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A New Flavonoid Compound (Rosmarinic Acid) Isolated from Methanolic Extract of *Desmodium triflorum*

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ABSTRACT

This study evaluated the new chemical compound from the methanolic extract of *Desmodium triflorum* (L.). The leaves of *D. triflorum* extracted with solvent methanol and the methanolic extract was purified by chromatographic methods such as Thin Layer Chromatography, HPTLC, and column chromatography. Isolation and characterization of the purified fraction was done with spectroscopic methods such as UV, IR, NMR and Mass spectroscopy. The advantage of our method was the isolation and characterization of the new compound from *Desmodium triflorum.*

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INTRODUCTION

Desmodium triflorum, a medicinal plant commonly known as Jangali methi from the Fabaceae family. This plant is a slender, prostrate, branched, somewhat hairy herb, the stems of which are 10 to 30 centimeters long. The leaves are 3-foliolate and small. The leaflets are oblong-elliptic to obovate and 7 to 15 millimeters long. The flowers are purplish about 6 millimeters long, axillary, solitary, or 2 or 3 together, with slender pedicles about 1 centimeter long. The pods are 5 to 12 millimeters long, and of 2 to 6 joints[1].

The plant is acrid, sweet, cooling, expectorant, and galactagogue, and used in vitiated condition of pitta, cough, bronchitis, wounds, abscess, sores, pruritus, dysentery, flatulence and burning sensation[1]. This plant is also used in ache (stomach), dermatosis, dysentery, abscess, diarrhea, ophthalmia, rheumatism, sore, tonic, diuretic and tumor [2].

Reported activities are antioxidant and antiproliferative activity[3], analgesic and anti-inflammatory activity[4], anthelmintic action[5], anticonvulsant activity[6], antibacterial activity[7] and antinociceptive activity[8].

Phytochemical investigations of previous studies on *D. triflorum* have been able to isolate astragalin, cosmossiin, tectorigenin[9],vitexin, genistin, aliphatic alcohols, aliphatic acids, urosolic acid, oleanolic acid, campesterol, stigmasterol, *β*-sitosterol, campesterol-3-O-*β*-D-glucose, stigmasterol-3-O-*β*-D-glucose, sitosterol-3-O-*β*-D-glucose and (+)-pinitol[10].2-O-glucosylvitexin[11], 2-O-*β*-xylosylvitexin[12].Alkaloids such as hypaphorine, N,N-dimethyltryptophan, betaine, choline, *β*-phenethylamine, and N,N-dimethyl tryptamineoxide have also been isolated from *D. trflorum*[13].

MATERIAL AND METHODS

The leaves of *Desmodium triflorum* were collected from outfield medicinal garden near to Gwalior (M.P.). The plant leaves were washed thoroughly in tap water, dried in shade, finely powdered and used for extraction. The plant was identified and a voucher specimen has been deposited in the herbarium at Department of Pharmacognosy for future references. A voucher specimen number is BU/Bot/10/06 for *Desmodium triflorum*.

Extraction of plant material

Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. The extraction was done by following general procedure. Powdered material (leaves) was packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was subjected to further extraction process by dichloromethane. The extract was concentrated under vacuum. After completion of the total, the extracted powder was discarded and the extracts so obtained were further processed. The excess solvent in the extracts was removed by distillation and the concentrated extract so obtained was further dried at a temperature not exceeding 40^0C in a water bath. The extract was then collected, kept inapetri dish and stored in desiccators at room temperature[14].

TLC of methanolic extract of *Desmodium triflorum*

TLC was performed for the separation of various bioactive compounds from bioactive extract; methanolic was developed to find out the probable number of compounds present in them. On the pre-coated TLC plate, test samples (after dissolving in respective solvents) were applied in the form of spots with the help of fine capillary. Spots were marked on the top of the plate for their identification. Rectangular glass chambers were used for chromatography. To avoid insufficient chamber saturation and undesirable edge effect, a smooth sheet of filter paper was placed in TLC chamber and was allowed to be in the developing solvent. A number of developing solvent systems were tried during the study. Each time plate was sprayed with vanillin-sulphuric acid and heated at 115^0C for 5 minutes. The solvent system in which there is a satisfactory resolution was taken as a final solvent system. Solvent systems; Ethyl acetate: methanol: water (76:20:4) was found to be the most satisfactory solvent system. After the development of plates, they were air-dried and a number of spots, color, and R_f values were recorded [14].

TLC plate: Precoated TLC plate silica gel 60 F_{264}

Bioactive extract: methanolic extract

Solvent system: Ethyl acetate: methanol: water (76:20:4)

Spraying agents: Vanillin sulphuric acid

R_f Value=Distance traveled by solute/Distance traveled by solvent

HPTLC analysis of methanolic extract of *Desmodium triflorum*

HPTLC fingerprint profile was established for methanolic extract of *Desmodium triflorum*. TLC plate was kept in an ascending mode and activated at 120^0C for 1 hour prior to application of sample bands. The sample was dissolved in 10 ml of methanol and sonicated for 10 minutes, filtered and applied on TLC plates (5×10 cm) in 1 tracks (4µl for methanolic extract) in the form of a band and allowed to equilibrate for 10 minutes. The spotted plate was then dipped in the mobile phase and the solvent front was allowed to travel about 70-80% distance on the plate vertically. Plate was then removed from the chamber and dried.

Plate was then scanned at 254 nm for methanolic extract. A number of spots, color, R_f values and % relative areas were recorded [15].

TLC plate: Precoated silica gel 60 plates F_{264}

Solvent system: Ethyl acetate: methanol: water (76:20:4)

Bioactive fraction: Methanolic extract

Concentration: 4 µl

Detecting wavelength: 254 nm

Isolation of Active compounds from fractions of *Desmodium triflorum*

Column chromatography

Preparation of column, sampling and loading was same as described in bioactivity-guided isolation of phytoconstituents from *Desmodium triflorum*. The combinations of solvent systems developed for TLC was used as a mobile phase for column chromatography and column was eluted by gradient elution methods. Column was first eluted with pure ethyl acetate and then gradually with increasing quantity (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35 and finally 50:50) of methanol. Total 190 fractions were collected of 20 ml elutes. The column was eluted

till 90 % of fraction loaded eluted out. All the collected fractions were monitored simultaneously on a TLC plate using ethyl acetate: methanol: water (76:20:4) as solvent system[16]. The fractions showing same TLC pattern were pooled together and finally 5 fractions (F1-F5) were obtained.

Characterization and Identification of compounds

The structure was characterized by means of UV, IR, NMR, and Mass spectral analysis for the structure determination and their identity.

The UV spectrum of rosmarinic acid [Graph No (2)] exhibited two absorption maxima (in MeOH) at λ=290 and 330 nm.

The FTIR spectrum [Graph No (3)] showed distinguishable absorption bands at: 3165.4, 1707.2, 1617.4, 1515.6, 1348.7,1285.1,1260.4,1231.5,1200.5,1154.0,1113.4,1075.9,972.3,851.7, 818.8, 781.4cm^{-1}

The^{1}H-NMR spectrum [Graph No (4)] shows11 signals. The aliphatic region has two signals with chemical shifts at δ=2.92 ppm and δ=3.08 ppm and can also be assigned to the methylene group of the 3-(3,4-dihydroxyphenyl)-2hydroxypropanoicacidstructure unit. The six protons of the two aromatic rings have the chemical shifts. Additionally, the protons H-20 and H-30 of the double bond in the caffeic acid and appear as two doublets (δ=6.26 ppm and δ=7.49ppm) in the ^{1}H-NMR spectrum.

The^{13}C-NMR spectrum [Graph No (5)] shows 18 signals for 18 carbons. The peaks between δ=114.09 ppm and δ= 149.12 ppm are located in the aromatic region, corresponding to two aromatic rings. The two signals δ=177.41 ppm and δ=168.88 ppm in the carbonyl region are defined through the both carbonyl groups of the acid and ester functions in the moleculer osmarinic acid.

The Mass spectrum [Graph No(6)] revealed the a quasi molecular peak$ [M- H]- at m/z = 359.0 (100) (i.e., base peak). Further ions at m/z = 197.0 (10) and m/z = 161.1 (36) resulted from the loss of caffeic acid (163) and of 3-(3, 4- dihydroxyphenyl)-2-hydroxypropanoic acid (197). On the basis of the molecular mass at m/z = 360.1 and the structural information obtained by

NMR analysis, the molecular formula $C_{18}H_{16}O_8$ was attributed to compound rosmarinic acid.

RESULT AND DISCUSSION

Chromatographic Studies of Bioactive extract

TLC study has shown the presence of different components present in the methanolic extract of *Desmodium triflorum* when the extracts were run on the specific solvent system. Before reaching to the most optimum solvent system a number of systems were employed.

Table No1: TLC of methanolic extract of *Desmodium triflorum*

Sr. No.	Fractions	Solvent system	Detecting reagent	Color	No. of spots	R_f value of Spots
1	Methanolic	Ethyl acetate: methanol: water (76:20:4)	Vanillin sulphric acid, heated at 110^0C for 5 min	Green & yellow	5	0.20, 0.30, 0.53, 0.58, 0.68.

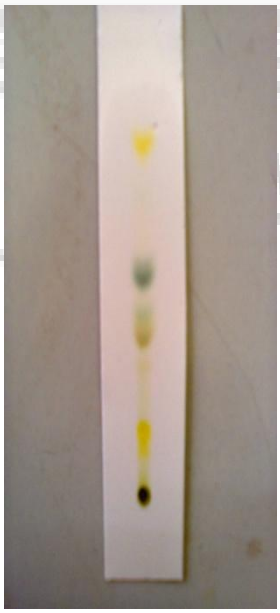
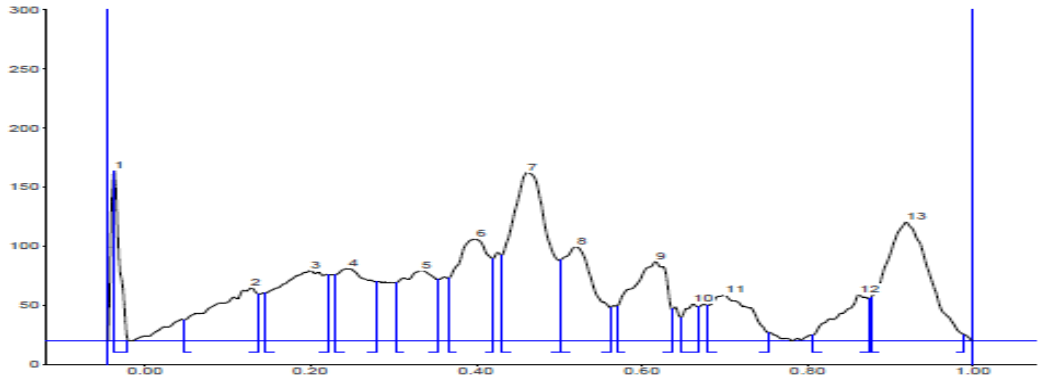


Figure No1 TLC of methanolicextracts

HPTLC analysis of methanolic extract of *Desmodium triflorum:*

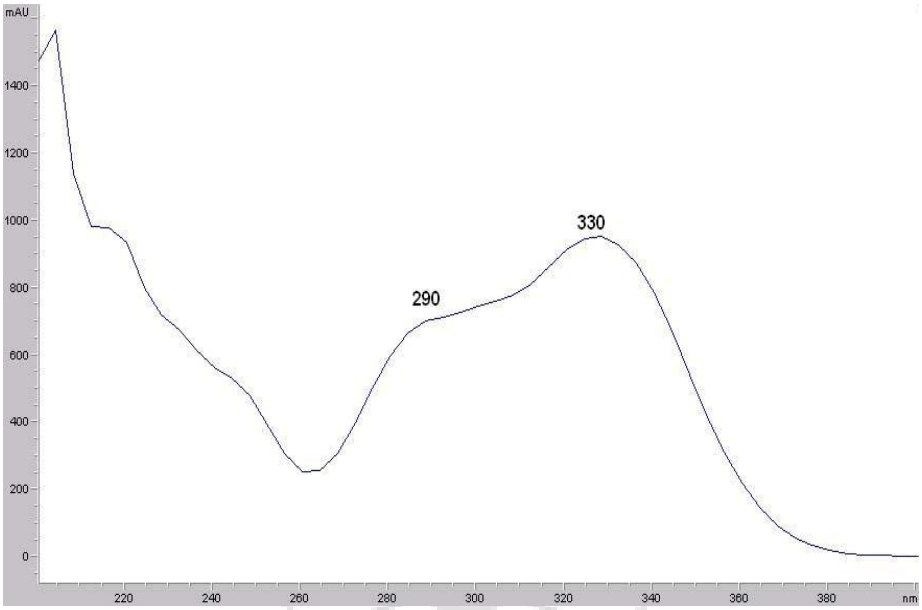


Graph No 1 HPTLC Fingerprint profile of methanolic extract

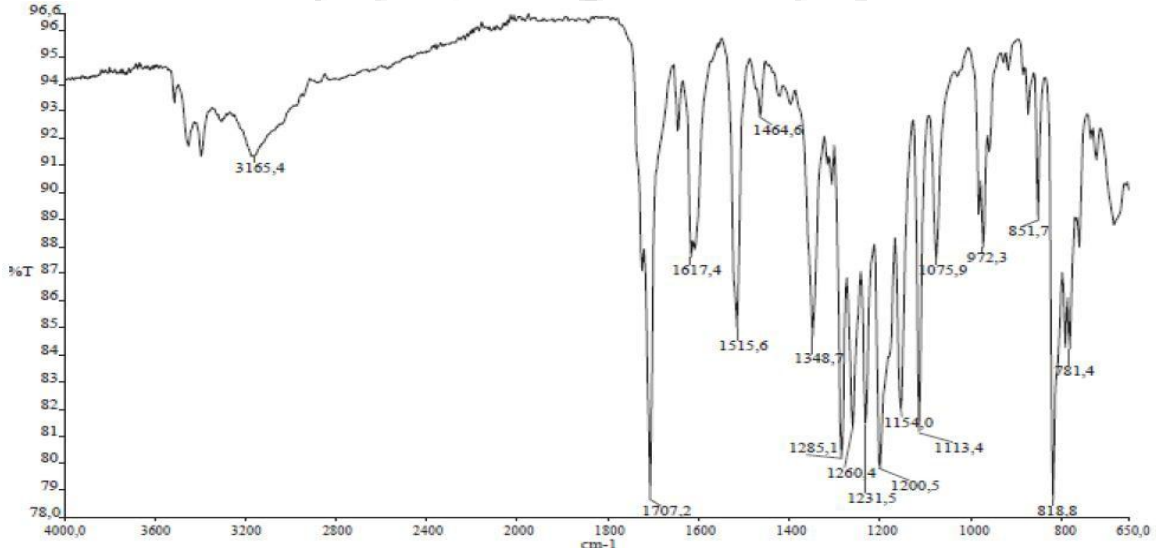
Table No 2: $R_f$valuesand relative percentage of compounds from methanolic extract

Sr.No.	Volume	Peak	Start R_f values	Start Height	Max Height	Max %	% Area	Assigned substance
1	4 µL	1	0.04	143.0	143.9	15.08	2.99	Sub 1
2	4 µL	2	0.5	17.5	44.8	4.70	6.77	Sub 2
3	4 µL	3	0.15	40.5	59.4	6.23	9.19	Sub 3
4	4 µL	4	0.23	55.9	61.2	6.41	6.59	Sub 4
5	4 µL	5	0.30	49.1	59.5	6.24	6.64	Sub 5
6	4 µL	6	0.37	52.5	86.1	9.03	9.50	Sub 6
7	4 µL	7	0.43	72.3	142.5	14.94	17.62	Sub 7
8	4 µL	8	0.50	68.4	79.8	8.37	8.22	Sub 8
9	4 µL	9	0.57	29.3	67.1	7.03	8.01	Sub 9
10	4 µL	10	0.65	19.7	31.4	3.29	1.40	Sub 10
11	4 µL	11	0.68	29.8	38.6	4.05	4.90	Sub 11
12	4 µL	12	0.81	5.0	39.3	4.11	3.82	Sub 12
13	4 µL	13	0.88	36.0	100.5	10.53	14.76	Sub 13

Characterization and Identification of compounds



Graph No (2) UVspectrumofrosmarinic acid

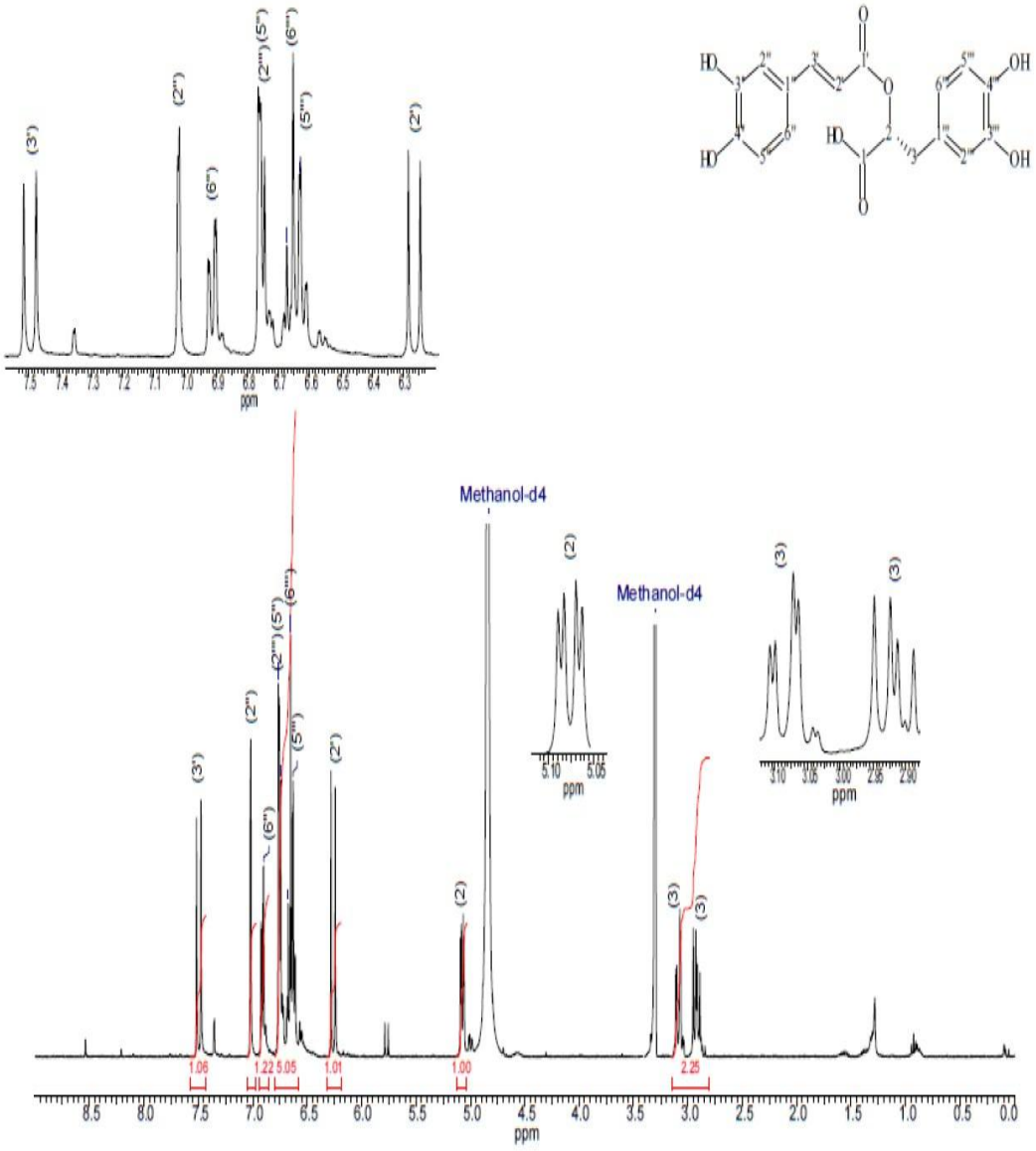


Graph No (3) IR spectraof rosmarinic acid

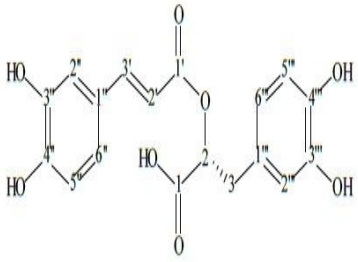
Table No 3: Chemical shifts of rosmarinic acid and standard

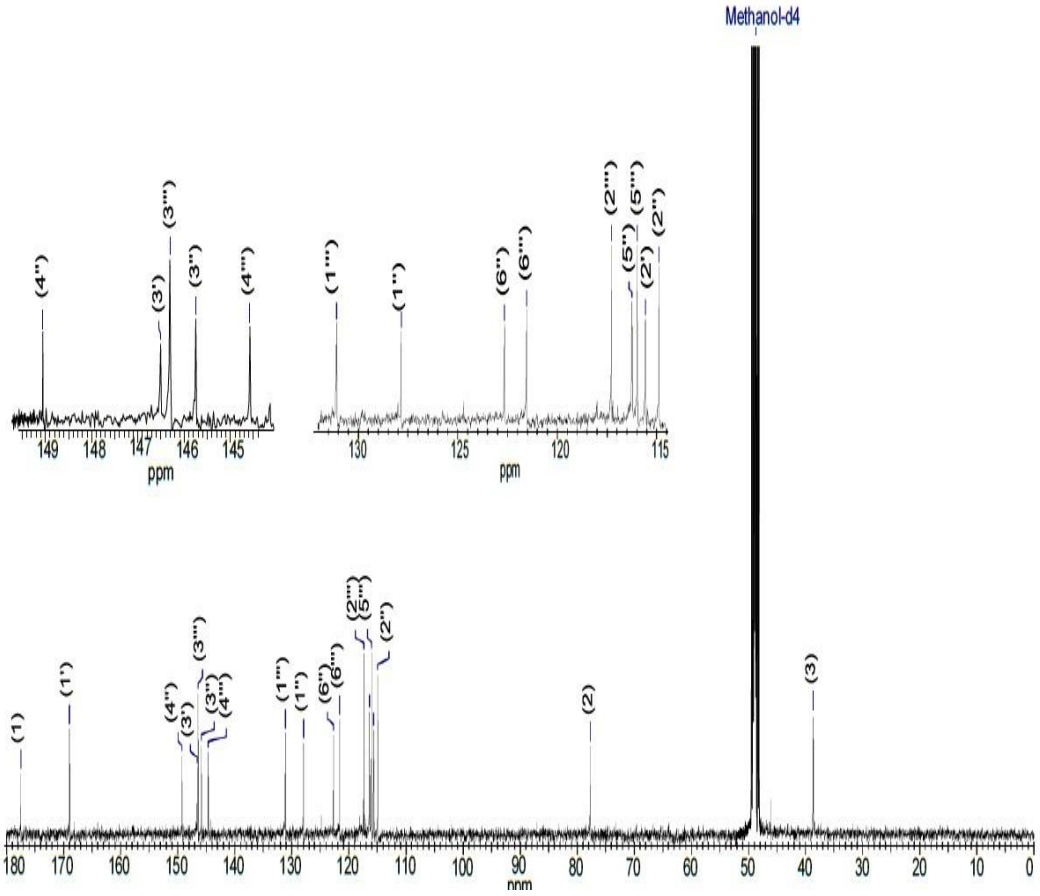
Atom numbers	$^{13}C^{*}$ δ/ppm	$^{13}C^{**}$ δ/ppm	$^{1}H^{*}$ δ /ppm(Mult.,J(Hz),H)	$^{1}H^{**}$ δ/ppm(Mult., J(Hz),H)	$(^{1}H\text{-}^{1}H)^{*}$ COSY
1	177.41	177.67	-	-	-
2	77.61	77.79	5.07(dd;9.9,3.3Hz;1H)	5.09 (dd; 10.0, 3.5 Hz;1H)	3
3	38.66	38.93	3.08 (dd; 9.9, 3.3 Hz; 1H);2.92(dd;14.31,9.9 Hz;1H)	3.10 (dd; 14.5, 3.5 Hz; 1H); 2.94 (dd; 14.5,10.0Hz;1H)	2, 3
1	168.88	169.24	-	-	-
2’	115.56	115.77	6.26(d;15.8Hz;1H)	6.27 (d; 15.5 Hz; 1H)	3’
3’	146.51	146.79	7.49(d;15.8Hz;1H)	7.51 (d; 15.5 Hz; 1H)	2’
1’’	127.84	128.12	-	-	-
2’’	114.90	115.27	7.02(d;1.8Hz;1H)	7.03(d;2.0Hz;1H)	6’’
3’’	145.75	146.85	-	8.75(s;OH)	-
4’’	149.12	149.50	-	9.20(s;OH)	-
5’’	116.25	116.60	6.75(d;7.9Hz;1H)	6.77 (dd; 8.0, 2.0 Hz;1H)	6”
6’’	122.66	123.04	6.91 (dd, 8.36, 1.8 Hz, 1H)	6.91 (dd; 8.0, 2.0 Hz;1H)	5’’, 2’’
1’’’	131.09	131.29	-	-	-
2’’’	117.28	117.63	6.67(brs;1H)	6.77(d;2.0Hz;1H)	6’’’
3’’’	146.31	146.08	-	8.81(s;OH)	-
4’’’	144.58	144.93	-	9.68(s;OH)	-
5’’’	115.98	116.60	6.62(d;7.5Hz;1H)	6.68(d;8.0Hz;1H)	6’’’
6’’’	121.54	121.89	6.65(m; 1H)	6.63 (dd; 8.0, 2.0 Hz;1H)	2’’’

*InMeOH-*d*4.**Dataof thestandard.

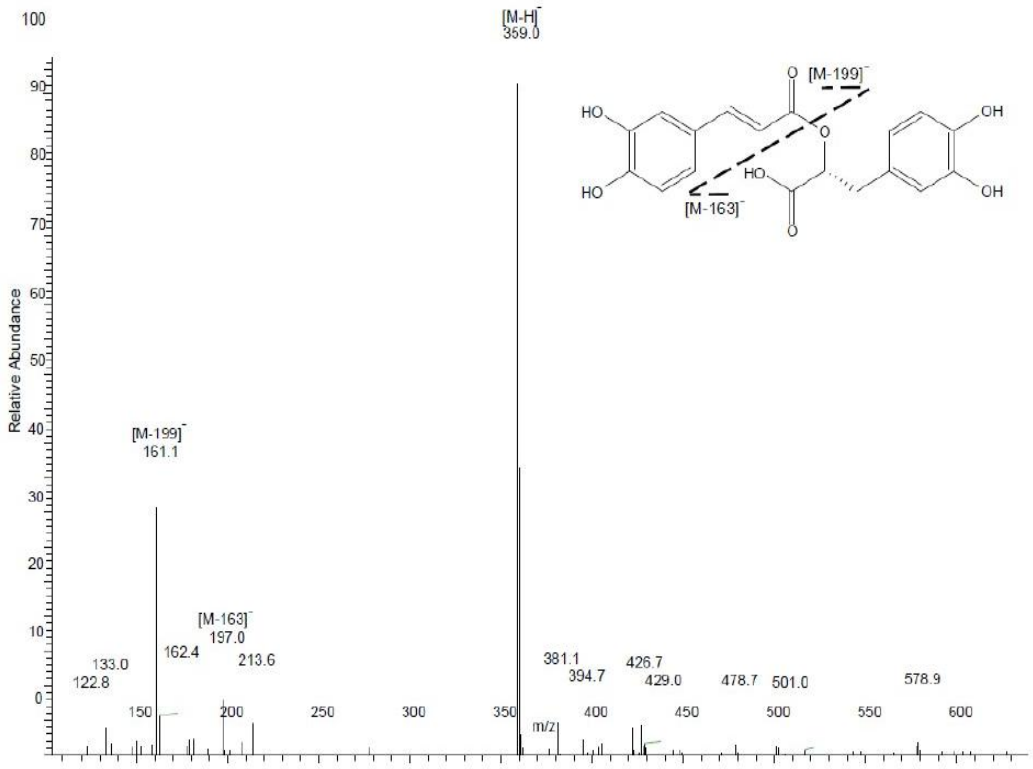


Graph No (4) ^{1}HNMR Spectra of rosmarinic acid



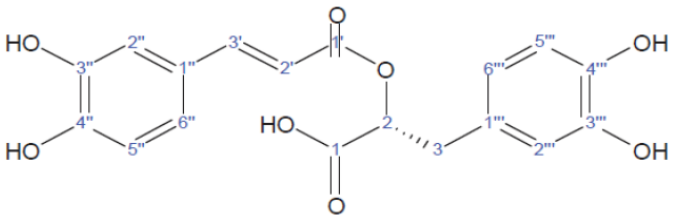


Graph No (5) ^{13}C –NMR Spectra of rosmarinic acid



Graph No (6) Mass Spectra of rosmarinic acid

Based onthe U V , IR, NMR and Mass analysis, the isolated compound was identified as rosmarinic acid



Structure of rosmarinic acid

CONCLUSION

The leaves of *D. triflorum* extracted with solvent methanol and the methanolic extract was purified by chromatographic methods as Thin Layer Chromatography, HPTLC and column chromatography. Isolation and characterization of the purified fraction was done with

spectroscopic methods such as UV, IR, NMR and Mass spectroscopy. The advantage of our method was the isolation and characterization of the new compound from *Desmodium triflorum.* In the present study, we found a new compound rosmarinic acid. It is a polyphenol flavonoid purified by methanolic extract of *Desmodium triflorum.* We further studied it for their biological activity.

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REFERENCES

1. Kirtikar KR, and Basu BD. Indian medicinal plants, National book distributors, Dehradun. 1995, Vol I, 335-336.
2. Khare CP, *et al.* Indian medicinal plant: An illustrated Dictionary, Springer-Verlag Heidelberg publication, 2007, 210.
3. Lai SC, Ho YL, Huang SC, Huang TH, Lai ZR, Wu CR, Lian KY, Chang YS. Am J Chin Med. 2010; 38(2):329-42.
4. Lai SC, Peng WH, Huang SC, Ho YL, Huang TH, Lai ZR, Chang YS. Am J Chin Med. 2009;37(3):573-88.
5. Raj RK. Indian J Physiol Pharmacol. 1975 Jan-Mar;19(1)
6. Bhosle V. Rev. Bras. Farmacogn. Braz. J. Pharmacogn. 2013, 23 (24)
7. Ethanobatanical Leaflet 12 : 2008, 227-230
8. Daya RW. *et al.* International Research Journal of pharmacy, 2 (7) 2011, 120-123.
9. Ogbeide O. and Parvez M. Identification of the flavonoids in Papilionaceae flowers using paper chromatography. J. Liq. Chromatogr. 1992, 15: 2989–2996.
10. Chio L.C. and Huang K F. Studies on the constituents of *Desmodium triflorum* (L.) DC. Master thesis, Providence University, Taiwan, 1995, pp. 1–14.
11. Adinarayana D, and Syamasundar K V. Occurrence of a rare diholosylflavone 2"-O- glucosylvitexin in *Desmodium triflorum*. Curr. Sci. 1982, 51: 936–937.
12. Sreenivasan K K, and Sankarasubramanian S. Chemical investigation of *Desmodium triflorum.* J.Health Sci. 1984, 10: 156–158.
13. Ghosal S, Shreivatava R S, Banerjee PK and Dutta SK. Alkaloids of *Desmondium triflorum*. Phytochemistry. 1971, 10(12): 3312–3313.
14. Mukherjee PK. Quality Control of Herbal Drugs, Business Horizons Pharmaceutical Publishers, 1st Edn., 2002, 186-189, 193, 256-370.
15. Srivastava MM. High-Performance Thin-Layer Chromatography (HPTLC). 2011, New York, Springer Heidelberg Dordrecht London.
16. Shukla R, Ishola IO, Agbaj OE, Narender T, Olufunmilayo OA. Bioactivity guided isolation of analgesic and anti-inflammatory constituents of *Cnestis ferruginea* Vahl ex DC (Connaraceae) root. Journal of Ethnopharmacology, 2012, Vol. 142, Issue 2, 383–389.