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Phytochemical Screening and GC-MS Analysis of Methanolic leaf Extract of an Endemic Plant *Kayea assamica.*

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Abstract*:Kayea assamica is an endemic plant species of Clusiaceae family found only in Padumoni Park and Dullung Reserved Forest of Lakhimpur district of Assam, India. It has significant ethnopharmacological evidence, but still now has not been scientifically validated. In this present study, the phytocompounds from K. assamica leaves were extracted using methanol as polar solvent and subjected to qualitative phytochemical screening followed by Gas chromatography-Mass spectrometry (GC-MS) analysis. The result revealed the presence of important phytochemicals including alkaloids, phenols, tannins, flavonoids, terpenoids, coumarins and cardiac glycosides which comprises thirty different compounds. These identified compounds reported to have important bio-activities like antibacterial, antifungal, anti-inflammatory, anticancer, antioxidant, antimutagenic etc. Many of the identified compounds are recognized as major constituent of the essential oil producing plants, which are Copaene, Beta-Caryophyllene, δ-Cadinene etc. More interestingly some compounds like δ-Cadinene, Lanceol, Methyl palmitate, Linolenic acid have unique therapeutic properties. Further these identified compounds of the plant extract possess as baseline in determining the possible health benefits in future. Therefore, these identified bioactive compounds explores the goodness of the endemic plant species and advised for its conservation.*

Keywords*:Kayea assamica, endemic, GC-MS, phytochemical screening, bioactive compounds, medicinal plant.*

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I. Introduction

Various plants have been used for many years in daily life to treat diseases in all over the globe. Plants produce wide range of bioactive compounds, which makes them a rich source of different types of medicines. According to the World Health Organization (WHO) in 2008, more than 80 % of the world's population relies on traditional medicine for their primary healthcare needs [1]. From over 3, 00,000 species of higher plants occur in nature, only about 2 percent have been screened. At least 12,000 compounds have been isolated so far; a number estimated to be less than 10% of the total [10]. Natural products provide crucial, unmatched chemical diversity to modern drug discovery programs. Natural products play significant role in drug development programs in the pharmaceutical [2]. The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and traditional medicine [3]. Therefore, search for newer drugs (with minimum side effects) obtained from traditional medicine continues [4].

The genus *Kayea* belongs to the subfamily Calophylloidea of the family Clusiaceae [5] [6]. It consist of three species namely *K. assamica*, *K. floribunda* and *K. manii*, occurring in India [5]. *Kayea assamica* is a slow growing tall good looking ever-green tree of 20-25 m height that is found only in Padumoni Park and Dullung Reserved Forest of Lakhimpur district of Assam (27°24'39.6"N 94°11'32.0"E), India [5]. It is endemic in Lakhimpur district of Assam, India. The fruits of *K. assamica* are used as fish poisoning in that particular region by the local people [5][6]. The stem bark also used in treatment of malaria by the ethnic people. Locally it is known as "Sia-nahor" and *K. assamica*is a synonym of *Mesua assamica* (King& Prain) [11]. Literature survey shows that only a few works have been done on this particular plant species due its unavailability in all habitat. In 2008, Nwet N. Win and his coworkers were isolated two novel anticancer compounds kayeassamins A and B from the flower of *K. assamica* of Myanmar [7]. Manabjyoti Bordoloiand his coworkers isolated one new and two known alkylated coumarin derivatives whose structures were determined through extensive use of NMR spectroscopy and chemical transformation [5]. The aim of the present work was to identify the phytocompounds present in the methanolic extract of the leaves of *Kayea assamica* by qualitative photochemical screening and Gas chromatography-Mass spectrometry (GC-MS) analysis.

(a) (b)

Fig. 1. (a) The plant *K. assamica*and(b) Leaf of *K. assamica*.

II. Materials and methods

2.1 Plant materials

The leaves were collected from Podumoni, Lakhimpur district of Assam, India during the month of April 2017. Identification of plant materials was done by Dr. Chitta Ranjan Bora,assistant professor (Taxonomy) of department of botany, North Lakhimpur College (autonomous) Assam, India. The leaves were cut into small pieces and were dried in a room having temperature 20-30^{0}C for one week, then it were powdered in a mechanical grinder and stored in well closed container until use.

2.2 Extraction

The leaf powder was suspended in 95% methanol solution in a ratio of 1:5 w/v and kept in magnetic stirrer for 24 hours. After 24 hours the suspension was filtered by using sterile muslin cloth and the filtrate was centrifuged at 2000 rpm for 20 mins. The supernatants were dried in a digital water bath till a dark orange residue was obtained. The percentage yield of the extract was calculated using the following formula –

Percentage yield = Final weight of the dried extract / Initial weight of the powder *100

The percentage yield was 8% W/W. The extract was collected and stored at -20^{0}C until experimentation.

2.3 Phytochemical screening

Thephytochemicals of methanol extract of the leaves of *Kayea assamica* were qualitatively analyzed in detail as per the standard protocols [8][9][2].

2.3.1 Test for phenols

0.02 g of the extract, .08 ml of 1M ferric chloride was added. A reddish brown colouration at the interface indicates the presence of phenols.

2.3.2 Test for alkaloids

0.02g of extract was dissolved in 2ml of methanol. Few drops of 1% HCl added to it. Then the mixture was heated, kept in steam and filtered after cooling. The mixture was divided into two portions. 0.2ml of mixture was treated with few drops of Wagner's reagent. The sample was observed for turbidity or precipitation. 0.8 ml of the filtrateand 0.28 ml of diluted ammonia were mixed followed by addition of 0.7ml of chloroform and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 1.4ml of acetic acid. Then the mixture was treated with few drops of Mayer's reagent. The formation of a cream was regarded as positive for the presence of alkaloids.

2.3.3 Test for tannins

About 0.02 g of the extract was boiled in 1 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.3.4 Test for flavonoids

0.02g of extract was dissolved in 1ml of distilled water mixed by vortex followed by centrifugation. The supernatant was collected and treated with 0.5ml diluted ammonia solution followed by addition of 1ml concentrated sulfuric acid. A yellow colour indicated the presence of flavonoids which get disappeared on allowing the solution to stand.

2.3.5 Test for terpenoids (Salkowski test)
0.02g of extract was dissolved in 1ml of chloroform and 1ml of concentrated sulfuric acid was added to it. A reddish brown discolouration at the interface showed the presence of terpenoids.

2.3.6 Test for Coumarins
0.02g of extract was dissolved in 2ml NaOH (10%). A yellow colour indicated the presence of Coumarins.

2.3.7 Test for Cardiac Glycosides
0.02 of extract was dissolved in 1ml of glacial acetic acid and 1-2 drops of ferric chloride solution was added. 0.5ml of concentrated sulfuric acid was slowly added. A brown ring at the interface indicated a deoxy sugar characteristic of cardenolides**.**

2.4 GC-MS analysis

The phytochemicals of *Kayea assamica* leaf was analyzed by GC-MS. It was carried out at Central Analytical Instrumentation Facility, Guwahati Biotech Park Incubation Center, Guwahati,Assam, India. The analysis was carried out on Perkin Elmer Turbo Mass Spectrophotometer (USA) of model Claurus 680 Gas chromatography/Claurus 600 Mass spectrometer (GC having Liquid Auto sampler). The column used was Perkin Elmer Elite-5MS capillary Column of length 60m and internal diameter 0.25mm composed of 5% diphenyl 95% dimethyl polysiloxane (low bleed). Pure helium gas (99.99%) was used as the carrier gas with flow rate of 1ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 200^0 C. The oven temperature was programed initially at 50^0C for two min then an increase to 260^0 C with increasing rate of 5^0 C/min and holding time of about 2 min. The MS transfer line was maintained at a temperature of 200^0 C and the source temperature was maintained at 180^0 C. 1µl of the prepared 1% of extract diluted with methanol was injected in a split less mode. Total run time was 55 minutes. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion chromatogram (TIC) for compound identification and quantification. The identity of the components present in the methanol extract was assigned by the comparison of their respective retention time (RT) and mass spectra fragmentation patterns with those stored in the computer library National Institute of Standard and Technology (NIST) and also with published literatures.

III. Results and Observations

Preliminary phytochemical screening of the methanolic extract of the leaves of *Kayea assamica* are shown in the Table 1.

Table 1. Phytochemical screening of the methanolic extract of the leaves of *Kayea assamica*

Phytochemical	Methanol extract
phenols	Present
alkaloids	Present
tannins	Present
flavonoids	Present
terpenoids	Present
Coumarins	Present
Cardiac Glycosides	Present

The compounds present in the methanol leaf extract of *Kayea assamica* was identified by GC-MS analysis (Figure 2).The active compounds with their retention time (RT), Molecular formula, common name, Molecular weight (MW), Molecular structure, nature and bioactivity in the methanol extract of the leaves of *Kayea assamica* are presented in Table 2. Some of the GC-MS peaks remained unidentified due to lack of authentic samples and library data of corresponding compounds.Thirty different compounds were identified according to their RT of Mass Spectrum in the methanol extract of *Kayea assamica*. Out of them, seventeen compounds have completed, three compounds have partial information reported in literatures, NIST Chemistry WebBook and NCBI PubChem and rest of them have no reported information.

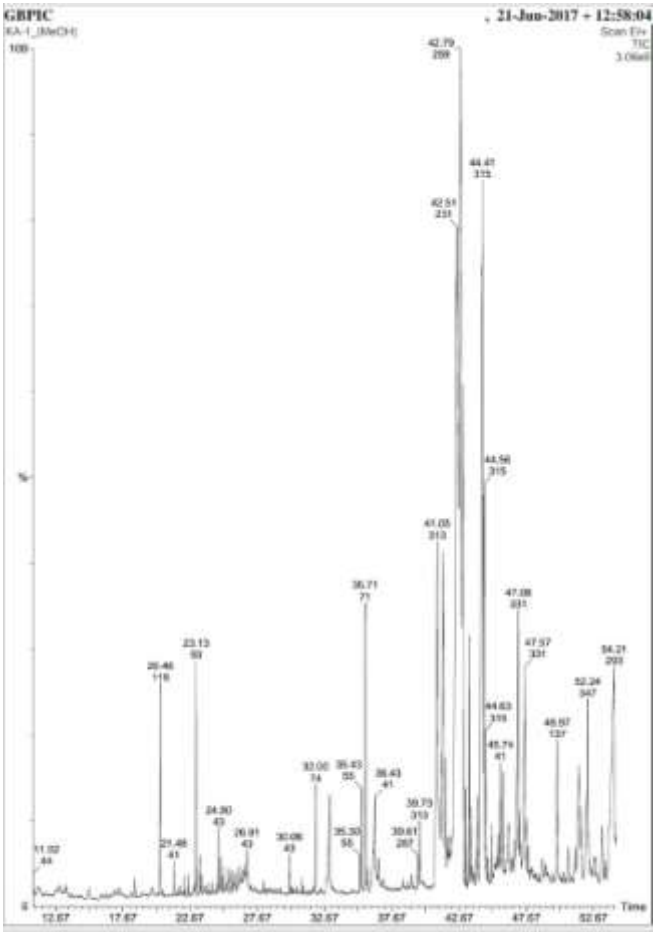
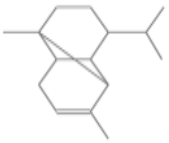
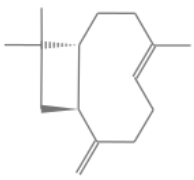
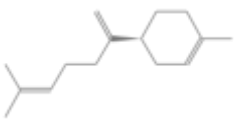
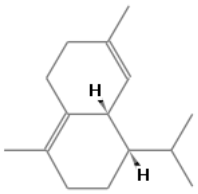
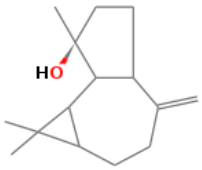
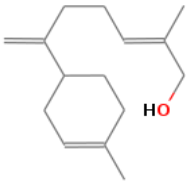
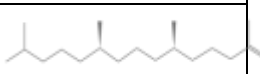
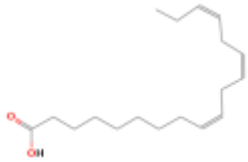
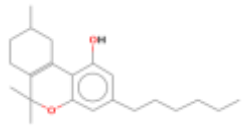
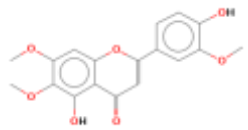
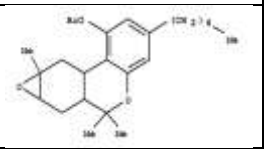


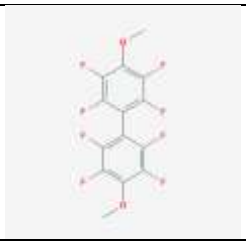
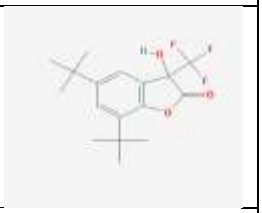
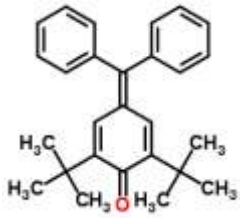
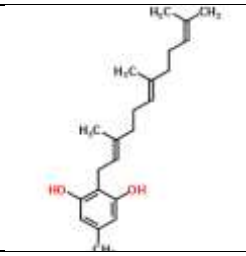
Fig. 2.GC-MS Total Ion Chromatogram of the methanol extract of the leaves of *Kayea assamica*

Table 2. Compounds present in the methanol extract of the leaves of *Kayea assamica*

peak no	R time	Compound name; chemical formula; Common name	MW g/mol	Nature and Bioactivity of compound	chemical structure
1	20.46	COPAENE, $C_{15}H_{24}$	204	Terpene; Antimicrobial, Anti-proliferative, antioxidant, anti-genotoxic and cytotoxic activities [12][13].	
2	21.48	BICYCLO [7.2.0] UNDEC-4-ENE, 4, 11, 11-TRIMETHYL-8-METHYLENE-, [1R-(1R*, 4Z, 9S*)]-; $C_{15}H_{24}$; Beta-Caryophyllene.	204	A dietary cannabinoid [16]; Pale yellow oily liquid with an odor midway between odor of cloves and turpentine; Antimicrobial compounds, non-steroidal anti-inflammatory, antinociceptiveand neuroprotective activity[14][15][16].	
3	23.13	CYCLOHEXENE, 1-METHYL-4-(5-METHYL-1-METHYLENE-4-HEXENYL)-, (S)-; $C_{15}H_{24}$; β-Bisabolene.	204	Terpene; Pungent and anti-ulcer activity [19], antibacterial [18] and antifungal activities, cytotoxic effect against human carcinoma [17]and use as food additives [20].	

4	23.41	NAPHTHALENE, 1, 2, 3, 5, 6, 8A-HEXAHYDRO-4, 7-DIMETHYL-1-(1-METHYLETHYL)-, (1S-; $C_{15}H_{24}$; δ-Cadinene.	204	Terpene; Antibacterial activity, nutrient, stabilizers, surfactants and emulsifiers. Novel larvicides against Malaria, Dengue and Filariasis mosquitoes, repellent activity, antiproliferative and apoptotic effect on human ovary cancer (OVCAR-3) cells [21][22][23].	
5	24.81	1H- CYCLOPROP [E] AZULEN-7-OL, DECAHYDRO-1, 1, 7-TRIMETHYL-4-METHYLENE-; $C_{15}H_{24}O$; Spathulenol.	220	Terpene; Colorless to pale yellow clear liquid; anti-hepatoma activity, antibacterial and antioxidant activity, anti-inflammatory, neuroprotective, cytotoxic activity [24][25][26][27][28][29].	
6	26.91	LANCEOL, CIS; $C_{15}H_{24}O$.	220	Alcohol [31]; Anti-Helicobacter pylori activity [30].	
7	30.06	3, 7, 11, 15-TETRAMETHYL-2-HEXADECEN-1-OL; $C_{20}H_{40}O$; Phytol.	296	Terpene; toxic, cytotoxic and genotoxic, antioxidant activity, antibacterial, schistosomicidal activity, neuroprotective, antiedematogenic activity, antitumorous, antimutagenic, anti-teratogenic, antibiotic-chemotherapeutic, antidiabetic, lipid lowering, antispasmodic, anticonvulsant, antinociceptive, anti-inflammatory, anxiolytic, antidepressant, immunoadjuvancy, hair growth facilitator, hair fall defense and antidandruff activities [32][33][34][35][36][37][38].	
8	32.00	HEXADECANOIC ACID, METHYL ESTER; $C_{17}H_{34}O_2$; METHYL PALMITATE.	270	SaturatedFatty acid; prevents Kupffer cell activation**,** hepatoprotective**,**anti–inflammatory, hypocholesterolemic, antifibrotic, universal macrophage inhibitor, nematicide, and antioxidant [39][40][41][42][43][44][59].	
9	33.07	N-HEXADECANOIC ACID; $C_{16}H_{32}O_2$;Palmitic Acid.	256	Saturated fatty acid; increased milk fat content and yield in cow, apoptotic activity, and cytotoxic effect against melanoma cell growth, anti –inflammatory, hypocholesterolemic, nematicide and antioxidant [39][40][41][42][43][44].	
10	35.30	9, 12-OCTADECADIENOIC ACID, METHYL ESTER; $C_{19}H_{34}O_2$; Methyl Linolelaidate.	294	Lipid peroxide; use as antifouling agent, protective against metabolic syndrome and cardiovascular disease risk factors, Catechol-O-Methyl-Transferase-Inhibitor and Methyl-Guanidine-Inhibitor, antioxidant, catalase activator [45][46][47].	
11	35.43	11, 14, 17-EICOSATRIENOIC ACID, METHYL ESTER; $C_{21}H_{36}O_2$.	320	Unsaturated fatty acid ester; antiarthritic, anticoronary, anti-inflammatory[48].	
12	35.71	PHYTOL; $C_{20}H_{40}O$	296	Terpene; toxic, cytotoxic and genotoxic, antioxidant activity, antibacterial, schistosomicidal activity, neuroprotective, antiedematogenic	

				activity, antitumorous, antimutagenic, anti-teratogenic, antibiotic-chemotherapeutic, antidiabetic, lipid lowering, antispasmodic, anticonvulsant, antinociceptive, anti-inflammatory, anxiolytic, antidepressant, immunoadjuvancy, hair growth facilitator, hair fall defense and antidandruff activities [32][33][34][35][36][37] [38].It can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. In ruminants, the gut fermentation of ingested plant materials liberates phytol, a constituent of chlorophyll, which is then converted to phytanic acid and stored in fats [58].	
13	36.43	9, 12, 15-OCTADECATRIENOIC ACID, (Z, Z, Z)-; $C_{18}H_{30}O_2$; Linolenic acid.	278	Polyunsaturated omega-3 fatty acid; Cardio protective, partially control type 2 diabetes, anti-inflammatory activity, effective against*Mycobacteriumtuberculosis* [49] [50] [51] [52].	
14	39.61	ANTHRA[2,3-D]-1,3-DIOXOLE-5,10-DIONE, 3A,4,11,11A-TETRAHYDRO-9-HYDROXY-7	344	No information reported	No structure reported
15	39.73	6H-DIBENZO(B,D)PYRAN-1-OL, 3-HEXYL-7,8,9,10-TETRAHYDRO-6,6,9-TRIMETHYL-; $C_{22}H_{32}O_2$; Parahexyl.	328	Benzopyran; use in treatment of alcoholic and drug withdrawal conditions [53].	
16	41.05	2-(4-HYDROXY-3-METHOXYPHENYL)-3, 7-DIMETHOXY-4H-CHROMEN-4-ONE; $C_{18}H_{16}O_6$; Cirsilineol.	328	Flavonoid; immunomodulatory properties, Ant proliferative activity, Anti-inflammatory activity [54] [55].	
17	41.49	6H-OXIRENO[4,5]BENZO[1,2-C][1]BENZOPYRAN-1-OL, 6A,7,7A,8A,9,9A-HEXAHYDRO; $C_{23}H_{32}O_4$;	372	No information reported	
18	42.49	PROPANOIC ACID, (4-PROPIONIYLAMINO-6-METHYL-1-FURO[2,3-H]COUMARINYL)	343	No information reported	No structure reported
19	42.51	PROPANOIC ACID, (4-PROPIONIYLAMINO-6-METHYL-1-FURO[2,3-H]COUMARINYL)	343	No information reported	No structure reported
20	42.79	ACETIC ACID, 6,6,9-TRIMETHYL-10-OXO-3-PENTYL-6A,7,8,9,10,10A-HEXAHYDRO-6H	372	No information reported	No structure reported
21	43.19	2-BUTOXY-6-(4-TRIFLUOROMETHYL-PHENYL)-NAPHTHALENE	344	No information reported	No structure reported
22	43.43	SILANE, DIMETHYLOCTYLOXYNONYLOXY-	330	No information reported	No structure reported
23	44.03	BENZOIC ACID, 2,4,6-TRIS(1,1-DIMETHYLETHYL); $C_{28}H_{48}O_2$	290	No information reported	No structure reported
24	44.41	SILANE, DIMETHYLOCTYLOXYNONYLOXY	330	No information reported	No structure reported

25	44.51	2,2',3,3',5,5',6,6'-OCTAFLUORO-4,4'-DIMETHOXYBIPHENYL; $C_{14}H_6F_8O_2$; 4,4'-Dimethoxyoctafluorobiphenyl.	358	No information reported	
26	44.56	SILANE, DIMETHYLOCTYLOXYNONYLOXY	330	No information reported	No structure reported
27	44.63	3H-BENZOFURAN-2-ONE, 5, 7-DI-TERT-BUTYL-3-HYDROXY-3-TRIFLUOROMETHYL; $C_{17}H_{21}F_3O_3$; RAC BHFF.	330	Anxiolytic activity [56].	
28	45.74	2, 6- BIS (1, 1-DIMETHYLETHYL)-4-DIPHENYLMETHYLENECYCLOHEXA-2, 5-DIEN-1-ON; $C_{27}H_{30}O$; NSC81685	370	No reported activity	
29	46.36	7-[4-(1-ACETAMIDO-1-ETHOXYCARBONYL-2,2,2-TRIFLUOROETHYL)-1-PIPERAZINYL	542	No information reported	No structure reported
30	47.08	1H-INDOLE-2,3-DIONE, 1-(TERT-BUTYLDIMETHYLSILYL)-5-HEXYL-, 3-(O-ETHYLOXI	388	No information reported	No structure reported
31	47.57	1H-INDOLE-2,3-DIONE, 1-(TERT-BUTYLDIMETHYLSILYL)-5-HEXYL-, 3-(O-ETHYLOXI	388	No information reported	No structure reported
32	47.97	1, 3-BENZENEDIOL, 5-METHYL-2-(3, 7, 11-TRIMETHYL-2, 6, 10-DODECATRIENYL); $C_{22}H_{32}O_2$; Grifolin.	328	A potent antitumor natural product [57].	
33	51.6	ACETAMIDE, 2,2,2-TRIFLUORO-N-(1,2,3,4-TETRAHYDRO-1-ACETYL-2,2,4-TRIMETHY	404	No information reported	No structure reported
34	52.24	ACETAMIDE, 2,2,2-TRIFLUORO-N-(1,2,3,4-TETRAHYDRO-1-ACETYL-2,2,4-TRIMETHY	404	No information reported	No structure reported

IV. Discussion

To explore the significance of any medicinal plant species, the primary step is to screen for its phytochemicals, as it gives an immense idea concerning the nature of compounds present in it. In the present study, the endemic plant species *Kayea assamica* was preliminarily screened for the phytochemicals present in the methanol extract of its leaves. This phytochemical screening assist as a beginning step for future determination of its bioactivity like antibacterial, antifungal, anti-inflammatory, anticancer, antioxidant, antimutagenic etc. The methanol extract of *Kayea assamica* leaves was subjected to qualitative screening as well as Gas Chromatography-Mass spectrometry (GC-MS) analysis for its phytochemical investigation. The result revealed the presence of all important phytochemicals including alkaloids, phenols, tannins, flavonoids, terpenoids, coumarins and cardiac glycosides which comprises thirty different compounds with more than 90 %

match with the library data. From the table 2 the major compounds identified against peak nos. 1, 2, 3, 7, 12 reported to have antimicrobial activity, peak nos. 2, 5, 7, 8, 9,11,12, 13, 16 reported to have anti-inflammatory activity, peak nos. 1, 7, 12, 8, 9, 10 reported to have antioxidant activity, peak nos. 3, 4, 7, 12, 9, 32 reported to have anticancer as well as antitumor activity, peak nos. 2, 5, 7,12 reported to have neuroprotective activity, peak nos. 1, 3, 5,7, 9,12 reported to have cytotoxic activity against cancer cell, peak nos. 7, 12, 16 reported to have immunomodulatory activity, peak nos. 1, 4, 16 reported to have antiproliferative activity, peak nos. 5, 8 reported to have hepatoprotective, peak nos. 10, 13 reported to have cardioprotective and peak nos. 4, 9 reported to have apoptotic activity. Among the identified compounds, some has interesting significant activity *i.e.* the compound copaene has anti-genotoxic activity [12], δ-Cadinene is a novel larvicides against Malaria, Dengue and Filariasis mosquitoes and has repellent activity[22], Lanceol has antibacterial activity against *H. pylori* which was previously isolated from *Santalum album*[30], Phytol has hair growth facilitator, hair fall defense and antidandruff activities which can be use as cosmetic purpose in future[37], it was reported that Methyl palmitate prevents Kupffercell activation and improves survival after orthotopic liver transplantation in the rat, it inhibit the phagocytosis by Kupffercell and reduce carbon uptake to about one-third to one-half of control values in unstored and stored livers, where the oxygen uptake increases in these livers[39], 11, 14, 17-eicosatrienoic acid, methyl ester has antiarthritic, anticoronary, anti-inflammatory activities[48], it was also reported that Linolenic acid isolated from *Sutherlandia frutescens* plant extracts has inhibition potential against shikimate kinase (a drug target for *M. tuberculosis*) a good drug target for *M. tuberculosis*[49]. Many of the identified compounds are recognized as major constituent of the essential oil producing plants, such compounds are Copaene from rhizome of *Piper boahmeriaefolium* [13], Beta-Caryophyllene from the essential oils of many different spice and food plants, such as oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.) and black pepper (*Piper nigrum* L.) [16], δ-Cadinene from *Kadsura heteroclita* leaf essential oil [22], Spathulenol from Brazilian spice *Xylopia sericea* [25], Phytol from *Citrus aurantifolia* and *Citrus grandis* L[60], Methyl palmitate and methyl linolenate from *Lawsonia inermis* L. [61] etc.

V. Conclusion

This type of phytochemical investigation is the first step towards understanding the nature of active principles in this endemic plant species. These bioactive compounds present in *Kayea assamica* leaf extract support the therapeutic application of the plant. Identification of these compounds in the plant serves as the starting point in determining the probable health benefits of the plant leading to future biotic and phytopharmaceutical importance. As this plant species endemic in nature, therefore it needs to be conserved for future purpose.

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