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RESEARCH ARTICLE

GAS CHROMATOGRAPHY- MASS SPECTROSCOPIC (GC-MS) ANALYSIS OF GANDHARVAHASTA-TAILA: A POPULAR COMPOUND FORMULATION OF AYURVEDA

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ABSTRACT

The present study deals with the GC-MS analysis of one Ayurvedic formulation Gandharvahasta-Taila. This preparation is used to treat many neurological as well as inflammatory disorders in Ayurveda. The GC-MS analysis results indicated the presence of several biomolecules of which some of them like Eicosatrienoic acid, palmitic acid, ricinolic acid, stearidienoic acid, linolic acid and oleic acid were present in higher quantities. The study tries to correlate the medicinal activity of the medicine with the some important biomolecules known for their similar medicinal roles.

Key words: Gandharvahasta-Taila, GC-MS, Ricinolic Acid, Ayurveda, Fatty Acids.

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INTRODUCTION

Ayurveda – the science of life as its ancient origin in India during Vedic period. Rigveda and Aharvaveda are considered the compendia of knowledge in many disciplines. Ayurveda is considered the subdivision of Rigveda and Atharvaveda. The medicinal plants described in those Vedic compendia are also found in original text of Ayurveda i.e. Charaka Samhita and Susruta Samhita. The origin of natural substances and their therapeutic uses are described in Charaka Samhita and Susruta Samhita. These natural substances are used to prepare single and compound formulation for the beneficial of patients. The following compound formulations are still in use to alleviate the disorders such as Taila, Ghrita, and Vati etc. Of them medicated oil preparation is still popularly used. The medicated oil preparation are prescribed to alleviate the disorder have their origin of vitiation of Vata. Gandharvahasta-Taila[1] is one of the popular medicated oil formulation and still in use in various part of our country. The World Health Organization has requested to the member state to utilize their medical knowledge for well being of society. The Gandharvahasta-Taila is a compound formulation consisting of following ingredients Erandamula (root of *Ricinus communis*), Yava (*Hordeum vulgare*), Shunthi (*Zingiber officinale*), Eranda taila (castor oil), Godugdha (Cow's milk). For the quality, safety and efficacy, World Health Organization emphasize that standardization of such preparations are needed. Many methods are developing to determine the quality parameters of Oils such as iodine value, peroxide value, Thin Layer Chromatography, High Performance Thin Layer Chromatography etc. in various pharmacopoeias. Gas Chromatography- Mass Spectroscopy (GC-MS) analysis is one of them. It is a versatile tool to separate, quantify and identify volatile organic compounds and gases. It is an integrated composite analytical instrument combining GC which is an excellent in its ability for separation with mass spectrometery ideal in identification and elucidate structure of separate component. In this connection, Gandharvahasta- Taila has been prepared and to identify the presence of fatty acids by Gas Chromatography- Mass Spectroscopy (GC-MS) to establish a standardization parameter to confirm quality, purity and safety.

MATERIALS AND METHODS

Preparation of medicated oil

Gandharvahasta-Taila as per the classical reference has been prepared by Kerala Ayurveda, Limited, Aluva, Kerala, India.

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GC-MS analysis

GC-MS analysis of the methanol extract of Gandharvahasta-Taila (GHT) was carried out using a Clarus 680 PerkinElmer (Auto system XL) Gas chromatograph equipped and coupled to a mass detector SQ8T- PerkinElmer, 30m x 0.25mm x 0.25mm of capillary column. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. The instrument was set to an initial temperature of 50^0 C and maintained at this temperature for 6 min. Then oven temperature was raised up to 180^0 C at the rate of an increase 10^0 C/min and maintained for 6 min. At the end of this period, temperature was raised up to 280^0 C and maintained for 8min. Helium gas was used as carrier gas at constant flow rate of 1ml/min and 3microlitre of GHT sample was injected using split mode injection system. The injector temperature was maintained at 250^0 C. Mass spectra were taken at 70 eV. The solvent delay was 0 to 3 min. and the total GC-MS running time was 40 min. Sample were injected in split mode. Mass spectral scan range was set at 30 to 512 m/z. Finally obtained chromatograms were compared with the NIST (National Institute of Standards and Technology) and Wiley which provided sufficient information regarding fatty acids in GHT.

RESULTS

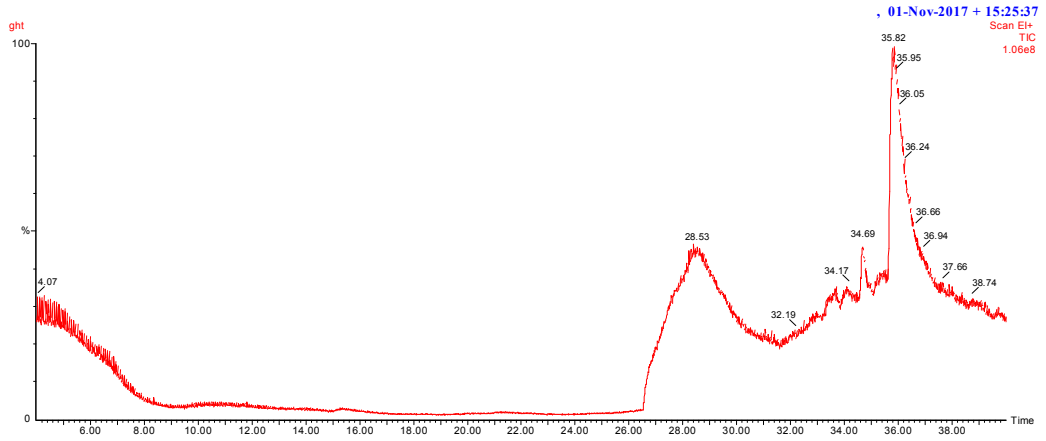

Figure 1. GC-MS total ionic chromatogram (TIC) of Gandharavahasta-Taila

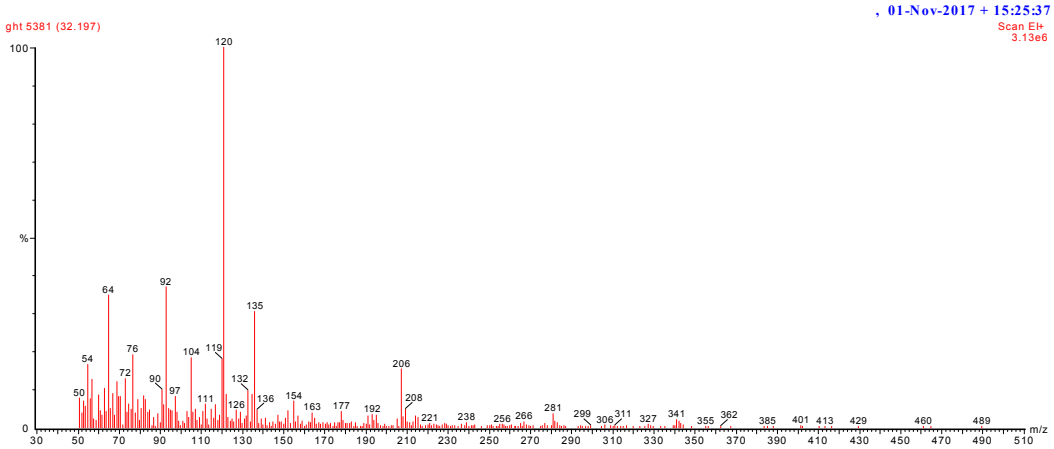

Figure 1. (A) MS of hexadecanoic acid

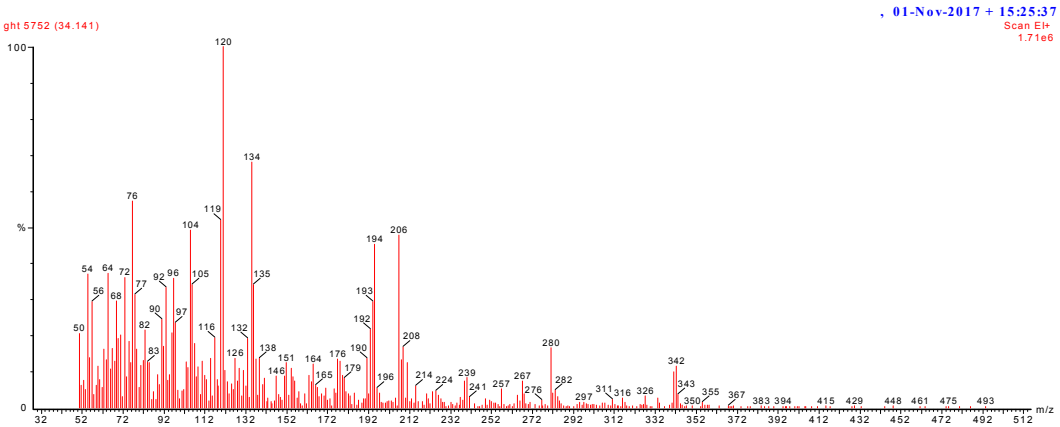

Figure 1. (B) MS of linoleic acid

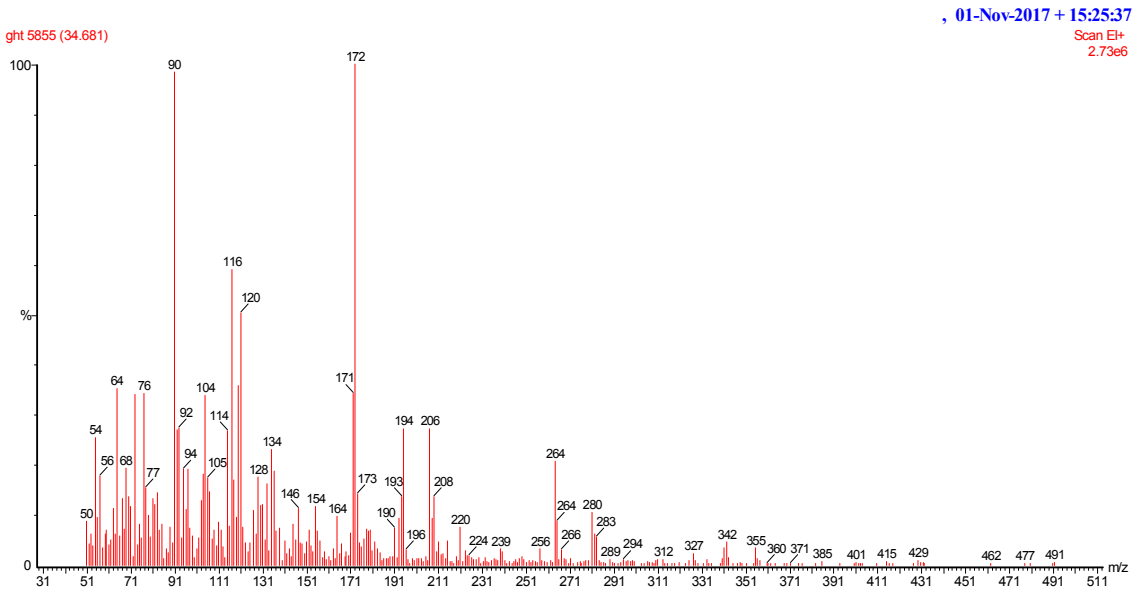

Figure 1. (C) MS of eicosanoic acid

Figure 1.(D) MS of stearic acid

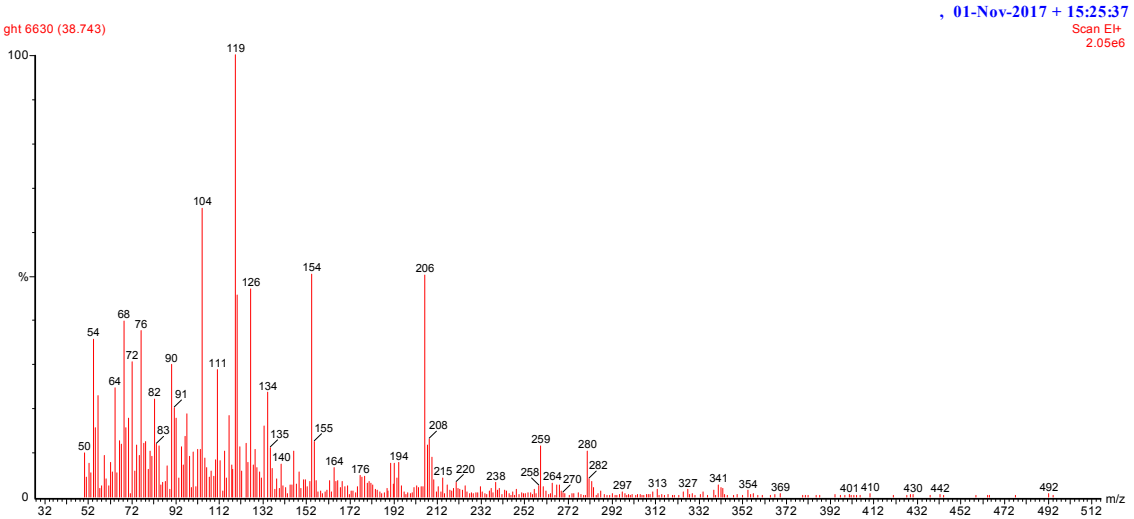

Figure 1 (E) MS of oleic acid

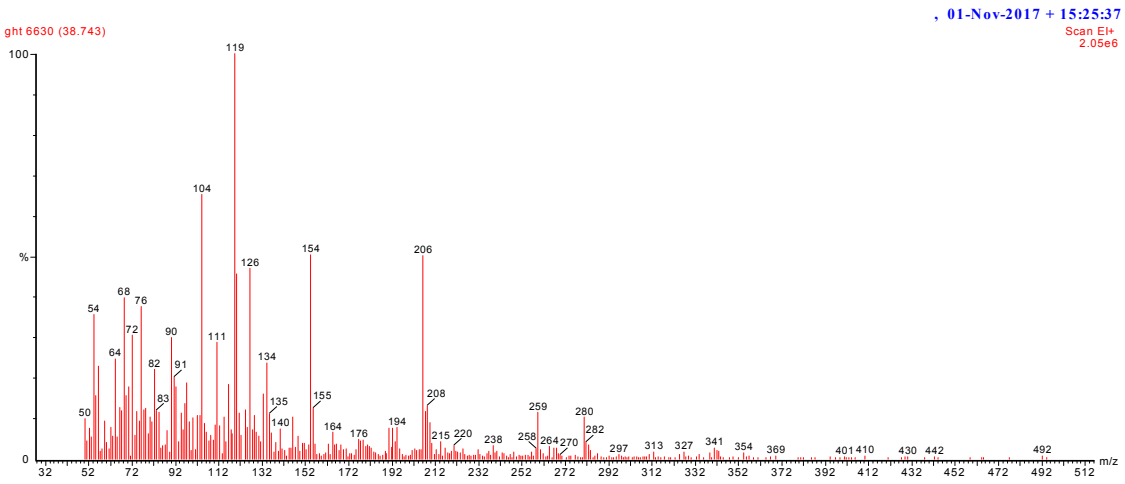

Figure 1 (E) MS of ricinoleic acid

Table. 1 Fatty Acid Composition And Fragmentation Pattern Of Gandharvahasta-Taila

Peak No.	Retention Time (min.)	Fatty Acid	Molecular Formula	Molecular Weight	Base peak	Characteristic ions
	32.19 a	Eicosatrieneoic Acid	$C_{20}H_{34}O_2$	306		
2	32.19 b	Palmitic Acid	$C_{16}H_{32}O_2$	256	74	74, 227,270
	34.17 a	Dihydroxystearic Acid	$C_{18}H_{36}O_2$	316		
3	34.17 b	Ricinoleic Acid	$C_{18}H_{34}O_3$	297		
	34.17 c	Linoleic acid	$C_{18}H_{32}O_2$	280	67	67, 263, 294
	34.17 d	Stearidonic Acid	$C_{18}H_{28}O_2$	276		
4	34.69 a	Eicosanoid Acid	$C_{20}H_{40}O_2$	312	55	55, 292, 324
	34.69 b					
5	35.82	Stearic acid	$C_{18}H_{36}O_2$	284	74	74, 255, 298
	38.74 a	Iso ricinolic Acid	$C_{18}H_{34}O_3$	409		
6	38.74 b	Ricinolic Acid	$C_{18}H_{34}O_3$	297	55	55, 183, 279, 297
	38.74 c	Oleic Acid	$C_{18}H_{34}O_2$	282	55	55, 264, 296

DISCUSSION

The fatty acids obtained from the GC-MS fragments are; (1) 11, 14, 17-Eicosatrienoic acid ($C_{20}H_{34}O_2$) is a rare polyunsaturated fatty acid of the omega-3-series. (2) Palmitic acid or n-hexadecanoic acid ($C_{16}H_{32}O_2$) is a saturated long chain fatty acid with a 16-carbon backbone, occurs in the form of esters (glycerides) in oils and fats of vegetable and animal origin. It is reported to have activities like antioxidant, antiandrognic, antibacterial and cytotoxic and as 5 alpha reductase inhibitior (Lalitharani *et al.,* 2009; Dinesh kumar and Raja kumar, 2015 and Rajeswari *et al.,* 2013). Retinyl palmitate is an antioxidant and a source of vitamin A added to low fat milk to replace the vitamin content lost through the removal of milk fat. Palmitate is attached to the alcohol form of vitamin A, retinol, to make vitamin A stable in milk. Rats fed a diet of 19% palmitic acid and 56% carbohydrate for extended periods showed alterations in central nervous system control of insulin secretion and suppression of the body's natural appetite-suppressing signals from leptin and insulin (the key hormones involved in weight regulation).[7] Palmitic acid strongly boosts metastasis in mouse models of human oral cancer cells. Among all fatty acids, it has the strongest effect in boosting the metastatic potential of CD36+ metastasis-initiating cells (Benoit *et al.,* 2009) (Pascual *et al.,* 2016) (3) Ricinolic acid or 12-hydroxy-9-*cis*-octadecenoic acid ($C_{18}H_{34}O_3$) is a polyunsaturated omega-9 fatty acid (Sánchez, Lucas, 2016). It is a major component of the seed oil obtained from mature *Ricinus communis* L. (castor plant). About 90% of the fatty acid content in castor oil is the triglyceride formed from ricinoleic acid (Frank D. Gunstone *et al.,* 2007) (https://en.m.wikipedia.org).

In an experimental study Ricinoleic acid exerts analgesic and anti-inflammatory effects. [12] In other study Ricinoleic acid specifically activates the EP3 prostanoid receptor for prostaglandin E2 (Vieira *et al.,* 2000). (4) Linoleic acid or 9, 12 Octadecadienoic acids ($C_{18}H_{34}O_3$) is a doubly unsaturated fatty acid, also known as an omega-6-fatty acid, occurring widely in plant glycosides. It is an essential fatty acid in human nutrition because it cannot be synthesized by humans. It is used in the biosynthesis of prostaglandins (via arachidonic acid) and cell membranes (Tunaru *et al.,* 2012). In a study Linoleic acid lipid radicals can be used to show the antioxidant effect of polyphenols and natural phenols. Experiments on linoleic acid subjected to 2,2'-Azobis (2-amidinopropane) dihydrochloride induced oxidation of linoleic acid; hence producing lipid radicals and then the use of different combinations of phenolics show that binary mixtures can lead to either a synergetic antioxidant effect or to an antagonistic effect towards the lipid radicals. Research like this is useful in discovering which phenols prevent the autoxidation of lipids in vegetable oils.[15] (5) Stearidonic Acid or 6, 9, 12, 15-Octadectetraenoic acid ($C_{18}H_{34}O_3$) also known as Moroctic acid isa polyunsaturated fatty acid of n 3 series, found in dietary plant oils. It may be used as a precursor to increase the eicosapentanoic acid (EPA) content of human lipids and that combination of gamma-linolenic acid and stearidonic acid. eicosapentanoic acid can be used to manipulate the fatty acid compositions of lipid pools in subtle ways. Such effects may offer new strategies for manipulation of cell compositions in order to influence cellular responses and functions in desirable ways(Peyrat-Maillard *et al.,* 2003). It is a anti-inflammatory, 5-alpha reductase inhibitor, antiandrogenic, antiarthiritic (Dandekar *et al.,* 2015) (6) Oleic

acid In chemical terms, oleic acid is classified as a monounsaturated omega-9 fatty acid. It has the formula ($C_{18}H_{34}O_2$). The term "oleic" means related to, or derived from, oil or olive, the oil that is predominantly composed of oleic acid, that is the most widely distributed and abundant fatty acid in nature. It is used commercially in the preparation of oleates and lotions, and emulsifying agent in foods and pharmaceuticals (Stedman, 26th ed) (Stedman *et al.,* 1995). Oleic acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol. However, its ability to raise HDL is still debated. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil. Adverse effects also have been documented, however, since both oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer, (Teres *et al.,* 2008) although the consumption of oleate in olive oil has been associated with a decreased risk of breast cancer (Pala *et al.,* 2001) (Martin-Moreno *et al.,* 1994).

Conclusion

From the results of the GC-MS Analysis of Gandarvahasta Taila, it can be concluded that the this medicated oil has potential to use in Vidradhi (abcess), Pliha (spleenomegaly), Gulma (abdominal lump), Udavarta (upward movement of gas), Sopha (inflammation), Udara roga (abdominal diseases), Mahavata Roga (Major neurological disorders).

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