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GC-MS ANALYSIS OF PHYTOCOMPONENTS IN THE ETHANOL EXTRACT OF *DENDROPHTHOE FALCATA* (L.F.) ETTINGSH COLLECTED FROM *ARTOCARPUS HETEROPHYLLUS* HOST TREE.

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ABSTRACT

The GC-MS analysis for the active principles in the ethanol extract of *Dendrophthoe falcata* indicated the presence of 12 compounds in the leaf harvested from *Artocarpus heterophyllus* host tree, one compound in bark sample and one compound in the tender shoot sample. Among the compounds identified, in the ethanol extract of *D. falcata* leaf sample, 1-terpine alcohol, 1-diterpine, 2-triterpines, 1-fatty acid ester, 1-linolenic acid, 1-plasticizer, 2-palmitic acid, 1-vitamin, 2-steroids compounds. Most of these compounds reported to have various bioactivities. No activity was reported in the fatty acid eater compound. On the other hand, one compound (plasticizer compound) was identified in the ethanol extract of *D. falcata* bark and tender shoot samples harvested from *A. heterophyllus* host tree which was reported to have antifouling and antimicrobial activities. Most of the identified compounds reported to have bioactivities are varied in nature.

KEYWORDS: Hemi-parasite, Mistletoe, *Dendrophthoe falcata*, Host tree, *Artocarpus heterophyllus*, Phytocomponents, GC-MS analysis.

INTRODUCTION

Phytochemicals are natural bioactive compounds found in plant parts and act as a defense system against diseases, more accurately to protect against disease causing microbes. Previous reports indicated that a large number of these plants and their extracts have shown beneficial therapeutic effects. *Dendrophthoe falcata* (Syn., *Loranthus longiflorus* Desr.), a hemiparasitic Mistletoe plant belongs to Loranthaceae, is one of the medicinally important used traditionally in different countries for curing various diseases. The hemiparasite *D. falcata* possesses remarkable potentials as a medicinal plant evident from the wound healing, anti-microbial, anti-oxidant, antinociceptive, anti-inflammatory, anti-diabetic and anti-hyperlipidaemic properties of its ethanol extracts.[1-8]

GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants. Chromatographic screening of fruit extracts of the Indian Ayurvedic plant, *Dendrophthoe falcata*, carried out by Uppuluri Venkata Mallavadhani *et al*.[9] Chandrakasan and Neelamegam.[10,11] carried out GC-MS analysis in the ethanol extracts of *Loranthus longiflorus* (Syn. *Dendrophthoe falcata*) leaf and bark samples collected from *Casuarina equisetifolia* and *Ficus religiosa* host trees. However, the perusal of literature reveals that GC-MS analysis of *D. falcata* is very little and that also obtained from very few host plants when compared to its wide host range. There was no report available on GC-MS analysis of *D. falcata* collected from *Artocarpus heterophyllus* host tree. Therefore the main aim of the present study is to identify the possible phytoconstituents present in the ethanol extract of *Dendrophthoe falcata* (L.f.) Ettingsh. Leaf, tender shoot and bark sample collected from *Artocarpus heterophyllus* host tree.

MATERIALS AND METHODS

Plant Material

The hemiparasitic mistletoe plant, *Dendrophthoe falcata* (L.f.) Ettingsh was collected from the host tree of *Artocarpus heterophyllus*, at Marthandam area, Kanyakumari district, Tamil Nadu in the month of November, 2011. The plant was identified by BSI, Coimbatore, Tamil Nadu, and the voucher specimen is preserved in the Department of Botany, S.T. Hindu College, Nagercoil, Kanyakumari District, Tamil Nadu.

Preparation of dry powder samples

Fresh leaf, bark and tender shoot samples of *Dendrophthoe falcata* collected from *A. heterophyllus* host tree were washed to remove the dust and dried separately for about two weeks at room temperature (30ºC±2ºC) to get a constant weight. The dried plant

materials (leaf, bark & tender shoot) were ground to powder separately by mechanical device, stored and used in this work throughout the study period.

Preparation of ethanol extracts for GC-MS

Required quantity of the leaf, bark and tender shoot dry powder of *Dendrophthoe falcata* was weighed, transferred to a stoppered flask, treated with ethanol until the powder was fully immersed, incubated over night and filtered through Whatmann No. 41 filter paper. Before filtering, the filter paper was wetted with sodium sulphate along with absolute alcohol to remove the sediments and traces of water in the filter paper. Then the filtrate was concentrated to 1ml by bubbling nitrogen gas into the solution. About 2µl sample solution was employed in GC-MS analysis for different compounds.

Dendrophthoe falcata infected *Artocarpus heterophyllus* host -Habit.

Plate 01: The Hemiparasite, *Dendrophthoe falcata* infected *Artocarpus heterophyllus* host tree –Habit (Photos -1 to 6).

GC-MS Analysis of Phytocomponents

GC-MS analysis of the ethanol extract of *Dendrophthoe falcata* leaf, bark and tender shoot samples collected from *A. heterophyllus* was performed using a Perkin Elmer GC Clarus 500 system comprising AOC-20i auto-sampler and a gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% Diphenyl / 95% Dimethyl Poly Siloxane) fused capillary column (30 x 0.25µm ID x 0.25µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70eV. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1ml/min, and an injection volume of 2µl was employed (split ratio of 10:1). The injector temperature was maintained at 250ºC, the ion -source temperature was 200ºC, the oven temperature was programmed from 110ºC (isothermal for 2min), with an increase of 10ºC/min to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC.Mass spectra were taken at 70eV; a scan interval of 0.5seconds and fragments from 45-450Da. The solvent delay was 0 to 2min and the total GC/MS running time was 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Identification of phytocomponents

Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the test materials were ascertained. The biological activities of the phytocompounds identified in the ethanol extract of *D. falcata* leaf, tender shoot and bark samples harvested from *A. heterophyllus* host tree were based on Dr. Duke's Phytochemical and Ethanobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/ USDA.

RESULTS AND DISCUSSION

GC-MS Analysis Leaf Sample of *Dendrophthoe falcata*

Figure 1-a show GC-MS chromatogram of the phytocomponents present in the ethanol leaf extract of *D. falcata* infested on *A. heterophyllus* host tree and the figures 2 (1-12) shows the mass spectrum and structure of phytocomponents identified. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the ethanol leaf extract of *Dendrophthoe falcata* collected from *A. heterophyllus* host tree were presented in table 1.

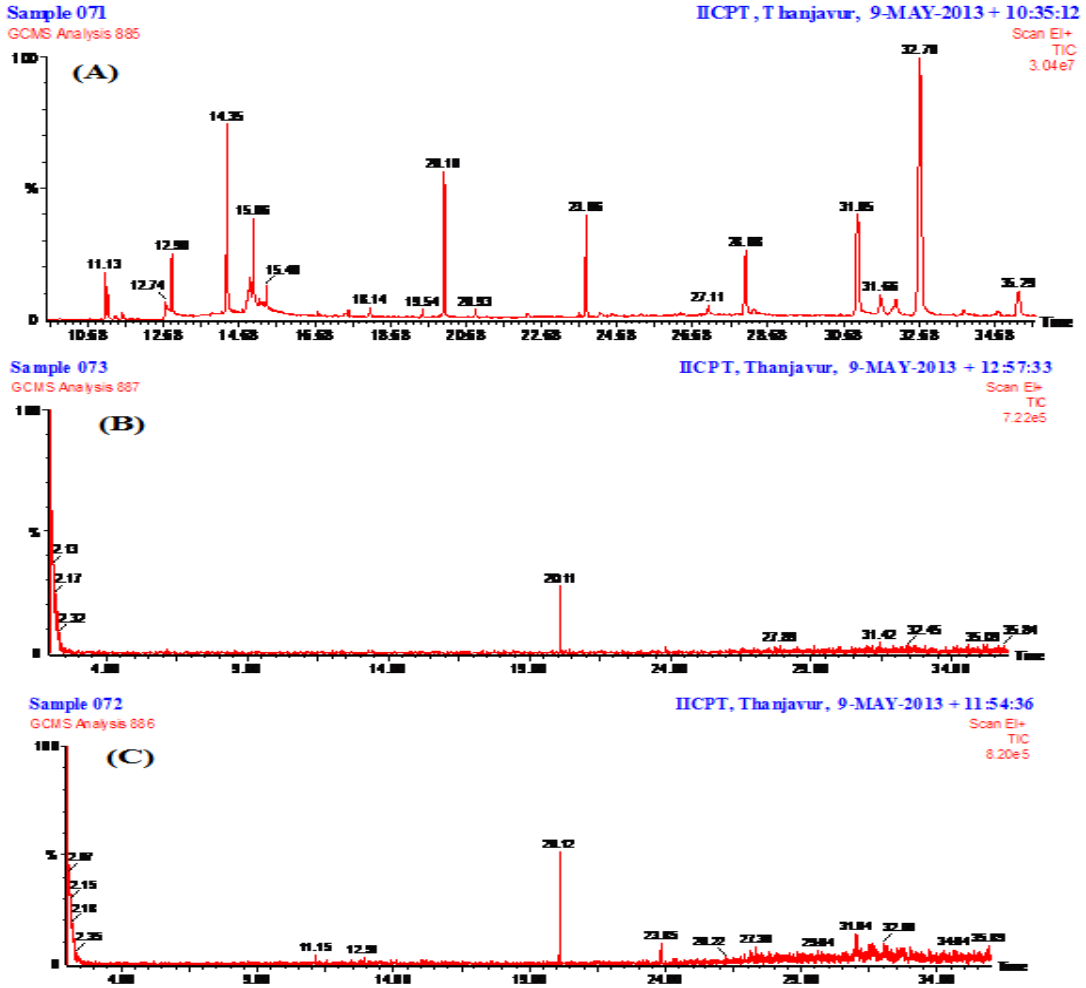

Figure 1: GC-MS chromatogram of ethanol extracts of *Dendrophthoe falcata* leaf (A), tender shoot (B) and bark (C) collected from *Artocarpus heterophyllus* host tree.

Table 1: Phytocomponents identified in the ethanol extract of *Dendrophthoe falcata* leaf sample collected from *Artocarpus heterophyllus* host plant by GC-MS.

Sl. No.	Name of the Compound	Formula	Molecular MW	Peak Area %	RT
1.	3, 7, 11, 15-Tetramethyl-2- hexadecen-1-ol	$C_{20}H_{40}O$	296	2.30	11.13
2.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.43	12.74
3.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	4.23	12.90
4.	Phytol	$C_{20}H_{40}O$	296	9.99	14.35
5.	9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	$C_{19}H_{32}O_2$	292	6.79	15.06
6.	Pentadecanoic acid, 2, 6, 10, 14-tetramethyl-, methyl ester	$C_{20}H_{40}O_2$	312	2.43	15.40
7.	1, 2-Benzenedicarboxylic acid, disooctyl ester	$C_{24}H_{38}O_4$	390	8.19	20.10
8.	Squalene	$C_{30}H_{50}$	410	6.02	23.86

9.	Vitamin E	$C_{29}H_{50}O_2$	430	5.51	28.08
10.	Diazoprogesterone	$C_{21}H_{30}N_4$	338	11.91	31.05
11.	Lupeol	$C_{30}H_{50}O$	426	36.49	32.70
12.	Urs-12-en-24-oic acid 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468	3.71	35.29

*Parameters tested are not covered under the scope of NABL accreditation.

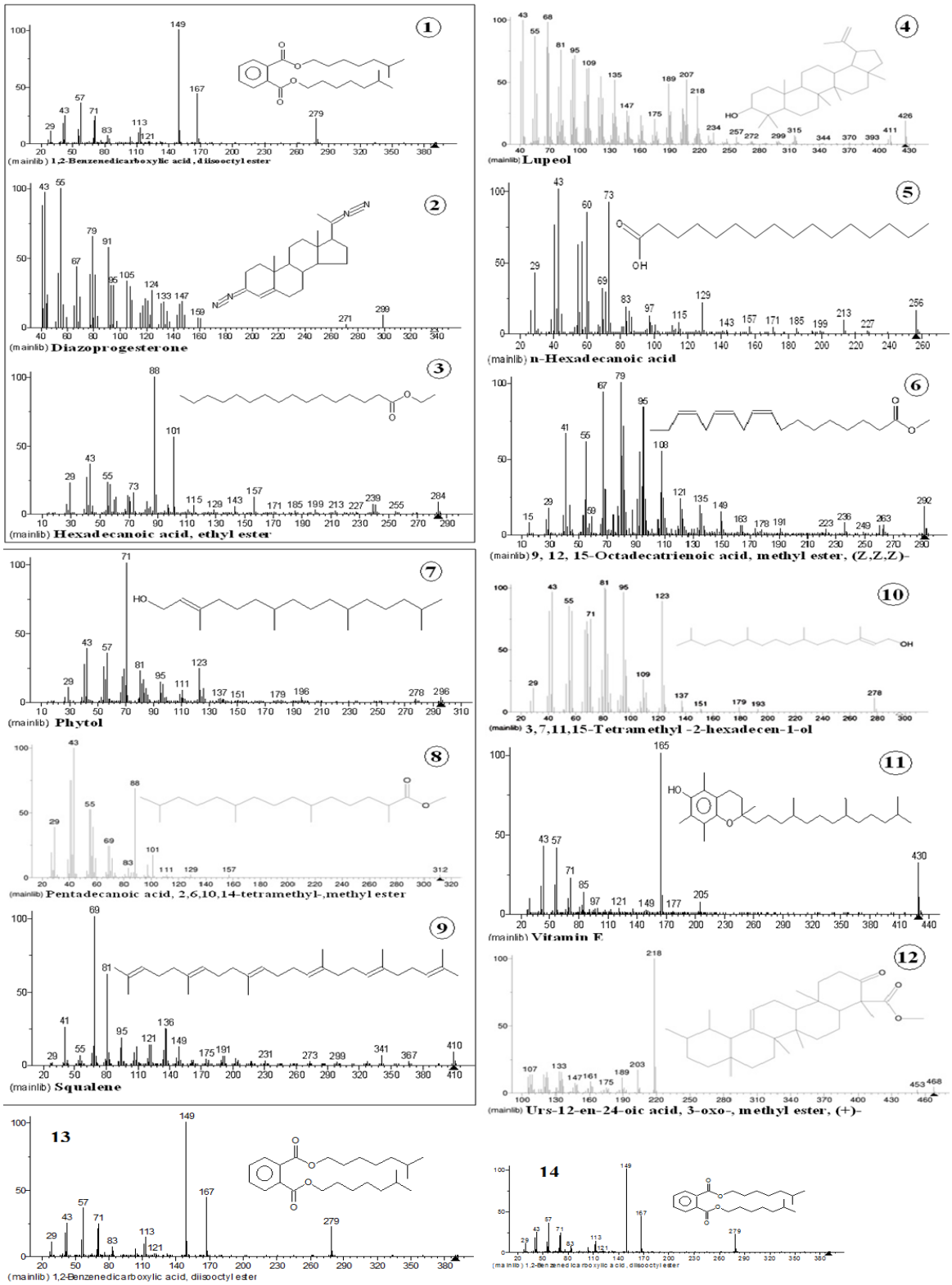

Figure 2: Mass spectrum and structure of phytocomponents identified by GC-MS in the ethanol extracts of *Dendrophthoe falcata* leaf (1-12), tender shoot (13) and bark (14) samples cillected from *Artocarpus heterophyllus* host tree.

GC-MS analysis for the active principles in the ethanol extract of *Dendrophthoe falcata* leaf sample indicates the presence of twelve compounds (Table 1). Among the 12 compounds identified. Lupenol ($C_{30}H_{50}O$) shows maximum peal area (36.49%) with a retention time (RT) of 32.70. and is followed by diazoprogestrone ($C_{21}H_{10}N_4$) with a peak area of 11.97% and 31.05 RT; phytol ($C_{20}H_{40}O$) with a peak area of 9.99% and RT 14.35; 1,2-benzenedicarboxilic acid, disooctyl ester with 8.19% peak area and 20.10 RT; 9,12,15-octadecatrienoic acid ($C_{19}H_{32}O_2$), methyl ester, (ZZZ)- shows 6.79% peak area and 15.06 RT; squalene ($C_{30}H_{50}$) with 6.02% peak area and 23.86 RT; and vitamin-E ($C_{29}H_{50}O_2$) with 5.51% peak area and 28.08 RT.

Table 2: Bioactivity of phytocomponents identified in the ethanol leaf extract of *Dendrophthoe falcata* collected from *Artocarpus heterophyllus* host plant by GC-MS.

Sl. No.	Name of the Compound	Nature of Compound	Activity
1.	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial, Anti-inflammatory.
2.	n-Hexadecanoic acid	Palmitic acid	5-Alpha Reductase Inhibitor, Antioxidant, Antiandrogenic, Flavor, Hemolytic, Pesticide, Hypocholesterolemic, Lubricant, Nematicide.
3.	Hexadecanoic acid ethyl ester,	Palmitic acid	5-Alpha Reductase Inhibitor, Antiandrogenic, Antioxidant, Flavor, Hypocholesterolemic, Hemolytic, Lubricant, Nematicide, Pesticide,
4.	Phytol	Diterpene	Anticancer, Anti-inflammatory, Antimicrobial, Diuretic.
5.	9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-ester	Linolenic acid	5-Alpha Reductase Inhibitor, Antiinflammatory, Antiacne, Antihistaminic, Antiandrogenic, Anticoronary, Antieczemi, Antiarthritic, Cancer preventive, Hypocholesterolemic, Hepatoprotective, Insectifuge, Nematicide,
6.	Pentadecanoic acid, 2, 6, 10, 14-tetramethyl-, methyl ester	Fatty acid ester	No activity reported.
7.	1, 2-Benzenedicarboxylic acid,disooctyl ester	Plasticizer compound	Antimicrobial, Antifouling.
8.	Squalene	Triterpene	Antibacterial; Antioxidant, Antitumor, Cancer preventive, Chemo preventive, Immunostimulant, Lipoxygenase- inhibitor, Pesticide.
9.	Vitamin E	Vitamin Compound	Antiageing, Anticoronary, Analgesic, Antidiabatic Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Antiulcerogenic, Antispasmodic, Antibronchitic, Hepatoprotective, Hypocholesterolemic, Vasodilator.
10.	Diazoprogesterone	Steroid	Anticancer, Antiarthritic, Antiasthma, Diuretic.
11.	Lupeol	Sterol compound	Antimalarial, Anti-inflammatory, Antioxidant, Antitumor, Antiviral, Antihyperglycemic, Antiflu, Antiangiogeni, Cytotoxic, Pesticide.
12.	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	Triterpenoids	Cytotoxic, Immunosuppresent, Inhibition of HIV1 protease.

All the identified compounds were reported to have various bioactivities except one ester compound (pentadecanoic acid 2, 6, 10, 14-tetramethyl-, methyl ester) in which no activity was reported (Table 2). The compounds identified in the ethanol leaf extract of *D. falcata* shows 1-terpine alcohol, 1-diterpine, 2-triterpines, 1-fatty acid ester, 1-linolenic acid, 1-plasticizer, 2-palmitic acid, 1-vitamin, 2-steroids compounds. Most of these compounds reported to have various bioactivities including antimicrobial, anti-inflammatory, antioxidant, antiandrogenic, anticancer, antiacne, antihistaminic, anticronary, antieczemi, antiarthritic, antifouling, antitumor, antiageing, antidiabatic, antidermatic, antilukemic, antiulcerogenic, antispasmodic, antibronchitic, antiasthma, antimalarial, antiviral, antihyperglycemic, antiflu, antiangiogeni, 5-alfa-reductase inhibitor, chemopreventive, cytotoxic, diuretic, hemolytic, hepatoprotective, hypochloresterolemic, immunostimulant, immunosuppresent, inhibition of HIV-1 protease, insectifuge, lipoxigenase-inhibitor, lubricant, nemeticide, pesticide and vasodilator properties. No activity was reported in the fatty acid eater compound (Table 2).

Among the twelve compounds identified, five compounds were reported to have antiinflammatory activity in 3,7,11,15-Tetramethyl 2-hexadecen-1-01, phytol, 9,12,15-Octadeca-trienoic acid methylester (z,z,z)-,Vitamin E, and Lupeol. Antimicrobial activity also were reported in some compound is 3, 7, 11, 15,-Tetramethyl -2-hexadecen-1-01, 1, 2, Benzene dicarboxylic acid, disooctyl ester and squalene. Most of

the compounds were identified, activities like, Antioxidant, Anticancer, Antimalarial, Aantidermatitic, Antifouling, hemopreventive, Pesticide, Antiandrogenic, Antiarthritic activity etc. were also reported in leaf sample of *Dendrophthoe falcata* collected from *A. heterophyllus* host tree.

GC-MS Analysis in the Tender Shoot Sample of *Dendrophthoe falcata*

The phytocompounds and its bio activity of ethanol tender shoot extract of *Dendrophthoe falcata* collected from *A. heterophyllus* host tree were identified by GC-MS analysis (Table 3). The chromatogram, the mass spectrum and structure of phytocomponents identified by GC-MS in the ethanol tender shoot extract of *Dendrophthoe falcata* collected from *A. heterophyllus* host tree were recorded in the figures 1b and 2(13). Only one compound (1,2-Benzenedicarboxylic acid, disooctyleaster) was identified in the ethanol extract of tender shoot sample collected from *A. heterophyllus* host tree. The bioactivity of the plasticizer compound was reported to have antimicrobical and antifouling activities (Table 3).

Table 3: Phytocomponents identified in the ethanol tender shoot of *Dendrophthoe falcata* collected from *Artocarpus heterophyllus* host plant by GC-MS.

1	**Name of the Compound identified**	**1, 2-Benzenedicarboxylic acid, diisooctyl ester**
2	**Formula**	$C_{24}H_{38}O_4$
3	**Molecular MW**	390
4	**Peak area %**	----
5	**RT**	20.11
6	**Nature of compound**	Plasticizer compound
7	**Bioactivity**	Antifouling, Antimicrobial.

*Parameters tested are not covered under the scope of NABL accreditation

GC-MS Analysis in the Bark Sample of *Dendrophthoe falcata*

The figure 1c shows GC-MS chromatogram of the phytocomponents identified in the ethanol bark extract of *Dendrophthoe falcata* infested on *A. heterophyllus* host tree and the figure 2(14) shows the mass spectrum and structure of phytocomponents identified by GC-MS in the ethanol extracts of *Dendrophthoe falcata* bark harvested from the *A. heterophyllus* host tree. The phytocompounds present in the ethanol bark extract of *Dendrophthoe falcata* collected from *A. heterophyllus* host tree were identified by GC-MS analysis (Table 4).

Table 4: Phytocomponents identified in the ethanol extract of *Dendrophthoe falcata* bark sample collected from *Artocarpus heterophyllus* host plant by GC-MS.

1	**Name of the Compound identified**	**1, 2-Benzenedicarboxylic acid, diisooctyl ester**
2	**Formula**	$C_{24}H_{38}O_4$
3	**Molecular MW**	390
4	**Peak area %**	----
5	**RT**	20.12
6	**Nature of compound**	Plasticizer compound
7	**Bioactivity**	Antifouling, Antimicrobial.

*Parameters tested are not covered under the scope of NABL accreditation

The nature of compounds and their bioactivity of the active principles with their retention time (RT), molecular formula and molecular weight (MW) in the ethanol extracts of *Dendrophthoe falcata* bark samples are also presented in the table 4. In this sample, only one compound was identified as 1, 2, Benzenedicarboxylic acid, disooctyleaster. The plasticizer compound identified in the ethanol bark extract of *Dendrophthoe falcata* collected from *A. heterophyllus* host tree, were reported to have antimicrobial and antifouling activity.

There are few reports on GC-MS analysis of *Dendrophthoe falcata* (Syn. *Loranthus longiflorus*) obtained from different host plants indicate the presence of phytocompounds that are varied depends on nature and their host plant. Uppuluri Venkata Mallavadhani *et al*.[9] carried out extensive chromatographic screening of fruit extracts of the Indian Ayurvedic plant, *Dendrophthoe falcata* and the study resulted in the isolation of three new triterpenes along with nine known compounds. GC-MS analysis in the bark ethanol extracts of *Loranthus longiflorus* showed 6 compounds in the bark sample collected from *Casuarina equisetifolia* and 9 compounds in the bark sample collected from *Ficus religiosa*[10]. The leaf sample of *L. longiflorus* collected from *Casuarina equisetifolia* shows 5 compounds, whereas 6 compounds identified in the leaf sample obtained from the *Ficus religiosa* host tree.[11]

Among the phytochemicals identified, n-hexadecanoic acid and squalene have the property of antioxidant; 9, 12, 15-Octadecanoic acid, methyl ester, (ZZZ)-ester, have the property of anti-inflammatory and antiarthritic as reported by the earlier worker.[12] Squalene has

antioxidant activity and recently it has been found that squalene possess chemo-preventive activity against the colon carcinogenesis.[13]

Vitamin-E is thought to be important chain breaking antioxidant, which plays an important role in various stages of carcinogenesis through its contribution and immune-competence, membrane and DNA repair and decreasing oxidative DNA damage.[14] In vitro studies showed that vitamin-E can prevent oxidation of DNA by inhibiting activated neurophils. Vitamin-E can protect the conjugated double bond of β-carotene from oxidation.[15]

Phytol, a bioactive principle, is also found to give effective preventive and therapeutic results against arthritis. The reactive oxygen species-promoting substances like phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases.[16] Phytol was noted to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membrane as a result there is a leakage of potassium ions from bacterial cells. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamin-E and –K1. It is used along with simple or corn syrup as a hardener in candies.[17, 18] Phytol act as effective adjuvants and also increases the titers of all major immunoglobulin-G (IgG) subclass and is also capable of including specific cytotoxic effectors T-cell responses.[19]

Steroid compounds are well known for their anticancer activity by inhibition of cancer cell proliferation, angiogenesis and induction of apoptosis[20] Terpenoids constitute an important class of phytochemical with antioxidant and anticancer activities (activities.[21-23] Certain terpenoids have shown antitumour activity in pre-clinical studies with minimum cytotoxicity on normal cells.[24]

All these compounds, found in the leaf of *Dendrophthoe falcata* collected from *Artocarpus heterophyllus*, which are being used for the pharmacological work. This study helps to identify the compounds present in the leaf, bark and tender shoot samples of *D. falcata* obtained from *A. heterophyllus* host tree, a hitherto uninvestigated species. Further study is necessary to purify the active compounds responsible for therapeutic activity and animal study to evaluate the dosage of the identified chemical compounds.

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