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PHYTOCHEMICAL EXAMINATION OF FLOWERS OF *JASMINUM AURICULATUM* VAHL

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ABSTRACT

The aim of the present study was phytochemical investigation of extracts of flowers of *Jasminum auriculatum* Vahl. (Oleaceae). The alcoholic extract (AlcE, 10% w/v) of dried coarse powdered flowers was prepared using 70% v/v alcohol by soxhlet method at a temperature of 60-70°C (yield 5.4% w/w). The alcoholic extract (500 g) was divided into two equal portions. Each portion was suspended in distilled water (500 ml) and then extracted with petroleum ether (60 – 80°C, 8 X 500 ml) and ethyl acetate (8 X 500 ml) in succession. All the fractions were then washed with distilled water (30 ml), dried over anhydrous sodium sulphate and freed of solvent by distillation. The ethanol extract was thus fractioned into petroleum ether soluble fraction (40 g) and ethyl acetate soluble fraction (68 g). Chemical investigation of the flowers led to the isolation of three compounds namely lupeol, apigenin and quercetin. The constituents isolated and characterized from the *Jasminum auriculatum* flowers can be categorized under the triterpenes flavonoids. Triterpenes like lupeol were isolated from petroleum ether extracts and flavonoids like quercetin was isolated from the ethyl acetate extract and characterized by spectral data. In conclusion, Flavonoids like quercetin are believed to act as diuretic hence hastens the process of dissolving the preformed stones in curative regimen and prevention of new stone formation in urinary system on prophylactic treatment and as antioxidant agents, therefore may be useful in the treatment of urolithiasis, where lipid peroxidation is an important constituent. This investigation could conclude the antiurolithiatic property of *J. auriculatum* flowers due to the presence of flavonoids like

quercetin.

KEYWORDS: *Jasminum auriculatum*, Alcoholic Extract, Fractionation, Triterpenes, Flavonoids.

INTRODUCTION

Despite technological and conceptual developments in the practice of medicine, the formation and growth of renal calculi continue to afflict mankind. The incidence of kidney stones has increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, calcium oxalate (CaOx), representing up to 80% of all analyzed stones.[1]

A large number of plant drugs have been used in India since ancient times which claim efficient cure of urinary stones.[2] Amongst the medicinal plants used in urolithiasis are 'patharphor' (*Didymocarpus pedicellata*), several Bergenia species, three species of Tribulus (*T. systoides, T. terrestris and T. alatus*), 'manjit' (*Rubia cordifolia* and *Rubia tinctorum*), 'varuna' (*Crataeva nurvala*) and 'imli' (*Tamarindus indica*).[3] *Costus spiralis.*[4] *Raphanus sativus.*[5] *Moringa oleifera.*[6] *Crataeva adansonii.*[7] *Melia azedarach.*[8] *Jasminum auriculatum.*[9, 10] *Eleusine coracana.*[11]

Jasminum auriculatum Vahl. (Oleaceae) grows throughout south India, on the dry slopes of the Western Ghats. It is cultivated throughout India, especially in Uttar Pradesh and Tamil Nadu. In Uttar Pradesh it is cultivated on commercial scale in Ghazipur, Jaunpur, Farrukhabad and Kannauj for its fragrant flowers which yield an essential oil. Indian Materia Medica describes the use of flowers of *J. auriculatum* has been claimed in traditional literature to be valuable against a wide variety of diseases. The roots are useful in skin diseases especially for ringworm. The flowers are fragrant, bitter, acrid, sweet, refrigerant, astringent, cardiotonic, diuretic and depurative. They are useful in burning sensation, hyperdipsia, ulcers, odontalgia, stomatopathy, ophthalmopathy, cardiopathy, urolithiasis, nephrolithiasis, strangury and dermatopathy.[12,13]

A literature survey indicated that the oximes of jasmone and both methyl trans and cis jasmonates have been identified in the flower of absolute of *Jasminum auriculatum* Vahl. from India.[14] Comments on the structure and synthesis of jasminol, a triterpene reported from *Jasminum auriculatum.*[15] The alcohol free defatted extract of *J. auriculatum* leaves

have been reported to contain lupeol and jasminol.[16] Qualitative analysis of volatile compositions of Dok Puthachad (*Jasminum auriculatum*) by using HS-SPME/GC-MS is done.[17] Isolation and Identification of constituents of *Jasminum auriculatum* Vahl. leaves is also reported.[18]

However, there are no records of systematic phytochemical studies that support its antiurolithogenic effect. Due to continuation of our research work on *J. auriculatum.* [9, 10] it was considered of interest to perform a phytochemical study of the alcoholic extract, so as to contribute with data that determine phytoconstituents responsible for its antiurolithogenic effect.

MATERIALS AND METHODS

Plant Material

The fresh flowers of *Jasminum auriculatum* Vahl. were collected from local areas of Belgaum, Karnataka, India during May-2008 and authenticated at Botanical Survey of India (BSI), Dehradun, India. A voucher specimen of the plant was deposited in the Botanical Survey of India herbarium under the number BSD/DD/Tech/572 for further reference.

Preparation of the extract

The flowers were dried in shade and were ground to get a coarse powder (40 mesh size). The aqueous extract (AqE, 10% w/v) of dried flowers was prepared using chloroform water I.P., by maceration method for 7 days at room temperature (yield 8.6% w/w) and alcohol extract (AlcE, 10% w/v) of dried flowers was prepared using 70% (v/v) alcohol by soxhlet method at a temperature of 60-70ºC (yield 5.4% w/w). The extracts were then filtered, concentrated under vacuum and freeze-dried.[19]

Fractionation of ethanolic extract

The extract (500 g) was divided into two equal portions. Each portion was suspended in distilled water (500 ml) and then extracted with petroleum ether (60 – 80°C, 8 X 500 ml) and ethyl acetate (8 X 500 ml) in succession. All the fractions were then washed with distilled water (30 ml), dried over anhydrous sodium sulphate and freed of solvent by distillation. The ethanol extract was thus fractioned into petroleum ether soluble fraction (40 g) and ethyl acetate soluble fraction (68 g).

Petroleum ether extract

The petroleum ether extract (40 g) was saponified with 20% ethanolic KOH (300ml) for 2h. The contents of the flask were then evaporated to remove all traces of EtOH, the lost volume being replaced by water from time to time. The unsaponifiable portion was then extracted with ether (5 X 300ml). All the ethereal fractions were combined, washed with distilled water (40ml), dried over anhydrous sodium sulphate and the solvent was evaporated to afford a yellow residue (8 g). The residue (8 g) was dissolved in $CHCl_3$ (10 ml) and adsorbed onto neutral alumina (20 g). After evaporation of the solvent it was loaded onto a neutral alumina column (150 g) prepared in petroleum ether (60–80°C). The column was eluted first with petroleum ether (60-80°C), petroleum ether (60-80°C): benzene graded mixtures (95:5, 90:10, 80:20 and 50:50), then with benzene followed by graded mixtures of benzene: chloroform (95:5, 90:10, 80:20 and 50:50), chloroform and finally chloroform : methanol (95:5,90:10, 80:20 and 50:50). The elution was monitored by TLC (Silica gel G; visualization: vanillin sulphuric acid reagent heated at 110°C). Each time 5 ml were collected and identical eluates (TLC monitored) were combined and concentrated to 5 ml and kept in a refrigerator.

Elutions carried out with petroleum ether (60-80°C): benzene graded mixture (50:50) resulted in a single component. After removing the solvent a residue resulted, this was isolated as pure component by recrystallising with petroleum ether. The product was designated as compound I (38 mg). The concentration of other eluates gave only brown resinous masses which were not processed further.

Ethyl acetate extract

The Ethyl acetate extract (68 g) as a solution in MeOH (10ml) was loaded on to the silica gel column (150 g) prepared in EtOAc. The column was eluted first with ethyl acetate and then with ethyl acetate:methanol graded mixtures (99:1, 95:5 and 90:10). The elutions were monitored by TLC (Silica Gel G; EtOAc : MeOH (95:5), visualization ; UV / Iodine Vapours). Elutions carried out with EtOAc : MeOH (95:5) and EtOAc : MeOH (90:10), resulted in a two spots on TLC (Silica gel G; EtOAc : MeOH (95:5), visualization; UV / Iodine Vapours). These were combined and concentrated to about 5 ml when it yielded yellow crystals which responded to Shinoda's test for flavonoids (designated as compound II and Compound III).

RESULTS AND DISCUSSIONS

Chemical investigation of the flowers led to the isolation of three compounds namely lupeol, apigenin and quercetin. The constituents isolated and characterized from the *Jasminum auriculatum* flowers can be categorized under the triterpenes and flavonoids. Triterpenes like lupeol were isolated from petroleum ether extracts, flavonol like apigenin and quercetin was isolated from the ethyl acetate extract and characterized by spectral data.

In conclusion, the presented data indicates that administration of the AlcE of *J. auriculatum* flowers to rats with ethylene glycol induced lithiasis caused diuresis.[10] and hastened the process of dissolving the preformed stones and preventing new stone formation in the urinary system, supporting folk information regarding antiurolithiatic activity of the plant part.

Extensive work was carried out on the antiurolithiatic activity of flavonoids as a class of compounds.[20] In addition, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet. In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane. The diuresis and lowering of urinary concentrations of stone forming constituents by flavonoids like quercetin is well documented in literature. The protective effect against oxalate induced lipid peroxidation may be contributory to the recovery of renal damage.

Though their mechanism of action has not been clearly understood, flavonoids like quercetin are believed to act as diuretic hence hastens the process of dissolving the preformed stones in curative regimen and prevention of new stone formation in urinary system on prophylactic treatment and as antioxidant agents, therefore may be useful in the treatment of urolithiasis, where lipid peroxidation is an important constituent.

This investigation could conclude the antiurolithiatic property of *J. auriculatum* flowers due to the presence of flavonoids like quercetin.

Characterization

Analysis of compound-I

Physical state : Pearl white crystals

R_f value : 0.71 (solvent system; petroleum ether: benzene 50:50)

Colour of spot : Pink (Spraying reagent; vanillin-sulphuric acid, heated at 110°C)

Melting point : 213 -215°C

Spectral Characteristics of compound-I

IR(KBr)

3326.49cm^{-1} (br, OH)

2937.42 cm^{-1}, 2869.48 cm^{-1} (C-H str. in CH_3 and CH_2)

1640.51 cm^{-1} (C=C str.)

1449.07 cm^{-1} (C-H deformation in CH_2/ CH_3)

1374.96 cm^{-1} (C-H deformation in gem dimethyl)

1041.47 cm^{-1} (C-O str. of secondary alcohol)

890 cm^{-1} (Exocyclic CH_2)

^{1}HNMR: ($CDCl_3$)

δ 0.7 to δ 1.026 (m, 18 H,6X CH_3)

δ 1.76 (s, 3H**,**-CH_3 –C=CH_2) OH,1 H)

δ 3.322(s, 1H, OH)

δ 4.581 (s, 1 H, H-3)

δ 4.700 (s, 1H, β-C-24H)

δ 4.88(s, 1H, α –C-24 H)

δ 0.8 to δ 1.74 (m, 25H for CH_2 and CH protons)

Mass spectra (EI-MS)

Molecular formula : $C_{30}H_{50}O$

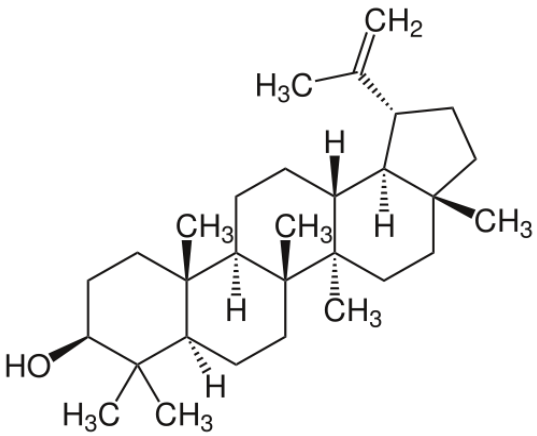
Molecular weight : 426

EIMS (M/z) : Mass spectrum shows molecular ion peak at m/z 426(15.2%). The other peaks appeared at 409(28.5%), 395(34.9%), 318(47.6%), 218(40%), 207(52.3%), 187(67.6%), 175(26.6%), 161(31.4%), 147(36.1%), 125(65.7%), 121(65.7%) and 81 (100%).

Acetylation of compound I

Compound I (5 mg) was taken up in dry pyridine (0.2 ml) and freshly distilled Ac_2O (1 ml) was added to it. The mixture was kept at room temperature overnight, then added to ice water, stirred, kept for 2 h, filtered and dried. The solid obtained was crystallized from C_6H_6 as white flakes. m.p. 217 - 219°C (lit 218°C).[21]

From the m.p., IR, ^{1}HNMR and mass spectral data, compound I was identified as **Lupeol.** It was further confirmed by co – chromatography with an authentic sample (sigma chemical company, USA) on silica gel, solvent system petroleum ether: benzene 50:50; spraying reagent; vanillin – sulphuric acid, heated at 110°C.



Lupeol

Analysis of compound-II

Physical state	: Yellow crystals
R_f value	: 0.52 (solvent system: EtOAc : MeOH (95:5)
Melting point	: 345°C

Spectral Characteristics of compound-II

IR(KBr)

3326.93 cm-1 (br, OH str.)

3092. 28 cm^{-1} (Ar C-H str.)

1604.12 cm^{-1} (C --- C str.)

1653.52 cm^{-1} (C=O str.)

^{1}HNMR(DMSO-d6)

δ10.84 (s, 1H,OH)

δ10.37(s, 1H, OH)

δ12.93(s, 1H OH)

δ 7.97(d, 2H, H-2^{1}, H-6^{1})

δ 6.93(d, 2H, H-3^{1}, H-5^{1})

δ 6.8 (d, 1H, H-8)

δ 6.5 (d, 1H, H-6)

δ 6.2 (s, 1H, H-3)

^{13}CNMR (DMSO –d_6)

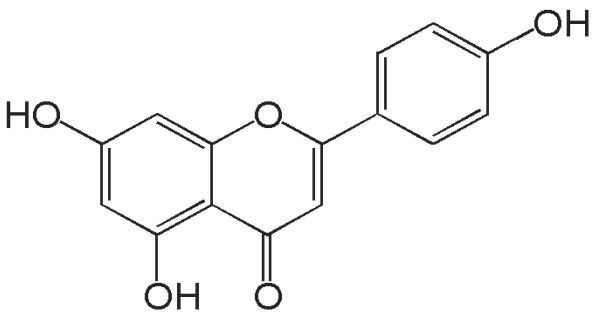
182.110 (C-4), 164.88 (C-2), 164.101 (C-7), 161.818 (C-5), 161.530 (C-4^{1}), 157.671 (C-9), 128.835 (C-6^{1}, C-2^{1}), 121.541(C-1^{1}), 116.318 (C-3^{1}, C-5^{1}), 103.208 (C –10), 99.195 (C-6), 94.321(C-8), 103.510 (C-3).

Mass spectra (GC-MS)

Molecular formula : $C_{15}H_{10}O_5$

Molecular weight : 270

Mass spectrum shows molecular ion peak at m/z 270 (M^{+}, $C_{15}H_{10}O_5$, 100%), other significant peaks were at 242 (22%), 213(4.8%), (30.48%), 121 (37.78%), 96 (9.52%), 78 (4.8%), 69 (7.9%). From the melting point, IR, ^{1}HNMR and mass spectral data, compound II was identified as Apigenin. It was further confirmed by co– chromatography with an authentic sample (sigma chemical company, USA) on silica gel, solvent system; EtOAc: MeOH, 95:5, visualization; UV / Iodine vapours.



Apigenin

Analysis of compound-III

Physical state : Yellow crystals

R_f value : 0.92 (solvent system: EtOAc : MeOH (95:5)

Melting point : 316 °C

Spectral Characteristics of compound-III

IR(KBr)

3411 cm-1 (O-H stretching vibration of Phenols)

1663.1 cm-1 (C=O Aryl Ketonic stretch)

1608.8 cm-1, 1523.5cm -1, 1496cm-1 (C ---C Aromatic ring stretch)

1383.1 cm-1 (In plane O-H bending of Phenols)

1318.9 cm-1 (In plane bending of C-H bond in Aromatic Hydrocarbon)

1265 cm-1 (C-O stretch of Aryl ether)

1203 cm-1 (C-O stretch of Phenol)

1167 cm-1 (C-CO-C stretch and bending in Ketone)

940.6, 821.4, 677, 602.3 cm-1 (Out of plane C-H bending of Aromatic Hydrocarbon)

^{1}HNMR(DMSO-d6)

δ12.93(s, 1H OH)

δ 10.87 (1H, s),

δ 6.93(d, 2H, H-31, H-51)

δ 6.8 (d, 1H, H-8)

δ 6.5 (d, 1H, H-6)

δ 6.2 (s, 1H, H-3)

δ 7.06 (1H, d, J = 2.18)

^{13}CNMR (DMSO –d_6)

182.110 (C-4), 164.88 (C-2), 164.101(C-7), 161.818 (C-5),

161.530 (C-4^1), 157.671(C-9), 128.835 (C-6^1, C-2^1), 116.318

(C-3^1, C-5^1), 103.208 (C –10), 99.195 (C-6), 94.321(C-8).

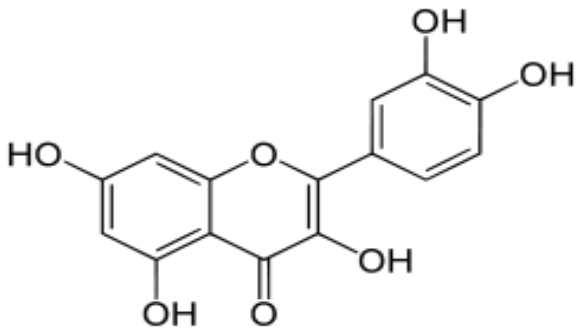
Mass spectra (EI-MS)

Molecular formula : $C_{15}H_{10}O_7$

Molecular weight : 302.236

Mass spectrum shows molecular ion peak at m/z 301 (M^+, $C_{15}H_{10}O_7$, molecular ion peak) (parent peak), other significant peaks were at 273 (22.7%), 257 (09.09%), 193 (06.63%) 179 (100%), 155 (63.63%), 109 (50.48%).

From the melting point, IR, ^{1}HNMR, ^{13}CNMR and mass spectral data, compound III was identified as **Quercetin.** It was further confirmed by co– chromatography with an authentic sample (sigma chemical company, USA) on silica gel, solvent system; EtOAc: MeOH, 95:5, visualization; UV / Iodine Vapours.



Quercetin

Table No. 01 Physicochemical Constants of *Jasminum auriculatum* Vahl. Flowers

S.No.	Parameters	I.P./B.P/As per literature Limit	Observation
I	Physical Tests		
	a) Nature	Coarse powder	Coarse powder
	b) Colour	Yellowish brown	Yellow
	c) Odour	Characteristic	Characteristic
	d) Taste	Astringent & Bitter	Astringent & Bitter
II	Loss on drying	*NMT 10 %w/w	7.235 % w/w
III	Ash values		
	a) Total ash	---	9.664 % w/w
	b) Acid insoluble ash	---	7.200 % w/w
	c) Water soluble ash	---	1.011 % w/w
IV	Fluorescence Analysis		Yellow fluorescence
V	Total Microbial Count (TMC)		
	TMC	*NMT 10,000 CFU/G	Confirms
VI	Heavy Metals		
	a) Mercury	*NMT 10ppm	Confirms
	b) Lead	*NMT 10ppm	Confirms
	c) Lithium	*NMT 10ppm	Confirms
	d) Cadmium	*NMT 10ppm	Confirms

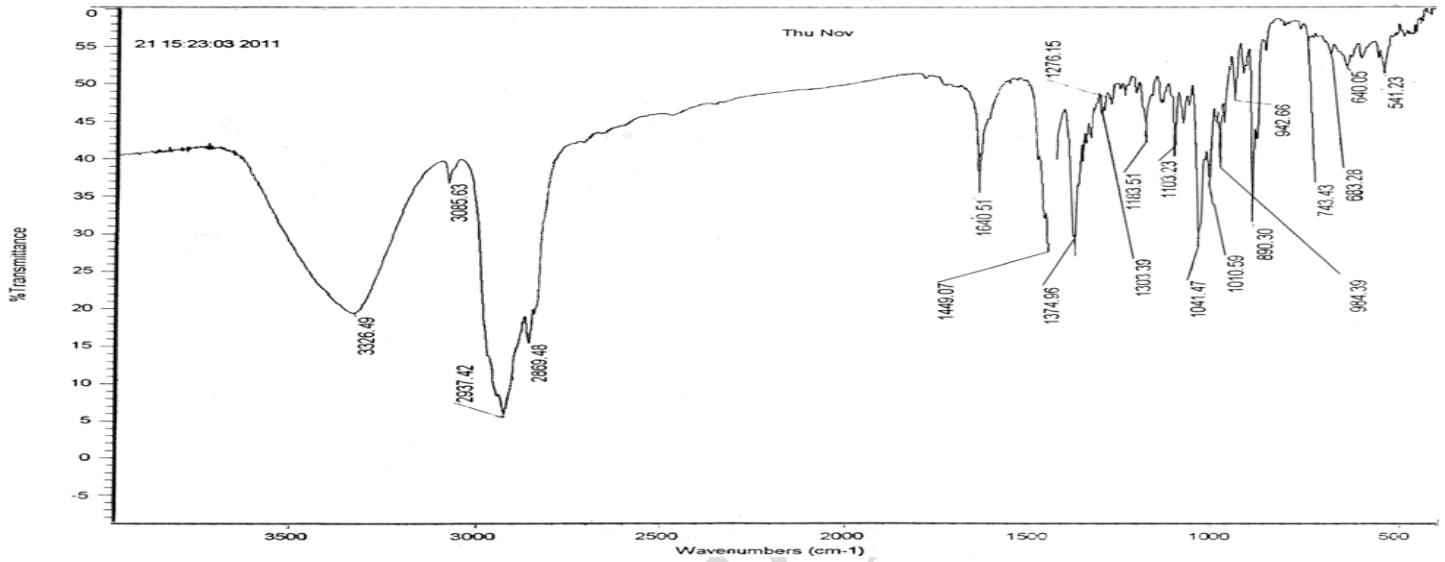
*** Not more than**

Table No. 02 Preliminary qualitative tests of various extracts of flowers of *Jasminum auriculatum* Vahl.

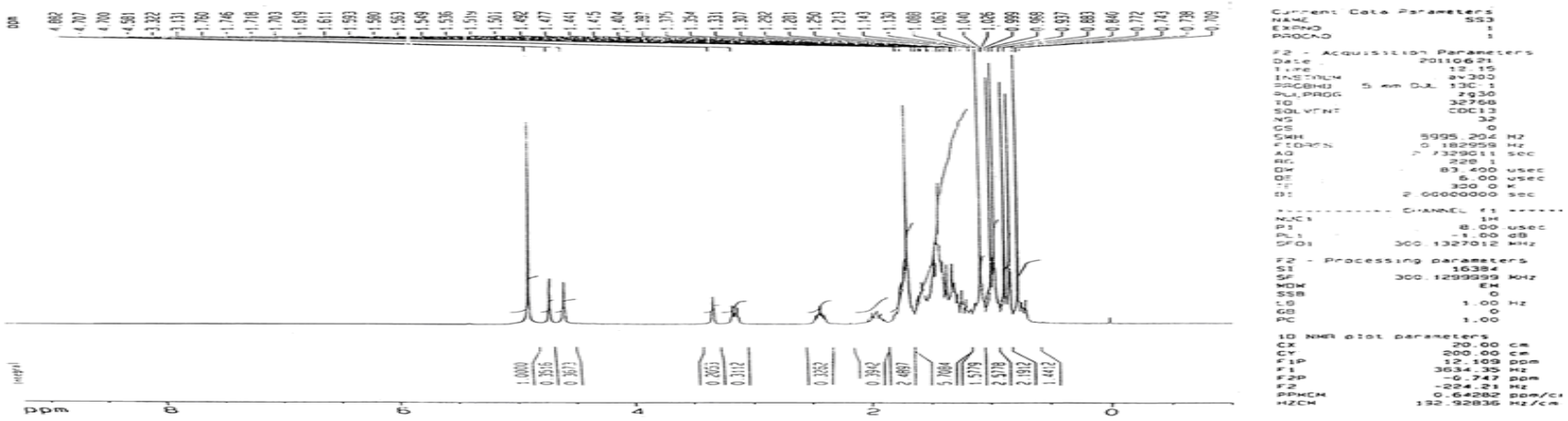
Phytoconstituents	Flowers		Successive extracts of Flowers		
	AlcE	AqE	PEE	CE	BE
Alkaloids	-	-	-	-	-
Amino acids	-	-	-	-	-
Carbohydrates	+	+	-	-	+
Fats and oils	-	-	+	-	-
Flavonoids	+	+	-	-	-
Glycosides	+	+	-	+	-
Gums and mucilage	-	-	-	-	-
Proteins	-	-	-	-	-
Saponins	-	+	-	-	-
Steroids	+	+	+	+	+
Tannins	+	+	-	-	-
Triterpenoids	+	+	-	-	-
Vitamins	-	-	-	-	-
Organic acids	-	-	-	-	-

PEE = Petroleum Ether (60-80^{0}C) extract ; CE = Chloroform extract

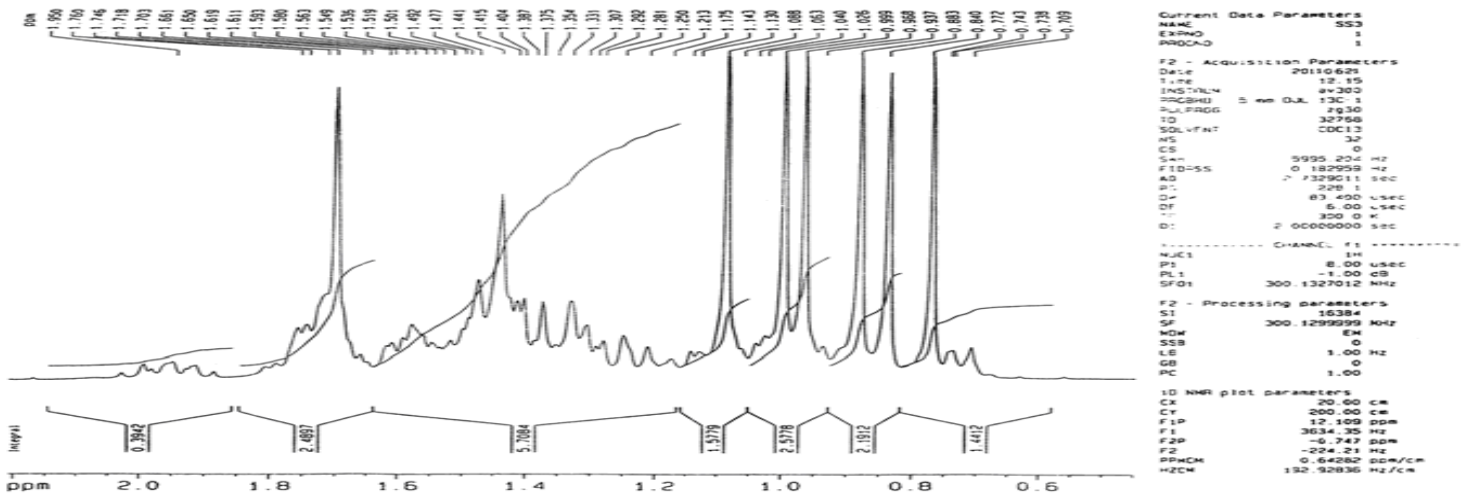
BE = Benzene extract ; AlcE= Alcoholic extrac AqE= Aqueous extract ; '+' = Present '-' = Absent



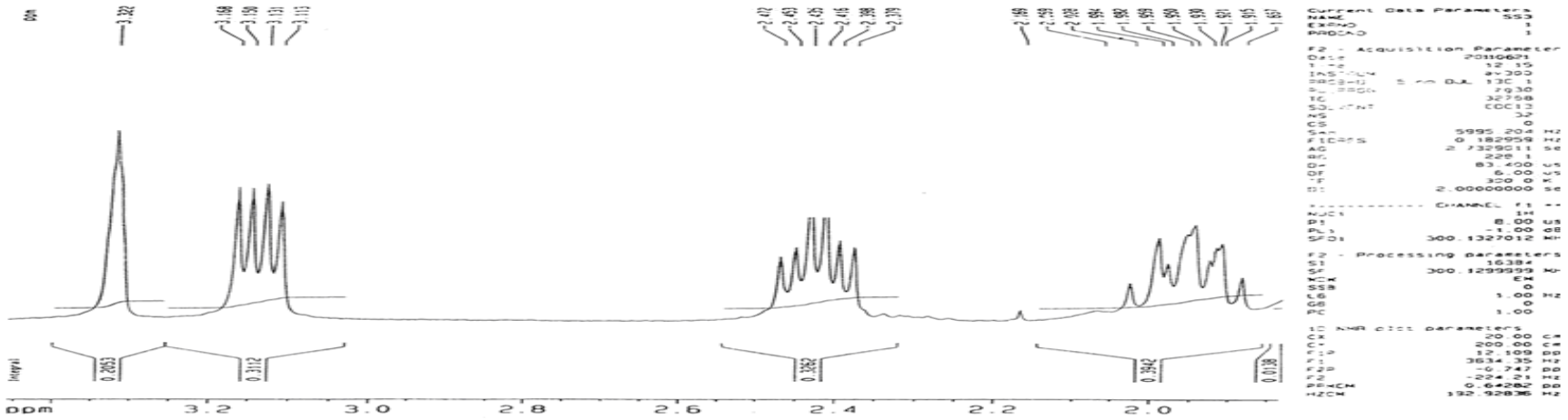
IR of Compound I (Lupeol)



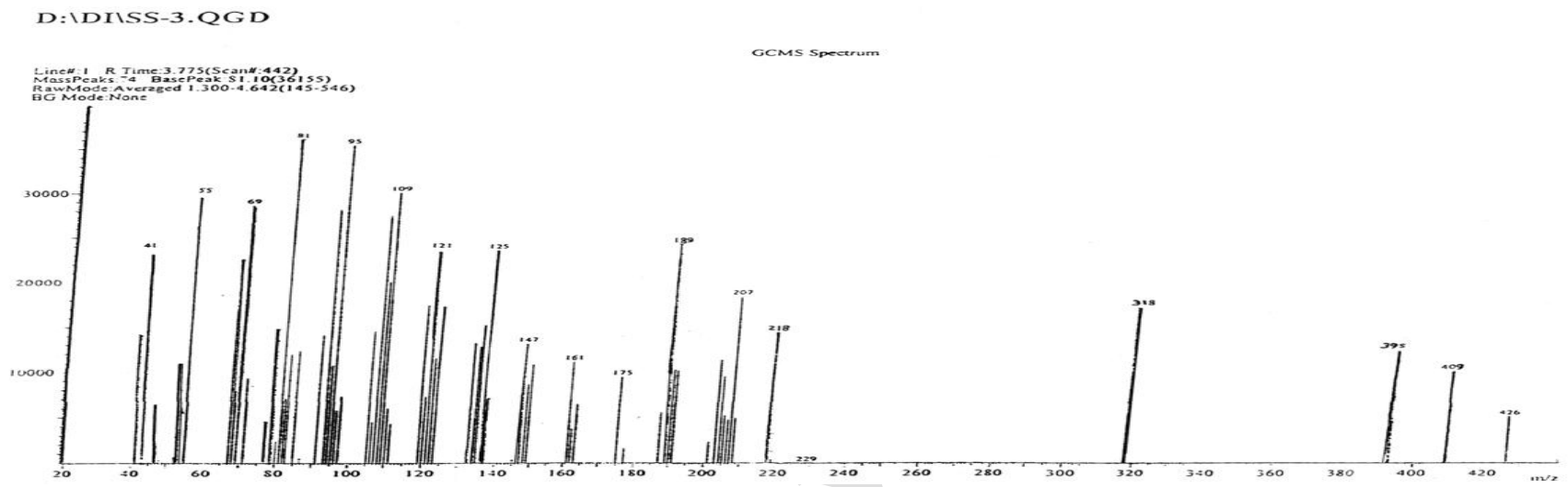
^{1}HNMR of Compound I (Lupeol)



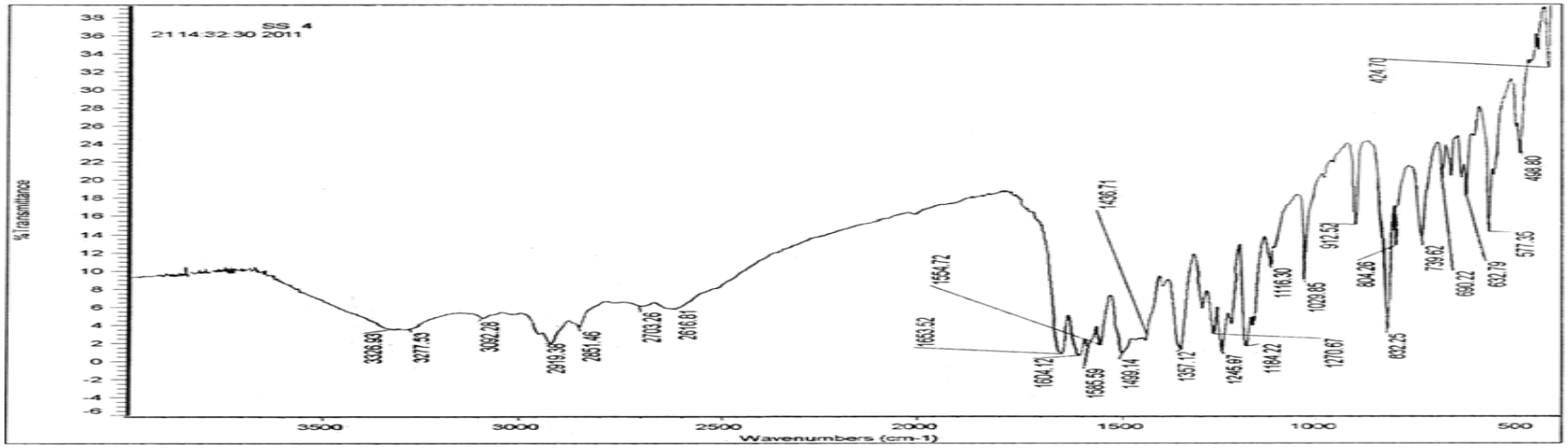
^{1}HNMR of Compound I (Lupeol)



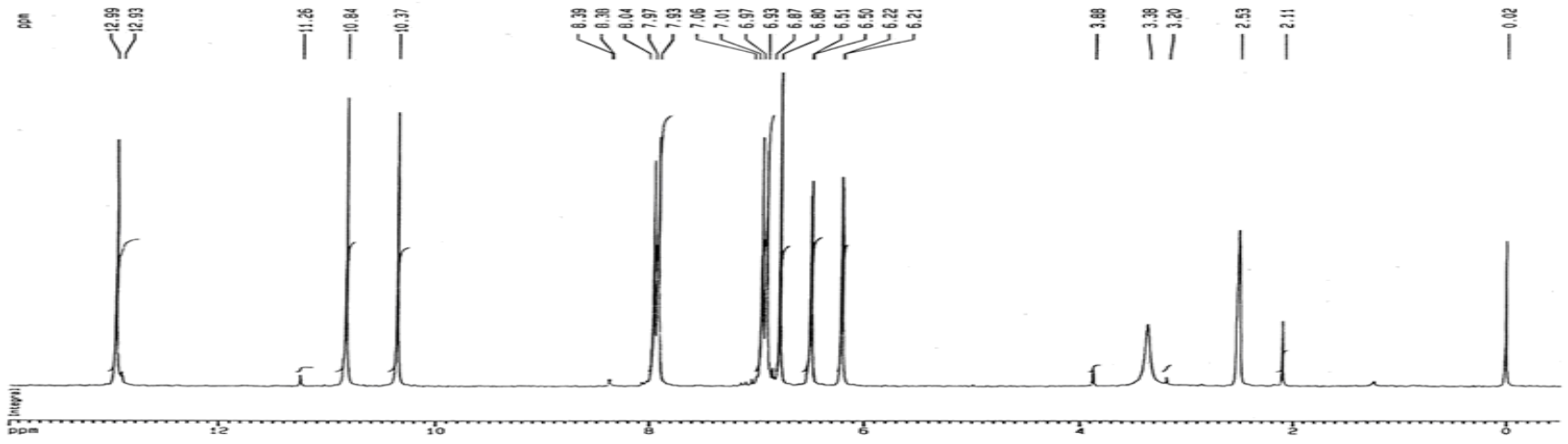
^{1}HNMR of Compound I (Lupeol)



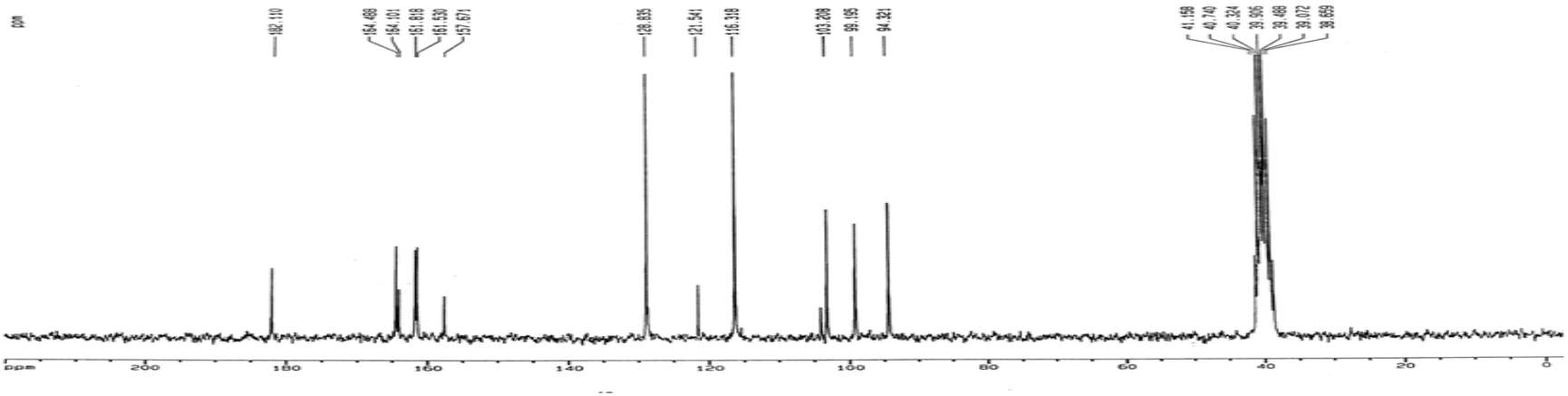
Mass Spectra of Compound I (Lupeol)



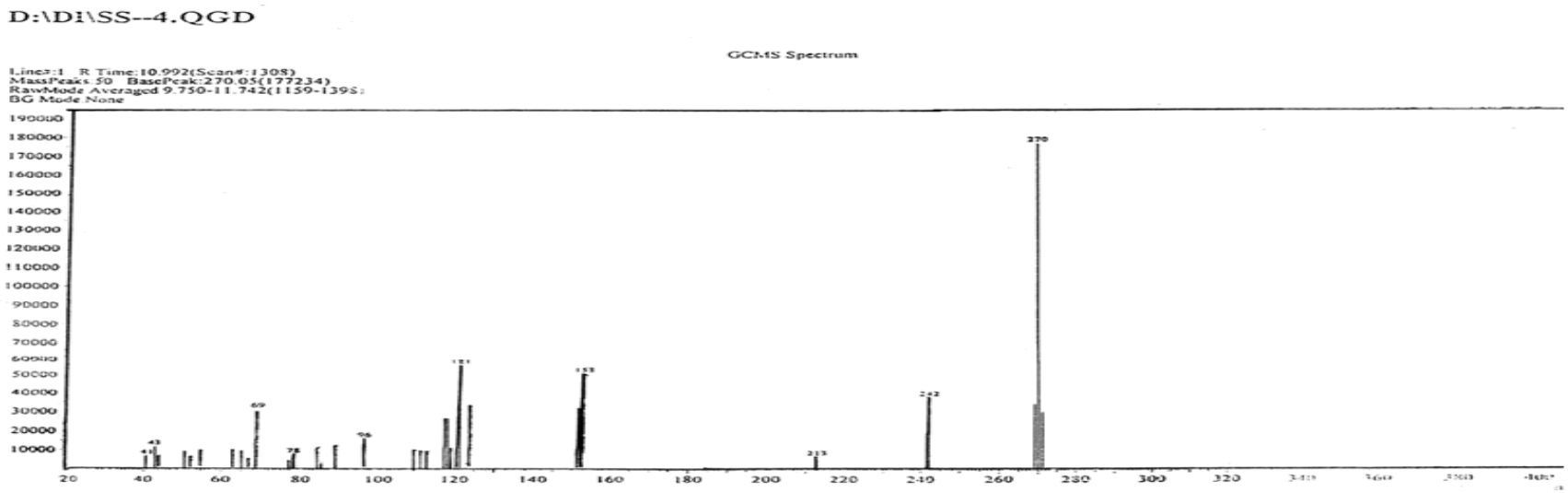
IR of Compound II (Apigenin)



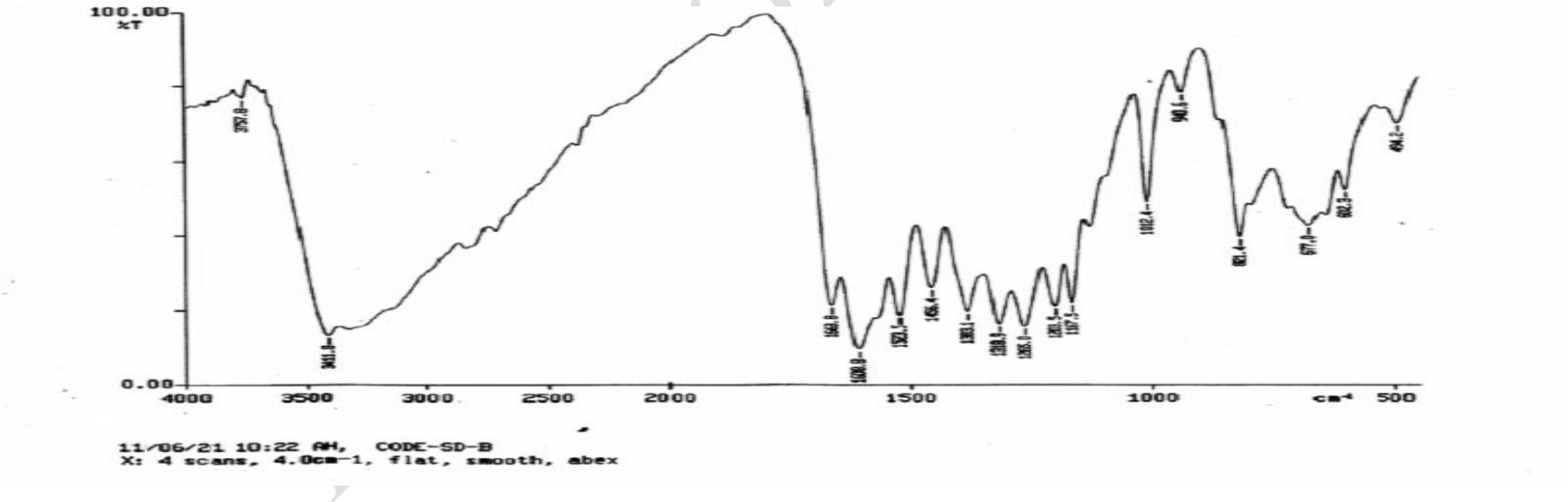
[1]HNMR of Compound II (Apigenin)



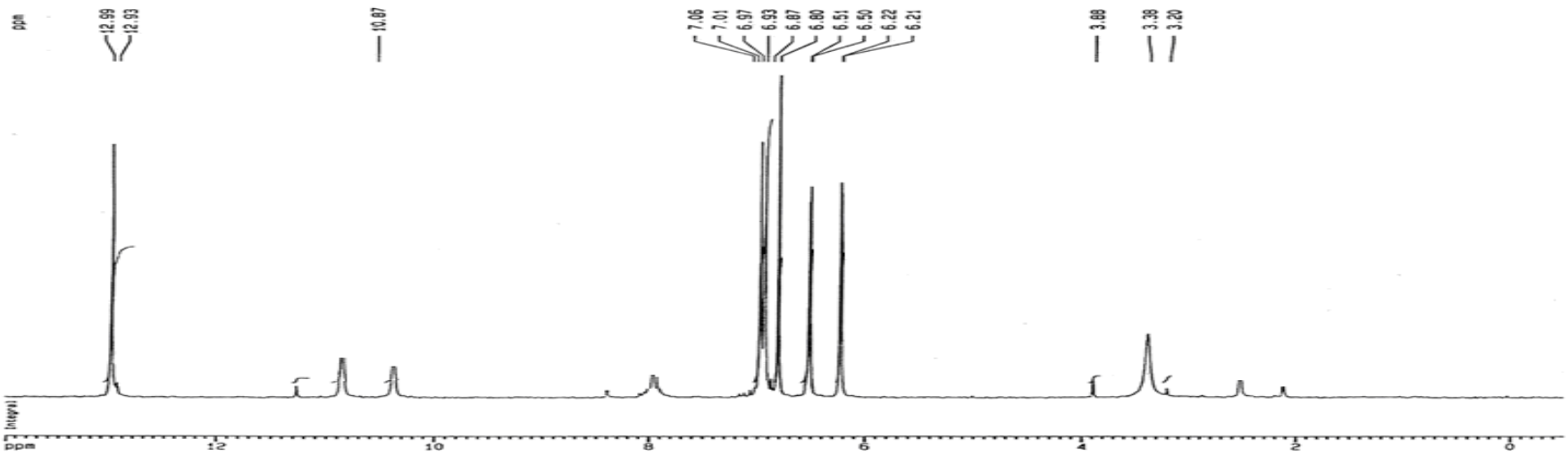
[13]CNMR of Compound II (Apigenin)



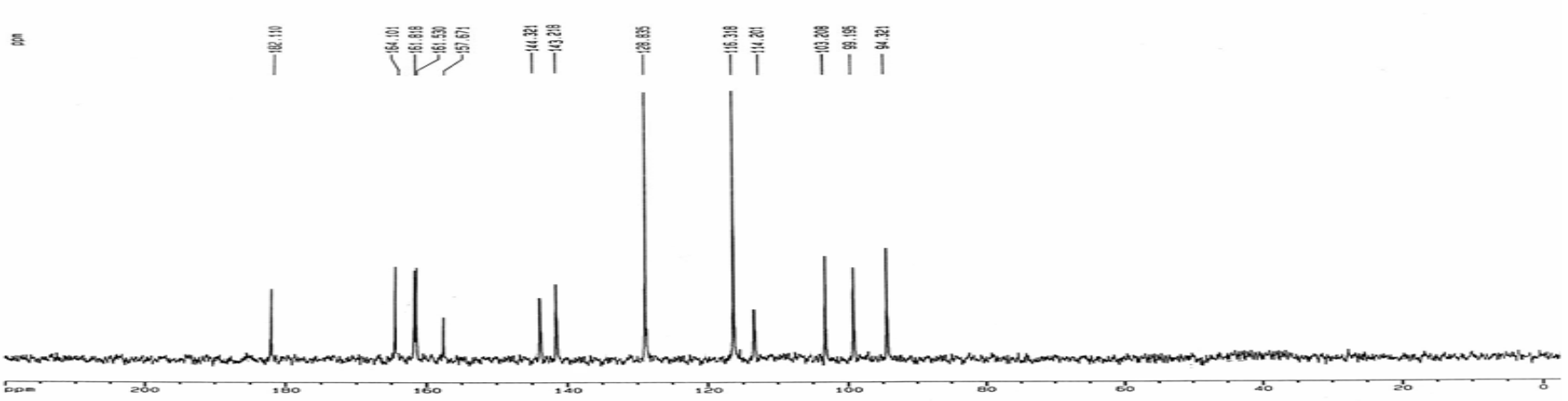
Mass Spectra of Compound II (Apigenin)



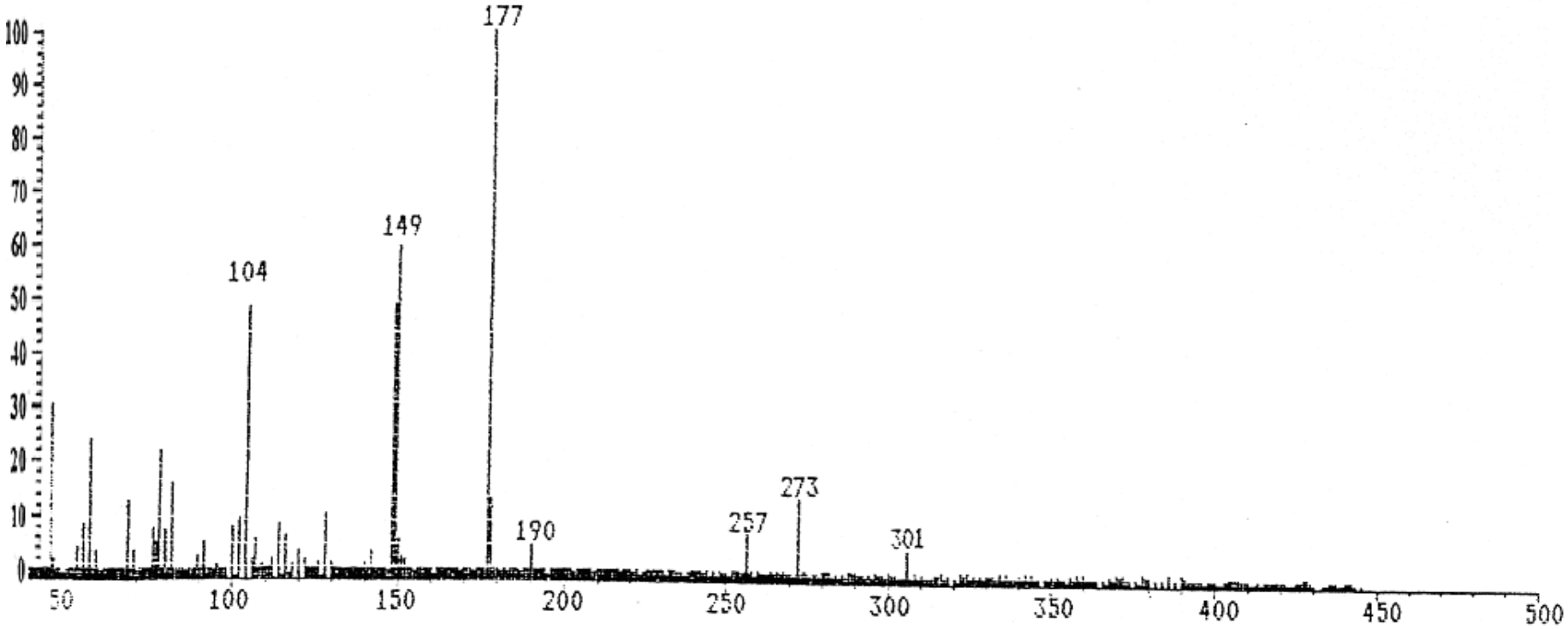
IR of Compound III (Quercetin)



^{1}HNMR of Compound III (Quercetin)



^{13}CNMR of Compound III (Quercetin)



Mass Spectra of Compound III (Quercetin)

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