Phytocompound and Heavy Metal Analysis of Purified *Corallocarpus epigaeus* Benth.ex.hook. (*Aagasagarudan Kizhangu*) in the aspect of Siddha System of Medicine

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ABSTRACT

Aim and objective: The aim of the study is to reveal the phytochemical and heavy metal studies of Purified *Corallocarpus epigaeus* in the aspect of siddha system of medicine. **Methods:** *Corallocarpus epigaeus (Aagasagarudan kizhangu)* was purified according to the principles of siddha literature ("***Agathiyar Gunavaagadam"***) and then a study regarding validation of the phytocompounds and heavy metal analysis of purified *corallocarpus epigaeus* was carried out using GC-MS, ICP-OES and FT-IR. **Results:** *Corallocarpus epigaeus* Benth.ex.hook is a familiar herb used in the siddha system of medicine because of its potent medicinal effect, which is being in practice by many siddha physicians and traditional siddha healers all over Tamilnadu, India in various forms such as powder (*Chooranam*) and oil (*Thailam*). There is a lacking in scientific evaluation of purified *Corallocarpus epigaeus*. **Conclusion:** The methanolic extract of purified *Corallocarpus epigaeus* revealed six phytocompounds which are reported with several activities and effective medicinal properties. This study point outs the significance of detoxification process of this sample and it innovate platform for future researchers to go ahead with much more research in this regard.

[Keywords: Siddha, Agathiyar, Corallocarpus epigaeus, *Aagasagarudan kizhangu*, phytocompounds]

I. INTRODUCTION

Herbals are always considered as very safe for medicinal uses. Though it is considered as safe, it was not safe always at every time in all doses and also due to some damage to our body internal environment. Nowadays due to the over use of herbals worldwide, we have to ensure the safety of herbal medicines[1]. The ultimate motive is to use only the highly potent medicinal value without causing any adverse effects. In siddha medical system, some detoxification methods were described by the siddhar's for safe use of the structures[2]. Here the purified *Corallocarpus epigaeus* powder was subjected into analysis to gain knowledge regarding the structural and functional components through some modern sophisticated analytical equipment's such as FTIR, GCMS & ICP-OES and also the properties of purified *Corallocarpus epigaeus* was comparatively analyzed to describe the detoxificatory effectiveness of siddha medicines.

II. MATERIALS AND METHODS

The plant *Corallocarpus epigaeus* was purified as per the siddha concept of purification method for detoxification of tubers which indicated in text ***"Sikicha Rathna Deepam"***[3]. After it was purified the drug was subjected into many characteristic analyses such as FTIR, ICP-OES and GCMS to find out the structural components present in it. ICP-OES analysis for this purified herbal has taken to know about the presence of heavy metals in this sample. All these analysis was carried out in IIT Madras.

Figure 1: Corallocarpus epigaeus – with skin

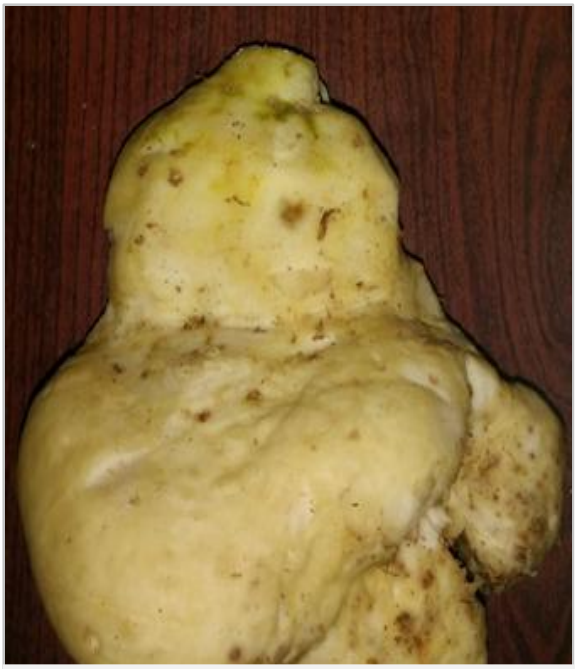

Figure 2: Corallocarpus epigaeus – without skin

FT-IR:

FT-IR spectra were recorded at SAIF, IIT Madras, India. The Perkine Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FT IR Spectra of *Corallocarpus epigaeus* in Potassium Bromide (KBr) matrix with scan rate of 5 scan per minute at the resolution 4cm-1 in the wave number region 450-4000cm-1. The samples were grounded to fine powder using agate motor and pestle and the mixed with KBr[4]. They were then Pelletized by applying pressure to prepare the specimen (the size of specimen about 13 mm diameter and 0.3 mm in thickness) to recorded the FT- IR Spectra under Standard conditions

FT- IR Spectra were used to determine the presence of the functional groups and bands in the *Corallocarpus epigaeus*. The recorded spectrum shows in figure 1.

GC-MS:

Preparation of Plant Extracts:

The dried purified tuber powder of *Corallocarpus epigaeus* (100gm) was extracted in soxhlet apparatus using methanol for 6 hours. The methanolic extracts were dried under reduced pressure using rotary evaporator to get the crude and were stored below 4ºC until further used. The extract contains polar components of the plant material, and 2 µl of the sample of the solutions was employed in GC-MS for analysis of different compounds.

GC-MS Analysis:

GC-MS analysis of the methanol extract of Purified *Corallocarpus epigaeus* was performed using a Agilant GC system comprising an 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 a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1).

The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 36 min. The relative percentage

amount of each component was calculated by comparing its average peak area to the total areas. . The mass-spectrometer used in this analysis was JEOL GC-MATE-II, and the software adopted to handle mass spectra and chromatograms was a JEOL Ver.2.0 and NIST library Ver.2.0 was used.

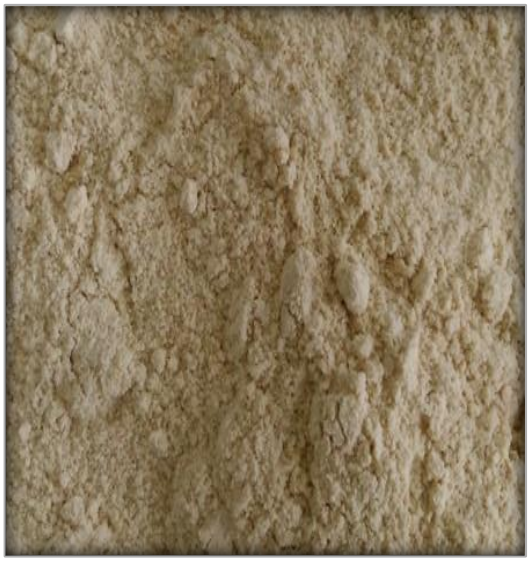

Figure 3: Purified Corallocarpus epigaeus sample

ICP-OES:

The Inductively Coupled Plasma Optical Emission Spectrometric (ICP-OES) analysis was done at SAIF in IIT MADRAS, Chennai - 36 using Perkin Elmer Optima 5300 DV. The digestion sample was prepared by using 100 mg of Purified *Corallocarpus epigaeus* added with 3 ml of Nitric acid and 25 ml of Distilled water.

III. RESULT

FTIR Spectra of purified Corallocarpus epigaeus

In the FT-IR Spectra analysis, this purified *Corallocarpus epigaeus* sample exhibits the peak value shows in Figure:4 at the wave number of 3509, 3357, 2162, 1682,1652, 1423, 1356, 1252, 1132,1077,1031,999,947,465,706,421,540,83. This indicates the presence of some organic functional groups such as alcohols, phenols,1`, 2` amines, amides, Alkynes, Alkenes, Alkyl halides, Nitro compound, Alcohols, carboxylic acids, esters, ethers, Aliphatic amines, Carboxylic acids, Aromatics.

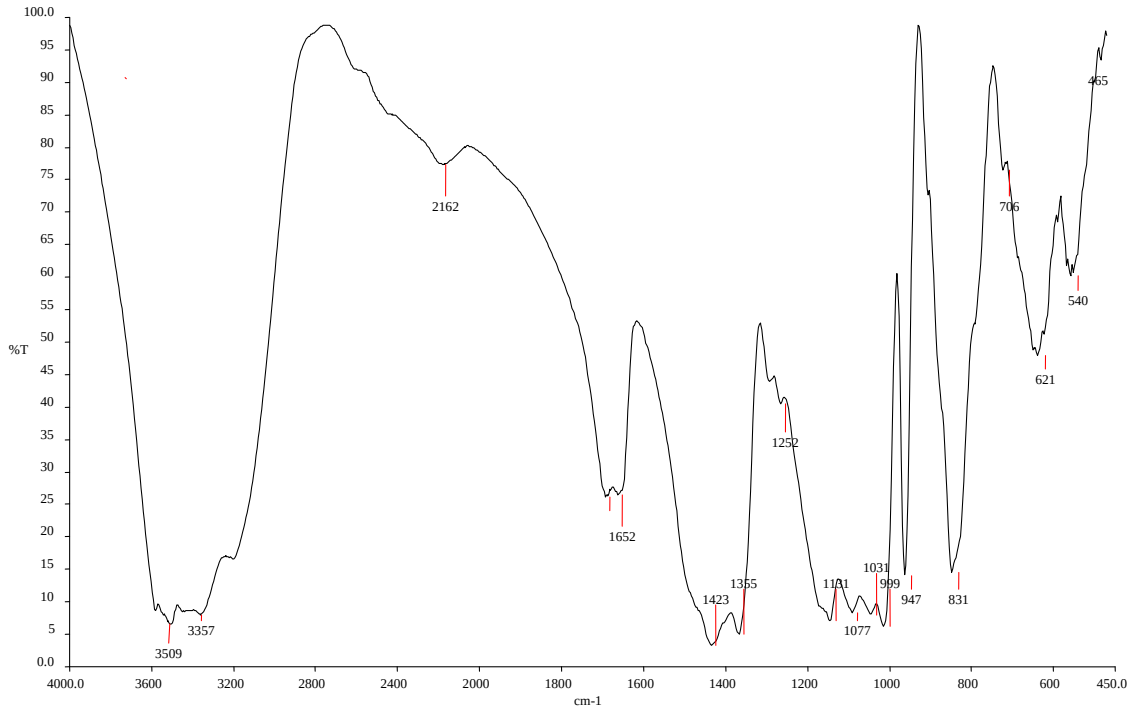

Fig 4: FTIR Spectra of purified *Corallocarpus epigaeus*

Table 1: FTIR interpretation

Sl.no.	Wavelength	Vibrational modes	Functional groups
1	3509	-OH-Stretch-H-bonded	Alcohols, phenols
2	3357	N-H stretch	1`, 2` amines, amides
3	2162	-C≡C-stretch	Alkynes
4	1682	-C=C-stretch	Alkenes
5	1652	-C=C-stretch	Alkenes
6	1423	C-C stretch (in-ring)	Aromatics
7	1356	N-O symmetric stretch	Nitro compound
8	1252	C-O Stretch	Alcohols, carboxylic acids, esters, ethers
9	1132	C-N Stretch	Aliphatic amines
10	1077	C-N Stretch	Aliphatic amines
11	1031	C-N Stretch	Aliphatic amines
12	999	= C-H Bend	Alkenes
13	947	O-H Bend	Carboxylic acids
14	831	C-Cl Stretch	Alkyl halides
15	706	-C≡C-H=C-H bend	Alkynes
16	421		
17	540	C-Br stretch	Alkyl halides
18	465		

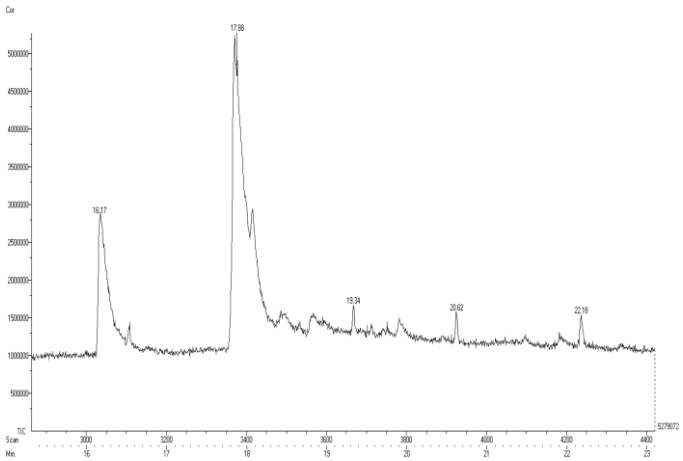

Fig 5: GCMS

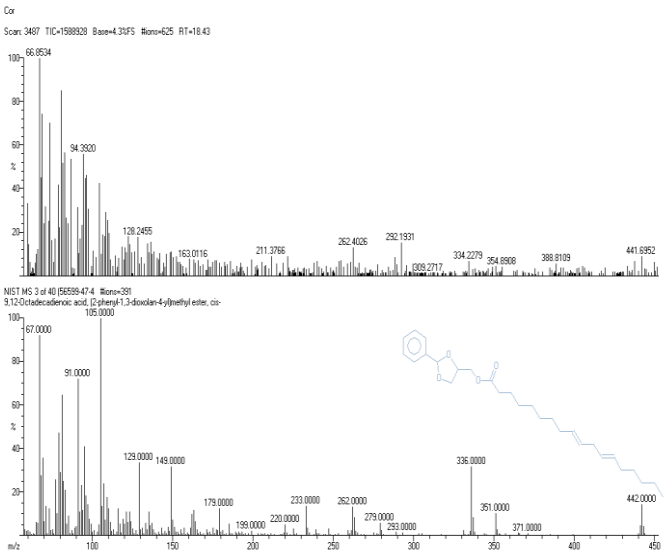

Fig 6: GCMS

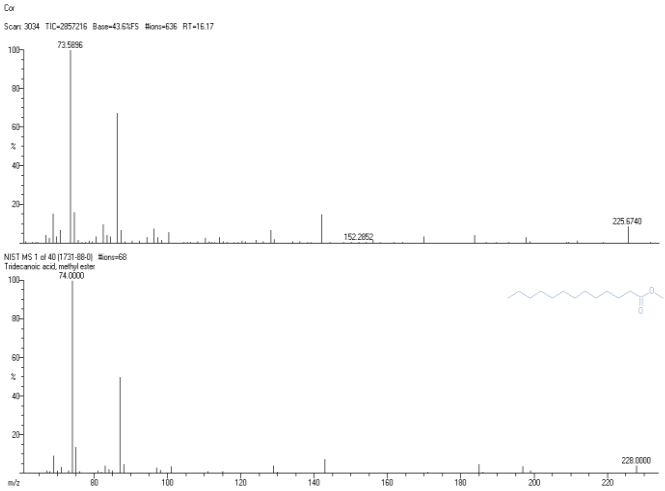

Fig 7: GCMS

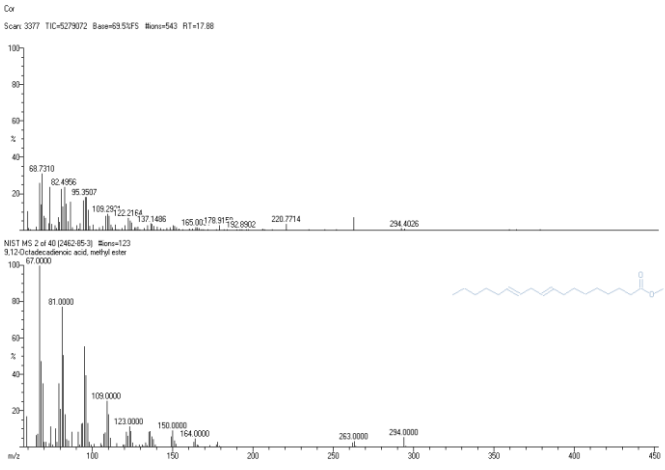

Fig 8: GCMS

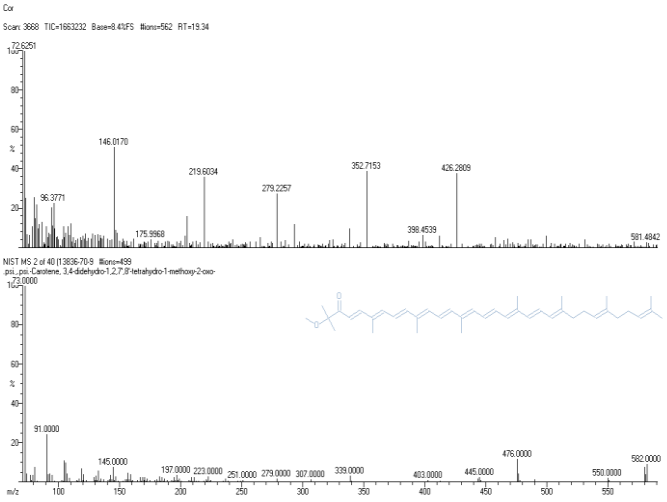

Fig 9: GCMS

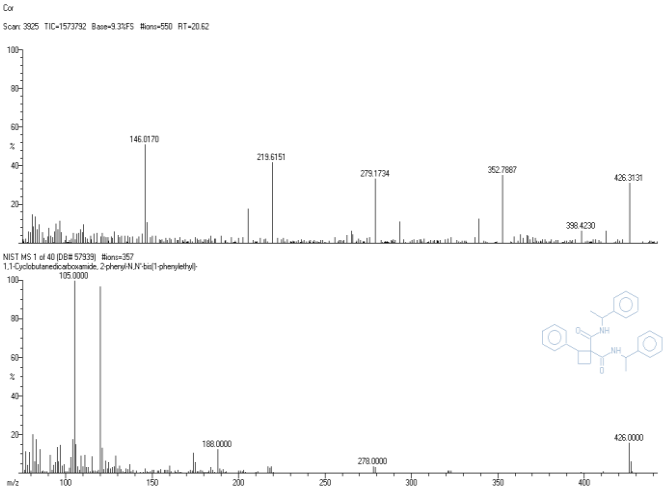

Fig 10: GCMS

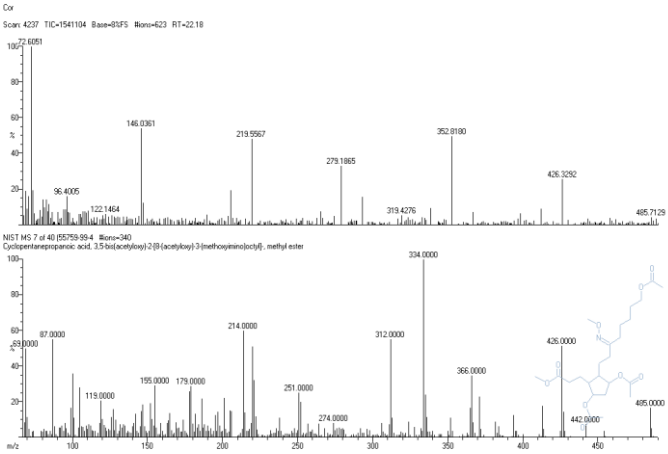

Fig 11: GCMS

This Gas chromatogram analysis shows the characteristics of purified drug *corallocarpus epigaeus*. In this sample, 6 phytocompounds were identified, they are as follows

Table 2: Gas chromatogram analysis

S. NO	Rt	Name of the compound	Molecular formula	Molecular weight (g/mol)
1	18.43	9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	$C_{28}H_{44}O_4$	444.656
2	16.17	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228.376
3	17.88	9, 12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.479
4	19.34	Psi, psi carotene, 3,4 didehydro-1, 2, 7′8′ – tetrahydro-1-methoxy-2-oxo	$C_{41}H_{58}O_2$	582.913
5	20.62	1,1-Cyclobutanedicarboxamide, 2-phenyl-N-N′-bis(1-phenylethyl)-	$C_{28}H_{30}N_2O_2$	426.56
6	22.18	Cyclopentanepropanoic acid, 3,5 bis(acetyloxy)-2-[-(acetyloxy)-3-(methoxyimino)octyl]-, methyl ester		

Table 3: ICP-OES Interpretation

Sl.No	Element name	Standard value	Obtained value
1	As	188.979	BDL
2	Ca	315.807	24.150 mg/L
3	Cd	228.802	BDL
4	Cu	327.393	BDL
5	Fe	238.204	2.340 mg/L
6	Hg	253.652	BDL
7	K	766.491	120.821 mg/L
8	Mg	285.213	01.020 mg/L
9	Na	589.592	13.110 mg/L
10	Ni	231.604	BDL
12	Pb	220.353	BDL
13	P	213.617	58.541 mg/L
14	Zn	213.856	01.587 mg/L

The presence of some metals such as Arsenic, Calcium, Cadmium, Copper, Iron, Mercury, Potassium,

Manganese, Sodium, Nickel, Lead, Phosphorus and Zinc were detected in the sample of Purified *Corallocarpus epigaeus*. Refer Table:3. The most important heavy metals such as lead, mercury, arsenic and cadmium are presence of BDL as per the WHO permissible levels in this purified sample.

IV. DISCUSSION

One research study was confirmed that the extract of unpurified tuber of *Corallocarpus epigaeus* have the functional groups of Amines, Unsaturated nitrogen compounds, C≡N stretching vibration isocyanates, alkene Carboxylic acids, Carboxylate , azides, Alkene[5].

This purified *Corallocarpus epigaeus* extract confirms the presence of some organic functional groups such as alcohols, phenols,1`, 2` amines, amides, Alkynes, Alkenes, Alkyl halides, Nitro compound, Alcohols, carboxylic acids, esters, ethers, Aliphatic amines, Carboxylic acids, Alkyl halides, Aromatics. Refer Table:1. These identified functional groups have more significance in the field of medicine. From this, can confirm that the purified *Corallocarpus epigaeus* has more functional and altered functional groups when compare with the extract of unpurified tuber of *Corallocarpus epigaeus*. This clearly indicates the process of purification plays a major role.

Through GCMS Analysis, the identified compounds in Purified *Corallocarpus epigaeus* are 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-, Tridecanoic acid, methyl ester,9, 12-Octadecadienoic acid, methyl ester, Psi, psi carotene, 3,4 didehydro-1, 2, 7'8' – tetrahydro-1-methoxy-2-oxo, 1,1-Cyclobutanedicarboxamide, 2-phenyl-N-N'-bis(1-phenylethyl)-, Cyclopentanepropanoic acid, 3,5 bis(acetyloxy)-2-[-(acetyloxy)-3-(methoxyimino)octyl]-, methyl ester. Refer Table:2. These identified compounds like 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, having so many medicinal values such as Hypocholesterolemic 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Hypocholesterolemic, Antieczemic, anti oxidant, anti microbial activity[6].

The heavy metals estimation clears that the purified *Corallocarpus epigaeus* is free from toxic and considered as safe for clinical use.

V. CONCLUSION

From this research study, the presence of heavy metals are evaluated that they are within the normal WHO Permissible limits so it is considered as safe for clinical use. These FT-IR characterization on purified *Corallocarpus epigaeus* creates the fingerprints for standardization of this sample. If further research findings will followed on these identified compounds of this purified sample through GC-MS can help the scientific community to develop the new drug to treat diseases such as virulent poison, leprosy which is indicated in siddha science. The compounds identified through the GCMS analysis and the functional groups which were identified through the FTIR characterization having more medicinal effect. **Fourier transform infrared spectroscopy** (**FTIR**)[7] is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique. GC is used to separate the volatile and thermally stable substitutes in a sample whereas **GC-MS** fragments the analyte to be identified on the basis of its mass[8]. So the process of ***'Suddhi'*** (intoxification method) plays a leading role in the elimination of toxicity and elucidates the potency of medicinal property. This research work will helps to identify the lead molecule in future from this purified sample and can make a pathway for safe and better therapeutic use.

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