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THE GC-MS STUDY OF MADHYAMA PANCHAMOOLA SADHITA GHRITA

Nagendra Chary M.[1]*, Lalitha B. R.[2] and T. Anil kumar[3]

[1]Ph.D Scholar, [2]Professor and HOD, Department of Dravyaguna
Government Ayurveda Medical College, Bengaluru, Karnataka, India.
[3]Professor and HOD, Department of Medicine, M.S Ramaiah Medical College, Bengaluru, India.

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***Corresponding Author**
Dr. Nagendra Chary M.
Ph.D Scholar, Department of Dravyaguna
Government Ayurveda Medical College, Bengaluru, Karnataka, India.

ABSTRACT

Background: In Ayurveda, medicated ghee is one the dosage form which focuses on the male infertility issues. Madhyama panchamoola is a group of five drugs consist of Bala (Sida cordifolia) Punarnava (Boerhavia diffusa), Eranda (Ricinus communis) Mudgaparni (Vigna trilobata) and Mashaparni (Teramnus labialis). Each of these drugs possess Rasayana (Rejuvenation), Vrushya, (Aphrodisiac) and Shukrajanana (Spermatogenic) activities. Madhyama panchamoola sadhita ghrita helps in treating Oligospermia. Gas chromatography – Mass Spectrometry is the proven analytical method which is highly effective and versatile to identify different components in a ghee based samples. **Objective:** The present study deals with the Gas Chromatography Mass Spectrometry (GC–MS) analysis of Madhyama panchamoola sadhita ghrita. **Materials and Methods:** The Madhyamapanchamoola sadhita ghrita was prepared by following Ayurveda formulary of India with GMP certified company and subjected to GC–MS analysis. **Results:** GC-MS revealed total 19 compounds. Glycerol, Squalene, Lanosterol (Lupeol) were experimentally proved to have Antioxidant and Spermatogenic activities. **Conclusion:** The Phytochemical constituents identified in the GC–MS profile revealed Madhyama panchamoola sadhita ghrita has a potential Shukra janana activity.

KEYWORDS: Madhyama panchamoola sadhita ghrita, GC-MS, Spermatogenic activity.

INTRODUCTION

Ayurveda Pharmaceutics utilizes medicinal plants for many dosage forms in order to obtain expected pharmacological actions.[1] Ghrita or medicated ghee one type of dosage form in which decoction or paste of crude drug boiled in the ghee media so that active principles of drug get transferred into ghrita.[2] Drugs processed in Ghrita like Phala ghrita, Apathyakara ghrita are well known in the management of male infertility specially Oligospermia.[3,4] Madhyama panchamoola Sadhita Ghrita is a novel ghee preparation. It comprises of drugs Bala (Sida cordifolia), Punarnava (Boerhavia diffusa), Eranda (Ricinus communis), Mudgaparni (Terminus labialis), Mashaparni (Vigna trilobata) and Ghee. Each of these possess Balya (Strength promoter), Rasayana (Rejuvenation), Vrushya (Aphrodisiac) Jeevaniya (Life promoting) and Shukrajanana (Spermatogenic) properties. Combined effect of these will rectify the Shukradosha and enhances Shukra (semen and sperm)[5] GC-MS is normally used to analyze of components existing in medicinal plants. This technique has been proved to be a valuable method of analysis for non-polar components and volatile oil, fatty acids, lipids and alkaloids.[6] Hence present study highlights the bioactive components of Madhyama panchamoola Sadhita ghrita through Gas chromatography mass spectrometry. (GC-MS).

Madhyma panchamoola

Madhyama panchamoola consist of Bala, Punarnava, Eranda, Mudgaparni and Mashaparni.[7] Different dosage forms and therapeutic combinations are not referred to this group in the classics. Relevant Phytoconstituents and pharmacological activities of Madhyama panchamoola, properties of ingredients of Madhyama panchamoola sadhita ghrita for Shukra janana(spermatogenic) activities are briefly described in the table 1 and 2.

Table no. 1: Phyto constituents and Pharmacological activities of drugs of Madhyama Panchamoola related to Shukrajanana.

Sl. no	Name of drugs	Phyto constituents	Pharmacological activities
1	*Bala (Sida cordifolia)*	Ecdysterone	Improves the sexual functions and sperm quality.[8]
2	*Punarnava (Boerhaavia diffusa)*	Boeravinone B and G Rotenoids	Anti-stress activity, Antioxidant.[9]
3	*Eranda (Ricinus communis)*	Octacosanol	Improves Volume, Sperm concentration, Motility, in rams[10]
4	*Mudgaparni (Vigna trilobata)*	Vitexin, Quercetin	Vitexin- Antioxidant, ameliorates sexual dysfunction and fertility impairments in male diabetic mice. Quercetin improves

			sperm morphology and functions[11,12]
5	*Mashaparni (Teramnus labialis)*	Vitexin, Bergenin	Bergenin-Antioxidant, Improves sperm concentration, diabetic testicular complications Reduce the sperm DNA Damage in Wistar albino rats[13]

Table no. 2: Pharmacological profile of Madhyama panchamoola sadhita ghrita.

Sl no	Name of the ingredient	Properties				
		Rasa	Guna	Veerya	Vipaka	Karma
1	Bala	Madhura	Snigdha Pichhila	Sheeta	Madhura	Vatapittashamaka, Brumhana, Balya, Shukrala,, Ojovardhana [14]
2	Punarnava	Madhura Tikta	Laghu, Rooksha	Ushna	Madhura	Tridoshahara,Deepana Anulomana, Vrushya Rasayana [15]
3	Eranda	Madhura	Snigdha, Teekshna Sukshma	Ushna	Madhura	Vatahara,Balya,Vrushya, Shukrashodhana[16]
4	Mudgaparni	Tikta Madhura	Laghu Rooksha	Sheeta	Madhura	Tridoshahara, Deepana, Anulomana, Jeevaneeya Shukrala, Vrushya[17]
5	Mashaparni	Tikta Madhura	Laghu, Rooksha Snigdha	Sheeta	Madhura	Vata pitta shamaka Deepana,Anulomana Balya, Jeevaniya Shukrajanana.[18]
6	Ghrita	Madhura	Snehotthama	Sheeta	Madhura	Agnivardhana, kapha vardhana, Vata pitta shamaka, shukra vardhana[19]

OBJECTIVES

- To carry out GC-MS analysis for Madhyama panchamoola sadhita ghrita.
- To evaluate phyto constituents for spermatogenesis

MATERIALS AND METHODS

Plants of Madhyama panchamoola were collected from their habitat and identified from botanist from Foundation for Revitalisation of Local Health Traditions (FRLHT), Bengaluru. Madhyama panchamoola sadhita ghrita is a ghee preparation it was prepared as per general principles of Ayurveda formulary of India (AFI) at Aashirwad Ayurveda Pharmacy, India Pvt Ltd, Coimbatore and subjected to GC–MS analysis by standard procedure to evaluate the phytoconstituents in the given sample.

Instrument: GC (Agilent: GC: [G3440A] 7890A. MS- MS: 7000 Triple Quad GC–MS) was equipped with MS detector.

Sample preparation: 1 ml of sample is diluted to 5ml using n-hexane. Mixed well transferred to HPLC vial and injected for scanning.

Instrument condition

GC-condition: Injection volume : 1 µl

Liner type : spit less

Inlet temperature : 280 ºC

Column : HP-5ms Ultra Inert: 30 m x 250 µm x 0.25 µm

Mass condition : Sample analyzed in scan mode

GC-MS protocol: The column used for analysis is HP-5ms Ultra Inert: 30 m x 250 µm x 0.25 µm is composive of (5%-phenyl)-methylpolysiloxane. Electron impact mode at 70 eV. Helium (99.999%) was used as a carrier gas at constant flow of 1 ml/min. injector temperature- 280 ºC with spitless mode, ion source temperature is 280 ºC. Oven programmed is initially 130 ºC increased to 180 ºC with rate of 10 ºC/min withhold time of 10 min. Then increased to 250 ºC with rate of 5 ºC/min withhold time of 5 min. Then increased to 280 ºC with rate of 5 ºC/min withhold time of 2 min. Total run time is 32 minutes. The compounds are identified by GC-MS Library -NIST.

RESULTS

Table 3: GC-MS profile of Madhyama panchamoola sadhita ghrita showing the type of Compound, Retention Time, Molecular weight, and Molecular formula and Pharmacological impact on spermatogenesis of each compound.

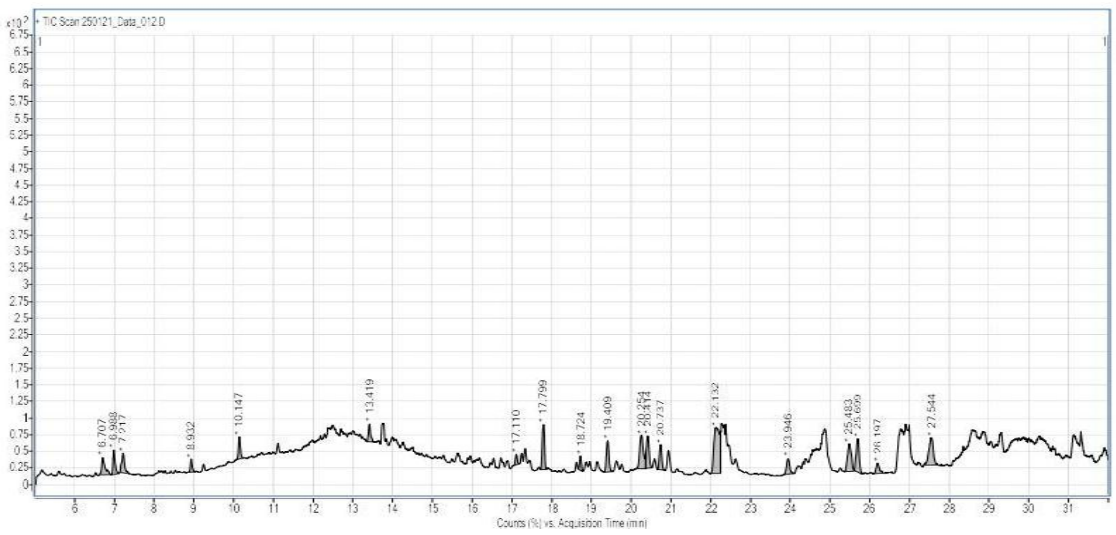
Sl. n	Compound name	RT	MW g/mol	MF	Pharmacological impact on spermatogenesis.
1	aR-Turmerone	6.78	216.31	$C_{15}H_{20}O$	-
2	2-Pentadecanone	7.02	-	-	-
3	3-Hydroxy-dodecanoic acid, pyrrolidide	7.21	241.37	$C_{14}H_{27}NO$	-
4	3,7,11-Trimethyldodecylacetate	8.93	264.49	$C_{17}H_{28}O_2$	-
5	2H-Pyran-2-one, tetrahydro-6-nonyl-	10.147	226.35	$C_{14}H_{26}O_2$	-
6	Oleic Acid	13.419	282.59	$C_{18}H_{32}O_2$	-
7	Spiro[furan-2(5H),2'(1'H)-naphtho[2,1-b]furan]- 5-one,	17.799	318.4	$C_{20}H_{30}O_3$	-

	3'a,4',5',5'a,6',7',8',9',9'a,9'b-decahydro- 3,3'a,6',6',9'a-pentamethyl-, [2'R-(2'α,3'aβ				
8	2,6,9,12,16-Pentamethylheptadeca-2,6,11,15-tetraene-9-carboxylic acid	19.409	_	_	-
9	Pregn-5-en-20-one, 12-(acetyloxy)-3,8,14,17-tetrahydroxy-, (3β,12β,14β,17α)-	20.254	_	_	-
10	Tetradecanoic acid,2,3-dihydroxypropyl ester	20.414	228.37	$C_{14}H_{28}O_2$	-
11	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	22.132	414.57	$C_{23}H_{42}O_6$	Glycerol act as metabolic re router linking major pathways glucose metabolism, lipid metabolism. Provides proper nursing environment for spermatogenesis.[20]
12	1-Heptatriacotanol	20.737	537	$C_{37}H_{76}O$	-
13	Ingol 12-acetate	25.483	408.5	$C_{22}H_{32}O_7$	-
14	Squalene	25.699	422.89	$C_{30}H_{50}$	Boars fed on Squalene at 20 or 40 mg/kg/day for 60 days had higher semen volume and higher sperm motility which increase the performance.[21]
15	9,12,15-Octadecatrienoic acid, 2,3- dihydroxypropyl ester, (Z,Z,Z)-	23.946	278.4	$C_{18}H_{30}O_2$	-
16	Lanosterol (lupeol)	27.544	426.7	$C_{30}H_{50}O$	Oral administration of lupeol at a dose of 50mg/kg for two weeks significantly decreased the oxidative stress and Neuro inflammatory effect.[22]
17	Hexadecanoic acid, 1-(hydroxymethyl)-1,2- ethanediyl ester	26.197	-	-	-
18	Hexadecanoic acid, 1-(hydroxymethyl)-1,2- ethanediyl ester	17.110	-	-	-
19	Myristin 2,3-deaceto-1-	18.724	-	-	-

RT- Retention time, MW – Molecular weight, MF- Molecular formula

DISCUSSION

GC-MS which elute the volatile compounds in the sample. 19 compounds identified through GC-MS along with their Retention time. Their molecular weight and molecular formula as searched by NIST (National institute of standard technology). Three compounds namely Glycerol, Squalene and Lanosterol have action on spermatogenesis. Out of which compounds Glycerol and Squalene, proven for their Spermatogenic activity where as Lanosterol is antioxidant and which support the spermatogenesis process. Other compounds like Ecdysterone ,Vitexin, Bergenin, Quercetin etc which also have Spermatogenic activity have not been identified because of limitation in GC-MS. Madhyama panchamoola drugs have Deepana , Balya, Rasayana, Jeevaniya, Bhrumhana, Shukra janana activities, in specific Bala -Vrushya, Punarnava- Rasayana, Eranda- Vrushya and Vatahara and Mudgaparni and Mashaparni)-Shukra janana activities. Except Bala all four drugs possess Madhura, Tikta rasa. These properties include in the treatment principle for treating Shukra dosha.[23] All drugs having madhura vipaka. Madhura vipaka is Kaphavardhaka and Shukrala.[24] Ghee consist of essential fatty acids and fat soluble vitamins like A.D,E and K. Vitamin A and E are antioxidants.[25] Ghee is suitable vehicle and dosage form for treating male infertility. Madhyama panchamoola have anti-oxidant, Hepatoprotective, anti-inflammatory, anti diabetic activities and by virtue of its phyto constituents like Squalene, Glycerol and Lanosterol may correct the Shukra dosha and enhances the count, motility and morphology of a sperm.



Qualitative compound Report

Figure 1: Gas chromatography mass spectrometry profile of Madhyama panchamoola sadhita ghrita.

CONCLUSION

Madhyama panchamoola sadhita ghrita has potential Shukra janana activity by virtue of compounds like Squalene, Glycerol and Lanosterol which helps in treating Ksheenashukra (Oligospermia).

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