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GC-MS screening of bioactive compounds from extracts of phylloplane fungi of *Markhamia lutea* (Benth.) K. schum

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Abstract
Phylloplane fungi are fungi growing on the surface of the leaf. Leaves forms a nutritive substrate for growth of fungi. Leaf and fungi grow in association with each other either symbiotically or symbiotically. Bioactive compounds are the frontline potent agents in both nutraceutical and pharmaceutical industries. Bioactive compounds gain higher importance in enhancing resistance to various diseases in plants as well as humans. (Dinesh Kumar *et al*., 2018). The present investigation was undertaken to explore the potential bioactive compounds present in the extracts of leaf of *Markhamia lutea* and their phylloplane fungi. Thus, the present study was focused mainly on the screening, analysis and comparison of these phytochemical and biochemical compounds using Gas chromatography higher resolution mass spectrometry after the isolation and identification of phylloplane fungi from leaves of *Markhamia lutea*. *Aspergillus flavus, Aspergillus niger, Alternaria alternata, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium oxysporum, Paecilomyces lilacinus, Penicillium sp., Phoma chrysanthemicola, Phomopsis sp., Rhizopus stolonifer, Trichoderma sp., Stachybotrys sansevieriae* were the phylloplane fungi isolated from the leaf of *Markhamia lutea*. The GC-MS analysis of DCM, Ethyl acetate and Methanol extracts of *Markhamia* leaf, its dominant phylloplane fungal mat and culture filtrates of *Alternaria alternata, Colletotrichum gloeosporioides, Paecilomyces lilacinus and Phoma chrysanthemicola* showed 44, 43 and 29 bioactive compounds, respectively. The major compounds identified were alcohols, alkaloids, carboxylic acids, chlorine containing, esters, ethers, hydrocarbons, iodine containing, oxalic acid, phenols & terpenoids. In a mass spectrum, each compounds were identified based on their retention time and peak area. The results of this study would offer a platform of using extracts of phylloplane fungi of *Markhamia lutea* as herbal alternative for some diseases.

Keywords: *Markhamia*, phylloplane fungi, SEM analysis, CFU count, phytochemical & biochemical compounds and Gas chromatography

1. Introduction
The mycoflora is an important link between biotic and abiotic components of ecosystem. Fungi associated with leaves which may influence the growth of the plants or could be useful for defense are known as "phylloplane" fungi. These belong to two groups including 'residents' and 'casuals'. Plants are rich source of traditional medicine and produce a diverse array of bioactive molecules. *Markhamia lutea* is a useful tropical plant belonging to family Bignoniaceae commonly called as Siala. The tree grows rapidly in good forest soils. It provides useful shade and acts as a windbreak. It is attractive and worth planting as a screen or background tree for gardens and on golf courses. It provides mulch, which enhances soil-moisture retention and increases organic matter. The tree also provides good bee forage. Leaves are known to have medicinal value for tooth-ache (as a gargle) and for convulsion in children. (Tropical. the ferns, 2018) [11]. The plant have been reported to have *antifungal*, antiprotozoal, analgesic, anti-inflammatory, and cytotoxic *activities*. The plant has also been used in the treatment of diarrhea, dysentery, pain, and inflammation in veterinary patients (Ibrahim MB *et al*., 2016) [9]. The therapeutic value of plants used in traditional medicine is due to the presence of phytochemical compounds that are found in parts of the plants. It is moreover, a medicinal plant whose biological activity has been ethnobotanical reported and scientifically established. The primary metabolites are mainly important to the plants, while the secondary metabolites are of medicinal value for humans. (Ibrahim MB *et al*., 2016) [9]. Leaves and phylloplane fungi consist of major biochemical constituents which can be similar or different in leaves and phylloplane fungi. Biochemical analysis using GC-MS were employed to analyse the substances found in the fungi & the chemical reactions underlying their life processes in comparision and support of leaf chemical constituents.

2. Materials and Methods
2.1 Collection
Leaf material without distinct symptoms were collected from the tropical forests of Western Ghats. The uninfected leaf were dried, stored as herbarium and authenticated at Blatter Herbarium, St. Xavier's, Mumbai.

2.2 Leaf impression technique (Ainsworth 1971 & K.R. Aneja, 2003)
Uninfected leaf were subjected to leaf impression technique. The fungi were grown on PDA media. Pure colonies were isolated from mixed culture plates. The colony and morphological characters of the pure cultures was noted. These fungi were identified upto the species level using standard flora and manuals (Gilman, 1957; Burnett and Hunter, 1972; Bhat, 2010). The data of phylloplane fungi isolated from uninfected leaf were stored as digital images, micro slides and master slants. These fungi were authenticated and deposited at Agharkar Research Institute, Pune.

2.3 Colony forming units (CFU) (Ogwu, 2014)

Number of fungi present on the surface of the leaf was calculated as colony forming units per gram dry leaf taking into account leaf area index and the seasonal occurrence of the fungi of total mycoflora was determined by counting the number of colonies developing on the plates. The relative abundance of each phylloplane fungi was calculated using the formula;

Relative abundance or CFU %

$$= \frac{\text{Total number of CFU of the individual fungi}}{\text{Total number of CFU in the plate}} \text{ x } 100$$

2.4 Frequency (John C. Zak, 2004)

Frequency of individual microbial species was calculated in percentage as follows;

Microbial frequency (%)

$$\frac{\text{number of colony of the species appeared}}{\text{Total number of all colony isolated from each sample}} \times 100$$

Total number of all colony isolated from each sample
The frequency of occurrence of each isolates was noted.

2.5 SEM analysis (Olive. H, 2002)

Scanning electron microscopy was performed for confirmation of direct observation of the leaf surface fungi on dried uninfected leaves at SAIF-IIT, POWAI. The dried small leaf segments (2 x 10 mm) were mounted dorsal and ventral side up on aluminum stub mounts using 12-mm carbon adhesive tabs coated with carbon-conducting glue and sputter coated with 6 nm of platinum using a Hummer 6.2 sputtering system. Images were obtained in high-vacuum mode with accelerating voltages at or around 2.0 kV as increasing mode of powers.

2.6 Data collection

The data of the phylloplane fungi isolated from uninfected leaf of *Markhamia* were stored as digital images, micro slides and master slants. The dominant phylloplane fungi were authenticated and deposited at Agharkar Research Institute, Pune. These cultures were studied further for their biochemical composition using GC- HRMS analysis.

2.7 Preparation of leaf extract

The leaves were washed with water, shade dried and ground to powder using an electronic blender, sieved and the fine powder was stored in air tight container for further study. 100 gram of powder was subjected to methanolic extraction by hot percolation method through Soxhlet apparatus. Thereafter, the extract was filtered through whatman filter paper no. 1. This leaf extract was concentrated using rotary evaporator with hot water bath at 40°C and dried. The concentrated extracts were used for further analysis.

2.8 Fungal cultivation

The fungi were cultivated on 800ml PD broth and incubated for 21 days at room temperature in 1000ml conical flask. 1% streptomycin was added to the broth medium to prevent bacterial contamination.

2.9 Extraction of fungal cultures

The cultures were filtered through 3 layered muslin cloth and were extracted in DCM & Ethyl acetate. These extracts were then evaporated to dryness under vacuum in rotary evaporator. The dried organic extract were reconstituted with 10ml of the same solvents. The culture mats were weighed before and after drying. These mats were then extracted in methanol and dried in rotary evaporator.

2.10 GC-HRMS analysis

A gas-chromatograph coupled with mass spectrometer (GC-HRMS) was used as a combined analyzer that has a superior ability in analyzing organic compounds qualitatively and quantitatively. Gas Chromatograph with high resolution Mass Spectrometry of model Agilent 7899 Jeol, AccuTOF GCV of source EI / CI with Time of Flight Analyser runned at Mass range 10 - 2000 amu at mass resolution - 6000 was used. Head Space injector were used to inject solvent samples of DCM, ethyl acetate and methanol. The graphs were detected by FID detector. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology MS database. The percentage of each component was calculated from relative peak area of each component in the chromatogram.

2.11 Identification of compounds

Interpretation of mass spectrum of 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 component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test material is mentioned in this paper.

3. Results

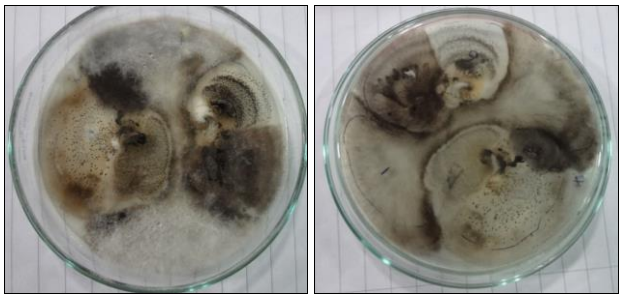
3A) Figures

i) Plant description

Markhamia lutea belongs to family- Bignoniaceae. It is commonly called as Nile Tulip or Siala tree. It is a subtropical small tree or shrub of 4-5 m height has lush tropical looking foliage and striking yellow trumpet flowers, often with red veining or spots, in panicles. Leaves are hairy, rough, large, growing over 1 ft long. (Flora of Maharashtra).

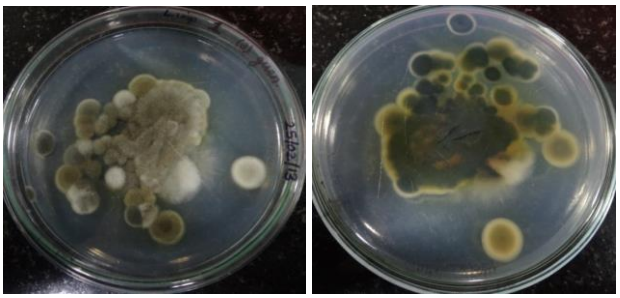
Fig a: *Markhamia lutea* (Benth.) K.Schum (=Blatter Herbarium specimen No. SU-1)

ii) Leaf Impressed Fungal Colonies of *Markhamia* leaf



b **c**

Fig b & c: surface and reverse view of leaf impressed fungal colonies in rainy season;



d **e**

Fig d & e: surface and reverse view of leaf impressed fungal colonies in winter season

f **g**

Fig f & g: surface and reverse view of leaf impressed fungal colonies in summer season.

iii) SEM images of dried leaf sample of *Markhamia* showing phylloplane mycelial forms and spores

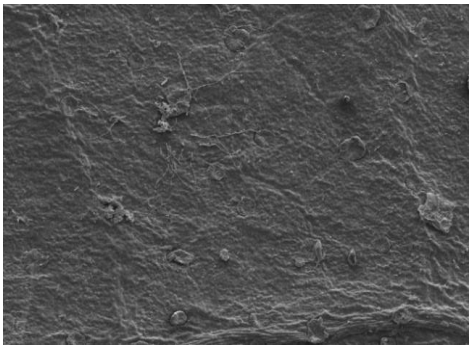


Fig h: magnification X 100, bar indicate100um,wd 8.4mm;

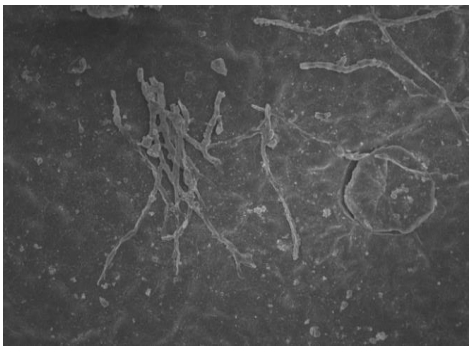


Fig i: magnification X 500, bar indicate10um, wd 8.3mm;

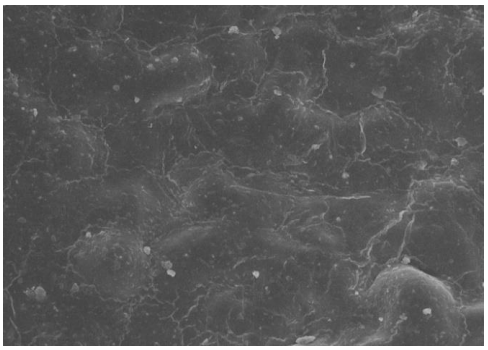


Fig j: magnification X 2,000, bar indicate10um,wd 8.3mm;

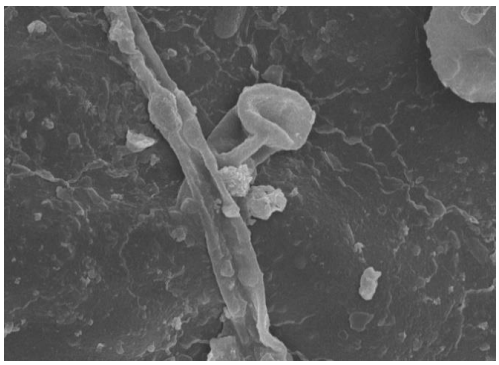


Fig k: magnificationX 5,000, bar indicate1um,wd 8.3mm.

3B) Tables

Table 1: Biodiversity of phylloplane fungi on *Markhamia* leaf

Name of the fungus	Types of leaves	Types of surface	Seasonal occurrence (+, -) on leaf surface		
			June-Sept	Oct-Jan	Feb-May
Aspergillus flavus	Tender	Dorsal	-	+	+
		Ventral	-	-	+
	Mature	Dorsal	-	-	-
		Ventral	-	+	+
Aspergillus niger	Tender	Dorsal	+	+	-
		Ventral	+	+	-
	Mature	Dorsal	-	-	-
		Ventral	-	-	-
Alternaria alternata	Tender	Dorsal	+	+	+
		Ventral	+	+	+
	Mature	Dorsal	+	+	+
		Ventral	+	+	+
Colletotrichum gloeosporioides gr.	Tender	Dorsal	+	+	+
		Ventral	+	+	+
	Mature	Dorsal	+	+	+
		Ventral	+	+	+
Curvularia lunata	Tender	Dorsal	+	+	+
		Ventral	-	-	-
	Mature	Dorsal	-	-	+
		Ventral	-	+	+
Fusarium sp.	Tender	Dorsal	-	-	+
		Ventral	-	-	-
	Mature	Dorsal	+	+	+
		Ventral	-	+	+
Mucor sp.	Tender	Dorsal	-	-	-
		Ventral	-	-	-
	Mature	Dorsal	-	-	-
		Ventral	-	-	-
Monilia sp.	Tender	Dorsal	-	-	-
		Ventral	-	-	-
	Mature	Dorsal	-	-	-
		Ventral	-	-	-
Paecilomyces lilacinus	Tender	Dorsal	+	+	+
		Ventral	+	+	+
	Mature	Dorsal	+	+	+
		Ventral	+	+	+
Penicillium sp.	Tender	Dorsal	-	-	-
		Ventral	-	+	+
	Mature	Dorsal	+	+	+
		Ventral	-	-	-
Phoma chrysanthemicola	Tender	Dorsal	+	+	+
		Ventral	+	+	+
	Mature	Dorsal	+	+	+
		Ventral	+	+	+
Phomopsis sp.	Tender	Dorsal	+	+	+
		Ventral	-	-	-
	Mature	Dorsal	-	-	+
		Ventral	-	-	-
Rhizopus stolonifer	Tender	Dorsal	+	+	+
		Ventral	-	+	-
	Mature	Dorsal	+	-	-
		Ventral	-	+	+
Torula sp.	Tender	Dorsal	-	-	-
		Ventral	-	-	-
	Mature	Dorsal	-	-	-
		Ventral	-	-	-
Trichoderma sp.	Tender	Dorsal	+	+	+
		Ventral	-	-	+
	Mature	Dorsal	-	-	-
		Ventral	-	-	-
Stachybotrys sansevieriae	Tender	Dorsal	-	-	+
		Ventral	-	-	+
	Mature	Dorsal	-	-	-
		Ventral	-	-	-

Table 2: CFU % and Frequency of fungi isolated from leaf surface of *Markhamia*

Seasons →	June-Sept				Oct-Jan				Feb-May			
	Total CFU count=43		Total plant sample units = 4		Total CFU count=62		Total plant sample units = 4		Total CFU count=148		Total plant sample units = 4	
Isolated Fungi ↓	CFU count	% of CFU	NOSU	% frequency	CFU count	% of CFU	NOSU	% frequency	CFU count	% of CFU	NOSU	% frequency
Aspergillus flavus	0	0	0	0	2	3.57	2	50	4	3.14	3	75
Aspergillus niger	1	4.34	2	50	3	5.35	2	50	2	1.57	0	0
Alternaria alternata	4	17.39	4	100	9	16.07	4	100	15	11.81	4	100
Chaetomium sp.	0	0	0	0	2	3.57	1	25	3	2.36	1	25
Colletotrichum gloeosporioides gr.	5	21.73	4	100	7	12.50	4	100	17	13.38	4	100
Curvularia lunata	2	8.69	1	25	3	5.35	2	50	5	3.93	3	75
Fusarium sp.	1	4.34	1	25	2	3.57	2	50	4	3.14	3	75
Paecilomyces lilacinus	1	4.34	4	100	11	19.64	4	100	44	34.64	4	100
Penicillium sp.	1	4.34	1	25	1	1.78	2	50	2	1.57	2	50
Phoma chrysanthemicola	3	13.04	4	100	10	17.85	4	100	21	16.53	4	100
Phomopsis sp.	1	4.34	1	25	2	3.57	1	25	2	1.57	2	50
Rhizopus stolonifer	3	13.04	2	50	3	5.35	3	75	4	3.14	2	50
Trichoderma sp.	1	4.34	1	25	1	1.78	1	25	2	1.57	2	50
Stachybotrys sansevieriae	0	0	0	0	0	0	0	0	2	1.57	2	50

Where; NOSU = Number of occurrence of fungi on sample units.

GC - HRMS

The 0.5% of DCM, Ethyl Acetate & Methanol extracts of *Markhamia leaf*, phylloplane fungal mat and culture filtrates revealed the presence phytochemical and biochemical compounds as the active principles with their peak, retention time (RT) and molecular weight mentioned in the Table 3, 4 & 5. A comparative analysis of chemical components of the extracts of leaf, fungal mats & culture filtrates of *Markhamia* were noted as phylloplane association between leaf and the phylloplane fungi.

Table 3: GC-MS analysis showing presence of bioactive compounds of the DCM extracts of leaf & dominant fungi

Peak name	DCM extract					RT	Mol wt.
	Ml	*Aa*	*Cg*	*Pl*	*Pc*		
Aerofroth 88	-	+	-	+	-	9.35	130
2,4,6- Cycloheptatriene-1-one	-	-	-	+	-	9.57	106
1-Hexanol-2-ethyl	-	+	-	+	-	9.95	130
Decane	-	+	-	-	-	10.15	142
1-Iodo-2- methylnonane	-	+	-	-	-	13.65	268
3- Tetradecane	-	+	+	+	-	13.96	196
Dimethyl Phthalate	-	-	-	+	-	14.40	194
1-Iodo-2- methylundecane	-	-	+	-	-	15.64	296
Diphenylamine	-	-	-	+	-	15.66	169
Tetradecane	-	+	-	+	-	17.38	256
Oxalic acid; allyl nonyl ester	-	+	-	-	-	17.38	256
2- Hexadecanol	-	+	-	-	-	17.74	242
Tert-Hexadecanethiol	-	-	+	+	-	18.31	258
Octadecane-6-methyl	-	-	+	+	+	18.46	268
Hexadecane	-	+	+	+	-	18.52	296
Heptadecane -2,6,10,14-tetramethyl	-	+	-	-	-	18.52	296
10,18- Bisnorabiela-8,11,13-triene	-	-	+	-	-	18.57	242
1-Hexadecanol,2-methyl	-	-	+	-	-	18.90	256
Nonadecane	+	-	+	+	-	19.10	268
Hexadecenoic acid	-	+	+	+	+	19.68	256
Palmitic acid, vinyl ester	-	+	-	-	-	19.68	282
Hexadecanoic acid,2-oxo,methyl ester	-	+	-	-	-	20.14	284
Octadecane-1-etheny(oxy)	-	-	+	-	-	20.75	296
11-Octadecenoic acid, methyl ester	-	-	-	+	-	20.85	296
17- Octadecenoic acid	-	-	-	+	-	21.65	280
Octadecenal-2-bromo	+	-	-	-	-	22.31	346
1-Chloroeicosane	+	-	-	+	+	22.34	316
Heptadecane,9-hexyl	-	-	-	+	-	22.43	324
1,3- Dioxocane, 2- pentadecyl	-	-	-	-	+	23.90	326
Hexadecanoic acid,(3-bromoprop-2-ynyl) ester	-	+	-	-	-	23.91	372
Octadecane, 3-ethyl-5-(2-ethylbutyl)	-	-	-	+	-	24.13	366
Oxalic acid, allyl hexadecyl ester	-	+	-	-	-	24.33	354
3α,5α - Cycloergosta	+	-	-	-	-	25.65	394
E-(13)- Docosenoic acid	+	-	-	-	-	25.72	338
9- Octadecenoic acid (z), hexyl ester	-	-	-	-	+	25.82	366
Phthalic acid, butyl undecyl ester	-	+	-	-	-	27.32	376
Cholesta	+	-	-	-	-	27.57	424
9-Octadecenoic acid (z), phenylmethyl ester	-	+	-	-	-	27.58	372

Phthalic acid, bis(7-methyloctyl)ester	-	+	-	-	-	29.02	418
Cycloartenol acetate	+	-	-	-	-	29.56	468
Pivalate	+	-	-	-	-	29.73	-
Betulin	+	-	-	-	-	30.90	442
17- Pentatriacontene	+	+	-	+	+	35.38	490
Retinol-β-glucuronide-6,3-lactone	+	-	-	-	-	35.55	458

Table 4: GC-MS analysis showing presence of bioactive compounds of the ethyl acetate extracts of leaf & dominant fungi

Peak name	Ethyl acetate					RT	Mol wt.
	Ml	*Aa*	*Cg*	*Pl*	*Pc*		
Oleic acid	+	-	-	-	-	_	282
1-Hepten-4-ol	+	-	-	-	-	8.69	114
1-Octene	-	-	-	-	+	8.77	140
1,2,3- Propanetriol,monoacetate	+	-	-	-	-	10.91	134
1-Octanal,2,7-dimethyl	-	-	-	+	-	11.30	158
1-Dodecyne	+	-	-	-	-	12.12	166
Decyl trifluoroacetate	-	-	-	-	+	12.16	254
2,4,6,8- Tetramethyl-1- undecane	+	-	-	-	+	12.87	210
Tridecene	+	-	+	-	-	13.13	184
1-Iodo-2- methylnonane	+	-	+	-	-	13.65	268
3- Tetradecane	+	-	-	-	-	13.96	196
Phenol-2,4-bis(1,1-dimethylethyl)	+	-	-	-	-	15.55	206
1-Iodo-2- methylundecane	+	-	+	+	-	15.64	296
Octyloxy	-	-	-	+	-	15.65	206
3- Hexadecene,(Z)	-	-	-	+	-	16.20	224
2- Piperidinone,N-(4-bromo-n-butyl)	-	-	+	+	+	16.35	233
1-Bromo-3(-2-bromoethyl)-nonane	-	-	-	-	+	16.43	312
3-Trifluoroacetoxypentadecane	-	-	-	-	+	16.48	324
Oxalic acid, allyl nonyl ester	+	-	-	+	+	17.38	256
Tert-Hexadecanethiol	+	-	+	+	-	18.31	258
Octadecane,6-methyl	-	-	+	+	-	18.64	269
Dodecanoic acid-2-penten 1-yl-ester	+	-	-	-	-	18.86	268
1- Hexadecanol,2-methyl	-	-	-	+	-	18.90	256
Hexadecenoic acid,2-oxo-methyl ester	-	-	+	+	-	20.14	284
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	+	-	-	-	-	20.45	296
Phytol	+	-	-	-	-	20.45	296
Stearyl vinyl ethrt	-	-	-	-	+	20.75	296
Oxalic acid; allyl tridecyl ester	+	+	-	-	-	21.35	312
n-Nonadecanol	+	+	-	-	-	21.53	284
Octadecenal,2-bromo	-	-	-	+	-	22.31	346
1-Chloroeicosane	-	-	+	+	-	22.34	316
Oxalic acid, allyl pentadecyl	+	-	-	-	+	23.34	340
Oxalic acid; allyl pentadecyl ester	-	-	-	+	-	23.34	340
1,3-Dioxocane,2- pentadecyl	-	-	+	-	-	23.90	326
Oxalic acid, allyl hexadecyl ester	+	+	-	+	-	24.33	354
Heneicosane,11-(1-ethylpropyl)	-	-	-	+	-	24.77	366
9- Octadecenoic acid (z), hexyl ester	+	-	-	+	-	25.82	365
Palatinol	-	-	+	+	-	27.04	390
9- Octadecenoic acid, phenyl methyl ester	-	-	-	+	+	27.58	372
Dodecanoic acid,1(hydroxymethyl)-1,2-ethanedyl ester	+	+	-	-	-	32.18	456
Laurin	+	+	-	-	-	43.36	638
Trimyristin	+	+	-	-	-	49.32	722
Octadecenoic acid,23-bis(1-oxotetradecyl)oxylpropyl ester	+	+	-	-	-	53.30	778
Digitoxin	-	-	+	-	-	57.75	764

Table 5: GC-MS analysis showing presence of bioactive compounds of the Methanol extracts of leaf & dominant fungi

Peak name	Methanol extract					RT	Mol wt.
	Ml	*Aa*	*Cg*	*Pl*	*Pc*		
Vitamin E	-	-	+	-	-	-	430
4- Methyl-2- Hexanol	-	-	-	-	+	8.15	116
(2S,3S)-3- propyloxiranemethanol	-	-	+	+	-	9.12	116
Epoxyhexanol	-	-	-	-	+	9.12	116
Hydroperoxide, 1-methylhexyl	+	-	-	-	-	10.13	132
2- Heptanol	+	-	-	-	-	10.14	144
1,6-Anhydro-2,4-dideoxy-β-D-arabo-hexopyranose	+	-	-	-	-	10.28	130
Cyclohexanol,4-(1-methylethyl)	+	-	-	-	-	11.03	142
Benzene, 1-methyl-2-propyl	+	-	-	-	-	11.06	134
3- Decyn-2-ol	-	-	-	-	+	11.95	154
Geranyl vinyl ether	+	-	-	-	-	12.50	180
Pyrogallol	+	-	-	-	-	12.79	154

2-Methoxy-4 vinylphenol	+	-	-	-	-	12.93	150
10-Undecen-1-al,2-methyl-	+	-	-	-	-	13.28	182
Methoxyeugenol	+	-	-	-	-	15.81	196
1,2- 15,16- Diepoxyhexadecane	-	-	+	-	-	17.92	254
Hexadecenoic acid, methyl ester	+	-	-	-	-	18.78	270
3,7,11,75- Tetramethyl-2-hexadecen-1-ol	+	-	-	-	-	20.45	270
Phytol	+	-	-	-	-	20.45	296
Linolenic acid	+	-	-	-	-	21.01	292
17- Octadecenoic acid	-	+	-	-	-	21.65	280
1-Gala-1-iodo-octose	+	-	-	-	-	22.21	240
Oxalic acid; allyl pentadecyl ester	-	-	+	-	-	23.34	340
Hexadecanoic acid, 2,3- dihydroxypropyl ester	+	-	-	-	-	24.82	330
9- Octadecenoic acid	-	-	-	+	-	25.82	365
Tetrahydroactinidiolide	+	-	-	-	-	26.58	316
9-Octadecenoic acid (Z), phenylmethyl ester	-	-	+	+	-	27.58	372
9, 12, 15 - Octadecetrienoic acid	+	-	+	-	-	29.68	436
Lactose	+	-	-	-	-	31.31	342

Where Ml = Markhamia leaf, *Aa = Alternaria alternata, Cg= Colletotrichum gleosporioides, Pl = Paecilomyces lilacinus & Pc = Phoma chrysanthemicola,* RT = Retension Time & Mol.Wt = Molecular Weight.

3C) Chromatograms

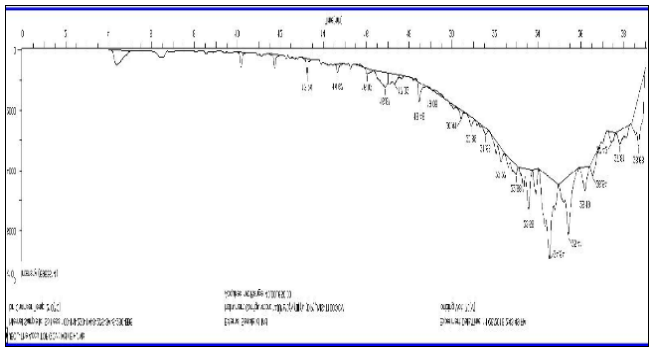


Fig l: GC-MS chromatogram of DCM extracts of Markhamia leaf

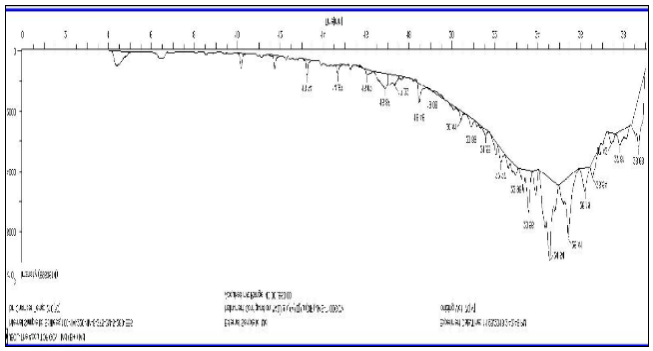


Fig m: GC-MS chromatogram of DCM extracts of *Alternaria alternata*

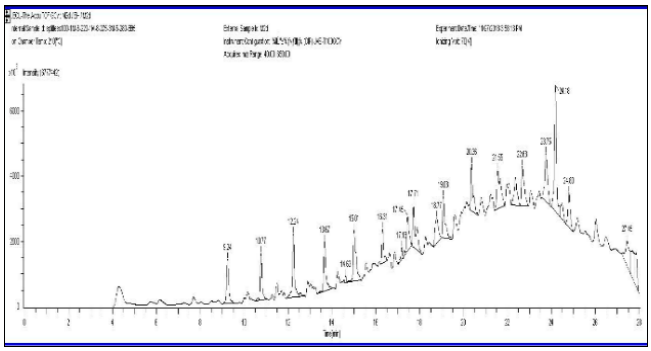


Fig n: GC-MS chromatogram of DCM extracts of *Colletotrichum gleosporioides*

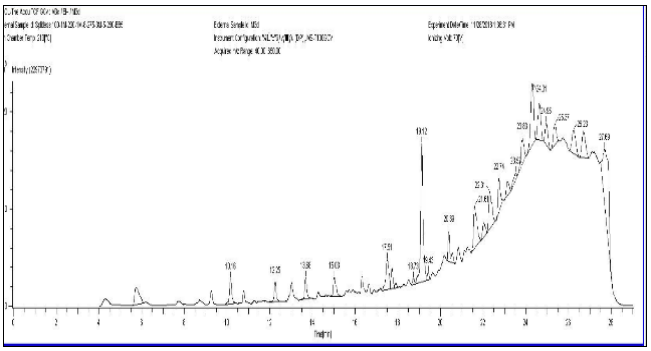


Fig o: GC-MS chromatogram of DCM extracts of *Paecilomyces lilacinus*

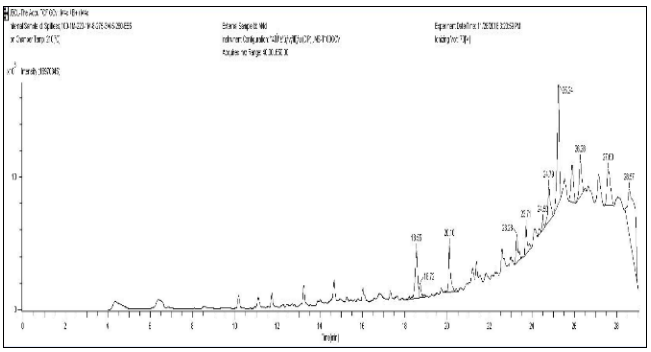


Fig p: GC-MS chromatogram of DCM extracts of *Phoma chrysanthemicola*

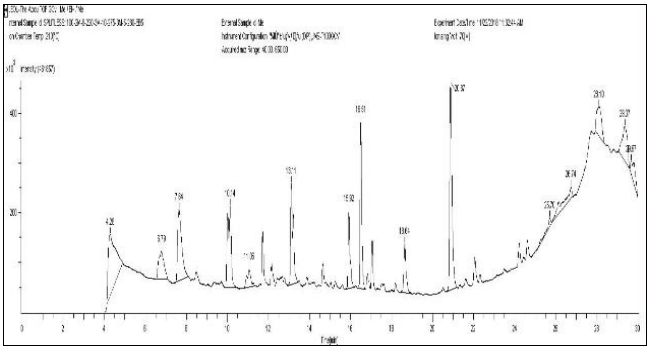


Fig q: GC-MS chromatogram of Ethyl acetate extracts of Markhamia leaf

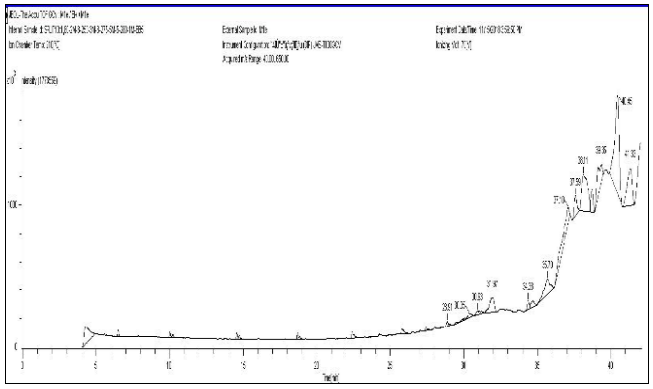


Fig r: GC-MS chromatogram of Ethyl acetate extracts of *Alternaria alternata*

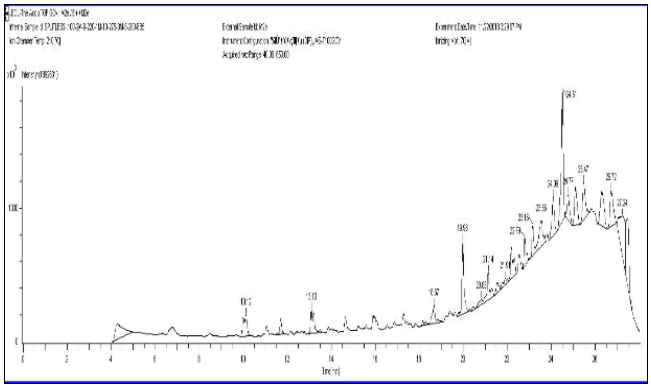


Fig s: GC-MS chromatogram of Ethyl acetate extracts of *Colletotrichum gleosporioides*

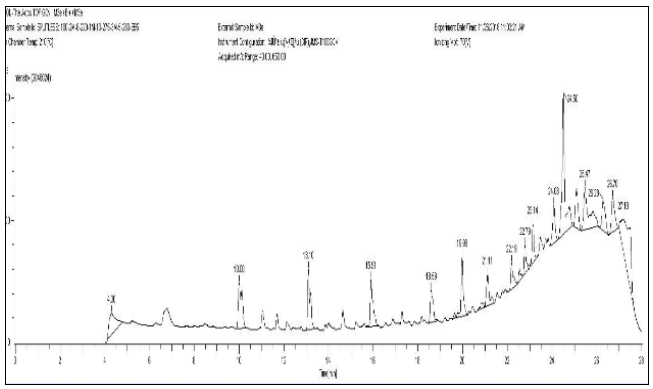


Fig t: GC-MS chromatogram of Ethyl acetate extracts of *Paecilomyces lilacinus*

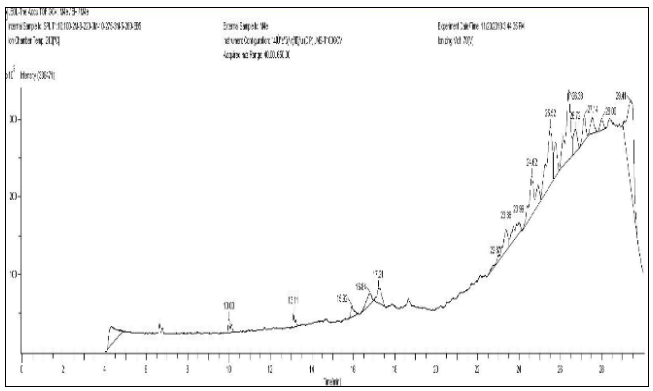


Fig u: GC-MS chromatogram of Ethyl acetate extracts of *Phoma chrysanthemicola*

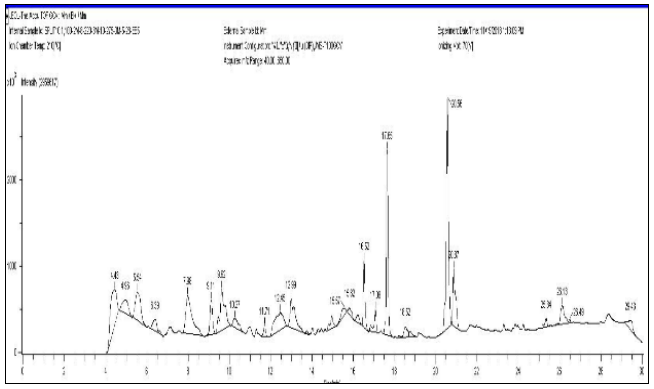


Fig v: GC-MS chromatogram of Methanol extracts of Markhamia leaf

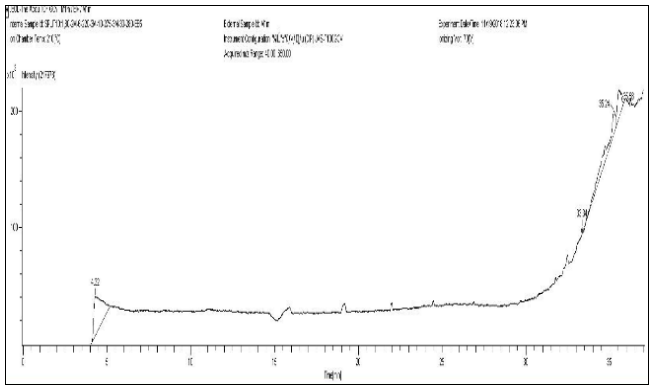


Fig w: GC-MS chromatogram of Methanol extracts of *Alternaria alternata*

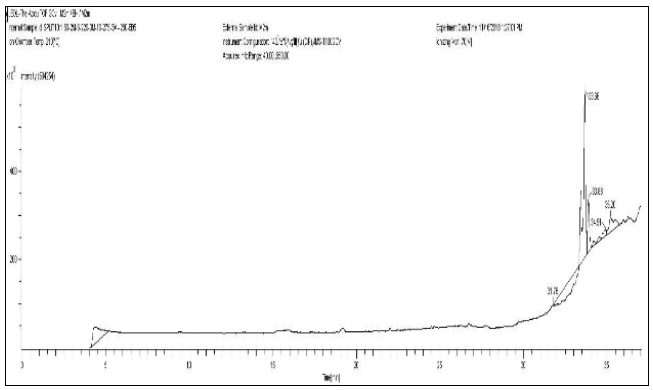


Fig x: GC-MS chromatogram of Methanol extracts of *Colletotrichum gleosporioides*

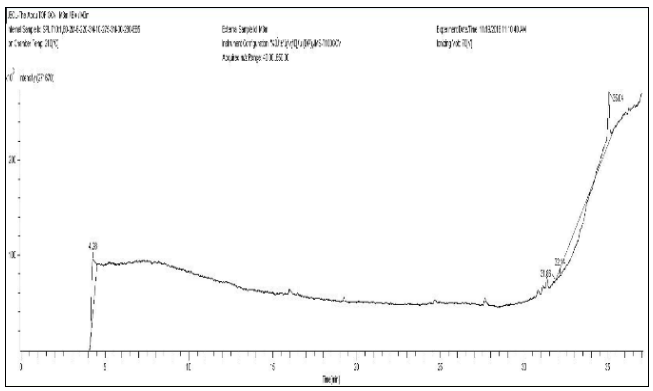


Fig y: GC-MS chromatogram of Methanol extracts of *Paecilomyces lilacinus*

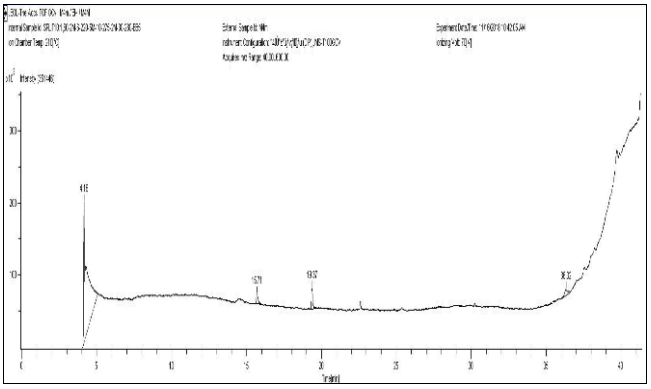


Fig z: GC-MS chromatogram of Methanol extracts of *Phoma chrysanthemicola*

4) Discussion

Phylloplane fungi on *Markhamia lutea*

Aspergillus flavus, Aspergillus niger, Alternaria alternata, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium oxysporum, Paecilomyces lilacinus, Penicillium sp., Phoma chrysanthemicola, Phomopsis sp., Rhizopus stolonifer, Trichoderma sp., Stachybotrys sansevieriae were the phylloplane fungi isolated from the leaf of *Markhamia lutea*. Out of these fungi, *Alternaria alternata, Colletotrichum gloeosporioides, Paecilomyces lilacinus and Phoma chrysanthemicola* were found to be dominant in all the season. The cfu count and the frequency of these dominant phylloplane were found to be more than other phylloplane fungi. SEM analysis detected the presence of mycelial forms and spores on the surface of the *Markhamia* leaf.

GC - HRMS

GC-MS was used for identifying different metabolites present in the leaf and fungal extract. The leaf extract of *Markhamia* and the four dominant phylloplane isolates of *Alternaria alternata, Colletotrichum gloeosporioides, Paecilomyces lilacinus and Phoma chrysanthemicola* analysed under GC – MS showed the presence of 44, 44 and 29 biochemical compounds in all the three extracts of DCM, Ethyl acetate and Methanol respectively. Nonadecane was the similar biochemical found in the DCM extracts of leaf as well as *Colletotrichum gleosporioides* & *Paecilomyces lilacinus*, 1-Chloroeicosane was similar to DCM extracts of leaf, *Paecilomyces lilacinus & Phoma chrysanthemicola*, and 17- Pentatriacontene was also found in leaf and DCM extracts of *Alternaria alternata, Paecilomyces lilacinus & Phoma chrysanthemicola.* In ethyl extracts; 2, 4, 6, 8-Tetramethyl-1- undecane was the similar biochemical in leaf as well as *Phoma chrysanthemicola*. Tridecene & 1-Iodo-2-methylnonane were common in ethyl acetate extracts of leaf and *Colletotrichum gleosporioides.* 1-Iodo-2-methylundecane, Tert-Hexadecanethiol & Octadecane, 6-methyl was common in ethyl acetate extracts of leaf and *Colletotrichum gleosporioides* & *Paecilomyces lilacinus;* Dodecanoic, Laurin, Trimyristin, Octadecenoic acid, 23-bis(1-oxotetradecyl)oxylpropyl ester were the biochemicals similar in ethyl acetate extracts of leaf and *Alternaria alternata.* 9, 12, 15 - Octadecetrienoic acid was the only biochemical compound screened similar in Methanol extracts of leaf and *Colletotrichum gleosporioides.* All other metabolites present in Methanol extract of all four dominant fungi were independent of the leaf metabolites. However, the similar phytochemicals of the leaf extract and biochemicals of the fungal extracts shows some association of the fungi living on the surface of the leaf. The biochemicals of fungi showing similar to the phytochemicals of the leaf and the one having their own identification other than the phytochemicals of the leaf can discover the novel drugs by their further isolation and characterization.

5. Acknowledgement

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6. References

1. Noad T, Birnie A. Trees of Kenya. General Printers, Nairobi, 1989.
2. ICRAF. A selection of useful trees and shrubs for Kenya: Notes on their identification, propagation and management for use by farming and pastoral communities, 1992.
3. ICRAF. Katende AB *et al.*, Useful trees and shrubs for Uganda. Identification, Propagation and Management for Agricultural and Pastoral Communities. Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA), 1995.
4. Hong TD, Linington S, Ellis RH. Seed storage behaviour: a compendium. Handbooks for Genebanks: No. 4. IPGRI, 1996.
5. Birnie A. What tree is that? A beginner's guide to 40 trees in Kenya. Jacaranda designs Ltd, 1997.
6. Khan MR. Cytotoxicity assay of some Bignoniaceae. Fitoterapia. 1998; 69:538-40.
7. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestree Database:a tree reference and selection guide version 4.0
8. Shabana MH, Hashem FA, Singab A, Khaled S, Farrag A. Protective and therapeutic activities of *Mayodendron ignem* Kurz against paracetamol induced liver toxicity in rats and its bioactive constituents. J Applied Pharma Sci. 2013; ;3:147-55.
9. Ibrahim MB, Kaushik N, Sowemimo AA, Odukoya OA. Review of the Phytochemical and Pharmacological Studies of the Genus Markhamia. *Pharmacogn Rev*. 2016; 10(19):50-9.
10. Dinesh Kumar G, Karthik M, Rajakumar R. GC-MS analysis of bioactive compounds from ethanolic leaves extract of *Eichhornia crassipes* (Mart) Solms. and their pharmacological activities. 2018; 7(8):459-462.
11. Tropical Plants Database, Ken Fern. tropical.theferns.info, 2018, 12-21.