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ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS IN PETROLEUM ETHER EXTRACTS FROM *CISSUS PALLIDA* LEAVES

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ABSTRACT

The study was aimed to scientifically explore the leaves of plant *Cissus pallida* for their novel Phytochemicals and Therapeutic uses, as very less data was available for this specific plant. A study was performed on Petroleum Ether extracts of leaves of *Cissus pallida* in search of the therapeutically active phytochemicals which might be present in it and serve as potentially active therapeutic compound. The value of medicinal plants, herbs and spices as herbal remedies is being lost due to lack of awareness. In the present study the leaves of *Cissus pallida* were examined. Specimens were collected from Nallamala forest region and were identified and authenticated. Three steroids were isolated from the petroleum ether extract and were characterized and identified by using spectral analytical techniques.

KEYWORDS: Petroleum ether, *Cissus pallida*, Medicinal plants.

1. INTODUCTION TO GENUS-*CISSUS*

There is interest in identifying plants or groups of plants that are used in traditional medicines around the world. However, scientific backing authenticates the proper use of these plants and also removes any medicines that may cause more harm than good to patients. It is encouraging to see that recently, many researchers are interested in giving scientific authentication and explanation to the activity of plants used in traditional medicine around the world. One such group of plants that is used in all the continents and is implicated to treat different ailments is plants belonging to the genus '*Cissus'*. It belongs to the *Vitaceae* family

which includes the common fruit- grapes (*Vitis vinifera*) and the medicinal properties of resveratrol, an active ingredient in grapes, are well established. It is anti-diabetic.[1] antineurogenerative diseases[2] anti-cancer,[3] protects from cardiovascular disease,[4] increases longevity[5] to name a few.

Plant description

As per Bentham & Hooker's classification, the plant is classified as

1. Kingdom : Plantae
2. Clade : Angiosperms
3. Order : Vitales
4. Family : Vitaceae
5. Genus : *Cissus*
6. Species : C. pallida
7. Synonym: Cissus adnata, Vitis simplex, Vitis adnata, Cissus compressa.

2. METHODOLOGY

Materials uses: Round bottomed flaks, soxhlet apparatus, heating mantle, Pet. Ether, china dishes, TLC plates, silica gel, glass slides, column for column chromatography, capillary tubes, hot air oven, UV chamber, Chloroform, Sulfuric acid, acetic anhydride/acid, Hexane, Acetic acid, Diethyl ether etc.

- The fresh plant materials were collected from Nallamalla forest region. During the collection of plant, it was kept in mind that the specimens to be studied were healthy.
- The collected plant material was identified and their authenticity was confirmed by Botanist.
- Both the processes – hot (soxhlet) and cold extraction (maceration) procedures were performed and in results of TLC profile of the extracts no significant change was observed. So, Soxhlet extraction was preferred as method of extraction as the yield was good.

Dried and powdered leaves weighing 150 gms was extracted using soxhlet apparatus with solvents of increasing polarity starting from Petroleum ether followed by Chloroform and Methanol etc.

2.1. Physical characteristics of extracts

The physical characteristics of the extracts like consistency, color, appearance of the extracts and their percentage yield were recorded.

Successive extract	Colour	Consistency	% Yield (w/w)
Petroleum Ether	Greenish black	Sticky mass	1.76

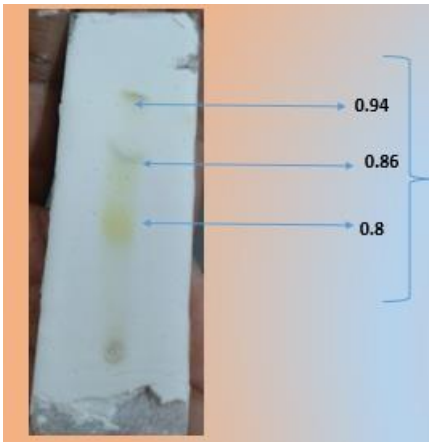
	Test for Steroids :	
PET. ETHER EXTRACT		
1	1. Chemical test was Positive.	Liebermann-Burchard test: 1 ml of the extract was treated with 2 ml of chloroform, 10 drops of acetic anhydride and 2 drops of H2SO4. Appearance of rose red colour which quickly changes through blue to green indicated the presence of sterols.
	Liebermann-Burchard test	

2.2. Thin Layer Chromatography

Sl. No.	Solvent System	Solvent distance	Solute distance	Rf Value
1	Hexane : Acetic acid : Diethyl ether (8:1:1)	4.7	1.4	0.29
2	Hexane : Acetic acid : Diethyl ether (7:1.5:1.5)	4.8	2.5	0.5
3	Hexane : Acetic acid: Diethyl ether (6:2:2)	5	4.8 2.9	0.9 0.5
4	Hexane : Acetic acid: Diethyl ether (6:3:1)	5.0	4 4.3 4.7	0.8 0.86 0.94

2.3. Column chromatography

The descended liquid was tested for different chemical test and fractions were collected and TLC was performed on them. Then the fractions with similar Rf values were mixed and the fractions have different Rf values were further separated with column chromatography.



TLC of Pet. Ether

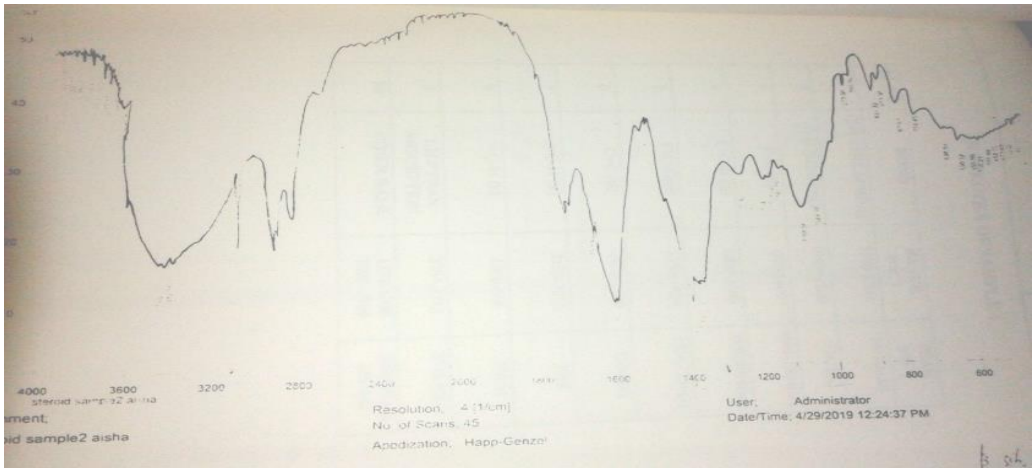
3. Characterization of Phytochemicals By Spectral Analysis

Finally, after the complete separation of the extract which was obtained by solvent extraction with petroleum ether, three different compounds were observed to be present based on their Rf values.

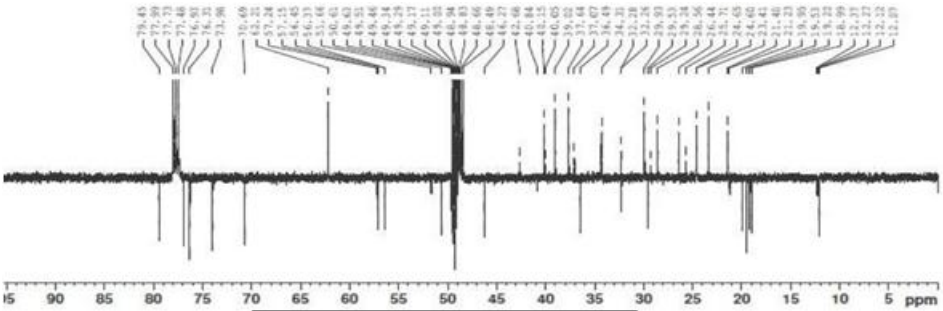
Sample No.	Phytochemical class (Test positive for)	Rf Value	Extract
1	STEROID	0.94	PET ETHER
2	STEROID	0.86	PET ETHER
3	STEROID	0.8	PET ETHER

3.1. Sample 1

3.1.1. Ir spectra of sample 1



SL. NO.	BOND	RANGE (cm^{-1})	PEAK (cm^{-1})
1	O-H (Stretching)	3500-3200	3411(m,b)
2	O-H BENDING	1440=1220	1361(m)
3	C-C (S)	600-1400	1068-1204(st)
4	C-C-H (S)	2800-3000	2850,2924(m)
5	C-C-H(B)	1350-1480	1334,1336(s)
6	C=C (S)	1620-1680	1630(m)
7	=C-H (S)	3100-3010	3098
8	=C-H (b)	1000-666	636.51
9	T.H.Pyran Absorption	2764-2713	2738.92(s)
10	Ether R-O-R	1320-1000 1000-1300	1008.56(m) 1361(st)



C^{13} NMR Spectra of sample 1

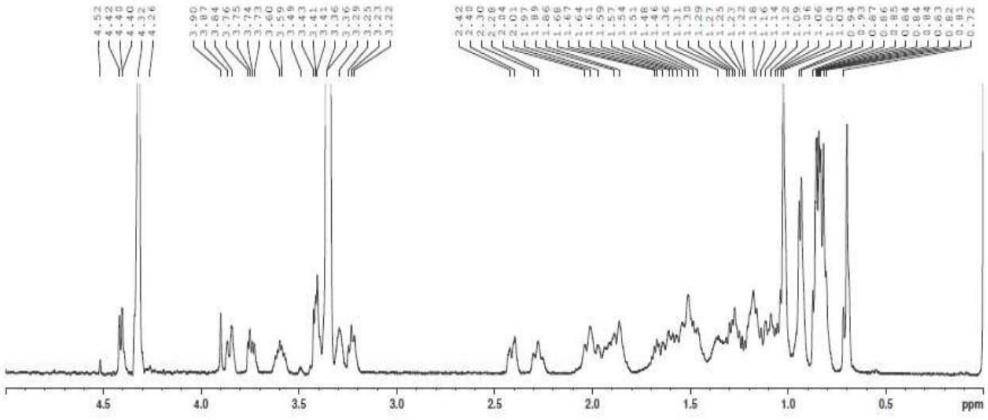
3.1.2. C^{13} NMR SPECTRA OF SAMPLE 1

SL.NO	BOND	RANGE	VALUE
1	C-C	0-50	30.14 (t)
2	C-C	0-50	27.85(d)
3	C-C	0-50	76.95 (d)
4	C-C	0-50	39.83 (s)

5	C=C	100-150	140.32(d)
6	C=C	100-150	121.26(d)
7	C-C	0-50	31.45(t)
8	C-C	0-50	49.63(s)
9	C-C	0-50	36.25(d)
10	C-C	0-50	38.33(s)
11	C-C	0-50	20.64(t)
12	C-C	0-50	40.00(d)
13	C-C	0-50	41.89(s)
14	C-C	0-50	55.46(s)
15	C-C	0-50	23.91(t)
16	C-C	0-50	29.30(t)
17	C-C	0-50	56.22(s)
18	C-C	0-50	42.05(d)
19	C-C	0-50	46.7(t)
20	C-C	0-50	36.49(s)
21	C-C	0-50	19.76(t)
22	C-C	0-50	33.37(t)
23	C-C	0-50	25.43(q)
24	C-C	0-50	45.17(q)
25	C-C	0-50	22.63(q)
26	C-C	0-50	11.71(q)
27	C-C	0-50	28.72(q)
28	C-C	0-50	18.97(s)
29	C-C	0-50	18.66(q)

SL.NO	BOND	RANGE	VALUE
1'	-O-C-O-	85-100	96.2 (d)
2'	C-O	50-100	74.4 (d)
3'	C-O	50-100	78.9 (d)
4'	C-O	50-100	71.5 (d)
5'	C-O	50-100	79.5 (d)
6'	C-O	50-100	61.11

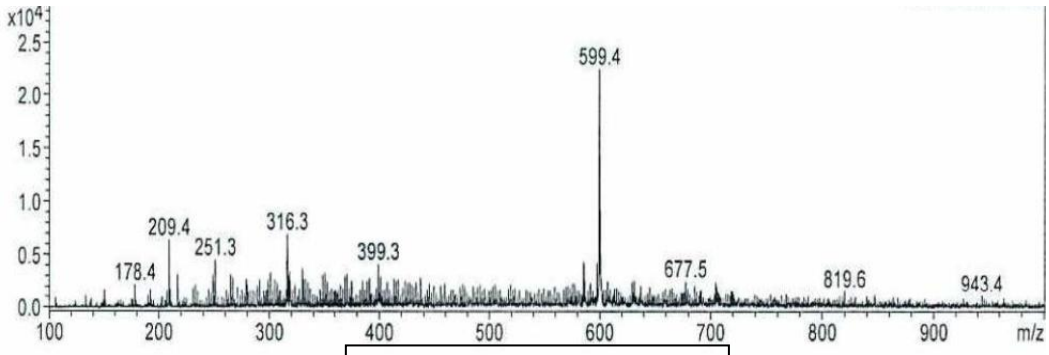
3.1.3. H^1 NMR SPECTRA OF SAMPLE 1



H^1 NMR Spectra of sample

SL. NO.	Number/Position	Protontype	δ	J
1	H-1	2H, m	1.724	
2	H-2	1H, t	2.50	J = 11.76
3	H-2	1H,dd	2.75	J = 2., 2.5Hz
4	H-3	1H, m	3.89	
5	H-4	2H, m	1.41	
6	H-6	1H, bs	5.37	
7	H-7	2H, bd	2.15	J = 11.3 Hz
8	H-8	1H, bd	1.94	J = 11.3 Hz
9	H-9	1H, t like	0.94	
10	H-11	2H, dt	1.10	J=13.07 Hz
11	H-12	2H, m	1.85	
12	H-14	1H, d	1.15	J = 13.02 Hz
13	H-14	1H, d	1.15	J = 13.02 Hz
14	H-15	2H, m	1.56	
15	H-16	2H, m	1.27	
16	H-17	1H, d	1.15	J = 13.20 Hz
17	H-18	3H, s	0.68	
18	H-19	3H, s	0.85	
19	H-20	1H, m	1.95	
20	H-21	3H, d	1.01	J = 7.0
21	H-22	2H, m	1.40	
22	H-22	2H, m	1.40	
23	H-23	2H, m	1.10	
24	H-24	1H, m	1.09	
25	H-25	1H, m	1.70	
26	H-26	3H,d	0.91	J = 6.42 Hz
27	H-27	3H, d	0.87	J = 6.42 Hz
28	H-28	2H, m	1.30	
29	H-29	3H, t	0.90	J = 6.5 Hz

3.1.4. Mass Spectra of Sample 1



Mass spectra of sample 1

Molecular ion peak: 677.5 Base Peak: 599.4

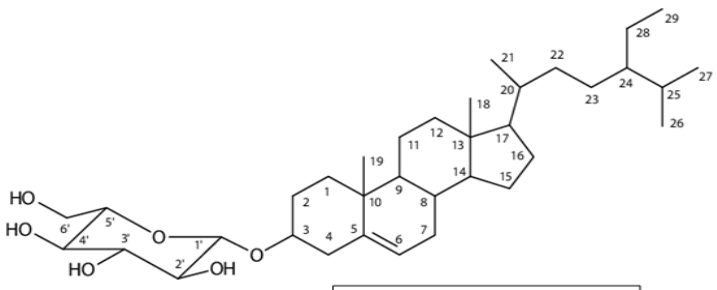
Fragment ion peak: 178.4

From the above data showed the sample 1 was solid, was interpret as Daucosterol (S1), a novel Sterol.

USES OF DAUCOSTEROL

In preparing medicines and health care products for promoting proliferation of neural stem cells. Daucosterol promotes the application in the cell proliferation of nerve cord medicine in preparation

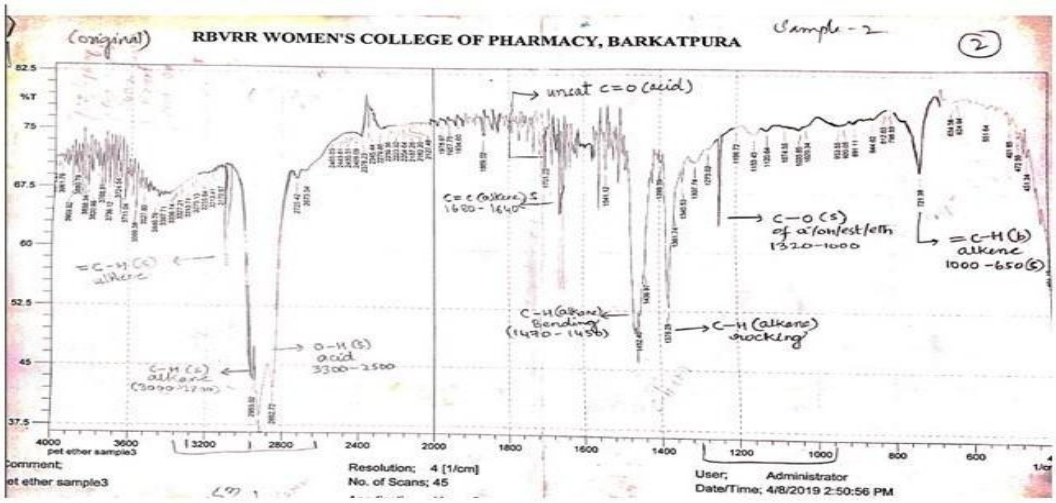
- Inhibits the cell proliferation
- Hypoglycemic activity
- Hepatoprotective activity
- Cardiovascular effects
- Anti-filarial effect
- Mosquito larvicidal effect



Structure of Daucosterol

3.2. Sample 2

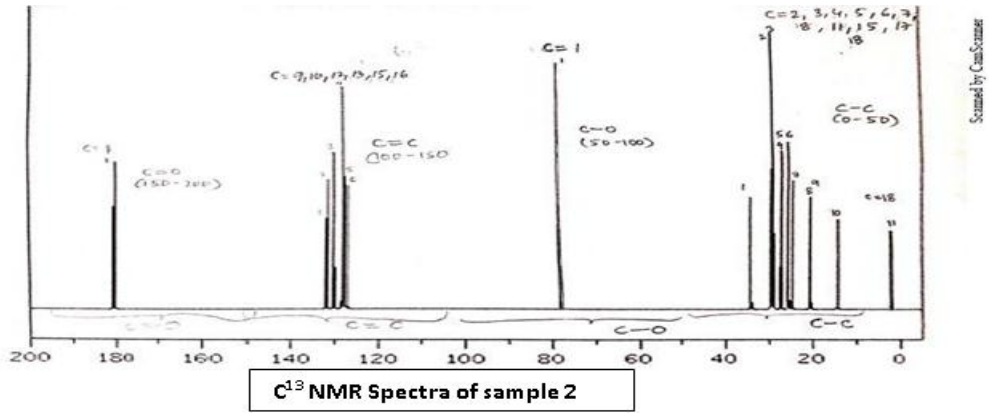
3.2.1. Ir spectra of sample 2



IR Spectra of sample 2

SL.NO	BOND	RANGE (cm^{-1})	PEAK (cm^{-1})
1	C-C (S)	600-1400	725.0 cm^{-1} (st)
2	C-C-H (S)	2800-3000	2953 (m)
3	C-C-H(B)	1350-1480	1336(s)
4	C=C (S)	1620-1680	1680(m)
5	C-C (S)	600-1400	790-1307(m)
6	C-C-H (S)	2800-3000	3200-3600 (m). broad
7	=C-H (S)	3100-3010	3200-3600
8	=C-H (b)	1000-666	721.55
9	C-O	1320-1000	1340
10	C=O	1760-1690	1701.22
11	O-H (acids)	3300-2500	2900

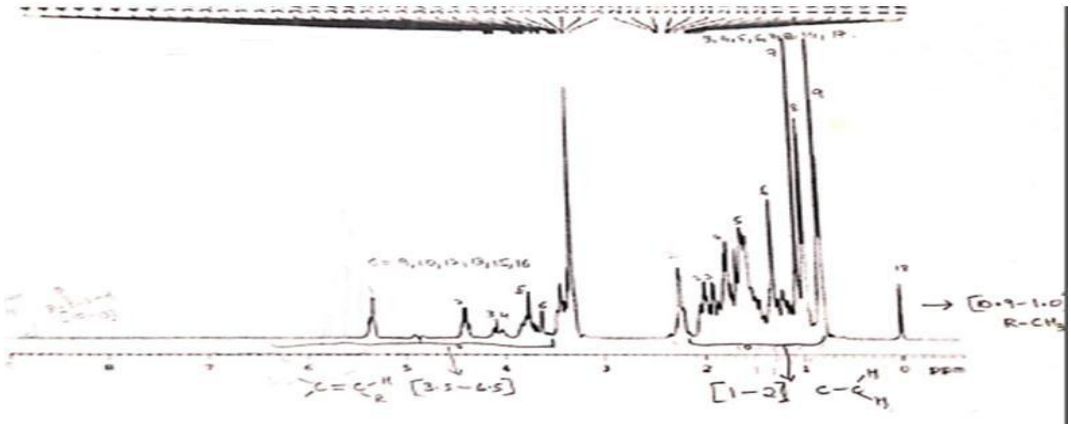
3.2.2. C^{13} NMR SPECTRA OF SAMPLE 2



C^{13} NMR Spectra of sample 2

SL.NO	BOND	RANGE	VALUE
1	O-C=O	150-200	40.12
2	=C-C	0-50	39.92
3	C-C	0-50	39.71
4	C-C	0-50	39.30
5	C-C	0-50	38.61
6	C-C	0-50	38.31
7	C-C	0-50	35.21
8	C-C	0-50	32.65
9	C=C	100-150	101.99
10	C=C	100-150	114.71
11	=C-C-	0-50	40.54
12	C=C	100-150	117.27
13	C=C	100-150	134.33
14	=C-C-	0-50	99.98
15	C=C	100-150	139.55
16	C=C	100-150	144.01
17	=C-C-	0-50	41.25
18	C-C	0-50	39.87

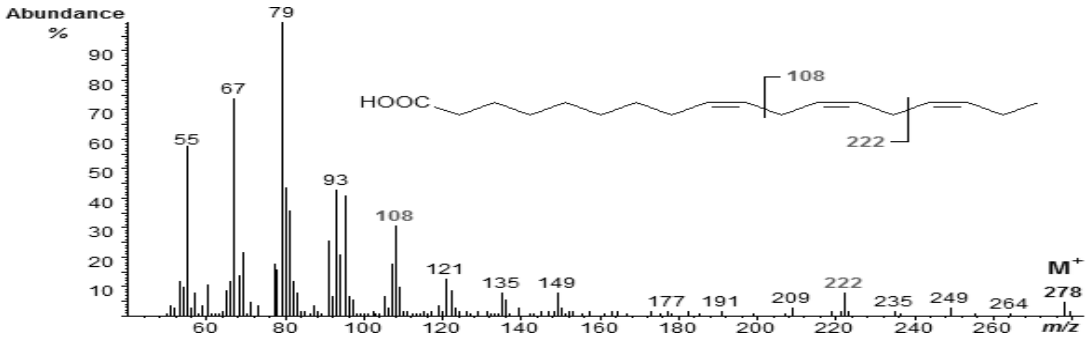
3.2.3. H^1 NMR SPECTRA OF SAMPLE 2



H^1 NMR Spectra of sample 2

POSITION	PROTON TYPE	δ VALUE	J
2	t,2,11	2.3	
3	t,2,13	1.62	
4	m,2.5	1.30	
5	m,2.5	1.31	
6	d 4.3	1.30	
7	s, 3	3.66	
8	m, 3.2	5.34	
9	m, 3.2	5.35	J = 11.6 Hz
10	m,2.5	1.31	
11	t, 1.3	2.77	
12	m, 3.2	5.37	J = 11.4 Hz
13	m, 3.2	5.36	
14	q, 2.7	2.06	
15	t, 2.3	1.34	J = 11.6 Hz
16		1.25	
17	d, 4.3	1.30	
18	m, 2.7	0.88	J = 6.42 Hz

3.2.4. MASS SPECTRA OF SAMPLE 2



Mass spectra of sample 2

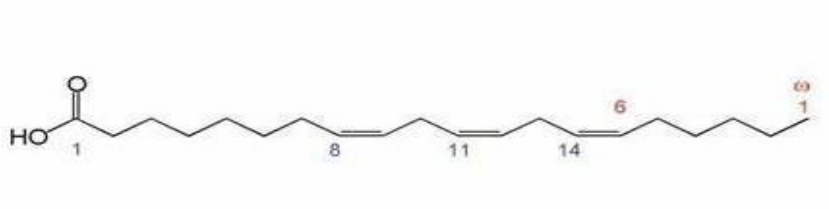
Molecular ion peak: 278;

Base peak: 278; Fragment ion peak: 55

From the above data showed the sample 1 was solid, was characterized as Linolenic acid.

Uses

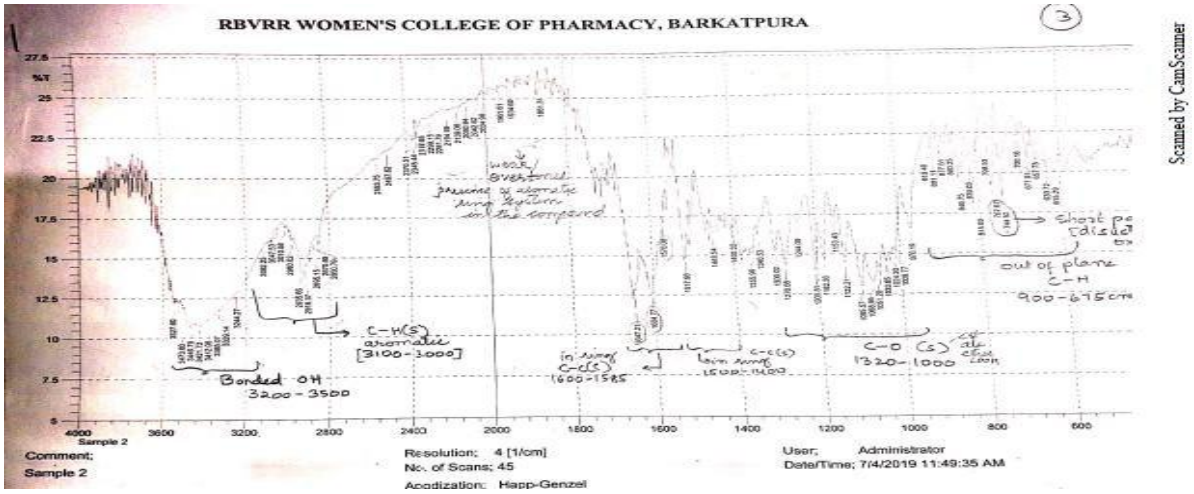
- **Used in systemic sclerosis**
- **Psoriasis**
- **Eczema**



Structure of Linolenic

3.3. SAMPLE 3

3.3.1. IR SPECTRA OF SAMPLE 3

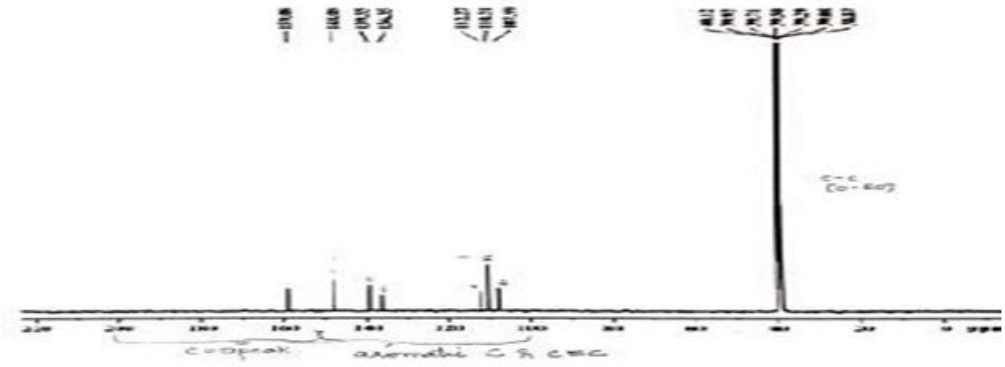


IR Spectra of sample 3

SL.NO	BOND	RANGE (cm^{-1})	PEAK (cm^{-1})
1	C-C (S)	600-1400	(st)
2	C-C-H (S)	2800-3000	2953 (m)
3	C-C-H(B)	1350-1480	1336(s)
4	C=C (S)	1620-1680	1680(m)
5	C-C (S)	600-1400	790-1307(m)
6	C-C-H (S)	2800-3000	3200-3600 (m). broad
7	=C-H (S)	3100-3010	3200-3600

8	=C-H (b)	1000-666	721.55
9	C-O	1320-1000	1340
10	C=O	1760-1690	1701.22
11	O-H (acids)	3300-2500	2900

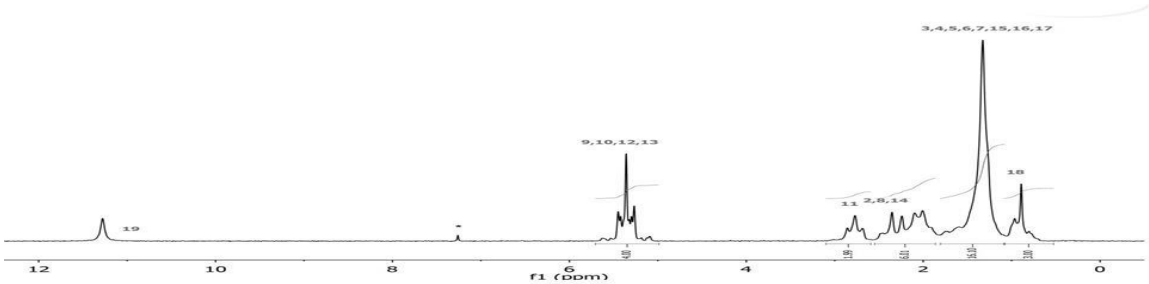
3.3.2. C^{13} NMR SPECTRA OF SAMPLE 3:



C^{13} NMR Spectra of sample 3

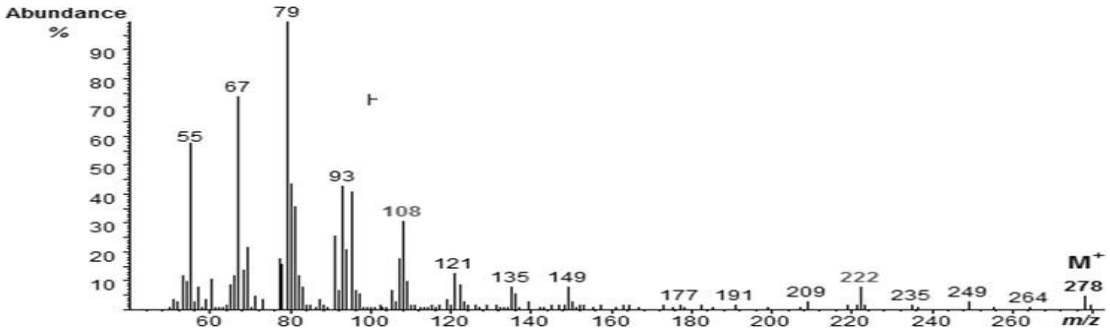
SL.NO	BOND	RANGE	VALUE
1	O-C=O	150-200	39.12
2	=C-C	0-50	36.92
3	C-C	0-50	38.8
4	C-C	0-50	39.30
5	C-C	0-50	37.61
6	C-C	0-50	38.31
7	C-C	0-50	38.21
8	C-C	0-50	34.65
9	C=C	100-150	98.99
10	C=C	100-150	109.61
11	=C-C-	0-50	42.44
12	C=C	100-150	117.27
13	C=C	100-150	132.73
14	=C-C-	0-50	101.98
15	C-C	100-150	137.55
16	C-C	100-150	146.01
17	C-C	0-50	45.25
18	C-C	0-50	39.87

3.3.3. H^1 NMR SPECTRA OF SAMPLE 3



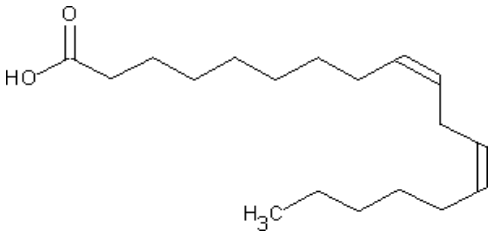
POSITION	PROTON TYPE	δ VALUE	J
2	t,2,11	2.4	
3	t,2,13	1.82	
4	m,2.5	1.30	
5	m,2.5	1.11	
6	d 4.3	1.27	
7	s, 3	3.82	
8	m, 3.2	5.44	
9	m, 3.2	5.47	J = 11.4Hz
10	m,2.5	1.42	
11	t, 1.3	2.85	
12	m, 3.2	5.42	J = 10.8Hz
13	m, 3.2	5.36	
14	q, 2.7	2.19	
15	t, 2.3	1.45	
16		1.37	
17	d, 4.3	1.43	
18	m, 2.7	0.74	J = 6.42 Hz

3.3.4. MASS SPECTRA OF SAMPLE 3



Mass spectra of sample 3

From the above data showed the sample 1 was solid, was characterized as Linolenic acid. Linoleic acid.



Structure of Linolenic

USES

- In immune function.
- In blood platelet aggregation.
- In synthesis of harmone like agents.
- From the above data showed the sample 1 was solid, was characterized as Linolenic acid.

4. CONCLUSION

The leaves of plant *Cissus pallida* were not extensively studied so the study was aimed to explore the phytochemical that are present in the leaves of *Cissus pallida* which might be therapeutically usefull. For this purpose extraction was carried out and the petroleum ether extracts were subjected for isolation through column chromatography. By using gradient elution system totally three compounds were isolated in pure form, out of which two were liquid remaining one was solid.Further these isolated compounds were characterized by Infra Red, Nuclear Magnetic Resonance (C^{13},H^{1}) and Mass spectroscopy. The spectras so obtained were matched with those of library standards to reveal their identity and if not to look for newer compounds. Those three isolated compounds were identified by spectral analysis as Daucosterol, Linolenic acid and Linoleic acid which are medicinally useful phytochemicals.

5. FUTURE SCOPE: The plant *Cissus pallida* is a potential candidate with novel phytochemicals present in it. There is a lot of scope for the other parts of the plants to be explored for the presence of novel phytochemical in it. The plant can be regarded as a potential drug candidate.

REFERENCES

1. Szkudelski T, Szkudelska K Anti-diabetic effects of resveratrol. Ann. N. Y. Acad. Sci., 2011; 1215: 34-39.
2. Sun AY, Wang Q, Simonyi A, Sun GY Resveratrol as a therapeutic agent for neurodegenerative diseases. Mol. Neurobiol., 2010; 41(2-3): 375-383.
3. Athar M, Back JH, Kopelovich L, Bickers DR, Kim AL Multiple molecular targets of resveratrol: Anti-carcinogenic mechanisms. Arch. Biochem. Biophys., 2009; 486(2): 95-102.
4. Bertelli AA, Das DK Grapes, wines, resveratrol, and heart health. J. Cardiovasc. Pharmacol., 2009; 54(6): 468-476.
5. Das DK, Mukherjee S, Ray D Resveratrol and red wine, healthy heart and longevity. Heart Fail. Rev., 2010; 15(5): 467-477.

6. https://patents.google.com/patent/CN102755343A/en Application of daucosterol in preparing medicines for promoting proliferation of neural stem cells.