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Phytochemical Investigation of *Dendrophthoe trigona (Wt. and Arn.) Danser.*

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Abstract
Dendrophthoe trigona parasitic on *Ficus racemosa* is an important medicinal plant belonging to family Loranthaceae. Phytochemical investigation was carried out by using HPTLC and LC-MS/MS analysis. The result of phytochemical investigation shows the presence of quercetin, epicatechin in ethyl acetate fraction. The ethyl acetate fraction of *D. trigona* contains 195.0µg/mg of quercetin and 157.30µg/mg of epicatechin. Result confirms the major amount of polyphenolics in *Dendrophthoe trigona.*

Key Words
Loranthaceae, Quercetin, Epicatechin, Polyphenolics.

Introduction
Dendrophthoe trigona parasitic on *Ficus racemosa* is an important medicinal plant belonging to family Loranthaceae. *Dendrophthoe* is a genus of evergreen, shrubby, partial parasites, till recently include under Loranthus, distributed in the tropical and sub-tropical regions of the old world. It comprising some 30 species from tropical Africa to Australia with its center of diversity in W. Malaysia and 7 species of the genus found in India. Plants under genus are reported to have anti-oxidant (Hidalgo, 1994), anti-microbial (Daud, 2005), anticancer (Cerda, 2005), anti-diabetic (Osadebe, 2004) activities. However, practically no further progress has been made thereafter in order to characterize the chemical substances, let alone to establish their biological role. Since, this is a newly identified medicinal plant not much work is reported on its phytochemical studies. Therefore, this plant is selected for the phytochemical investigation.

Materials and methods
Plant material
The plant material of *Dendrophthoe trigona* (Wt. and Arn.) Danser was collected from Western Ghat region of Maharashtra (16° 41' 60N Latitude and 74° 13' 0E Longitude and 1000m Altitude) in November 2010**.** The plant specimen was authenticated by Dr. P.S. N. Rao (Botanical Survey of India, Pune). Air-dried leaves were used for the investigation.

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All chemicals including solvents used for extraction were of analytical grade. Solvents used for chromatography were of HPLC grade.

Preparation of extracts
About 1 kg of powdered material of leaves and stems was first defatted with n-hexane and then extracted with ethanol (80% v/v, 2.5 L) by cold maceration method for 24 h. The extract was collected and combined and concentrated using rotary vacuum evaporator. The yield of extract was 6.5% w/w. The extract so obtained was then fractionated with ethyl acetate and n-butanol. The ethyl acetate fraction rich in polyphenolics was subjected to TLC analysis in Chloroform: Methanol: Formic acid (5: 1.5: 0.2) as a solvent system. The pattern obtained on the TLC plate gives the idea about the number of compounds, chemical nature of compounds present in the extracts. The ethyl acetate fraction was subjected to LC-MS/MS analysis.

LC-MS/MS analysis
It was done using Varian Polaris (50×2mm), 5µm column and PS-210 HPLC pump. The binary system of A (water 100 vol %) and B (acetonitrile 100 vol %) in a linear gradient time of 30 min, flow rate of 1ml/min was used as mobile phase. Detection was done using PS-210; INTGR 1 Detector. The MS spectra were obtained using, atmospheric pressure chemical ionization (APCI), at electron energy of 80V, at source temperature 190°C. The ion energy at 1.7V and at pressures < 1.7e – 4 Torr and 1.1e – 5 Torr penning was used for MS analysis. Mass

spectra of sample were matched with that of standard.

Quantification of epicatechin and quercetin by HPTLC

It was carried out by using HPTLC (Camag, Switzerland) using pre-coated silica gel plates as stationary phase. (Merck). Chloroform: Methanol: Formic acid (5: 1.5: 0.2) was used as mobile phase. The chromatogram was evaluated densitometrically using wincat software. The flavonoids were traced after treatment of natural product reagent at 366nm and the proanthocyanidins were traced by using Vanillin-HCl at 280 nm.

Results and Discussion

LC-MS/MS analysis of ethyl acetate fraction of *D. trigona* demonstrated the presence of two compounds. The LC-MS pattern showed protonated molecular ion at 303 ($M+ H^+$), m/z 291 ($M+ H^+$) respectively. The mass fragmentation pattern of these compounds was compared with standard compounds as reported by Tsimogiannis et al. (2007) (Table 1). The MS-MS fragmentation pattern of these compounds confirmed the presence of quercetin, (-) - epicatechin and/or (+)-catechin in ethyl acetate fraction of *D. trigona*. Further, quercetin and epicatechin were quantified by HPTLC. The ethyl acetate fraction of *D. trigona* contains 195.0µg/mg of quercetin and 157.30µg/mg of epicatechin.

Conclusion

Results revealed the presence of major amount of polyphenolics in *D. trigona*. This will help in future pharmacological investigation of *D. trigona*.

References

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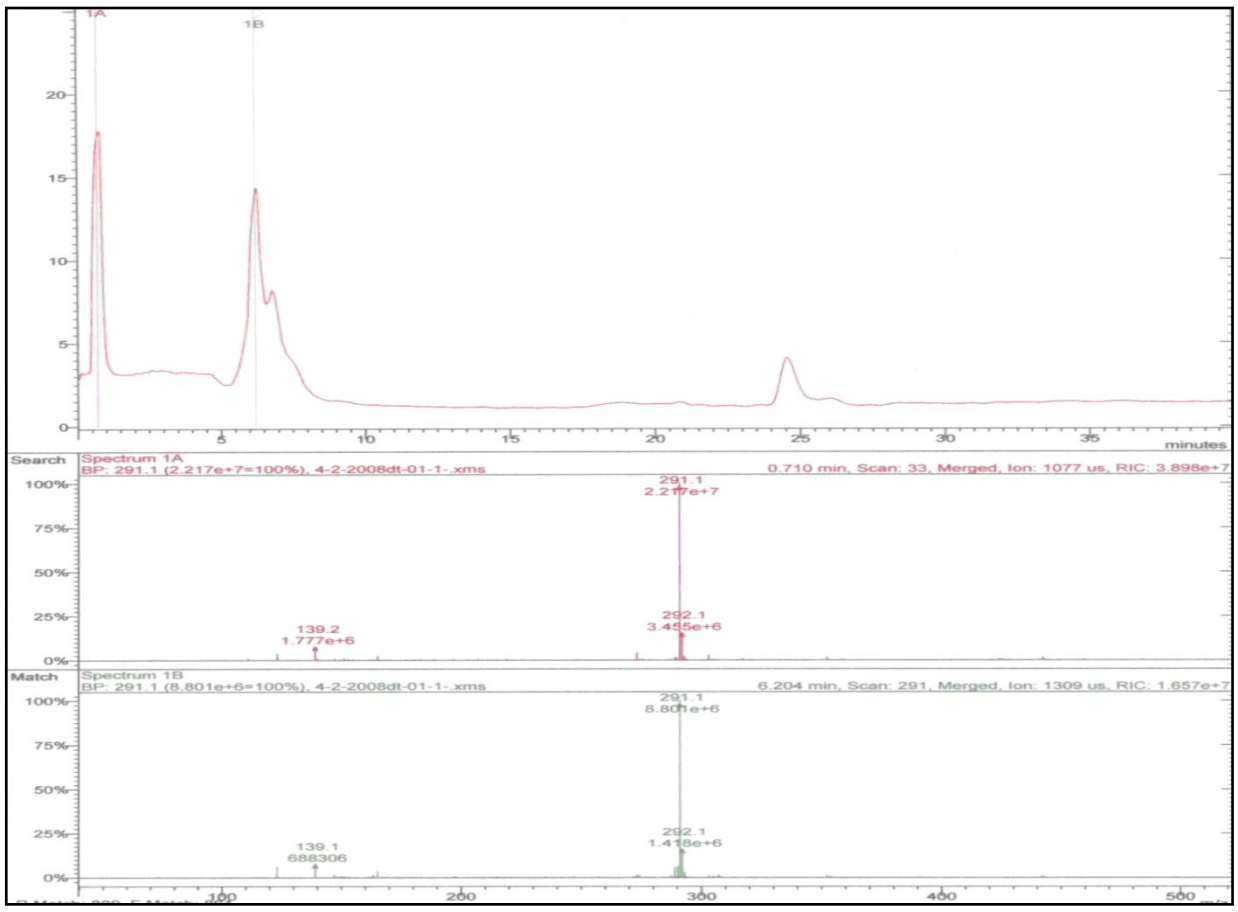

Fig.1: LC-MS of ethyl acetate fraction of *D. trigona.*

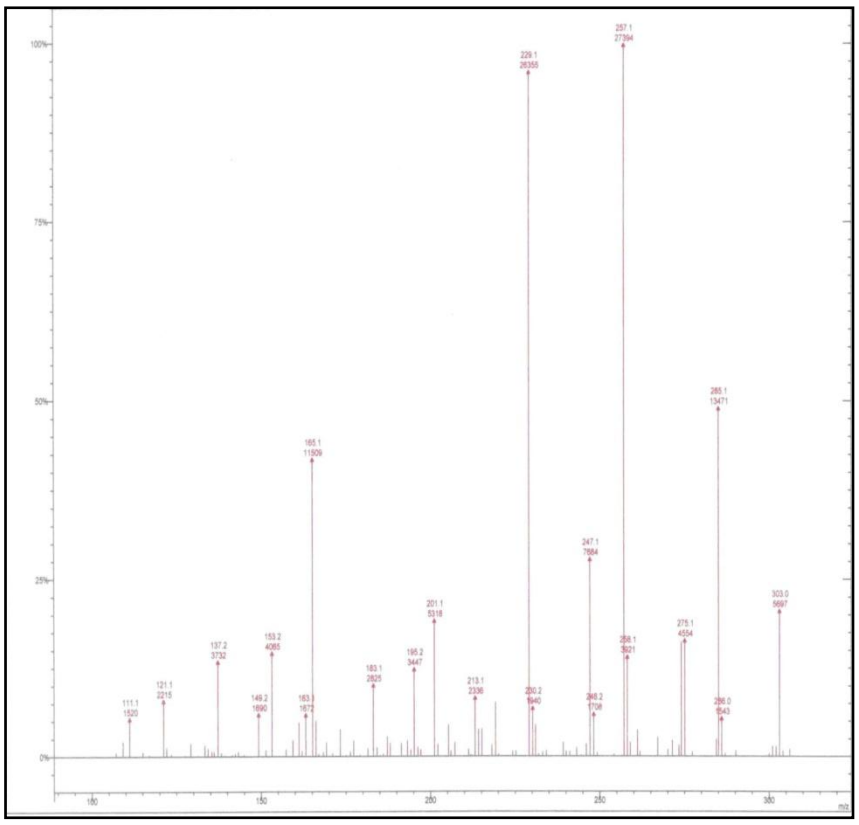

Fig.2: MS/MS of Compound 1.

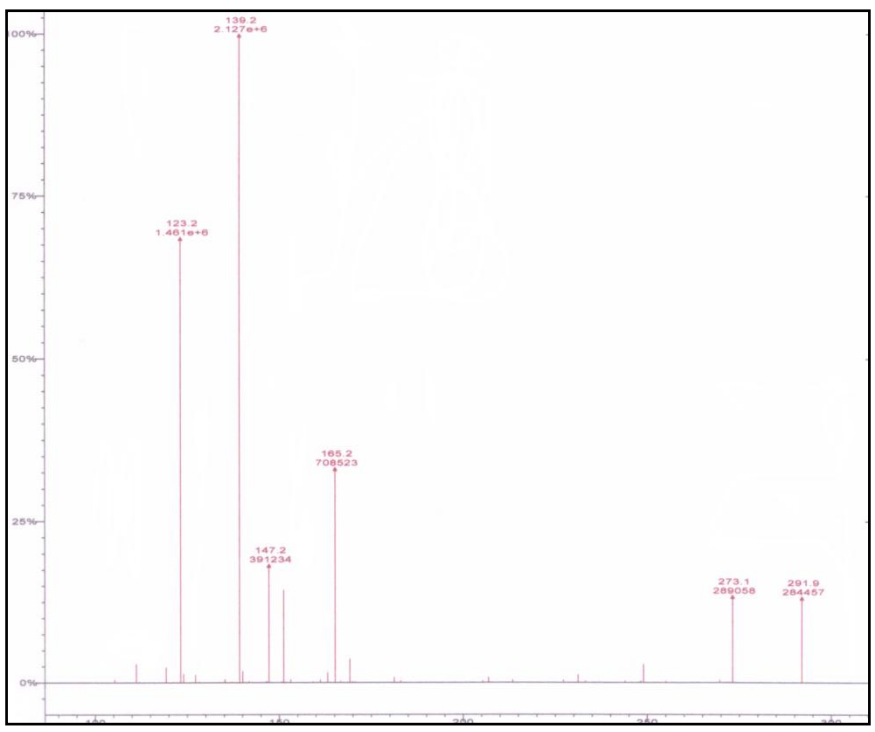

Fig.3: MS/MS of Compound 2.

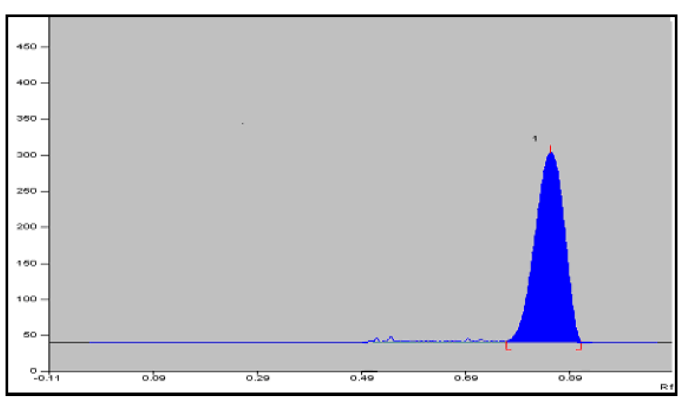

Fig. 4A: HPTLC of reference standard epicatechin.

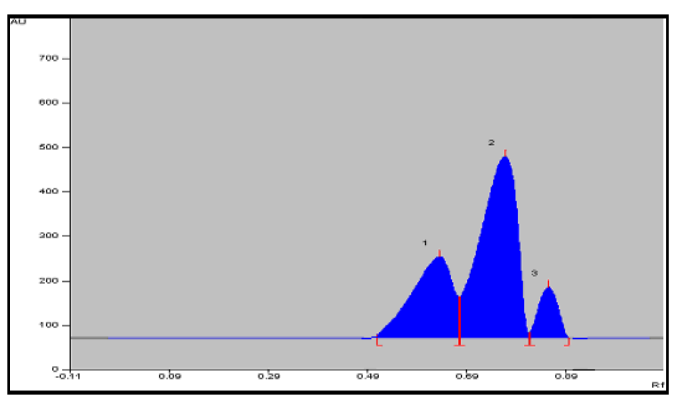

Fig.4B: HPTLC of ethyl acetate fraction of *D. trigona* parasitic on *Ficus racemosa*.

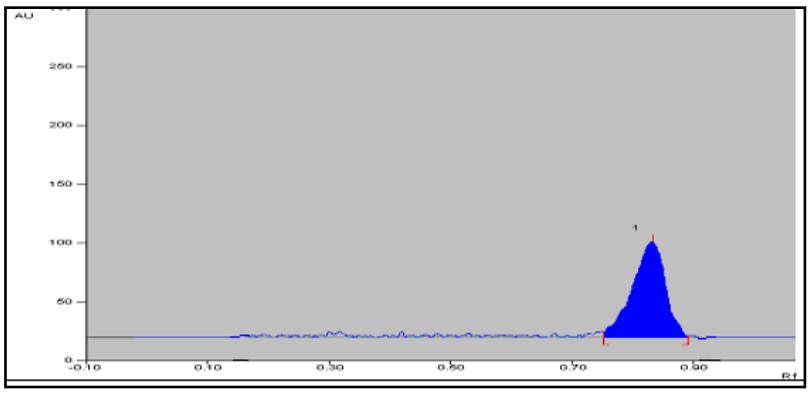

Fig. 5A: HPTLC of reference standard Quercetin.

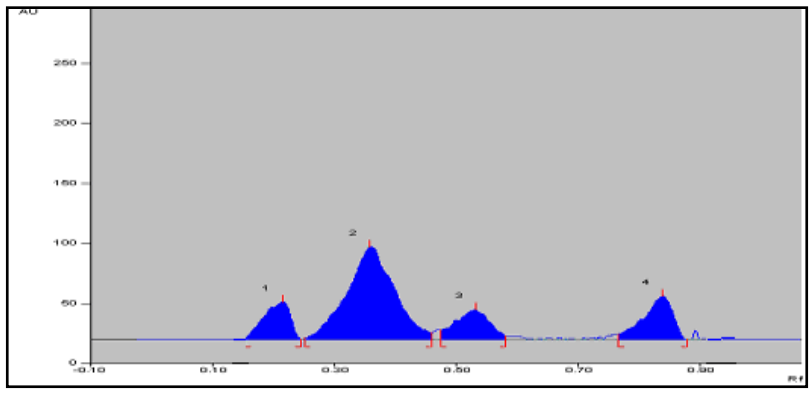

Fig. 5B: HPTLC of ethyl acetate fraction of *D. trigona* parasitic on *Ficus racemosa*

Table 1: MS-MS fragmentation pattern of flavonoids.

Assessed Fragments	m/z				
	Quercetin	(+) –Catechin	(–) -Epicatechin	Isolated compounds	
[M+H]+	303	291	291	303	291
[M+H-H_2O]+	285	-	-	285	-
[M+H-H_2O-CO]+	257	273	273	257	273
[M+H-H_2O-2CO]+	229	-	-	229	-
[M+H-CO]+	275	-	-	275	-
[M+H-2CO]+	247	-	-	247	-
[M+H-CH_2CO]+	-	249	249	-	249
-	165 (35.5)	-		165	
-	137 (11)	123	123	137	123
-	153 (7)	139	139	153	139
-	149 (5.5)		-	149	-

Table 2: R_f values and peak areas of epicatechin in HPTLC of ethyl acetate fraction of *Dendrophthoe trigona*.

Track no.	Sample	Conc.(µg/ml)	Max. R_f	AUC	Mean ±SEM
1	DT-01	10	0.87	3833	3821.56±90.10
2	DT-01	10	0.86	3971.6	
3	DT-01	10	0.86	3660.1	

Table 3: R_f values and peak areas of Quercetin in HPTLC of ethyl acetate fraction of *Dendrophthoe trigona*.

Track no.	Sample	Conc.(µg/ml)	Max. R_f	AUC	Mean ±SEM
1	DT-01	10	0.84	1410.6	1541.43±80.23
2	DT-01	10	0.85	1687.3	
3	DT-01	10	0.85	1526.4	

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