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GC-MS ANALYSIS OF BIOACTIVE COMPONENTS OF KARISALAI KARPA CHOORANAM- A SIDDHA POLY HERBAL FORMULATION

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ABSTRACT

Karisalai Karpa Chooranam (KKC) is a Polyherbal formulation. It consists equal quantity of dried whole plant powders of *Eclipta prostrata*, *Wedelia chinensis*, *Centella asiatica*, *Acalypha indica*, *Indigofera tinctoria* and *Sphaeranthus indicus*. In the siddha system of medicine, KKC has been used as an Immunomodulator, Hepatoprotective and Anti-ageing drug. In the present study, the qualitative analysis of ethanolic extract of KKC was carried out using GC-MS for the identification of bioactive components. The nature and structure of the bioactive compounds were identified at different time intervals using mass spectrometer. The finger prints of the compound were identified from The National Institute of Standard and Technology (NIST) library database. The result of GC-MS analysis of KKC revealed the existence of Phenols, Saturated fatty acids, Alkane, Ketone, Diterpenoids, Esters and other bioactive components.

Key words: Karisalai Karpa Chooranam, GC-MS, NIST.

INTRODUCTION

It is a well-known fact that Traditional Systems of medicines always played important role in meeting the global health care needs (Ravishankar, 2007) India has the unique distinction of having six recognized systems of medicine. They are Ayurveda, Siddha, Unani, Yoga, Naturopathy and Homoeopathy. Botanicals are the vital part of these traditional medicines. Although the traditional Indian system of medicine has a long history of use, they required adequate scientific validation (WHO, 1998). Siddha system of medicine is one of the oldest systems of medicine practiced in South India especially in Tamil Nadu. Karisalai Karpa Chooranam (KKC) is a Siddha polyherbal formulation which consists equal quantity of dried whole plant powders of *Eclipta prostrata* (*Asteraceae*), *Wedelia chinensis* (*Asteraceae*), *Centella asiatica* (*Apiaceae*), *Acalypha indica* (*Euphorbeaceae*), *Indigofera tinctoria* (*Fabaceae*) and *Sphaeranthus indicus* (*Asteraceae*). The method of preparation and therapeutic uses of this formulation is mentioned in ancient text *Bogar 7000*. Another name of Bogar *7000* is *Bogar Sapthakandam* (Loganathan 2007). This polyherbal formulation traditionally used for the treatment of liver enlargement, hepatitis, leg swelling, anemia, ascites, black patches on the skin, eye disorders and it increases immunity (Siddha Formulary of India, 1992). The present research work is designed to identify the bioactive components which are present in the polyherbal formulation.

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MATERIALS AND METHODS

Plant collection

The botanical species of *Eclipta prostrata*, *Wedelia chinensis*, *Centella asiatica*, *Acalypha indica*, *Indigofera tinctoria* and *Sphaeranthus indicus* were collected from rural areas of Thanjavur and Herbal garden, PRIST University, Thanjavur, Tamil Nadu, India. All of the plant materials are authenticated by scientist

Dr.G.V.S.Murthy, Botanical Survey of India (BSI), Ministry of Environment and Forests, Coimbatore, Tamilnadu, India. Voucher specimens (BSI/SRC/5/ 23/2011-12/Tech-1046, 47, 48, 49, 50, 51) of the same have been deposited in the Department of Pharmacy, PRIST University for future reference.

Preparation of the sample

In house formulation of sample was prepared by the method described in the classical Siddha literature *'Bogar 7000'*. As mentioned in the text, entire part of each plant including leaf, stem, root, flower and seeds were collected and dried under shade for 7 days. After drying, each plant material was finely powdered and sieved (40#). After sieving, equal quantity of each plant powders mixed together. Finally it was stored in airtight container and used for further studies.

Extraction

The powdered sample of KKC was extracted with 500ml of 90% ethanol by using soxhlet apparatus until the extraction was completed. After the completion of the extraction process, the extract was filtered and the solvent was removed by distillation under reduced pressure (Raaman, 2006) Dark green colour residue was obtained. The percentage yield of the extract was 18 %. It was used for GC-MS analysis.

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

GC-MS analysis was carried out using Shimadzu GC-MS QP 2010 Plus consist of a Automatic operation controller (AOC-20i auto injector) and Gas Chromatograph interfaced to a Mass Spectrometer. Restek Rtx- 5MS capillary column (Diameter 0.32mm, length 30m, thickness 0.50μm) employing for separation of components and operating in electron impact Ionization mode at 70eV. Helium was used as carrier gas at a constant flow of 1.73 ml/min and an injection volume of 0.5μl was employed. The temperature of Injector and ion source were maintained at 270^0C and 200^0C respectively. The oven temperature was programmed from 50^0C for 2 min, inversed to 150^0C at 8^0C/min and to 240^0C at 8^0C/min, and held at 270^0C for 20min. Mass spectra were obtained at 70eV. A scan interval is 0.5 seconds and fragments from 40 to 450 Da. The amount of each component was calculated on relative percentage basis comparing its average peak area to the total areas. The Total Ion Chromatogram (TIC) was created by summing up intensities of all mass spectral peaks. The TIC was compared with GC chromatogram. Turbo Mass Version 5.2.0 Software was adopted to handle mass spectra and chromatograms (Karpagasundhari, 2014).

Identification of compounds

The compounds were identified by interpretation of the spectrum of the unknown compounds with the spectrum of the known compounds mentioned in The National Institute of Standard and Technology (NIST) library database.

RESULTS AND DISCUSSION

Table 1 represents the Compounds Identified in Ethanolic extract of *Karisalai Karpa Chooranam* using GC-MS Analysis. In this analysis, 25 compounds were identified. Figure 1 shows the peak values identified in the ethanolic extract of *Karisalai Karpa Chooranam*. The results of GC-MS investigation of ethanolic extract of KKC led to the recognition of many compounds. These compounds were recognized through mass spectrum attached with Gas chromatogram. The components with their retention time (Rt), molecular formula, molecular weight, and peak area (%) are mentioned in table 1. 2,2' Bioxirane (0.20%), Butanoic acid (0.55%), Methyl 2-oxopropanoate (0.23%), Phenol (3.03%), 2,4 dihydroxyacetophenone -(2.44%), D-Erythro-Pentose, 2-Deoxy- (0.88%), Cyclopentasiloxane, decamethyl-(0.26%), 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethyl siloxy) tetrasiloxane (0.25%), Dodecanoic acid (1.87%),2 (3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-4a-methyl-(0.29%), Myristic acid (7.20%),9-Octadecanoic acid (Z) (0.22%),2,6,10-Trimethyl,14-Ethylene-14-Pentadecen- (0. 55%),2-Pentadecanone,6,10,14-Trimethyl- (0.47%),Penta decanoic acid(0.65%), 3,7,11,15-Tetramethyl-2-Hexa decen -1-ol (0.26%),Hexadecanoic acid, methyl ester (0.67%),cis-10-Nonadecenoic acid (1.29%),1-(+)-Ascorbic acid 2,6-dihexadecanoate (31.30%), Andro grapholide (1.23%),Methyl 9-cis,11-trans-octade cadienoate (0.54%),11- Octadecanoic acid, methyl ester (1.53%),Methyl stearate (0.81%),Cyclopentadecanone,2-hydroxy- (29.89%), Octadecanoic acid (13.36%).

The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The heights of the different peaks indicate the relative concentration of the different components present in the sample. The finger prints of the compound which can be identified from The National Institute of Standard and Technology (NIST) library database.

The result exposed that the presence of 2, 2' Bioxirane, is recommended to be a Diepoxybutane. Butanoic acid is recommended to be a butyric acid and it might act as an anti mutagenic. Methyl 2-oxopropanoate is recommended to be a methyl ester and it might act as an insulin stimulator (Lembert *et al.*,2001), (Zawalich, 1997). Phenol is recommended to be an aromatic organic compound and it might act as an antioxidant, immunomodulator, Anti carcinogenic and Anti-inflammatory, 2, 4 dihydroxyacetophenone is recommended to be a ketone and it might act as an Anti Oxidant (Basher *et al.*, 2002). D-Erythro-Pentose, 2-Deoxy- is recommended to be a deoxy sugar and it might act as a Platelet activator (Phillips, 1986),

Cyclopentasiloxane, decamethyl is recommended to be a siloxane D5. 3-Isopropoxy-1, 1, 1, 7, 7, 7- hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane is recommended to be a Siloxane. Dodecanoic acid is recommended to be a saturated fatty acid and it might act as an antimicrobial and increases HDL cholesterol level (Nakatsuji, *et al.*, 2009), (Yang *et al.*, 2009), (Mensink *et al.*, 2003). 2(3H)-Naphthalenone, 4, 4a, 5, 6, 7, 8-hexahydro-4a-methyl is recommended to be a ketone and it might act as an Anti-inflammatory (Murugesan, 2014). Tetradecanoic acid is recommended to be a saturated fatty acid and it might act as a lipid anchor in biomembranes, Anxiolytic(Carlos M. Contreras *et al.*, 2014). 9-Octadecanoic acid (Z) is recommended to be a saturated fatty acid and it might act as an anti hypertensive and increases HDL cholesterol decreases LDL level (Teres *et al.*, 2008). 2,6,10-Trimethyl,14-Ethylene-14-Pentadecen is recom mended to be a alkane. 2-Pentadecanone,6,10,14-Trimethyl- is recommended to be a ketone ,Pentadecanoic acid is recommended to be a saturated fatty acid. 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol is recommended to be an alcohol. Hexadecanoic acid, methyl ester is recommended to be a saturated fatty acid and it might as act as an Antioxidant, hypocholesterolemic, anti androgenic, hemolytic and alpha reductase inhibitor (Sermakkani, 2012). cis-10-Nonadecenoic acid is recommended to be a monosaturated fatty acid and it might act as an Antitumor (Fukuzawa *et al.*, 2008). 1-(+)-Ascorbic acid 2,6-dihexadecanoate is recom mended to be a ester and it might act as an Antioxidant, Vitamin C and Immunomodulator (Elizebeth Thomas *et al.*, 2013). Andrographolide is recommended to be a diterpenoid and it might act as an anti-inflammatory and Anti cancer (Lim JC, 2012). Methyl 9-cis,11-trans-octadecadienoate is recommended to be a ester and it might act as an anti tumor (Belury *et al.*, 1996). 11- Octadecanoic acid, methyl ester is recommended to be a saturated fatty acid and it might act as an anti cancer. , Methyl stearate is recommended to be methyl ester. Cyclopentadecanone,2-hydroxy is recommended to be a ketone. Octadecanoic acid is recommended to be a saturated fatty acid and it might act as a cholesterol lowering agent (Hunter, 2009). Table 2 shows the nature and biological activities of the predicted compounds.

Table 1. Compounds Identified in Ethanolic extract of *Karisalai Karpa Chooranam*

S.No	R_t	Name of the Compound	Molecular Formula	Mol. weight	Peak area %
1	3.033	2,2' Bioxirane	$C_4H_6O_2$	86	0.20
2	3.667	Butanoic acid	$C_4H_8O_2$	88	0.55
3	3.782	Methyl 2-oxopropanoate	$C_4H_6O_3$	102	0.23
4	6.820	Phenol	C_6H_6O	94	3.03
5	6.880	2,4 dihydroxyacetophenone	$C_{14}H_{24}O_3Si_2$	296	2.44
6	8.828	D-Erythro-Pentose, 2-Deoxy-	$C_5H_{10}O_4$	134	0.88
7	9.475	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	370	0.26
8	15.615	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576	0.25
9	16.811	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.87
10	18.978	2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-4a-methyl-	$C_{11}H_{16}O$	164	0.29
11	19.638	Myristic acid	$C_{14}H_{28}O_2$	228	7.20
12	20.537	9-Octadecanoic acid (Z)	$C_{18}H_{34}O_2$	282	0.22
13	20.608	2,6,10-Trimethyl,14-Ethylene-14-Pentadecen-	$C_{20}H_{38}O$	278	0.55
14	20.713	2-Pentadecanone,6,10,14-Trimethyl-	$C_{18}H_{36}O$	268	0.47
15	20.874	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.65
16	21.124	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{20}H_{40}O$	296	0.26
17	21.615	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.67
18	21.835	cis-10-Nonadecenoic acid	$C_{19}H_{36}O_2$	296	1.29
19	22.022	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	31.30
20	23.101	Andrographolide	$C_{20}H_{30}O_5$	350	1.23
21	23.492	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294	0.54
22	23.539	11- Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296	1.53
23	23.792	Methyl stearate	$C_{19}H_{38}O_2$	298	0.81
24	23.987	Cyclopentadecanone,2-hydroxy-	$C_{15}H_{28}O_2$	240	29.89
25	24.219	Octadecanoic acid	$C_{18}H_{36}O_2$	284	13.36

Table 2. Pharmacological activities of components identified in the ethanolic extract of KKC

S.No	Name of the Compound	Compound Nature	Activity reported
1	2,2' Bioxirane	Diepoxybutane	Antineoplastic
2	Butanoic acid	Butyric acid	Anti cancer
3	Methyl 2-oxopropanoate	Methyl ester	Increases Insulin secretion
4	Phenol	Aromatic organic	Antioxidant,anticarcinogenic, antiinfammatory
5	2,4 dihydroxyacetophenone	Ketone	Antioxidant
6	D-Erythro-Pentose, 2-Deoxy-	Deoxy sugar	Involved Platelet Activation
7	Dodecanoic acid	Saturated fatty acid	Antimicrobial, increase HDL level,
8	2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-4a-methyl-	Ketone	Anti inflammatory
9	Tetradecanoic acid	Saturated Fattty acid	Act as lipid anchor in biomembranes, Anxiolytic.
10	9-Octadecanoic acid	Oleic acid	Antihypertensive, Increase HDL and decrease LDL Cholesterol.
11	Hexadecanoic acid, methyl ester	Palmittic acid	Antioxidant, hypocholesterolemic , Anti androgenic , hemolytic, Alpha reductase inhibitor.
12	cis- 10 Nondecanoic acid	Saturated Fatty acid	Antitumor
12	1-(+)-Ascorbic acid 2,6-dihexadecanoate	Ester	Vitamin c, antioxidant, immunomodulator
13	Andrographolide	Diterpenoid	Cell signaling, immunomodulator, used in stroke.
14	Methyl 9-cis,11-trans-octadecadienoate	Ester	Anti cancer
16	11- Octadecanoic acid, methyl ester	Ester	Anti tumour
17	Octadecanoic acid (stearic acid)	Saturated fatty acid	Lower LDL Cholesterol level

Figure 1. The GC-MS Chromatogram of ethanolic extract of *Karisalai Karpa Chooranam*.

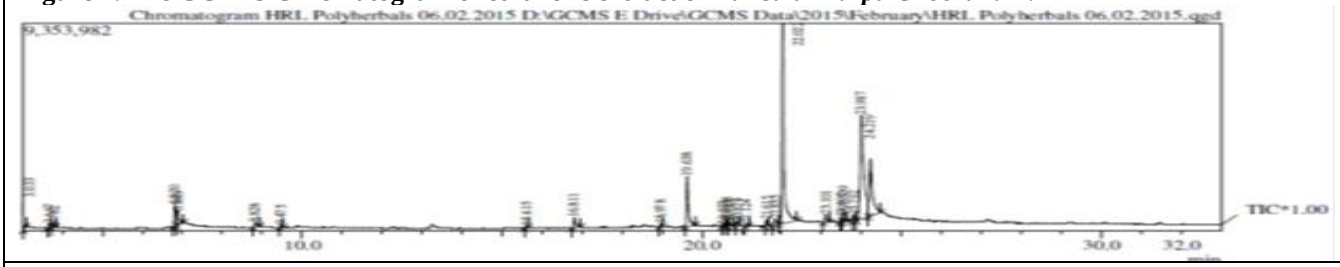


Figure 2-6. The individual fragmentation pattern of the important compounds present in the *Karisalai Karpa Chooranam*.Figure 2: Phenol

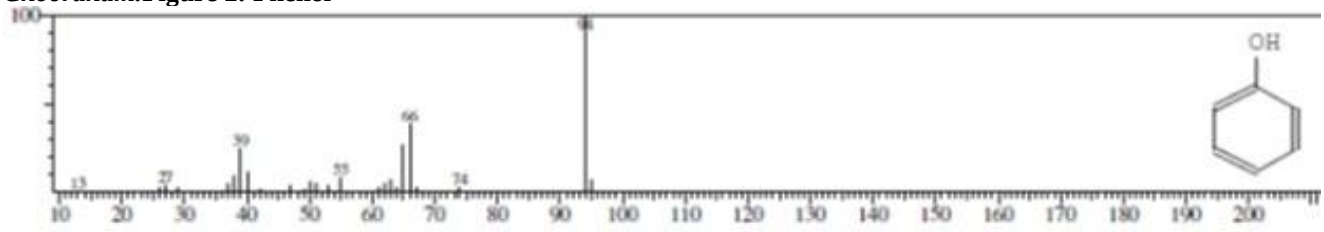


Figure 3. 2, 4 dihydroxyacetophenone

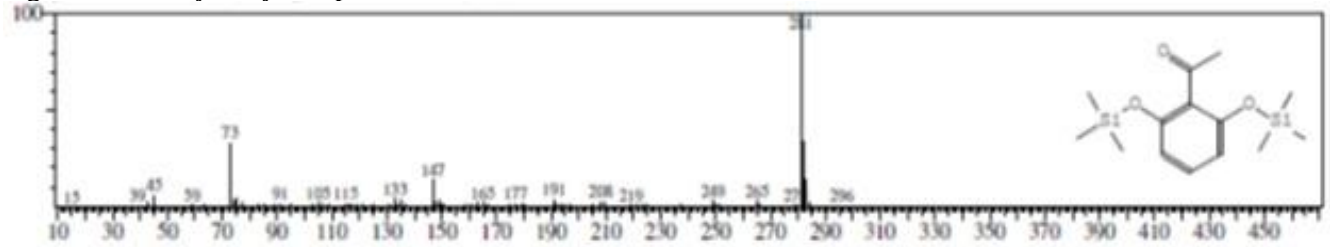


Figure 4. 1-(+)-Ascorbic acid 2,6-dihexadecanoate

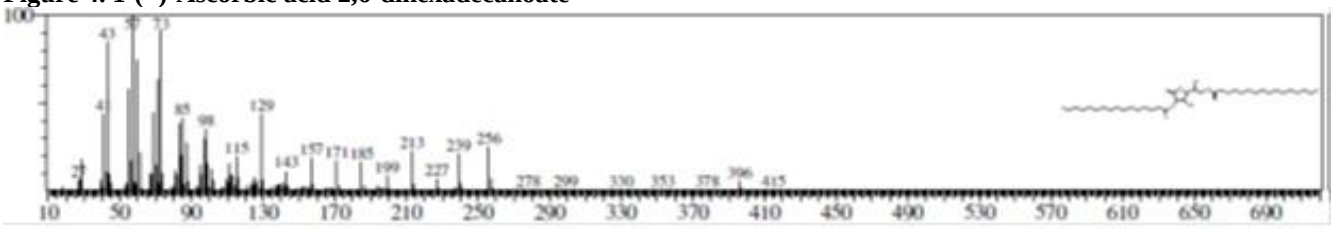
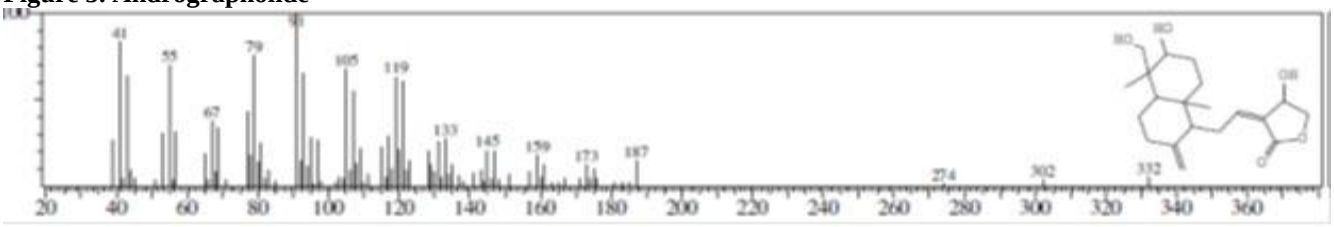


Figure 5. Andrographolide



CONCLUSION

The present study characterized the phytochemical profile of the polyherbal formulation Karisalai Karpa Chooranam using GC-MS. The chromatogram shows the comparative concentration of different components getting eluted as a purpose of retention time. The heights of the different peaks indicates the relative concentration of the compounds exist in the ethanolic extract of KKC. The mass spectrometer analyses the compounds which were eluted at different time intervals to recognize the nature and structure of the compounds. These spectrum are finger print of the compound which can be identified from the NIST library. The identification of various bioactive compounds confirms the therapeutic application of KKC for a variety of diseases. Further research is in progress for the pharmacological evaluation of KKC.

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