Research Article

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CHEMICAL INVESTIGATIONS OF A SIDDHA HERBOMINERAL DRUG BY GC-MS ANALYSIS

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ABSTRACT

Siddha medicines are prepared from herbs, metals / minerals and animal products. Kandhaga rasayanam is a Siddha herbo mineral drug containing fifteen herbs and one mineral. Sulphur is the main ingredient of Kandhaga Rasayanam. The aim of the present study was to identify the bioactive compounds of the drug using Gas Chromatography-Mass Spectroscopy. The GC- MS analysis was performed using the instrument GC-MS-5975C [AGILENT]. Twenty four compounds were identified. Furfural, benzoic acid, benzene methanol, hexadeconic acid, oleic acid, triazoles are the few compounds that are present in Kandhaga rasayanam. Imidazole, Hexadecanoic acid have antifungal property. Hexadecanoic acid and Octadecanoic acid is an antimicrobial agent with antioxidant property. These properties of the compounds in Kandhaga rasayanam justifies its usage in skin diseases and infections.

Keywords: Benzene methanol, oleic acid, furfural, Kandhaga Rasayanam, Siddha, Gas Chromatography-Mass Spectroscopy.

INTRODUCTION

Siddha system of medicine is one of the indigenous systems of medicine practised in India. The exponents of this system are the Siddhars. The unique nature of this system is its continuous service to humanity for more than thousand years in combating diseases and in maintaining the physical, mental and moral health[1]. Kandhaga rasayanam is a compound herbo-mineral formulation containing sulphur as the sole mineral ingredient. Kandhaga rasayanam is used for skin diseases, venereal diseases, urinary tract infection, vatha diseases etc[2]. Though this classical Siddha drug has been in use for many years, so far no preclinical studies have been done. The present study was aimed to identify the chemical components of Kandhaga Rasayanam by GC-MS analysis.

MATERIALS AND METHODS

Botanical authentication

Herbal drugs were purchased from an authorised dealer. They were identified and authenticated by Dr.D.Aravindh, Assistant professor, Botany department, National Institute of Siddha, Chennai, India.

Chemical authentication

Kandhagam was purchased from an authorised dealer. It was identified and authenticated by Mrs. Shakila, Research officer, Department of Chemistry, SCRI, Chennai, India.

Purification of raw drugs

All the raw drugs were purified as per the methods defined in Siddha literature.

Purification and detoxification of sulphur

Sulphur was melted in an iron spoon. A small quantity of cow's butter was added and the spoon was heated till the butter melts; this mixture was immersed in inclined position in cow's milk. This position was repeated for 30 times to get purified sulphur. Eachtime fresh milk has to be used[2].

Purification of *Withania somnifera* and *Smilax china*

It was dried, powdered and then purified using aviyandhiram (Parboiling machine-steam cooking machine). The liquid inside the bottom vessel was milk; boiling time: 3 hours[3].

Purification of *Zingiber officinale*

The outer skin was peeled off. Lime stone was applied to it and then dried.

Purification of *Piper nigrum*

It was soaked in sour butter milk for 3 hours and then fried[3].

Purification of *Piper longum*

It was soaked in lime juice for 24 minutes and then dried in sunlight.

Purification of *Terminalia chebula, Phyllanthus emblica, Terminalia bellerica*

The seeds were removed and only the outer portion was used.

Purification of *Embelia ribes*, *cardamomum* and *Cinnamomum zeylanicum*

The impurities were removed and then dried in sunlight.

Purification of *Santalum album*

The centre part (vaira pagam) alone was used in the wood[3].

Method of preparation of Kandhaga rasayanam

Tamil name	Botanical name/ Chemical name	Quantity
Kandhagam	Sulphur	350 grams
Amukkara kizhangu	*Withania somnifera.* Dunal	175 grams
Parangi chakkai	*Smilax china* Linn	70 grams
Kadukkai	*Terminalia chebula.* Retz	35 grams
Nellikai	*Phyllanthus emblica* Linn	35 grams
Thandrikkai	*Terminalia bellerica* Roxb	35 grams
Chukku	*Zingiber officinale.* Roscoe	35 grams
Thippili moolam	*Piper longum.* Linn	35 grams
Milagu	*Piper nigrum.* Linn	35 grams
Vaividangam	*Embelia ribes,* Burm	35 grams
Ealam	*Elataria cardamomum.* Linn	35 grams
Kirambu	*Cinnamomum zeylanicum.* Breyn	35 grams
Chandhanam	*Santalum album,* Linn	35 grams
Kadalai	*Cicer arietinum,* Linn	35 grams
Senkottai	*Semecarpus anacardium.* Linn	35 grams
Chithiramoolam	*Plumbago zeylanica,* Linn	35 grams[4]

Sugar, Honey and Ghee

The quantity of sugar, honey and ghee is not prescribed. So, sufficient quantity was added. The above mentioned ingredients were powdered separately and mixed together. Sufficient quantity of Sugar, honey and ghee were then added.

GC- MS analysis

The GC- MS analysis was performed using the instrument - GC-MS-5975C [AGILENT]. The GC conditions are given below:

Column Oven Temperature: 70˚C. Injector Temperature: 280˚C. Injection Mode: Split. Split Ratio: 50. Flow Control Mode: Linear Velocity. Column Flow: 1.2 ml/min. Carrier Gas: Helium 99.9995 % purity. Injection volume: 1 micro litre.

Column Oven Temperature Program

Rate Temperature (˚C) Hold Time (min) - 70.0 3.0 10 300 9.0[35.0 mts total]

Column: DB-5MS-Agilent Length: 30.0 m Diameter: 0.25 mm Film Thickness: 0.25 um

MS Condition

MS Ion source temp: 200°C. Interface temp: 300°C. Scan range: 40 – 700 m/z. Solvent cut time: 3.5 minutes. MS start time: 3.5 (min). MS end time: 35 (min). Ionization: EI (-70ev). Scan speed: 2000.

Identification of compounds

Database of National Institute Standard and Technology (NIST 11) was used for interpretation of GC-MS compounds. The spectrum of unknown compounds was compared with the spectrum of the known compounds recorded in the NIST library. The details such as the name of the compound, its molecular weight and structure were ascertained.

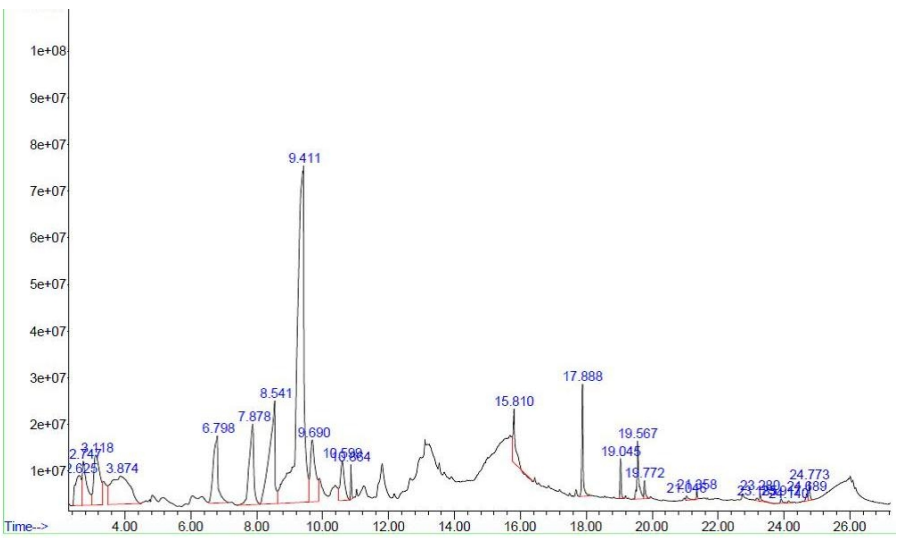

Figure 1: GC-MS graph of Kandhaga Rasayanam

Table 1: Compounds detected in Kandhaga rasayanam

S. No.	RT	Area %	Compound name
1.	2.621	2.94	Furfural, 1H-Imidazole, 4,5-dimethyl- Dimethylpyrazole
2.	2.752	3.39	2-Furanmethanol.
3.	3.115	5.22	Propanoic acid, 2-methyl-, methyl ester Aziridine-2-carbothioamide Ethanamine,
4.	3.870	8.86	4- methyl-1,3,2-dioxaathiane 2- oxide Isoxazolidine, Pentaborane (11)
5.	6.803	5.46	1,3,5-Triazine-2,4,6-triamine 4,5-Diamino-2-hydroxypyrimidine, Maltol
6.	7.878	6.16	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl, 2,3,1-Benzodiazaborine, 1,2- dihydro-1-methyl.
7.	8.546	9.73	Benzoic acid
8.	9.418	40.85	Benzene methanol, 3-fluoro-4-Fluorobenzyl alcohol, Benzene methanol, 3-fluoro
9.	9.694	5.22	1,2,3- Propanetriol, Heptanoic acid
10.	10.594	2.75	1- Napthalenol, 4-Hydroxy -N-methylpiperidine, 2-Napthalenol
11.	10.870	0.50	Eugenol, Phenol , 2- methoxy-3- (2- propenyl)
12.	15.808	2.15	Tetradecanoic acid
13.	17.884	2.29	n- Hexadecanoic acid
14.	19.046	0.80	2,3-Diazabicyclo[3.2.0]hept-2-ene,1,6,6,-trifluoro-4-spirocyclopropane-Cyclic octaatomic sulfur 7-Amino-7H-S-triazolo[5,1-c]-S-triazolo(5,1-c)- S- triazole-3-thiol.
15.	19.569	1.81	Oleic acid, 9- Octadecenoic acid
16.	19.773	0.45	Octadecanoic acid
17.	21.051	0.20	3-(6,6-Dimethyl-5-oxohept-2-enyl)- cyclohexanone, Estra-1,3,5(10)-trien-17.beta.-olZ-9-Pentadecenol
18.	21.356	0.12	Morpholine, 4-(1-oxo-3-phenyl-2-pr openyl)-1-Penten-3-one, 4-methyl-1-phenyl-2-Propenamide, N,N-diethyl-3-phenyl
19.	23.185	0.09	3-(6,6-Dimethyl-5-oxohept-2-enyl)-cyclohexanone Hepten-6-one, 5-phenyl-, (E)-7-Tetradecanol acetate
20.	23.287	0.23	Heptadecane, 4-methyl-Pentadecane, 4-methyl-Diaziridinone, bis(1,1-dimethylpropyl)-
21.	23.912	0.14	(4-Dimethylaminomethyl-5-hydroxy-benzofuran-3-yl)(2,4-dimethyl-oxazo l-5-yl)methanone, Podocarpa-8,11,13-trien-3-one, isopropyl-13-methoxy-[1,2,4]Oxadiazole, 5-(4-tert-butyl phenoxymethyl)-3-(thiophen-2-yl)-
22.	24.144	0.07	Cinnamaldehyde
23.	24.696	0.21	o-Acetyl-3-(trimethylsilyl)propioohydroximamide 1,3,5-Trisilacyclohexane, 1,1-dimethyl-13-Methylpentadec-14-ene-1,13-diol
24.	24.768	0.38	Phytol, 1,2,4-Triazolo[4,3-a]pyrimidine-6- carbonitrile, 7,8-dihydro-5-(2-fluorophenyl)-8-methyl-7-oxo- Octanoic acid, nonyl ester

RT: Retention Time

RESULTS AND DISCUSSION

The chemical compounds present in the drug Kandhaga Rasayanam were identified by GC-MS analysis. The active principles with their retention time and peak area % were tabulated in Table 1. The GC-MS graph is given in Figure 1. Imidazole, one of the components of Kandhaga rasayanam is an organic compound. Its molecular formula is $C_3H_4N_2$. It is classified as an alkaloid. Imidazole is related to histamine[5]. Many antifungal drugs contain imidazole ring[6]. 1-H imidazole has been screened for *in-vitro* antimicrobial activity against *E. coli, S. aureus, C. albicans* and *A. niger*[7]. Anshul chawla *et al* in a review article has compiled the action of imidazole. Imidazole and its derivatives have anti anthelmintic activity[8], cardiovascular activity[9,10], analgesic and anti-inflammatory activity[11-14], antineoplastic activity[15], antifungal activity[15,16], enzyme inhibition activity, anti filarial agent, antiviral activity and antiulcer activity. Benzoic acid $C_{11}H_{14}O_3$, which occurs naturally in many plants, was found to have antifungal activity by Salkowski in 1875. It was used for preservation of cloudberry fruits[17]. It has anticandidal activity[18]. Benzoic acid with salicylic acid is used in the treatment of Tinea pedis and Tinea capitis[19]. Oleic acid is a common monounsaturated fat in human diet. It decreases LDL and increases HDL[20]. It is a very good antioxidant[21-25]. Tetradecanoic acid (Myristic acid) $C_{14}H_{28}O_2$ is a fatty acid which has antifungal, antioxidant, cancer preventive and has nematicide activity. Hexadecanoic acid $C_{17}H_{34}O_2$ has antioxidant and cholesterol lowering activity. Heptadecanoic acid $C_{17}H_{34}O_3$ is an antimicrobial agent. Octadecanoic acid and hexadecanoic acid have antimicrobial activity[26]. Many naphthalene derivatives are studied for antimicrobial and antifungal activity in recent years[27-31]. 1-Napthol has both antifungal and antimicrobial activity[32]. Propanoic acid, $C_3H_2O_2$ inhibits the growth of moulds and some bacteria at the levels between 0.1 and 1 % by weight. It is used by veterinarian to treat dermatomycoses.[33] Cinnamaldehyde C_9H_8O and Morpholine derivatives are used as fungicide in agriculture[34]. The pyrazole compound 4, 5-Dimethyl pyrazole is seen in Kandhaga Rasayanam. The pyrazole nucleus has been reported to possess anti-inflammatory[35], antibacterial[36], analgesic[37], antifungal[38] and antiviral activity[39]. In the present study, twenty four compounds have been identified. Most of the components possess antimicrobial, antifungal and antioxidant property. The presence of these compounds justifies its usage in skin infections, urinary tract infections and venereal diseases.

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