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Research Article

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Assessment of heavy metals, phytocompounds and functional groups in the Siddha herb *Aagasagarudan Kizhangu*

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Abstract

Background: *Aagasagarudan kizhangu* (*Corallocarpus epigaeus*) is a familiar herb used in the Siddha system because of its potent medicinal effect which is being practised by many Siddha physicians and traditional Siddha healers all over Tamilnadu in various forms such as *chooranam* and *thailam*. This study is to reveal the phytocompounds, organic functional groups and heavy metal analysis of *Aagasagarudan kizhangu*. **Methods:** This tuber was purified according to the general purification method mentioned in Siddha literature *"Sikitcha Rathna Deepam"* and an evaluation study have been carried out in SAIF, IIT Madras, Chennai. **Results:** The most important heavy metals such as lead, mercury, arsenic and cadmium are present BDL (Below Detection Level) as per the WHO permissible levels in this sample. The Methanolic extract of purified *Aagasagarudan kizhangu* revealed six phytocompounds such as 9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-, Tridecanoic acid, methyl ester etc. and organic functional groups such as Alkenes, Aromatics, 1', 2' amines, amides etc which are reported with several beneficial activities and effective medicinal properties. **Conclusion:** This study also point outs the significance of purification process of this herb along with sophisticated analysis and hence this drug is medicinally beneficial and safe consumption for public.

Keywords: Siddha, Corallocarpus epigaeus, Tridecanoic acid methyl ester, Alkenes]

Introduction

Herbals are always considered as very safe for medicinal uses. Though it is not considered as harmless at every time and it too creates some damage to our body because of excessive consumption. 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 to the mankind. In siddha medical system, some purification methods for herbals were described by the siddhars for safe use [2]. Here the purified *Aagasagarudan kizhangu (AGK)*sample 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 *AGK* was comparatively analyzed to describe the detoxificatory effectiveness of siddha medicines.

Materials and Methods

The plant *Aagasagarudan kizhangu* was purified as per the siddha concept of purification method for tubers which was indicated in text ***"Sikitcha Rathna Deepam"***[3]. After it is 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. The ICP-OES, FTIR and GCMS analysis for this purified herbal has taken to know about the presence of heavy metals, organic functional groups and phytocompounds respectively in this sample. All these analysis was carried out in IIT Madras.

Figure 1: AGK – with skin

Figure 2: AGK – without skin

GC-MS:

Preparation of Plant Extracts:

The dried purified *AGK* powder (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.

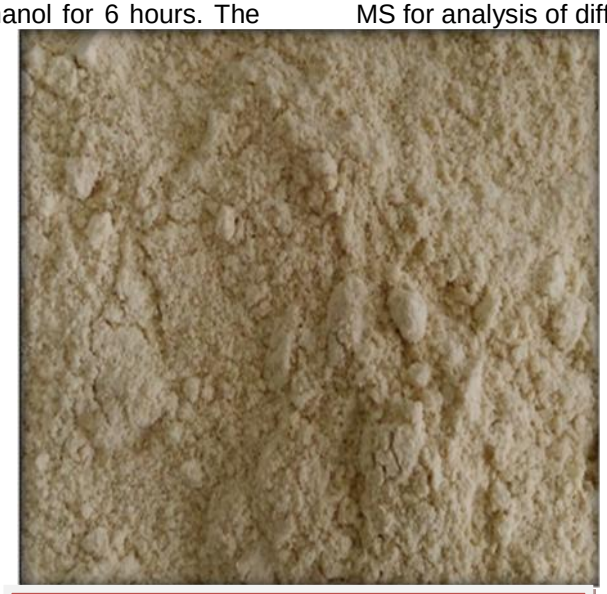


Figure 3: Purified AGK sample

GC-MS Analysis:

GC-MS analysis of the methanol extract of Purified *AG* was performed AGK 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.

ICP-OES Analysis:

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 *AGK* added with 3 ml of Nitric acid and 25 ml of Distilled water.

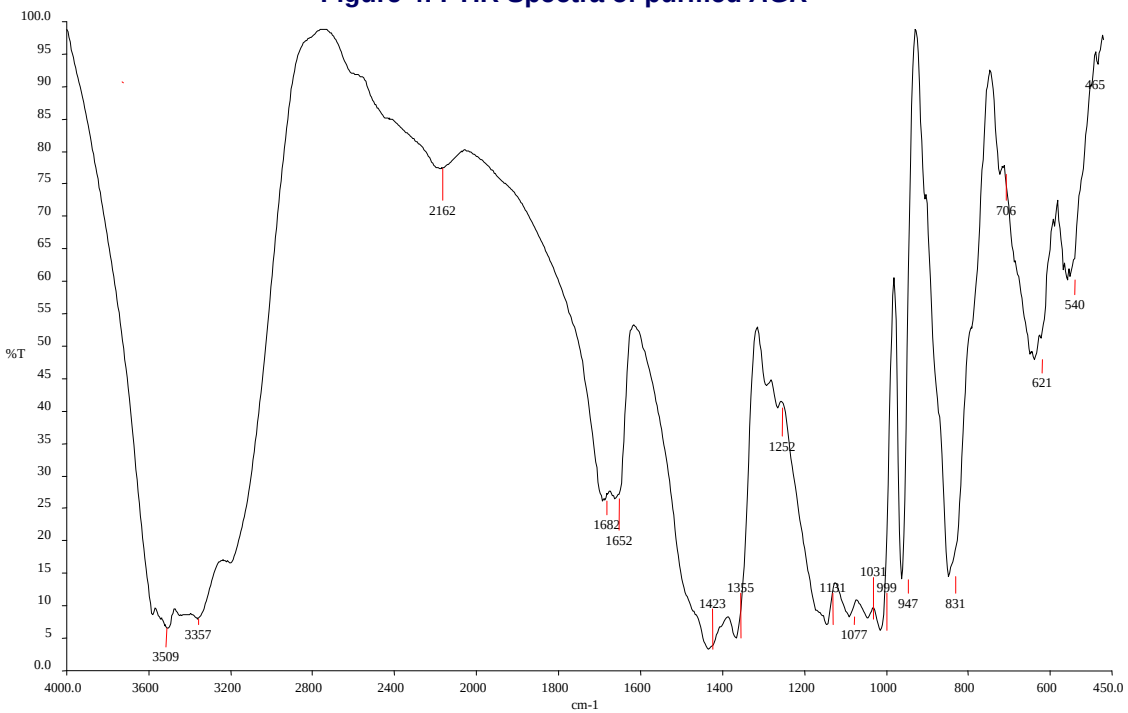
FT-IR Analysis:

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 *AGK* 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 *AGK*. The recorded spectrum shows in figure 1.

Results

FTIR Analysis:

Figure 4: FTIR Spectra of purified *AGK*



FTIR interpretation:

Table 1:

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(400-500 CM-1)	Zn-O bond	Zinc
17	540	C-Br stretch	Alkyl halides
18	465		

In the FT-IR Spectra analysis, this purified *AGK* 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, Zinc, Alkyl halides, Nitro compound, Alcohols, carboxylic acids, esters, ethers, Aliphatic amines, alkenes, Carboxylic acids, Aromatics.

GCMS Analysis:

Figure 5: GCMS spectra of purified *AGK*

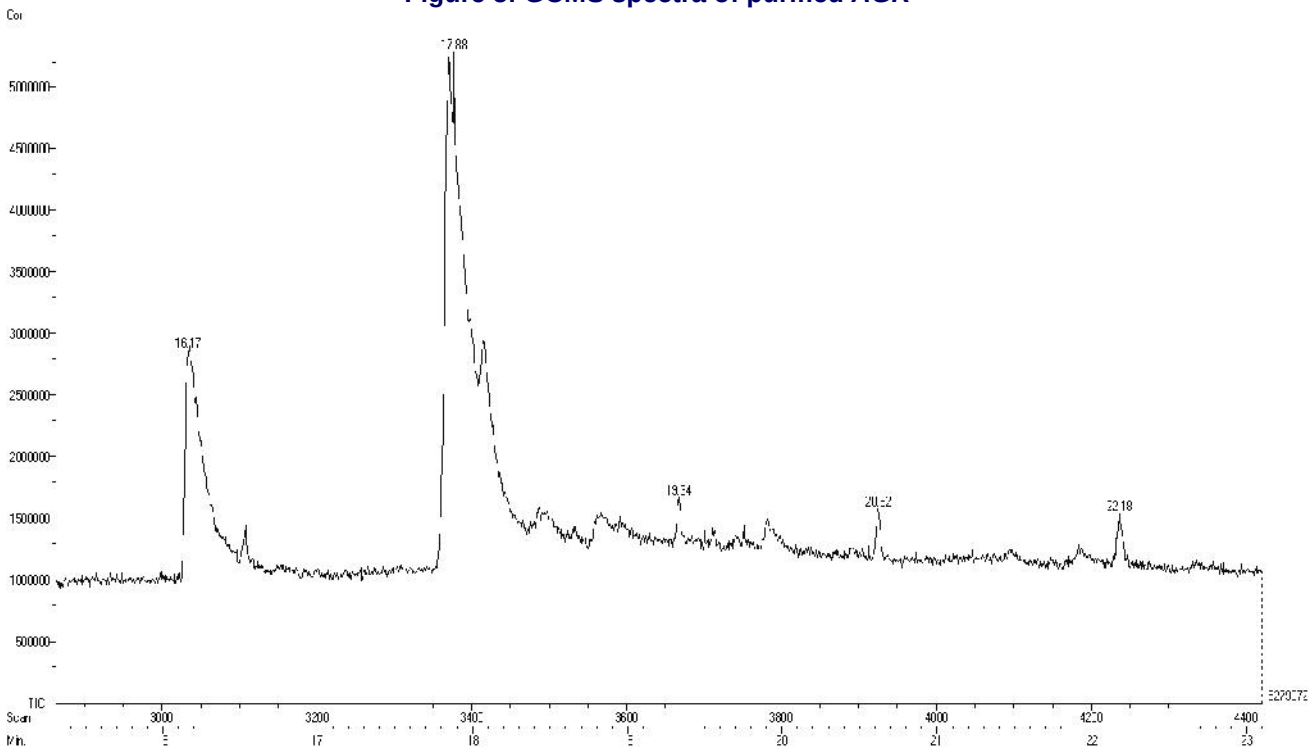


Figure 6: Phytocompound name - 9, 12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-

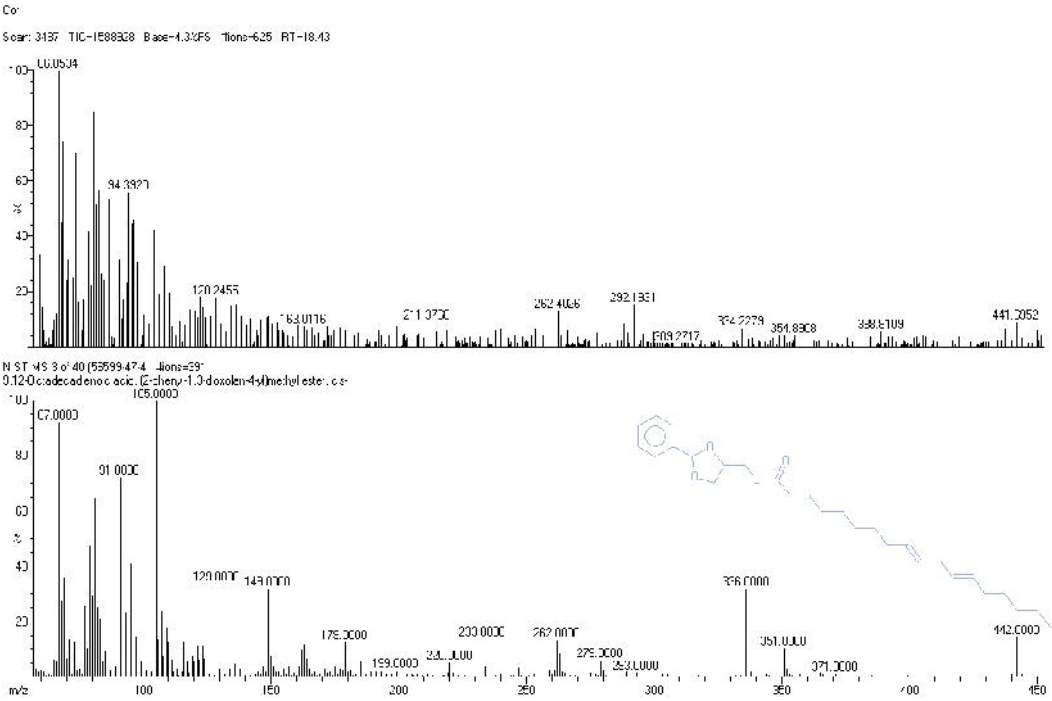


Figure 7: Phytocompound name - Tridecanoic acid, methyl ester

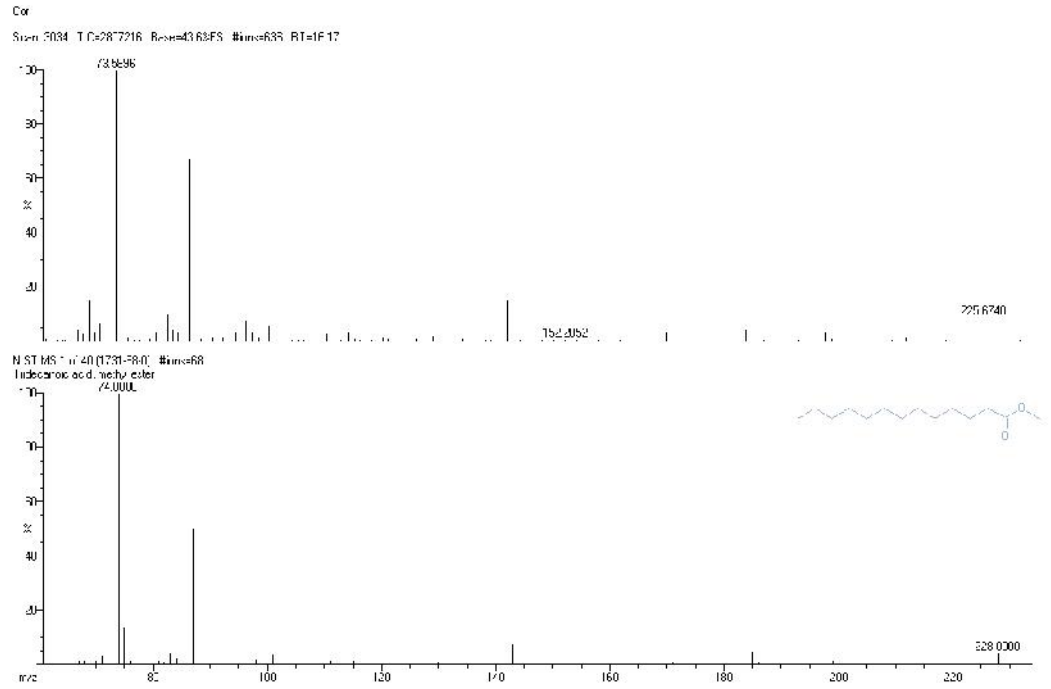


Figure 8: Phytocompound name - 9, 12-Octadecadienoic acid, methyl ester

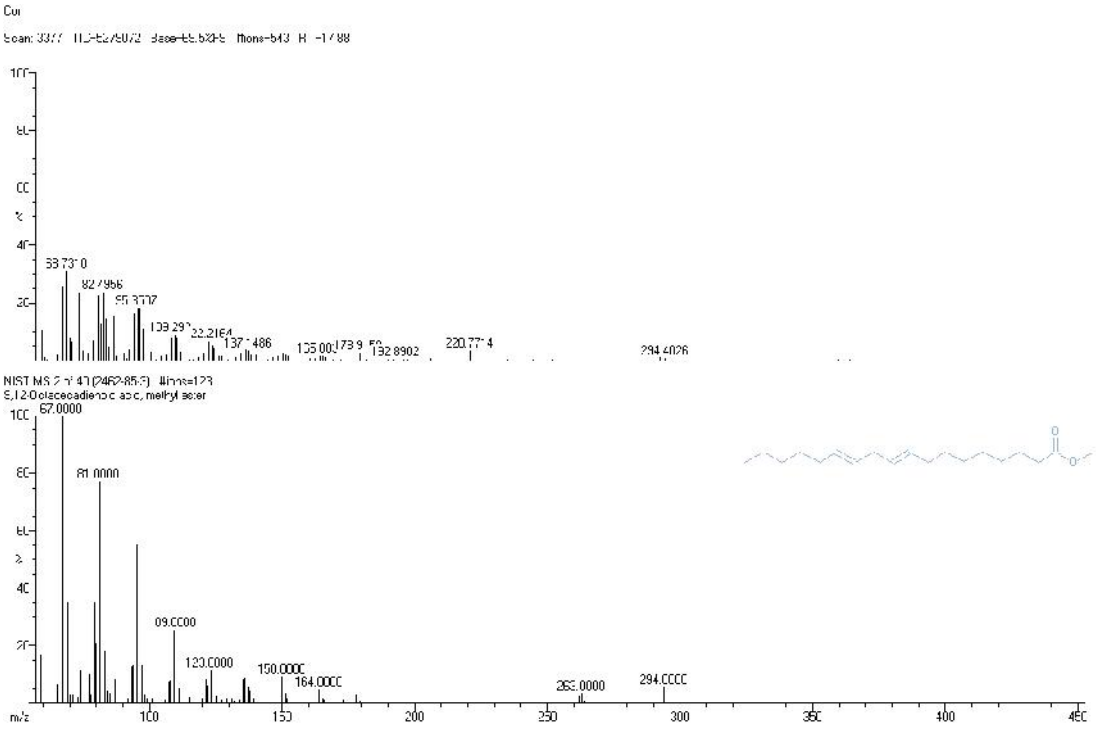


Figure 9: Phytocompound name - Psi, psi carotene, 3,4 didehydro-1, 2, 7'8' – tetrahydro-1-methoxy-2-oxo

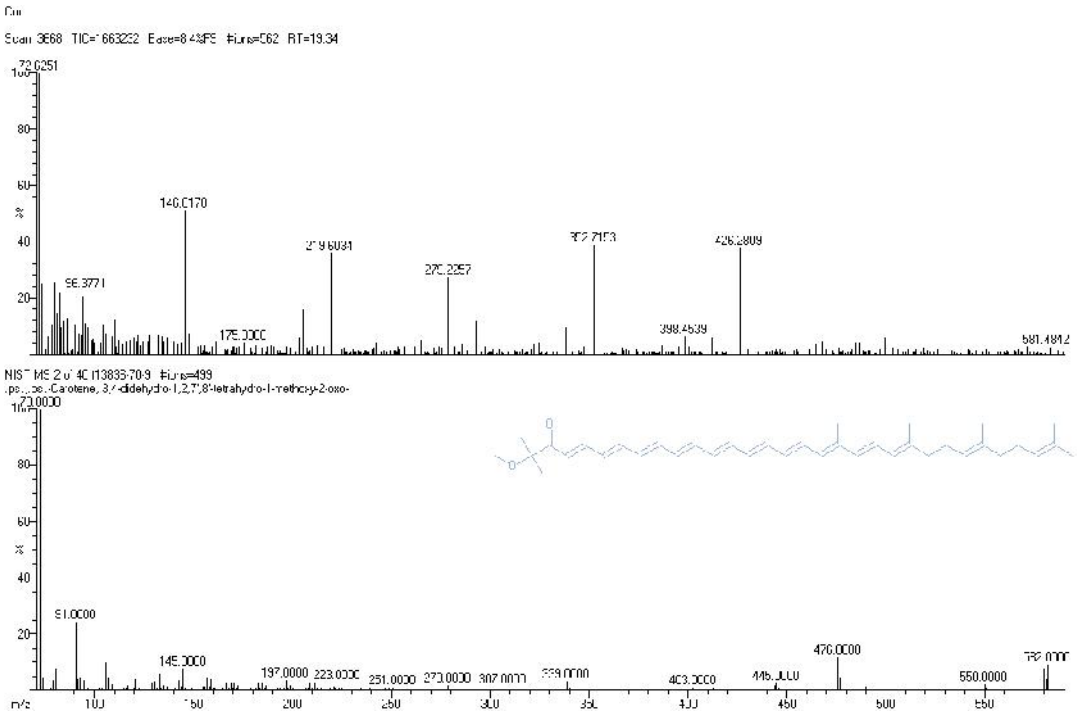


Figure 10: Phytocompound name - 1,1-Cyclobutanedicarboxamide, 2-phenyl-N-N'-bis(1-phenylethyl)-

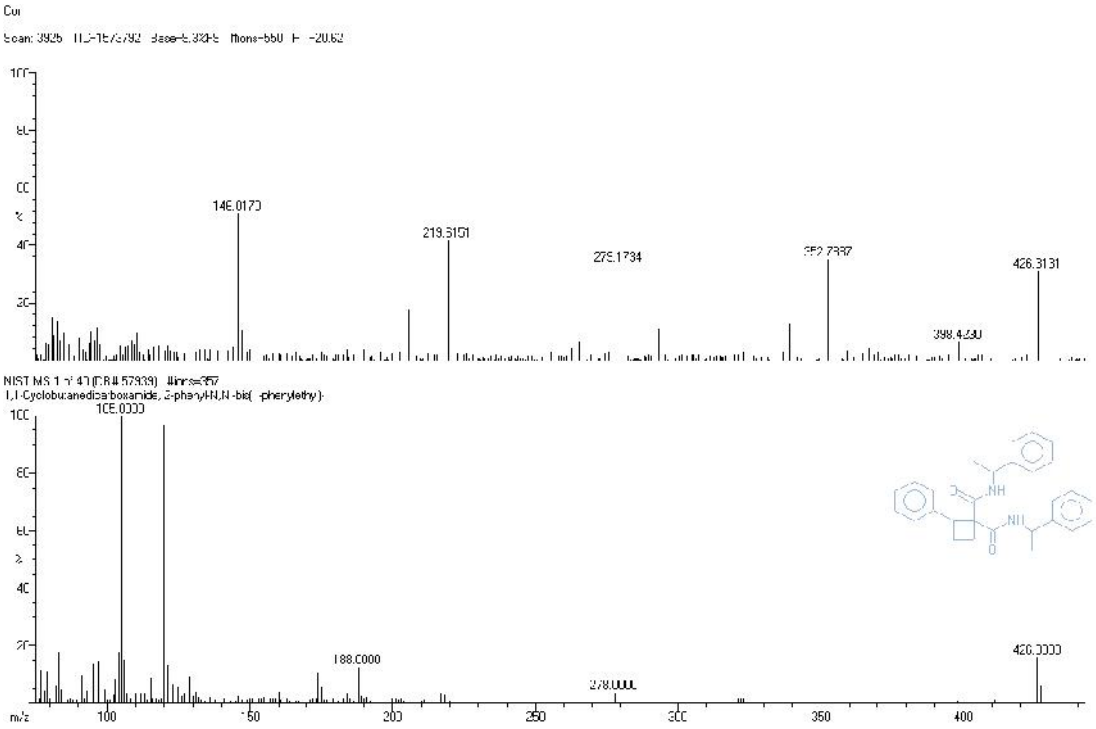
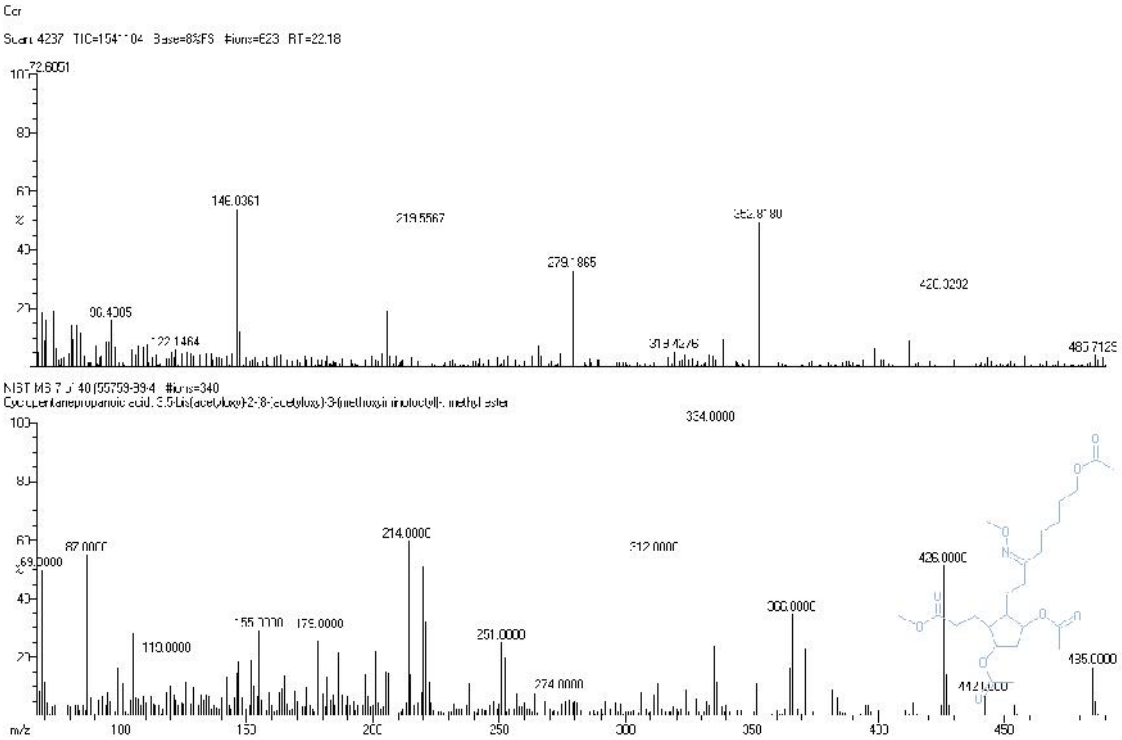


Figure 11: Phytocompound name - Cyclopentanepropanoic acid, 3,5bis(acetyloxy)-2-[-(acetyloxy)-3-(methoxyimino)octyl]-, methyl ester



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

Table 2:

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		

ICP-OES:

Table 3:

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

BDL*- Below detection level

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 *AGK*. 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 sample.

Discussion

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

This purified *AGK* extract reveals the presence of some organic functional groups such as alcohols, phenols,1‘, 2‘ amines, amides, Alkynes, Alkenes, Zinc, 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, it is confirmed that the purified *AGK* has more functional and altered functional groups when compare with the extract of unpurified tuber of *AGK*. This clearly indicates the process of purification plays a major role.

Through GCMS Analysis, the identified compounds in Purified *AGK* 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, Antihistamic, Anticoronary, Insectifuge, Hypocholesterolemic, Antieczemic, anti oxidant, anti microbial activity[6].The heavy metals assessment clears that the *AGK* is free from toxic and considered as safe for clinical use.

Conclusion

From this research study, using ICP-OES analysis 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 FTIR characterizations on purified *AGK* create the fingerprints for standardization of this sample. If further research studies on these identified compounds of this purified sample through GCMS could help the scientific community to develop the new drug to treat diseases such as virulent poison, leprosy etc., which is indicated in Siddha science. The compounds identified through the GCMS analysis and the functional groups which were identified through the FTIR spectra having more medicinal effect. Fouriertrans form 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 (GCMS) is a hyphenated analytical technique. GC is used to separate the volatile and thermally stable substitutes in a sample whereas **GCMS** fragments the analyte to be identified on the basis of its mass [8]. Hence the process of ***'Suddhi'*** (Purification method) plays a leading role in the elimination of toxicity and elucidates the potency of medicinal property. This research work will helps to make a pathway for safe and better therapeutic use for the public.

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References

[1]. Junhua Zhang, IghoJ.onakpoya, Paul posadzki, Mohamed Eddouks, The Safety of Herbal Medicine: From Prejudice to Evidence**,** Evidence-Based Complementary and Alternative Medicine Volume 2015 (2015), Article ID 316706, 3 pages

[2]. *Sarakku Sudhi Muraikal*, Aanaivaari Aanandan, Department of Indian medicine and homoepathy department, 2008

[3]. C.Kannusamipillai, *Sikitcharathna Deepam*, Rathnanaicker & sons, 2007, Page no.32

[4]. Northern Illinois University, Chemistry Analytical lab, FT-IR sample preparation. 2007.http://www.niu.edu/ANALYTICALLAB/ftir/samplepreparation.shtml (06 Oct 2013)

[5]. Saranya N[1*], Rangabhashiyam S , Rubini D Sivaranjani C R, Screening of Functional Groups, DNA Quantification and Determination of Antimicrobial Potency of Corallocarpus epigaeus Tubers, International Journal of Pharmaceutical and Clinical Research 2015; 7(1): 1-4

[6]. Never Zekeya, Musa Chacha, Francis Shahada and Abdul Kidukuli, Analysis of phytochemical composition of Bersamaabyssinica by gas chromatography – mass spectrometry, Journal of Pharmacognosy and Phytochemistry 2014; 3(4): 246-252

[7]. Griffiths, P.; de Hasseth, J.A., Fourier Transform Infrared Spectrometry (2nd ed.), https://en.wikipedia.org, Wiley-Blackwell. (18 May 2007), ISBN 0-471-19404-2.

[8]. Ashish Chauhan, Manish Kumar Goyal and Priyanka Chauhan, GC-MS Technique and its Analytical Applications in Science and Technology, Journal of Analytical & Bioanalytical Techniques, Volume 7, Issue 6, November 17, 2014.

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