Chemoprofile Investigation of *Dendrophthoe falcata* (L.f.) Ettingsh growing on *Boswellia serrata* Roxb. ex. Coleb.

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ABSTRACT

Dendrophthoe falcata (L.f.) Ettingsh is an angiospermic hemi-parasite which grows on a number of host species. Also, it has medicinal importance and used in traditional medicine practices by the tribals of the Melghat Region. Present paper deals with the chemo profiling of Dendrophthoe falcata (L.f) Ettingsh, plant parts growing on the host plant Boswellia serrata Roxb. ex. Coleb. belonging to Family: Burseraceae which is a common host in this region. Preliminary study revealed the presence of Carbohydrates, Anthraquinone glycosides, Cardiac glycosides, Coumarins, Quinones, Steroids, Alkaloids, Flavonoids, Phenolics, Tannins, Saponins and Terpenoids while quantitative study showed ample presence of Alkaloids, Flavonoids, Phenolics and Saponins in the plant. Alkaloids, Flavonoids and Phenolics showed prominent spots when separated through Thin layer chromatography and LC-MS analysis confirmed the presence of alkaloids, flavonoids and glycosides.

Keywords: *Dendrophthoe falcata, Boswellia serrata, tribals, Melghat Region, Alkaloids, Flavonoids, Phenolics, Saponins, thin layer chromatography, LC-MS, etc.*

INTRODUCTION:

The genus *Dendrophthoe* is evergreen, shrubby, partial parasites, distributed in the tropical and sub-tropical regions of the old world. The whole parasitic plant *Dendrophthoe falcata* is used in indigenous system of medicine as cooling, bitter, astringent, aphrodisiac, narcotic and diuretic and is also useful in pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, renal and vesicle calculi and vitiated conditions of kapha and pitta. The decoction of *Dendrophthoe falcata* is used by women as antifertility agent. The Dendrophthoe falcata also have anticancer activity (Hikino, 1991). *Dendrophthoe falcata*, belongs to the family Loranthaceae; is a branched angiospermic hemiparasite, most frequently observed on hosts like *Mangifera indica* (Anacardiaceae), *Chloroxylon swietenia* (Meliaceae), *Madhuca longifolia* (Sapotaceae), etc. Barks of *Dendrophthoe falcata* are grey, its leaves are thick, coriaceous, much variable in shape usually opposite, 7.5-18 x 2-10 cm and its flowers are stout, unilateral racemes, often two from an axil pedicel. The flowers are ovate, sub-acute, concave and scarlet or orange in colour. Anthers are linear, equal in length, to the free portion of the filament. Berries of *Dendrophthoe falcata* are 8-13 mm long ovoid oblong, pink, smooth crowned by a cup- shaped calyx (Chopra *et al*., 1956). The genus *Dendrophthoe* comprises of 20 species and about 7 species are found in India.

MATERIALS AND METHODS:

Plant material: The *Dendrophthoe falcata* leaves and stem was collected from the host *Boswellia serrata* Roxb. ex. Coleb. during March- April of 2015 from Melghat forest region of West Vidarbha, Maharashtra, India and were authenticated by taxonomist, Dr. S. P. Rothe, Head, Department of Botany. The herbarium specimens were given voucher number and deposited in the herbarium of Department of Botany, Shri Shivaji College of Arts, Commerce and Science, Akola, Maharashtra, India.

Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary and secondary metabolites in 5 solvents viz., Acetone, Distilled water, Ethanol Ethyl Acetate and Methanol. (Harborne, 1973).

Quantitative Phytochemical Analysis

All plant parts in which preliminary qualitative analysis showed presence of specific groups of secondary metabolites like Flavonoids, Alkaloids, Saponins and Phenolics were subjected to quantitative analysis. The crude quantification of major phytochemicals present was done using precipitation and spectrophotometric method as per suitability. Each sample was analyzed in triplicates. Only Alkaloids, Flavonoids, Saponins and Phenolics were quantified by the standard procedures given below.

Flavonoids: 10 gm of sample was extracted repeatedly in 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman paper no. 42. The filtrate then transferred to a crucible and evaporated to dryness over a water bath and weighed (Bohm and Kocipai- Abyazan, 1994).

Alkaloids: 5 gm of sample was weighed in 250 ml beaker and 200 ml 20% acetic acid in ethanol was added and covered to stand for about 4 hrs. This was filtered and extract was concentrated using water bath to 1/4th of original volume. Concentrated Ammonium hydroxide was added drop wise to the extract till its complete precipitation. The whole solution was allowed to settle and precipitate was collected and weighed (Harborne, 1973).

Saponins:10 gm of plant powder was taken in 200 ml 20% ethanol to make a suspension. This was heated for about 4 hrs over hot water bath (55^{o}C) continuous stirring. The mixture was filtered and the residue was re-extracted with 200 ml 20% ethanol. The combined extract was reduced to 1/10th of the original volume. The concentrate was taken into 250 ml separating funnel, to this added 20 ml diethyl ether and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated for 2-3 times. Then 60 ml n-butanol was added to it. The combined solution was then washed twice with 10 ml 5% aqueous sodium hydroxide. The remnant was heated in a water bath for complete evaporation and dried. This dried content was calculated as Saponin percentage in a sample (Obdoni & Ochuko, 2001).

Phenolics: The total phenolics in the extract were determined using Folin-Ciocalteu method. To each sample solution (1.0 ml) and standard (Gallic acid) was added 5 ml of Folin-Ciocalteu and 4 ml sodium carbonate (7 % w/v). The mixture was shaken and allowed to stand for 30 min in the dark at room temperature; after which absorbance was measured at 765 nm using a spectrophotometer. The amount of total phenolics was expressed as Gallic acid equivalent (GAE) in milligram per gram dry plant extract using the expression; C = c x (V/m); (where C= Total phenolics content of plant extract in mg/g GAE, c= concentration of Gallic acid established from calibration curve mg/g, V= volume of the extract (ml) and m= weight of pure plant extract (g) (Vermerris *et al*., 2006).

Chromatographic Analysis: The Ethyl acetate, Chloroform, Methanol and Ethanol extracts of *Dendrophthoe falcata* (L.f.) Ettingsh, leaf and stem collected from *Boswellia serrata* Roxb. ex. Coleb. were subjected to thin layer chromatographic analysis to find out the presence of number of chemical constituents. TLC fingerprinting was carried out only for Alkaloids, Flavonoids and Phenolics to study the variation in these compounds in different solvents as well as variation in the phytochemicals in the parasite when host change occurs. The details of the procedure are as follows. The qualitative evaluation of the plate was done by determining the migration behaviour of the separated substances given in the form of Retardation factor (R_f) value.

$$\text{Retardation factor } (Rf) = \frac{Distance\ travelled\ by\ the\ solute\ from\ the\ origin}{Distance\ travelled\ by\ the\ solvent\ from\ the\ origin}$$

Two Different solvent systems were utilized to study the phytoconstituents viz. Alkaloids, Flavonoids and Phenolics. The solvent systems employed during the experimentation were, for Alkaloids, Toluene: Acetone: Ethanol: Ammonia (40:40:6:2) and Toluene: Methanol (86:14) and Dragenfroff's Reagent was used as spraying reagent; for Flavonoids, Chloroform: Ethyl Acetate (60:40) and Toluene: Ethyl acetate: Formic Acid (50:40:10) were used as solvent systems and sprayed with 5% Alc. $FeCl_3$; for Phenolics, Ethyl acetate: Formic Acid: Acetic Acid: Dist. Water (100:11:11:26) and Toluene: Acetone (9:1) were used as solvent systems and sprayed with 5% Alc. $FeCl_3$.

Liquid Chromatography-Mass Spectroscopy (LC-MS) Analysis

The analysis of the Methanol extract of leaf and stem of *Dendrophthoe falcata* (L.f) Ettingsh growing on *Boswellia serrata* Roxb. ex. Coleb. was carried out using Liquid chromatography-Mass spectrometer by standard method. Samples were analysed SAIF, Punjab University, Chandigarh, India. Methanol extracts of Leaf and stem of Dendrophthoe falcata were subjected for LC-MS analysis and the Mass spectra were obtained. The samples were diluted with acetone and injected 10 µl of the same, Mobile phase used was MPA: MPB:: 5 mM Ammonium formate:0.1 % formic acid in water: Methanol::30 :70 v/v.

Identification of compounds: Interpretation on mass spectrum of GC-MS was done using the National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of unknown compounds was compared with the spectral data of known compounds present in spectral libraries (NIST). The name, molecular weight and molecular formula of the identified molecules were ascertained.

OBSERVATION AND RESULTS:

The qualitative phytochemical screening of *Dendrophthoe falcata* (L.f.) Ettingsh growing on *Boswellia serrata* Roxb. ex. Coleb. in all the undertaken five extracts i.e., 5 solvents viz., Acetone, Distilled water, Ethanol, Ethyl Acetate and Methanol showed that there is presence of phytoconstituents like Alkaloids, Flavonoids, Phenols, Saponins, Steroids and Terpenoids. Table 1 and Table 2.

Table 1: Qualitative Phytochemical screening of *Dendrophthoe falcata* (L.f.) Ettingsh leaf growing on *Boswellia serrata* Roxb. ex. Coleb.

S.N.	Constituents	Chemical Tests	Extract solvent				
			Ac	Aq.	E	EtAc	M
1	Carbohydrates	**Benedict's Reagent**	+	+	+	+	+
2	Proteins	**Millon's Reagent**	-	-	-	-	-
3	Anthraquinone glycosides	**Borntrager's Reagent**	+	+	+	-	+
4	Cardiac Glycosides	**Keller-Killiani Test**	-	-	-	-	-
5	Coumarins	**Extract + 10% NaOH**	+	+	+	-	+
6	Quinone	**Extract + Conc. H_2SO_4**	+	+	+	+	+
7	Steroids	**Salkowski Test**	+	-	-	+	-
8	Alkaloids	**Dragendroff's Reagent**	-	+	+	-	+
		Wagner's Reagent	-	+	+	-	+
9	Flavonoids	**Shinoda Test**	+	+	+	-	+
		Lead Acetate Test	+	+	+	-	+
10	Phenolics & Tannin	**$FeCl_3$ Test**	+	+	+	-	+
		Lead Acetate Test	+	+	+	-	+
11	Saponins	**Foam Test**	-	+	-	-	+
12	Lignins	**Furfuraldehyde Test**	-	-	+	-	-
13	Fixed Oil & Fat	**Spot Test**	-	-	-	-	-
14	Terpenoids	**$CHCl_3$ + H_2SO_4**	+	-	-	+	-

Ac- Acetone; Aq.- Distilled water; E- Ethanol; EtAc- Ethyl Acetate; M- Methanol.

Table 2: Qualitative Phytochemical screening of *Dendrophthoe falcata* (L.f.) Ettingsh stem growing on *Boswellia serrata* Roxb. ex. Coleb.

S.N.	Constituents	Chemical Tests	Extract solvent				
			Ac	Aq.	E	EtAc	M
1	Carbohydrates	**Benedict's Reagent**	+	+	+	+	+

2	Proteins	**Millon's Reagent**	-	-	-	-	-
3	Anthraquinone glycosides	**Borntrager's Reagent**	+	+	+	-	+
4	Cardiac Glycosides	**Keller-Killiani Test**	-	-	-	-	-
5	Coumarins	**Extract + 10% NaOH**	+	+	+	-	+
6	Quinone	**Extract + Conc. H_2SO_4**	+	+	+	+	+
7	Steroids	**Salkowski Test**	+	-	-	+	-
8	Alkaloids	**Dragendroff's Reagent**	-	+	+	-	+
		Wagner's Reagent	-	+	+	-	+
9	Flavonoids	**Shinoda Test**	+	+	+	-	+
		Lead Acetate Test	+	+	+	-	+
10	Phenolics & Tannin	**$FeCl_3$ Test**	+	+	+	-	+
		Lead Acetate Test	+	+	+	-	+
11	Saponins	**Foam Test**	-	+	-	-	+
12	Lignins	**Furfuraldehyde Test**	-	-	+	-	-
13	Fixed Oil & Fat	**Spot Test**	-	-	-	-	-
14	Terpenoids	**$CHCl_3$ + H_2SO_4**	+	-	-	+	-

Ac- Acetone; Aq.- Distilled water; E- Ethanol; EtAc- Ethyl Acetate; M- Methanol.

Quantitative Phytochemical Analysis:

The secondary metabolites in plant contain appreciable concentrations of Alkaloids, Flavonoids, Phenolics and Saponins. Table shows the concentration of bioactive compounds in leaf and stem. Leaf: In the quantification of leaf, the following concentration was observed, the Alkaloids 7.2 (± 0.12) mg/100g, Flavonoids 8.3 (± 0.21) mg/100g, Phenolics 8.7 (± 0.18) mg/100g and Saponins 10.1 (± 0.17) mg/100g. Stem: Quantification of stem, showed concentrations as for, the Alkaloids 3.5 (± 0.11) mg/100g, Flavonoids 4.7 (± 0.16) mg/100g, Phenolics 2.3 (± 0.13) mg/100g and Saponins 11.3 (± 0.15) mg/100g. The quantification studies of leaf and stem of *Dendrophthoe falcata* (L.f) Ettingsh collected from *Boswellia serrata* Roxb. ex. Coleb. shows that, concentration of Alkaloids, Flavonoids and Phenolics is higher in leaf than to amount in stem, whereas, the concentration of Saponins is higher in stem than to that in leaf.

Table 3: Quantitative Phytochemical analysis of *Dendrophthoe falcata* (L.f) Ettingsh leaf and stem growing on *Boswellia serrata* Roxb. ex. Coleb.

Sr. No.	Phytochemicals	Leaf (mg/100g)	Stem (mg/100g)
1	**Alkaloids**	**7.2 (± 0.12)**	**3.5 (± 0.11)**
2	**Flavonoids**	**8.3 (± 0.21)**	**4.7 (± 0.16)**
3	**Phenolics**	**8.7 (± 0.18)**	**2.3 (± 0.13)**
4	**Saponins**	**10.1 (± 0.17)**	**11.3 (± 0.15)**

Chromatographic Analysis: Thin Layer Chromatography

TLC profile is an important aspect in determining chemo profile of bioactive constituents. Well resolved TLC profiles were recorded for the future reference and to identify the phytochemicals in the plant material. TLC profile was carried out to quantify the number of Alkaloids, Flavonoids and Phenolics in two solvent systems for each for leaf and stem extracts of *Dendrophthoe falcata* (L.f.) Ettingsh collected from

Boswellia serrata Roxb. ex. Coleb. extracted in 4 solvents viz. Ethyl acetate, Chloroform, Methanol and Ethanol. Table 4 and Table 5.

Table 4: TLC study of Leaf extracts of *Dendrophthoe falcata* (L.f.) Ettingsh growing on *Boswellia serrata* Roxb. ex. Coleb.

S. N.	Chemical Constituent	Solvent System	Extracting Solvent	Total Bands	Rf Values	Spraying Reagent
1	Alkaloids	Toluene: Acetone: Ethanol: Ammonia solution (40:40:6:2)	Ethyl acetate	2	0.36, 0.66	Dragendroff's
			Chloroform	2	0.27, 0.33	
			Methanol	2	0.30, 0.76	
			Ethanol	2	0.33, 0.61	
		Toluene: Methanol (86:14)	Ethyl acetate	1	0.75	Dragendroff's
			Chloroform	2	0.51, 0.75	
			Methanol	--	--	
			Ethanol	1	0.76	
2	Flavonoids	Chloroform: Ethyl acetate (60:40)	Ethyl acetate	2	0.48, 0.74	5% $FeCl_3$ (Alc.)
			Chloroform	5	0.18, 0.31, 0.42, 0.49, 0.83	
			Methanol	2	0.23, 0.42	
			Ethanol	2	0.23, 0.42	
		Toluene: Ethyl acetate: Formic Acid (50:40:10)	Ethyl acetate	5	0.13, 0.25, 0.36, 0.46, 0.81	5% $FeCl_3$ (Alc.)
			Chloroform	1	0.79	
			Methanol	5	0.16, 0.25, 0.36, 0.46, 0.66	
			Ethanol	5	0.14, 0.22, 0.33, 0.41, 0.66	
3	Phenolics	Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)	Ethyl acetate	1	0.90	5% $FeCl_3$ (Alc.)
			Chloroform	--	--	
			Methanol	2	0.16, 0.84	
			Ethanol	2	0.18, 0.77	
		Toluene: Acetone (9:1)	Ethyl acetate	1	0.37	5% $FeCl_3$ (Alc.)
			Chloroform	2	0.68, 0.77	
			Methanol	3	0.43, 0.74, 0.80	
			Ethanol	3	0.43, 0.74, 0.80	

Table 5: TLC study of Stem extracts of *Dendrophthoe falcata* (L.f.) Ettingsh growing on *Boswellia serrata* Roxb. ex. Coleb.

S. N.	Chemical Constituent	Solvent System	Extracting Solvent	Total Bands	Rf Values	Spraying Reagent
1	Alkaloids	Toluene: Acetone: Ethanol: Ammonia solution (40:40:6:2)	Ethyl acetate	2	0.17, 0.23	Dragendroff's
			Chloroform	--	--	
			Methanol	1	0.12	
			Ethanol	2	0.11, 0.17	
		Toluene: Methanol (86:14)	Ethyl acetate	--	--	Dragendroff's
			Chloroform	1	0.7	
			Methanol	--	--	
			Ethanol	--	--	
2	Flavonoids	Chloroform: Ethyl acetate (60:40)	Ethyl acetate	--	--	5% $FeCl_3$ (Alc.)
			Chloroform	--	--	
			Methanol	--	--	
			Ethanol	--	--	
		Toluene: Ethyl acetate: Formic Acid (50:40:10)	Ethyl acetate	2	0.29, 0.54	5% $FeCl_3$ (Alc.)
			Chloroform	--	--	
			Methanol	--	--	
			Ethanol	--	--	
3	Phenolics	Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)	Ethyl acetate	--	--	5% $FeCl_3$ (Alc.)
			Chloroform	--	--	
			Methanol	--	--	
			Ethanol	--	--	
		Toluene: Acetone (9:1)	Ethyl acetate	--	--	5% $FeCl_3$ (Alc.)
			Chloroform	--	--	
			Methanol	--	--	
			Ethanol	--	--	

The TLC separation studies revealed that, the number of Alkaloids, Flavonoids and Phenolics are more in the leaf than in the stem of *Dendrophthoe falcata* (L.f.) Ettingsh.

Liquid Chromatography - Mass Spectroscopy Analysis

LC-MS Analysis was performed for Leaf and Stem of Dendrophthoe falcata (L.f.) Ettingsh collected from *Boswellia serrata* Roxb. ex. Coleb. in Methanol.

Figure 1: Mass Spectrum of Methanol extract of Leaf of *Dendrophthoe falcata* (L.f.) Ettingsh on *Boswellia serrata* Roxb. ex. Coleb.

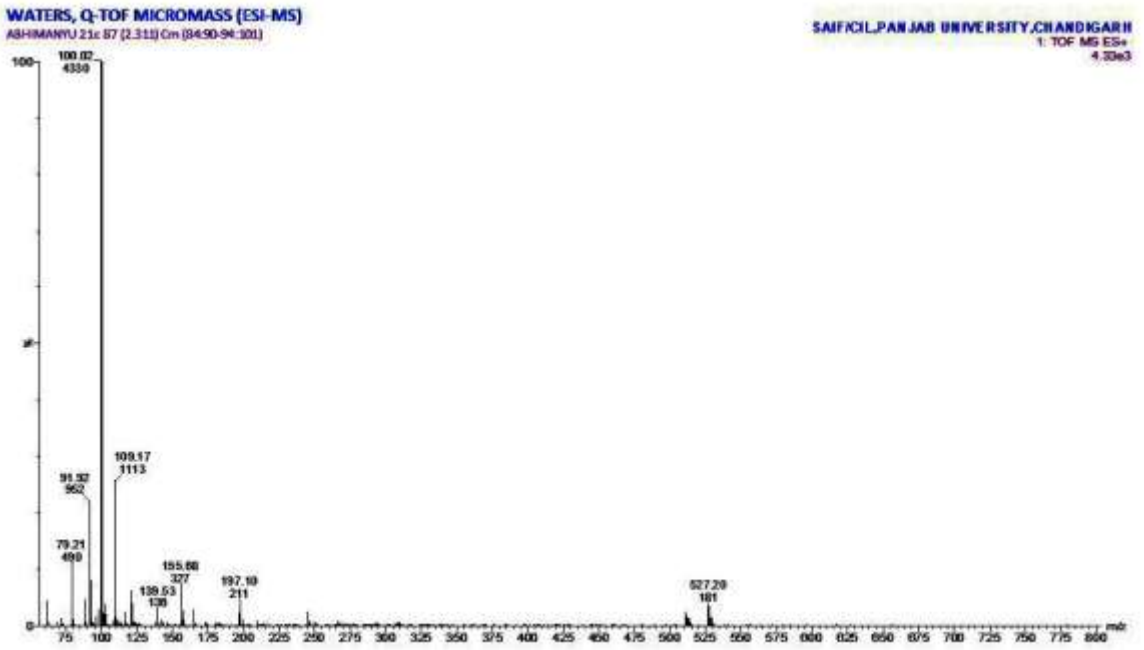

Table 6: Compounds identified in the Methanol extract of Leaf of *Dendrophthoe falcata* (L.f.) Ettingsh on *Boswellia serrata* Roxb. ex. Coleb.

Sr. No.	m/z	Name of the Metabolite	Molecular Formula
1	79.21	Azobenzene	C_5H_5N
2	91.92	Phenylamine	$C_6H_5NH_2$
3	100.02	N-Nitropyrolidine	$C_4H_8N_2O$
4	109.17	Hypotaurine	$C_2H_7NO_2S$
5	139.53	2-Methyl-4-amino-6-methoxy-s-triazine	$C_5H_8N_4O$
6	155.68	3-Indoleacetonitrile	$C_{10}H_8N_2$
7	197.10	3,4-Dihydroxy-L-phenylalanine	$C_9H_{11}NO_4$
8	527.20	Daunorubicin	$C_{27}H_{29}NO_{10}$

Figure 2: Mass Spectrum of Methanol extract of Stem of *Dendrophthoe falcata* (L.f.) Ettingsh on *Boswellia serrata* Roxb. ex. Coleb.

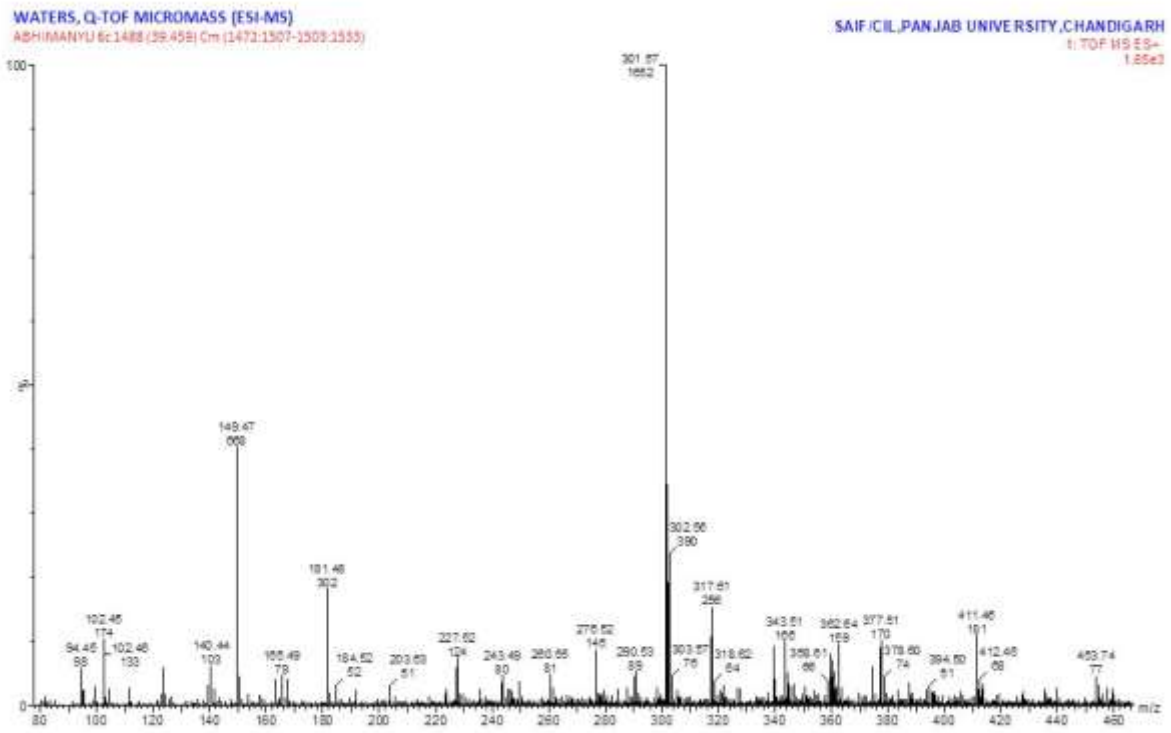

Table 7: Compounds identified in the Methanol extract of Stem of *Dendrophthoe falcata* (L.f.) Ettingsh on *Boswellia serrata* Roxb. ex. Coleb.

Sr. No.	m/z	Name of the Metabolite	Molecular Formula
1	94.45	2-Aminopyridine	$C_5H_6N_2$
2	102.45	Betaine aldehyde	$C_5H_{12}NO$
3	140.44	4-Methoxy-6-methyl-1,3,5-triazin-2-amine	$C_5H_8N_4O$
4	149.47	L-Methionine	$C_5H_{11}NO_2S$
5	165.49	Hordenine	$C_{10}H_{15}NO$
6	181.48	Etilefrin	$C_{10}H_{15}NO_2$
7	184.52	Phosphocholine	$C_5H_{15}NO_4P$
8	203.63	3-Indolebutyric acid	$C_{12}H_{13}NO_2$
9	227.52	Flindersine	$C_{14}H_{13}NO_2$
10	243.49	Cytosine arabinoside	$C_9H_{13}N_3O_5$
11	260.55	Glucose 6-phosphate	$C_6H_{13}O_9P$
12	276.52	Triethyl citrate	$C_{12}H_{20}O_7$
13	290.53	Catechin	$C_{15}H_{14}O_6$
14	301.57	N-Acetyl-α-D-glucosamine 1-phosphate	$C_8H_{16}NO_9P$
15	302.56	Quercetin	$C_{15}H_{10}O_7$
16	317.61	Petunidin	$C_{16}H_{13}O_7$
17	343.51	Dibucaine	$C_{20}H_{29}N_3O_2$
18	356.61	Hycanthone	$C_{20}H_{24}N_2O_2S$

19	362.64	Piretanide	$C_{17}H_{18}N_2O_5S$
20	377.51	Iodo-5'-deoxyadenosine	$C_{10}H_{12}IN_5O_3$
21	394.50	Rotenone	$C_{23}H_{22}O_6$
22	411.46	Fluvastatin	$C_{24}H_{26}FNO_4$
23	412.46	Decanoyl-2-hydroxy-sn-glycero-3-phosphocholine	$C_{18}H_{39}NO_7P$

DISCUSSION:

The Preliminary phytochemical screening of *Dendrophthoe falcata* (L.f) Ettingsh growing on *Boswellia serrata* Roxb. ex. Coleb. revealed the presence of major phytoconstituents viz., Anthraquinone glycosides, Cardiac glycosides, Coumarins, Quinones, Steroids, Alkaloids, Flavonoids, Phenolics, Tannins and Terpenoids. Quantitative estimation showed ample concentration of Alkaloids, Flavonoids, Phenolics, and Saponins. From the present study, the ethnomedicinal values of *Dendrophthoe falcata* (L.f.) Ettingsh can be justified, by the presence of the bioactive compounds. The bioactivity of plant extracts is attributed to phytochemical constituents. Alkaloids in plants generally, show antimicrobial properties (Ahmed *et al*., 2010). Tannin content shows remarkable antibacterial potential due to the basic character, that allows them to react with proteins, to form stable water-soluble compounds, thereby killing the bacteria directly by damaging its cell membrane (Mohamed *et al*., 2010). Flavonoids are known to show, antiviral properties (Mehrangiz *et al*., 2011).

CONCLUSION

Dendrophthoe falcata (L.f) Ettingsh growing on *Boswellia serrata* Roxb. ex. Coleb. was evaluated qualitatively, quantitatively, chromatographically and also by mass spectrum studies. The study revealed that the plant D. falcata constitutes phytochemicals like Alkaloids, Flavonoids, Phenolics, etc. which attributes to its traditional medicinal use.

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