IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"Phytopharmacological screening of *Jasminum multiflorum* as potent Bronchodilator"

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Abstract:

The use of medicinal plants as a source of relief from illness can be traced back over five millennia to written documents of the early civilizations in India for a long period of time. Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies on natural therapies. Medicinal plants play an important role in the development of potent therapeutic agents. Natural products from plant, animal and minerals havebeen the basis of the treatment of human disease. Drug discovery from medicinal plants has playedan important role in the treatment of antihistaminic, bronchodialator activties and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating allergies. Extraction of *jasminum multiflorum* was carried out by successive extraction techniques. Further phyto-chemical tests were carried out followed by *in-vitro* pharmacological activity by using isolated goat trachea. Then resulted acetone extract were allowed to evaporation of solvent, concentrate further isolation of active secondary metabolite from extract was done by Column Chromatography, Preparative TLC and TLC. Spectral analysis of Extract and compound was carried out by IR, NMR, GSMS-MS were performed. The standardization parameters, TLC and Column Chromatography profiles were used for deciding the identity and purity of herbal extract. Chromatographic and spectroscopic techniques (NMR, IR, UV) proved its usefulness in isolation and proper identification of Flavonoids. Target compound i.e Jasmanolactone B may be the possible structure of isolated compound.

Keywords: Extraction, Jasminum *multiflorum,* Histamine, Retention factor.

Introduction:

Jasminum is a genus of vines and shrubs in the olive family (Oleaceae) that is commercially grown in tropical and subtropical countries for its flowers and essential oil production. *Jasminum multiflorum, Jasmin pubescens* is a plant native to India. It is also known as furry jasmine or downy jasmine. The Western Ghats and Sub-Himalayan forests up to 1500 m, Southeast Asia, and parts of Europe and Africa are the most popular. *Jasminum mutiflorum* is an evergreen ornamental plant with velvety leaves and white flowers that bloom profusely during the winter. It is commercially grown for its essential oil of flower. It is a spreading shrub or climber of one foot or hedge of 5 to 6 feet with velvety appearance of leaves and white flowers blooming profusely during winters and commercially cultivated for its essential oil of flower. *J. multiflorum* flowers are bitter, cooling, laxative, cardiotonic, alexipharmic, depurative, and digestive, and can help with vitiated pitta, inflammation, rheumatism, and cephalalgia. The leaves and flowers are said to have pharmacological properties that are both coronary vasodilating and cardiotropic.

Botanical Classifications:

Botanical name:	*Jasminum multiflorum*
Kingdom:	Plantae (Angiosperms)
Order:	Lamiales
Family:	Oleaceae
Genus:	*Jasminum*
Species:	*J. multiflorum*
Clade:	Tracheophytes

Figure 1. Plant of ***Jasminum multiflorum* (Oleaceae)**

Method & Material:

Plant Collection:

During the months of December and January, the healthy parts of the experimental plant were collected from the cultivated fields of Charegaon, Tal- Karad Dist- Satara. The voucher specimen (No. PMD -1) has been deposited in the same department's herbarium.

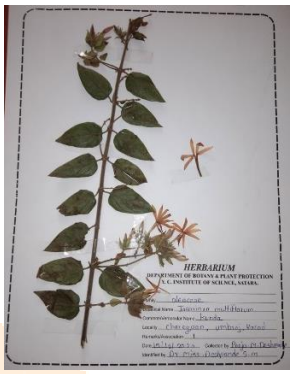

Figure 2. Herbarium ("*Jasminum multiflorum*") PMD-1

Extraction procedure:

Leaf powder was made by air drying and pulverizing the leaves**.** The leaves of "Jasminum multiflorum" were air dried and ground into a powder. In the main jar of the Soxhlet, 1000 g of the powdered plant material from which the extract must be extracted was packed. The 400ml solvent was poured into the round bottom flask, and condensation was extracted under reduced pressure and at a controlled temperature below the boiling point of the solvent, which was set to boil via a regulated heating mantle. In a rotary evaporator, the filtrate was evaporated to dryness at 40 C. And the preceding procedure was repeated several times until a sufficient amount of extract was produced. The concentrated plant extract was kept at 4 degrees Celsius until it was needed. Solvents were chosen based on their polarity, which ranged from non-polar to polar.

1. Petrochemical ether

2. Chloroform

3. Acetone

4. Ethyl acetate is a type of acetate.

5. Methanol

6. Hydroalchol

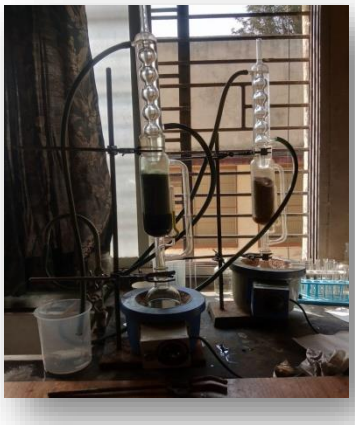

Figure 3. Extraction of *"Jasminum multiflorum" by* Soxhlet Apparatus

Solvent drying:

Pouring an extract onto a petry plate and allowing it to evaporate at room temperature resulted in solvent evaporation.

Figure 4: Evaporation of solvent in petri plates

Phytochemical Analysis of *"Jasminum multiflorum''*

The aqueous extract was freshly prepared and divided into different test tubes, after which the various chemical constituents were analysed using the methods described. Steroids, triterpenes, alkaloids, flavonoids, saponin, tannins, glycosides, lignin, and volatile oil were among the chemical constituents examined.

In-vitro Assay for Antihistaminic & Bronchodilator Activities:

Animals were obtained from a slaughterhouse and isolated adult goat tracheal tissue was obtained. Trachea was collected in an oxygenated Krebs' solution that was kept at room temperature.

Methodology Histamine induced contraction of isolated goat trachea preparation & bronchodilator effect of acetylcholine using goat trachea:

The goat tracheal tissue was obtained immediately after the animals were slaughtered. Trachea fragments were placed in freshly prepared ice-cold oxygenated Kreb's solution. The goat trachea was then cut into individual rings and linked together in a chain. It was suspended in a bath containing Kreb's solution and kept at 37 ± 0.5ºC while a stream of air (1 bubble/sec) was bubbled through the organ tube. The tracheal muscle was attached to an S-shaped aerator on one end and an isotonic frontal writing lever to a drum on the other. Under a 1g load, the tissue was allowed to equilibrate for 45 minutes. The contractile responses of tracheal strip to histamine (3000µg/ml) with doses of 0.1ml, 0.2ml, 0.4ml, 0.8ml and 1.6ml were recorded in absence and presence of methanolic extract of "Jasminum multiflorum" L.(200µg/ml) by using Sherrington's Recording Drum with a frontal writing lever. In the presence of the standard drug Chlorpheniramine Maleate (10g/ml), a similar concentration-effect curve was obtained. To express percentage inhibition, the height of the response curve was measured. The graph was created by plotting log dose versus response curve height. The bronchodilator effect of acetylcholine (2000 g/ml) was measured using a tracheal strip with doses of 0.1ml, 0.2ml, 0.4ml, 0.8ml, and 1.6ml in the absence and presence of methanolic extract of "Jasminum multiflorum" L (200g/ml) using Sherrington's Recording Drum with a frontal writing lever. A similar concentration-effect curve was obtained in the presence of the standard drug aminophylline (10g/ml). The height of the response curve was measured to express percentage inhibition. The graph was created by plotting log dose verses response curve height.

Table no. 1: Krebs solution composition

Sr. No	Ingredients	Quantity (gm) for liter
1	Sodium chloride	6.9
2	Potassium chloride	0.35
3	Calcium chloride	0.28
4	Magnesium sulphate	0.28
5	Sodiunm bicarbonate	2.1
6	Potassium dihydrogen phosphate	0.16
7	Glucose	2.0

The constituents from acetone extract were then separated by column chromatography.

Column chromatography with silica gel 60-120

Column Packing:

Acetone was poured into a 20 cm column. Silica gel (mesh size 60-120) slurry was made with petroleum ether and poured into the column. During the setting process, the excess solvent was drained and an additional amount of the slurry was added. This procedure was repeated until the column reached a height of 45cm. To prevent disruption of the packed silica gel during solvent addition, a circle of filter paper was placed over the surface of the silica gel, and a small amount of acid washed sand was placed over the filter paper.

Figure 5: Isolation by Column Chromatography

Elution

The dried extract of "*Jasminum multiflorum*" leaves (5 g) was ground with a little amount of silica gel and was made into slurry. The column was loaded with this powdered slurry. Eluents included petroleum ether, chloroform, acetone, ethyl acetate, methanol, and hydroalchol. During this process, the eluate flow rate was reduced to 1ml/ 2 min. For elution, pure petroleum ether was used, followed by the addition of chloroform, ethyl acetate, acetone, methanol, and distil water in the following concentration ratios: 80-20%, 60-40%, 40-60%, 80-20%, and 100%. The fractions (each 15ml) were collected individually and tested for its purity by TLC. The identical fractions were mixed and concentrated.

Partial Characterization of Phytoconstituents from the leaves of *Jasminum multiflorum* by Column Chromatography:

The acetone extract from the plant was subjected to silica gel column chromatography by using solvents with increasing polarity gives 15 major fractions.

Isolation was done using silica gel open-column chromatography, eluting with Chloroform: Ethyl acetate gradient (80-20- broad fraction 1),

Ethyl acetate : acetone (80:20 - broad fraction 2),

Ethyl acetate : acetone (50:50 broad fraction 3),

Ethyl acetate : acetone (30:70 - broad fraction 4),

Ethyl acetate : acetone (20:80 - broad fraction 5),

Ethyl acetate : acetone (10:90 – broad fraction 6),

Acetone (100 % - broad fraction 7),

Acetone : methanol (80:20 - broad fraction 8), Acetone : methanol (60:40 - broad fraction 9), Aacetone : methanol (50:50 - broad fraction 10)

Acetone : methanol (30-70 - braod band 11).

Fractions with similar spots were mixed together and concentrated at reduced pressure and temperature.

Figure 6: Separation by Column Chromatography

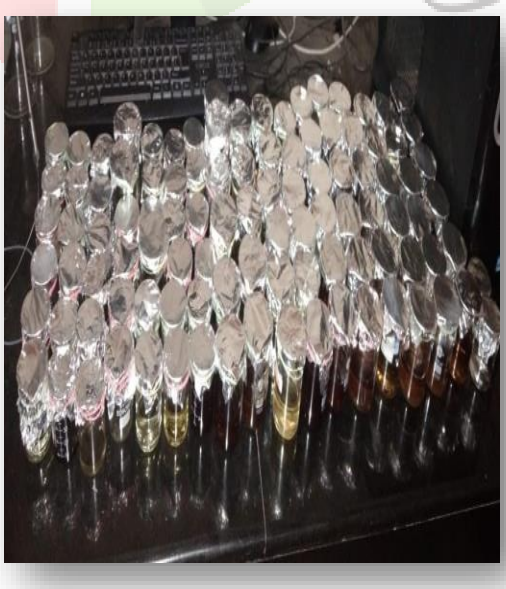

Figure 7: Collection of fractionated eluent

Preparative Thin Layer Chromatography

For preparative thin layer chromatography, glass plates (20 x 20 cm) were thickly coated (0.4-0.5 nm) with silica gel 'G' (45 gm/80 ml water), activated at 100°C for 30 minutes, and cooled at room temperature (PTLC). The plant extract was applied to a plate and developed in a solvent system (mobile phase) of (chloroform: ethyl acetate: formic acid) 2.5:2:0.5 v/v/v. The chromatogram plate spots were air dried and visualised in an iodine chamber. The spots were marked and the Rf values were found to be 0.8.

$$\text{Retention factor} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Partial Characterization of Phyto Constituents from the Leaves of *Jasminum multiflorum* by TLC:

The acetone extract of jasminum multiflorum loaded on silica gel coated TLC plates and developed with a solvent system of chloroform: ethyl acetate: formic acid in the ratio of 2.5:2:0.5 v/v/v was efficient to extract the antihistaminic compound. The extracts were used for further general studies.

Spectroscopic Analysis:

A UV Shimadzu UV-1800 visible spectrophotometer from Japan was used to obtain UV spectra. The IR spectra were measured using an ATR-Bruker spectrometer. In column chromatography, silica gel was used (60-120 mesh). The GCMS data were collected using a series 6540 LC-QTOFMS/MS mass spectrometer. NMR spectra were captured using PicoSpin 80 NMR spectrometers.

Results and Discussion:

Phytochemical Screening of Acetone Extract:

The phytochemical screening of the *Jasminum multiflorum* studied presently showed the presence of alkaloids**, flavonoids, saponin, tannins, steroids & volatile oil.**

Table no. 2: Phytochemical Test Results

Extract	Test No	For steroids	Tri-terpenoids	alkaloid	flavonoid	saponin	tannin	glycoside	Volatileoil
Diethyl Ether	1	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
	2	-ve	+ve	+ve	+ve	+ve	+ve		
Pet-ether	1	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve
	2	+ve	-ve	+ve	+ve	+ve	+ve		
Chloroform	1	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
	2	-ve	-ve	+ve	+ve	+ve	+ve		
Ethyl-acetate	1	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve
	2	+ve	+ve	+ve	+ve	+ve	+ve		
Acetone	1	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve

	2	+ve	-ve	-ve	+ve	+ve	+ve		
Methanol	1	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve
	2	+ve	-ve	-ve	+ve	-ve	+ve		
Hydroalcoholic	1	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve
	2	+ve	-ve	-ve	+ve	-ve	+ve		

Preliminary Screening for Antihistaminic and Bronchodilator activity:

Table no.3: Results of In-vitro pharmacological evaluation

Sr.no	Sample	Conc [µg/ml]	Dose [ml]	Height [mm]
1	acetylcholine	2000	0.1	3
			0.2	4
2	Me extract +ach	100	0.1+0.1	3
			0.1+0.2	5
		200	0.1+0.1	2.0
			0.1+0.2	2.5
3	Acetone extract +ach	100	0.1+0.1	1.5
			0.1+0.2	2.5
		200	0.1+0.1	1.5
			0.1+0.2	2.5
4	Ethyl acetate+ach	100	0.1+0.1	1
			0.1+0.2	3
		200	0.1+0.1 0.1+0.2	1.5 2.5

Conc. of histamine: 2000µg /10ml
Conc. of acetylcholine: 2000µg/10ml
Conc. of extract: 1: 100µg /ml
2: 200µg/ml

Dose of histamine was taken: 0.2, 0.4, 0.6, 0.8, 1.2ml

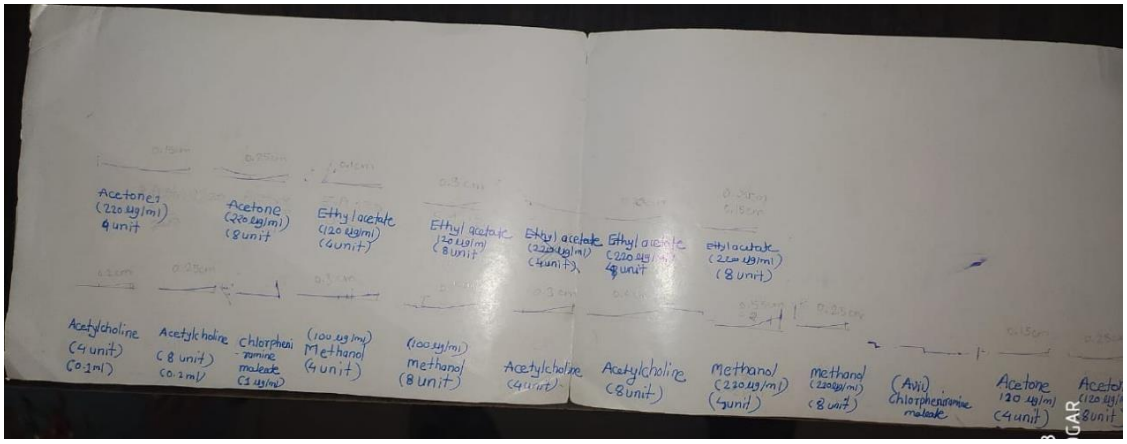

Figure 8: Graph log dose /height of response

At the different Concentration, **acetone** samples showed good activity

Partial Characterization of Phytoconstituents:

From the leaves for *Jasminum multiflorum* by Column Chromatography:

The acetone extract from the plant was subjected to silica gel column chromatography by using solvents with increasing polarity gives 15 major fractions.

Isolation was done using silica gel open-column chromatography, eluting with Chloroform: Ethyl acetate gradient (80-20- broad fraction 1),

Ethyl acetate: acetone (80:20 - broad fraction 2), Ethyl acetate : acetone (50:50 broad fraction 3), Ethyl acetate : acetone (30:70 - broad fraction 4), Ethyl acetate : acetone (20:80 - broad fraction 5), Ethyl acetate : acetone (10:90 – broad fraction 6), Acetone (100 % - broad fraction 7),

Acetone : methanol (80:20 - broad fraction 8),

Acetone : methanol (60:40 - broad fraction 9), Aacetone : methanol (50:50 - broad fraction 10) Acetone : methanol (30-70 - braod band 11).

Fractions with similar spots were mixed together and concentrated at reduced pressure and temperature.

Figure 9: Column Chromatography of acetoneextract of *Jasminum multiflorum*

Partial Characterization of Phytoconstituents from the leaves of Jasminum multiflorum by Preparative TLC:

The extract of plant was applied on plate and developed in solvent system chloroform: ethyl acetate: formic acid (mobile phase) 2.5:2:0.5 v/v/v. Spots obtained chromatogram plate was air dried and visualized under iodine chamber. The spots were marked and calculated the Rf values found to be 0.8 which resembles Jasmanolactone B.

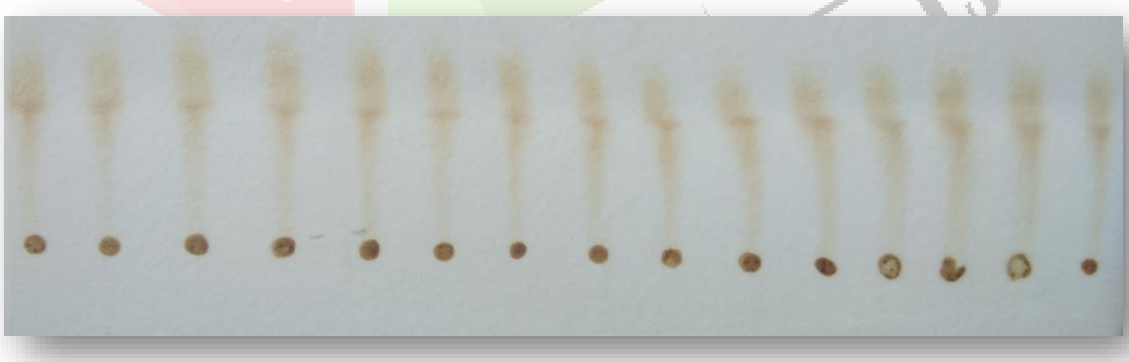

Figure 10: Preparative Thin Layer Chromatography of acetone extract of *Jasminum multiflorum*

Rf value –0.8

Stationary phase – Silica gel,

Mobile phase - chloroform: ethyl acetate: formic acid (2.5:2:0.5 v/v/v)

Table no. 4 : Retention Factor

Sr. no.	Concentration	Retention Factor
1	80-20	0.81
2	50-50	0.75
3	30-70	0.6
4	20-80	0.77
5	10-90	0.85
6	100	0.8
7	80-20	0.78
8	60-40	0.6
9	50-50	0.7
10	30-70	0.82

Characterization of *Jasminum multiflorum* extract by Infra Red Spectrum.

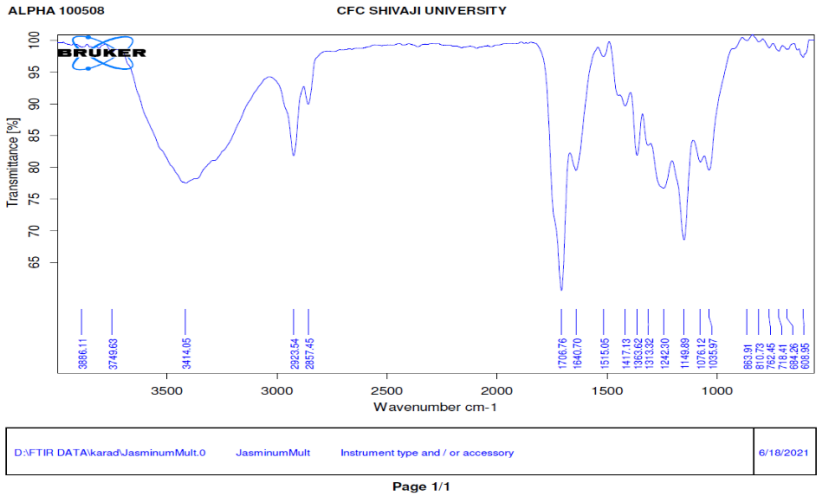

Figure 11: IR spectra of Acetone extract of *Jasminum multiflorum*

Table no.5: IR interpretation observations

Functional Groups	appearance	Standard Frequencies (cm-1)	Observed Frequencies (cm-1)	Compound class
-OH streching	Strong, broad	3550-3200	3414.05	alcohol
-OH streching	Strong, broad	3300-2500	2923.54	Carboxylic acid
C=O streching	weak	1725-1705	1706.76	Aromatic ketone
C=C	medium	1662-1626	1640.70	alkene
C-O	strong	1275-1200	1242.30	Alkyl arylether
C-O streching	strong	1205-1124	1149..89	Tertiaryalcohol
C-H bending	strong	755+/-20	762.45	1,2-disubstituted

Characterization Jasminum multiflorum compound by 1H-NMR Spectrum:

The isolated compound was dissolved in denatured acetone solvent and the spectra was recorded in AVANCE 11-300 Bruker model at the temperature 27ºC and chemical shift were recorded based on δ (ppm) or δ TMS=0 and coupling constants are in hertz .

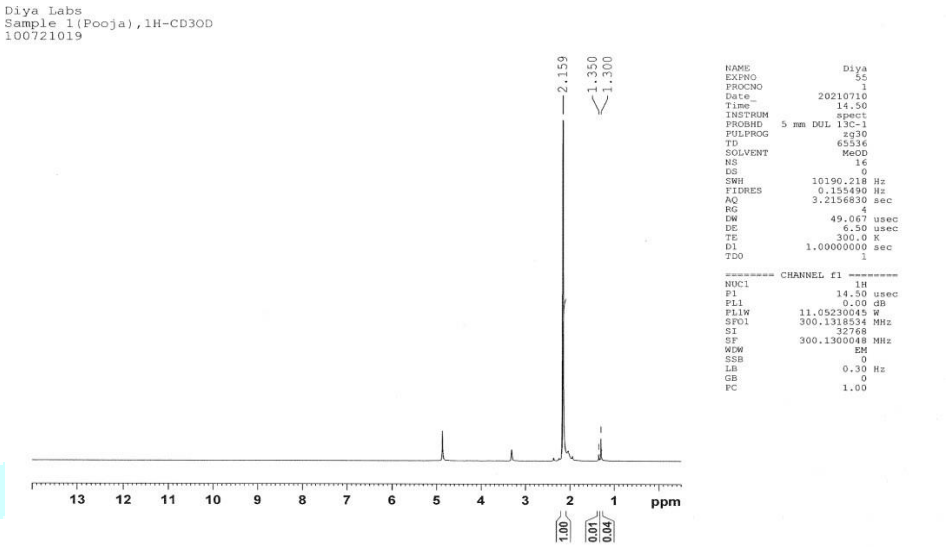

Figure 12: 1H-NMR Spectrum of isolated compound

Table no.6: NMR interpretation observations

Proton	Δvalues	Observed values	functional group
R-NH_2	0.5-5.0	4.9	amine
R-CH_2-OR	3.3-4.0	3.4	alcohol
R-C=O-CH_3	2.2-2.6	2.2	ketone
Ar-CH_3	2.2-2.5	2.2	Aromatic group
Ar-CH_2R	2.3-2.8	2.2	Aromatic group
R-CH_2-R	1.4-1.7	1.3	Aliphatic group

Characterization of *Jasminum multiflorum* analysis extract and compound by Gas Chromatography Mass Spectrum (GCMS):

Fraction extract with acetone was further studied through LCMS. The molecular formula could not be established just because the extract may contain many compounds and each compound will exhibit its own pattern of ionization and also may interfere the other peak.

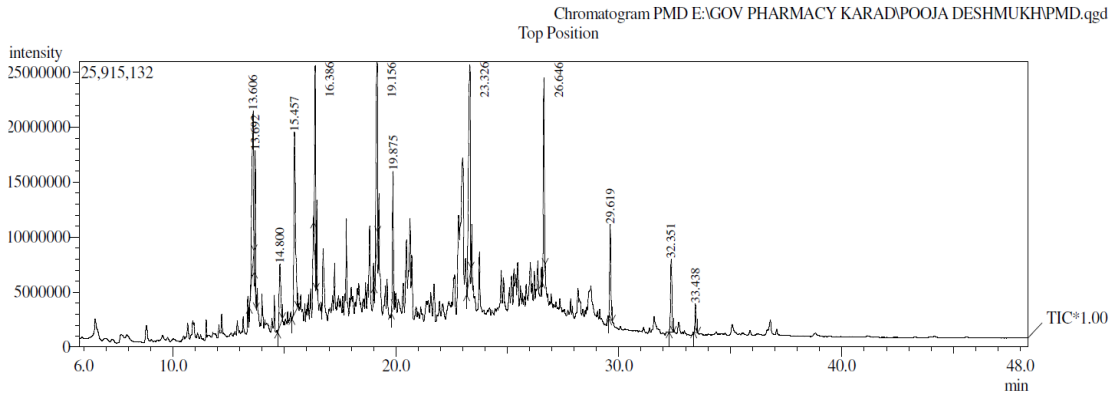

Figure 13: GC-MS Spectrum

Table no: 7 Interpretation by Gas chromatography -Mass Spectrum

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	13.606	13.430	13.635	73001889	12.07	2H-Pyran-2-one, 3-acetyl-4-hydroxy-6-methyl
2	13.692	13.655	13.740	24497778	4.05	1-Tridecene
3	14.800	14.700	14.915	27265514	4.51	4-Hydroxy-2,6-dimethyl-nicotinic acid ethyl es
4	15.457	15.335	15.600	81475123	13.47	2,4-Di-tert-butylphenol
5	16.386	16.320	16.425	52307760	8.65	n-Pentadecanol
6	19.156	19.030	19.195	76960016	12.73	1-Nonadecene
7	19.875	19.800	19.925	39185316	6.48	Neophytadiene
8	23.326	23.180	23.380	105887222	17.51	1-Octadecanol, methyl ether
9	26.646	26.560	26.705	56694307	9.37	Octacosanol
10	29.619	29.545	29.715	29989008	4.96	1-Hexacosanol
11	32.351	32.255	32.450	28618669	4.73	Phthalic acid, di(2-propylpentyl) ester
12	33.438	33.355	33.540	8887005	1.47	1-Hexacosanol
				604769607	100.00	

Conclusion:

The potential anti-histaminic activity of jasmanolactone B(Flavonoid compound) of *jasminum multiflorum*. The leaves of *jasminum multiflorum* contain considerable amounts of the Flavonoid compounds used in the studies. Screening of the phytochemical analysis of leaf extracts of the plants studied exhibited the presence of alkaloids, flavonoids, saponin, tannin, volatile oil. The Flavonoid compound, characterized by infra-red spectroscopy 1H-NMR and gas chromatography-mass spectrum studies.

Acknowledgements:

The authors of this research article are thankful to the Principal, Government College of Pharmacy, Karad for allowing us to publish this paper. We are also thankful to the AICTE for providing research fellowship to complete our research work and convert our research work into the publications. Also thankful to my guide Dr. R. D. Chakole for constant support and guidance for publication of this manuscript.

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