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ASSESSMENT OF SIGNIFICANCE OF SAMSKARA IN THE PREPARATION OF SUKUMARA GHRITA BY CHROMATOGRAPHICAL TECHNIQUES

Ayurveda

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ABSTRACT

Sukumara Ghrita is used as Shamana (alleviating treatment) Aushadhi (medicine) and in Purvakarma (preceding procedure) of Virechana (purgation therapy) or Vamana (emesis therapy), Vibandha (constipation), Udara (diseases of abdomen / enlargement of abdomen), Gulma (abdominal lump), Pleeharoga (splenic disease), Vidradhi (abscess), Shopha (oedema), Yonisula (pain in female genital tract), Arsha (Haemorrhoids), Vriddhi (Hydrocele), Vatavyadhi (diseases due to Vata Dosha), Vatarakta (gout). Murchana Samskara (processing of ghee) is mentioned in Bhaishajya Ratnavali for both Taila (oil) and Ghrita Kalpana (ghee formulations) to reduce Amadosa, Durgandhata (bad odour) etc. Dosha (impurities) and to enhance the Viryata (potency) of Sneha (lipid). Physico-chemical analysis of Sukumara Ghrita prepared with Murchita Ghrita (processed ghee) sample has shown increase in the specific gravity, saponification value, Iodine value, Ester value, total fatty acids and decrease in acid value, viscosity, Density etc. Murchana process imparts changes in good colour, odour, minimizing rancidity & increasing stability facilitates better dissolution of bio constituents in Ghrita (ghee). So, to evaluate the changes after Murchana with advanced method like GC-MS study. The samples of Amurchita (plain ghee) and Murchita Ghrita (processed ghee) were subjected for analysis. In GC-MS study Ghrita (ghee) sample shown increase in number of unsaturated fatty acids like oleic acid, gondoic acid, 9,12-Octadecadienoic acid (Z,Z) methyl ester etc. may contribute increase in HDL Cholesterol and decrease in LDL Cholesterol. Other effects of Murchana Samskara (processing of ghee) are beneficial in decreasing risk of developing cardiovascular diseases, advantageous in impact on metabolism, in reducing risk of male infertility & growth retardation and addition of new components.

KEYWORDS

Sukumara Ghrita (ghee), Ghrita Murchana, Murchita Ghrita (processed ghee), GC-MS

INTRODUCTION

Different types of extraction are mentioned in Ayurvedic classics like Kashaya (decoction), Hima (cold infusion), Phanta (Hot infusion), Asava Arista (hydroalcoholic extraction) etc. To extract fat soluble active principles with different medias like Ghrita (ghee) and Taila (oil) are mentioned. Many Samskara (procedures) are mentioned in the Ayurvedic classics for preparation of Ghrita Kalpana (ghee formulation) and Taila Kalpana (oil formulations). Murchana samskara (procedure) is one among them mentioned for removal of undesired effects and enhancement of therapeutical properties[1].

Sukumara Ghrita is mentioned in Ayurveda classical books2,3,4. Sukumara Ghrita is indicated in Vibandha (constipation), Udara (diseases of abdomen / enlargement of abdomen), Gulma (abdominal lump), Pleeharoga (splenic disease), Vidradhi (abscess), Shopha (oedema), Yonisula (pain in female genital tract), Arsha (Haemorrhoids), Vriddhi (Hydrocele), Vatavyadhi (diseases due to VataDosha), Vatarakta (gout) and also as a Snehapana (oleation therapy) in Purvakarma (preceding procedure) of Virechana (purgation therapy) & Vamana (emesis therapy).

In analysis of Aushadhi Siddha Ghrita (medicated ghee) prepared with Amurchita Ghrita (unprocessed ghee) and Murchita Ghrita (processed ghee) shown Murchana Samskara (processing of ghee) is beneficial in stability and enhancement of therapeutical properties. So, an effort was made to know the changes in the components of Sukumara Ghrita prepared with Murchita Ghrita (processed ghee) sample by advanced techniques like chromatography.

Gas chromatography (GC) is a widely applied technique in many branches of science and technology. For over half a century, GC has played a fundamental role in the determination of components (in number & proportion) exist in a mixture. However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system. The most used, is the mass spectrometric detector (MSD), which allows obtaining the "fingerprint" of the molecule, i.e., its mass spectrum. Mass spectra provide information on the molecular weight, elemental composition. If a high-resolution mass spectrometer is used, functional groups present, in some cases, the geometry and spatial isomerism of the molecule5. Generally used for a) Identification and quantitation of volatile and semi volatile organic compounds in complex mixtures. b) Determination of molecular weights and elemental compositions of unknown organic compounds in complex mixtures. c) Structural determination of unknown organic compounds in complex mixtures both by matching their spectra with reference spectra and by a prior spectral interpretation

Aims & objectives

To analyse the changes in terms of fatty acids in Sukumara Ghrita prepared with Amurchita Ghrita (processed ghee) and Murchita Ghrita by GC-MS To provide additional evidence by comparing the GC-MS results of Amurchita Ghrita (ghee) and Murchita Ghrita (processed ghee)

Materials And Methods

Preparation of Sukumara Ghrita and Murchita Ghrita was done at Rasashastra and Bhaishajya Kalpana practical laboratory, S.D.M. College of Ayurveda, Hassan as per the reference of Ayurveda Formulary of India and Bhaishajya Ratnavali. Chromatographical study was conducted at Bureau Veritas, Chennai Method of sample preparation for GCMS analysis

Esterification: 0.2 g of sample is taken in 50 ml of F.B Flask. Then added 2.4 ml of methanolic-HCl and 12 ml of methanol. Reflux it for 1 hour at 1000 C. Then check the TLC for the completion of the reaction and reaction mixture was extracted with hexane. Hexane layer was taken, dried it at room temperature and sample given for GC-MS analysis

Observations And Results

Table-1 showing the components of GCMS report of Ghrita samples

Sl no	Components	Functional group	Sukumara Ghrita prepared with Amurchita Ghrita	Sukumara Ghrita prepared with Murchita Ghrita
1.	Cholesterol	alcohol	2	0
2.	Z-11-Pentadecenol	alcohol	1	0
3.	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11, 12,13,14, 15,16,17-tetradecahydro-1H-cyclopenta{a}phenanthren-3-ol	alcohol	1	0

		Total alcohol	4	0
4.	Methyl tetradecanoate	ester	3	2
5.	Methyl 9-tetradecenoate	ester	1	1
6.	Methyl Z-11-tetradecenoate	ester	1	0
7.	Methyl 13-methyltetradecanoate	ester	2	2
8.	Methyl 9-methyltetradecanoate	ester	1	3
9.	Methyl hexadec-9-enoate	ester	1	1
10.	Methyl 9-heptadecenoate or 9-17:1	ester	1	1
11.	Methyl 18-methylnonadecanoate	ester	1	1
12.	Methyl 12-oxo-octadec-9-enoate	ester	0	1
13.	Methyl 12-hydroxy-9-octadecenoate	ester	1	1
14.	Cyclododecanecarboxylic acid, methyl ester	ester	1	0
		Total ester	13	13
15.	Pyrazole,3-nitro-	ether	0	1
		Total ether	0	1
16.	Octanoic acid, methyl ester	saturated	3	0
17.	Decanoic acid, methyl ester	saturated	3	5
18.	Dodecanoic acid, methyl ester	saturated	2	0
19.	Undecanoic acid, 10-methyl –methyl ester	saturated	1	1
20.	Tridecanoic acid, 12 –methyl-methyl ester	saturated	3	1
21.	Pentadecanoic acid, methyl ester	saturated	5	5
22.	Pentadecanoic acid, 14-methyl,methyl ester	saturated	2	3
23.	Hexadecanoic acid, methyl ester	saturated	4	3
24.	Hexadecanoic acid, 15-methyl-, methyl ester	saturated	3	2
25.	Hexadecanoic acid, 14-methyl-, methyl ester	saturated	3	4
26.	Heptadecanoic acid, methyl ester	saturated	3	4
27.	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	saturated	1	1
28.	Eicosanoic acid, methyl ester	saturated	2	1
29.	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	saturated	0	1
30.	Nonadecanoicacid ,methyl ester	saturated	3	1
31.	Tetradecanoic acid, 12-methyl-,methyl ester	saturated	1	0
32.	Methyl myristoleate	saturated	1	1
33.	11-Hexadecenoic acid, 15-methyl-,methyl ester	saturated	1	0
34.	Methyl stearate	saturated	3	4
35.	10-Nonadecenoic acid, methyl ester	saturated	1	0
		Total saturated	45	37
36.	Methyl 9-cis, 11-trans-octadecadienoate	unsaturated	0	1
37.	Methyl 10-trans, 12-cis -octadecadienoate	unsaturated	2	1
38.	2,5-Pyrrolidinedione,3-methyl-4-propyl-	unsaturated	0	1
39.	Cis-10-Nonadecenoic acid,methyl ester	unsaturated	1	0
40.	9-Hexadecenoic acid, methyl ester	unsaturated	0	3
41.	9,12-Octadecadienoic acid (Z,Z) methyl ester	unsaturated	1	3
42.	9,12-Octadecadienoic acid,methyl ester, (E,E)	unsaturated	2	1
43.	9-Octadecenoic acid, methyl ester, (E)-	unsaturated	2	2
44.	9-Octadecenoic acid (Z)-, methyl ester	unsaturated	3	3
45.	8-Octadecenoic acid, methyl ester	unsaturated	1	1
46.	7-Hexadecenoic acid, methyl ester,(Z)-	unsaturated	1	1
47.	11-Octadecenoic acid, methyl ester	unsaturated	1	1
48.	9-octadecenoic acid,12-hydroxy-methyl ester,{R-(Z)}-	unsaturated	1	2
49.	9-octadecenoic acid,12-hydroxy-methyl ester,(Z)-	unsaturated	1	1
50.	9-Hexadecenoic acid,methyl ester,(Z)-	unsaturated	3	1
51.	9-octadecenoic acid, methyl ester	unsaturated	1	0
52.	Cis-10-Heptadecenoic acid, methyl ester	unsaturated	1	1
53.	Cyclohexane,1(cyclohexylmethyl)-4-methyl-, cis-	unsaturated	0	1
		Total unsaturated	21	24

Table-2 showing the classification of components of GCMS report of Ghrita samples

Sl no	Components	Sukumara Ghrita prepared with Amurchita Ghrita	Sukumara Ghrita prepared with Murchita Ghrita
1.	Alcohol	4	0
2.	Aldehyde	0	0
3.	Ester	13	13
4.	Ether	0	1
5.	Ketone	0	0
6.	Saturated fatty acids	45	37
7.	Unsaturated fatty acids	21	24
	Total	83	75

Table-3 Showing Area% of major components detected in Ghrita samples by GCMS report

	COMPONENTS		Type of fatty acid	Sukumara Ghrita prepared with Amurchita Ghrita	Sukumara Ghrita prepared with Murchita Ghrita
Sl no	Systematic name	Common name		Area%	Area%
1.	Decanoic acid, methyl ester	Capric	saturated	0.76	0.26
2.	Dodecanoic acid, methyl ester	Lauric	saturated	1.83	0.71
3.	Hexadecanoic acid, methyl ester	Palmitic	saturated	17.42	15.68
4.	Heptadecanoic acid, methyl ester	margaric	saturated	0.7	
5.	Methyl stearate		saturated	8.43	7.31
6.	Eicosanoic acid, methyl ester	arachidic	saturated	---	---
7.	Cis-11-Eicosenoic acid, methyl ester	gondoic	unsaturated	---	---
8.	9,12-Octadecadienoic acid (Z,Z) methyl ester	linoleic	unsaturated	5.02	3.33
9.	9-Octadecenoic acid, methyl ester, (E)-	oleic	unsaturated	14.5	
10.	9-Octadecenoic acid (Z)-, methyl ester	oleic	unsaturated	---	2.68
11.	9-octadecenoic acid,12-hydroxy-methyl ester,{R-(Z)}-	oleic	unsaturated	32.02	46.96

Table -4 Showing numbers of major components detected in Ghrita samples by GCMS report

	COMPONENTS		Type of fatty acid	Sukumara Ghrita prepared with Amurchita Ghrita	Sukumara Ghrita prepared with Murchita Ghrita
Sl no	Systematic name	Common name			
1.	Decanoic acid, methyl ester	Capric	Saturated	3	5
2.	Dodecanoic acid, methyl ester	Lauric	Saturated	2	0
3.	Hexadecanoic acid, methyl ester	Palmitic	Saturated	4	3
4.	Heptadecanoic acid, methyl ester	margaric	Saturated	3	4
5.	Eicosanoic acid, methyl ester	arachidic	Saturated	2	1
6.	9,12-Octadecadienoic acid (Z,Z) methyl ester	linoleic	unsaturated	1	3
7.	9-Octadecenoic acid, methyl ester, (E)-	oleic	unsaturated	2	2
8.	9-Octadecenoic acid (Z)-, methyl ester	oleic	unsaturated	3	3
9.	8-Octadecenoic acid, methyl ester		unsaturated	1	1
10.	9-octadecenoic acid,12-hydroxy-methyl ester,{R-(Z)}-	oleic	unsaturated	1	2

DISCUSSION

Total 83 number of components are detected in Sukumara Ghrita prepared with Amurchita Ghrita and 75 in Sukumara Ghrita prepared with Murchita Ghrita. Number of components are decreased in Sukumara Ghrita.

4 alcohol compounds detected in in Sukumara Ghrita prepared with Amurchita Ghrita and are absent in Sukumara Ghrita prepared with Murchita Ghrita. After Murchana samskara, alcohol components decrease when prepared with Murchita Ghrita in Sukumara Ghrita.

13 esters are detected each in Sukumara Ghrita prepared with Amurchita Ghrita & Sukumara Ghrita prepared with Murchita Ghrita. It suggests that there is no change in the esters in case of Sukumara Ghrita when prepared with Murchita Ghrita.

1 ether compound is detected in Sukumara Ghrita prepared with Murchita Ghrita and is absent in Sukumara Ghrita prepared with Amurchita Ghrita. There is increase of ether group in case of Sukumara Ghrita when prepared with Murchita Ghrita.

45 fatty acids are detected in Sukumara Ghrita prepared with Amurchita Ghrita and 37 in Sukumara Ghrita prepared with Murchita Ghrita. Saturated fatty acids are decreased in Sukumara Ghrita when prepared with Murchita Ghrita. Saturated fats increase Low Density Lipoproteins (LDL or bad cholesterol) &very low-density lipoproteins (VLDL's). So Murchana samskara is proved beneficial therapeutically.

Decanoic acid increased in Sukumara Ghrita prepared with Murchita Ghrita and decreased in Area%. Decanoic acid, also known as “Capric acid,” occurs naturally in coconut oil and palm kernel oil, as well as in the milk and animal fats of some mammals6. According to study results published in 1998 in the "American Journal of Clinical Nutrition." Capric acid together with lauric acid and caprylic acid, other medium-chain fatty acids help to increase levels of high-density lipoproteins (HDL), relative to low-density lipoproteins (LDL).

Dodecanoic acid, methyl ester which is known as Lauric acid7 is decreased in area% and number in Sukumara Ghrita prepared with Murchita Ghrita. Lauric acid, as a component of triglycerides, comprises about half of the fatty acid content in coconut oil, laurel oil, and palm kernel oil (not to be confused with palmoil), Otherwise, it is relatively uncommon. It is also found in human breastmilk (6.2% of total fat), cow's milk (2.9%), and goat's milk (3.1%).

Lauric acid increases total serum cholesterol more than many other fatty acids. But most of the increase is attributable to an increase in high-density lipoprotein (HDL) (the "good" blood cholesterol). As a result, lauric acid has been characterized as having "a more favorable effect on total HDL cholesterol than any other fatty acid.

Palmitic acid i.e. Hexadecanoic acid, methyl ester is decreased area% and number in Sukumara Ghrita prepared with Murchita Ghrita. According to the World Health Organization, evidence is "convincing"

that consumption of palmitic acid increases risk of developing cardiovascular diseases8.

Heptadecanoic acid is increased in number in Sukumara Ghrita when prepared with Murchita Ghrita and absent in area%. Heptadecanoic acid, also called margaric acid or C17:0, is found in dairy fat, rye, and some fish and could help reverse the early stages of diabetes in humans.

Researchers studied the fatty acid blood levels in 49 dolphins as well as their dietary fish shows that, of the 55 fatty acids studied, the saturated fat heptadecanoic acid appeared to have had the most beneficial impact on metabolism9.

9-Octadecenoic acid, methyl esters are also called as oleic acid are increased in number when prepared with murchita ghrita in sukumara ghrita.

Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fatsandoils. In chemical terms and is classified as a monounsaturated omega-9 fatty-acid10.

Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol11.

21 unsaturated fatty acids detected in Sukumara Ghrita prepared with Amurchita Ghrita and 24 in Sukumara Ghrita prepared with Murchita Ghrita. Unsaturated fatty acids are increased in Sukumara when prepared with Murchita Ghrita. Unsaturated fats increase High-Density Lipoprotein (HDL) and decrease Low Density Lipoproteins (LDL).

It shows that Murchana samskara proved beneficial in all the three Sukumara Ghrita when prepared with Murchita Ghrita.

CONCLUSION

Increased Decanoic acid which is called as capric acid in Sukumara Ghrita prepared with Murchita Ghrita proved Murchana samskara beneficial in increasing HDL cholesterol. Decreased Palmitic acid which is known as Hexadecanoic acid, methyl ester in Sukumara Ghrita when prepared with Murchita Ghrita proved beneficial in decreasing risk of developing cardiovascular diseases. Increased Heptadecanoic acid, also called margaric acid in Sukumara Ghrita prepared with Murchita Ghrita proved that Murchana Samskara is advantageous in beneficial impact on metabolism. Increased number of 9-Octadecenoic acid, methyl esterswhich are also called as oleic acid in Sukumara Ghrita prepared with Murchita Ghrita proved advantageous in decreasing LDL and increasing HDL cholesterol.

Increased unsaturated fatty acids in Sukumara Ghrita prepared with Murchita Ghrita provide evidence base to the statement mentioneed in Ayurveda classic for quality, stability & therapeutic efficacy. Murchana Samskara is benficial to human health by increasing the HDL.

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