ruonong F. J. Pharm. Biomed. Anal 2007; 44: 464.
9. De-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV, Kohn LK, de-Carvalho JE. Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design. Curr. Med. Chem 2006; 13: 3371-3384.
10. Deshpande SM, Upadyaya RR.Chemical studies of *Jasminum auriculatum* Vahl leaves.Current.Science 1967; 36-233.
11. Duke JA. Handbook of Phytochemical constituents of GRAS herbs and other economic plants, *Boca Raton 1992 FL.CRC Press*.
12. Ghosh MN. Fundamental of experimental pharmacology scientific Book Agency Calcutta, Edition IInd 1984; 156-157.
13. Green RJ.Antioxidant activity of peanut plant Tissues. Master's thesis, North Carolina state University 2004; USA.
14. Harbone JB. Phytochemical methods, London Chapman and Hall, Ltd 1973; Pp.49-188.
15. Haznagy-Radnai E, Czigle S, Mathe I. J. Planar. Chromatogram 2007; 20: 189.
16. Kaushik JC, Arya Sanjay, Tripathi NN, Arya S. Antifungal properties of some plant extracts against the damping off fungi of forest nurseries. Indian Journal of Forestry 2002; 25: 359-361.
17. Lalitha Rani S, Mohan VR, Regini GS, Kalidass C. GC-MS analysis of ethanolic extract of Pathos *scandens* leaf. J. Herb. Medi. Toxicology 2009; 3: 159-160.
18. Lampronti I, Saab AM and Gambari R. Int. J. Oncol 2006; 29: 989.
19. Lee S J, Umano K, Shibamoto T and Lee KG. Food Chem 2005; 91: 131.
20. Merlin NJ, Parthasarathy V, Manavala R and S Kumaravel S. Chemical investigation of aerial parts of *Gmelina asiatica* Linn by GC-MS. Pharmacog. 2009; 1(3): 152-156
21. Muniappan Ayyanara, Savarimuthu Ignacimuthu. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats of India. Journal of Ethnopharmacology2011; 134:851-864.
22. Palomba EA, Semple SJ. Antibacterial activity of traditional Australian medicinal plants. Journal of Ethanopharmacol 2001; 77: 151-157.
23. Rajinder Raina, Shahid Parwez (2008). Studies on the alcohol free defatted extract of *Jasminum auriculatum* leaves has been reported to contain *lupeol* and *Jasminol* (Deshpande& Upadyaya, 1967).
24. Trease GE, Evans WC. Pharmacognosies 11th Ed. Brailliar Tiridel can. Macmillan Publishers 1989; P.530.
25. Uniyal SK, Singh KN, Jamwal P, Lal B.Traditional use of medicinal plants among the communities of chhota Bhangal, western Himalayan. Journal of Ethanobiol. Ethnomed 2006; 2:1-14.
26. Vaidyaratanam P S. Indian Medicinal Plants- A compendium of 500 species. Orient Longman Private Ltd: Hyderabad 2003; Vol. 3: 164- 165.
27. Yeong LH, Nancy KG, Micheal WP. Newly recognized nticarcinogenic fatty acids: identification and quantification in natural and processed cheeses. J. Agric. Food Chem 1989; 37:75-81
28. Yuenyongsawad S, Tewtarkul S. Essential oil components and biological activities of *Coleus parvifolius* leaves, *Songklanakarin.* Journal of Science and Technology 2005; 27(2): 497-502