ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

THE GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF AN AYURVEDIC FORMULATION *AMRUTHADI KASHAYA*

Asha Kumari L S[1]*, S Anand[2]

*[1]PG Scholar, [2]Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Government Ayurveda College, Thiruvananthapuram, Kerala, India.

Article info

Article History:
Received: 11-01-2024
Accepted: 12-02-2024
Published: 05-03-2024

KEYWORDS:
Amruthadi kashaya, GCMS, *Pandu*, *Kamala, Rakthapitha*, a-Terpineol.

ABSTRACT

Amruthadi Kashaya is a *Ayurvedic* formulation mentioned in the book Arogya Kalpadruma for the treatment of *Pandu* (anaemic syndrome), *Kamala* (jaundice) and *Raktapitha* (bleeding disorders). The *Yoga* contains six drugs namely *Amrutha (Tinospora cordifolia), Vasa (Adhatoda vasica), Yashtimadhu (Glycyrrhiza glabra), Tiktha (Solanum anguivi), Ela (Elettaria cardamomum), Pichumantha (Azadirachta indica).* This *Kashaya* is rich in phytoconstituents, such as phenolics, terpenoids, and lipids, which are known for their many biological benefits, including anti-inflammatory, anti-cancer, and antioxidant effects. The purpose of the current study is to use Gas Chromatography Mass Spectrometry (GCMS) analysis to identify the different types of bio molecules that are present in this *Kashaya*. *Kashaya* was prepared according to standard protocol. It was concentrated in the rotary vacuum evaporator, and the total soluble solid (TSS) obtained was used for GCMS analysis. The GCMS of *Kashaya* showed the presence of bio molecules such as 7 Hexadecanoic acid, methyl ester, Methyl tetradecanoate, Diethyl Phthalate, Dodecanoic acid, methyl ester, Methyl stearate, à-Terpineol, Methyl 8-methylnonanoate, 3-Cyclohexene-1-methanol, à, à,4-trimethyl-, acetate, Decanoic acid, methyl ester, 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-, (E)-, Ethyl iso-allocholate, Spiro [2,4] hepta-4,6-diene, Butylated Hydroxytoluene, 9-Hexadecenoic acid, methyl ester, (Z) etc. à-Terpineol, 7-Hexadecenoic acid, methyl ester, Butylated hydroxytoluene, Nerolidyl acetate have antioxidant activity likewise some of the bio molecules have anti-inflammatory, antibacterial, anticancer activity. It is concluded that *Ayurvedic* formulation *Amruthadi Kashaya* contains many bio molecules having therapeutic actions.

INTRODUCTION

Between 2500 and 500 BC, the Ayurvedic science originated in India[1]. Since then, Indian medicinal practices have included Ayurveda as a system of medicine and adopted its dosage forms like *Swarasa kalka, Kashaya, Churna, Avaleha, Arishta, Asava* and *Gritha* into practice. These dosage forms face limited recognition internationally due to a lack of scientific evidence and proven efficacy, so there is an urgent need to validate and standardize Ayurvedic formulations according to modern parameters in order to bring these traditional medicinal practices back to the forefront of research.

There are only a few hepatoprotective drug which are available in contemporary sciences and surplus of medicines are mentioned in *Ayurveda* under *Pandu* (anaemic syndrome) *Kamala* (jaundice), *Yakrit roga* (liver diseases) *Adhikarana*. *Amruthadi kashaya* is one among them mentioned in *Pandu prakarana* of *Arogya Kalpadruma* book by Kaikulangara Ramawarrier [2] which is indicated for *Pandu, Kamala* and *Raktha pitha*.

The ingredients of the *Yoga* are *Amrutha (Tinospora cordifolia), Vasa (Adhatoda vasica), Yashtimadhu (Glycyrrhiza glabra), Tiktha (Solanum anguivi), Ela (Elettaria cardamomum), Pichumantha (Azadirachta indica)*. *Amrutha* one of the best rejuvenator and tonic have proven anti-inflammatory, anti-arthritic and hepatoprotective activity[3], *Vasa* for

Access this article online

Quick Response Code

https://doi.org/10.47070/ijapr.v12i2.3098

hepatoprotective, antibacterial, anti-inflammatory, antioxidant activity[4], *Yashtimadhu* for hepato-protective, antiviral, anti-carcinogenic, antioxidant and anti-inflammatory activity,[5] *Tikta* for antioxidant activity[6], *Ela* for hepatoprotective[7], antioxidant, anti-inflammatory, gastro protective activity.

The study formulation *Amruthadi kashaya*, is also need to be scientifically analyzed for its acceptance in the present era. So the present study deals with the GCMS analysis of *Amruthadi kashaya* for understanding the presence of various bio molecules which could give a clue to the mechanism of action of this medicine.

MATERIALS AND METHODS

Preparation of *Kashaya*: *Kashaya* was prepared according to the method mentioned in Sharangadhara Samhitha Eight gm of each of the cleaned, washed, dried, crushed drugs of root of *Vasa*, root of *Yashtimadhu*, seed of *Ela*, root of *Tikta*, and stem bark *Nimba*, and fresh *Guduchi* (outer covering removed) were taken for the preparation of *Kashaya*. All the ingredients were taken in a steel vessel, and 96 ml of water was added and marked with a measuring scale. Then, 672 ml of water was added. Then the vessel is placed over an ignited stove, and the *Kashaya* is boiled until it is reduced to 96 ml (up to the marked level). The *Kashaya* was filtered into another clean stainless-steel vessel through a double-layered muslin cloth. Then the *Kashaya* was concentrated in a rotary vacuum evaporator, and the total soluble solid was collected.

Fig no 1: Raw drugs of *Amruthadi Kashaya*

A Fresh stem of *Amrutha*, B Dried root of *Vasa*, C Dried root of *Yashtimadhu*, D Dried root of *Tiktha*, E Dried seed of *Ela*, F Stem bark *Pichumantha*

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis: It was conducted in the Central Instruments Laboratory, CoVAS, Mannuthy using M/S Shimadzu GC-MS Model Number: QP2010S (Software: GCMS Solutions) equipped with ELITE-SMS Capillary column (30 mx 0.25 mm ID, 0.25 um thickness) for analysis of the chemical composition of methanolic extract of TSS of *Kashaya*. One milligram TSS of *Kashaya* was dissolved in one millilitre of methanol and filtered. The column temperature was held at 80°C for 4 min and then increased to 280°C at the rate of 5°C/min and held at 280°C for 6 min. The injector and interface temperature were 200°C and 280°C respectively. The ion source temperature was 200°C. For GC-MS detection, an electron ionization system with ionization energy 70 eV was used over a scan range of 50-500 m/z. Carrier gas was Helium at flow rate of 1.00 ml/ min in split 1:50 with injection volume of 1 µ. Libraries used were NIST 11 & WILEY 8.

RESULT AND DISCUSSION

Figure 2 indicates the GCMS chromatogram representing the different peaks corresponding to each molecule present in *Amruthadi kashaya* and the Table 1 indicates the GCMS profile of *Amruthadi Kashaya.*

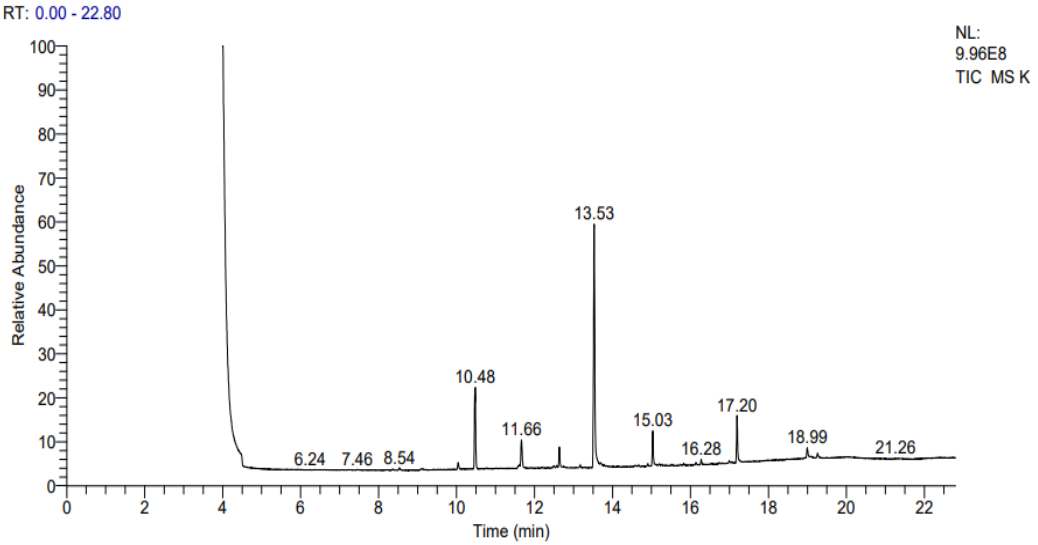

Fig 2: Shows the GC MS graph of *Amruthadi Kashaya*

Table 1: The GC MS analysis results of *Amruthadi Kashaya* indicating the name of the possible compounds, Retention time, percentage of area, molecular weight

Selected compound Name	RT	Area %	Mol Wt
Spiro [2,4] hepta-4,6-diene	4.48	2.10	92
1,3,5-Cycloheptatriene	4.48	2.10	92
Toluene	4.48	2.10	92
Cyclobutene, 2-propenylidene	4.48	2.10	92
Spiro [3.3] hepta-1,5-diene	4.48	2.10	92
à-Terpineol	8.54	0.17	154
L-à-Terpineol	8.54	0.17	154
5,7-Octadien-2-ol, 2,6-dimethyl	8.54	0.17	154
Methyl 8-methyl-nonanoate	10.04	0.99	186
Decanoic acid, methyl ester	10.04	0.99	186
Methyl 8-methyl-decanoate	10.04	0.99	200
Undecanoic acid, 2-methyl	10.04	0.99	200
Cyclopentane undecanoic acid, methyl ester	10.04	0.99	268
3-Cyclohexene-1-methanol, à, à,4-trimethyl-, acetate	10.48	16.04	196
Isobutyl 2-(4-methylcyclohex-3-enyl) propan-2-yl carbonate	10.48	16.04	254
Bicyclo[3.1.0]hexane, 6-isopropylidene-1-methyl-	10.48	16.04	136
2-Methylbicyclo [4.3.0] non-1(6)-ene	10.48	16.04	136
3-Cyclohexene-1-methanethiol, à, à,4-trimethyl-	10.48	16.04	170
Propane, 2,2-bis(methylthio)	11.66	6.67	136
Silane, (2-methoxyethyl) trimethyl	11.66	6.67	132
1-(1-Propen-1-yl)-2-(2-thiopent-3-yl) disulfide	11.66	6.67	194
L-5-Propylthiomethylhydantoin	11.66	6.67	188
Methanethioamide, N, N-dimethyl-	11.66	6.67	89
Butylated Hydroxytoluene	12.49	0.13	220
Ethanone,1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-4H-cyclohepta [b]furan-5-yl)	12.49	0.13	220

Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-	12.49	0.13	220
4,6-di-tert-Butyl-m-cresol	12.49	0.13	220
Spiro [5.5] undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-	12.57	0.16	204
á-Bisabolene	12.57	0.16	204
1H-Benzocycloheptene,2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)	12.57	0.16	204
Guaia-1(10),11-diene	12.57	0.16	204
Undecanoic acid, methyl ester	12.64	3.22	200
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	13.17	0.25	222
Nerolidyl acetate	13.17	0.25	264
Diethyl Phthalate	13.53	52.43	222
Methyl myristoleate	14.91	0.24	240
Methyl tetradecanoate	15.03	5.82	242
Ethyl iso-allocholate	16.14	0.18	436
9-Hexadecenoic acid, methyl ester, (Z)	16.99	0.19	268
7-Hexadecanoic acid, methyl ester (Z)	17.20	8.32	270
Methyl stearate	19.26	0.63	298
trans-13-Octadecenoic acid, methyl ester	18.99	1.75	296
9-Octadecenoic acid (Z) methyl ester	18.99	1.75	296
Tridecanoic acid, methyl ester	17.20	8.32	228
Methyl Z-11-tetradecenoate	14.91	0.24	240
Methyl hexadec-9-enoate	16.99	0.19	268
Methyl E-11-hexadecenoate	14.91	0.24	240
Z-11-Tetradecenoic acid	14.91	0.24	226

Among the bio molecules present a few have known medicinal activities. à-Terpineol is a monoterpineol which is known for its antioxidant, anticancer, anticonvulsant, anti-ulcer, antibacterial, anti-nociceptive activity, cardiovascular and anti-hypertensive effect, anticonvulsant and sedative activity, skin penetrating and enhancing activity, insecticidal activity[8] 7-Hexadecenoic acid methyl ester, and butylated hydroxytoluene are reported to have antioxidant activities[10,11] Methyl 8 methyl decanoate, Methyl myristoleate, Methyl Z-11-tetradecenoate, Methyl tetradecanoate, Methyl hexadec-9-enoate, Methyl E 11-hexadecenoate have Catechol-O-Methyltransferase-Inhibitor, Methyl-Guanidine-Inhibitor, Methyl-Donor activities. Methyl 8-methyl-nonanoate have antimicrobial and anti-inflammatory activity [12] catechol-O-methyltransferase -inhibitor, methyl-guanidine-inhibitor, methyl-donor activities. [13]

Researchers reported that Methyl stearate to have anti-inflammatory, nematicidal, anti-nociceptive, antioxidant, intestinal lipid metabolism regulation and anti-fungal activities[13,14] and also have catechol-O-methyltransferase-inhibitor, methyl-guanidine-inhibitor, methyl-donor activities[9].

9-Octadecenoic acid (Z) methyl ester and Trans -13-octadeconoicacid methyl ester have anti-inflammatory, anti-androgenic, cancer preventive, dermatitigenic, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge activity[9]. Tridecanoic acid methyl ester has proven anti-enteric activity and anti-bacterial activity [15]. Nerolidyl acetate also known as Nerolidol has antimicrobial, anti-parasitic, antibiofilm, antioxidant, antinociceptive, anti-inflammatory, antiulcer, skin penetration enhancer, insect repellent and anticancer properties [16]. Decanoic acid, methyl ester has antibacterial[17] and Undecanoic acid 2 methyl has antifungal activity[18]. Cyclopentane undecanoic acid methyl ester has antioxidant and antibacterial activity[19]. L-5-Propylthiomethylhydantoin has 5-lipoxygenase-inhibitor, antitumor (Liver) lipase-inhibitor, lipoxygenase-inhibitor, inhibit 12-lipoxygenase, anti-LDL activity[9]. 1H-Benzocyclo-heptene, 2,4a,5,6,7,8,9,9 a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis) is a diterpenoid has proven antioxidant activity[20] and also has hemagglutinator, hepatoprotective, hepato-

regenerative, hepatotonic, hypercholesterolemic, hypolipidemic, hydrochole-rectic, hydrogen-peroxide-inhibitor activity[9]. Z-11-Tetradecenoic acid increase zinc bioavailibility[9]. The study formulation *Amruthadi kashaya* is endowed with various medicinal properties maybe due to the presence of all these compounds described.

CONCLUSION

The presence of some significant biomolecules in *Amruthadi Kashaya*, as shown by the GC MS analysis, suggests that the therapeutic activity may be due to these biomolecules. The combined action of these biomolecules results in the therapeutic actions of the formulation. For a better knowledge of the therapeutic activity of this formulation higher sophisticated methods of analysis and clinical studies are needed.

ACKNOWLEDGEMENT

I express my heartfelt gratitude to all faculties in the Department of Rasasatra and Bhaishajya Kalpana especially Dr.Neethu Indukumar (Assistant professor), Dr.Sujith S, (Assistant Professor Department of Veterinary Pharmacology and Toxicology), College of Veterinary and Animal Sciences (CoVAS), Mannuthy Thrissur, friends and family members for their constant support throughout the study. I would like to acknowledge Central Instruments Laboratory, (CoVAS), Mannuthy, Thrissur for providing facilities for the GCMS analysis.

REFERENCES

1. Subhose V, Srinivas P, Narayana A. Basic principles of pharmaceutical science in Ayurveda. Bull Indian Inst Hist Med Hyderabad. 2005;35 (2):83-92.
2. Kaikulangara Ramawarrier, Arogya kalpa druma, Thrissur, Samarat publishers 2011 p. 103.
3. Upadhyay AK, Kumar K, Kumar A, Mishra HS. Tinospora cordifolia (Willd.) Hook. f. and Thoms. (Guduchi)- Validation of the Ayurvedic pharmacology through experimental and clinical studies. Int J Ayurveda Res. 2010; 1(2):112–21.
4. Shoaib A. A systematic ethnobotanical review of Adhatoda vasica (L.), Nees. Cellular and Molecular Biology 2022 [cited 2023 Sep 10].Available from: https://www.cellmolbiol.org/index.php/CMB/article/view/4058
5. Sharma V, Katiyar A, Agrawal RC. Glycyrrhiza glabra: Chemistry and Pharmacological Activity. In: Mérillon JM, Ramawat KG, (eds). Sweeteners. Reference Series in Phytochemistry Cham: Springer International Publishing; 2018 [cited 2023 Sep 10]. Available from: http://link.springer.com/10.1007/978-3-319-27027-2_21
6. Mohan B, Kakkar A. Evaluation of phenolic, flavonoid contents and antioxidant activity in leaves, stem and roots of Solanum indicum Linn. Environment Conservation Journal. 2020 10; 21(1&2):167-72.
7. Aboubakr M, Abdelazem AM. Hepatoprotective effect of aqueous extract of cardamom against gentamicin induced hepatic damage in rats. Int J Basic Appl Sci. 2015 [cited 2023 Sep 9]. Available from: http://www.sciencepubco.com/index.php/ijbas/article/view/5435
8. Khaleel C, Tabanca N, Buchbauer G. α-Terpineol, a natural monoterpene: A review of its biological properties. Open Chem. 2018[cited 2023 Sep 10]. Available from: https://www.degruyter.com/document/doi/10.1515/chem-2018-0040/html
9. Duke, James A. Dr. Duke's Phytochemical and Ethnobotanical Databases. Ag Data Commons. Dataset. 2016 https://doi.org/10.15482/ USDA. ADC/1239279.
10. Reza ASMA, Haque MdA, Sarker J, Nasrin MstS, Rahman MdM, Tareq AM, et al. Antiproliferative and antioxidant potentials of bioactive edible vegetable fraction of Achyranthes ferruginea Roxb. in cancer cell line. Food Sci Nutr. 2021[cited 2023 Oct 20]. Available from: https://onlinelibrary.wiley.com/doi/10.1002/fsn3.2343
11. Yehye WA, Rahman NA, Ariffin A, Abd Hamid SB, Alhadi AA, Kadir FA, et al. Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review. Eur J Med Chem. 2015 [cited 2023 Nov 20]. Available from: https://linkinghub.elsevier.com/retrieve/pii/ S022352341530101X
12. Kaur S, Sharma P, Bains A, Chawla P, Sridhar K, Sharma M, et al. Antimicrobial and Anti-Inflammatory Activity of Low-Energy Assisted Nanohydrogel of Azadirachta indica Oil. Gels 2022 [cited 2023 Oct 20]. Available from: https://www.mdpi.com/2310-2861/8/7/434
13. Adnan Md, Nazim Uddin Chy Md, Mostafa Kamal ATM, Azad M, Paul A, Uddin S, et al. Investigation of the Biological Activities and Characterization of Bioactive Constituents of Ophiorrhiza rugosa var. prostrata (D.Don) & Mondal Leaves through In Vivo, In Vitro, and In Silico Approaches. Molecules. 2019 [cited 2023 Oct 20]. Available from: https://www.mdpi.com/1420-3049/24/7/1367
14. Pinto MEA, Araújo SG, Morais MI, Sá NP, Lima CM, Rosa CA, et al. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. An Acad Bras Ciênc. 2017 [cited 2023 Nov 20]. Available from: https://doi.org/10.1590/0001-3765201720160908
15. Misra D, Ghosh NN, Mandal M, Mandal V, Baildya N, Mandal S, et al. Anti-enteric efficacy and mode of action of tridecanoic acid methyl ester isolated from Monochoria hastata (L.) Solms leaf. Braz J

Microbiol. 2022 [cited 2023 Nov 20]. Available from: https://link.springer.com/10.1007/s42770-022-00696-3

16. Chan WK, Tan L, Chan KG, Lee LH, Goh BH. Nerolidol: A Sesquiterpene Alcohol with Multi-Faceted Pharmacological and Biological Activities. Molecules. 2016 [cited 2023 Oct 20]. Available from: http://www.mdpi.com/1420-3049/21/5/529
17. Damiano F, De Benedetto GE, Longo S, Giannotti L, Fico D, Siculella L, et al. Decanoic Acid and Not Octanoic Acid Stimulates Fatty Acid Synthesis in U87MG Glioblastoma Cells: A Metabolomics Study. Front Neurosci 2020 [cited 2023 Sep 20]. Available from:https://www.frontiersin.org/article/10.3389/ fnins.2020.00783/full
18. Rossi A, Martins MP, Bitencourt TA, Peres NTA, Rocha CHL, Rocha FMG, et al. Reassessing the Use of Undecanoic Acid as a Therapeutic Strategy for Treating Fungal Infections. Mycopathologia.2021 [cited 2023 Nov 20].Available from: https://link.springer.com/10.1007/s11046-021-00550-4
19. Daniels A, Temikotan T. Fatty acid profile, antioxidant and antibacterial effect of the ethyl acatate extract of cleistopholis patens. Bulletin of Scientific Research. 2021; 3(1):21-31.
20. Shaheena S, Chintagunta AD, Dirisala VR, Sampath Kumar NS. Extraction of bioactive compounds from Psidium guajava and their application in dentistry. AMB Express c2019 [cited 2023 Dec 21].Available from: https://amb-express.springeropen.com/articles/10.1186/s13568-019-0935-x

Cite this article as:
Asha Kumari L S, S Anand. The Gas Chromatography Mass Spectrometry Analysis of an Ayurvedic Formulation Amruthadi Kashaya. International Journal of Ayurveda and Pharma Research. 2024;12(2):1-6.
https://doi.org/10.47070/ijapr.v12i2.3098
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. Asha Kumari L S
PG Scholar,
Department of Rasashastra and Bhaishajya Kalpana,
Government Ayurveda College,
Thiruvananthapuram, Kerala.
Email:
ashakumarils1234@gmail.com