ABSTRACT
Ashokarishtam is a proven Ayurvedic medicine for the treatment of disorders of female reproductive system, particularly, menorrhagia, metrorrhagia and amenorrhea. In order to understand the various biomolecules present in this medicine, GC MS analysis 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 like 1-Propanol, Oxalic acid, cyclobutyl heptyl ester, Propanoic acid, 2-oxo-, methyl ester, 1,3-Butadiene-1-carboxylic acid, 1, 2-Cyclopentanediene, 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-nitropropanionate. 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

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
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 antihyperglycemic 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, amenorrhea, 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 (Dubeys et al, 2014). [12] The leaves have sedative properties and the juice of fresh flowers is applied on the forehead relieves 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, anthelmintic, 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]

_Santhish (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 chooram, _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, anti diarrhoeal, antihypertensive, antispasmotic, hypo lipidemic, Antispasmodic, Immuno-modulatory and antiviral activities. [27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38]

_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, antiflatulence properties. 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 antimicrobial 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). 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 aristata)**
*Berberis aristata* is ethnomedicinally 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 hyperlipemic and anti hepatotoxic 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 phytocompounds 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°C for 1 min, and then increased linearly to 70°C to 220°C held for 3 min followed by linear increased temperature 100°C up to 290°C and held for 10min. The injection port temperature was 290°C and the GC/MS interface was maintained at 29°C, 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

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Retention Time (Min)</th>
<th>Compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Peak %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.306</td>
<td>9-Octadecanamide</td>
<td>C18H35NO</td>
<td>281</td>
<td>0.426</td>
</tr>
<tr>
<td>2.</td>
<td>2.606</td>
<td>2-Amino-1,3-propanediol</td>
<td>C3H9NO2</td>
<td>91</td>
<td>3.414</td>
</tr>
<tr>
<td>3.</td>
<td>2.781</td>
<td>1-Propanol</td>
<td>C3H8O</td>
<td>60</td>
<td>14.830</td>
</tr>
<tr>
<td>4.</td>
<td>2.919</td>
<td>2-Propanone, 1-hydroxy-</td>
<td>C3H8O</td>
<td>74</td>
<td>0.790</td>
</tr>
<tr>
<td>5.</td>
<td>3.275</td>
<td>Threoctic acid</td>
<td>C2H4OS</td>
<td>76</td>
<td>0.547</td>
</tr>
<tr>
<td>6.</td>
<td>3.332</td>
<td>Oxalic acid, cyclobutyl heptyl ester</td>
<td>C13H22O4</td>
<td>242</td>
<td>1.799</td>
</tr>
<tr>
<td>7.</td>
<td>3.407</td>
<td>2-Hexanonic acid, 2-methyl-1,3-oxo-, ethyl ester</td>
<td>C6H12O3</td>
<td>144</td>
<td>0.524</td>
</tr>
<tr>
<td>8.</td>
<td>3.588</td>
<td>2,3-Butanediol, [R-(R*,R*)]-</td>
<td>C4H10O2</td>
<td>90</td>
<td>12.476</td>
</tr>
<tr>
<td>9.</td>
<td>3.657</td>
<td>2,3-Butanediol, [R-(R*,R*)]-</td>
<td>C4H10O2</td>
<td>90</td>
<td>4.491</td>
</tr>
<tr>
<td>10.</td>
<td>4.314</td>
<td>2-Furanmethanol</td>
<td>C5H8O</td>
<td>98</td>
<td>1.882</td>
</tr>
<tr>
<td>11.</td>
<td>4.733</td>
<td>Propanoic acid, 2-oxo-, methyl ester</td>
<td>C3H6O3</td>
<td>102</td>
<td>8.987</td>
</tr>
<tr>
<td>12.</td>
<td>5.083</td>
<td>Propane, 1,1-diethoxy-</td>
<td>C7H16O2</td>
<td>132</td>
<td>0.420</td>
</tr>
<tr>
<td>13.</td>
<td>5.402</td>
<td>2,3-Hexanone</td>
<td>C4H10O2</td>
<td>94</td>
<td>1.218</td>
</tr>
<tr>
<td>14.</td>
<td>5.577</td>
<td>1,3-Butadiene-1-carboxylic acid</td>
<td>C5H8O2</td>
<td>98</td>
<td>1.131</td>
</tr>
<tr>
<td>15.</td>
<td>5.696</td>
<td>1,2-Cyclopentanediencnone</td>
<td>C5H10N</td>
<td>135</td>
<td>0.338</td>
</tr>
<tr>
<td>16.</td>
<td>6.084</td>
<td>1,3-Dioxepane, 2-heptyl-</td>
<td>C12H24O2</td>
<td>200</td>
<td>0.836</td>
</tr>
<tr>
<td>17.</td>
<td>6.434</td>
<td>Dihydroxyacetone</td>
<td>C3H8O</td>
<td>90</td>
<td>2.192</td>
</tr>
<tr>
<td>18.</td>
<td>6.684</td>
<td>2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one</td>
<td>C6H8O4</td>
<td>144</td>
<td>7.700</td>
</tr>
<tr>
<td>19.</td>
<td>6.753</td>
<td>2-Octanedicarboxylic acid</td>
<td>C8H16O4</td>
<td>146</td>
<td>0.536</td>
</tr>
<tr>
<td>20.</td>
<td>7.135</td>
<td>2H-Pyrane-2,6(3H)-dione</td>
<td>C5H6O2</td>
<td>112</td>
<td>0.413</td>
</tr>
<tr>
<td>21.</td>
<td>7.654</td>
<td>Benzenemethanamine, N-ethyl-</td>
<td>C9H13N</td>
<td>135</td>
<td>0.338</td>
</tr>
<tr>
<td>22.</td>
<td>7.873</td>
<td>2-Cyclopenten-1-one, 2-hydroxy-3-methyl-</td>
<td>C6H10O2</td>
<td>112</td>
<td>0.357</td>
</tr>
<tr>
<td>23.</td>
<td>8.136</td>
<td>Ethyl 2-nitropropanate</td>
<td>C5H9N</td>
<td>147</td>
<td>2.725</td>
</tr>
<tr>
<td>24.</td>
<td>8.324</td>
<td>Furfyl hydroxymethyl ketone</td>
<td>C6H8O3</td>
<td>126</td>
<td>3.185</td>
</tr>
<tr>
<td>25.</td>
<td>8.800</td>
<td>2,5-Dimethyl-4-hydroxy-3(2H)-furanone</td>
<td>C6H12O3</td>
<td>129</td>
<td>1.473</td>
</tr>
<tr>
<td>26.</td>
<td>14.585</td>
<td>Thiophene, 2-buty1-5-ethyl-</td>
<td>C10H16S</td>
<td>168</td>
<td>0.723</td>
</tr>
<tr>
<td>27.</td>
<td>15.298</td>
<td>Phenol, 2,4-bis(1,1-dimethylthylidene-</td>
<td>C14H26O2</td>
<td>206</td>
<td>5.209</td>
</tr>
<tr>
<td>28.</td>
<td>15.492</td>
<td>Benzoc acid, 4-ethoxy-, ethyl ester</td>
<td>C11H16O3</td>
<td>194</td>
<td>0.519</td>
</tr>
<tr>
<td>29.</td>
<td>16.512</td>
<td>Ethyl N-(o-anisyl)formimidate</td>
<td>C10H13N</td>
<td>179</td>
<td>0.952</td>
</tr>
<tr>
<td>30.</td>
<td>17.700</td>
<td>2-Hexanonic acid, 2-methyl-3-hexyl-5-ethyl-3,5-dimethyl-2(3H)-furanone</td>
<td>C6H14O3</td>
<td>126</td>
<td>3.185</td>
</tr>
<tr>
<td>31.</td>
<td>18.626</td>
<td>Name: 3-Hexadecanol</td>
<td>C16H34O2</td>
<td>242</td>
<td>0.932</td>
</tr>
<tr>
<td>32.</td>
<td>18.851</td>
<td>Isopropyl myristate</td>
<td>C17H34O2</td>
<td>270</td>
<td>0.807</td>
</tr>
<tr>
<td>33.</td>
<td>19.940</td>
<td>7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione</td>
<td>C3H17O3</td>
<td>276</td>
<td>0.283</td>
</tr>
<tr>
<td>34.</td>
<td>20.134</td>
<td>Malonic acid, 4-heptyl undecyl ester</td>
<td>C21H40O4</td>
<td>356</td>
<td>0.275</td>
</tr>
<tr>
<td>35.</td>
<td>20.246</td>
<td>n-Octadecanoic acid</td>
<td>C18H36O2</td>
<td>286</td>
<td>3.949</td>
</tr>
<tr>
<td>36.</td>
<td>21.341</td>
<td>Hexanoic acid, 2-ethyl-4-hydroxy-3-carboxylic acid</td>
<td>C6H12O3</td>
<td>270</td>
<td>1.418</td>
</tr>
<tr>
<td>37.</td>
<td>22.135</td>
<td>Octadecanoic acid</td>
<td>C20H40O2</td>
<td>330</td>
<td>1.418</td>
</tr>
<tr>
<td>38.</td>
<td>25.113</td>
<td>Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester</td>
<td>C20H40O2</td>
<td>330</td>
<td>4.100</td>
</tr>
<tr>
<td>39.</td>
<td>27.046</td>
<td>Octadecanoic acid, 2,3-dihydroxypropyl ester</td>
<td>C21H42O2</td>
<td>358</td>
<td>0.191</td>
</tr>
</tbody>
</table>

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-dimethylthylethyl)- 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), hypcholesterolemic, 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, CNS depressant, etc. (Chaudhury et al., 2012).[88] Furyl hydroxymethyl ketone: A related compound 5-Hydroxymethylfurfural is reported to have antiapoptotic (Gu et al, 2013) and cytotoxic (Wanning et al, 2010). [89, 90] It is also reported to work against oxidative stress (Kao et al, 2013). [91] Dihydroxyacetone has cytotoxic properties (Peterson et al 2004) [92] 2-Furanmethanol is reported to have antioxidant activity (Wei et al., 2001) [93]. 2, 5- Dimethyl-4-hydroxy-3(2H)-furanone is an antimicrobial compound (Sung et al, 2007) [94]. Furanes 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 like 1-Propanol, Oxalic acid, cyclobutyl heptyl ester, Propanoic acid, 2-oxo-, methyl ester, 1,3-
Butadiene-1-carboxylic acid, 1. 2-Cyclopentanediene, 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 throw some light on the medicinal efficacy of Ashokarishtam. This is a preliminary work towards understanding more about the medicinal efficacy of Ashokarishtam.

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