ELUCIDATION OF ANTIOXIDANT FRACTIONS IN LEAVES OF *Dendrophthoe falcata* L. AND STANDARDISATION OF TAPE METHOD OF CONTROL

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THESIS

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2021

DECLARATION

I hereby declare that the thesis entitled **"Elucidation of antioxidant fractions in leaves of *Dendrophthoe falcata* L. and standardisation of tape method of control"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that the thesis entitled **"Elucidation of antioxidant fractions in leaves of *Dendrophthoe falcata* L. and standardisation of tape method of control"** is a bonafide record of research work done independently by **Ms. Aswathi Gopal (2019-11-255)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Aswathi Gopal (2019-11-255)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Physiology**, agree that the thesis entitled **"Elucidation of antioxidant fractions in leaves of *Dendrophthoe falcata* L. and standardisation of tape method of control"** may be submitted by **Ms. Aswathi Gopal** in partial fulfillment of the requirement for the degree.

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Aswathi Gopal

DEDICATED TO

MY PARENTS

MY ADVISOR

FARMERS

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LIST OF ABBREVIATIONS

ºC	Degree celcius
%	Percent
Wt.	Weight
mM	Milli Molar
µL	Microlitre
M	Molar
D. falcata	*Dendrophthoe falcata*
Jack	Jackfruit
P.E	Petroleum ether
E.A	Ethyl acetate
W	Water
et al.	Co-author/ Co-worker
eV	Electron volt
amu	Atomic mass unit
mL	Milli litre
Chlor	Chloroform
SAP	Super absorbent polymer
SAC	Super absorbent cotton
SAT	Super absorbent paper

INTRODUCTION

INTRODUCTION

Dendrophthoe falcata is a partial stem parasite belonging to Loranthaceae family which is considered as a destructive pest of many economically important fruit trees such as mango, sapota, sugar apple and guava. It causes damage to the host plants by depleting nutrients, releasing toxins, and limiting their growth (Subhashini *et al*., 2019). Though several management strategies are suggested by previous researchers which include pruning of its branches (Perry 1995; Torngren *et al*. 1980), base banding using 2,4-D (Mathew and Habeeburrahaman, 2013) and spray of ethrel in combination with organosilicone (non-ionic surfactant) (Garggi, 2021), the latter gave a promising response in comparison to others without affecting the host. Because of its destructive nature, some of the potential pharmaceutical features of the parasite often go unnoticed; if prospectively exploited it will automatically open up avenues for targeted removal of the parasitizing aerial parts of *D. falcata*.

Plants are thought to be one of the most important sources for discovering and developing pharmaceuticals that are both effective and safe compared to currently available synthetic drugs. Traditional and folk medicines are gaining favour over contemporary therapy due to fewer side effects and a higher safety margin. *D. falcata*, a widely distributed plant in India, is one of nature's many plants with medicinal characteristics. The potential medicinal property of Loranthus has been reported in Ayurveda. Medicinal properties of mistletoes are host specific (Girija *et al*., 2009). It is reported to have diuretic, wound healing, anti-microbial, anti-helminthic, anti-fertility, antioxidant, anti-cancer, anti-diabetic, anti-hyperlipidemic and anti-hypersensitive activities (Manthri *et al*., 2011). In humans, high quantities of free radicals and reactive oxygen species (ROS) play a key role in the onset of diseases like cancer, rheumatoid arthritis, diabetes and Alzheimer's disease. Hence, natural antioxidants have received much scientific attention now a days as they exhibit no side effects when compared to synthetic antioxidants. The antioxidant property of these parasitic plants might be due to their phenolic compounds including tannins and flavonoids (Cook and Samman, 1996). However, it will be very

interesting to identify the novel antioxidant principles in the context of specific host associations.

With the view towards developing an effective management strategy, the current strategy developed for control of Loranthus using foliar application of ethrel @ 25 mL/L requires fine tuning to reduce the regrowth of the parasite on the host. The use of additives/surfactants along with the chemical shows promising responses and delay in re-growth of the parasite (Garggi, 2021). Tape method of ethrel+surfactant application can be tried as an alternative technique which needs further refinement, aimed to be standardised as part of this study.

With this outlook, the major aim of the study can be encompassed in two main sub objectives i) characterisation and elucidation of bioactive antioxidants from *D. falcata* leaves by *in-vitro* assays and identification of bioactive compounds through GC-MS/MS with prospective application in pharmaceutical sector, and ii) standardization of efficient and non-regenerative method to control *D. falcata* .

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

In the Indian Ayurvedic System of Medicine, *Dendrophthoe falcata* is known as "Vanda." *Dendrophthoe falcata* is a parasitic perennial climbing woody plant. Its a hemiparasitic plant found primarily in tropical areas and widely dispersed throughout India, whose entire plant is utilised in traditional medicine as a tonic. It is beneficial in the treatment of pulmonary tuberculosis, asthma, menstrual irregularities, and oedema sores, ulcers, calculi in the kidneys and vesicles, and kapha and pitta vitiated circumstances (Manthri *et al*., 2011).

Antioxidants have been shown to protect beta-cells and other cells from oxidative stress and to suppress oxidative processes in the human body. This study aims at finding the bioactive compounds from Loranthus with different host contexts, with maximum antioxidant potential and also for standardization of Loranthus control. This chapter examines the relevant literature on several elements of this subject.

2.1 Origin and distribution

Dendrophthoe falcata (L. f.) Ettingsh is a parasitic perennial climbing plant belonging to the family of Loranthaceae with over 401 plant hosts, including sugar apple (*Annona squamosa*) and epicortical roots such as those found on sapota (*Achras zapota*), guava (*Psidium guajava*), pomegranate (*Punica granatum*), Jackfruit (*Artocarpus heterophyllus*) and mango (*Mangifera indica*) (Mantri *et al*., 2011 ; Priya and Neelamegam, 2016). It is native to the tropics, particularly India, Sri Lanka, Thailand, China, Australia, Bangladesh, Malaysia, and Myanmar (Russell & Nickrent 2007).

2.2 Plant taxonomy and morphology

According to Bentham and Hookers (1890) classification, *Dendrophthoe falcata* is positioned into different taxa.

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Santalales

Family : Loranthaceae

Genus : *Dendrophthoe*

Species : *falcata*

Evergreen shrubs with many branching, often jointed branches, usually aerial, hemiparasitic or other seed plants. Petioles flattened above and rounded beneath, inflorescence racemose and spicate, sub umbellate, sometimes pubescent; leaves 7.6–25.4 cm long and 1.3—12.7 cm wide, alternate or sub opposite. Flowers are 2.5–10.2 cm long, brilliantly coloured (red–orange), regular, and bisexual, bracteates often have 2 or more bracteoles, stamens are many, anthers are basified or dorsifixed, ovary inferior and single celled, style short or long, stigma simple. Fruit is a single-seeded berry or drupe with a fleshy pericarp and viscid mesocarp. Seeds are single, without distinguishing testa, with fleshy albumen and a diameter of around 1.3cm (Pattanayak *et al*., 2008).

2.3 Physicochemical properties of *Dendrophthoe falcata*

The earthy matter or inorganic composition, as well as other contaminants present were determined using physicochemical studies. Total ash (10.11%), water soluble ash (7.20%), acid soluble ash (4.37%), and sulphated ash (8.86%) are the different types of ash present in *D. falcata*. Methanol extract 4.25 (% w/w), ethanol extract 4.18 (% w/w), ethyl acetate extract 3.81 (% w/w), benzene 2.40 (% w/w), and petroleum ether extract 7.60 (% w/w) were shown to be having the most effective extractive values (Kodithala *et al*., 2013)

2.3.1 Phytochemical compounds in *Dendrophthoe falcata*

Phytocompounds such as phenols, flavanoids, alkaloids, saponins and tannins are abundant in *Dendrophthoe falcata*. Flavonoids like quercetin and quercetrin are found in the leaves, as well as tannins such gallic and chebulinic acid are commonly found. The stem includes β-amyrin-0-acetate, oleonolic acid, its methyl ester acetate, β-

sitosterol, and stigmasterol, while the young shoots contain almost 10% tannins. The bark contains catechin and leucocynidin (API, 2015).

According to Sinoriya *et al.* (2011) carbohydrates, alkaloids (leaf), phytosterols, fixed oils, tannins, proteins, gums, mucilages and phenolic substances were found in the preliminary phytochemical screening of leaves of *D. falcata*. Gallic, ellagic, and chebulinic acids, as well as quercetin, have been discovered in *D. falcata* growing on *Terminalia tomentosa* (Indrani *et al*., 1980). From the leaves of *D. falcata* growing on *Nerium oleander*, three cardiac glycosides – strospeside, odoroside F, and neritaloside were identified (Boonsong and Wright, 1961). The stems of *D. falcata* grown on *Mangifera indica* were used to extract oleanolic acid, its acetate and methyl ester acetate, β-sitosterol, and stigmasterol (Anjaneyula *et al*., 1993).

Three new triterpenes, 3β-acetoxy-1β-(2-hydroxy-2-propoxy)-11α-hydroxyolean-12-ene (1), 3β-acetoxy-11α-ethoxy-1β-hydroxyolean-12-ene (2), and 3β-acetoxy-1β-hydroxy-11α-methoxyolean-12-ene (3), were discovered after extensive chromatographic screening of extracts from the fruits of the Indian Ayurvedic plant *Dendrophthoe falcata* along with compounds such as 3β-acetoxy-1β,11α-dihydroxy-olean-12-ene (4), 3β-acetoxy1β,11α-dihydroxy-urs-12-ene (5), 3β-acetoxy-urs-12-ene-11-one (6), 3β-acetoxy-lup-20(29)-ene (7), 30-nor-lup-20(29)-ene (8), 30-nor-lup-20(29) 3β-acetoxy-20-one (8), 3b-acetoxy-20-one (8), 3b-acetoxy-20 (20S) -3b-acetoxy-lupan-29-oic acid (9), kaempferol-3-O-a-L-rhamnopyranoside (10), quercetin-3-O-a-L-rhamnopyranoside and gallic acid (Mallavadhani *et al*., 2006).

Atun *et al.* (2019) qualitatively screened phenols and flavonoids from ethanolic extracts of leaves of *D. falcata* inhabiting host tree *Melia azedarach*. Total phenols and flavonoids were found to be higher in case of ethyl acetate fractions. Pattanayak and Mazumder (2010) carried out phytochemical screening of hydroalcoholic extract from aerial parts of *D. falcata* and spotted the presence of polyphenols, terpenes and steroids. Gond *et al*. (2018) characterized the phytochemical constituents in methanolic extract of *D. falcata* parasitizing *Madhuca indica*. Positive tests for tannins, saponins, reducing sugars, alkaloids, terpenoids, flavonoids and anthraquinones were found in the extract that was subjected to phytochemical

screening. Cardiac glycosides and phenols were not found in the extract, indicating their absence.

Anthraquinone glycosides, cardiac glycosides, coumarins, quinones, steroids, alkaloids, flavonoids, phenolics, tannins and terpenoids were found in preliminary phytochemical screening of *D. falcata* growing on *Boswellia serrata* Roxb. ex. Coleb. Quantitative analysis revealed high levels of alkaloids, flavonoids, phenolics, and saponins (Maheshwari and Rothe, 2018). Total phenolics, total flavonoids, and total proanthocyanidin concentration in 70% methanolic extracts of hemiparasite *D. falcata* were found to be 720.9 ± 10.540, 390.5 ± 0.8330, and 327.1 ± 0.513g/mL, respectively, by Parmar *et al*. (2010).

Examination of the phytochemical composition of several solvents of *Dendrophthoe falcata* on host tree *Azadirachta indica* by Channabasava *et al*. (2013) found that aqueous solvent extracts provided more compounds than other solvents. Carbohydrates, cardiac glycosides, proteins, amino acids, polysterols, alkaloids, phenols, tannins, and reducing sugars were all found in higher concentrations in the aqueous extract. The hexane and ethyl acetate extracts, on the other hand, generated less phytochemicals. The aqueous extract had the most phenolic compounds, followed by the methanol extract. Jain *et al*. (2016) conducted a qualitative analysis of secondary metabolites in *D. falcata* growing on *B. serrata* leaf extracts and discovered that chloroform extract included phenols, but ethyl acetate extract and methanolic extract had more phenols, flavanoids, and saponins.

Dendrophthoe pentandra contained tannins, saponins, flavonoids, and alkaloids, but no terpenoids, according to qualitative phytochemical screening of *D. pentandra* ethyl acetate (DPEA) extract. Using tannic acid equivalent, quantitative phytochemical analysis of DPEA extract reveals the presence of 1.3±0.2 percent alkaloid, 2.7±0.1 percent flavonoid, and 14.9±0.2 % total phenolic content (Yee *et al*., 2017). Phytochemical evaluation of medicinal plants from Bangladesh by Asadujjaman *et al*. (2013) showed that *D. falcata* had the presence of alkaloids, glycosides, phenols, taninns and reducing sugars.

Chloroform (CEDL) and Petroleum ether (PEDL) extracts of *D. falcata* were subjected to a variety of qualitative phytochemical analyses. Alkaloids, tannins, saponins, flavonoids, steroids, reducing sugars, and terpenoids were found in the phytochemical screening. The total phenol and total flavonoid concentrations of CEDL and PEDL were measured in mg/g of gallic acid and quercetin, respectively. CEDL and PEDL had TPCs of 155.20 ± 6.38 mg/g Eq gallic acid and 182.69 ± 7.12 mg/g Eq gallic acid, respectively. CEDL and PEDL had TFCs of 109.96 ± 5.90 mg/g Eq of quercetin and 132.63 ± 8.20 mg/g Eq of quercetin, respectively (Haque, 2015).

Priya and Neelamegam (2016) carried out phytochemical and antimicrobial evaluation of *Dendrophthoe falcata* parasitizing on host, *Artocarpus heterophyllus* and found that except for sucrose, the leaf sample of *D. falcata* contained majority of the compounds analysed, and among the extracts, acetone and ethanol extracts contained a greater number of compounds than chloroform, ethyl acetate and water extracts. Anthocyanins, coumarins and phenols were abundant in the acetone leaf extract, while flavonoids and saponins were moderate, and alkaloids, proteins and sugars were absent. Ethanol leaf extract was high in flavonoids and phenols, but low in alkaloids, anthocyanins, coumarins, proteins and tannins, and contained little steroids. They also quantified various biomolecules and minerals in leaf of *D. falcata* which revealed presence of higher amount of starch and calcium respectively.

Pattanayak *et al*. (2011) measured the total phenolic content (TPC) and total flavonoid content (TFC) of *Dendrophthoe falcata* extracts in chloroform (CEDF), methanol (MEDF) and hydroalcohol (HEDF) were determined using Folin–Ciocalteau and aluminium chloride colorimetric techniques, respectively. HEDF had the highest concentration of total phenolic compounds (46.43 2.55 mg GAE/g extract), while CEDF had the lowest concentration (21.78 ± 3.145 mg GAE/g extract). The HEDF had the maximum flavonoid level of 33.42 ± 2.083 mg QRT/g extract, whereas the CEDF had the lowest level (9.74 ± 1.324 mg QRT/g extract). Steroids, terpenes, glycosides, tannins, proteins, flavonoids, carbohydrates and polysaccharides were detected in the HEDF during preliminary phytochemical screening. Steroids, terpenes and flavonoids were found in CEDF, while steroids, tannins, terpenes, glycosides, and flavonoids were observed in MEDF.

According to Pattanayak and Sunita (2008) preliminary phytochemical screening of the crude ethanolic extract and fractions prepared from petroleum ether (A), chloroform (B), and ethanol (C) revealed the presence of steroids and terpenes in all fractions, but glycosides, tannins, and proteins in fractions B and C. Furthermore, fraction C yielded positive results for flavonoids, while fraction A yielded positive results for fixed oil.

GC-MS study by Beulah *et al*. (2018) revealed twenty-six components in the ethanol extract of *D. falcata* leaf. The predominant compounds were Dibutyl phthalate (13.26%), n-Hexadecanoic acid (13.02%), 1,3,4,5-TetrahydroxyCyclohexan (8.43%), 2,4-Imidazolidinedione, 1 – [[5-Nitro-2-(uranyl)Methylene] Amino] (6.36%), Z,E-2-Methyl-3, 13-Octadecadien-1 5-methylhex-2-yl butyl ester (4.28%), 1,2,3-Benzenetriol (Pyrogallol) (4.17%), 1-Octadecanol (3.99%), 9-Octadecenoic acid(Z) (3.99%), Phthalic acid, bis-(10-hydroxydecyl ester) (3.71%), 2,6,10-Trimethyl, 14-Ethylene-14-pen (3.52%) (2.67 %), Di-noctyl phthalate (2.22%), 1,2,Dibromo -1-Chloro-1,2,2 - trifluoroethane (2.02%), 1,2-Benzenedicarboxylic acid and Dimethylester (1.96%).

Secondary metabolites such as alkaloids, flavonoids, saponins, tannins, sterols and triterpenes were identified in phytochemical screening of extracts dichloromethane:methanol (DM), methanol:water (MW) and ethanol:water (EW) of *Dendrophthoe falcata* infesting *Mangifera indica*. Analysis using GC–MS revealed 16 phytocompounds found in the DFDM extract which include diethetyl phosphonate (43.8%), benzyl oxy tridecanoic acid (44%), 9(2-phenyl ethyl) heptadecane (29.1%), hexadecanoic acid butyl ester (65.5%), linoleic acid, 2,3 bis (O-TMS) propyl esters (26.3 percent) (Bhagat and konduwara, 2021).

GC-MS was used to identify twenty phytocompounds in the aqueous extract of *D. falcata* leaves. The major compounds found were 1,2 Benzenedicarboxylic acid, phthalic acid derivatives, campestrol, octadecanoic acid, heptadecanoic acid, 9-octadecenoic acid, methyl ester, and cis-13,16- docasadienoic acid (Beschi *et al*., 2021).

2.3.2 Volatile bioactive compounds

GC-MS analysis of methanolic extract of seeds of *Origanam vulgare* has cis-vaccenic acid(RT=17.014) and phytol (RT=16.625) as the third set of peak with exhibited anti-carcinogenic and anti-inflammatory properties, respectively (Al-Tameme *et al*., 2015).

Chloroform:methanol (1:1) extract of leaves of *Camellia sinensis* when subjected to GC-MS analysis revealed the presence of cis-vaccenic acid (Gupta and Kumar, 2017). Methanolic extract of *Crateva adansonii* when analysed with GC-MS reported the presence of bioactive compound cis-vaccenic acid (Christiana *et al*., 2019).

The volatile chemical compositions of *Albizia adianthifolia* (heartwood) and *Pterocarpus angolensis* (stem bark) from n-hexane and chloroform extracts revealed the presence of n-hexadecanoic acid, which can be linked to their anti-inflammatory, antioxidant, and anti-androgenic properties (Abubakar and Majinda, 2016).

GC-MS study revealed distinct types of high and low molecular weight components in ethanolic crude extracts of weed, *Pistia stratiotes* L. and *Eichhornia crassipes* which include n-hexadecanoic acid, as common compound. Phytols were found in case of *E. crassipes* (Tyagi and Agarwal, 2017). According to Agyei *et al*. (2020) the GC-MS chromatogram of acetone and ethanol extract of *Daedalea elegans* showed dominant peaks of n-hexadecanoic acid which is considered to have anti-inflammatory and antioxidant activity.

GC-MS analysis of ethanolic extract of *Clerodendrum serratum* roots showed the presence of squalene (Reddy *et al*., 2021). It has been extensively researched and reported to have beneficial bioactivities such as antioxidant, cytoprotective and antitumor properties, so it is recommended for supplementation. It is thought to be responsible for tumour growth inhibition and the prevention of normal cells from mutating into tumour cells when exposed to oxidative stress (Kohno et al. 1995; Murakoshi et al. 1992; Amarowicz 2009). GC-MS study of essential oil from *Discocleidion rufescens* leaves by Jia *et al*. (2008) revealed the presence of phytol (39.30%) and n-hexadeconic acid (11.72%). Antinociceptive, antioxidant, anti-

inflammatory and anti-allergic properties are all found in phytol (de Moraes *et al*., 2014). Okoye *et al*. (2014) discovered that α-amyrin and β-amyrin acetate extracted from the *Alstonia boonei* stem bark have potent anti-inflammatory properties.

2.4 Pharmacological properties of *Dendrophthoe falcata*

Dendrophthoe falcata is thought to have medicinal virtues by the local people. It has diuretic, wound healing, antimicrobial, antihelminthic, antifertility, anti-oxidant, anti-cancer, anti-diabetic, anti-hyperlipidemic, and anti-hypertensive properties, according to reports (Manthri *et al*., 2011).

2.4.1 Antioxidant activity

According to Hasan *et al*. (2006) ethanolic extract of *D. falcata* exhibited very potent radical scavenging properties in a TLC-based qualitative antioxidant assay using DPPH, as indicated by a yellowish spot on the reddish purple background of the TLC plate. The main compound quercitrin or quercetin 3-*O*-α rhamnoside is responsible for the extract's potent antioxidant activity (50% inhibition concentration (IC_{50}) 5.1µg/mg).

With an IC_{50} of 77.8 µg/mL for DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity, Patil *et al*. (2011) proved that methanolic extract of *D. falcata* leaves is a more effective antioxidant than aqueous extracts.

When compared to ascorbic acid soluble fraction (ASA), the DPPH free radical scavenging capacity of crude extract and various fractions revealed substantial activity. ASA, methanol extract (ME), petroleum ether soluble fraction (PESF), chloroform soluble fraction (CSF) and aqueous soluble fraction (AQSF) had IC_{50} values of 43.04 ± 1.42 µg/ml, 305.00 ± 4.51 µg/ml, 136.52 ± 3.55 µg/ml, 245.80 ± 3.45 µg/ml, and 43.49 ± 1.12 µg/ml, respectively (Rafe *et al*., 2018). Atun *et al*. (2019) investigated antioxidant activity in *D. falcata* leaves and found that IC_{50} values less than 10 µg/mL indicated high antioxidant activity. The IC_{50} values for ethyl acetate and ethanolic fractions were 6.66 and 9.65 µg/mL, respectively.

Pattanayak *et al*. (2011) examined the scavenging effects of HEDF and ascorbic acid on DPPH free radicals. When compared to ascorbic acid, HEDF exhibited a reduced scavenging impact on the DPPH radical and had a significant scavenging effect with increasing concentrations in the range of 25-250 µg/mL. The IC_{50} values for HEDF, MEDF, and CDFE were 46.8, 81.4, and 100.07µg/mL, respectively. Lower IC_{50} values are associated with higher DPPH radical scavenging activity.

In the sodium nitroprusside/Griss reagent method, ethanol extract showed superior *in-vivo* antioxidant activity in both DPPH radical scavenging (IC_{50}=16.78 µg/ml) and nitric oxide radical scavenging (IC_{50}=54.5 µg/ml). The ethanolic extract of aerial portions of *Dendrophthoe falcata* had significant antioxidant action, suppressing lipid peroxidation, lowering glutathione levels, increasing catalase activity and lowering superoxide dismutase levels (Sinoria *et al*., 2011). According to Mohesh *et al*. (2018), DPPH radical scavenging activity of methanolic extract of *D. falcata* with IC_{50} and the control quercetin's IC_{50} were discovered to be 37.75 g/ml and 7.79 g/ml, respectively. In comparison to the quercetin compound, free radical scavenging activity was shown to be present, but the strength of the dosage of the extract necessary to bring about radical scavenging activity was found to be larger.

The percentage DPPH inhibition activity of DFDM extracts and ascorbic acid was measured at different concentrations between 25 and 200 g/ml, with higher DPPH free radical scavenging activity found to be 81.02 ± 3.76% for extract and 72.52 ± 1.15, 67.41 ± 2.49, 68.34 ± 2.33% for fractions, and 99.89 ± 2.56% for ascorbic acid (Bhagat and Konduwara, 2021).

D. falcata methanolic and aqueous extracts showed antioxidant activity in various *in vitro* models. IC_{50} values for total antioxidant capacity were 260 µg/mL and 180 µg/mL for methanolic and aqueous extracts, respectively, 18 µg/mL and 26 g/mL for DPPH and 22 µg/mL and 29 µg/mL for ABTS, and 62 g/mL and 75 g/mL for nitric oxide radical activity (Dashora *et al*., 2011).

Using the DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging experiment, Asadujjaman *et al*. (2013) evaluated the antioxidant activity of ethanol extracts of eight medicinal plants from Bangladesh. These plant extracts were reported to have potent antioxidant properties, which could explain and justify some of their traditional medical applications. *Ammannia multiflora*, *Caesalpinia pulcherrima*, *Dendrophthoe falcata* and *Syzygium cumini* extracts were shown to have substantial DPPH free radical scavenging activity, with IC_{50} values of 6.25 µg/ml, 7.46 µg/ml, 6.22 µg/ml, and 6.08 µg/ml, respectively, when compared to the IC_{50} value of 4.5 µg/ml of ascorbic acid in a dose dependent manner. Both chloroform and petroleum ether extracts of *D. falcata* contain DPPH radical scavenging activity that varies with concentration. The standard ascorbic acid's IC_{50} was 12.58±2.34g/ml, while CEDS and PEDS showed 36.38±5.33 and 33.42±3.15 g/ml, respectively (Haque, 2015).

According to Jadhav *et al*. (2005) total phenolics, total flavonoids, and DPPH free radical scavenging activity in methanolic extracts of three host plants show a definite pattern, with the greatest value for *M. indica*, followed by *P. guajava*, and *M. azadirachta*. These plants' hemiparasite samples revealed that the host had a substantial impact on total phenolics and total flavonoids. In the case of radical scavenging activity, however, no significant influence was seen. It is also clear that the hemiparasite's total flavonoids are either very similar to or higher than those of the host plants, which is reflected in lower IC50 values for DPPH radical scavenging activity. This trend was also seen in the HPTLC flavonoid pattern, where at least one extra peak was seen in the parasite plant that was not present in the host plant. Further, the presence of mangiferin was confirmed in *D. falcata* parasitizing on host mango.

2.5 Host parasite relation of Loranthus

Plant parasites are a type of flowering plants that grow on other living plants and rely on them for nutrients and water, either partially or totally. They connect to the host through specialised structures called haustoria. These parasites are classed as root or shoot parasites depending on where they attach to their hosts, and as hemiparasitic or holoparasitic based on whether or not their leaves have functional chloroplasts.

Mistletoes (*Loranthus sp*.) are one of the most common plant parasites in India, and they can be found in a variety of vegetative zones. When compared to any other member of the parasitic family of flowering plants, they cause significant economic loss to our fruit and timber trees. Plant parasites have a number of well-known consequences on their host trees, including reduced overall growth rate and vigour, low fruit and seed output, delayed branch drying, predisposition to other pests and diseases, and finally, premature death. Several botanical researchers have reported on mistletoe infestations, their prevalence in urban environments, and the potential impacts Loranthus *sp*. could have on a wide range of native and invasive species (Sreenivasan, 2020).

2.5.1 Host preference

Plants like *Achras zapota*, *Annona reticulata* L., *Annona squamosa* L., *Bombax ceiba* L., *Bauhinia purpurea* L., *Cassia fistula* L., *Casuarina equsetifolia*, *Eucalyptus globules, Ficus religiosa* L., *Nerium odorum* and *Psidium guajava* are most common host preferences of *D.falcata*. *D. falcata* was found in abundance on *Mangifera indica*, as well as *Annona squamosa* L. (Annonaceae) and *Achras zapota*, two other fruit-bearing plants (Sapotaceae). Due to the fierce effects of the stem parasite *D. falcata*, *M. indica* had the highest infestation and mortality rate of any host plant (Kuramana *et al*., 2020).

2.5.2 Life cycle of Loranthus

During the summer, *D. falcata* produces an abundance of berry-like fruits on its branches. Birds disperse the seeds after eating these berries, which are stuck to tree trunks at branching junctions of the host with the help of a sticky viscin known as "bird glue." On the commencement of monsoon, seeds on the host surface (tree trunk) germinate and penetrate directly into the host bark. The parasite's initial growth is gradual, but after it has penetrated the host, it produces a sucking apparatus called the haustorium. The haustorium enters host tissue, absorbing water and nutrients from the host's conducting tissue. The establishment of a parasitic connection results in the formation of a large knob or gall-like outgrowth, as well as a point of contact between

the parasite and the host known as the woodrose, which is a prominent feature of mistletoes. *D. falcata* possesses fully functional leaves, but it lacks a real root system, making it unable to survive in the absence of a host plant. Nutrients and water taken by the host plant's roots are transferred to the parasite's growth, resulting in a significant reduction in the host's growth above the point of penetration. Parasites develop at a faster rate at the same time. The host is entirely weakened by the development of multiple Loranthus branches. The host plant's vigour is significantly diminished, and the quantity and quality of the host plant's fruits are lowered as a result. Epicortical roots with haustoria aid in the parasitic plant's attachment to its host and allow it to grow larger (Kuramana *et al*., 2020).

2.5.3 Host mistletoe interaction

The mistletoe shoots, leaves, and seeds (transpiration and photosynthesis), the haustorium connecting mistletoe and host (adds frictional resistance), and the endophytic systems, epicortical runners, and secondary haustoria are three important components of the host–mistletoe system (Kuijt, 1991). Few members of the Loranthaceae and Santalaceae families have an endoparasitic life cycle with three distinct stages: lack of autonomous growth due to epicotyl (embryonic shoot apex) abortion; vegetative body development restricted to endophyte; and exophyte formed exclusively by reproductive structures. All endoparasitic Santalalean species have been found to exhibit epicotyl abortion (Costa and Davis, 2021).

When the host's photosynthesis was at its peak, the xylem water potential was found to be at its lowest. The mistletoe must endure a higher negative water potential than the host in order to maintain a flux gradient and avoid stomatal closure and withering. The hemiparasite is capable of collecting water from the host and delaying stomatal closure to significantly lower host xylem water potential compared to the host by tolerating lower water potential. Maintaining a high transpiration flux allowed holoparasites to maintain a continuous flow of photosynthate-rich phloem sap from the host to the parasitic (Glatzel and Geils, 2009).

Host parasitic dynamics between the parasites *D. falcata* and *H. elastica* and the host cocoa was studied using labelled radiolabelled ^{32}P by foliar and root application, revealed that there existed a two-way communication between the host and parasite. A prioritized partitioning of ^{32}P between host and parasite based on the sink demand was observed Garggi *et al*., 2020).

Gas exchange parameters such as photosynthetic rate and transpiration rate, and stomatal conductivity was higher for *D. falcata* and *H. elastica* compared to its host, cocoa. Consequently, carboxylation efficiency and light use efficiency was higher for parasites, whereas water use efficiency was higher in case of the host, cocoa (Garggi, 2021).

2.6 Management of Loranthus

Manual cutting of infested branches or complete removal of severely infested parts are recommended mistletoe management strategies, as cutting causes practical difficulty of removing the penetrated subepidermal haustoria from the host and the parasite's ability to re-emerge from any left-over portions (Perry 1995; Torngren *et al*., 1980). According to Kuramana *et al*. (2020) some of agricultural practices in mango orchards to control bird pollination, pollen development and seed dispersal of *D. falcata* are:

(1) The cultivation of some alternative crops/ fencing crops to attract birds.

Tickell's Flowerpecker pollinates the plant species *Muntingia calabura* L. (Muntingiaceae), *Sterculia colorata* Roxb. (Malvaceae), and *Woodfordia floribunda* L. (KURZ) (Lythraceae); therefore, they could be used as a fencing crop or an alternative to Tickell's Flowerpecker birds. (2) The use of small concentrations of chemicals or plant growth regulators to cause male sterility of *D. falcata*, at different stages of pollen growth, plant hormones such as ethrel, isothiazole, gibberellins, and abscisic acid may be used to cause male sterility in *D. falcata*. (3) The degradation of viscin tissue (bird glue) using ethylene diamine tetra acetic acid, sodium carbonate or sodium borohydride to control seed adhesion and seed dispersal of *D. falcata*.

Alternative strategies include foliar application of herbicides such as 40 percent diesel emulsion or 2,4-D (Baillon and Frochot 1987; Vidhyasekaran, 2004) or glyphosate (Baillon *et al*. 1988), as well as a technically difficult procedure of basal injection with a mixture containing seven parts $CuSO_4$ and one part 2,4-D (Prakash 2004). Hawksworth and Wiens (1996) look at a number of herbicides and growth regulators tested between 1970 and the early 1990s, including Dacamine, MCPA, Butyrac, Goal, Thistrol, D-40, Weedone, Emulsamine, DPX, Prime, and 2,4,5-T. The endophytic system is not killed by these compounds, despite the fact that they cause shoot mortality with minimum host harm. Experiments with systemic drugs have been inconclusive so far (Shamoun and DeWald 2002).

Base banding technique of managing Loranthus using 2,4-D (Mathew and Habeeburrahaman, 2013) was also attempted, but due to the reason of bark splitting in host a better alternative was attempted. Ethephon is a ripening hormone that works by releasing ethylene, which causes flowers, fruits, and shoots to abscise early. The use of ethephon at a concentration of 25 mL/L was found to be efficient in suppressing the parasite without affecting the host plant (Girija, 2015). When ethrel was used in combination with an organosilicone surfactant, Knoche *et al*. (1991) found that the growth regulator's efficacy was increased. Ethrel (25mL/L) and organosilicone sprayed at different concentrations (0.2mL/L, 0.5mL/L and 0.8mL/L) to Loranthus infesting cocoa proved that application of Ethrel and OS at 25mL/L and 0.5mL/L respectively lead to complete defoliation of Loranthus in three days (Garggi, 2021).

Super absorbent polymers, also known as hydrogels, are loosely cross-linked, three-dimensional networks of flexible polymer that can absorb and store hundreds of times their dry weight due to a small number of widthwise connections (Kiatkamjornwong, 2007). According to Li and Bai (2005), the biodegradation of phenanthrene, a model polycyclic aromatic hydrocarbons (PAH), was examined in saline micellar solutions of a biodegradable commercial alcohol ethoxylate, non-ionic surfactant. The solubility of phenanthrene in surfactant micellar solutions increased linearly with surfactant concentrations, whereas the biodegradability of phenanthrene in the

micellar solutions decreased with increasing surfactant concentrations at a given phenanthrene concentration.

Alcohol ethoxylates (AE) are usually more biodegradable and their degradation products are unobjectionable in terms of their aquatic toxicity. Toxicity appears to decrease with alkyl chain length for compounds with the same degree of ethoxylation, but toxicity appears to rise with alkyl chain length for chemicals with the same degree of ethoxylation (Bejarano and Wheeler, 2021). These findings are in line with previous research on AEs, which has shown that the chemical characteristics of the alkyl chain determine their toxicity, and that increased ethoxylation decreases lipophilicity and increases acute toxicity (Dorn *et al*. 2014; Wakabayashi *et al*. 1987).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study "Elucidation of antioxidant fractions in leaves of *Dendrophthoe falcata* L. and standardisation of tape method of control" was conducted in Department of Plant Physiology, College of Agriculture, Vellanikkara, Kerala Agricultural University during 2019-2021. The goal of study was to characterize bioactive antioxidant fractions in *Dendrophthoe falcata* through *in vitro* assays and standardisation of tape method of Loranthus control.

3.1 Experiment I

3.1.1 Location

3.1.2 Plant materials

Hemi parasitic plant, Loranthus belonging to genera *Dendrophthoe* was used as the study material . Leaves of *Dendrophthoe falcata* infesting the host plants *Mangifera indica, Manilkara zapota, Artocarpus heterophyllus* and *Theobroma cacao* were collected from College Orchard, Veterinary Institute of KVASU, Mannuthy and Instructional Farm of Kerala Agricultural University, Thrissur.

3.1.2 Laboratory chemical and glasswares

Chemicals like ethyl acetate, chloroform, methanol and petroleum ether for extraction were procured from Chemind. Diphenyl-1- picrylhydrazly (DPPH) for antioxidant assay, picric acid for phytochemical screening were also purchased from Sigma Aldrich Company. Super absorbent polymer, paper, cotton and adhesive tape were procured through Amazon. Organo silicon and Alcohol ethoxylate were obtained from Victus Laboratory, Coimbatore.

3.1.3 Equipment and machinery

Equipments available at Department of Plant Physiology, College of Agriculture, Vellanikkara were used for the study.. Tape method of control of the parasitic species,

Loranthus was undertaken at the Cocoa Research Centre of Kerala Agricultural University.

3.1.3 Sample Preparation

3.1.3.1 Leaf collection

Leaves of *Dendrophthoe falcata* infesting mango, jackfruit, sapota and cocoa were collected from the premises of Instructional Farm of Kerala Agricultural University, Kerala Veterinary and Animal Science University and Karippal, Kannur.. Leaves characteristically had lesser width and were longer in length with oblong shape and coriaceous.

Plate 1: *Dendrophthoe falcata* inhabiting the hosts a) Mango b) Cocoa c) Sapota d) Jack

3.1.3.2 Drying of leaves

The Collected leaves were washed well in water, wiped with sterile cloth and shade dried at room temperature for about two weeks until it could be easily crushed between hands. About 100 gram each of the leaves were spread on a paper for drying.

3.1.3.3 Powdering of leaves

After drying, the leaves were ground into fine powder in a mixer. The powder was transferred into an air tight container and stored.

3.1.4 Extraction and isolation of antioxidant fraction from Loranthus

Hundred gram of the powder from each host species was subjected for defatting in 150 mL of petroleum ether for about 2 hours in a shaker. Twenty five gram of the defatted powder was dissolved in 100 ml of chloroform, methanol, ethyl acetate and water and subjected to boiling till the solvent and powder attained a concentrated condition. After extraction, the extract was transferred to a standard flask and residue was kept open for evaporation and dried. Weight of the residue after extraction was recorded and extract yield was computed using the formula:

$$\text{Extract yield (\%)} = \frac{\text{Weight of extract in grams}}{\text{Weight of sample in gram}} x\ 100$$

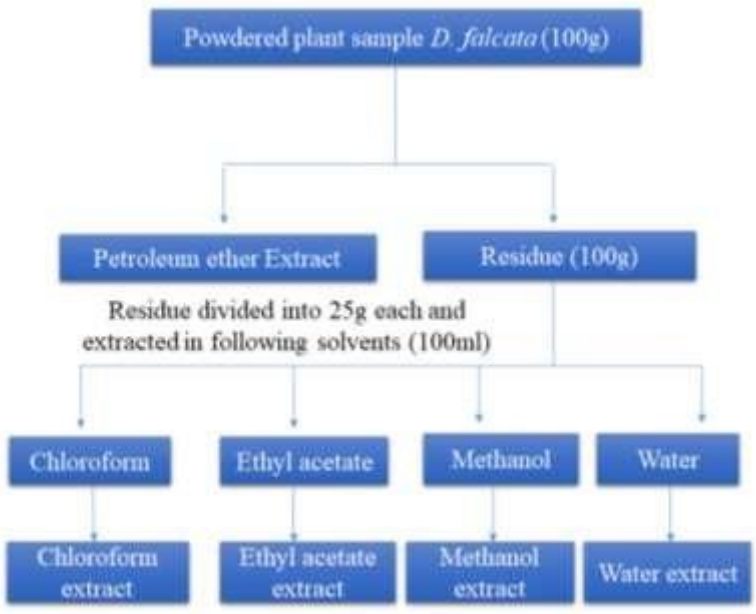


Plate 2: Flow chart illustrating differential extraction in *D. falcata*

3.1.5 Antioxidant assay

An antioxidant assay was performed on the extract to determine its ability to scavenge reactive oxygen species (ROS).

DPPH solution of 0.2 mM was prepared by dissolving in 99.5% ethanol and stored in dark for 2 h until absorbance was stabilized. Absorbance of DPPH was recorded by adding 1mL DPPH to 200 µL ethanol and 800 µL of 0.1 M Tris-HCl buffer (pH 7.4). Sample absorbance was recorded for three replicates at intervals such as immediately, half an hour, 1 hour and 2hour by adding 1mL DPPH to tubes containing 200 µL of sample and 800 µL of Tris - HCl buffer. The tubes were placed in dark and absorbance was read at 517nm. A solution of 1.2 mL of ethanol and 800 µL of Tris-HCl buffer was used as blank.

Absorbance of sample was expressed as A_S and that of DPPH as A_C. Inhibition ratio was calculated using the equation:

Inhibition ratio (%) = $[(A_C – A_S)/A_C] \times 100$

3.1.6 Phytochemical screening of extracts eluted in various organic solvents

Qualitative phytochemical screening of solvent soluble fractions was carried out for testing alkaloids, tannins, saponins, flavonoids, terpenoids, steroids and phenols using a standard approach outlined by Harborne (1973) and Sofowora (1993)

3.1.6.1 Alkaloids

Extracts were aggressively combined with 70 percent HCl solution before being filtered. The filtrate was treated with Hager's reagent (picric acid saturated solution). Presence of alkaloids is indicated by the yellow colour of the precipitate.

3.1.6.2 Saponins

Foam forming method was used to test for saponin. 0.5 mL sample and 5 mL distilled water were added to the test tube. The mixture was violently shaken, and the foam that resulted was noted.

3.1.6.3 Phenols

To the extracts, 2 to 3 drops of 0.1 percent FeCl3 solution was added. The presence of phenol is indicated by the formation of a black or bluish tint.

3.1.6.4 Flavonoids

Extracts were combined with 10 mL ethyl acetate, boiled on a water bath for a few minutes, and then filtered. To 4 mL filtrate, 1 mL dil. ammonia solution was added. Presence of flavonoids is indicated by the yellow colour of the precipitate.

3.1.6.5 Terpenes

Three to four drops of ethyl acetate was added to 0.5 mg of extract and 5 mL water. Presence of terpenes is indicated by the formation of a brilliant green colour.

3.1.6.6 Steroids

Ten drops of CH_3COOH and 2 drops of H_2SO_4 concentrated in 2 drops were added to 2 mL of each *D. falcata* fraction. The solution was gently shaken and let aside for several minutes. Steroids produce blue or green colour in positive tests, whereas triterpenoid gives red or purple colour.

3.1.6.7 Tannins

The extracts were warmed in a water bath for five minutes with 20 mL distilled water and then filtered while still hot. Then 1 mL of cool filtrate was combined with 5 mL distilled water, along with a few drops (2-3) of 10% ferric chloride. Presence of tannins is indicated by a bluish-black or brownish-green precipitate.

3.1.7 Identification of bioactive compounds through GC-MS/MS

GC MS analysis was conducted at Central Instruments Laboratory, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Science University, Mannuthy, Thrissur.

3.1.7.1 Sample preparation for GC- MS/MS

Samples of 1 mL each were taken as duplicates in 1.5 mL eppendorf tubes and placed in cold trap at -118ºC followed by lyophilization of the samples in two batches. The

samples were reconstituted with 750 µL of acetonitrile and placed in vortex mixer for enabling the compounds to dissolve in acetonitrile. 2 mL of the reconstituted samples was taken using disposable syringes for each sample and filtered through syringe filters and transferred to GC vials.

3.1.7.2 Gas chromatography mass spectrophotometry (GC-MS/MS) analysis

GC-MS/MS analysis of *D. falcata* extracts in chloroform, ethyl acetate, petroleum ether, methanol, and water was done using a TSQ 8000 MS/MS (Thermo Fisher Scientific) with a triple quadrupole mass spectrometer detector. The Agilent DB – 5MS +DG column (30m length, 0.250mm diameter, and 0.25m film filter) was used in the GC-MS system. Helium was employed as the carrier gas, with a flow rate of 1mL/min. Initial temperature was 110°C (hold 2 minutes), ramped at 15°C/min to 150°C (wait 1 minute), ramped at 10°C/min to 250°C (hold 5 minutes) with a total experiment run length of 21 minutes. A sample of about 2 µL was injected. The injector and detector were both kept at 250ºC. Electron ionisation at 70eV was used to acquire mass spectra with a spectral range of m/z 50-700 amu.

3.2 Experiment II- Tape method of control of Loranthus

3.2.1 Location

The experiment for Loranthus management was conducted on the cocoa plants maintained by the Cocoa Research Centre, at the instructional farm, Vellanikkara

3.2.2 Details of the experiment

Dendrophthoe falcata infected cocoa trees were spotted and labelled. To control the parasite 25mL/L of ethrel was used. In the experiment two non-ionic surfactants were mixed with 25mL/L of ethrel and tested on Loranthus. Organosilicone (OS) and alcohol ethoxylate (AE) were the two non-ionic adjuvants utilized (Table 1). These adjuvants mixed with ethrel were impregnated on three materials namely super absorbent polymer, super absorbent cotton and super absorbent paper and was developed as a sticky tape (Plate 3). One month dried, one week dried and freshly prepared tapes were used for the study. The outer bark of the parasite was scrapped to

a length of 2 cm at the point of attachment to host. This portion was then covered with the sticky tapes with ethrel and the adjuvants.

Plate 3: Materials used for the study : Super absorbent polymer, super absorbent cotton and super absorbent paper

Table 1: Treatment combinations for Loranthus control

SL.NO.	MATERIAL	TREATMENT COMBINATIONS
1.	Super absorbent polymer	25mL/L Ethrel + 0.5mL/L O.S 25mL/L Ethrel + 0.5mL/L AE
2.	Super absorbent cotton	25mL/L Ethrel + 0.5mL/L O.S 25mL/L Ethrel + 0.5mL/L AE
3.	Super absorbent paper	25mL/L Ethrel + 0.5mL/L O.S 25mL/L Ethrel + 0.5mL/L AE

3.2.3 Observations recorded

The sticky tapes were stuck on the branches of Loranthus on 16.08.2021 and observations were made on weekly basis for about five weeks:

a) Before treatment

   Stage of the parasite (Vegetative/Flowering)

b) After treatment

1. Extent of drying and defoliation
2. Time to start regrowth, if any

3.2.4 Statistical Analysis

Design of experiments followed for the study was Factorial Completely Randomised Design (CRD). The means of treatments and treatment combinations were analysed for significance using GRAPES software (Gopinath *et al*., 2020).

RESULTS

4. RESULTS

The findings of the study "Elucidation of antioxidant fractions in leaves of *Dendrophthoe falcata* L. and standardisation of tape method of control" is reported in this chapter under various sections.

4.1 EXPERIMENT I

Comparative elucidation of antioxidant fractions in leaves of *Dendrophthoe falcata* infesting different hosts such as mango, sapota, cocoa and jackfruit was attempted as part of this study.

Coarse dried samples of *Dendrophthoe falcata* from four main hosts namely mango, sapota, cocoa and jackfruit were extracted in organic solvents such as petroleum ether, chloroform, ethyl acetate, methanol and water (Plate 4).

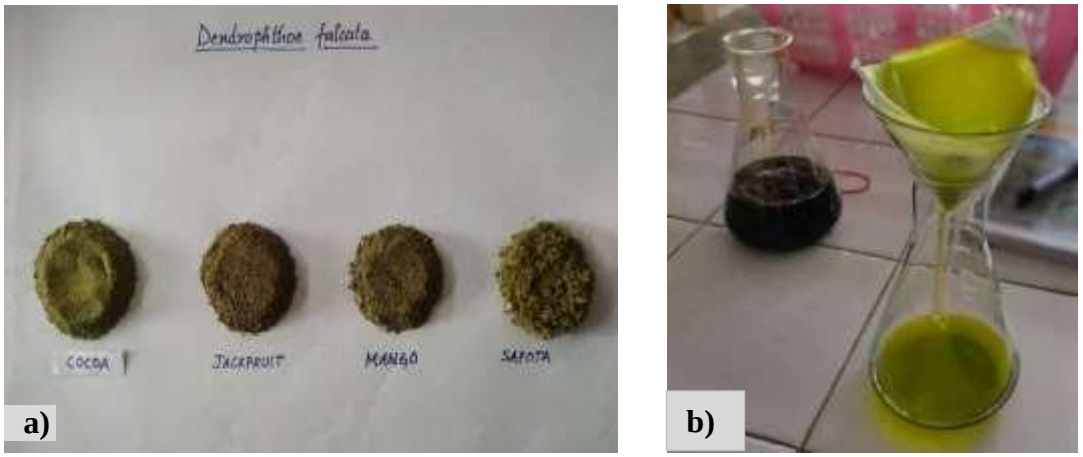


Plate 4: a) Powdered samples of *Dendrophthoe falcata* on different hosts, b) Extraction in different solvents

Extract yields of samples in various solvents were computed. Aqueous extracts of *D. falcata* inhabiting all the hosts showed significantly higher yields compared to other solvents. Extract yield in case of petroleum ether and ethyl acetate were reported to be lowest compared to other solvents. D(Sapota) showed lowest extract yield in petroleum ether (1.6%) and ethyl acetate (1.7%), whereas D(Jack) and D(Cocoa) had higher extract yields of 14.8% and 14.4% in water respectively as tabulated in Table 2.

Table 2: Extract yield of *Dendrophthoe falcata* on different host in various solvents

Samples	Intitial wt (g)	P.E (%)	Chloroform (%)	E.A (%)	Methanol (%)	Water (%)
D(Mango)	100	2.2	4.8	2.8	7.6	13.6
D(Jack)	100	2.9	4	2.8	5.6	14.8
D(Cocoa)	100	2.4	10.8	2.4	10.8	14.4
D(Sapota)	100	1.6	2.7	1.7	12.3	10.2

D(Host) indicates *D. falcata* infesting different hosts; P.E: Petroleum ether; E.A: Ethyl Acetate

4.1.2 Antioxidant assay

Extracts obtained for each sample were subjected to 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay to know the ability of the extract(s) to scavenge the reactive oxygen species (ROS) present in the sample followed by computation of percent inhibition ratio. Inhibition activity of different hosts, solvents and their interaction effects were analysed at various intervals such as immediately, half an hour, one hour and two hours after initiation of radical scavenging reaction respectively.

Different organic solvents were taken as first factor and host infested with *D. falcata* as second factor. Inhibition activity recorded immediately indicated that petroleum ether extract had highest (68.03%) and water (28.97%) had the least (Table 3). Among the hosts infested with *D. falcata*, D(jack) showed highest inhibition activity of 58.689% and least activity was observed in D(sapota) (36.39%) (Table 4). Interaction effect of host infested with *D. falcata* with various extracts revealed that chloroform extract of D(cocoa) (75.66%), petroleum ether (77.01%), ethyl acetate (58.3%), methanolic (57.90%) and water (42.42%) extract of D(Jack) showed maximum inhibition activity (Table 5).

Observation taken after half an hour incubation of radical scavenging reaction, indicated that inhibition activity varied with respect to both solvent, infested host and their interaction. Among the solvents, chloroform extract showed highest percent inhibition activity and least was seen in water extract (Table 3). Sapota (44.34%) and jack (43.78%) infested with *D. falcata* showed highest percent inhibition activity which were not significantly different, whereas *D. falcate* inhabiting cocoa (35.94%)

recorded the least percent inhibition activity (Table 4). Interaction effect proved that *D. falcata* infesting sapota extracted in chloroform showed highest inhibition activity (68.03%) and methanolic extract of *D. falcata* from jack had the lowest (5.76%) (Table 5).

Inhibition activity recorded one hour after incubation showed that chloroform extract performed better (65.22%) than other solvents, while methanolic extract had the lowest value (19.19%) (Table 3). Jack infested with *D. falcata* showed highest percentage inhibition (46.02%) and *D. falcata* growing on cocoa possessed the least (32.83%) (Table 4). *D. falcata* infesting jack extracted in chloroform exhibited the highest inhibition activity (85.00%) and those infesting cocoa extracted in petroleum ether recorded the lowest (13.50%) (Table 5).

The inhibition activity after two hours of incubation was analyzed and the results revealed that the antioxidant potentials of water (75.86%) and chloroform (72.21%) extracts were not significantly different. Similarly, petroleum ether (42.60%) and methanolic (42.25%) extracts were also not significantly different (Table 2). *D. falcata* inhabiting mango extracted in chloroform (88.05%) and *D. falcata* infesting cocoa extracted in water (8.87%) possessed highest percent inhibition activity, though not significantly different. *D. falcata* infesting mango extracted in ethyl acetate recorded the least inhibition activity (10.62%) (Table 5).

Table 3: Inhibition activity (%) of antioxidant fractions of *D. falcata* leaf extract in different solvents at various time intervals

Treatments	Immediate	Half hour	One hour	Two hours
Chloroform (1)	53.06^{b}	61.16^{a}	65.22^{a}	72.21^{a}
P.E (2)	68.04^{a}	38.07^{c}	27.38^{d}	42.60^{b}
E.A (3)	47.03^{c}	47.96^{b}	47.81^{b}	18.32^{c}
Methanol (4)	37.35^{d}	24.89^{e}	19.19^{e}	42.25^{b}
Water (5)	28.97^{e}	32.14^{d}	34.51^{c}	75.85^{a}
CD (0.05)	2.14	3.21	2.67	4.87

: Chloroform, (2) : P.E- Petroleum ether, (3) : E.A- Ethyl acetate, (4) : Methanol, (5) : Water

Table 4: Inhibition activity (%) of antioxidant fractions of *D. falcata* leaf extract in different hosts at various time intervals

Host	Immediate	Half hour	One hour	Two hours
C	48.852^{b}	35.94^{c}	32.83^{c}	51.17
J	54.689^{a}	39.30^{b}	46.02^{a}	47.72
M	47.624^{b}	43.78^{a}	39.37^{b}	50.03
S	36.388^{c}	44.34^{a}	37.06^{b}	52.08
CD	1.919	2.87	2.39	NS

C: Cocoa, J: Jack, M: Mango, S: Sapota

Table 5: Inhibition activity (%) of antioxidant fractions of *D. falcata* leaf extract in different solvents at various time intervals across different treatment combinations

Treatment combination	Immediate	Half hour	One hour	Two hours
1_C	75.66^{a}	66.63^{a}	56.01^{cd}	79.98^{ab}
1_J	37.80^{fg}	43.82^{cde}	85.00^{a}	57.99^{cde}
1_M	65.32^{b}	66.14^{a}	68.45^{b}	88.05^{a}
1_S	33.44^{h}	68.03^{a}	51.40^{de}	62.82^{c}
2_C	50.49^{d}	23.64^{g}	13.50^{l}	29.41^{ghi}
2_J	77.01^{a}	49.67^{c}	26.35^{i}	52.14^{de}
2_M	68.53^{b}	47.77^{c}	28.08^{i}	39.04^{fg}
2_S	76.11^{a}	31.21^{f}	41.59^{gh}	49.83^{e}
3_C	45.88^{e}	48.35^{c}	50.49^{ef}	20.34^{ij}
3_J	58.32^{c}	57.82^{b}	57.33^{c}	28.41^{hi}
3_M	41.59^{ef}	41.26d^{e}	40.11^{h}	10.62^{j}
3_S	42.33^{e}	44.39^{cde}	43.32^{gh}	13.92^{j}
4_C	44.15^{e}	14.82^{h}	19.52^{jk}	37.23^{fgh}
4_J	57.90^{c}	5.76^{i}	20.01^{j}	39.20^{f}
4_M	34.84^{gh}	16.72^{h}	14.25^{kl}	31.54^{fgh}
4_S	12.52^{k}	62.27^{ab}	22.98^{ij}	61.03^{cd}
5_C	28.08^{i}	26.27^{fg}	24.62^{ij}	88.87^{a}
5_J	42.42^{e}	39.45^{e}	41.43^{gh}	60.87^{cd}
5_M	27.84^{i}	47.03^{cd}	45.96^{fg}	80.88^{ab}
5_S	17.54^{j}	15.81^{h}	26.02^{i}	72.81^{b}
CD	**2.15**	**6.42**	**5.34**	**9.74**

1_C, 1_J, 1_M, 1_S: *D. falcata* inhabiting cocoa, jack, mango and sapota extracted in chloroform;

2_C, 2_J, 2_M, 2_S: *D. falcata* inhabiting cocoa, jack, mango and sapota extracted in petroleum ether;

3_C, 3_J, 3_M, 3_S: *D. falcata* inhabiting cocoa, jack, mango and sapota extracted in ethyl acetate;

4_C, 4_J, 4_M, 4_S: *D. falcata* inhabiting cocoa, jack, mango and sapota extracted in methanol;

5_C, 5_J, 5_M, 5_S: *D. falcata* inhabiting cocoa, jack, mango and sapota extracted in water.

4.1.3 Phytochemical screening of *Dendrophthoe falcata*

Chloroform extract of D(Mango) tested positive for the presence of flavonoids, terpenes and steroids. D (Jack) possessed alkaloid, flavonoid and steroid fractions. D(Cocoa) exhibited the presence of alkaloids, phenols, flavonoids and steroids, while D(Sapota) possessed alkaloid, saponin, flavonoid and steroid fractions (Table 6). Ethyl acetate extract of the hosts namely mango, jack and sapota infested with *D. falcata* confirmed the presence of terpenes and steroids, whereas D(Cocoa) possessed alkaloids in addition to terpenes and steroids (Table 6).

Methanolic extract of D(Mango) contained alkaloids, phenols, saponins, flavonoids and steroids. D(Jack) possessed all other compounds except phenols and tannins. D(Cocoa) analysis revealed the presence of saponins, phenols, flavonoids, terpenes and steroids. D(Sapota) had significantly higher amounts of flavonoids in addition to phenols, tannins and steroids as shown in Table 6.

The analysis of aqueous extract of D(Mango) showed the presence of alkaloids, saponins and phenols. D(Sapota) possessed alkaloids, phenols, saponins, flavonoids and tannins, while D(Jack) and D(Cocoa) consisted mainly of alkaloids and phenols (Table 6).

Table 6: Comparative qualitative phytochemical characterization of different fractions extracted in different solvent of *D. falcata* leaves infesting different hosts

Samples	Alkaloid	Saponin	Phenol	Flavanoid	Terpene	Tannin	Steroid
Chloroform							
D (Mango)	-	-	-	+	+	-	+
D (Jack)	+	-	-	+	-	-	+
D (Cocoa)	+	-	+	+	-	-	+
D (Sapota)	+	+	-	+	-	-	+
Ethyl acetate							
D (Mango)	-	-	-	-	+	-	+
D (Jack)	-	-	-	-	+	-	+
D (Cocoa)	+	-	-	-	+	-	+

D (Sapota)	-	-	-	-	+	-	+
Methanol							
D (Mango)	+	+	+	+	-	-	+
D (Jack)	+	+	-	+	+	-	+
D (Cocoa)	-	+	+	+	+	-	+
D (Sapota)	-	-	+	++	-	+	+
Water							
D (Mango)	+	+	+	-	-	-	-
D (Jack)	+	-	+	-	-	-	-
D (Cocoa)	+	-	+	-	-	-	-
D (Sapota)	+	+	+	+	--	+	-

+ : Present, - : Absent, ++ : Present a lot

Qualitative phytochemical screening of different extracts of *D. falcata* on the four hosts namely mango, jackfruit, cocoa and sapota were carried out to detect the presence of alkaloids, phenols, saponins, flavonoids, terpenes, tannins and steroids. There was a differential display of possession of different classes of phytochemicals by *D. falcata* across different host associations which varied with the nature of solvent used for extracting the antioxidant fractions (Plate. 5-11).

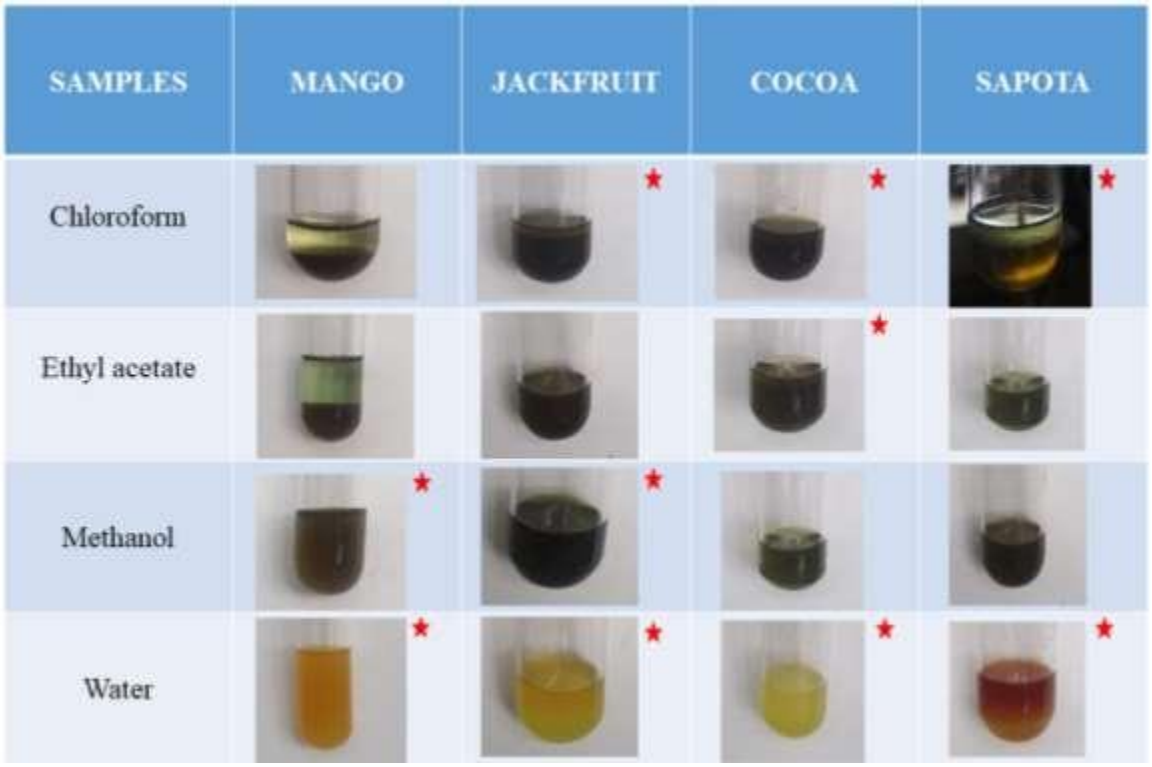


Plate 5: Phytochemical screening of *Dendrophthoe falcata* for alkaloids

Plate 6: Phytochemical screening of *Dendrophthoe falcata* for saponins

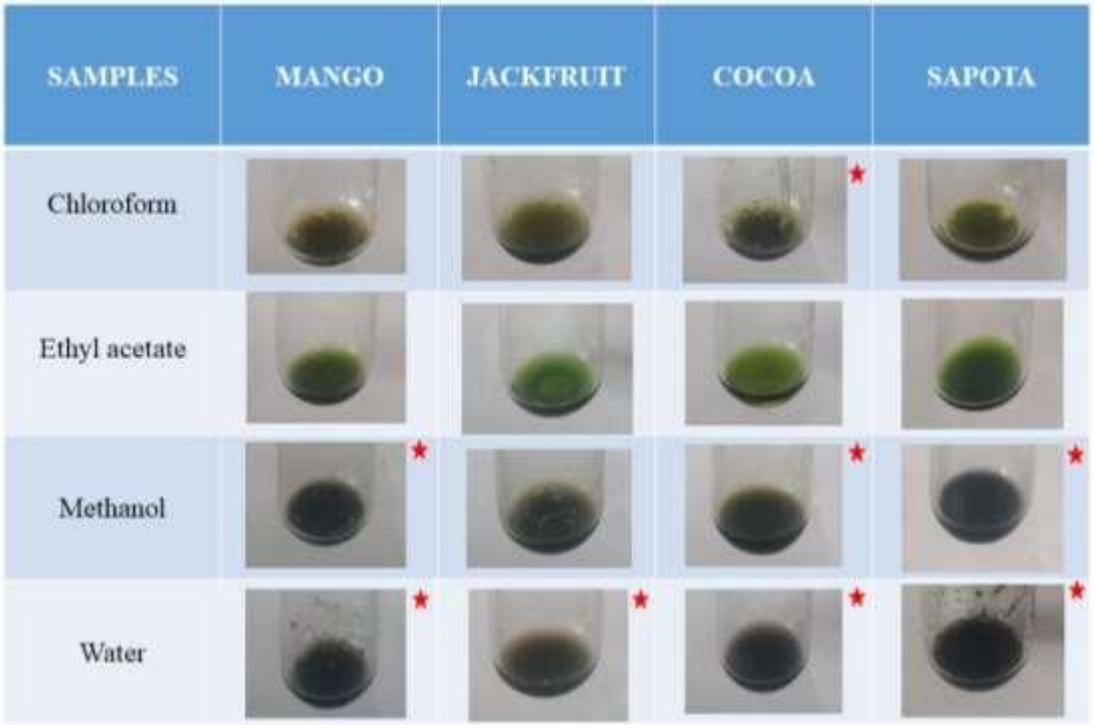


Plate 7: Phytochemical screening of *Dendrophthoe falcata* for phenols

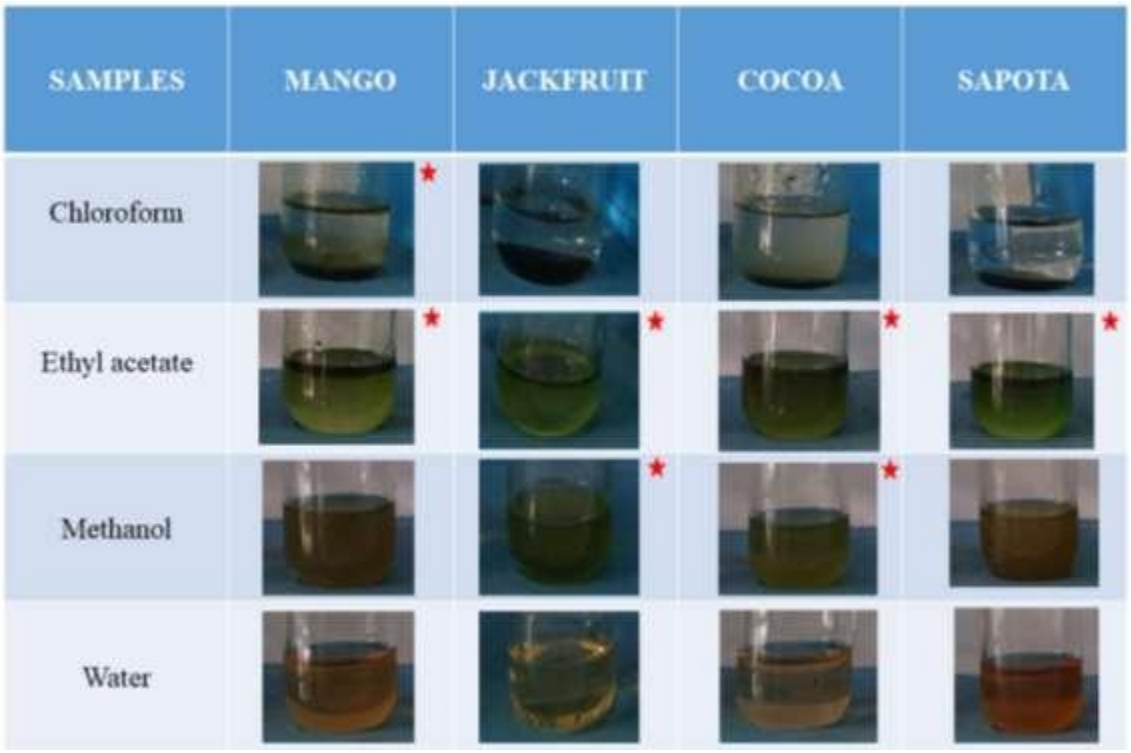


Plate 8: Phytochemical screening of *Dendrophthoe falcata* for terpenes

SAMPLES	MANGO	JACKFRUIT	COCOA	SAPOTA
Chloroform				
Ethyl acetate				
Methanol				
Water				

Plate 9 : Phytochemical screening of *Dendrophthoe falcata* for flavonoids

SAMPLES	MANGO	JACKFRUIT	COCOA	SAPOTA
Chloroform				
Ethyl acetate				
Methanol				
Water				

Plate 10 : Phytochemical screening of *Dendrophthoe falcata* for steroids

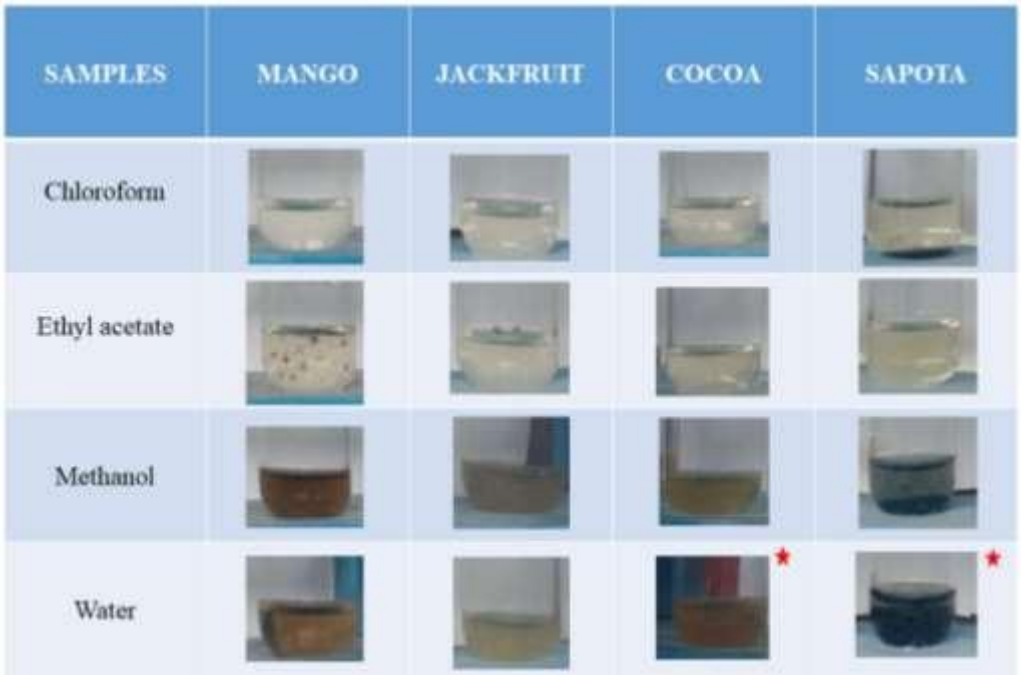


Plate 11 : Phytochemical screening of *Dendrophthoe falcata* for taninns

4.1.4 Identification of bioactive compounds through GC-MS/MS

Based on the antioxidant potential of the different extracts and host specificities, eight samples namely *D. falcata* on mango and sapota extracted in chloroform, *D. falcata* on jack and sapota extracted in petroleum ether, *D. falcata* on cocoa and sapota extracted in water and also *D. falcata* on jack and sapota extracted in ethyl acetate and methanol respectively were shortlisted for GC-MS/MS analysis as given in Table 7.

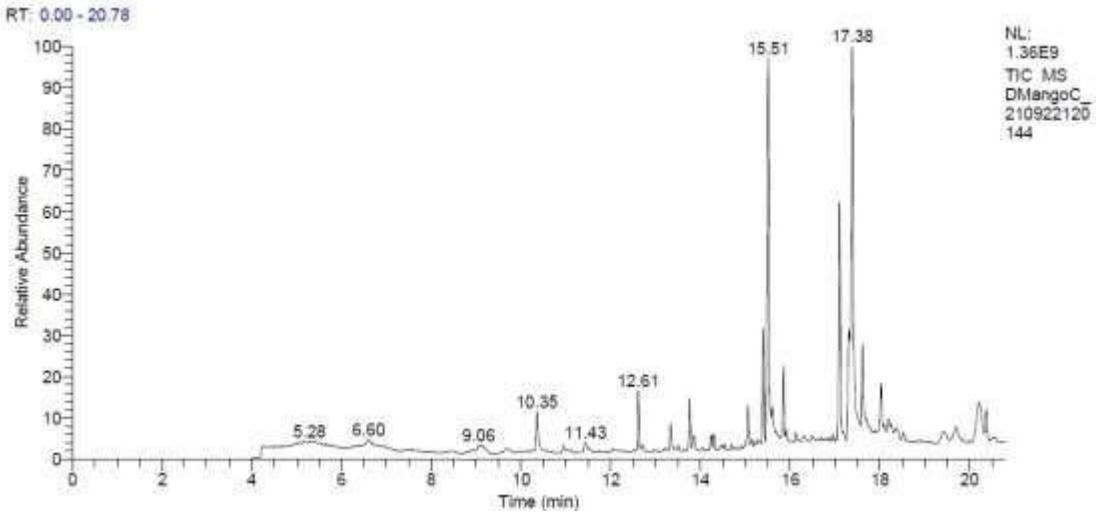
Table 7: Name of samples and its code for GC-MS/MS analysis

Sl. No	Sample	Code	Sl. No	Sample	Code
1	*D. falcata* on Mango in chloroform	DMangoC	5	*D. falcata* on Jack in ethyl acetate	DJackE.A
2	*D. falcata* on Sapota in chloroform	DSapotaC	6	*D. falcata* on Sapota in methanol	DSapotaM
3	*D. falcata* on Jack in P.E	DJackPE	7	*D. falcata* on Cocoa in water	DCocoaW
4	*D. falcata* on Sapota in P.E	DSapotaPE	8	*D. falcata* on Sapota in water	DSapotaW

P.E: Petroleum ether, E.A: Ethyl acetate, M: Methanol, W: Water, C: Chloroform, D: *Dendrophthoe falcata*

The GC-MS/MS analysis was performed in anticipation of detecting volatiles and limited to certain classes of metabolites alone due to lesser derivatization steps during sample extraction and preparation. Bioactive substances were detected in different extracts of *D. falcata* during GC-MS analysis. Each extract revealed its characteristic peaks and the National institute of standards and technology (NIST) database was used to identify the compounds corresponding to the respective peaks. The retention time was used to validate the chemical compounds' identity; the molecular weight, peak area in percentage and probability were also detected. The GC-MS/MS chromatogram of different extracts of *D. falcata* on all the four hosts were obtained as a function of relative abundance and time and are represented in Plate 12-16.

a)



b)

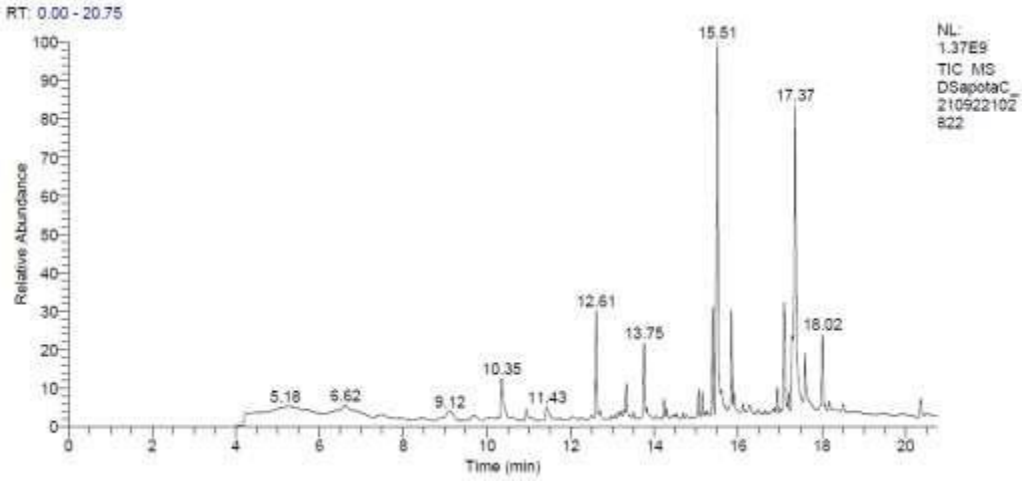
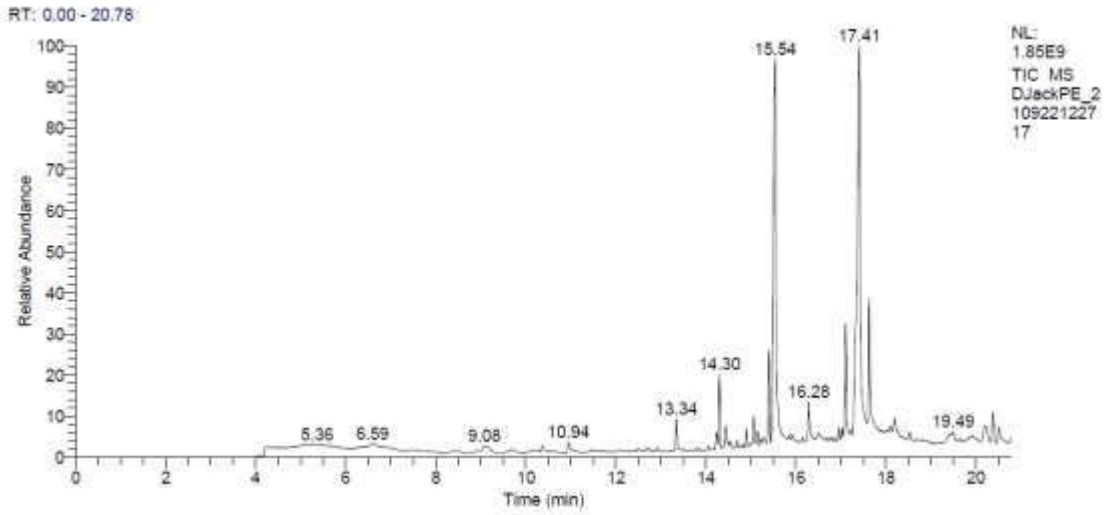


Plate 12 : GC-MS/MS chromatogram of chloroform extract of *D. falcata* on a) Mango b) Sapota

a)



b)

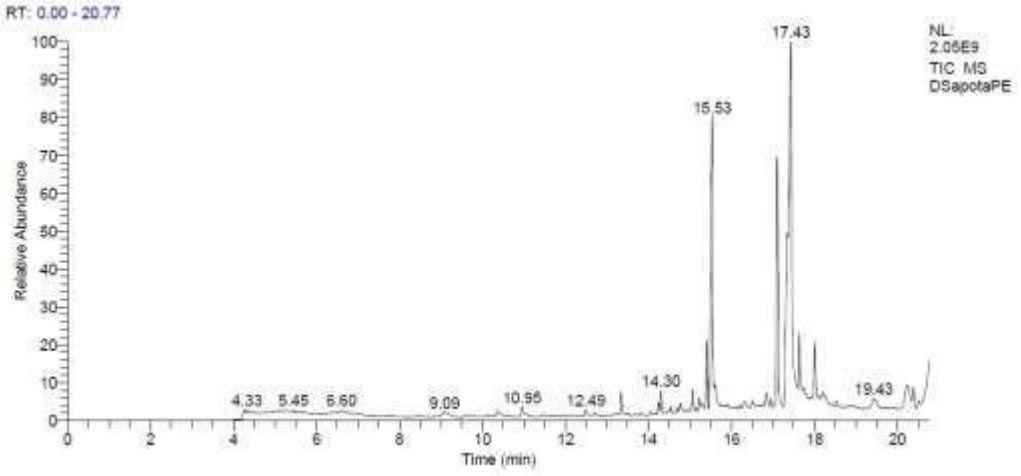


Plate 13 : GC-MS/MS chromatogram of P.E extract of *D. falcata* on a) Jack b) Sapota

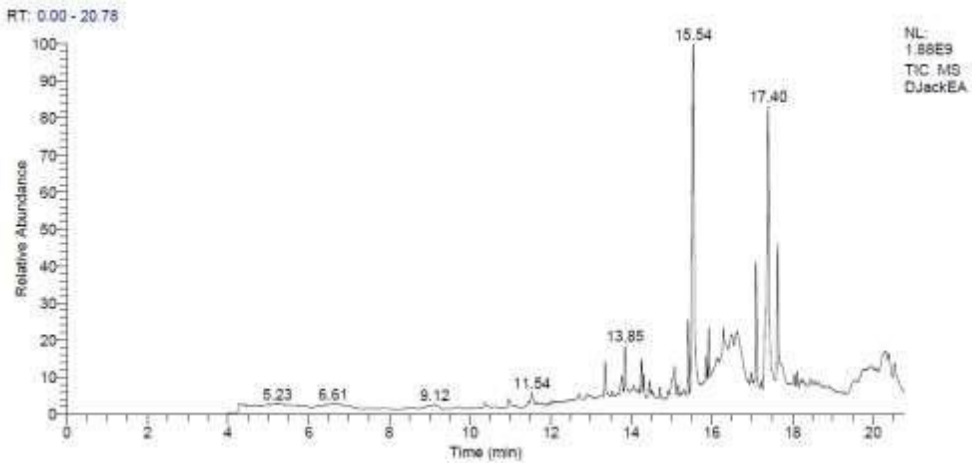


Plate 14 : GC-MS/MS chromatogram of E.A extract of *D. falcata* on Jack

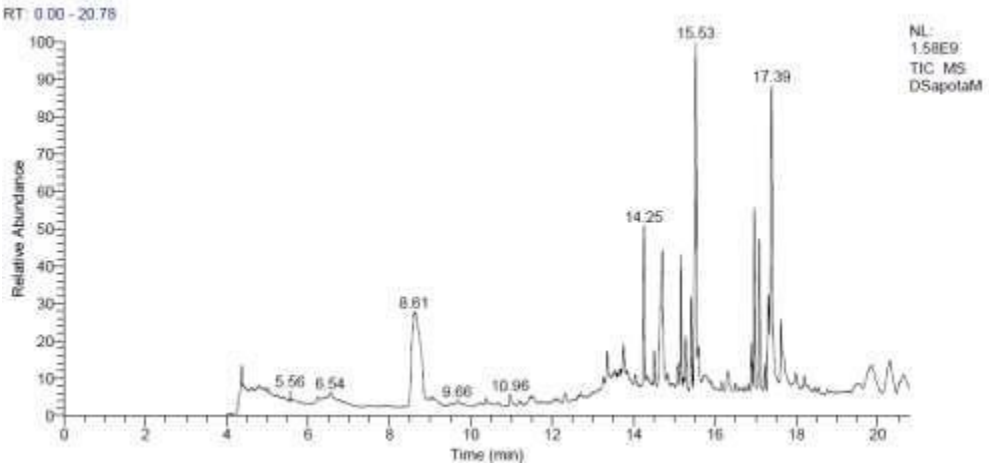
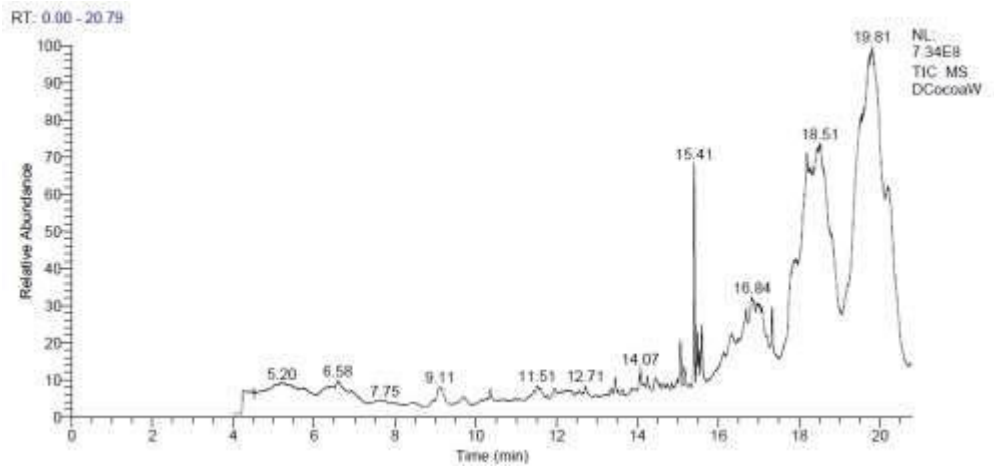


Plate 15 : GC-MS/MS chromatogram of Methanol extract of *D. falcata* on Sapota

a)



b)

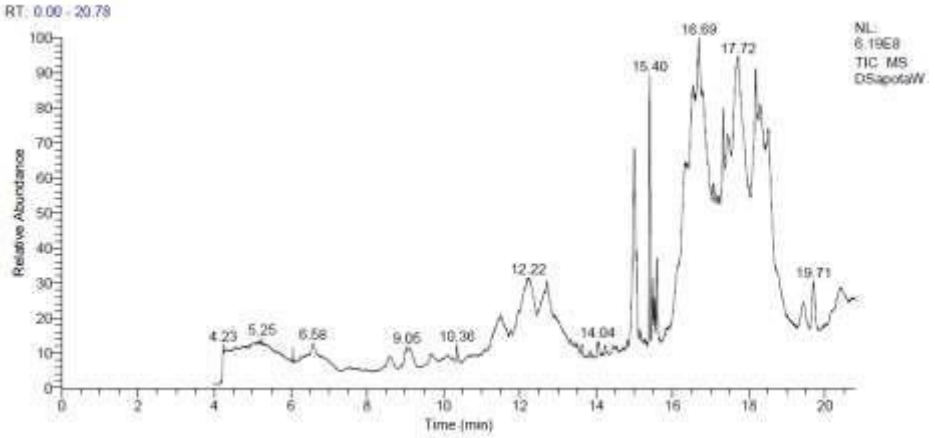


Plate 16 : GC-MS/MS chromatogram of water extract of *D. falcata* on a) Cocoa b) Sapota

The bioactive compounds identified in chloroform extract of D(Mango) and D(Sapota) are enlisted in Table 8 based on the peak area occupied by a compound and certain unique peaks that varied with respect to host. The chloroform extract of D(Mango) had the following compounds with highest peak area viz. cis-Vaccenic acid (21.4%) and n-Hexadecanoic acid (19.4%), whereas the compounds possessing the unique peaks are Dodecyl acrylate (2.02%), 9Nonadecene (2.03%), 9-Hexacosene (2.27%), E-8-Methyl-9-tetradecen-1-ol acetate (1.11%), 1-Heptatriacotanol (1.13%) and Squalene (4.12%). Chloroform extract of D (Sapota) had compounds with highest peak area viz. n-Hexadecanoic acid (22.55%) and cis Vaccenic acid (18.92%). Compounds within unique peaks include 1- Hexadecanol (1.14%), 2-Propenoic acid, tridecyl ester (4.54%) and 3-Eicosene,(E) (3.89%).

Table 8 : Bioactive compounds identified in chloroform extracts based on peak area

Sample	Compound names	RT	Prob	Area %	PT
DMangoC	Phenol, 2,4bis(1,1dimethylethyl)	10.35	64.64	2.17	
	Dodecyl acrylate	12.61	10.38	2.02	U
	9Nonadecene	13.76	6.67	2.03	U
	7,9Ditertbutyl1oxaspiro(4,5)deca6, 9diene2,8dione	15.06	95.19	1.61	
	Diphenyl sulfone	15.4	93.43	4.22	U
	nHexadecanoic acid	15.51	57.1	19.4	HP
	Benzothiazole, 2(2hydroxyethylthio)	15.6	69.72	1.43	
	10-Heneicosene(c,t)	15.85	5.82	2.29	
	Phytol	17.1	63.28	11.49	
	9-Octadecynoic acid	17.31	6.64	4.6	
	cisVaccenic acid	17.38	8.48	21.4	HP
	Octadecanoic acid	17.61	55.47	3.56	
	9- Hexacosene	18.03	3.77	2.27	U
	E-8-Methyl-9-tetradecen-1-ol acetate	19.41	15.38	1.11	U
	1-Heptatriacotanol	19.69	22.19	1.13	U
	Squalene	20.21	33.49	4.12	U
	4,8,12,16-Tetramethylheptadecan-4-olide	20.37	1.84	1.24	
DSapotaC	Phenol, 2,4bis(1,1dimethylethyl)	10.35	57.76	3.48	
	1- Hexadecanol	11.43	4.38	1.14	U
	2Propenoic acid, tridecyl ester	12.61	6.11	4.54	U
	10-Heneicosene(c,t)	13.75	5.2	3.57	
	Diphenyl sulfone	15.4	93.31	4.58	

	n-Hexadecanoic acid	15.51	46.64	22.55	HP
	3-Eicosene,(E)	15.85	6.06	3.89	U
	Phytol	17.1	61.02	6.73	
	9-Octadecynoic acid	17.29	9.26	3.66	
	cisVaccenic acid	17.37	18.21	18.92	HP
	Octadecanoic acid	17.6	51.29	2.59	

RT: Retention time, Prob: Probability, PT: Peak type

Bioactive compounds obtained from petroleum ether (P.E) extract of D(Jack) showing highest peak area are n-Hexadecanoic acid (27.86%) and cis Vaccenic acid (30.97%). Compounds within the unique peaks are Tetradecanoic acid (1.31%), 2-Pentadecanone,6,10,14trimethyl (2.6%), Pentadecanoic acid (1.14%), 7,9Ditertbutyl1oxaspiro(4,5)deca6,9diene2,8dione (1.18%), iPropyl 12methyltetradecanoate (1.32%), Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate (1.54%), 4,8,12,16-Tetramethylheptadecan-4-olide (1.6%) and Eicosanoic acid (1.2%).

P.E extract of D(Sapota) had n-Hexadecanoic acid (19.9%) and cis Vaccenic acid (37.22%) with highest peak area and trans-Geranylgeraniol (3.03%), Decanedioic acid, bis(2ethylhexyl)ester (1.3%) and Squalene (2.35%) were the compounds corresponding to unique peaks (Table 9).

Table 9 : Bioactive compounds identified in petroleum ether extracts based on peak area

Sample	Compound names	RT	Prob.	Area %	PT
DJackPE	Tetradecanoic acid	13.34	60.73	1.31	U
	2-Pentadecanone,6,10,14trimethyl	14.3	73.14	2.6	U
	Pentadecanoic acid	14.44	60.53	1.14	U
	7,9Ditertbutyl1oxaspiro(4,5)deca6, 9diene2,8dione	15.07	69.84	1.18	U
	Diphenyl sulfone	15.4	95.27	3.64	
	n Hexadecanoic acid	15.54	54.76	27.86	HP
	Phytol	17.1	68.87	5.79	
	cis Vaccenic acid	17.41	20.19	30.97	HP
	Octadecanoic acid	17.63	59.81	5.42	
	iPropyl 12methyltetradecanoate	18.19	4.61	1.32	U
	Z-(13,14-Epoxy)tetradec-11-en-1-ol	20.19	5.23	1.54	U

	acetate				
	4,8,12,16-Tetramethy lheptadecan-4-olide	20.38	74.25	1.6	U
	Eicosanoic acid	20.51	64.2	1.2	U
DSapotaPE	Diphenyl sulfone	15.4	92.68	2.94	
	n-Hexadecanoic acid	15.53	43.16	19.9	HP
	Phytol	17.1	68.21	15.46	
	cis Vaccenic acid	17.43	12.18	37.22	HP
	Octadecanoic acid	17.63	45.93	2.47	
	trans-Geranylgeraniol	18.01	54.95	3.03	U
	Decanedioic acid, bis(2ethylhexyl)ester	19.43	56.25	1.3	U
	Squalene	20.23	38.04	2.35	U

RT: Retention time, Prob: Probability, PT: Peak type

Ethyl acetate (E.A) extract of D(Jack) possessed n-Hexadecanoic acid (24.83%) and cis Vaccenic acid (19.14%) with highest peak area. Compounds within unique peaks are ç-Sitosterol (1.89%), dl α Tocopherol (4.6%), 9,19-Cyclolanostane-3,7-diol (1.63%) and Lupeol (2.74%) (Table 10).

Table 10 : Bioactive compounds identified in ethyl acetate extracts based on peak area

Sample	Compound names	RT	Prob.	Area %	PT
DJackEA	Tetradecanoic acid	13.35	66.4	1.45	
	Eicosane	13.85	7.35	2.01	
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.26	28.14	1.4	
	7,9 Ditertbutyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	15.05	88.88	1.42	
	Diphenyl sulfone	15.42	95.73	2.72	
	n-Hexadecanoic acid	15.54	21.27	24.83	HP
	ç-Sitosterol	16.29	41.79	1.89	U
	dl à Tocopherol	16.64	45.5	4.6	U
	Phytol	17.1	66.35	5.39	
	cis Vaccenic acid	17.4	21.67	19.14	HP
	Octadecanoic acid	17.64	65.41	7.41	
	1-Heptatriacotanol	19.75	9.54	1.18	
	9,19Cyclolanostane3,7diol	19.95	8.48	1.63	U
	Lupeol	20.32	30.6	2.74	U

RT: Retention time, Prob: Probability, PT: Peak type

Bioactive compounds in case of methanolic extract of D(Sapota) with highest peak area include 1,2,3 Benzenetriol (13.75%) and n-Hexadecanoic acid (13.49%). Unique peaks compounds are 9,10Dimethyltricyclo[4.2.1.1(2,5)]decane9,10diol (1.62%), Benzene-propanoic acid, 3,5bis(1,1dimethylethyl)4hydroxy,methyl ester (1.64%), Docosanoic acid, methyl ester (1.1%) and Methyl 8,11,14heptadecatrienoate (4.34%) (Table 11).

Table 11 : Bioactive compounds identified in methanolic extracts based on peak area

Sample	Compound names	RT	Prob.	Area %	
DSapotaM	1,2,3 Benzenetriol	8.61	92.45	13.75	HP
	Tetradecanoic acid	13.35	64.79	1.35	
	9,10Dimethyltricyclo[4.2.1.1(2,5)] decane9,10diol	13.75	10.38	1.62	U
	3,7,11,15-Tetramethyl2hexadecen1ol	14.25	35.35	4.38	
	Benzoic acid, 3,4,5trihydroxy,methyl ester	14.71	45.11	7.51	
	Hexadecanoic acid, methyl ester	15.71	64.9	2.79	
	Benzenepropanoic acid, 3,5bis(1,1dimethylethyl)4hydroxy, methyl ester	15.28	96.94	1.64	U
	Diphenyl sulfone	15.42	94.47	2.28	
	n-Hexadecanoic acid	15.53	52.53	13.49	HP
	Docosanoic acid, methyl ester	16.31	31.45	1.1	U
	9,12 Octadecadienoicacid, methyl ester	16.9	13.34	1.03	
	Methyl 8,11,14heptadecatrienoate	16.97	7.17	4.34	U
	Phytol	17.09	64.42	3.79	
	9 Octadecynoic acid	17.31	14.14	2.86	
	cis Vaccenic acid	17.39	17.86	11.25	
	Octadecanoic acid	17.62	58.47	2.67	
	1,6,10,14Hexadecatetraen-3-ol,3,7,11,15-tetramethyl,(E,E)	19.86	19.6	2.85	
	Squalene	20.3	52.65	2.84	
	Hexadeca2,6,10,14tetraen1ol,3,7,11,16tetramethyl	20.63	14.34	1.83	

RT: Retention time, Prob: Probability, PT: Peak type

Aqueous extract of D(Cocoa) detected certain compounds with highest peak area such as 9,19Cycloergost24(28)en3ol,4,14dimethyl,acetate, (3á,4à,5à) (19.26%) and Lupeol (8.12%). iPropyl12methyltridecanoate (1.05%), 2(1Methyl1Hindol3yl) 2oxoacetamide (1.06%), Dibutyl phthalate (1.09%) and 2,2,4Trimethyl3 (3,8,12,16 tetramethylheptadeca 3, 7,11,15tetraenyl)cyclohexanol (6.23%).

Bioactive compounds in aqueous extract of D(Sapota) with highest peak area included Obtusifoliol (13.79%), α Amyrin (12.72%) and 4H1Benzopyran4one, 2,3dihydro5,7dihydroxy2phenyl,(S) (10.81%). Unique peak compounds are 1,1,3,3Tetramethyl1,3disilaphenalane (1.83%),Friedelan3one (2.48%),ç Sitosterol (2.92%) and Estra1,3,5(10)trien17áol (1.07%) (Table 12).

Table 12 : Bioactive compounds identified in water extracts based on peak area

Sample	Compound names	RT	Prob.	Area %	PT
DCocoa W	Dodecanal	9.11	32.95	1.77	
	iPropyl12methyltridecanoate	14.07	45.44	1.05	U
	2(1Methyl1Hindol3yl)2oxoacetamide	14.43	26.33	1.06	U
	7,9 Ditertbutyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	15.07	94.43	1.73	
	Diphenyl sulfone	15.41	96.58	8.09	
	n Hexadecanoic acid	15.48	61.06	1.48	
	Dibutyl phthalate	15.53	9.31	1.09	U
	Benzothiazole, 2(2hydroxyethylthio)	15.59	94.88	2.5	
	á Amyrin	16.84	24.75	3.22	
	1Heptatriacotanol	17.09	17.07	1.75	
	Lup20(29)en3ol,acetate, (3á)	17.79	28.18	6.49	
	Lupeol	18.53	59.08	8.12	HP
	9,19Cycloergost24(28)en3ol,4,14dimethyl, acetate, (3á,4à,5à)	19.81	44.5	19.26	HP
	2,2,4Trimethyl3(3,8,12,16tetramethylheptadeca 3,7,11,15tetraenyl)cyclohexanol	20.23	10.19	6.23	U
DSapota W	1,1,3,3Tetramethyl1,3disilaphenalane	9.05	48.71	1.83	U
	Friedelan3one	12.22	38.92	4.28	U
	ç Sitosterol	12.71	31.09	2.92	U

	4H1Benzopyran4one,2,3dihydro5, 7dihydroxy2phenyl,(S)	15	93.2	10.81	HP
	Diphenyl sulfone	15.4	97.29	5.88	
	Estra1,3,5(10)trien17áol	15.48	19.59	1.07	U
	Benzothiazole, 2(2hydroxyethylthio)	15.59	75.66	2.04	
	á Amyrin	16.69	63.4	12.72	HP
	9,19Cyclolanost24en3ol,acetate, (3á)	17.33	6.29	2.22	
	Obtusifoliol	17.72	27.29	13.79	HP
	Lupeol	18.18	49.68	6.29	
	1Heptatriacotanol	19.71	32.35	2.39	

RT: Retention time, Prob: Probability, PT: Peak type

With an aim to attribute the role of the detected compounds in conferring the medicinal property/antioxidant potential of leaf extracts from *Dendrophthoe* inhabiting different hosts, the biological properties of the bioactive compounds detected corresponding to the highest peak area were collected from available literature and enlisted in Table 13.

Table 13 : Bioactive compounds from different host and solvents with highest peak area and its reported biological properties

	Solvent (Area %)						Biological properties
Metabolite	**Host**	**Chlor**	**PE**	**EA**	**Methanol**	**Water**	
cis-Vaccenic acid	Mango	21.4					Antioxidant as well as tyrosinase inhibitory activity, induces differentiation and up-regulates gamma globin
	Sapota	22.55	37.22				
	Jack		30.97	19.14			

Metabolite	Host	Solvent (Area %) Chlor	PE	EA	Methanol	Water	Biological properties
	Cocoa						synthesis in K562, JK1 and transgenic mice erythroid progenitor stem cells, antibacterial andhypolipidemic effects in rats
n-Hexadecanoic acid	Mango	19.4					Antioxidant, antimicrobial, act as cytotoxic by inhibiting DNA topoisomerase-I and prevents cell proliferation
	Sapota	22.55	19.9		13.49		
	Jack		27.86	24.83			
	Cocoa						
1,2,3 Benzenetriol	Mango						Antioxidant, anti-inflammatory, anticancer(polyphenol compound)
	Sapota				13.75		
	Jack						
	Cocoa						
9,19Cycloergost24(28) en3ol,4,14dimethyl, acetate, (3á,4à,5à)	Mango						No activity
	Sapota						
	Jack						
	Cocoa					19.26	
Obtusifoliol	Mango						Steroid, anti-inflammatory and chemopreventive
	Sapota					13.79	
	Jack						
	Cocoa						
α Amyrin	Mango						Triterpene, Anti-inflammatory
	Sapota					12.72	
	Jack						
	Cocoa						
4H1Benzopyran4one, 2,3dihydro5,7dihyd	Mango						Antioxidant, Flavonoid
	Sapota					10.81	
	Jack						

	Solvent (Area %)						Biological properties
Metabolite	**Host**	**Chlor**	**PE**	**EA**	**Methanol**	**Water**	
roxy2phenyl,(S)	Cocoa						
Lupeol	Mango						Antioxidant
	Sapota						
	Jack						
	Cocoa				8.12		

Chlor: Chloroform, PE: Petroleum, EA: Ethyl acetate

4.2 EXPERIMENT II

Cocoa trees infested with *Dendrophthoe falcata* at Cocoa farm of Cocoa Research Centre, Kerala Agricultural University, Thrissur were managed by impregnating the super absorbent polymer, super absorbent cotton and super absorbent paper with a combination of Ethrel and OS/AE and developed as a tape.

4.2.1 Effect of fresh application of Ethrel and OS/AE on defoliation of Loranthus

Fifth week after sticking the tape impregnated with Ethrel and OS, a maximum defoliation of 8.917% was achieved. The percent defoliation (8.00%) was not significantly different in the fourth week, from that of the fifth week. While the percent defoliation was least (2.08%) in the first week after sticking the tape, SAP performed better than other materials due to higher rate of defoliation (9.75%) and percent defoliation was least in case of SAT (1.75%) (Tables 14 and 15).

Interaction effects revealed that the percent defoliation was highest during the fifth week of sticking the SAP (16.25%). There was no significant difference between the fourth (14.50%) and fifth week of sticking SAP. The percent defoliation was found to be least during the first week of sticking SAT (Table 16).

Fresh application of Ethrel and AE as tape showed that maximum defoliation was observed in the fifth week (5.33%) after sticking the tape though the defoliation in the fourth week (5.17%) was not significantly different from that of fifth. SAP (7.85%) resulted in highest defoliation whereas SAC (1.95%) and SAT (1.15%) were not significantly different (Tables 14 and 15).

Fifth week after sticking SAP (12%) maximum defoliation was observed, though there was no significant difference with that of the fourth week (11.75%). First (0.75%) and second week (0.75%) after sticking the SAT showed least percent defoliation and were not significantly different (Table 16).

Table 14 : Number of loranthus leaves defoliated by fresh application of Ethrel and OS/AE on loranthus at different weeks

Treatment	No. of leaves defoliated	No. of leaves defoliated
	E + OS (25mL/L+ 0.5mL/L)	E + AE (25mL/L + 0.5mL/L)
1w	2.08^{c}	1.42^{c}
2w	2.9^{c}	2.42^{c}
3w	5.17^{b}	3.92^{b}
4w	8.00^{a}	5.17^{a}
5w	8.92^{a}	5.33^{a}
CD	1.14	1.07

E: Ethrel, OS: Organosilicone, AE: Alcohol ethoxylate, 1w: 1 week, 2w: 2 week, 3w: 3 week, 4w: 4 week, 5w: 5 week

Table 15 : Number of loranthus leaves defoliated by fresh application of Ethrel and OS/AE at different week across different materials

Materials	No. of leaves defoliated	No. of leaves defoliated
	E + OS (25mL/L + 0.5mL/L)	E + AE (25mL/L + 0.5mL/L)
SAC	4.75^{b}	1.95^{b}
SAP	9.75^{a}	7.85^{a}
SAT	1.75^{c}	1.15^{b}
CD	0.884	0.83

E: Ethrel, OS: Organosilicone, AE: Alcohol ethoxylate, SAC: Super absorbent cotton, SAP: Super absorbent polymer, SAT: Super absorbent paper

Table 16 : Number of loranthus leaves defoliated by fresh application of Ethrel and OS/AE at different weeks across different treatment combination

Treatment combinations	No. of leaves defoliated	No. of leaves defoliated
	E + OS (25mL/L + 0.5mL/L)	E + AE (25mL/L + 0.5mL/L)
1w_SAC	1.75^{f}	1.25^{d}
1w_SAP	3.00^{ef}	2.25^{d}

1w_SAT	1.50^{f}	0.75^{d}
2w_SAC	2.25^{ef}	1.75^{d}
2w_SAP	5.00^{d}	4.75^{c}
2w_SAT	1.50^{f}	0.75^{d}
3w_SAC	3.75^{de}	2.00^{d}
3w_SAP	10.00^{b}	8.50^{b}
3w_SAT	1.75^{f}	1.25^{d}
4w_SAC	7.50^{c}	2.25^{d}
4w_SAP	14.50^{a}	11.75^{a}
4w_SAT	2.00^{ef}	1.50^{d}
5w_SAC	8.50^{bc}	2.50^{d}
5w_SAP	16.25^{a}	12.00^{a}
5w_SAT	2.00^{ef}	1.50^{d}
CD	1.98	1.86

E: Ethrel, OS: Organosilicone, AE: Alcohol ethoxylate, 1w: 1 week, 2w: 2 week, 3w: 3 week, 4w: 4 week, 5w: 5 week, SAC: Super absorbent cotton, SAP: Super absorbent polymer, SAT: Super absorbent paper

4.2.3 Effect of ethrel and OS (1 week dried) on defoliation of Loranthus

There was no significant difference between second (1.33%), third (1.67%), fourth (1.67%) and fifth week (1.75%) after sticking the tape and least defoliation observed during first week (0.83%). Among the materials SAP (2.65%) performed better than SAT (0.6%). No significant interaction effect was observed among different weeks and materials (Tables 17 and 18).

There was no significant effect on defoliation between different weeks in this case also. Among the materials used, there was no significant difference between SAP and SAC. Similiarly, no significant interaction effect on defoliation between treatments and materials was observed (Table 19).

Table 17 : Number of loranthus leaves defoliated by sticking one week dried tape of Ethrel and OS/AE across different weeks

Treatment	No.of leaves defoliated	No. of leaves defoliated
	E + OS (25mL/L + 0.5mL/L)	E + AE (25mL/L + 0.5mL/L)
1w	0.833[b]	1.50
2w	1.333[a]	1.67
3w	1.667[a]	1.67
4w	1.667[a]	1.75
5w	1.750[a]	1.92
CD	0.46	NS

E: Ethrel, OS: Organosilicone, AE: Alcohol ethoxylate, 1w: 1 week, 2w: 2 week, 3w: 3 week, 4w: 4 week, 5w: 5 week

Table 18 : Number of loranthus leaves defoliated by sticking one week dried tape of Ethrel and OS/AE at different week across different materials

Materials	No. of leaves defoliated	No. of leaves defoliated
	E + OS (25mL/L + 0.5mL/L)	E + AE (25mL/L +0.5mL/L)
SAC	1.10[b]	1.95[a]
SAP	2.65[a]	2.20[a]
SAT	0.60[c]	0.95[b]
CD	0.36	0.32

E: Ethrel, OS: Organosilicone, AE: Alcohol ethoxylate, SAC: Super absorbent cotton, SAP: Super absorbent polymer, SAT: Super absorbent paper

Table 19 : Number of loranthus leaves defoliated by sticking one week dried tape of Ethrel and OS/AE at different week across different treatment combinations

Treatment combination	No.of leaves defoliated	No. of leaves defoliated
	E + OS (25mL/L + 0.5mL/L)	E + AE (25mL/L + 0.5mL/L)
1_SAC	0.75	1.75
1_SAP	1.75	2.00
1_SAT	0.00	0.75
2_SAC	1.00	2.00
2_SAP	2.25	2.00
2_SAT	0.75	1.00
3_SAC	1.25	2.00
3_SAP	3.00	2.00
3_SAT	0.75	1.00
4_SAC	1.25	2.00
4_SAP	3.00	2.25
4_SAT	0.75	1.00
5_SAC	1.25	2.00
5_SAP	3.25	2.75
5_SAT	0.75	1.00
	NS	NS

E: Ethrel, OS: Organosilicone, AE: Alcohol ethoxylate, 1w: 1 week, 2w: 2 week, 3w: 3 week, 4w: 4 week, 5w: 5 week, SAC: Super absorbent cotton, SAP: Super absorbent polymer, SAT: Super absorbent paper

4.2.5 Effect of treatments on Loranthus control expressed as percentage defoliation

Comparison between treatments (E+OS and E+AE) on percent defoliation revealed that there is no significant difference between 20% and 30% defoliation, while both 40% (24 days) and 50% (31.75 days) defoliation took longer time in case of T2 (E+AE) than T1 (E+OS). T2 is significantly different from T1 (Table 20).

Table 20 : Percentage defoliation and number of days taken for defoliation after five weeks of sticking the tape impregnated with Ethrel and OS/AE

Treatments	Number of days for defoliation			
	20% Defoliation	30% Defoliation	40% Defoliation	50% Defoliation
T1- E+OS	12.25	16.75	21.5[b]	29.25[b]
T2- E+ AE	14.00	17.75	24.0[a]	31.75[a]
CD	NS	NS	S	S

E: Ethrel, OS: Organosilicone, AE: Alcohol ethoxylate

Plate 17 : Sticking the tape impregnated with Ethrel + OS/AE on to Loranthus branches

Plate 18: Status of the Loranthus branch after defoliation

DISCUSSION

5. DISCUSSION

Dendrophthoe falcata is a hemiparasite colonizing most of the fruit trees of commercial importance. There have been concerted efforts to devise a control measure, which is attempted to be refined as part of this study. Besides its destructive nature on the host, the leaves possess potential medicinal properties that pave way for drug discovery. All these aspects involving the tapping of available medicinal properties and checking the efficacy of the tape method of control measure are discussed in this chapter.

5.1.1 Extraction and isolation of antioxidant fraction from Loranthus

Phytochemical constituents that could be present in leaves of *Dendrophthoe falcata* inhabiting different hosts namely mango, sapota, jack and cocoa were assessed in order to understand the parasite-host specificities. In this context, the efficiency of employing different solvents to extract the respective phytochemicals assumed significance. The use of different solvents varying in their polarity and chemical nature such petroleum ether, chloroform, ethyl acetate, methanol and water were used to extract the phytochemicals. The results of the study indicated that the extract yields were higher in case of aqueous extract of *D. falcata* inhabiting the four different hosts and the lowest in organic solvents such as petroleum ether, ethyl acetate *etc*. (Table 2). High extract yield can be due to higher solubility of major components present in the leaves of *D. falcata* in water. The yield of chloroform extracts of aerial parts of *D. falcata* was lowest compared to methanol and ethanol-water extract due to low solubility of primary components of aerial section *D. falcata* (Pattanayak *et al*., 2011). Solvent extraction yield of *D. pentandra* showed almost same yield in petroleum ether and ethyl acetate compared to chloroform and diethyl ether (Yee et al., 2017). The hierarchy in extraction yields across different solvents observed in our study were in conformity with earlier reports as mentioned above.

5.1.2 Antioxidant assay

A lipophilic radical is thought to be modelled after the DPPH radical. The lipid autoxidation starts a cascade of lipophilic radicals. At normal temperature, DPPH is a stable free radical that accepts an electron or hydrogen radical to form a stable

diamagnetic molecule. A drop in DPPH's absorbance at 517 nm, which is generated by antioxidants, was used to measure its reduction capabilities. The presence of a positive DPPH test in the samples indicated that they were free radical scavengers. Chloroform and water extracts of *D. falcata* showed an increasing trend in free radical inhibition activity with time (Table 3). Ethyl acetate extract initially showed almost constant reading later the inhibition activity reduced at two hour incubation. According to Mothana *et al*. (2011), the most potent portion of a methanolic extract of *Loranthus regularis* was discovered to be the ethyl acetate fraction, which showed the greatest suppression of inflammation (67%) and oxidation of β-carotene (92%). Aqueous extract of *Dendrophthoe falcata* leaves was found to be more effective than methanolic extract of *D. falcata* leaves in acute and chronic inflammation, and aqueous extract of *D. falcata* leaves was shown to be less potent as an antioxidant agent than methanolic extract of *Dendrophthoe falcata* leaves (Patil *et al*., 2011). Atun et al. (2019) studied that IC_{50} of ethyl acetate and the chloroform fractions from *D. falcata* leaves was less than 10 g/mL, indicating that antioxidants were particularly active. Previous research revealed that *D. falcata* plants contained high levels of phenolics and flavonoids which is responsible for high antioxidant activity.

5.1.3 Phytochemical screening of *Dendrophthoe falcata*

Chloroform extract of all the four hosts infested with *D. falcata* contained alkaloids, phenols, flavonoids, terpenes and steroids (Table 6). Alkaloids have antioxidant and anti-bacterial properties. The alkaloid in *Phoebe declinata* leaf extract was reported to be responsible for its antioxidant properties *in vitro* (Agarwal *et al*., 2013). Many flavonoids and terpenoids are powerful antioxidants with anti-inflammatory, antibacterial, antiviral, and cancer-fighting properties. Flavonoids and flavonols have an important role in medications that lower cholesterol and fat, as well as those that help lower the risk of coronary heart disease (Kumar and Pandey, 2013). Phenolics have anti-oxidant, anti-diabetic, anti-carcinogenic, anti-microbial, anti-allergic, anti-inflammtory and anti-mutagenic properties, and they have a strong influence on neurodegenerative illnesses. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989). The phenolic compounds may contribute directly to antioxidative action (Duh *et al*.,

1999). Steroids are recognised for their cardiotonic characteristics, as well as their insecticidal and antimicrobial capabilities (Kaurinovicco *et al*., 2012).

Ethyl acetate extract had comparatively lesser phytochemicals, whereas methanolic extract had alkaloids, saponins, phenols, flavonoids, terpenes and steroids. Water extract yielded tannins along with other phytochemicals. According to Govindappa *et al*. (2013) methanolic and aqueous extract yielded alkaloids, saponins, flavonoid and phenols in higher concentration compared to other solvents in *D. falcata* on *Azadirachta indica*. Saponins are used to treat hypercholesterolemia and hyperglycemia, as well as being antioxidants, anticancers, antifungals, antibacterials, anti-inflammatory, and weight loss agents (Garai, 2014). Terpenes, terpenoids, flavonoids, saponins, alkaloids, and certain proteins, as well as phenolic compounds and tannins, have insecticidal effect through a variety of molecular targets (Mostafa *et al*., 2018).

5.1.4 Identification of bioactive compounds through GC-MS/MS analysis

GC-MS/MS analysis of different extracts of *D. falcata* from different hosts revealed certain bioactive compounds with highest abundance. The careful scrutiny of the bioactive principles thus identified helped us to relate to the medicinal/pharmacological properties of the extracts with high antioxidant potentials in the context of parasite-host specificities.

The bioactive compound, cis-vaccenic acid was present in chloroform extract of D (mango) and sapota, petroleum ether extract of sapota and jack and, ethyl acetate extract of jack. Cis-vaccenic acid was reported to be responsible for antioxidant as well as tyrosinase inhibitory activity (Panda *et al*., 2018), which induces differentiation and up-regulates gamma globin synthesis in K562, JK1 and transgenic mice erythroid progenitor stem cells, antibacterial and hypolipidemic effects in rats (Aimola *et al*., 2016). It is also a potent skin whitening agent attributed by its anti-tyrosinase which is involved in melanin synthesis (Pillaiyar *et al*., 2017)

n-hexadecanoic acid was abundant in chloroform extract of mango and sapota, petroleum ether extract of sapota and jack, ethyl acetate extract of jack and, methanol extract of sapota. n- hexadecanoic acid is a palmitic acid with antioxidant and antimicrobial activity which also confers cytotoxicity by inhibiting DNA

topoisomerase-I thereby preventing cell proliferation, It is also acts as a hypo cholesterolemic, nematicide, pesticide, lubricant, antiandrogenic and hemolytic which is also used in conferring flavor and also acting as a 5-alphareductase inhibitor (Ravi and Krishnan 2017).

Methanolic extract of sapota and cocoa contained 1,2,3 benzenetriol and lupeol respectively. 1,2,3 benzenetriol (pyrogallol) is a polyphenol compound with antioxidant, anti-inflammatory, anticancer, analgesic, insecticidal properties (Bhagat and Konduwara, 2021). Lupeol is a lupane type triterpene present in fungi, plants and animal kingdom and possesses anti-protozoal, anti-cancerous, chemopreventive and anti-inflammatory properties (Gallo and Sarachine, 2009).

Aqueous extract of sapota showed the presence of obtusifoliol, α-amyrin and 4-H-1-Benzopyran-4-one,2,3dihydro5,7dihydroxy2phenyl,(S) whereas of that of cocoa had 9,19Cycloergost24(28)en3ol,4,14dimethyl,acetate, (3á,4à,5à) as the bioactive compounds (Table 18). Obtusifoliol is a lipid molecule that is classified as a sterol, with evening primroses have its highest content. Obtusifoliol has also been found in a variety of foods, including common chokecherries, jicama, pepper (*C. frutescens*), avocado, and pecan nuts, which makes it an ideal biomarker for the ingestion of these foods. It is a product of the CYP51A1 enzyme, which catalyses the production of cholesterol (Lamb *et al*., 1998). Obtusifoliol has anti-inflammatory and chemopreventive potential (Elisabete and Jorge, 2020). Alpha-amyrins are triterpene with anti-inflammatory properties and has potential in imposing anti-cancer properties (Okoye *et al*., 2014; Ranjbar *et al*., 2016). 4-H-1-Benzopyran-4-one is a flavonoid that prevents cellular damage (Khadem and Marles, 2012) and an antioxidant that acts as an aldose reductase inhibitor (Costantino *et al*, 1999).

Some of the other unique compounds in various solvents include squalene, phytol, dibutyl phthalate, friedelan-3-one, ξ-sitosterol, geranyl-geraniol and diphenyl sulfone. All these unique bioactive compounds have been reported in the very first report on GC-MS analysis in *D. falcata* (Beulah *et al*., 2018). However it will be interesting to learn more about the presence of these bioactives in the context of host specificities. Phytol is a diterpene with anti-microbial, anti-inflammatory, anticancer and diuretic properties. Squalene is a triterpene compound having antibacterial, antioxidant,

antitumor, cancer preventive, immune stimulant, chemo preventive, lipoxygenase-inhibitor and pesticide attributes. Dibutyl phthalate is a plasticizer compound acting as an antimicrobial and antifouling agent. Geranyl-geraniol, a terpene alcohol with fragrance possesses anti-microbial and anti-inflammatory properties (Beulah *et al*., 2018).

Among the many phytosterols, ξ-sitosterol (SIT) is the most abundant substance in plants. Many in-vitro and in-vivo studies have shown that SIT has a variety of biological actions, including anxiolytic and sedative effects, analgesic, immunomodulatory, antimicrobial, anticancer, anti-inflammatory, lipid lowering effect, hepatoprotective, protective effect against NAFLD and respiratory diseases, wound healing effect, antioxidant, and anti-diabetic properties (Babu and Jayaraman, 2020).

Friedelan-3-one is a flavonoid with anti-microbial, anti-convulsant properties along with hepatoprotective activity (Odeh *et al*., 2016). Diphenyl sulfones are anti-inflammatory, anti-microbial, anticancer, anti-HIV, anti-malarial and anti-proliferative principles which also acts as agonists for thyroid receptor and cyclic nucleotide-gated receptors (Ahmad and Shagufta, 2015).

The overall analysis revealed the presence of many novel medicinal attributor phytochemicals in the leaves of *D. falcata* with distinct contexts to the host on which they inhabit. Looking into the two common and abundant bioactive principles viz. cis-vaccenic acid and n-hexadecanoic acid, it was interesting to note that abundance was higher in the petroleum ether extracts of D (sapota) and D(jack) for the former and D(jack) for the latter (Plate 19). However, the higher yields of n-hexadecanoic acid from D(sapota) was obtained by chloroform extraction. Petroleum ether extraction resulted in the best profiling for both the bioactive compounds from D(jack), indicating the association of *D. falcata* with jack as the most potential one to be tapped for pharmacological uses (Plate 19). *D. falcata* inhabiting cocoa neither accumulated cis-Vaccenic acid nor n-hexadecanoic acid (Plate 19).

Metabolite	Host	Solvent (Area %)					Sparkline-Line Trend	Sparkline-Column Trend
		Chloroform	PE	EA	Methanol	Water		
cis-Vaccenic acid	Mango	21.4						
	Sapota	22.55	37.22					
	Jack		30.97	19.14				
	Cocoa							
n-Hexadecanoic acid	Mango	19.4						
	Sapota	22.55	19.9		13.49			
	Jack		27.86	24.83				
	Cocoa							

PE : Petroleum ether, EA: Ethyl acetate

Plate 19: Trend of cis vaccenic acid and n-hexadecanoic acid in different extracts and hosts

5.2 Tape method of control of Loranthus

Development of sticky tape by impregnating Ethrel and Organosilicone (OS)/Alcohol ethoxylate (AE) on three different materials namely super absorbent polymer, cotton and paper followed by sticking it onto the scraped branches of Loranthus was attempted to check the rate of defoliation. Fresh application and 1 week dried tape were stuck of which fresh application performed better than dried. The rate of defoliation also increased with time and after a while the number of leaves defoliated remained constant. Among the applicant material used, super absorbent polymer was found to be superior to super absorbent cotton and paper (Tables 14-19). Percent defoliation due to both surfactants revealed that AE performed better than OS (Table 20). According to Knoche *et al*. (1991) the efficacy of growth regulator enhanced when ethrel was combined with surfactant such as organosilicone. On application to the adaxial leaf surface of perennial ryegrass (*Lolium perenne* L.), the addition of the organosilicone surfactant 'Silwet L77' at 1-5 ml litre^{-1} to formulated glyphosate resulted in total surface wetting. The solution's wetting qualities were linked to rapid foliar uptake and near maximal uptake in 3 hours, compared to more than 5 hours in the absence of 'Silwet L77.' It was also demonstrated that 'Silwet L77' solutions rapidly penetrated stomata (Field and Bishop, 1988). SAP-Microsphere (sodium acrylic acid-vinyl alcohol copolymer) polymer was reported to absorb fluids in a

matter of minutes and expand its diameter (Jiaqi *et al*., 1996). Super absorbent polymers, also known as hydrogels, are loosely cross-linked, three-dimensional networks of flexible polymer that can absorb and store hundreds of times their dry weight due to a small number of widthwise connections (Kiatkamjornwong, 2007). Studies using plant growth-promoting rhizobacteria (*A. lipoferum* + *Pseudomonas putida*) in combination with super absorbent polymers have revealed that super absorbent polymers aid to increase soil cationic exchange capacity and improve water and nutrition material absorption (Moselmi *et al*., 2011).

The use of alcohol ethoxylate as an alternative to organosilcone would also ensure ecosafety. The most powerful adjuvants and super-penetrants accessible to growers are organosilicone surfactants at the same time it is ecotoxic and kills honey bee population (Mullin *et al*., 2016). Alcohol ethoxylates are usually more biodegradable and their degradation products are unobjectionable in terms of their aquatic toxicity. Performance properties of alcohol ethoxylates depend on the alkyl chain length, the alkyl branching and the polyethylene glycol chain (Bejarano and Wheeler, 2021). Hence, the study revealed that use of ethrel along with surfactant alcohol ethoxylate as a sticky tape using superabsorbent polymer as the impregnating material can be used as an effective management strategy. The tape method can form an alternative to spray method of control although it would comparatively take more time for defoliation than the latter, provided they confer the similar no regrowth potentials as demonstrated in previous studies (Garggi, 2021), which needs to be carefully examined. It will be worthwhile to investigate the number of sticky tapes needed for effective control as well as comparatively assess the difficulty, time, energy, benefit cost ratio, labour requirement, and effectiveness compared to spray.

SUMMARY

6. SUMMARY

Hemiparasitic weeds like *Dendrophthoe falcata* require a comprehensive addressal as to how best it can be managed without causing economic losses to trees, they infest. With the major aim of meeting this requirement, the study titled "Elucidation of antioxidant fractions from leaves of *Dendrophthoe falcata* L. and standardization of tape method of control" was carried out at the Department of Plant Physiology, College of Agriculture, Kerala Agricultural University during 2019 – 2021. The objectives of the study were to characterize bioactive antioxidant fractions in *Dendrophthoe falcata* through *in vitro* assays and standardisation of tape method of Loranthus control.

The salient findings of the study are summarised in this chapter.

- The extract yields were highest in case of aqueous extract of *D. falcata* inhabiting the four different hosts and lowest in organic solvents such as petroleum ether and ethyl acetate, indicating polar inorganic extraction to be advantageous, although novel and unique bioactive compounds could be effectively extracted in the latter.
- Chloroform and water extracts of *D. falcata* showed an increasing trend in inhibition activity with time. However, ethyl acetate extract marked a reduction in inhibition activity after two hours of incubation.
- Chloroform extract of all the four hosts infested with *D. falcata* contained alkaloids, phenols, flavonoids, terpenes and steroids. Ethyl acetate extract had comparatively lesser phytochemicals, whereas methanolic extract had alkaloids, saponins, phenols, flavonoids, terpenes and steroids. Water extract yielded tannins along with other phytochemicals.
- Two common and abundant bioactive principles viz. cis-vaccenic acid and n-hexadecanoic acid were extracted in higher abundance in the petroleum ether extracts of *D. falcata* inhabiting sapota and jack for the former and that on jack for the latter.
- *D. falcata* inhabiting cocoa neither accumulated cis-Vaccenic acid nor n-hexadecanoic acid

- Petroleum ether extraction resulted in the best profiling for both the bioactive compounds from D(jack), indicating the association of *D. falcata* with jack as the most potential one to be tapped for pharmacological uses.
- Ethrel along with surfactant alcohol ethoxylate (AE) as a sticky tape using superabsorbent polymer as the impregnating material can be used as an effective management strategy as AE is more eco-friendly than organosilicone. The tape method can also be an alternative to spraying even in the event its longer time for defoliation, provided if it ensures similar regrowth restriction efficiencies.

Future line of work

- Ideal solvent – host combinations can be used for tapping specific metabolites. Most active fractions can be screened by LC-MS/MS. Molecular docking can help in identifying specific ligand and receptors pertaining to different health conditions.

- Time and stage of application of Ethrel and AE can be standardized along with development of specific devices that can aid in attaching the tape to branches of parasites infesting host trees.

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ELUCIDATION OF ANTIOXIDANT FRACTIONS IN LEAVES OF *Dendrophthoe falcata* L. AND STANDARDISATION OF TAPE METHOD OF CONTROL

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ABSTRACT OF THE THESIS

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ABSTRACT

Dendrophthoe falcata is a partial stem parasite belonging to Loranthaceae family, which is considered as a destructive pest of many economically important fruit trees such as mango, sapota, sugar apple and guava. They cause damage to the host plants by depleting nutrients, releasing toxins, and limiting their growth. Though several management strategies have been suggested by previous researchers which include pruning of its branches, base banding using 2,4-D and spray of ethrel in combination with organosilicone (non-ionic surfactant), the latter gave a promising response in comparison to others without affecting the host. Because of its destructive nature, some of the potential pharmaceutical features of the parasite often go unnoticed; if prospectively exploited it will automatically open up avenues for targeted removal of the parasitizing aerial parts of *D. falcata.*

Plants are thought to be one of the most important sources for discovering and developing pharmaceuticals that are both effective and safe compared to currently available synthetic drugs. Traditional and folk medicines are gaining favour over contemporary therapy due to fewer side effects and a higher safety margin. *Dendrophthoe falcata*, a widely distributed plant in India, is one of nature's many plants with medicinal characteristics. The potential medicinal property of Loranthus has been reported in Ayurveda. It is reported to have diuretic, wound healing, anti-microbial, anti-helminthic, anti-fertility, antioxidant, anti-cancer, anti-diabetic, anti-hyperlipidemic and anti-hypersensitive activities. Medicinal properties of mistletoes are host specific. The antioxidant property of these parasitic plants might be due to their phenolic compounds including tannins and flavonoids. However, it will be very interesting to identify the novel antioxidant principles in the context of specific host associations.

The present study was envisaged with a major aim of characterisation and elucidation of bioactive antioxidants from *D. falcata* leaves by *invitro* assays and identification of bioactive compounds through GC-MS/MS with prospective pharmaceutical applications, and standardization of efficient and non-regenerative method to control Loranthus.

The experiments were carried out in Department of Plant Physiology, College of Agriculture, Vellanikkara, Thrissur. In the first experiment the leaf samples of *Dendrophthoe falcata* inhabiting different hosts namely mango, jackfruit, cocoa and sapota were collected, powdered and extracted in various organic solvents such as chloroform, petroleum ether, ethyl acetate, methanol and water followed by computation of extract yields. Aqueous extracts of *D. falcata* inhabiting all the hosts showed significantly higher (10.2 to 14.8%) yields compared to other solvents. Extract yield in case of petroleum ether and ethyl acetate were reported to be lowest (1.6 to 2.9% respectively) compared to other solvents indicating polar inorganic extraction to be advantageous, although novel and unique bioactive compounds could be effectively extracted in the latter. D(Sapota) showed lowest extract yield in petroleum ether (1.6%) and ethyl acetate (1.7%), whereas D(Jack) and D(Cocoa) had higher extract yields of 14.8% and 14.4% in water respectively. Free radical inhibition activity of *D. falcata* were assessed using DPPH radical scavenging assay in different hosts across various solvent extractions at different time intervals. Chloroform and water extracts of *D. falcata* showed an increasing trend in inhibition activity with time. However, ethyl acetate extracts marked a reduction in inhibition activity after two hours of incubation. Qualitative phytochemical screening of different extracts of *D. falcata* on the four hosts namely mango, jackfruit, cocoa and sapota were carried out to detect the presence of alkaloids, phenols, saponins, flavonoids, terpenes, tannins and steroids. There was a differential display of possession of different classes of phytochemicals by *D. falcata* across different host associations which varied with the nature of solvent used for extracting the antioxidant fractions. Chloroform extract of all the four hosts infested with *D. falcata* contained alkaloids, phenols, flavonoids, terpenes and steroids. Ethyl acetate extract had comparatively lesser phytochemicals, whereas methanolic extract had alkaloids, saponins, phenols, flavonoids, terpenes and steroids. Water extract yielded tannins along with other phytochemicals. Based on the antioxidant potential of the different extracts and host specificities, eight samples were shortlisted for GC-MS/MS analysis. Bioactive substances were detected in different extracts of *D. falcata* during GC-MS/MS analysis. Two common and abundant bioactive principles viz. cis-vaccenic acid and n-hexadecanoic acid were extracted in higher abundance in the petroleum ether extracts of D(sapota) and D(jack) for the former and D(jack) for the

latter. Petroleum ether extraction resulted in the best profiling for both the bioactive compounds from D(jack), indicating the association of *D. falcata* with jack as the most potential one to be tapped for pharmacological uses.

The second experiment was carried out at Cocoa farm of Cocoa Research Centre, Kerala Agricultural University, Thrissur where the cocoa trees infested with Loranthus were tested for efficient management by impregnating the super absorbent polymer, super absorbent cotton and super absorbent paper with a combination of Ethrel and surfactants like oraganosilicone (OS)/alcohol ethoxylate (AE) @ 25mL/L of Ethrel + 0.5 mL/ L OS/AE and developed as a tape. Fresh and 1 week dried tapes were comparatively assessed, of which fresh application performed better than dried. The rate of defoliation also increased with time and after a while the number of leaves defoliated remained constant. Among the applicant material used, super absorbent polymer was found to be superior to super absorbent cotton and paper. Percent defoliation due to both surfactants revealed that AE performed better than OS. Ethrel along with surfactant alcohol ethoxylate (AE) as a sticky tape using superabsorbent polymer as the impregnating material can be used as an effective management strategy as AE is more eco-friendly than organosilicone. The tape method can also be an alternative to spraying even in the event its longer time for defoliation, provided if it ensures similar regrowth restriction efficiencies.