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GC MS analysis of an Ayurvedic medicine "Ashokarishtam"

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ABSTRACT

Ashokarishtam is a proven Ayurvedic medicine for the treatment of disorders of female reproductive system, particulary, menorrhagia, metrorrhagia and amenorrhea. In order to understand the various biomolecules present in this medicine, GC MS analaysis was performed. It was found that some very important biomolecules like 2, 3-Butanediol, [R-(R, R*)], Phenol, 2,4-bis(1,1-dimethylethyl)- derivatives, n- Hexadecanoic acid, 2-Amino-1,3-propanediol or Thiophene, Furyl hydroxymethyl ketone, Dihydroxyacetone, 2-Furanmethanol, 2, 5- Dimethyl-4-hydroxy-3(2H)-furanone were found which have some very effective medicinal roles. It is of interest to find that there is no biological activity reports available for some of the major components found in the GC MS study like1-Propanol, Oxalic acid, cyclobutyl heptyl ester, Propanoic acid, 2-oxo-, methyl ester, 1,3-Butadiene-1-carboxylic acid, 1, 2-Cyclopentanedione, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Hexanoic acid, 2-ethyl-anhydride, 1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol, Ethyl 2-nitropropionate. Further investigations are warranted to identify the biological functions of these components to get some clues as to the medicinal role of Ashokarishtam.*

Key words: Ashokarishtam, GC MS, Hexadecanoic acid, 1-Propanol, Metrorrhagia, Amenorrhea

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INTRODUCTION

Ashokarishtam is an ayurvedic tonic used for treatment of gynecological disorders like menorrhagia, metrorrhagia and amenorrhea. It nourishes the endometrium, helps in proper ovulation and in fertility. The reference of this tonic is in the Ayurvedic treatise, Bhaishajya Ratnavali Streeroga context 114-116.The standard dosage is 1-2 table spoons twice a day with warm water. A number of Ayurvedic companies manufacture this tonic. It is made by an elaborate process. The following plants along with their proportion for the preparation are mentioned.

Ingredients of Ashokarishtam
Ashoka – *Saraca indica* – Bark – 4.8 kg

Dhataki – *Woodfordia fruticosa* – flower – 768 g
Musta – *Cyperus rotundus* – Rhizome – 48 g
Shunti – Ginger – *Zinziber officinale* -Rhizome – 48 g
Haritaki – *Terminalia chebula* – fruit – 48 g
Vibhitaki – *Terminalia bellerica* – fruit – 48 g
Amalaki – Amla – India Gooseberry- *Phyllanthus embelica* - fruit – 48 g
Amrasthi – Mango- *Mangifera indica*- seed – 48 g
Jeeraka – Cumin- *Cuminum cyminum* seed – 48 g
Vasa – *Adhatoda vasica* – Whole plant – 48 g
Chandana – *Santalum album* (Sandalwood) – 48 g
Ajaji – Kala Jeera- *Nigella sativa* – Fruit – 48 g
Daruharidra – *Berberis aristata* – Stem – 48 g
Utpala – *Nymphaea stellata* – flower – 48 g
Water for decoction – 49.152 liters
Kashayam – decoction – 12.288 liters
Guda – Jaggery – 9.6 kg

MATERIALS AND METHODS

Method of preparation

The herbs mentioned are coarsely powdered and kashaya (water decoction) is prepared. The kashaya is strained and kept in the fermentation pot, vessel or barrel. Jaggery is dissolved, boiled, filtered and added. All the plant parts mentioned above except Dhataki flowers are finely powdered and added. At the end, Dhataki flowers are added. The mouth of the pot, vessel or barrel is covered with an earthen lid and the edges sealed with clay-smeared cloth wound in seven consecutive layers. The container is kept in a heap of paddy fermentation at constant temperature.

After the specified period, the lid is removed, and the contents examined to ascertain whether the process of fermentation has been completed. The fluid is first decanted and then strained after two or three days. When the fine suspended particles settle down, it is strained again and bottled.

Ashokarishtam

Dushing *et al*, 2012, have compared the antioxidant activities of Ashokarishtam prepared by different sources. [1] Govindarajan *et al*, 2008 and Parihar *et al*, 2010 have standardized this medicine with different methods. [2] Modi *et al*, 2012 reported the clinical evaluation of Ashokarishtam on menopausal syndrome. [3]

Ashokarishtam is prepared by a number of plants and it is pertinent to mention about the medicinal aspects of each constituent plant.

Ashoka (*Saraca Indica*)

There are numerous reports on the medicinal and ethno-botanical aspects of *Saraca indica*. [4] It is reported to have activities like antihypreglycemic and antioxidant activity, antipyretic, antitumor, antioxidant and cytotoxic activity, anthelmintic activity, central nervous system depressant activity, analgesic and antipyretic activity, antimicrobial activity etc. [5,6,7,8,9,10,11] Ashoka is helpful in all cases of uterine bleeding. Ashoka is blood purifier and used in all skin diseases, ammenorhea, dysmenorrhea menopause, menorrhagia, painful menstruation blood circulation and purification, cancer, diarrhea, dysentery, edema, heart disease, hepatitis, herpes, jaundice, joint pain, kidney and gall stones, paralysis, skin problems, rheumatoid arthritis, obstructions in urinary passages.

Dhataki (Woodfordia fruticosa)

This medicinal plant is used in various Ayurvedic preparations. The butanolic leaf extract was reported to be antibacterial (Dubey *et al*, 2014). [12] The leaves have sedative properties and the juice of fresh flowers is applied on the forehead relives headache. The medicinal value of the this plant is due to the presence of important secondary metabolites like alkaloids, flavonoids, glycosides, saponins, sterols etc. [13]

Musta or Nagarmotha (*Cyperus rotundus)*

There are reports of the plant's medicinal values as diuretic, carminative, emmenagogue, anthelminthic, analgesic, anti-inflammatory, anti-dysenteric, anti rheumatic activities. [14] There are reports of the medicinal values of this

plant such as, antimicrobial, anti mutagenic and antioxidant activity, anti malarial, antispasmodic, anticonvulsant, anti platelet activity and lipid lowering activity. [15, 16, 17, 18, 19, 20, 21]

Sunthi* (Ginger) *Zinziber officinale
Ginger is also one of the household medicines used against common cold, cough and indigestion. Its medicinal values are well documented. (Zadeh and Ko, 2014; Abel and Prakash, 2014 have reported its antioxidant properties. [22, 23] Ginger controls vomiting and nausea during pregnancy. [24] It controls blood pressure by blocking calcium channels (Ghayur and Gilani, 2005). [25]

***Haritaki* (*Terminalia chebula*)**
One of the constituent of the common Triphala choornam, *T. chebula* is one wonder medicinal plant which has a range of curative properties and used extensively in Sidha and Ayurveda practice. [26] The various parts of the plant like bark, rind, galls etc have been found to have activities like antioxidant, antimicrobial, anti diabetic, hepato protective, anti-inflammatory and anti arthritic, anti mutagenic, anti proliferative, radio protective, cardio protective, hypo lipidemic, Antispasmodic, Immuno-modulatory and antiviral activities. [27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38]

***Vibhitaki* (*Terminalia bellerica*)**
Vibhitaki is also a part of the famous ayurvedic preparation, Triphala. This plant is endowed with a number of medicinal properties and used to cure various ailments in traditional folk practice all over India. A review of the medicinal values of this plant is reported by Saraswathi *et al*, 2012. [39] The plant has medicinal properties such as antibacterial, antioxidant, hypoglycemic, hepatoprotective, antidiorrhial, antihypertensive, antispasmodic and bronchodialatory. [40, 41, 42, 43, 44, 45] Khan and Gilani, 2010 have shown that this plant is a potent analgesic, antisecretory and antinociceptive. [46]

Amla* (*Phyllanthus embelica)
Amla is an age old home remedy for various ailments practiced all over the world. Various researchers have reported the medicinal role of Amla. Bhide *et al*, 2014 have reviewed the medicinal value of Amla. [47] Amla has multifarious medicinal properties such as antipyretic, analgesic and as skin care lotion. The juice of bark of Amla along with honey and turmeric prevents Gonorrhoea (Kumar *et al*, 2012). [48] Amla juice is used to stop nausea, vomiting, indigestion, nose bleeding (Dasaraju *et al*, 2014). [49] The liver stimulant activity of Amla was reported by Singh *et al*, 2011. [50] This helps to ward off jaundice and reduces cholesterol in the blood. Amla is an excellent antioxidant and used as a main ingredient of Ayurvedic preparation, Chavanaprash.

Mango (*Mangifera indica*) seeds
Mango has a special role in ayurveda not only because of its taste and variety but also because its multiferous medicinal values. [51] Mango seed kernel is used as anti diarrheal as a homemade medicine. The anti diarrheal, anti-inflammatory activity of mango seed kernel was reported by (Sairam *et al*, 2003). [52] Garrido *et al*, 2001 a, b, have claimed the presence of poly phenols present in mango seeds for the anti-inflammatory activity. [53, 54] The seeds are reported to be act as antibacterial (Rajan *et al*, 2011). [55]

Cumin seed (*Cuminum cyminum)*
One of the most used spice all over the world; cumin seeds have antioxidant, carminative, antiflatulance properities. They are good source of fibre in the food. The active principles like cuminaldehyde, pyrazines etc. help in digestion and gut mobility. It contains many important elements like iron, copper, zinc, potassium, manganese, selenium etc. and many important vitamins. It has got antmicrobial property (Dua and Garg, 2013). [56]

Vasa* (*Adhatoda vasika)
This plant is also one of the wonder drug in Ayurveda. Many reports are there on the medicinal value of this plant parts (Claeson *et al*, 2000). [57] This is known to antimicrobial, antiulcer, hepatoprotective and anti-inflammatory. [58, 59, 60, 61]

Chandana* (Sandal): *Santalum album
Sandal is an age old medicinal plant and it is used for many diseases. Rao *et al*, 2013, have reviewed the various medicinal properties of Sandal. [62] The anti hyperglycemic and anti hyperlipidemic effect of sandal on diabetes was reported by Kulkarni *et al*, 2012. [63] Cardio-protective role of sandal was studied by Khan *et al*, 2014. [64] It

functions as a brain tonic (Papaiah *et al*, 2010). [65] Its anti ulcerogenic property was demonstrated by Ahmed *et al*, 2013 in rats. [66]

Ajaji *(Nigella sativa)*
It is used as liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers, to fight parasitic infections and to support immune system. [67] Thymoquinone (TQ) which is a major active chemical component of its essential oil has most of its therapeutic properties. Black seeds are also used in food like flavoring additive in the breads and pickles because it has very low level of toxicity. [68] The therapeutic role of *N. sativa* has been reviewed by Ahmad *et al*, 2013. [66] It has anticancer potential also. [69]

***Daruharidra* (*Berberis aistata*)**
Berberis aristata is ethno botanically important herb that is used from time immemorial by mankind for the treatment of various ailments. Sharma *et al*, 2011 has reviewed this plant's therapeutic role. [70] This plant is known to be hepato-protective, hypoglycemic, anticancer, antimicrobial, anti-inflammatory, antioxidant etc. among many other medicinal values. [71, 72, 73, 74, 75, 76]

Utpala (Nymphaea stellata)
This plant has been reported to have anti hyper lipidemic and anti hepato toxic effect (Rajagopal and Sasikala, 2008). [77] The flowers are hepato protective (Bhandarkar and Khan, 2004). [78] The filament of plant is used as an astringent and a cooling agent in burning sensation of the body and in menorrhagia. The seeds are used as stomachic and restorative. [79] Das *et al*, 2012 have elucidated the medicinal importance of this plant. [80]

The present study envisages finding the active biomolecules present in Ashkoarishtam by GC MS analysis and tries to understand the medicinal efficacy of this drug due to these bio molecules. Since this Ayurvedic preparation was made with medicinal plants or plant parts as discussed above, which have already proven medicinal values. It is assumed that during the processing of this drug the constituent plants which contributed their various phyto-chemicals must have interacted to produce the bio molecules as observed by GC MS analysis.

The drug, Ashokarishtam was purchased from standard Ayurvedic pharmacy at Chennai, India. The medicine was subjected to GC Ms analysis by standard method after processing it suitably. 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

The GC MS results are tabulated in Table1.

Table 1. GC MS analysis report with the relevant details

Sl No.	Retention Time(Min)	Compound	Molecular Formula	Molecular Weight	Peak %
1.	2.306	9-Octadecenamide	C18H35NO	281	0.426
2.	2.606	2-Amino-1,3-propanediol	C3H9NO2	91	3.414
3.	2.781	1-Propanol	C3H8O	60	14.830
4.	2.919	2-Propanone, 1-hydroxy-	C3H6O2	74	0.790
5.	3.275	Thioacetic acid	C2H4OS	76	0.547
6.	3.332	Oxalic acid, cyclobutyl heptyl ester	C13H22O4	242	1.799
7.	3.407	Butanoic acid, 2-methyl-3-oxo-, ethyl ester	C7H12O3	144	0.524
8.	3.588	2,3-Butanediol, [R-(R*,R*)]-	C4H10O2	90	12.476
9.	3.657	2,3-Butanediol, [R-(R*,R*)]-	C4H10O2	90	4.491
10.	4.314	2-Furanmethanol	C5H6O2	98	1.882
11.	4.733	Propanoic acid, 2-oxo-, methyl ester	C4H6O3	102	8.987
12.	5.083	Propane, 1,1-diethoxy-	C7H16O2	132	0.420
13.	5.402	2(5H)-Furanone	C4H4O2	84	1.218
14.	5.577	1,3-Butadiene-1-carboxylic acid	C5H6O2	98	1.131
15.	5.696	1,2-Cyclopentanedione	C5H6O2	98	1.033
16.	6.084	1,3-Dioxepane, 2-heptyl-	C12H24O2	200	0.836
17.	6.434	Dihydroxyacetone	C3H6O3	90	2.192
18.	6.684	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C6H8O4	144	7.700
19.	6.753	2-Oxopentanedioic acid	C5H6O5	146	0.536
20.	7.135	2H-Pyran-2,6(3H)-dione	C5H4O3	112	0.413
21.	7.654	Benzenemethanamine, N-ethyl-	C9H13N	135	0.338
22.	7.873	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	C6H8O2	112	0.357
23.	8.136	Ethyl 2-nitropropionate	C5H9NO4	147	2.725
24.	8.824	Furyl hydroxymethyl ketone	C6H6O3	126	3.185
25.	8.880	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C6H8O3	128	1.473
26.	14.585	Thiophene, 2-butyl-5-ethyl-	C10H16S	168	0.723
27.	15.298	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	5.209
28.	15.492	Benzoic acid, 4-ethoxy-, ethyl ester	C11H14O3	194	0.519
29.	16.512	Ethyl N-(o-anisyl)formimidate	C10H13NO2	179	0.952
30.	17.700	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	C14H26O2	226	7.047
31.	18.626	Name: 3-Hexadecanol	C16H34O	242	0.932
32.	18.851	Isopropyl myristate	C17H34O2	270	0.307
33.	19.940	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	276	0.283
34.	20.134	Malonic acid, 4-heptyl undecyl ester	C21H40O4	356	0.275
35.	20.246	n-Hexadecanoic acid	C16H32O2	256	3.949
36.	21.341	Hexanoic acid, 2-ethyl-, anhydride	C16H30O3	270	1.418
37.	22.135	Octadecanoic acid	C18H36O2	284	0.378
38.	25.113	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	4.100
39.	27.046	Octadecanoic acid, 2,3-dihydroxypropyl ester	C21H42O4	358	0.191

The known biological roles and medicinal values of some of the important bio molecules that are shown in Table 1 are discussed.

2, 3-Butanediol, [R-(R*, R*)] - This molecule is reported as potent CNS depressant on rats. [81] Phenol 2, 4-bis (1, 1-dimethylethyl)- derivatives are known for their antibacterial and anti-inflammatory roles. [82, 83] Its role as antioxidant was reported by Ayayi *et al*, 2011) [84] n- Hexadecanoic acid is reported to have activities like antioxidant (Lalitharani *et al*, 2009), hypocholesterolemic, nematicide, pesticide and lubricant, anti androgenic, as flavoring agents, hemolytic, antibacterial and cytotoxic (Dinesh Kumar *et al*, 2015) and as 5-alpha reductase inhibitor. (Rajeswari *et al*, 2013). [85, 86, 87]. 2-Amino-1,3-propanediol or Thiophene and its various derivatives have been reported to have medicinal properties like Antimicrobial, anticancer, analgesic, antipyretic, cyto-toxic, CNS depressant, etc. (Chaudhury *et al*, 2012).[88] Furyl hydroxymethyl ketone: A related compound 5-Hydroxymethylfurfural is reported to have antiapopototic (Gu *et al*, 2013) and cyto protective (Wannng *et al*, 2010). [89, 90] It is also reported to work against oxidative stress (Kao *et al*, 2013). [91] Dihydroxyacetone has cytotoxic property (Peterson *et al* 2004) [92] 2-Furanmethanol is reported to have antioxidant property (Wei *et al*., 2001) [93]. 2, 5- Dimethyl-4-hydroxy-3(2H)-furanone is an antimicrobial compound (Sung *et al*, 2007) [94]. Furanones are potential antibacterial coatings on bio materials. (Baveja *et al*, 2004) [95]

It is of interest to find that biological activity reports are not available for some of the major components found in the GC MS study like1-Propanol, Oxalic acid, cyclobutyl heptyl ester, Propanoic acid, 2-oxo-, methyl ester, 1,3-

Butadiene-1-carboxylic acid, 1, 2-Cyclopentanedione, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Hexanoic acid, 2-ethyl-anhydride, 1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol, Ethyl 2-nitropropionate. Further investigations are warranted to identify the biological functions of these components to understand better about the medicinal role of Ashokarishtam.

CONCLUSION

From the above discussion it could be concluded that the biological and medicinal properties of the molecules present in Ashokarishtam, particularly, the antioxidant, anti-inflammatory and anti androgenic activities of some of the molecules could help in curing the menstrual disorders. The medicinal activities of some of the compounds are not known yet. It is quite possible that further research on these compounds could through some light on the medicinal efficacy of Ashokarishtam. This is a preliminary work towards understanding more about the medicinal efficacy of Ashokartishtam.

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