



Review Article

DECODING CLASSICAL AYURVEDIC FORMULATION: THE ROLE OF GC-MS IN UNVEILING BIOACTIVE COMPOUNDS

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ABSTRACT

Ayurvedic formulations such as *Ghrita*, *Asava*, *Arishta*, and *Churna* have been traditionally used for eras for their healing properties. They are complex mixtures of herbs, oils, and other natural materials intended to reestablish equilibrium and vitality rather than merely being treatments. Because of the complexity of their multi-herbal compositions, the pharmacological basis of their bioactivity is still poorly understood despite widespread use. Even while their effectiveness as a treatment is well known, the true question is still: what causes them to function at the molecular level? Modern science can help with this. As a "molecular detective," gas chromatography-mass spectrometry (GC-MS) deconstructs these intricate compositions and exposes the chemistry that is concealed within. By separating and identifying small components, GC-MS reveals an intriguing realm of flavonoids, terpenoids, tannins, fatty acids, and other bioactive compounds that work together to produce the medicinal effect that is discussed in traditional literature. Every composition has a unique chemical narrative. *Taila* displays special phytochemicals that explain its restorative and therapeutic properties, whereas *Ghrita* may have a unique composition of fatty acids and antioxidants. In addition to verifying conventional claims, GC-MS's mapping of these chemical fingerprints connects the precision of contemporary pharmacology with the knowledge of Ayurveda. This study's objective is to examine published GC-MS studies on Ayurvedic dosage forms, specifically focusing on *Ghrita* and *Taila*, and to demonstrate how interpreting their bioactive profiles can convert traditional knowledge into evidence-based treatment for the modern world.

INTRODUCTION

GC-MS analysis revealed the presence of several bioactive compounds present in the particular formulations. Gas chromatography-mass spectrometry (GC-MS) is a hyphenated technique that combines the structures of gas chromatography and mass spectrometry to differentiate numerous substances inside a sample. In this method, the GC is used to isolate the volatile and thermally stable substitutes in a sample wherein the MS isolates the compounds based on their mass. The technique is widely recognized for its effectiveness in identifying unknown compounds.

In order to both qualitatively identify and quantitatively evaluate the specific chemicals that are present in complex mixtures. This article represents the GC-MS technique used in the detection of the active compounds in various ayurvedic formulations, which include *Asava-Arista*, *Churna* and *Ghritas*, respectively. In this present study, we will be focusing on the decoding of classical ayurvedic formulations by using the GC-MS technique.

METHODOLOGY

Reviewed around 06 articles on *Asava-Arista*, 06 articles on *Churna*, and 06 articles on *Ghritas* published in open access journals on the GC-MS analysis of classical ayurvedic formulation. A review has been presented in this article on the active compounds present in the various complex ayurvedic formulations acting on various diseases. Moving forward to one-to-one formulations, respectively.

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**Asava-Arista****Gas chromatography-mass spectrometry analysis of one Ayurvedic anti-obesity medicine, *Lohasava*<sup>[1]</sup>**

GC-MS of *Lohasava* was done by dissolving the sample in the suitable solvents and executing the procedure in column DB5 MS: 7000 triple quad at temperature programming from 50°C with an increase of 40°C/min till it reaches 170°C, then increased by 10°C/min till 310°C, with fragments from 45 to 450 Da. The total time of run was 32.02 min.

After running the GC-MS, they got various molecules with their retention timing and molecular mass, some of which are mentioned below:

Phenylethyl alcohol, hydrocinnamic acid, ethyl ester piperine, 3-hydroxy-butanoic acid, etc. Of the compounds discovered, piperine exhibited the widest range of biological and beneficial medicinal properties, including cardioprotective, immunomodulatory, anti-tumor, anti-depressant, anti-convulsant, anti-nociceptive, and anti-arthritis activities<sup>[1]</sup>. The one that helps in the absorption of vitamin B, beta carotene, and other nutrients. Suggesting that the *Lohasava* is a potent anti-anemic tonic that can be used.

**Quality Control and Phytochemical Profiling of a Polyherbal Traditional Indian Medicine by the GC-MS Method<sup>[2]</sup>**

The article reveals the importance of *Gomutra* and the formulation made out of it, i.e., *Gomutrasava*, in various indications such as *Shivtra* (vitiligo), *Kushtha* (leprosy), and other skin diseases. Using *Ashtang Hrudya* as a guide, *Gomutrasava* was made before being put through GC-MS.

GC-MS was carried out in the quadrupole with a prefilter analyzer with a mass range of 20-620 Da and a 250 L/sec turbomolecular pump.<sup>[2]</sup>

Compounds obtained from GC-MS of *Gomutrasava* are P-Cresol, Phenol, 3-Methyl-, Furan, 2-(2-Propenyl)-, 2-Methyl-1-Phenylbut-3-En-1-ol

2-(2-Isopropenyl-5-methylcyclopentylmethoxy) tetrahydropyran and many more.

*Gomutrasava* has revealed the compounds having antibacterial, anticancer, and antifungal activity and anti-obesity agents. Geranyl isovalerate present in *Gomutrasava* starts apoptosis over oxidative stress-mediated mechanisms. Hence, through this analysis, we can use this formulation to treat leprosy, vitiligo, and other skin conditions effectively.

**GC-MS Analysis of an Ayurvedic Medicine “Modified *Arjunarishta*”<sup>[4]</sup>**

*Arjunarishta* is given as a stimulant and appetizer along with the main indication of nourishment and strengthens the cardiac muscle in various cardiovascular disorders.

GC-MS was a Perkin Elmer GC Clarus 680, which yields fragments ranging from 40 to 600 Da after two minutes at 60°C, three minutes at 300°C, and six minutes of holding. It ran for 32 minutes in total.

Using NIST tools, a total of eight chemicals were found in *Arjunarishta*. The compound acetamidoacetaldehyde 2-en-1-one, 4-ethyl-2-hydroxycyclopent-1,1-dichloro-2-propanone, 3,5-dihydroxy-6-methyl-2H-pyran-4-one 2,3-dihydro Butaneboronate, 2-amino-octadec-7-ene-1,3-diol, 2-hydroxy-2-cyclopenten-1-one, hexamethylcyclotrisiloxane, [4-(2-methyl-4-oxo-pentyl)phenoxy] trimethylsilane

Out of the eight compounds mentioned above, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one possesses the formulation's strongest synergistic impact.<sup>[4]</sup>

Hence, the formulation can be used as the cardioprotective due to the presence of the above compounds.

**GC-MS and Network Pharmacology Analysis of the Ayurvedic Fermented Medicine, *Chandanasava*, Against Chronic Kidney and Cardiovascular Diseases<sup>[5]</sup>**

In this article they have studied the phytochemicals present in the formulation. *Chandanasava* contains 24 different herbs and is responsible in treating the chronic kidney and cardiovascular diseases.

Perkin Elmer Clarus 500 instruments were used to run the GC-MS with a helium gas flow of 1 milliliter per minute at an initial temperature of 60 degrees Celsius. ramped to 300°C at 10°C min<sup>-1</sup>; a full scan was done at 40-450 Da.

The TCMSP database identified and separated all 62 chemicals. Around 27 targets for CKD and 35 targets for CVD were found from the DisGeNET and GeneCards databases, namely 40 active constituents: eugenol, stigmasterol, 1-methyl-4-phenyl-, ethyl ester, piperine, 5-hydroxymethylfurfural, 1,2-benzenedicarboxylic acid, dibutyl ester, etc., in which 10 bioactive mixes showed higher attraction towards the CKD and CVD target proteins.<sup>[5]</sup> Molecular docking presented piperine and melatonin as actual controllers of the hub gene of CKD and CVD.

**In Silico Probing of Anti-Arthritic Potential of Traditionally Fermented Ayurvedic polyherbal product *Balarishta* Reveals Lupeol and Desulphosinigrin as Efficient Interacting Components with ureC<sup>[7]</sup>**

The purpose of the paper is to quantify the anti-arthritis qualities of *Balarishta*, which is used to combat immunological disorders. e.g., RA caused by the Proteus UTI through in-silico analysis and examination of antimicrobial activity.

GC-MS was carried out in a PerkinElmer Clarus 500 instrument at the oven program started at 50°C with a ramp rate of 10°C/min to 150°C, followed by a ramp of 8°C/min to 280°C, where it was held for 10 minutes. It then to 60°C at 8°C/min, ramped again at 10°C/min to 200°C, and further increased to 300°C, where it was held for 5 minutes. The range of the scan was 40–450 Da.

For *Balarishta*, Gas Chromatography-Mass Spectroscopy (GC-MS) study was used to identify 42 bioactive compounds. These compounds were then tested in silico for their ability to bind strongly to the urease subunits and accessory proteins of *Proteus mirabilis*. Among them, the ureC subunit showed the strongest binding with the compound desulphosinigrin (-10.52 kcal/mol), followed closely by lupeol (-10.03 kcal/mol), with interactions occurring mainly between amino acid residues 308 and 327. Desulphosinigrin created six hydrogen bonds when it was linked to ureC, whereas lupeol generated four. Flexible docking simulations revealed that lupeol had a stronger binding affinity to ureC (-9.2kcal/mol) compared to desulphosinigrin (-6.0kcal/mol). In both cases, the binding involved conserved residues- Cys319, His320, and His321. It is suggested that *Balarishta* could be effective in managing urinary tract infections associated with complications of rheumatoid arthritis.<sup>[7]</sup>

#### **Influence of intrinsic microbes on phytochemical changes and antioxidant activity of the Ayurvedic fermented medicines *Balarishta* and *Chandanasava***<sup>[3]</sup>

The above article objective is to check the native microbes, phytochemical changes, and antioxidant activities of the mentioned formulae.

The mentioned formulae were firstly prepared, and then sent for the GCMS procedure of *Asava* and *Arishta* samples, in which the samples were frozen at -20°C for 1 day in a deep freezer and then frozen with the support of a vacuum evaporator at -80°C; the frozen samples were then dissolved in 10ml HPLC-grade methanol and GCMS was runned with helium gas flow at 1ml/min. Inserted port temperature was 280°C. Two type of oven temperature were followed. The temperature was first 50°C at 10°C/min, then 150°C at 8°C/min, reaching 280°C, and last 60°C at 8°C/min, 200°C at 10°C/min, reaching 300°C and The peaks were synchronized using the NIST MS search library.

There were a number of phytochemicals that were retained, disappeared, and newly formed in the process of fermentation, as especially the yeasts were known for their alcohol production skills. *Balarishta* and *Chandanasava* are known to contain a variety of beneficial. Therefore, the inherent bacteria also add to the medicinal benefits of *Arishtas* and *Asava* plant

compounds, also called secondary metabolites. Studies on different *Arishtas* and *Asavas* have found, interestingly, some of these- like flavonoids, saponins, and phytosterols- tend to form later during the fermentation process. This suggests that the microorganisms involved in fermentation play a key role in creating these compounds.

Hence, the intrinsic microorganisms contribute, moreover, to the therapeutic value of *Asava* and *Arishtas*.<sup>[3]</sup>

#### **Churna**

#### **Gas Chromatography Tandem Mass Spectrometry for Quantitative Analysis of Pesticides in *Sitopaladi Churna*: Multi-Residue Method Development**<sup>[9]</sup>

The article evaluates the pesticide remnants in the herbal formulation.

It is very crucial to find the pesticides present in the herbal formulae to prevent long-term consumer safety and also chronic toxicity. For which GC and MS were carried out to evaluate all pesticides contained in the formula by hydro-alcoholic and aqueous extracts of *Pistacia integerrima*.

*Quercus infectoria*, *Terminalia chebula*.

Common compounds present into it are (Z)-3-(Heptadec-10-en-1-yl) phenol, 1-(2-Furanyl)-2-hydroxyethanone, 1,2,3-Benzenetriol, 2,4-Di-tert-butylphenol, 2-hydroxybutanedioic acid diethyl ester, etc.<sup>[9]</sup>

The method that has been adopted by this study can also help in evaluating the market sample of churna to ensure food safety and excellence for consumers across the globe.

#### **Chemical Profiling of *Trijata Churna*: A Herbal Formulation**<sup>[10]</sup>

The article evaluates the phytochemical, physiochemical, organoleptic, and fluorescence properties, as well as thin-layer and gas chromatography screening of the *Trijata Churna*, to see the properties of it in various therapeutic effects that are coated in the classical text.

GCMS was carried out in 1ml ethanol extract and was analyzed as per standard procedures, in which the eugenol content was zero in *Ela* and was 134.24mg/g in *Twak*, 152.51mg/g in *Patra*, and 268.88 mg/g in the *Trijata Churna*.<sup>[10]</sup>

The study shows various bioactive components in the chromatographic scan, which can be linked with the action of these drugs in various therapeutic benefits and importance as mentioned in the classical text.

#### **The gas chromatography-mass spectrometry study of one herbal formulation, *Trikatu Churnam***<sup>[11]</sup>

The GCMS analysis of *Trikatu Churna* for the treatment of digestive issues is the subject of this study.



GCMS was allowed to run, and the identification of the metabolites was fulfilled by evaluating the retention time and fragmentation pattern with MS in NIST.

The presence of some biomolecules gives us the efficacy of *Trikatu Churna* in digestion-related disorders, and we concluded that the formulation shows its accuracy in Ayurveda as a digestive formulation.

#### **The GC-MS Study of One Ayurvedic Formulation, *Navayasa Churnam*<sup>[12]</sup>**

The present study shows the importance of the *Navayasa Churnam* in treating disorders like heart disease, piles, jaundice, skin disease, and anemia by performing GCMS and finding the bioactive compounds, which helps the formulation to show the result in the above-mentioned disorders.

GCMS was equipped with a DB5 MS column prepared with 5% phenyl and 95% methyl polysiloxane with electron impact mode 70 eV and helium flow of 1ml/min; the temperature of the injector was set at 280°C, auxiliary at 290°C, ion source at 280°C, and oven at 50°C with a 1 min isothermal hold and raised at 40°C/min to 170°C and held for 4 mins- ramped till 10°C/min to 310°C.

Overall procedure duration was 32.02 mins, and compounds were identified for their significant antioxidant, anti-inflammatory, antiviral, and antimicrobial properties.<sup>[12]</sup>

Thus, the above-identified biomolecules give several important medicinal roles that are aligned with the traditional use of this medication, sustaining the efficacy and expected as a therapeutic negotiator.

#### **The gas chromatography-mass spectrometry study of one Ayurvedic medicine *Ashtachurnam*<sup>[13]</sup>**

The present study shows the importance of the *Ashtachurnam* in treating disorders like appetite, abdominal colic, abdominal lumps, incomplete evacuation, and irritable bowel syndrome by performing GCMS and finding the bioactive compounds, which helps the formulation to show the result in the above-mentioned disorders.

GCMS was performed with DB5 MS, electron mode 70 eV, and helium flow at 1ml/min with injector temperature 280°C, ion-source temperature 280°C, and auxiliary temperature 290°C. oven temperature 50°C with an increase of 40°C/min to 170°C, then 10°C/min to 310°C. Total time taken was 32.02 mins.<sup>[13]</sup>

The molecules that were identified were showing diverse activity of homeostasis of the digestive system and body; hence, the *Churam* is not only restricted to treating the digestive problem but also upholds the overall health of the body.

#### **The GC-MS Study of One Ayurvedic Preparation: *Amrithamehari Churnam*<sup>[14]</sup>**

The above-mentioned titled article shows the efficacy of *Amrithamehari Churnam* in treating diabetes, bladder disorder, and weight loss.

GCMS of the following *Churnam* was carried out to see the presence of biomolecules responsible for the effective therapeutic outlook of the formulation.

About 50 peaks were seen in the GCMS of this formulation, which adds value and gives several important medicinal roles that are aligned with the traditional use.<sup>[14]</sup>

#### ***Ghrita***

#### ***Bhallatakadi Ghrita*: Development and evaluation with reference to *Murchhana* and *Shata-Dhauta* process<sup>[17]</sup>**

In this article 3 sample preparations were done: one simply with polyherbal *Bhallataka Ghritas*, another with the *Murchita Ghritas*, and then with the help of the *Shata-dhauta* process. After preparation, they were given for the assessment of various physiochemicals present in the formulation with the help of the GC-MS technique.

There were almost 18 major saturated fatty acids, like capric, lauric, stearic, and caprylic, and unsaturated.<sup>[17]</sup>

The most plentiful polyunsaturated fatty acid islinoleic acid, which is present in this formulation.

#### **Product development and characterization of a lipid-based Ayurvedic polyherbal formulation: *Kalyanaka Ghrita*<sup>[18]</sup>**

The *Kalyanaka Ghrita* was made with the help of 2 references, i.e., *Ashtanga Hrudaya* and AFI, then subjected to the GC-MS screening, which includes the ionization energy of 70 eV and 99.99% helium gas with a constant flow of carrier gas at the rate of 1.0 ml/min and a split rate of 10:1; oven temperature 40°C for 1 min, raised 20°C/min to 150°C, then raised by 3°C/min and held for 0 min, then again raised up to 300°C and held for 15 min. overall time to complete the whole process took 36 mins.

After screening the *Kalyanaka Ghritas*, 99 peaks were identified, in which 26 main peaks were seen. 0.17% of chebulagic acid and 0.02% of curcumin were present in all batches of *Kalyanaka Ghritas*.<sup>[18]</sup>

#### **The GC-MS Study of *Madhyama Panchamoola Sadhita Ghrita*<sup>[19]</sup>**

The present study evaluates the effect of *Madhyama Panchamoola Sadhita Ghritas* on spermatogenic activity with the help of GC-MS screening, which includes the ionization energy of 70 eV and 99.99% helium gas with a constant flow of carrier gas at the rate of 1.0ml/min and with spitless mode; oven temperature 130°C, raised to 180°C for 10 min, then raised by 5°C every minute, held for 5 minutes, and then raised once more to 280°C and held

for 2 minutes. overall time to complete the whole process took 32 mins.

19 compounds are present in total, in which squalene, glycerol, and lanosterol show spermatogenesis activity, as do other composites such as vitexin, quercetin, etc., which also show the spermatogenic action. [19]

Hence, the above formulation shows the *Shukra-Janana* activity.

#### **Determination of chemical composition of Panchagavya ghrita and garlic pill using gas chromatography and mass spectrometry (GC-MS)[20]**

In Above article the *Panchagavya Ghrita* and garlic pill were set for GC-MS analysis to see the compound present in it.

GC-MS screening that uses a 70 eV ionization energy and 99.99% helium gas with a split rate of 10:1 and a continuous 1.0ml/min carrier gas flow; oven temperature 40°C for 1 min, raised 20°C/min to 150°C, then increased by 3°C/min and held for 0 min, then again increased up to 300°C and held for 15 min. Overall time to complete the whole process took 36 mins.

In these 15 compounds were found in the *Panchagavya Ghrita*; they are:

Eicosane, 10-methyl, Ethyl iso-allocholate, Lauric acid methyl ester, Stearic acid methyl ester, etc., and in garlic pills. Propen-1-ol, Allyl trisulphide, Ethyl thio-penta-1,5-dien-3-ol, etc. [20]

Thus, it holds the activities like anti-inflammatory, anti-oxidant, anti-atherosclerotic, etc.

#### **Shata-dhauta-ghrita – A case study [21]**

The present study was carried out to see the changes taking place after the *Ghrita* has been washed by the water for one hundred times.

The GC-MS was carried out in the Shimadzu QP-2010 with Col. 30ml/0.32 mm; temp 50°C to 260°C at 5°C/min. [21]

It is seen that the fatty acids in *Shata Dhauta ghrilas* are almost decreased in their numbers and can be compared with the phenomenon of fat splitting due to the prolonged pressure applied to them.

#### **The GC-MS study of one Ayurvedic formulation Tiktaka Ghrilas[22]**

The GC-MS investigation on *Tikata Ghrita*, a remedy for skin conditions, is discussed in the article above.

GC-MS screening, which comprises a 70 eV ionization energy and 99.99% helium gas with a steady 1.0 ml/min carrier gas flow and a 10:1 split rate; oven temperature from 50°C to 170°C for 10°C/min, to 310°C; fragments from 45 to 450 Da. The overall time to complete the whole process took 32.02 mins. [22]

The compounds that are found in the *Tikata Ghrita* are mostly hormone-controlling and antioxidant properties and also glucose metabolism in the body, controlling the skin diseases.

#### **DISCUSSION**

The addition of gas chromatography-mass spectrometry (GC-MS) in the investigation of classical Ayurvedic formulations marks a remarkable development in bridging traditional medicine with modern. The study focused on the 4 traditional dosage forms mainly- *Asava*, *Arishta*, *Churna*, and *Ghrita*—aiming to reveal their bioactive constituents and provide a systematic basis for their therapeutic applications. The study reveals a diverse array of the phytoconstituents, which are known for their pharmacological activities, such as anti-inflammatory, antimicrobial, anti-obesity, and anticancer effects. These findings not only validate the therapeutic claims written in the classics but also offer deeper insights into the molecular basis of these old remedies.

By encoding specific bioactive markers, GC-MS facilitates the standardization and quality control of Ayurvedic preparations, a very important step as a global demand for evidence-based traditional medicine. Also, this characterization opens promising areas for new drug development and the advancement of integrative therapeutic models. Therefore, future investigation can blend advanced phytochemical techniques like GC-MS with pharmacological and clinical evaluations to fully understand the multi-dimensional nature of Ayurvedic therapeutics.

Hence, GC-MS serves as a powerful decoding tool that brings scientific clarity to the bioactive essence of classical formulation. Thus, this review strengthens the value of classical Ayurvedic preparations through current analytical validation, highlighting GC-MS as a main tool in detecting the bioactive basis of traditional therapeutics.

#### **CONCLUSION**

The encoding of GC-MS in the analysis of ayurvedic formulation has proven to be a priceless scientific tool for identifying and quantifying the various phytochemical constituents within complex herbal media. This technique offers high sensitivity, enabling accuracy to unravel the involved chemical profiles of traditional remedies. This encoding not only lends systematic credibility to the pharmacological claims of classical ayurvedic preparations but also supports essential aspects such as standardization, quality control, and safety evaluation- essential for Ayurveda's worldwide integration with contemporary healthcare systems.

Sustained research that includes GC-MS can bridge the gap between traditional knowledge and modern science, promoting a deeper understanding of the mechanisms in the Ayurvedic treatments.

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