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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(3): 449-457

www.thepharmajournal.com
Received: 26-01-2019
Accepted: 27-02-2019

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Detection of bioactive compound of Ayabirungaraja karpam GC-MS analysis

Nagalingam Varnakulendran and Veerayan Elango

Abstract
Ayabirungaraja karpam (ABK) is a synergistic poly herbo- metallic formulation. It consists iron, ferric oxide, Juice of lemon, Juice of birungarajam. ABK is recommended as an effective drug for Anemia, Anti- ageing, and premature grey as per reputed siddha literature. The present study was carried out to characterize the bioactive compound profile of ABK of extract using Gaschromatographie Mass spectrometry (GC-MS) analysis. This experiment revealed the biochemical components of the ethanol extract of ABK with distinctive peak. It is an ideal technique known for its higher resolution spectra of structurally similar compounds. The use of electron ionization in mass spectrometry produces distinctive mass spectral fragmentation pattern enabling mass spectra for unknown to be several against libraries, retention time, molecular formula, molecular weight, chemical structure and area percentage.
The aim of the study is to detect bioactive compound of ABK by GCMS analysis. In like that, the qualitative analysis of ethanol extract of ABK was carried out using GC-MS for the identification of bioactive compound of ABK. The nature and structure of the bioactive compounds were identified by the relative concentrations of various compounds getting eluted as function of retention time. The heights of the peak indicate the relative concentration of the components present in the ABK. The mass spectrometer analyses the compound eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different ratios. These mass spectra are finger print of that compound which can be identified from the data library.
It is concluded that the finger prints of the compound were identified 18 new compounds from the NIST library data base with various group of chemicals such as Terpene, Diterpene, Phenol, Coumarin and Fatty acid, Fatty alcohol and Plasticizer. The presence of various bioactive componenets enhance efficacy of ABK apart from the management of IDA.

Keywords: GCMS, kayakarpam, fingerprint, bioactive compound

Introduction
At present, plant-based medicines are widely employed in various public health practices throughout the globe as they are safe and cost-effective, and efficiently combat various deadly diseases and help in maintaining good health, (Kumaraswamy M *et al*. 2011) [1] many of the leading active drug molecules of plants and their derivatives used presently in allopathic medicine are mainly due to the understanding of traditional medical practices for curing diseases (Swamy M *et al*, 2015) [2]. Modern drug discovery researches governed by natural plant-based compounds and their products, followed by synthetic chemicaldrugs. Currently, natural products are considered as a major source of medicaments and, hence, they are extensively used by pharmaceutical industries. Plant and its products play a dominant role in the development of new drug which are beneficial for the preventive and curative aspect of management (Newman D *et al*, 2003) [3]. The phytochemical are present in a variety of plants, and as utilized as important components in the preparion and purification process of the siddha medicine. The phytochemicals are often referred as secondary metabolites of plants which are safer than the synthetic chemicals because phytochemicals in the plant extract target the biochemical pathway. (Mithraja M *et al*, 2012) [4].
Ayabirungaraja karpam (ABK) is a synergistic poly herbo-metallic formulation. It consists iron, ferric oxide, Juice of lemon, Juice of birungarajam. ABK is recommended as an effective drug for Anemia, Anti- ageing, premature grey as per reputed siddha literature (Kuppusamy Mudhaliyar. K N and Uthamroyan, K S, 1998) [5]. The siddha medicine is a unique system for kayakalpa preparation and its therapy which have dual perspective, one is to use as preventive to prolong life and avoid premature grey and other mode is as curative to treat degenerative diseases.

The aim of the study is to detect bioactive compound of ABK by GCMS analysis. In like that, the qualitative analysis of ethanol extract of ABK was carried out using GC-MS for the identification of bioactive compound of ABK. The nature and structure of the bioactive compounds are identified in different time interval using mass spectrometer. The finger prints of the compound were identified from the NIST library data base. The result of GCMS analysis of ABK revealed that the presence of terpene, Diterpene, Phenol, coumarin and fatty acid, fatty alcohol, Plasticizer.

Materials and Methods

Qualitative Preliminary photochemical screening

The phytochemical screening chemical test were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993) [6], Trease and Evans (1989) [7] and Harbone (1973) [8].

Preparation of extract- Digest 2.5g of sample in 50ml of alcohol and allowed to stand for 24h.Filter, Filtrate is evaporated for dryness over a water bath. Residue was collected to carry out the following phytochemical screening.

Procedures

1. Test for alkaloids

Test drug was extracted with 2mL of Hcl and added 2mL of Drgendroffs reagent, observation were red or orange precipitate indicates the presence of alkaloids

2. Test for Flavonoid

1g of ABK powdered sample was treated with alcohol and filtered, potassium hydroxide solution 10% were added. While yellow colour was produced confirming the presence of flavonoids.

3. Test for Tannins

The ABK extract 0.2g was taken in test tube, distilled water was added to it and wrmed the mixture on water bath, filtered it. Few drups of Ferric choride ($FeCl_3$) was added to it, the dark green color solution indicated the presence of tannin.

4. Test for carbohydrate (Fehling's Test)

The aqueous extract of powdered sample was treated with Fehling's solution I & II on heated on boiling bath for half an hour, appearance of yellow and then brick red precipitate indicated the carbohydrates.

5. **Test for protein (Biuret)**

1g of test drug ABK of aqueous and alcoholic extract in a 1mL of water was added 1% solution of copper sulphate followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

6. Test for sapponin –

The test sample ABK was boiled with 5mL of distilled water, filtered. To the filtrate about 3mL of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming indicates the presence of saponin.

7. Test for Glycosides (Borntrager's)

About 0.1g of ABK powdered sample was boiled with dilute HCL 2 min and few drops of ferric chloride solution were added, filtered while hot and cooled. The filtrate was then extracted with benzene and the layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well. No pink color was produced in the ammonial layer showing the anthroquinone glycosides.

8. Test for Sterol

A few drops of concentrated sulphuric acid were added to the ABK solution, shaken well and set aside. The lower layer of the solution turns red in colour indicating the presence of sterols.

9. Test for Terpinoids

The ABK test extract was mixed with 2mL of chloroform and concentrated H_2SO_4(3mL) is carefully added to form a layer. A reddish brown colouration of the interface is formed to show positive result of the presence of terpenoids.

10. Test for Mucilage

Took small amount of dry ABK powder, mount it on a slide with ruthenium red solution and observe it under microscope the pink color develops indication of presence of mucilage.

GC-MS analysis

Instrument Details

Name - GC–MS (Gas chromatography – Mass spectrometer)
Make : USA
Model : Clarus 500, Perkin Elmer GCMS
Software : Turbomass ver 5.2.0
Column type : Capillary column Elite 5 (Cross bond 5% Phenyl 95% dimethylpolysiloxane)
Column length : 30m
Column ID : 250µm

G C- Conditions:

Oven	Rate	Temp	Hold
Initial	---	60	0.00
1	6.0	150	2.00
2	4.0	280	5.00

Injector temp. : 280^0C
Carrier gas : Helium @flow rate 1mL/min
Split ratio : 1:10

MS condition

MS range : 40 -600amu
Type of ionization : Electron ironization
Electron energy : 70ev
Transfer line and source temperature : 200^0C, 160^0C
Sample injected : 1.0µL

Preparation of extract

10g powdered ABK sample was packed in a thimble and used for extraction by soxhlet apparatus at a temperature below the boiling temperature of ethanol solvent. A portion of the powdered ABK sample was soaked in the conical flask contains solvent, wrapped with aluminum foil and placed in arotatary shaker at 120-140 rpm for 48h.The extract wee filtered using whatman filter paper No 1.The solvent was evaporated and residue was dissolved in sterile dimethylsulphoxide DSMO 9:1 in 50mg/mL concentration. The extract was filered usng 0.22 microfilter (Type GV - Millipore) and store at 4^0C for analysis

Procedure

The GC–MS analysis of bioactive compounds from the Methanol extract of ABK was done using Perkin Elmer Gas Chromatography-Mass spectroscopy (Clarus 500, USA) equipped with Elite-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 2 min. Finally, the temperature was increased to 280 °C at the rate of 8°C/min holding for 10min. 1µL of the prepared extracts diluted with respective solvents was injected in a split mode of 1:10. Relative quantity of the chemical compounds present in each of the extracts of *ABK* was expressed as percentage based on peak area produced in the chromatogram. The resulted mass spectrum was compared with NIST library database containing more than 62,000 mass spectrums. (Parimaladevi ST, 2014) [9].

Identification of compounds

Identification of components Interpretation on mass spectrum of GC-MS was done using computer searches on a NIST Ver.2.1 MS data library which have more than 62,000 patterns and comparing the spectrum obtained through GC-MS compounds present were identified. The mass 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, peak area%, retention time, concentration of the test materials were established. (Sathyaprabha G, 2011) [10], (Nezhadali A, 2010) [11].

Results and Discussion

Table 1: Results of preliminary phytochemical screening

S, No	Parameters	Test	Results
1	Alkaloids	Dragendroffs' method	--
2	Flavinoids	Shinod's method	--
3	Tannins	Ferric-chloride test	--
4	Sterol	Salkowski's method	+
5	Terpinoids	Salkowski;s method	+
6	Glycosides	Borntrager's method	--
7	Mucilage	Rutheneum red test	--
8	Protein	Biuret method	+
9	Saponin	Froth method	--
10	Carbohydrate	Fehling method	--

Detected (+) Not detected (--)

Ethanolic extract of Aya Bhringraj Karpam was undergo for qualitative analysis of preliminary phytochemical investigation, the results was shown in table (Table 1) and it reveals the presence of sterol, terpinoids and protein. These compound contribute for therapeutic efficacy of test drug ABK such as terpinoids act as hepato protective (Dhanasegaran M *et al*, 2009) [12], protein indirectly influence the antioxidant defence and oxidative damage. (Halliwell B, Guttendge J M, 1984) [13], sterol was found to possess anti-inflammatory, anti-cholesteremic, anti-diabetic and anticancer. (Gupta N B *et al*, 1980) [14].

GCMS Analysis

The current trend has increased to determine the organic compounds from plants and its products. The combination of a best method of a separation technique gas chromatography (GC) with best identification technique mass spectrometry (MS) made GCMS. It is an ideal technique known for its higher resolution spectra of structurally similar compounds**.** (Grover N, 2013) [15] The use of electron ionization in mass spectrometry produces distinctive mass spectral fragmentation pattern enabling mass spectra for unknown to be several against libararies, (Nguyen H P, Kimaru I W, 2014) [16].

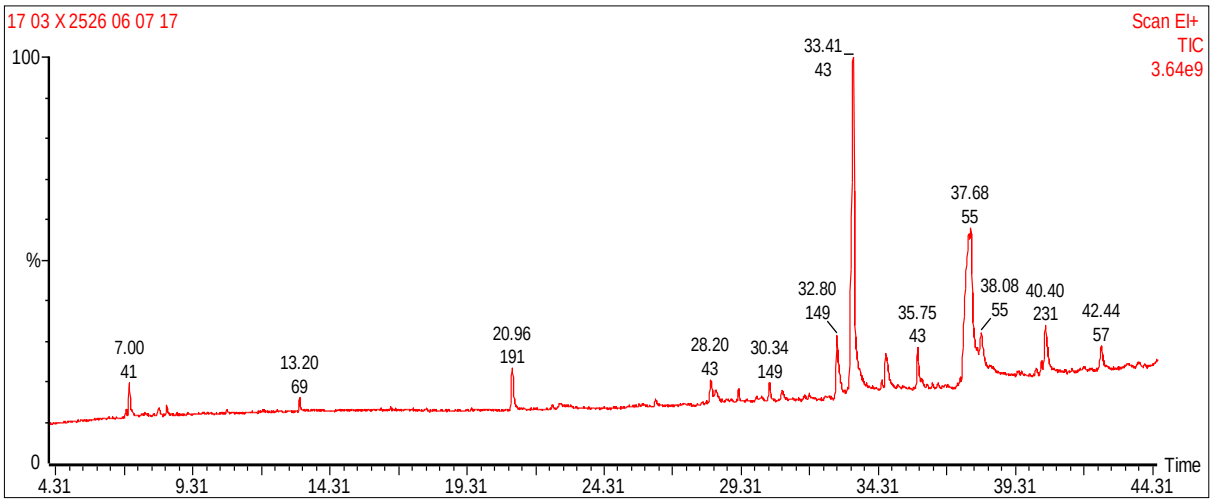

Fig 1: Chromatogram of Ethanolic extract of Aya Bhringraj Karpam

Chromatogram showed the relative concentrations of various compounds getting eluted as function of retention time. The heights of the peak indicate the relative concentration of the components present in the ABK. The mass spectrometer analyses the compound eluted at different times to identify the nature and structure of the compounds. The large compounds of fragment into small compounds giving rise to appearance of peaks at different mass-to-charge (M/Z) ratios. These mass spectra are finger print of that compound which can be identified from the data library (Szarka. S *et al*. 2008) [17].

Gas chromatography mass spectrometer is a method that combines the features of gas liquid chromatography and mass spectrometry to identify different substance of the test sample. Applications of GCMS include drug detection, environmental analysis, and explosives investigation and identify unknown sample. GCMS method proved to be very effective and

sensitive for separation and detection of complex mixture of phytochemicals. (Margl L *et al*, 2002 and Bicchi C *et al*, 1992) [18, 19] The GCMS analysis of ethanol extract ABK revealed the presence of phytochemical compounds (Table: 2) that enhance the activity of iron based herbometallic drug.

Table 2: Phytochemical compounds of ABK

S. No.	Peak Name	Retention time	Peak area	% Peak area	Type of compound
1.	Name: Bicyclo [3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)- Formula: C10H16 MW: 136	6.87	2689397	0.2059	Terpene
2.	Name: á-Myrcene Formula: C10H16 MW: 136	7.00	15634629	1.1969	Terpene
3.	Name: Cyclohexene, 1-methyl-5-(1-methylethenyl)- Formula: C10H16 MW: 136	8.08	8017630	0.6138	Terpene
4.	Name: Cyclohexene, 4-methylene-1-(1-methylethyl)- Formula: C10H16 MW: 136	8.36	5371764	0.4112	Terpene
5.	Name: 1,6-Octadien-3-ol, 3,7-dimethyl-, formate Formula: C11H18O2 MW: 182	13.20	7630650	0.5842	Terpene
6.	Name: Phenol, 2,4-bis(1,1-dimethylethyl)- Formula: C14H22O MW: 206	20.96	32271946	2.4707	Phenol
7.	Name: Tetradecanoic acid Formula: C14H28O2 MW: 228	28.20	21834800	1.6716	Fatty acid
8.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: C20H40O MW: 296	29.21	16609881	1.2716	Diterpene
9.	Name: 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester Formula: C16H22O4 MW: 278	30.34	13402380	1.0261	Plasticizer
10.	Name: Pentadecanoic acid Formula: C15H30O2 MW: 242	30.81	13836900	1.0593	Fatty acid
11.	Name: Dibutyl phthalate Formula: C16H22O4 MW: 278	32.80	58032804	4.4428	Plasticizer
12.	Name: n-Hexadecanoic acid Formula: C16H32O2 MW: 256	33.41	465354528	35.6264	Fatty acid
13.	Name: 2H-1-Benzopyran-2-one, 5,7-dimethoxy- Formula: C11H10O4 MW: 206	34.57	38300652	2.9322	Coumarin compound
14.	Name: 1-Octadecanol Formula: C18H38O MW: 270	35.75	24982054	1.9126	
15.	Name: 2-Methyl-Z,Z-3,13-octadecadienol Formula: C19H36O MW: 280	37.68	430991136	32.9956	Fatty alcohol
16.	Name: Octadecanoic acid Formula: C18H36O2 MW: 284	38.08	83727816	6.4100	Fatty acid
17.	Name: 7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy- Formula: C13H10O5 MW: 246	40.40	42006240	3.2159	Coumarin compound
18.	Name: Pentadecane, 2,6,10,14-tetramethyl- Formula: C19H40 MW: 268	42.44	25512616	1.9532	Hydrocarbon

This type of analysis is the first time towards the understanding of nature and availability of phytoconstituents of metallic drug since the ABK contain chiefly metallic components and treated with herbal extract in its manufacturing process. In this analysis,it is observed significant number of active compounds in ABK extract. Total of 18 structural chemical compound with various molecular weight among chiefly eight type of compounds in like that, five compounds of terpene group, one compound of diterpene group, one compound of phenol, four compounds of fatty acids, two compounds of fatty alcohol, two compounds of Coumarin, two compounds of Plasicizer and one hydrocarbon compound. The detail is shown in the [Table Z] In the present study, the compounds present in the ethanol

extract of ABK identified by GCMS analysis, and the prevailing compounds of ethanol extract were Bicyclo[3.1.0]hex-2-ene- 4-methyl-1-(1-methylethyl), á-Myrcene, Cyclohexene, 1-methyl-5-(1-methylethenyl), Cyclohexene, 4-methylene-1-(1-methylethyl), 1,6-Octadien-3-ol, 3,7-dimethyl- formate, Phenol, 2,4-bis(1,1-dimethylethyl), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Tetradecanoic acid, Pentadecanoic acid, n-Hexadecanoic acid, 1-Octadecanol, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Dibutyl phthalate, 2H-1-Benzopyran-2-one, 5,7-dimethoxy, 7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy, Octadecanoic acid, 2-Methyl-Z,Z-3,13-octadecadienol, Pentadecane, 2,6,10,14-tetramethyl.

Table 3: Biological activity of the extracted compound

No	Chemical Name	Compound Name	Type of compound	Bio efficacy
1	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-C10H16	β-Thujene	Terpene	Larvicidal, Bactericidal, Antioxidant [20, 21, 22]
2	á-Myrcene C10H16	Myrcene	Terpene	Repellent, Sedative, Analgesic activity, culinary and perfume uses [23, 24, 25]
3	Cyclohexene, 1-methyl-5-(1-methylethenyl)-C10H16	Sylvestrene	Terpene	Antibacterial activity, Insecticidal activity [26]
4	Cyclohexene, 4-methylene-1-(1-methylethyl)-C10H16	β-Phellandrene	Terpene	Antimicrbial activity particularly Antibacterial and Antifungal [27, 28, 29]
5	1,6-Octadien-3-ol, 3,7-dimethyl-, formate C11H18O2	Linallol/Linalyl formate	Terpene	Antimicrobial, Vitamin A synthesis, Food flavour component [30]
6	Phenol, 2,4-bis(1,1-dimethylethyl)-C14H22O	Phenol	Pheneol	Antimicrobial, Antioxidant [31]
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol C20H40O	Phytol	Diterpene	Anti -arthritic, Anti- diuretic, Antimicrobial and antioxidant, used as house hold cleaner, cosmetics, toilet soaps, and shampoos [32]
8	Tetradecanoic acid C14H28O2	Myristic acid	Fatty acid	Inhibition of ascetic tumour, Renal protection, Antibacterial [33]
9	Pentadecanoic acidC15H30O2	Pentadeconic acid	Fatty acid	Antioxidant, Lubricant and adhesive agent [34]
10	n-Hexadecanoic acid C16H32O2	Palmitic acid	Fatty acid	Antimicrobial,Anti-inflammatory,Antioxidant,Nematocidal,pesticide,Haemolytic, mosquito larvicidal [35, 36, 37]
11	1-Octadecanol C18H38O	Stearyl alcohol	Fatty alcohol	Sexpheromone [38]
12	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester: C16H22O4	Pthalic acid ester	Plasticizer	Anticancer, Ant diabetes, Anti-inflammatory, Antioxidant and Antimicrobial [39, 40]
13	Dibutyl phthalate C16H22O4	Dibutyl Pthalate	Plasticizer	Antifungal and Antibacterial activity [41, 42]
14	2H-1-Benzopyran-2-one, 5,7-dimethoxy-C11H10O4	Citropen	Coumarin	Antimicrobial activity [43]
15	7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy-C13H10O5	Isopimpenellin	Coumarin	Inhibit insulin stimulant lipogenesis, Block initiation of skin tumour [44]
16	Octadecanoic acid C18H36O2	Stearic acid	Fatty alcohol	Anti-inflammatory, hypocholesteremic, anti-eczemic, antihistamine, hepetoprotective, antiviral, cancer preventive [45]
17	2-Methyl-Z,Z-3,13-octadecadienol C19H36O	Octadecadienol	Fatty alcohol	Anti-protozoal, Anti-cancer activity [46, 47]
18	Pentadecane, 2,6,10,14-tetramethyl-C19H40	Pristane	Hydrocarbon	Antimicrobial, cytotoxic, Pathogens of Rheumatoid Arthritis and lupus [48]

There is growing awareness in correlating the phytochemical constituents and their biological activities according to past research literatures [49, 50]. Phytoconstituents exhibit a broad spectrum of effects which are used in the treatment of various diseases [51]. Among the identification of phytochemicals palmtic acid (n-Hexadecanoic acid) has the property of antioxidant which is reaction of oxygen species promoting substance, and also can be a hypercholesterolemic, nematocide, pesticide and lubricant acivities [52] Phytol (3,7,11,15-Tetramethyl-2-hexadecen-1-ol) considered as novel class of pharmaceutical agent for the treatment of rheumatoid arthritis and possibly the inflammatory diseases., antidiuretic and also used as house hold cleaners, toilet soaps, shampoos etc Phytol is the key acylicditerpene acohol that is a precursor for vitamin E and K1.

Pristane (Pentadecane, 2, 6, 10, 14-tetramethyl-) is a hydrocarbon group shows antimicrobial and cytotoxic effect. 2-Methyl-Z, Z-3, 13-octadecadienol is a fatty alcohol which produce antiprotozoal and cytotoxic effect. Phytol was observed to have antimicrobial activities against Staphlococcus aureus by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells [53]. Stearic acid (Octadecanoic acid) is under the group of fatty alcohol has the property of antifungal, antihistamine, antieczemic, hypocholesteremic, hepatoprotective, antiviral, cancer protective. Isopimpenellin (7H-Furo[3,2-g] [1] benzopyran-7-one, 4,9-dimethoxy-) is the coumarin class of compound which produce antimicrobial activity. Citropten (2H-1-Benzopyran-2-one, 5,7-dimethoxy-) is also coumarin group exhibits antifungal and antibacterial activity. Dibytyl

phthalate is a plasticizer has the property of antifungal and antibacterial. Pthalic acid ester (1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester:) is also the plasticizer has anticancer, antioxidant, antibacterial.

Pentadeconic acid has reports as antioxidant, lubricant and adhesive agent. Myristic (Tetradecanoic acid) acid shows inhibition of ascetic tumour, renal protective and antibacterial. Phenol (Phenol, 2,4-bis(1,1-dimethylethyl)-) shows antimicrobial and potent antioxidant. Linalool (1,6-Octadien-3-ol, 3,7-dimethyl-, formate) was observed to has antimicrobial, vitamin A synthesis, food flavour component.β-Thujene (Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-) has reported as larvicidal, antimicrobial and antioxidant acivity.

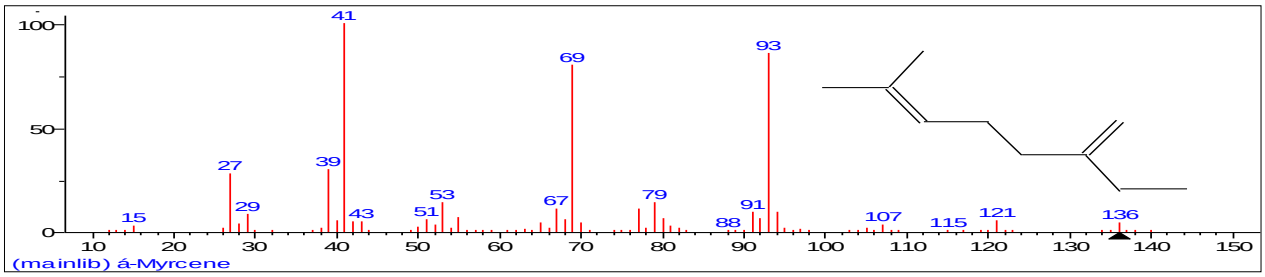

Fig 2: Mass Spectrum of á-Myrcene

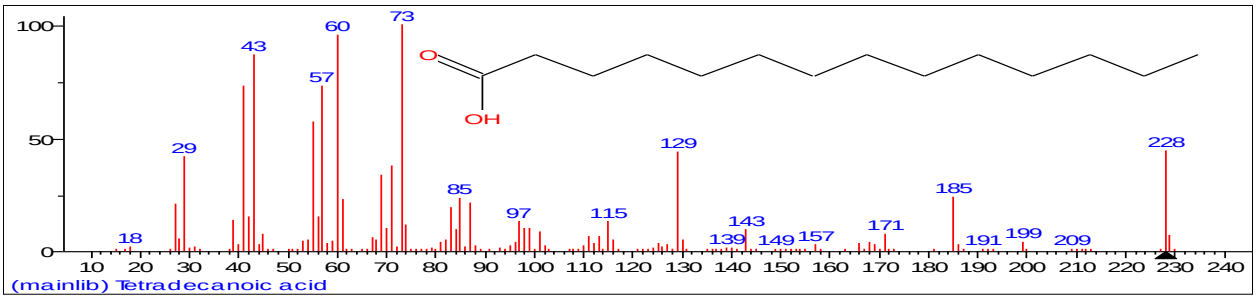

Fig 3: Mass Spectrum of Tetradecanoic acid

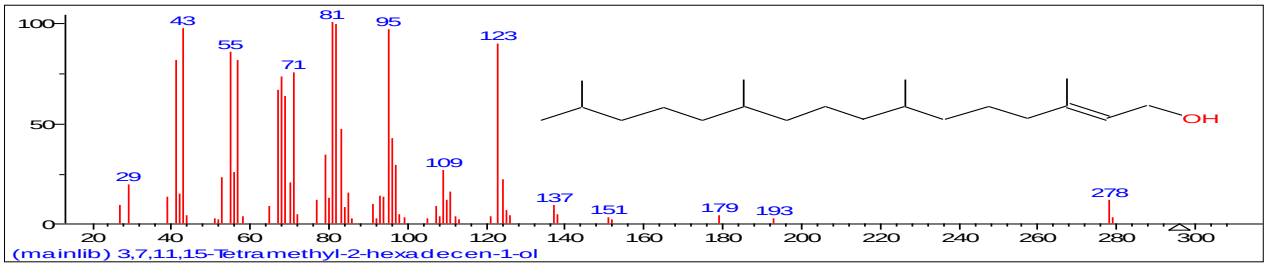

Fig 4: Mass Spectrum of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

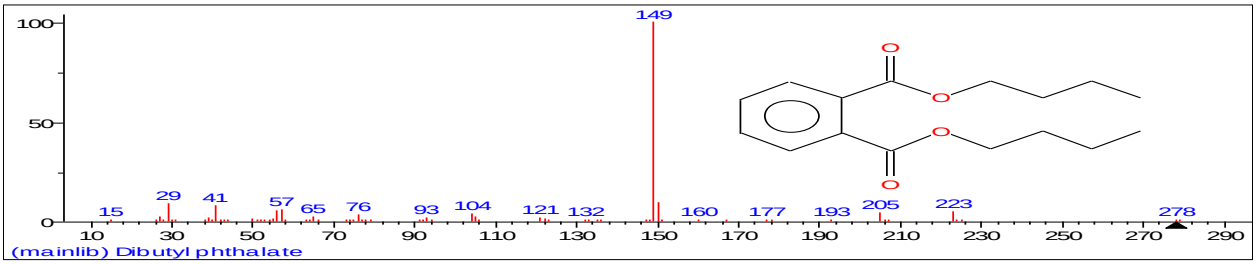

Fig 5: Mass Spectrum of Dibutylphthalate

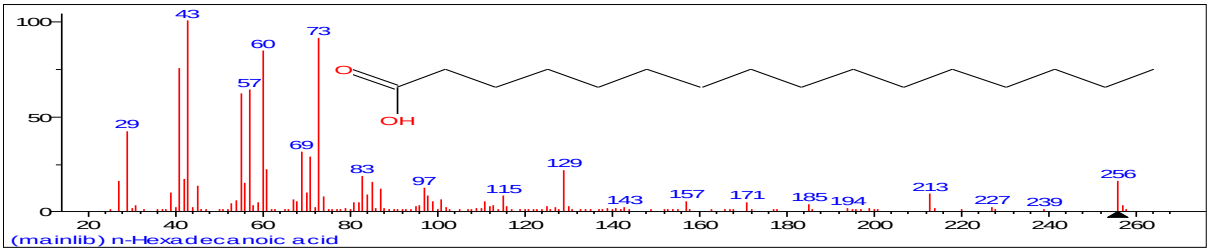

Fig 6: Mass Spectrum of n-Hexadecanic acid

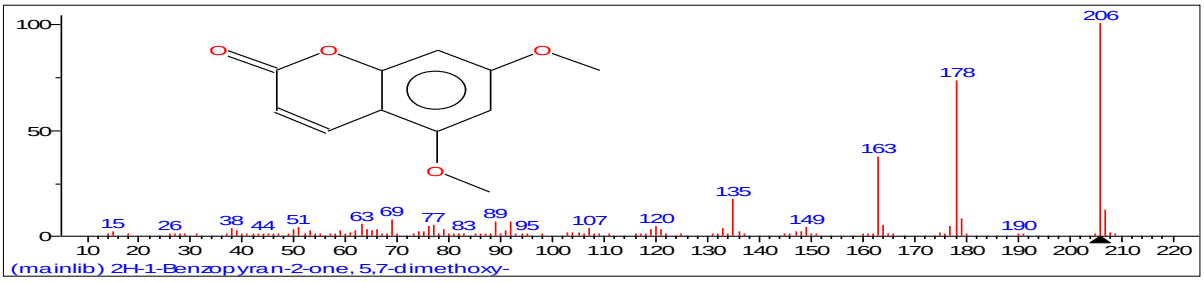

Fig 7: Mass Spectrum of 2H-1-Benzopyran-2-one,5,7-dimethoxy

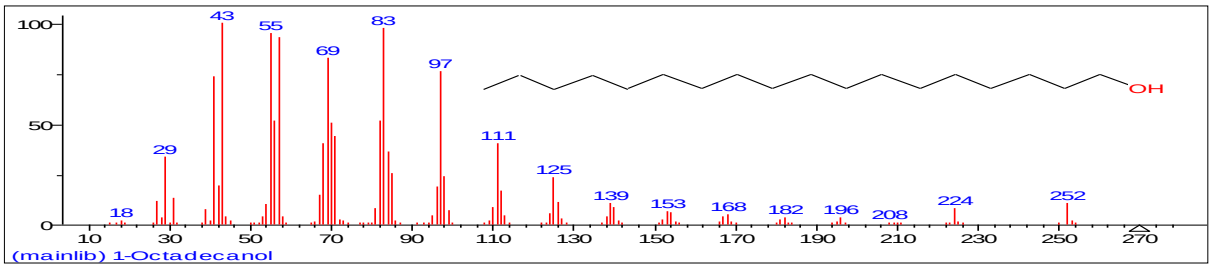

Fig 8: Mass Spectrum of 1-Octadecanol

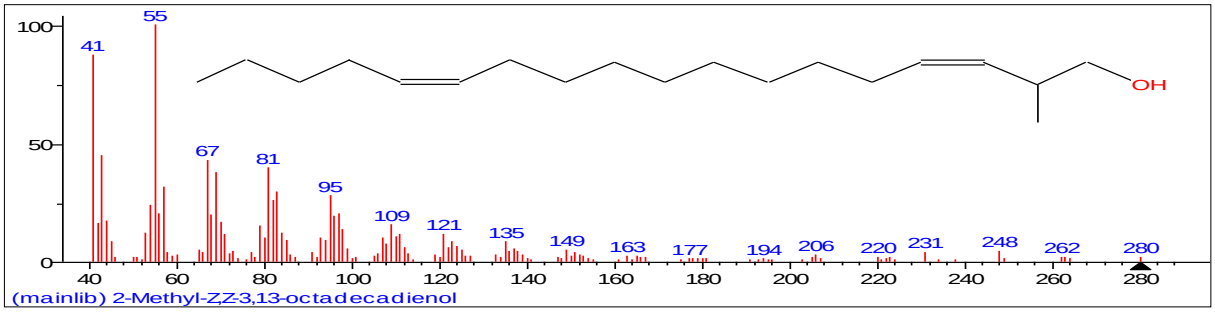

Fig 9: Mass Spectrum -2 methyl-z-z-3,13 octadienol

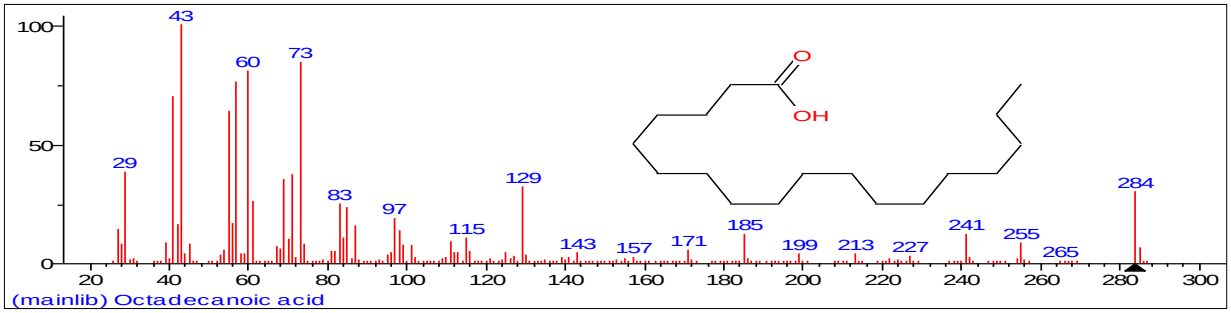

Fig 10: Mass Spectrum – Octadecanoic acid

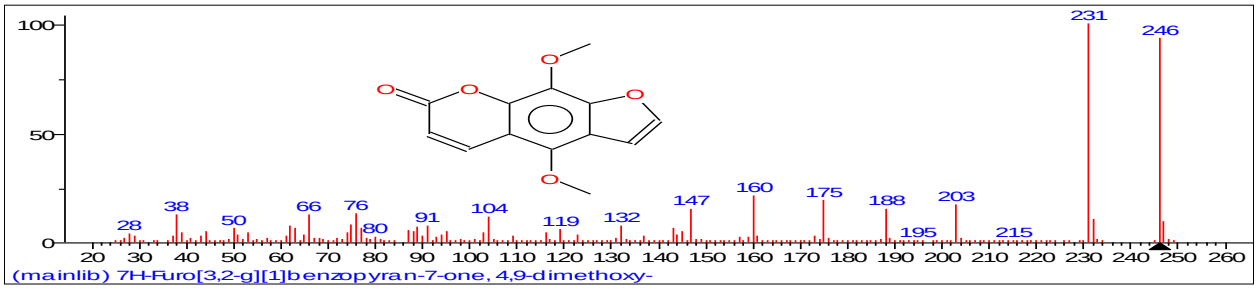

Fig 11: Mass Spectrum – 7H-Puro[3,2-0][1]-7-one,4,9-dimethoxy

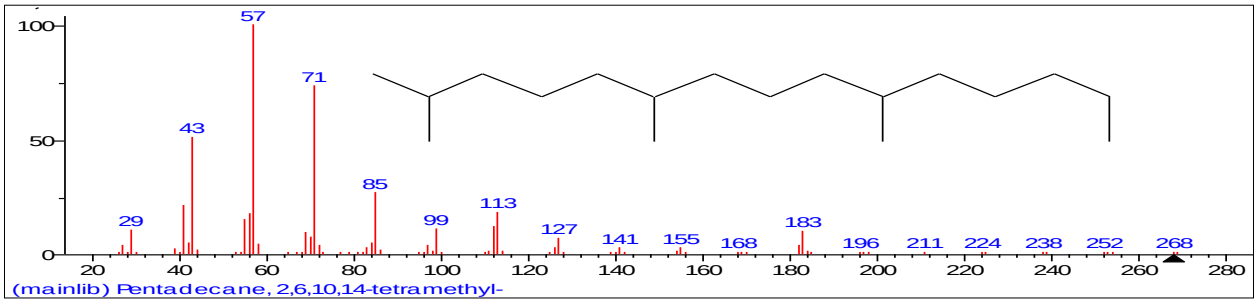

Fig 11: Mass Spectrum – Pentadecane, 2, 6, 10, 14-tetramethyl

Conclusion

"The boon given to our earth is the herbs" Therefore, the siddhamedical system drug preparation involved in metallic, mineral and animal products is processed with herbal materials in the different stage of processing. In like that, lime juice and Manjalkrisalai (*W. chinensis*) juices are used in the preparation of Aya Bhringraj Karpam, that contain numerous phytochemical constituents which are confirmed by the preliminary phytochemical testing and GCMS analysis of ABK these organic compounds which are account to a great extent of exhibiting the pharmacological activity of Herbometallic drug Aya Bhringraj Karpam.

It is concluded that the 13 compounds were identified by GCMS analysis. The presence of different bioactive components justify the use of ABK for various ailments other than the IDA which are claimed and reported of bioactive potentials of specific compounds by previous researchers. However the isolation of identical phytochemical and its bio efficacy should be screened in future.

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