International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 05-06-2021, Accepted: 17-06-2021, Published: 05-07-2021
Volume 6, Issue 4, 2021, Page No. 13-18

Comparative evaluation of antioxidant and antimicrobial activity of *Dendrophthoe falcata* leaves collected from two different host plants

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Abstract
Dendrophthoe falcate is well known for their medical properties, they have the ability to treat many diseases which were confirmed by the previous literature study. This study covers the antioxidant assay, antimicrobial assay and the plasmid DNA cleavage ability. The sample for this study was the leaves collected from *Mangifera indica* and *Achras sapota* which were the host plants for the hemiparasite (*Dendrophthoe falcate*) to grow on it. Therefore two traits of the sample were used for the study. From the collected leaf samples, the phytochemical assays were undergone qualitative for the presence of active chemical compounds like carbohydrate, protein, steroids, saponins, tannin, terpenoid, glycosides, alkaloid, flavonoid and phenol. Then the quantitative of the phytochemicals on the leaf samples was done to know the quantity of chemical compounds present. Two different antioxidant assays were performed are Ferrous ions chelating ability and Nitric oxide radical scavenging assay. The antimicrobial assay was performed against the bacteria and fungi that are antibacterial and antifungal experiments were done. Finally, the pUC18 plasmid DNA cleavage assay using agarose gel electrophoresis. They have a moderate ability to act against fungi while comparing their activity against bacteria. The ethanolic extract of the two traits shows cleavage property.

Keywords: *Dendrophthoe falcate*, antioxidant, antimicrobial, DNA cleavage

Introduction
Dendrophthoe falcata (D. falcata) is one of the hemiparasitic plants commonly attached to host plants such as *Mangifera indica* (Anacardiaceae), *Manilkara zapota* (Sapotaceae) and *Psidium guajava* (Myrtaceae). *Dendrophthoe falcata* commonly known as "Banda" is indigenous to tropical regions especially in India, Srilanka, Thailand, China, Australia, Bangladesh, Malaysia and Myanmar (Manthri *et al*., 2011) [12]. It is used in traditional medicine to cure various human health disorders (Warrier *et al*., 1993Various reports indicate that D. falcata is a potential source of phytochemicals and bioactive principles involved in various bioactivities (Dashora *et al*., 2011) [4]. Since, D. falcata is a hemiparasite, its phytoconstituents, bioactive principles, the range of bioactivities and other health care benefits are varied and influenced by its host plants.

D. *falcata* is known to be much effective treatment to bone fracture, rheumatic pains, leucorrhoea, wound healing, menstrual disorders, astringent, aphrodisiac, diuretic, antifertility, skin diseases, asthma abortion, schizophrenia, antilithiatic, antinociceptive, anti-hyperlipidaemic, anti-diabetic and psychological disorders (Nandkarni, 1996) [15].

The plant contains phytosterols, flavonoids, quercetin, phenolic compounds, tannin and terpins etc. The plant parts like leaves, stem and bark are known to possess antibacterial and antioxidant activities. Antibacterial and antifungal screening of aerial parts of *Dendrophthoe falcata* was studied by Pattanayak & Sunita (2008) [19] while Patil *et al*., (2012) [18] studied antibacterial sensitivity of different solvent of leaves of D. *falcata* growing on M. *indica*.

Since, D. *falcata* is a hemiparasite, its phytoconstituents, bioactive principles, the range of bioactivities and other health care benefits are varied and influenced by its host plants. Therefore, the present study was carried out to evaluate the qualitative and quantitative phytochemicals and minerals, and antimicrobial potential of *Dendrophthoe falcata* (L.f.) Ettingsh collected from Artocarpus heterophyllus host tree.

Thus the present study occurrence of D. falcate on two different host plants *Mangifera indica* (Mango) and *Achras sapota* (Sapodilla) generated interest to study the phytochemical, antioxidant and antimicrobial properties.

Materials and Methods
Collection of Plant Material
The leaves of *Dendrophthoe falcata* were collected from Nagercoil of Kanyakumari District, Tamilnadu, India. It was identified and authenticated by Dr P. Nagerndra Prasad, Head, Department of Biotechnology, Sri Paramakalyani College, Alwarkurichi, Tirunelveli. The plant was grouped into two groups based on the leaf size. The *Dendrophthoe falcata* plant from the mango tree is grouped as Trait 1 and from Sapodilla tree is grouped as Trait 2.

Preparation of Plant Extract
The collected samples were cleaned and dried to remove the moisture content. The dried samples were powdered to fine particles using the dry blender. Then the powdered samples were kept for solvent extraction successfully with three

different solvents namely ethanol, chloroform and aqueous by using the soxhlet apparatus and the extraction was carried out for 24 hours at temperature based on solvents. Then the extracts were filtered and concentrated at 45°C using the rotary vacuum evaporator and the concentrated extract was stored.

Qualitative Analysis of Phytochemicals

The leaf extracts of D. falcate (Trait 1mango tree and Trait 2 sapodilla tree) subjected to a qualitative phytochemical test to ensure the presence of chemical constitutes such as carbohydrate, protein, steroids, saponins, tannin, terpenoid, glycosides, alkaloid, flavonoid and phenol (Mukherjee 2002) [23].

Quantification of Phytoconstituents

Phytoconstituents were estimated by various methods like carbohydrate (Roe, 1955) [22] protein (Lowry *et al*., 1951) [11], steroids (Devanaboyina *et al*., 2013) [5], saponins (Sim, 2011) [23], tannin (Robert, 1971) [21], terpenoid (Ghorai *et al*., 2012) [8], glycosides (Solich *et al*.,1992) [24], alkaloid (Evans, 1966) [7], flavonoid and (Evans, 1966) [7] phenol (Evans, 1966) [7].

Determination of Antioxidant Activity

Ferrous ions Chelating Ability

The antioxidant assay of the extracts was evaluated by (Dinis *et al*. 1994) [6] method. The reaction mixture contained 1.0 ml of various concentrations of the extracts (2-10 mg/ml) and 0.05 ml of 2 mM $FeCl_3$. The reaction begins with the addition of 0.2 ml of 5 mM ferrozine. Then it was shaken thoroughly and allowed to stand at room temperature for 10 minutes and the absorbance was measured at 562 nm. Lower absorbance indicates the higher ferrous ion chelating ability.

$$\% \text{ Inhibition} = [(\text{Control- Test})/\text{control}] \times 100$$

Nitric Oxide Radical Scavenging Assay

Nitric oxide radical scavenging assay was done by the procedure described by (Green *et al*. 1982). The reaction mixture contained 3.0 ml of 10 mM sodium nitroprusside in phosphate-buffered saline (pH 7.4) and various concentration of (2-10 mg/ml) extracts. The resulting solution was then incubated at 25°C for 60 minutes. To the incubated sample 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% NEDD in 2% H_3PO_4) was added and the absorbance of the chromophore formed was measured at 546 nm against a reagent blank. Percentage inhibition of the nitrite ions generated is observed. The standard ascorbic acid and BHT were used for comparison. The antioxidant activity was determined by evaluating % inhibition.

Antibacterial Activity

Antibacterial activity of leaf extracts of D. *falcata* was determined by using the agar disc diffusion method on Muller Hinton agar (MHA) medium (Bauer *et al*., 1996) [1]. The bacterial strains were first cultured in a nutrient broth for 18 hours prior to use. Test organisms used are five Gram-positive, five Gram-negative and five fungi. 25 µl of the sample with 100 µg concentration is used as the test sample. Streptomycin 25 µg was used as positive control and sterile disc (Hi-media) was used as the negative control.

Cleavage of pUC18 DNA

The cleavage of supercoiled pUC18 DNA to its nicked circular form studied by using agarose gel electrophoresis. pUC18 DNA (0.3 µg) dissolved in 5 $mmolL^{-1}$Tris-HCl/50 $mmolL^{-1}$ NaCl buffer (pH 7.2), was treated with the complexes. The gel mixture was incubated at 37 °C for 1 hour and then mixed with the loading buffer. Each sample (10^{-3} M, 0.5 µL) was loaded into 1% (w/v) agarose gel. Electrophoresis was undertaken for 2 h at 100 V in Tris-acetate-EDTA (TAE) buffer (pH 8.0). Ethidium bromide present in the gel will help to view DNA under UV light and then photographed under a UV transilluminator. To improve the DNA cleaving activity of the complexes, hydrogen peroxide ($100\mu molL^{-1}$) was added to each sample.

Results and Discussion

Phytochemical Screening

The results of the preliminary phytochemical screening of various solvents (aqueous, ethanol and chloroform) extracts of *D. falcate* leaf was recorded and presented in Table 1. The result revealed that the presence of carbohydrate, protein, flavonoid, glycoside, tannin, steroid, saponin and phenol in the leaf sample extracts. The phytochemical screening of *D. falcata* T1 showed the presence of carbohydrate, protein, flavonoid, glycoside, tannin and terpenoid and T2 have carbohydrate, flavanoid, phenol, steroid, saponin and tannin (Table 1). Qualitative estimation of the phytochemicals is useful in the quantification process revealed the presence of carbohydrates, phytosterols, flavonoids, glycosides and phenolic compounds in D. *falcata*. Pattanayak *et al*. (2011) [20] reported that the chloroform extract of D. *falcata* had shown a positive result for steroids, terpenes, flavonoids, while methanol extract had revealed the presence of steroids, tannins, terpenes, glycosides and flavonoids. The results of the present study also are in agreement with the results of previous reports.

Table 1: Phytochemical analysis of *D. falcata* leaf extracts

Test Name	T1			T2		
	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform
Carbohydrate	+	+	-	+	+	-
Protein	+	+	-	-	-	-
Alkaloid	-	-	-	-	-	-
Flavanoid	+	+	+	+	+	
Glycoside	+	+	-	-	-	+
Steroid	-	-	+	+	-	-
Saponin	-	+	-	+	-	-
Phenol	+	+	-	+	+	-
Tannin	+	+	-	+	+	-
Terpenoid	+	+	-	-	-	+

Quantitative Estimation of Phytoconstituents

The quantitative estimation of different extracts was carried out and was tabulated in Table 2. Aqueous extract of T1 leaves showed the presence of carbohydrate (83 mg/g) and the maximum amount of saponin (143.6 mg/g) was present in T2 leaves. Whereas in ethanolic extracts, the maximum amount of carbohydrate (145 mg/g) was present in T2 leaves and T1 leaves showed the presence of protein 97 mg/g is the maximum amount. The phytochemical quantitative estimation carried out using different procedure for carbohydrate (Roe, 1955) [22] the aqueous and ethanol has the highest quantity of it. Protein content in Trait 2 is ethanol solvent was high and estimated by (Lowry *et al*., 1951) [11]. Phenol and saponin was high in Trait 2 aqueous and ethanol solvent. Saponin has the antioxidant effect, antiglycation, anti-inflammatory and surfactant activity due to amphipathic nature (Chen *et al*., 2008) [2], complexation with cholesterol and causes hemolysis in erythrocyte on iv, enhance proteins penetration through membranes and augment the cytotoxicity of targeted immunotoxins directed against human cancer cells. Although tannins have astringency and poor bioavailability, it is reported to possess the ability to reduce the risk of cardiovascular disease (Nagao *et al*., 2007) [14], antioxidant activity and anti-carcinogenic as well as anti-mutagenic properties (Strick *et al*., 2000) [25]. Phenolic compounds are well known for their antioxidant properties by acting either as free radical scavengers, reducing agents or metal chelators (Chua and Hidayathulla, 2017) [3].

Table 2: Quantitative estimation of phytoconstituents

Test	T1(mg / G)			T2(mg / G)		
	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform
Carbohydrate	83 ± 0.81	74.6 ± 1.1	-	119.6 ± 1.08	145 ± 0.70	
Protein	29.1 ± 0.62	97 ± 0.81	-			
Flavanoid	38.6 ± 0.62	43.9 ± 0.82	22.5 ± 0.41	19.1 ± 0.71	27.5 ± 0.93	
Glycoside	3 ± 0.16	1.6 ± 0.29	-			56.3 ± 1.08
Tannin	57.5 ± 1.08	52.4 ± 0.43	-	77.2 ± 0.77	27.3 ± 0.43	
Saponin	-	12.3 ± 0.44	-	143.6 ± 1.08		
Steroid	-	-	19.8 ± 0.68	60.1 ± 0.71		
Terpenoid	17.9 ± 0.69	47.4 ± 0.41	-			26.1 ± 0.54
Phenol	1.2 ± 0.2	2.1 ± 0.5	-	129.3 ± 1.08	59.6 ± 1.08	

Antioxidant Activity

Antioxidant activity was determined by nitric oxide scavenging assay and a ferrous iron chelating assay of all extracts at three different concentrations (25, 50 and 100 µg/ml) and the results of extracts are depicted in Table 3 and 4. The antioxidant activity increased with an increase in the concentration of the extracts. For nitric oxide scavenging assay, the ethanolic extracts of T1 leaves had maximal scavenging activity (64.5 ± 0.77) at 100 µg/ml and T2 plants showed 57.6 ± 0.56 in the aqueous extract. In the ferrous iron chelating assay, the concentration of 100 µg/ml aqueous extracts of T1 leaves showed higher ion chelating ability (46.1 ± 0.3) than the T2 leaves. The antioxidant property of the solvent extract was studied to know the radical scavenging activity. The nitric oxide scavenging activity and the ferrous ion chelating shows moreover similar percentage inhibition values for the Trait which confirms the antioxidant activity. The phenols and flavonoids in the sample which was confirmed by the phytochemical quantitative analysis show a good antioxidant property. The formation of the ferrous and ferrozine complex, suggest chelating activity and capture of ferrous ion before ferrozine. The absorbance of Fe–ferrozine complex was decreased based on concentration otherwise the activity increased with increasing concentration from 50 to 500 mg/ml (Dinis *et al*. 1994) [6]. The *D.falcata* also gives the result with increased inhibition at increased concentration. The presence of terpenes, vitamins and sterol compounds in the extract of *Dendropthoe falcata* leaves were found responsible for its antioxidant property. Pattanayak (2011) [20] in their study have confirmed the role of phenolic compounds present in the leaves of the plant to be the source of antioxidant properties. Another study revealed that the antioxidant activity of the plant to its polyphenol, flavonoid and phytosterol constituents (Dashora *et al*., 2011) [4].

Table 3: Nitric Oxide Scavenging Activity of *D. falcata* leaf extracts

Concentration	Percentage of Nitric oxide scavenged (%)						Standard (Ascorbic Acid)
	T1			T2			
	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform	
25 µg/ ml	26.7 ± 0.86	45 ± 0.40	18.3 ± 0.62	20.8 ± 0.54	18.9 ± 0.61	16.9 ± 0.28	56.3 ± 0.92
50 µg/ ml	35.1 ± 0.33	52.8 ± 0.57	26.1 ± 0.41	32.8 ± 0.84	31.7 ± 0.45	26.9 ± 0.67	69.5 ± 0.40
100 µg/ ml	42.6 ± 0.84	64.5 ± 0.77	34.1 ± 0.53	57.6 ± 0.56	54 ± 0.61	46.9 ± 0.71	92.5 ± 0.32

Table 4: Ferrous ion chelating activity of *D. falcata* leaf extracts

Concentration	Percentage of Ferrous Ion chelated (%)						Standard (GallicAcid)
	T1			T2			
	Aqueous	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform	
25 µg/ ml	25.6 ± 0.4	20.7 ± 0.5	18.5 ± 0.6	15.5 ± 0.43	15.0 ± 0.39	6.2 ± 0.24	55.4 ± 0.32
50 µg/ ml	38 ± 0.4	26.1 ± 0.4	25.5 ± 0.5	16.3 ± 0.39	20.5 ± 0.48	12.0 ± 0.35	74.9 ± 0.73
100 µg/ ml	46.1 ± 0.3	42.9 ± 0.09	36.2 ± 0.5	18 ± 0.70	21.7 ± 0.48	16 ± 0.35	93.4 ± 0.32

Antimicrobial Activity

Antimicrobial activities were carried out using *Dendrophthoe falcate* trait 1 and trait 2 on bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus mutans*, *Klebsiella pneumonia*, *Escherichia coli* and *Proteus vulgaris*. Fungi *Aspergillus niger* and *Aspergillus flavus*. The above-mentioned bacteria and fungi showed a certain diameter of zone of inhibition. The experiment was done with the positive and negative control. For trait 1 the aqueous extract showed the zone of inhibition for fungi and did not show any inhibition fungi. The ethanol and chloroform sample showed inhibition for *Klebsiella pneumonia* and *Aspergillus flavus*. Trait 2 gave the best result while comparing the trait 1 result, the ethanol and chloroform extract sample showed the zone of inhibition for all the bacteria and fungi. The aqueous extract showed inhibition in only three bacteria, not in fungi. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The zone of inhibition was studied for the sample and gave results with respect to the positive control used. The antibacterial activity observed in *D. falcata* might have arisen as a result of a number of phytoconstituents present in the plant.. The higher antibacterial activity might be due to the presence of high concentration of phytoconstituents such as phenols and saponins in the leaf extract of *D. falcata*. The varied phytochemical constituents (flavonoids, alkaloids, and saponins) present in the extracts were reported to possess biological activity against microbes (Narayana *et al*., 2001) [16]. Many authors have demonstrated the antibacterial activity of these phytochemicals (Takhi *et al*., 2011) [26]. Thus, the observed antibacterial activity in *D. falcata* may be due to synergy among such constituents like tannins, flavonoids, alkaloids, terpenoids and/or saponins as suggested by Ukwueze *et al*. (2013). As observed in this study, Orji *et al*. (2013) [17] reported that the ethanol leaf extract of Loranthus micranthus inhibited the growth of Aspergillus species and Penicillium species which are causative agents of infectious diseases as candidiasis, respiratory micosis, vaginosis, pelvic inflammatory disease, etc.

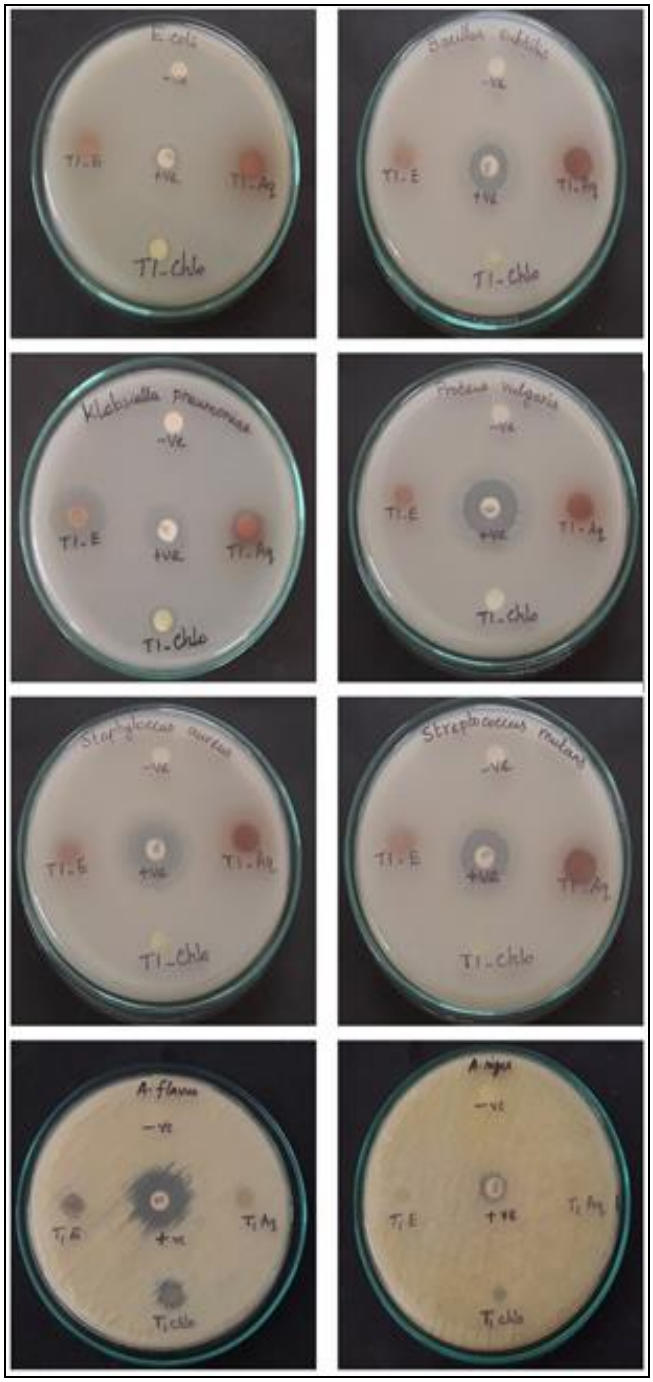

Fig 1: Antibaterial and Antifungal inhibition zone images of *Dendrophthoe falcata* trait1

Table 5: Antimicroial activity of different solvent extract of *Dendrophthoe falcata* (Trait 1) leaves against bacteria and fungi

Microbes		Zone of inhibition (mm)				
		T1 Aq	T1 E	T1 Chlo	Positive	Negative
Bacteria	*S. aureus*	10	-	-	19	-
	B. subtilis	10	-	-	15	-
	S. mutans	12	-	-	17	-
	E.coli	9	-	8	10	-
	K. pneumoniae	11	21	10	20	-
	P.vulgris	8	-	-	19	-
Fungi	*A. niger*	-	-	-	10	-
	A. flavus	-	7	8	19	-

Table 6: Antimirobial activity of different solvent extract of *Dendrophthoe falcata* (Trait 2) leaves against bacteria and fungi

Microbes		Zone of inhibition (mm)				
		T2 Aq	T2 E	T2 Chlo	Positive	Negative
Bacteria	*S. aureus*	-	21	11	25	-
	B. subtilis	-	22	10	15	-
	S. mutans	-	15	7	28	-
	E.coli	10	20	11	14	-
	K. pneumoniae	10	19	13	22	-
	P.vularis	9	16	12	18	-
Fungi	*A. niger*	-	15	13	31	-
	A. flavus	-	17	12	30	-

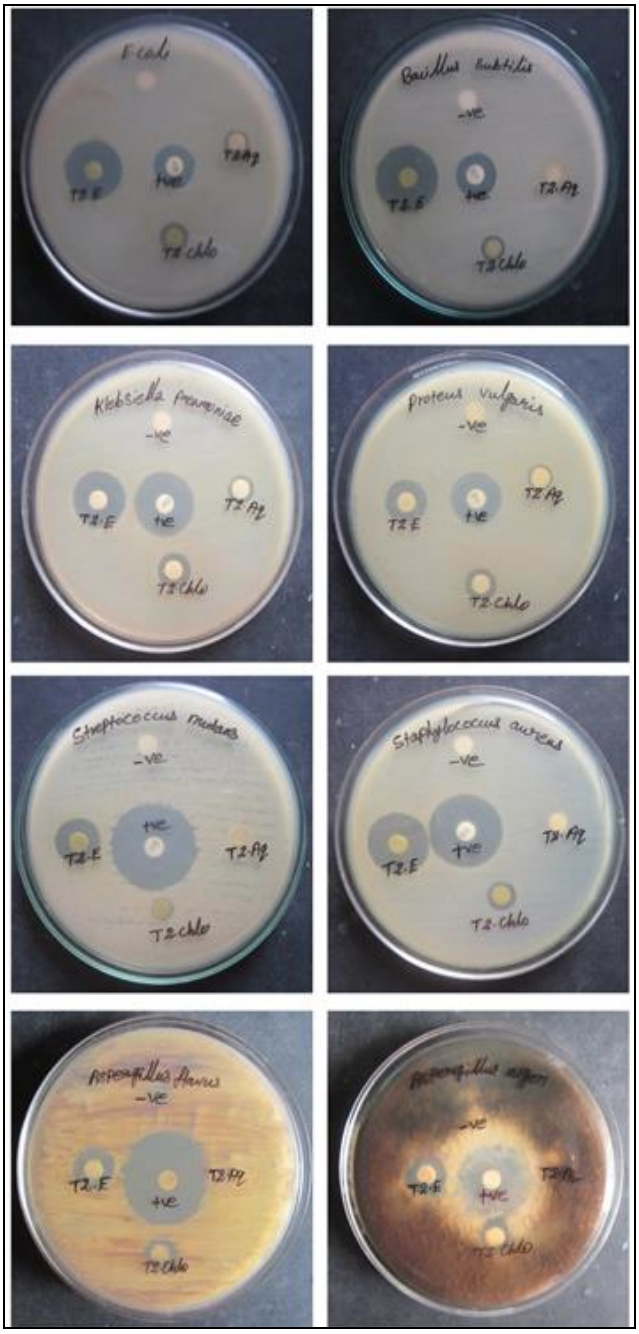

Fig 2: Antibaterial and Antifungal inhibition zone images of *Dendrophthoe falcata* Trait 2

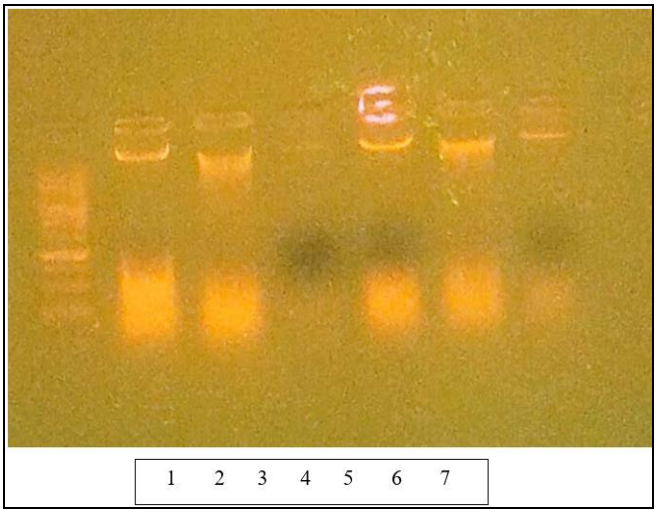

Fig 3: Electrophoresis gel image showing cleavage bond

Lane 1: Marker DNA

Lane 2: Plasmid DNA and Trait1 Aqueous- Not Cleaved the Plasmid DNA

Lane 3: Plasmid DNA and Trait1 Chloroform- Not Cleaved the Plasmid DNA

Lane 4: Plasmid DNA and Trait1 Ethanol - Completely Cleaved the Plasmid DNA

Lane 5: Plasmid DNA and Trait2 Aqueous- Not Cleaved the Plasmid DNA

Lane 6: Plasmid DNA and Trait2 Chloroform-Not Cleaved the Plasmid DNA

Lane 7: Plasmid DNA and Trait2 Ethanol - Completely Cleaved the Plasmid DNA

The image of electrophoretic gel reveals that the DNA was cleaved it was confirmed by the band formation. There is a total of seven lanes in the gel, lane 1 shows the DNA ladder which consists of DNA fragments with varying molecular weight. Lane 2 was loaded with plasmid DNA and trait 1 aqueous sample extract the result after the run shows that there was no cleavage happened. In lane 3 along with the plasmid DNA chloroform sample extract trait, 1 was added in the gel well which gives the result as no cleavage happened. Then about lane 4 which shows the complete cleavage of the plasmid DNA, it was loaded with ethanol sample extract trait 1and the plasmid DNA. Lane 5 did not show any cleaving property they are loaded with the mixture of plasmid DNA and trait 2 aqueous sample extract. Lane 6 was loaded with a mixture of plasmid DNA and trait 2 chloroform sample extract that does not show any cleavage in the plasmid DNA. Lane 6 shows cleavage of the plasmid DNA the well of this lane was loaded with plasmid DNA and trait 2 ethanol sample extract. The cleavage was done by the conversion of coiled DNA to linear nicked form (Justin *et al*, 2008). DNA cleavage activity of the extracts was analyzed by monitoring the conversion of supercoiled DNA to nicked circular DNA. In the agarose gel electrophoresis, supercoiled DNA quickly migrates than other forms. If one strand is broken supercoil form will turn open circular form. In this study control groups showed no effect on the plasmid DNA, but the ethanol extract of two traits only has the ability to cleave the plasmid DNA.

Conclusion

The qualitative and quantitative analysis of phytochemicals, antioxidant assay, antimicrobial assay and plasmid DNA cleavage assay study on Dendrophthoe falcata plant from mango tree are grouped as Trait 1 and from Sapodilla tree is grouped as Trait 2 concludes that they have good presence of phytochemicals like carbohydrate, proteins and saponins. The qualitative assay was way helpful in the quantitative assay of phytochemicals. They also have an antioxidant

property which was confirmed by two antioxidant assay Ferrous ions chelating ability and Nitric oxide radical scavenging assay. The antimicrobial activity was checked against bacteria and fungi. For the cleavage of the pUC18 plasmid DNA, the ethanol extract of both the trait has the cleaving property.

Acknowledgement
We are thankful to Biomeitez Research and Development Pvt. Ltd., and Staff Members for providing laboratory facilities and moral support.

Conflict of Interest
The authors declare that no conflict of interest exists in the course of conducting this research. All authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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