Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):393-400

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Preliminary GC-MS analysis of an Ayurvedic medicine "Kulathadi Kashayam"

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ABSTRACT

Kulathadi kasahaym is an ayurvedic formulation used for treatment of Amenorrhoea. The main ingredients of this medicine seeds of horse gram, sesame, errand and garlic. This is a liquid formulation. GC MS analysis of this medicine is done in the present study to know the various bioactive compounds present therein. It was found that major peaks represented Benzoic acid, Diethyl Phthalate, Tetratricontane, Octadecanoic acid, n-Hexadecanoic acid, Heptacosane 1-Chloro, Eicosane, Phenol, 2,4-bis(1,1-dimethylethyl)-, Eicosane, 2-methyl-, Nonadecane, 2-methyl-, Hexadecane 2,6,11,15- tetramethyl-, Heptadecane, 2,6,10,15-tetramethyl- and Eicosane, 10-methyl- derivative. The medicinal values of most of these compounds indicate that this medicine does have a scientific indication as an effective cure for Ammenorrhorea. Further work is in progress to prove the validity of this medicine.

Key words: Kulathadi Kashayam, GC MS, horse gram, Garlic, Errand, Sesame, Ammenorrhorea, Benzoic acid.

INTRODUCTION

The use of contemporary and alternative medicines among the population the world over is time tested. Ayurvead and Sidha in India are age old systems of treatment. The Ayurvedic formulations in the form of solids, semi solids, liquids, pastes and powders are in use for more than 3000 years in India. But these medicinal practices are not getting their due in the international scenario of Medical practice due to lack of scientific evidence and proven efficacy. Some of the factors for this state of Ayurveda and Sidhha medical system is discussed by Raj *et al*, 2011; Rao *et al*, 2015a, b; Ravi *et al*, 2015). [1, 2, 3, 4] Pordie' and Gaudillie're 2014, have discussed in datails about the factors involved in polyherbal formulation in drug discovery and related issues pertaining to Ayurveda as an industry. [5] The present article tries to understand the presence and role of the bioactive compounds present in one such liquid Ayurvedic formulation, namely, Kukathadi Kashayam by GC MS analysis.

Kulathadi kashayam is an Ayurvedic formulation to treat Amenorrhoea and Cryptomenorrhoea. This formulation is made up the following ingradients:

1. Kulatha - Horse gram (*Dolichos biflorus*) seeds
2. Lahsuna- Garlic (*Allium sativum*)
3. Eranda- *Ricinus communis* (seeds)
4. Tila- *Sesamum indicum* (Seeds)

One part each of the above mentioned ingredients are made to coarse powder and mixed througly. One part of this powder is boiled in 16 parts of water till the volume reduces to 4 parts. This is Kulathadi kashayam which is bottled, sealed and used as medicine. The dose is 5 to 10 ml before food in empty stomach once or twice or as advised by the medical practitioner. This formulation is prepared according to the Ayurvedic treatise, Yogagrantha. The manufacturer of this formulation is Arya Vaidya Sal, Kottakkal, Kerala State, India. The four plant ingredients which are used for the preparation of this formulation have many medicinal values. Some of the medicinal properties of each plant are discussed in the following paragraphs.

Dolichos biflorus

This is common vegetable of India belonging to Leguminosae family. The antilithiatic activity of the aqueous extracts of the seeds is a well known folklore medicine in India. The seeds contain a dimeric protein of 98 kD molecular weight which is a crystal growth inhibitor. (Bijamia *et al*, 2009). [6] Atodariya *et al*, 2013 have reported that the aqueous fractions of the seeds have very good capacity to dissolve calcium oxalate crystals. [7] The hypolipidemic role of the seed extract is shown in rats by Menaka and Helitha, 2013. [8]The seed extract is antihistamic (Sularkar *et al*, 2013 a, b) and the seed lecithin has lipoxygenase activity (Subbaiah *et al*, 2009). [9, 10, 11]

Garlic (*Allium sativum*)

Garlic is one of the oldest medicinal having various beneficial activities. The organo-sulphur compounds present in it are known to treat bacterial and fungal infections, cardiovascular diseases and cancer (Raghu *et al*, 2012). [12] In their review Thomson and Ali, 2003, have mentioned the role of garlic as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and as antitumor. [13]The anticancer activities of garlic are attributed to the organo-sulphur compounds present in Garlic (Karmakar *et al*, 2011). [14]

3. Erand (Castor)

The oil of Erand is commonly used in India as purgative for children and also as lamp oil. The medicinal role of Erand has been reported by many researchers. Rachhadiya *et al*, 2011, have reported the cytoprotective role of the oil of Erand on gastric mucosa thus reducing the risk of ulcer formation. [15]The castor oil is shown to have lypolytic (Lombard *et al*, 2001), antidiabetic (Shokeen *et al*, 2008), antibacterial (Verma *et al*, 2011, Islam *et al*, 2010), anti-inflammatory (Saini *et al* 2010; Valderramas *et al*, 2008), wound healing (Prasad *et al*, 2011) hepatoprotective Natu *et al*, 1977), antioxidant (Singh *et al*, 2010; Oloyede, 2012) activities. [16-25] Darmanin *et al*, 2009, have reported the cytotoxic and apoptotic activity of castor. [26]

4. Sesame

The nutritional, medicinal and industrial use of Sesame is elaborately reviewed by Raghavan *et al*, 2010. [27] It contains two compound sesamin and sesamolin, which control blood pressure by lowering cholesterol in the blood. Nigam *et al*, 2015 have shown the antioxidant and antibacterial properties of sesame. [28]The anti-hyperlipidemic activity of sesam was demonstrated by Asgary *et al*, 2010. [29]

EXPERIMENTAL SECTION

Kulatahdi Kashayam was procured from a standard local Ayurvedic shop at Chennai.

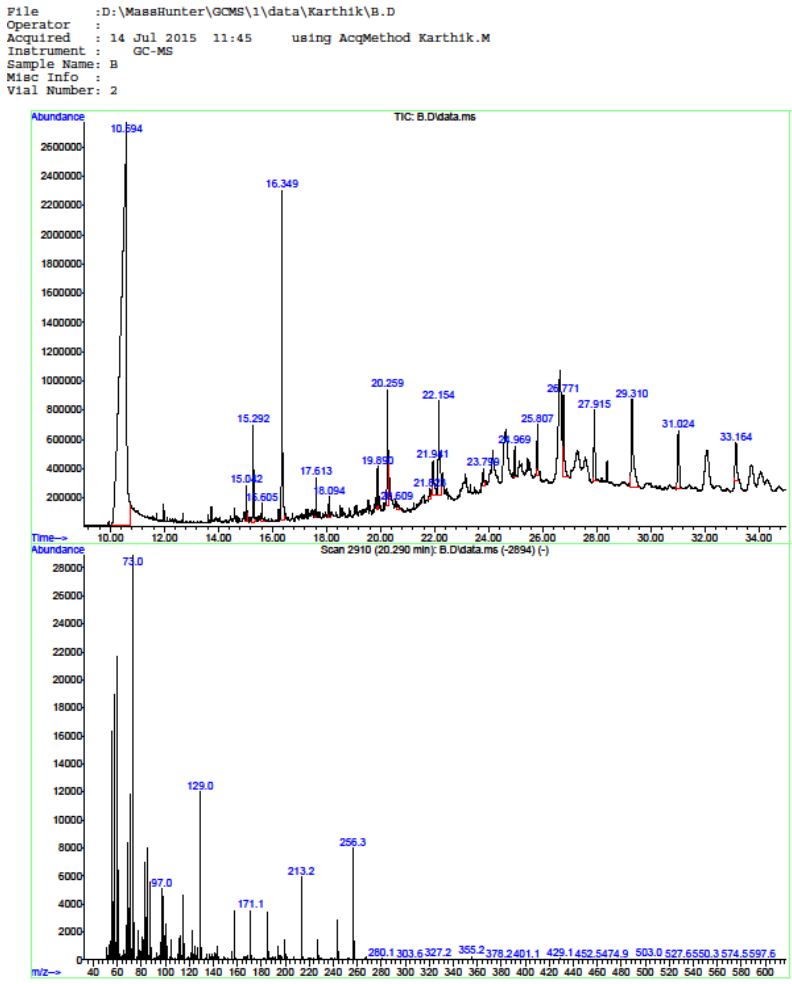
The medicine which is available in liquid form was subjected to GC MS analysis as per standard procedure. (Figure 1)

The metabolites in the samples were identified using a P2010 gas chromatography with thermal desorption system TD20 coupled with mass spectroscopy (Shimadzu). The ionization voltage 70ev and GC was conducted in the temperature programming mode with a Restek column (0.25mm, 60m, XTI-5). The temperature in the initial column was 80^0c for 1 min, and then increased linearly to 70^0c to 220^0c held for 3 min followed by linear increased temperature 100^0 c up to 290^0c and held for 10min. The injection port temperature was 290^0 c and the GC/MS

interface was maintained at 29^0c, the samples were introduced via an all glass injector working in the split mode with helium carrier gas low rate with 1.2 ml per minute. The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS. The relative percentage of each extract constituent was expressed with peak area normalization.

RESULTS AND DISCUSSION

Figure 1. The GC MS Graph of Kulathadi Kashayam



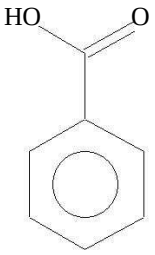
The GC MS data of Kulathadi Kashayam is shown in Table 1.

Table 1

Sl No.	Retention Time (In Min)	Name of the Compound	Molecular formula	Molecular Weight	Peak (%)	(%) Probability
1	10.594	Benzoic acid	C7H6O2	122	62.236	46.1
2	15.042	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	296	0.630	7.23
3	15.292	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	1.967	60.5
4	15.605	Hexadecane	C16H34	226	0.394	7.97
5	16.349	Diethyl Phthalate	C12H14O4	222	7.362	68.6
6	17.613	Hexadecane, 2,6,11,15-tetramethyl-	C20H42	282	0.665	7.53
7	18.094	Heptadecane	C17H36	240	0.337	7.86
8	19.890	Nonadecane, 2-methyl-	C20H42	282	0.782	6.48
9	20.259	n-Hexadecanoic acid	C16H32O2	256	2.882	75.4
10	20.609	Heptadecane, 9-hexyl-	C23H48	324	0.314	15.7
11	21.823	Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-	C39H80O2	496	0.401	7.79
12	21.941	Eicosane, 2-methyl-	C21H44	296	1.400	7.14
13	22.154	Octadecanoic acid	C18H36O2	284	3.519	73.3
14	23.799	Heptadecane, 9-hexyl-	C23H48	324	0.379	6.27
15	24.969	Eicosane, 10-methyl-	C21H44	296	0.564	10.5
16	25.807	Eicosane	C20H42	282	1.184	10.2
17	26.771	Heptacosane, 1-chloro-	C27H55Cl	414	2.801	16.5
18	27.915	Eicosane	C20H42	282	2.120	13.5
19	29.310	Tetratriacontane	C34H70	478	5.141	6.72
20	31.024	Eicosane	C20H42	242	2.529	7.65
21	33.164	Tetratriacontane	C34H70	478	1.822	9.52

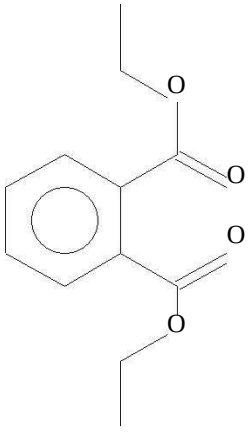
The important biomolecules present in Kulathadi kashayam are represented by Figure no. 2 to 13.

Figure 2. Benzoic Acid



Name: Benzoic acid
Formula: $C_7H_6O_2$
MW: 122

Figure 3. Diethyl Phthalate

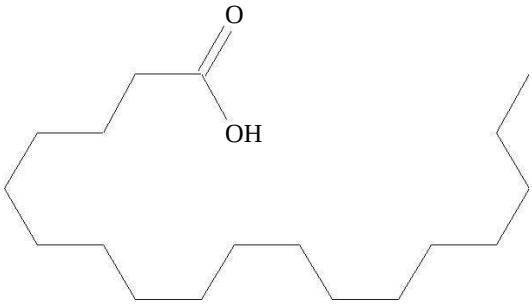


Name: Diethyl Phthalate
Formula: $C_{12}H_{14}O_4$
MW: 222

Figure 4. Tetratriacontane

Name: Tetratriacontane
Formula: $C_{34}H_{70;}$
MW: 478

Figure 5. Octadecanoic acid

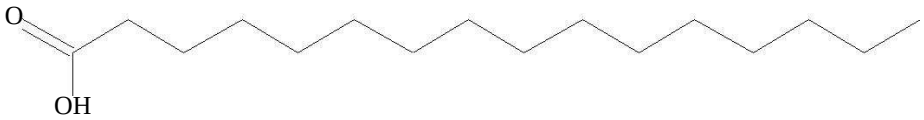


Name: Octadecanoic acid

Formula: C18H36O2

MW: 284

Figure 6. n-Hexadecanoic acid



Name: n-Hexadecanoic acid
Formula: $C_{16}H_{32}O_2$
MW: 256

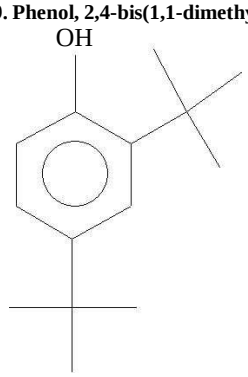
Figure 7. Heptacosane, 1-chloro-

Name: Heptacosane, 1-chloro-
Formula: $C_{27}H_{55}Cl$
MW: 414

Figure 8. Eicosane

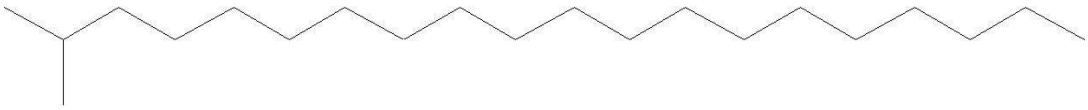
Name: Eicosane
Formula: $C_{20}H_{42}$
MW: 282

Figure 9. Phenol, 2,4-bis(1,1-dimethylethyl)-



Name: Phenol, 2,4-bis(1,1-dimethylethyl)-
Formula: $C_{14}H_{22}O$
MW: 206

Figure 10. Eicosane, 2-methyl-

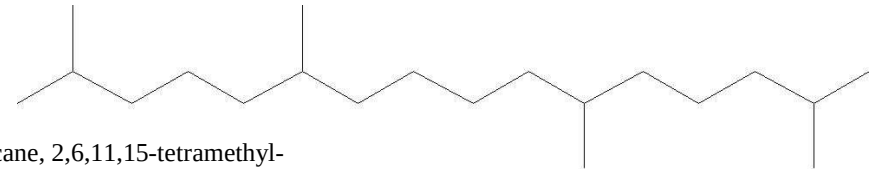


Name: Eicosane, 2-methyl-
Formula: $C_{21}H_{44}$
MW: 296

Figure 11. Nonadecane, 2-methyl-

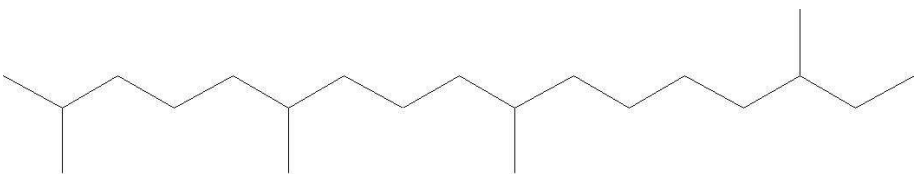
Name: Nonadecane, 2-methyl-
Formula: $C_{20}H_{42}$
MW: 282

Figure 12. Hexadecane, 2,6,11,15-tetramethyl-



Name: Hexadecane, 2,6,11,15-tetramethyl-
Formula: $C_{20}H_{42}$
MW: 282

13. Heptadecane, 2, 6, 10,15-tetramethyl-



Name: Heptadecane, 2,6,10,15-tetramethyl-
Formula: $C_{21}H_{44}$
MW: 296

The GC MS analysis results have shown the presence of the following biomolecules in abundance as shown by their peak value percentages: Benzoic acid, Diethyl Phthalate, Tetratricontane, Octadecanoic acid, n-Hexadecanoic acid, Heptacosane 1-Chloro, Eicosane, Phenol, 2,4-bis(1,1-dimethylethyl)-, Eicosane, 2-methyl-, Nonadecane, 2-methyl-, Hexadecane 2,6,11,15- tetramethyl-, Heptadecane, 2,6,10,15-tetramethyl- and Eicosane, 10-methyl-.

Phenol, 2, 4-bis (1, 1-dimethylethyl) - derivative is present in various plants and is known for its antibacterial and anti-inflammatory activities. (Mujeeb *et al*, 2014, Costantino *et al*, 1993) [30, 31]

Octadecanoic acid is Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic , antiacne , 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, antipsychotic and insectifuge (Dandekar *et al*, 2015). [32] n- Hexadecanoic acid is antioxidant, hypocholesterolemic, nematicide, pesticide, and lubricant, antiandrogenic, as flavoring agents, hemolytic and as 5-alpha reductase inhibitor. (Rajeswari *et al*, 2013). [33]

Eicosane, 2-methyl- derivative is a good antioxidant. Octadecanoic acid esters are reported to be antiviral, antibacterial and antioxidant activities. (Reagan *et al*, 2013, Sudharsan *et al*, 2010) [34, 35] Benzoic acid derivatives possess antibacterial, antifungal properties (Vidhu and Evans, 2015; Terreaux *et al*, 1998). [36, 37] Hexadecanoic acid and naphthalene are known to have anti- feedant and insect-repellent activities (Senthilkumar *et al* 2012). [38] Phenolic compounds, esters, alkanes, aldehydes, alkenes and ketones are the major volatile compounds which have anti-inflammatory, antiarthritic, antidiabetic, antiulcer, hypolipidemic, antiatherosclerotic, anti-HIV and cytotoxic activities (Safayhi and Sailer, 1997). [39] Heptacosane is anti corrosive/ antioxidant. Nonadecane, 2- methyl is antioxidant. Tetratricontane and other higher alkenes are antifungal particularly against fungal spores, germination and also are antioxidant and antitumour (Dandekar*et al*, 2015) [32].

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