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Comparison of *In Vitro* Antioxidant Activity, Total Phenolic Content, Total Flavonoid Content and Chromatographic Evaluation of Hemiparasite *Dendrophthoe Falcata* (L.f.) Ettingsh Growing on Four Different Medicinal Host Plants

Kaliyath Salasmi[1,*], Azhimala Bhaskaranpillai Remashree[2]

[1]Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, India
[2]Spices Board, Ministry of Commerce and Industry, India

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Abstract **Background:** *Dendrophthoe falcata* (L.f.) Ettingsh is a hemiparasitic medicinal plant of the family Loranthaceae. It is the most common mistletoe occurring throughout India, with a wide host range. The ethanol extracts of whole plant of *D. falcata* growing on *Anacardium occidentale* (DFA), *Lagerstroemia speciosa* (DFL), *Mangifera indica* (DFM) and *Strychnos nux-vomica* (DFS) were selected for the present investigation to evaluate the variation in antioxidant potential and quantitative determination of total phenolic and flavonoid content, which has significant role in biological activities. **Materials and Methods:** The four samples were subjected to assess antioxidant potential by DPPH and FRAP assays (*in vitro*) and chemical profiling using High Performance Thin Layer Chromatography (HPTLC) analysis. Folin-Ciocallteau reagent method and Aluminium chloride colorimetric assay were employed to determine total phenolic and flavonoid content respectively. HPTLC analysis is carried out using Gallic acid as the marker compound. **Results:** DFM and DFA exhibited potential antioxidant activity on DPPH and FRAP assays. The antioxidant potential of the extracts follows the order as DFM > DFA > DFS > DFL in both assays. In HPTLC analysis, specific band of Gallic acid was observed at R_f 0.30. Gallic acid is present only in two samples of *D. falcata* (DFA and DFM), which also showed quantitative variations as estimated from the peak area developed in the chromatogram. Phenolic content was high in DFM and DFL, while flavonoid content was high in DFM and DFA. **Conclusion:** The four samples exhibited a varying range of antioxidant activities. The study helped identify and establish chemotypes with respect to selected host plants. So host specificity is an important factor to consider while using *D. falcata* for therapeutic purposes.

Keywords Quercetin, DPPH, FRAP, HPTLC, Gallic Acid

1. Introduction

Dendrophthoe falcata (L.f.) Ettingsh is a hemiparasitic perennial shrub which bears creeping grey aerial roots and much branched shoot system with thick coriaceous leaves and pink stout flowers. The plant produces haustorium that penetrates the host tissues to withdraw water and nutrients from the vascular system of the living host plant. Most hemiparasites are shown to acquire both primary and secondary compounds using their absorptive structures from their hosts [1]. Usually the compounds taken up from the hosts remain as such within the hemiparasites. This accounts for the diverse nature of secondary compounds within the hemiparasites, growing on various hosts [2]. *D. falcata* has been recorded growing on 420 hosts distributed among 227 genera of 77 families [3]. The phytoconstituents observed in *D. falcata* include proteins, carbohydrates, glycosides, flavonoids, triterpenes, tannins, steroids, saponins and phenolic compounds, on preliminary analysis. Therapeutically important chemical constituents such as alkaloids, phytosterols, fixed oils, gallic acid, ellagic acid, chebulinic acid, quercetin, oleonolic acid, beta sitosterol, stigmasterol, quercitrin, rutin, catechin, beta-amyrin acetate etc. are also detected in D. falcata growing on diverse host plants [4].

Scientific studies conducted on D. falcata showed its potentiality having Antidiabetic [5-6], Antioxidant [7], Antinociceptive [8], Antimicrobial [9-10] Diuretic and Antilithiatic [11], Anticancerous [12-13], Anxiolytic [14], Anthelmintic [15], Hepatoprotective [16], Immunomodulatory [17], Contraceptive [18], Estrogen receptor binding [19], Cytotoxic [20], Analgesic and Antiinflammatory [21], Anticonvulsant and Muscle relaxant [22] activities. The chemical components determine the therapeutic value of D. falcata that may vary according to the host specificity. Because of this, health benefits may differ from one plant to another, as well. Therefore, a thorough biological screening is necessary to understand the therapeutic dynamics of D. falcata growing on different medicinal plants.

The present study is focused to evaluate the variation in the in vitro antioxidant activity of ethanol extract of whole plant of D. falcata growing on four different medicinal host plants. Antioxidants are certain compounds that can prevent or slow down the oxidation process of certain molecules, which otherwise form free radicals through chain reactions that may cause cell damage [23]. Thus antioxidants ensure protection to our body cells from the action of free radicals. The study also intended to evaluate the influence of phenolic and flavonoid content present in the samples on antioxidant activity. HPTLC analysis was carried out to determine the variation in the chemical profile of *D. falcata* growing on different host plants.

2. Materials and Methods

Plant Material and Identification

The whole plant of *D. falcata* (Honey Suckle Misletoe) growing on *Anacardium occidentale L.* (Cashew tree), *Lagerstroemia speciosa* (L.) Pers. (Banaba plant), *Mangifera indica* L. (Mango tree) and *Strychnos nux-vomica* L. (Strychnine tree) was collected from different places of Malabar region. The plants were identified with the help of taxonomic characters by Scientists of Botany division, Centre for Medicinal Plants Research, Kottakkal Arya Vaidya Sala.

Extract Preparation

Plants are dried under shade for three weeks and powdered in electric mill. Extraction is carried out with 150 g of powdered drug in 600 ml ethanol by using Soxhlet apparatus for 5 hours. Extract is filtered and concentrated to dryness in a water bath.

Evaluation of Antioxidant Activity

DPPH Free Radical Scavenging Assay

DPPH assay was done as per the reference with minor modifications. The electron donating ability of the ethanol extract of selected plant samples was determined by estimating the absorbance changes caused by the bleaching of the deep purple-coloured ethanol solution of stable radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) using a spectrophotometer. Add 50 µl of the sample extracts at each different concentration to 150 µl of a 0.004% ethanol solution of DPPH. Absorbance was measured at 517 nm after incubating the test mixture for 30 minutes in dark at room temperature. The standard used was Quercetin. Percentage Inhibition of free radicals by DPPH was calculated using the following equation [24].

$$\text{Percentage of radical scavenging activity} = \left(\frac{(\text{AC} - \text{AS})}{\text{AC}}\right) \times 100$$

AC: Absorbance of the Control
AS: Absorbance of the Sample

Extract concentration bringing about 50% inhibition (IC_{50}) of the reagent DPPH was determined through linear regression analysis. Analysis of four samples and standard was done in triplicate. Concentration of substrate resulting in 50% loss of the DPPH activity is thus obtained, known as the IC_{50} value. The results from the DPPH method are usually interpreted by checking the parameter IC_{50} [25].

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was conducted as per the reference with a few modifications. First step involves preparation of the FRAP reagent by mixing 2.5 ml of 10 mM 2,4,6-tris (2pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 2.5 ml of 20 mM $FeCl_3$ in 25 ml of 0.1 M acetate buffer at pH 3.6. The newly prepared reagent was incubated for 10 minutes at 37 °C. Add 200µl of sample extract at different concentrations (25, 50, 100 and 200 µg/ml) to 2 ml of the FRAP reagent and make up to 10 ml by adding distilled water up to 10 ml. A blank was prepared in the same manner as mentioned above using distilled water as a substitute to the extract. Analysis of each sample and the standard $FeSO_4$ was done in triplicate. Absorbance was measured at 593 nm against blank after 30 minutes of incubation [26].

Total Phenolic Assay

Folin-Ciocalteu assay is conducted to assess the total phenolic content. Mix 1 ml of various concentrations of the extracts or standard - Gallic acid (20, 40, 60, 80 and 100 µg/ml) with 9 ml of distilled water and 1 ml of Folin-Ciocalteu reagent in a 25 ml volumetric flask. Keep aside for 5 minutes after shaking well. Then add 10 ml of 7% Na2CO3 solution to the mixture and make up the volume to 25 ml. A blank is prepared by mixing reagent in distilled water. Absorbance is measured against the reagent (blank) at 550 nm in an UV-Visible spectrophotometer (Shimadzu 1700, Japan) after incubating for 90 minutes under room temperature [27].

Total Flavonoid Assay

Aluminium chloride colorimetric assay is employed to determine the total flavonoid content. Mix 1 ml of various concentrations of the extracts or standard - Quercetin (20, 40, 60, 80 and 100 µg/ml) with 4 ml of distilled water and 0.30 ml of 5% $NaNO_2$ in a 10 ml volumetric flask. The mixture is kept aside for 5 minutes and shaken well after adding 0.3 ml of 10% $AlCl_3$. Then again after five minutes, add 2 ml of 1M NaOH and make up the volume to 10 ml using distilled water. The absorbance is measured against the blank at 510 nm in an UV-Visible spectrophotometer (Shimadzu 1700, Japan) [28].

High Performance Thin Layer Chromatography (HPTLC) Analysis

Chromatographic Instrument and Conditions

Instruments required for sample application are Linomat IV automatic TLC sampler (CAMAG, Muttenz, Switzerland) and a Hamilton (Bonaduz, Switzerland) syringe (100 µL). Chromatography was carried out on HPTLC plates with pre-coated silica gel 60 F_{254} (E. Merck, Darmstadt, Germany) of 0.2 mm thickness. CAMAG ADC having a glass twin trough chamber (20 × 10 × 4 cm) was used for spotting the samples on HPTLC plates. Developed plates were observed through CAMAG TLC Visualizer at 254 nm and 366 nm for taking photographs. CAMAG TLC Scanner was used for densitometric scanning of the developed plates. Data analysis was carried out using CAMAG winCATS integrated software.

Spotting of all samples and the standard was done with ethanol on a pre-washed, dried silica plate. 5.0 µl of each test extract and 3.0 µl of standard solution of Gallic acid were applied on silica plates having a band length of 8 mm and keeping a distance of 14 mm between tracks. The mobile phase chosen was Toluene - Ethyl acetate - Methanol - Formic acid (5:5:1:0.1, v/v). The plates are developed only after saturation of the chamber with the mobile phase for 30 minutes. After spotting, the silica plates are kept aside for 10 minutes inside the chamber to let them dry. Development of plates were done up to a height of about 75 mm at temperature 25 ± 2°C and 45% relative humidity. The plates were again dried after development by keeping under air current for 10 minutes. Documentation of the plates was carried out by taking photographs under UV 254 nm and 366 nm. The bands for Gallic acid were obtained after scanning under the absorbance mode using a deuterium lamp source at UV 254 nm and 366 nm. Derivatization of the Plate was done using Anisaldehyde Sulphuric acid (ANS) and documented under visible light. The calibration curves were plotted after recording the peak areas and concentrations [29].

Statistical Analysis

The assays of all samples and the standard were done in triplicate. The results were indicated in terms of mean values and their standard deviations.

3. Results

DPPH Radical Scavenging Assay

The four samples of *D. falcata* growing on *A. occidentale* (DFA), *L. speciosa* (DFL), *M. indica* (DFM) and *S. nux-vomica* (DFS) showed a dose dependent free radical scavenging activity on DPPH assay. Quercetin, which is a flavonoid and a well-known antioxidant was taken as the positive control. The actual color of DPPH is deep purple. Upon addition of extracts and positive control, decolorization can be seen and its extent is measured by using a spectrophotometer.

Table 1. Percentage Inhibition of DPPH by Quercetin and four samples of *D. falcata*

Sl. No.	Concentration (µg/ml)	Percentage Inhibition of DPPH				
		Quercetin	DFA	DFL	DFM	DFS
1	100	83.22 ± 0.34	83.28 ± 0.84	81.98 ± 0.54	83.86 ± 0.59	81.90 ± 0.62
2	50	80.74 ± 0.56	75.07 ± 0.61	74.22 ± 0.63	78.79 ± 0.83	76.07 ± 0.81
3	25	75.77 ± 0.65	62.61 ± 0.65	59.94 ± 0.88	73.42 ± 0.71	61.96 ± 0.52
4	12.5	71.43 ± 0.47	54.71 ± 0.86	43.78 ± 0.42	55.06 ± 0.56	48.16 ± 0.48
5	6.25	54.35 ± 0.85	44.68 ± 0.81	36.02 ± 0.83	43.04 ± 0.54	37.42 ± 0.53
6	3.125	38.81 ± 0.64	39.21± 0.49	24.53 ± 0.43	33.54 ± 0.42	30.98± 0.38

*Each value is presented as Mean ± Standard Deviation (n=3)

Table 2. IC_{50} values of four samples of *D. falcata* and Standard (Quercetin)

Sl. No.	Standard and samples	IC50 (µg/ml)
1	Quercetin	5.5
2	DFA	10
3	DFL	17.5
4	DFM	9
5	DFS	14.5

The DPPH free radical scavenging activity of four samples was compared with each other and with that of Quercetin. The IC_{50} values of samples and Quercetin were calculated from the values of percentage of inhibition and are given in Table 2. The free radical scavenging assay by the extracts follows the order as DFM > DFA > DFS > DFL. The lowest IC_{50} value, close to the value of Quercetin was shown by DFM, thus indicating the high antioxidant potential compared to other samples. The highest IC_{50} value was shown by DFL, thereby indicating the least antioxidant activity among the four samples. The free radical scavenging activities of four test extracts, exhibited a concentration-dependent activity due to reduction of DPPH radicals to non- radical form, as presented in Table 1.

Ferric Reducing Antioxidant Power Assay

FRAP assay involves formation of blue colored ferrous complex (Fe^{2+} tripyridyltriazine) from colorless ferric complex (Fe^{3+} tripyridyltriazine) due to reduction caused by the action of electron donating antioxidants at acidic pH. Change in absorbance was recorded at 593 nm to assess the reducing capacity of the extract (Blank reading was taken using distilled water instead of the test sample). FRAP values are obtained by calculating the difference between the absorbance of the sample and the blank. FRAP values were indicated as µMol Fe2+/mg of sample.

In the present study, the reducing potential of the antioxidants present in ethanol extract of *D. falcata* growing on four different host plants, was estimated by FRAP assay. The FRAP value of the plant samples was assessed from the linear calibration curve of standard $FeSO_4$ ($y = 0.022x – 0.0238$, $R^2 = 0.9878$), which is created from the data obtained in addition of FRAP reagent to varying known concentration of Fe^{2+} solution, as shown in Figure 1. FRAP capacity was assessed with reference to the capacity for reducing ferric ions. The four samples of *D. falcata* exhibited concentration dependent increase in their FRAP capacities. DFM exhibited the highest FRAP value of 72.9 ± 0.50 µMol Fe^{2+}/mg at 200 µg/ml of the extract, which shows the good antioxidant potential among the selected samples of *D. falcata*. The lowest FRAP capacity of 47.9 ± 0.95 µMol Fe^{2+}/mg was given by DFL at 200 µg/ml, which shows the least antioxidant potential compared to other samples of *D. falcata*.

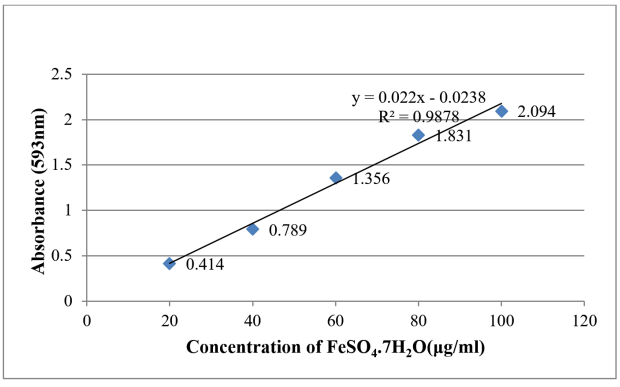


Figure 1. Standard Calibration Curve of $FeSO_4$ for the determination of FRAP

Table 3. Ferric Reducing Antioxidant Power of four samples of *D. falcata*

SL. No.	Concentration	DFA	DFL	DFM	DFS
1	25 µg/ml	27.4 ± 0.55	21.4 ± 0.80	21.1 ± 0.70	20.3 ± 0.85
2	50 µg/ml	44.4 ± 0.65	37.6 ± 0.55	37.6 ± 0.95	35.1 ± 0.55
3	100 µg/ml	61.7 ± 0.80	46.9 ± 0.60	62.6 ± 0.85	45.7 ± 0.80
4	200 µg/ml	64.0 ± 0.50	47.9 ± 0.95	72.9 ± 0.50	52.5 ± 0.60

*Each value is presented as Mean ± Standard Deviation (n=3)

The ferric reducing capacity of the extracts follows the order as DFM > DFA >DFS > DFL. FRAP capacities of four samples of *D. falcata* at different concentrations, expressed in terms of µMol Fe^{2+}/mg of plant extract are given in Table 3.

FRAP values obtained for the samples are compared to the FRAP values of known antioxidant Ascorbic acid, for evaluation of antioxidant potential. The FRAP values of *D. falcata* parasitic on *A. occidentale* (DFA) and *M. indica* (DFM) are almost close to the FRAP value of Ascorbic acid (Figure 2).

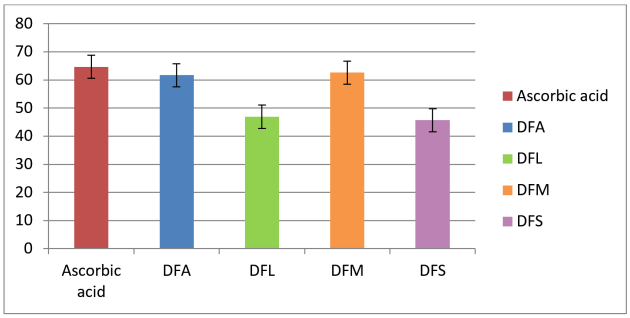


Figure 2. FRAP Values of four samples of *D. falcata* and Ascorbic acid at concentration 100µg/ml

Determination of Total Phenolic Content

The total phenolic content in the *D. falcata* samples was calculated from the linear calibration curve obtained for the standard Gallic acid (y = 0.0061x + 0.2778, R^2 = 0.9522) as shown in Figure 3. The total phenolic content of *D. falcata* samples was indicated as milligram Gallic acid equivalents (mg GAE/g of extract). The four samples of *D. falcata* showed a notable variation in the phenolic content ranging from 6.83 to 23.66 mg GAE/g. Phenolic content was high in DFM (23.66 ± 0.69 mg GAE/g) and low in DFS (6.83 ± 0.21 mg GAE/ g) (Table 4).

Determination of Total Flavonoid Content

The total flavonoid content in the *D. falcata* samples was calculated from the linear calibration curve obtained for the standard Quercetin (y = 0.0028x + 0.0748, R^2 = 0.9988) as shown in Figure 4. The total flavonoid content of *D. falcata* samples was indicated as milligram of Quercetin equivalents (mg QE/g of extract). The four samples exhibited marked variations in the flavonoid content, ranging from 3.0 to 12.5 mg QE/g. The highest flavonoid content was detected in DFM (12.5 ± 0.53 mg QE/g) and the lowest in DFS (3.0 ± 0.08 mg QE/g) (Table 5).

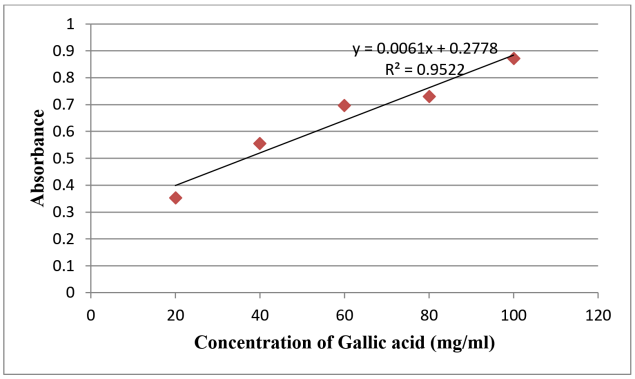


Figure 3. Standard Calibration Curve of Gallic acid for determination of total phenolic content

Table 4. Total phenolic content in the ethanol extract of *D. falcata* growing on different host plants

Sl. No:	Sample	Concentration of phenol in Gallic acid equivalent mg/g of extract
1	DFA	19.33 ± 0.52
2	DFL	21.33 ± 0.61
3	DFM	23.66 ± 0.69
4	DFS	6.83 ± 0.21

*Each value is presented as Mean ± Standard Deviation (n=3)

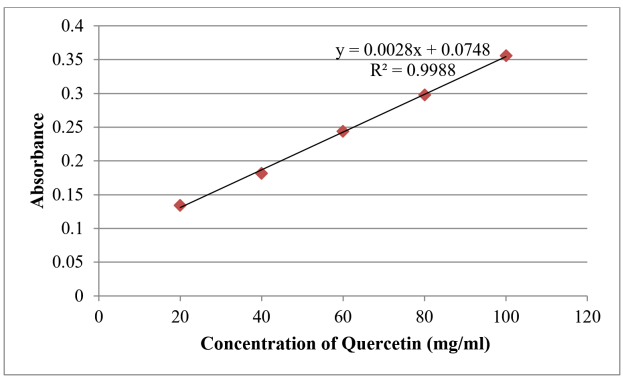


Figure 4. Standard Calibration Curve of Quercetin for determination of total flavonoid content

Table 5. Total flavonoid content in the ethanol extract of *D. falcata* growing on different host plants

Sl. No:	Sample	Concentration of flavonoid in Quercetin equivalent mg/g of extract
1	DFA	10.5 ± 0.36
2	DFL	8.0 ± 0.48
3	DFM	12.5 ± 0.53
4	DFS	3.0 ± 0.08

*Each value is presented as Mean ± Standard Deviation (n=3)

The content of different phytochemicals exhibited marked variations in selected samples of *D. falcata*, which signifies the need for host identification while taking the plant for therapeutic purpose.

HPTLC Analysis

HPTLC analysis was carried out to determine chemical variations among *D. falcata* growing on *M. indica*, *A. occidentale*, *L. speciosa* and *S. nux-vomica* with a special reference to the phenolic marker, Gallic acid. For the study, whole plant of *D. falcata* samples along with both infected and normal branches of the host plants was used. Ethanol is used as the solvent for extraction of both *D. falcata* and the host parts. The selected mobile phase Toluene (5): Ethyl acetate (5): Methanol (1): Formic acid (0.1) produced high resolution peaks for the standard Gallic acid at R_f 0.30 under UV 254nm and UV 366 nm. HPTLC profiles displayed notable variations, both in the quality and quantity of phytoconstituents, among the four samples. The two samples of *D. falcata*, DFA and DFM produced bands at R_f 0.30 corresponding to the marker Gallic acid (Figure 6- D & E). The HPTLC patterns of test samples vary greatly in terms of number of bands and its R_f values, indicating variations in the chemical composition (Tables 6, 7 & 8). The quantitative variations of components in the plant samples were estimated from the difference in the peak area corresponding to specific bands in the chromatogram.

Table 6. R_f values of *D. falcata* samples along with normal and infected part of Host plant at UV 254nm

Gallic acid	DFA	AON	AOI	DFL	LSN	LSI	DFM	MIN	MII	DFS	SNN	SNI
0.30	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.07
	0.13	0.19	0.20	0.21	**0.30**	0.23	0.14	0.21	0.21	0.21	0.10	0.22
	0.15	**0.30**	**0.30**	0.49	0.40	**0.30**	0.21	0.24	**0.30**	0.50	0.22	0.27
	0.21	0.35	0.35	0.65	0.48	0.41	**0.30**	**0.30**	0.35		0.32	0.33
	0.30	0.41	0.49		0.59	0.48	0.41	0.35	0.41		0.51	0.42
	0.42	0.49			0.65	0.51	0.52	0.41	0.50		0.67	0.47
	0.53	0.58			0.75	0.58		0.50	0.54		0.74	0.51
	0.65				0.89	0.64		0.54	0.57		0.78	0.62
					0.97	0.70		0.59	0.59		0.85	0.67
						0.74		0.66	0.66			0.75
								0.88	0.69			0.79
									0.88			0.86
									0.89			
									0.97			

Table 7. R_f values of *D. falcata* samples along with normal and infected part of Host plant at UV 366nm

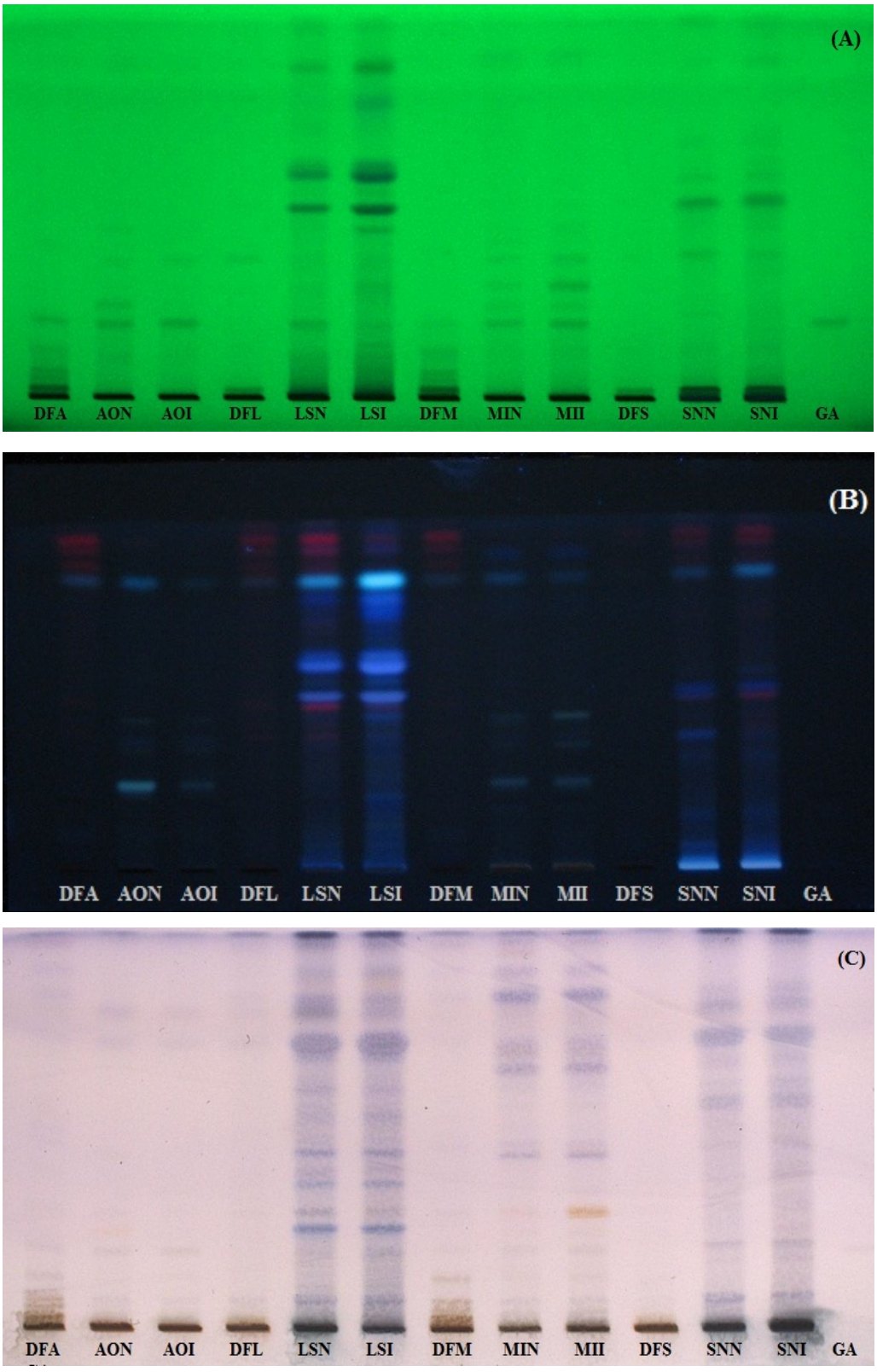
Gallic acid	DFA	AON	AOI	DFL	LSN	LSI	DFM	MIN	MII	DFS	SNN	SNI
0.30	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.07
	0.10	0.19	0.20	0.21	**0.30**	0.19	0.21	0.14	0.21	0.09	0.24	0.26
	0.13	**0.30**	**0.30**	0.49	0.39	**0.30**	**0.30**	0.21	**0.30**	0.21	0.51	0.46
	0.21	0.35	0.35	0.65	0.49	0.39	0.52	**0.30**	0.35	0.50	0.60	0.51
	0.30	0.40	0.48		0.55	0.49		0.35	0.41		0.66	0.59
	0.65	0.48	0.57		0.58	0.58		0.41	0.49		0.76	0.67
		0.58			0.61	0.64		0.50	0.58			0.77
					0.65	0.70		0.59				
					0.70	0.74						
					0.75							
					0.91							
					0.97							

Table 8. R_f values of *D. falcata* samples along with normal and infected part of Host plant after ANS Derivatization under visible light

Gallic acid	DFA	AON	AOI	DFL	LSN	LSI	DFM	MIN	MII	DFS	SNN	SNI
0.30	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.07
	0.11	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.17	0.18
	0.14	0.22	0.23	0.21	0.20	0.21	0.21	0.23	0.23	0.21	0.23	0.23
	0.16	**0.30**	**0.30**	0.25	0.24	0.23	**0.30**	**0.30**	**0.30**	0.36	0.28	0.29
	0.19	0.37	0.37	0.34	0.26	0.26	0.38	0.43	0.37	0.42	0.33	0.34
	0.21	0.42	0.50	0.42	**0.30**	**0.30**	0.42	0.50	0.42	0.48	0.41	0.38
	0.30	0.48	0.60	0.51	0.37	0.38	0.52	0.59	0.48	0.62	0.44	0.44
	0.41	0.53	0.66	0.6	0.42	0.43	0.61	0.62	0.59	0.74	0.48	0.46
	0.44	0.57	0.81	0.65	0.51	0.51	0.69	0.73	0.62	0.94	0.53	0.54
	0.60	0.58	0.92	0.80	0.54	0.55	0.75	0.79	0.73		0.63	0.56
	0.65	0.84		0.92	0.60	0.60	0.93	0.84	0.80		0.67	0.62
	0.67	0.92			0.62	0.63		0.89	0.85		0.68	0.69
	0.71				0.70	0.70		0.91	0.89		0.74	0.74
	0.75				0.79	0.78			0.91		0.8	0.76
	0.83				0.90	0.89					0.86	0.81
	0.94				0.93	0.92					0.95	0.88
												0.95

Variation of Gallic Acid in D. Falcata Samples

The quantity of Gallic acid in the samples was assessed by analyzing the area of the peak corresponding to the R_f value of Gallic acid (R_f 30). Even though two samples of *D. falcata* showed the presence of Gallic acid, they vary greatly. Peak area is high in DFA (2624.9 AU), which is almost close to the area of the marker Gallic acid (2861.5 AU) in the chromatogram whereas peak area is low in DFM (1090.8 AU). Gallic acid is completely absent in DFL and DFS. The infected and normal parts of the host plant *L. speciosa*, displayed bands corresponding to marker compound Gallic acid on HPTLC analysis confirming its presence, but Gallic acid is totally absent in *D. falcata* growing on the same plant (Figure 5).



AON: Normal part of Anacardium occidentale, **AOI**: Infected part of Anacardium occidentale

LSN: Normal part of Lagerstroemia speciosa, **LSI**: Infected part of Lagerstroemia speciosa

MIN: Normal part of Mangifera indica, **MII**: Infected part of Mangifera indica

SNN: Normal part of Strychnos nux-vomica, **SNI**: Infected part of Strychnos nux-vomica

Figure 5. HPTLC fingerprint profiles of *D. falcata* samples and their host parts with the marker Gallic acid (GA): (A) Under UV 254 nm (B) Under UV 366nm (C) Under visible light after ANS Derivatization

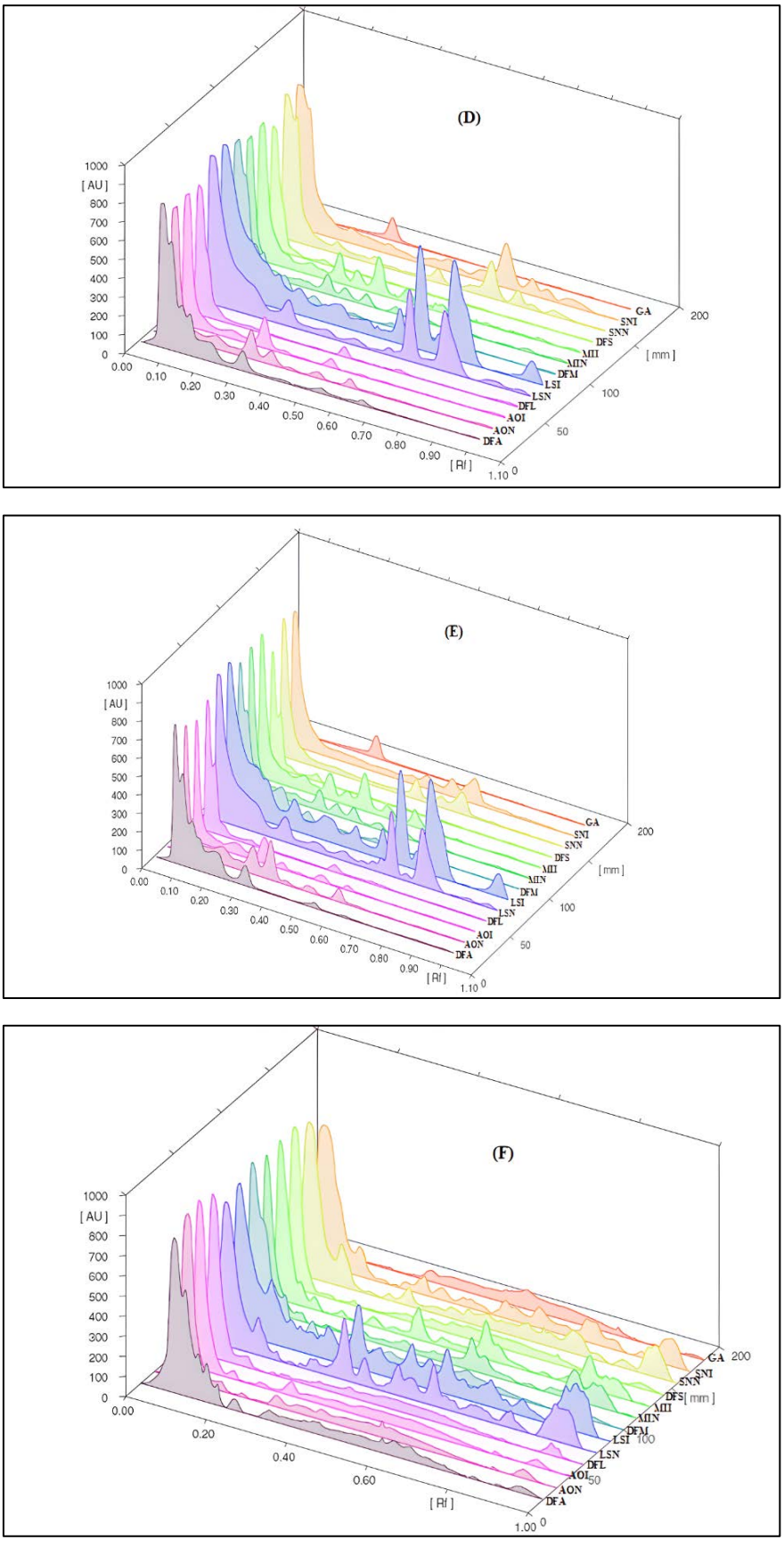


Figure 6. Densitometric scanning profiles of *D. falcata* samples and their host parts with Gallic acid: (D) Under UV 254 nm (E) Under UV 366nm (F) Under Visible light after ANS Derivatization

4. Discussion

The radical scavenging potential of the extracts in DPPH assay is found to be the most effective at concentration 100 µg/ml, where all test samples induced a percentage inhibition above 80% which is comparable to that of standard Quercetin. DFM showed the highest percentage inhibition (83.86 ± 0.59%), followed by DFA (83.28 ± 0.84%) which is even more than that induced by the standard Quercetin (83.22 ± 0.34%) at 100 µg/ml. In DPPH assay, the IC_{50} value of four samples was compared to that of Quercetin to recognize the varying nature of antioxidant potential in *D. falcata* grown on different host plants. The order of IC_{50} value is DFL (17.5 µg/ml) > DFS (14.5 µg/ml) > DFA (10 µg/ml) > DFM (9 µg/ml) > Quercetin (5.5 µg/ml). The variation in the IC_{50} values might be due to the presence of certain antioxidant components that are produced within *D. falcata* or transferred directly from the host as a result of interaction. Thus the four samples exhibited great differences in their antioxidant capacities. DFM and DFA having the lowest IC_{50} values close to that of Quercetin, exhibited the highest antioxidant capacities than other two samples. The antioxidant potential of the extracts follows the order as DFM > DFA > DFS > DFL.

In FRAP assay, antioxidant activity of the samples was assessed based on increase in the Fe^{2+} tripyridyltriazine complex, formed by reduction of Fe^{3+} tripyridyltriazine complex by the antioxidants present in the test extracts [30]. The four samples of *D. falcata* parasitic on different hosts exhibited noticeable variation in reducing capacities on FRAP assay, with FRAP values ranging from 47.9 to 72.9 µMol Fe^{2+}/mg of plant extract. The highest FRAP capacity was shown by DFM (72.9 ± 0.50 µMol Fe^{2+}/mg of plant extract) and lowest FRAP capacity was shown by DFL (47.9 ± 0.95 µMol Fe^{2+}/mg of plant extract). The most effective concentration of the extract in FRAP assay is observed as 200 µg/ml for all samples, which caused a significant reduction of ferric ions to ferrous ions. The difference in the FRAP values shown by the samples might be due to the qualitative and quantitative variations in the antioxidant components that impart different reducing capacities in *D. falcata* parasitic on different hosts. Such antioxidant compounds might have been either formed within *D. falcata* or directly transferred from the host as a result of host-parasite interaction.

The results of the antioxidant studies in the selected samples of *D. falcata* displayed an equivalent capacity on DPPH and FRAP assays, when compared to the standard antioxidants but to varying extents due to variations in the quality and quantity of chemical constituents in different samples with respect to host specificity. Variation in antioxidant activity may also occur with respect to different solvents used for extraction, due to the influence on phytochemical composition. Methanol extract of leaves of *D. falcata* parasitic on *M. indica* has shown a good anti-oxidant activity with IC_{50} value of 37.71 µg/ml while that of Quercetin was 7.79 µg/ml on DPPH assay [31]. Hydro alcoholic extract of *D. falcata* grown on *Azadirachta indica* exhibited a significantly high antioxidant activity than other solvent extracts on DPPH and FRAP assays [7].

Total phenolic and flavonoid content in DFM (23.66 ± 0.69 mg GAE/g and 12.5 ± 0.53 mg QE/g)) is about four -fold high to that present in DFS (6.83 ± 0.21 mg GAE/g and 3.0 ± 0.08 mg QE/g). Total phenolic content was high in DFM followed by DFL, while total flavonoid content was high in DFM followed by DFA. Both phenolic and flavonoid content was detected high in DFM, which might be the reason behind the high antioxidant potential of DFM than other three samples. The total phenolic and flavonoid contents in the selected samples of *D. falcata* vary greatly which is a clear indication of the quantitative differences in the active chemical components.

HPTLC analysis of selected samples of *D. falcata* using Gallic acid as the marker compound provided new information about the variation in the chemical constituents with respect to selected host plants. Gallic acid is significantly high in DFA which is almost halved in DFM. Gallic acid is totally absent in DFL and DFS. Apart from Gallic acid, variations are also observed in the number of bands obtained in the chromatogram of *D. falcata* samples, with different Rf values indicating the presence of different components. A band at R_f 21 was commonly seen in chromatograms of all four samples under UV 254 nm, UV 366 nm and visible light. A band at R_f 0.07, was observed in chromatograms of three samples except in DFS. Constituents at R_f 0.07, 0.21 and 0.65 were commonly seen in chromatograms of DFA and DFL (Tables 6, 7 & 8).

The infected and normal parts of the host plant were also subjected to HPTLC analysis, to detect the transferring nature of chemical components between the host and the hemiparasite. The normal and infected parts of the same host plant showed varying numbers of bands with different R_f values indicating the formation of new chemical components as a result of infection. DFL does not contain any trace of Gallic acid, while the infected and normal parts of host plant *L. speciosa* showed the presence of Gallic acid, revealing the selective uptake of compounds from the host**.** Thus the presence and quantity of Gallic acid content in the test samples of *D. falcata* collected from different host plants showed marked variations. The role of Gallic acid in radical scavenging effect was previously reported [32]. Gallic acid with tri-hydroxyl groups plays a significant role as an effective antioxidant, which was supported by a previous report mentioning the role of phenolic hydroxyl groups in a radical scavenging effect [33]. The high antioxidant activities shown by DFA and DFM might be due to the presence of Gallic acid.

Significant variations in the quality and quantity of phenolic compounds were observed in the leaves of *D. falcata*, parasitic on four diverse host plants through HPTLC analysis. Caffeic acid, (+)-epicatechin, and kaempferol were detected in varying quantities in *D.*

falcata growing on *M. indica*, *Melia azedarach*, *Wrightia tinctoria* and *Callistemon lanceolatus.* Ellagic acid was detected only in *D. falcata* parasitic on *M. indica*, while gallic acid was totally absent only in *D. falcata* parasitic on *M. azedarach* [34]. *D. falcata* parasitic on six diverse host plants, showed the presence of different flavonoids, of which Quercitrin and Quercetin were found to be the major common constituents [35]. Mangiferin (C-glucosyl xanthone) was detected in stem sample of *D. falcata* parasitic on *M. indica*, demonstrating the transfer of constituents from host to hemiparasite [36]. Thus host-parasite interaction plays a significant role in the presence of components shown by *D. falcata*. Majority of phenolic and flavonoid compounds reported in *D. falcata* parasitic on diverse hosts might have been transferred selectively from the host plants.

Thus host-parasitic interaction results in significant variations in the phytochemical composition of *D. falcata*, that affects the drug quality, accompanied with influential changes in the medicinal properties. The present study results reveal the antioxidant efficiency of selected samples of *D. falcata* parasitic on four different medicinal host plants, which may scavenge free radicals and ensure protection. However extensive research is absolutely necessary for the identification and isolation of different components in *D. falcata* imparting the antioxidant potential. Cytotoxicity assessment is also mandatory before using the plant for a therapeutic purpose.

5. Conclusions

The present study revealed that *D. falcata* parasitic on diverse hosts displayed significant variations in the antioxidant activity as evident by both DPPH and FRAP assays. Such variations in different samples of *D. falcata* arise due to host- parasite interactions. Thus it is very necessary to consider the host while choosing *D. falcata* for a medicinal purpose. The variation in the quantity of phenolic and flavonoid content, HPTLC fingerprint profiles and antioxidant activities in the selected samples of *D. falcata* parasitic on diverse host plants points out the necessity for performing qualitative and quantitative analysis of phytochemicals in different parts of the plant, before using the plant for therapeutic purpose.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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