

# "GC–MS Profiling of Rasnadi Ghrita and the Pharmacological Significance of Its Identified Phytoconstituents."

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## ABSTRACT

**Introduction:** Rasnadi Ghrita, a traditional Ayurvedic formulation based on ghee, is well-regarded for its effectiveness in managing various health conditions

### Materials & Methods:

This research employs advanced analytical techniques, specifically Gas Chromatography-Mass Spectrometry (GC-MS), to profile the phytoconstituents of Rasnadi Ghrita. The objective is to identify its active compounds and explore their possible mechanisms in managing various health conditions. The formulation was procured from a certified source, Vaidyaratnam Pharmacy, and subjected to GC-MS analysis using acetone-based extracts.

**Results:** The GC-MS analysis revealed the presence of 25 distinct phytochemical compounds in Rasnadi Ghrita.

**Conclusion:** The therapeutic efficacy of Rasnadi Ghrita can be attributed to the bioactive constituents identified via GC-MS, which exhibit diverse pharmacological properties, including antioxidant, anti-inflammatory, and analgesic effects.

**Keywords:** antioxidant, anti-inflammatory, analgesic, Rasnadi Ghrita, gas chromatography–mass spectrometry

## INTRODUCTION

Osteoarthritis (OA) is a joint disorder often seen in the elderly, marked by mild inflammation, cartilage breakdown, and reduced synovial fluid, leading to pain and disability (1). In India, osteoarthritis (OA) is the most prevalent joint condition, affecting 22–39% of the population, with knee OA being the predominant form. Globally, 250 million suffer from OA, mainly due to obesity and aging (2). Between 1990 and 2019, the number of symptomatic OA cases in India increased from 23.46 million to 62.35 million (3).

In Ayurveda, it corresponds to *Sandhivata*, a type of *Vatavyadhi* caused by aging, poor lifestyle, and diet, which lead to tissue degeneration (*Dhatukshaya*) (4). This disrupts the *Vata dosha*, impairing movement, while a decline in *Shleshaka Kapha* reduces joint lubrication and worsens symptoms.

This study uses gas chromatography-mass spectrometry (GC-MS) analysis to investigate the chemical and possible therapeutic effects of *Rasnadi Ghrita*, a traditional Ayurvedic ghee preparation.

Rasna, Dashamoola, Shatavari, Kulatha, Badara, Yava, Ajamamsa rasa, and Jeevaniya group herbs—known for their Madhura, Tikta, Guru, and Snigdha qualities—are among the constituents of *Rasnadi Ghrita*. By enhancing *Shleshaka Kapha*, these attributes aid in preventing joint deterioration. In *Vata Disorders*, this formulation is utilized as *basti* (enema), *navana* (nasal drops), and *pana* (oral consumption).

This GC-MS analysis of *Rasnadi Ghrita* was conducted to precisely identify and quantify its phytoconstituents, including those present in trace amounts that are often difficult to detect through traditional isolation and structural analysis techniques. The study aimed to deliver an in-depth insight into the chemical composition of the formulation, thereby highlighting its potential therapeutic properties.

## MATERIALS AND METHODS:

### Materials

*Rasnadi Ghrita* was obtained from the GMP-certified Vaidhyaratnam Oushadashala Private Limited (Batch No: 24A0440), and its analytical evaluation was carried out accordingly

### Methods:

*Rasnadi Ghrita* was prepared as per the reference of *Ashtangahridayam* (5). The method of *Ghrita* preparation is provided in supplementary material.

The GC-MS study was conducted at Amrith Private Lab, Nisargam Private Limited, Shivamogga, Karnataka. The Shimadzu model QP 2010SE GC-MS equipment, which offers high sensitivity and reliability and is extensively utilized for accurate qualitative and quantitative analysis in a variety of applications, was employed for the conduction

Table 1: Physicochemical analysis of *Rasnadi Ghrita*

| S.no | Parameters                            | Specification /Range         | Result   |
|------|---------------------------------------|------------------------------|----------|
| 1    | Colour                                | Pale yellow to yellow colour | -        |
| 2    | Odour                                 | Herbal ghee colour           | -        |
| 3    | Taste                                 | Herbal taste                 | -        |
| 4    | Loss on Drying                        | NMT 0.5                      | 0.21     |
| 5    | Refractive index at 30 degree Celsius | 1.455-1.458                  | 1.455    |
| 6.   | Acid Value                            | NMT 10                       | 3.10     |
| 7.   | Iodine value                          | 30-45                        | 34.85    |
| 8    | Saponification value                  | 225-240                      | 228.83   |
| 9.   | Rancidity                             | Negative                     | Negative |
| 10.  | Mineral Oil                           | Absent                       | Absent   |

### Sample Preparation of *Rasnadi Ghrita* for GC-MS study

The following actions are part of the analytical preparation process:

- 1. Preparation:** The formulation was thoroughly inspected and homogenized to ensure uniformity before extraction.
- 2. Acetone Extraction:** A 1 mL sample was diluted with 10 mL of acetone.
- 3. Chromatography and Mass Spectrometry Method**

A Shimadzu GC-MS-QP-2010SE system was used for the analysis, and helium was used as the carrier gas. A solution of 1 µL was injected into the device after a 1 mL sample was diluted with 10 mL of acetone. A split ratio of 10:1 was used when the injection was performed in split mode. The initial temperature of the column oven was set at 80.0 °C, and the injection port was heated to 280.0 °C.

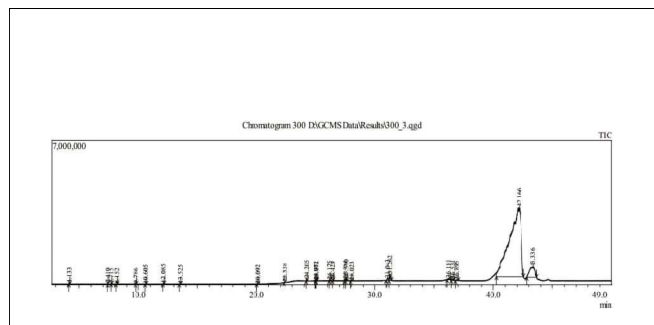
At a pressure of 24.2 kPa, the flow control was operated in linear velocity mode. With the column flow at 1.50 mL/min and a linear velocity of 45.1 cm/sec, the system was able to sustain a total flow of 19.5 mL/min. The purge flow rate was 3.0 mL/min. Features including splitter hold, carrier gas saver, and high-pressure injection were disabled.

After starting at 80.0 °C and holding for 2 minutes, the column temperature was increased to 280.0 °C at a rate of 10.0 °C / minute and held for 10 minutes. With a final hold time of five minutes, it then rose to 330.0 °C at a pace of 20.0 °C/min.

The interface temperature was fixed at 300.0 °C, and the ion source was kept at 200.0 °C for mass spectrometry. The cut-off time for the solvent was 1.40 minutes. With the tuning result, detection was carried out in scan mode with a threshold of 0 and a detector gain of 0.95 kV. The scan lasted from two minutes to thirty-three minutes. At a scan speed of 1666 and an event duration of 0.30 seconds, scans were performed over a mass range of 35.00 to 500.00 m/z. The GC-MS system inlet was used to introduce the sample.

# RESULT:

**Figure 1** displays a GC-MS chromatogram illustrating the distribution and relative abundance of these bioactive constituents. The findings of the GC-MS study of Rasnadi Ghrita extracted with acetone are shown in **Table 3**, where several phytocompounds were identified. **Table 4** summarizes the biological properties of these compounds, including their antioxidant, anti-inflammatory, antibacterial, and analgesic effects.



**Figure 1: GC-MS Chromatogram of Bioactive Compounds Detected in Rasnadi ghrita**

The GC-MS study revealed the detection of several bioactive compounds. Higher concentrations of butyric acid 3-pentadecyl ester, butyric acid 3-tetradecyl ester, hexadecanoic acid 2,3-bis (acetyloxy) propyl ester, dodecanoic acid, and 1,2,3-propanetriyl ester were detected. This means that they are important for the Rasnadi ghrita health benefits. The bioactive molecule of *Rasnadi Ghrita* has Anti-inflammatory, antioxidant and antimicrobial properties. Other phytoconstituents including Maltol, Heptadecane, Dodecasonic acid derivatives, exhibited a range of diverse biological effects, such as antimicrobial activity, antioxidant, and hypercholesterolemic activity

**Table 2: Phytocomponents detected in the acetone-extracted Rasnadi Ghrita using GC-MS:**

| Peak# | R.Time | Area   | Area% | Height | Height% | CAS#       | Name of compound                             |
|-------|--------|--------|-------|--------|---------|------------|----------------------------------------------|
| 1     | 4.133  | 100174 | 0.04  | 54730  | 1.05    | 123-42-2   | 2-Pentanone, 4-hydroxy-4-methyl-             |
| 2     | 7.410  | 46382  | 0.02  | 27186  | 0.52    | 118-71-8   | Maltol                                       |
| 3     | 7.715  | 38501  | 0.02  | 23616  | 0.45    | 28564-83-2 | 4 H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 |
| 4     | 8.152  | 19533  | 0.01  | 11875  | 0.23    | 112-31-2   | Decanal                                      |
| 5     | 9.786  | 42040  | 0.02  | 20739  | 0.40    | 112-54-9   | Dodecanal                                    |
| 6     | 10.605 | 34814  | 0.01  | 19618  | 0.38    | 2922-51-2  | 2-Heptadecanone                              |
| 7     | 12.085 | 111160 | 0.04  | 42526  | 0.82    | 124-25-4   | Tetradecanal                                 |
| 8     | 13.525 | 77039  | 0.03  | 23123  | 0.44    | 2345-28-0  | 2-Pentadecanone                              |
| 9     | 20.092 | 56184  | 0.02  | 16144  | 0.31    | 2721-22-4  | 2H-Pyran-2-one, tetrahydro-6-nonyl-          |
| 10    | 22.358 | 67972  | 0.03  | 30464  | 0.59    | 7501-44-2  | Glycidyl palmitate                           |
| 11    | 24.205 | 211582 | 0.09  | 111497 | 2.15    | 7501-44-2  | Glycidyl palmitate                           |
| 12    | 24.982 | 69699  | 0.03  | 21705  | 0.42    | 6946-24-3  | Tetracosane, 1-bromo-                        |
| 13    | 25.072 | 102213 | 0.04  | 39409  | 0.76    | 0-00-0     | Sulfurous acid, 2-pentyl tridecyl ester      |
| 14    | 26.175 | 152668 | 0.06  | 52572  | 1.01    | 0-00-0     | Glycidyl (Z)-9-Heptadecenoate                |
| 15    | 26.429 | 79702  | 0.03  | 34008  | 0.65    | 7501-44-2  | Glycidyl palmitate                           |
| 16    | 27.453 | 245411 | 0.10  | 48332  | 0.93    | 14473-55-3 | Myristin, 2,3-diaceto-1-                     |

|    |        |           |       |         |       |            |                                                   |
|----|--------|-----------|-------|---------|-------|------------|---------------------------------------------------|
| 17 | 27.590 | 515426    | 0.21  | 136967  | 2.64  | 0-00-0     | Butyric acid, 3-pentadecyl ester                  |
| 18 | 28.023 | 106801    | 0.04  | 27980   | 0.54  | 55429-67-9 | Eicosanoic acid, 2,3-bis(acetyloxy)propyl ester   |
| 19 | 31.043 | 859803    | 0.35  | 97549   | 1.88  | 55268-70-7 | Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester |
| 20 | 31.262 | 1646341   | 0.67  | 290410  | 5.59  | 0-00-0     | Butyric acid, 3-tetradecyl ester                  |
| 21 | 36.181 | 472567    | 0.19  | 61010   | 1.17  | 15677-71-1 | 9-Octadecene, 1,1-dimethoxy-, (Z)-                |
| 22 | 36.531 | 529850    | 0.21  | 72097   | 1.39  | 55268-70-7 | Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester |
| 23 | 36.895 | 380090    | 0.15  | 49323   | 0.95  | 0-00-0     | Oxalic acid, decyl neopentyl ester                |
| 24 | 42.166 | 225726259 | 91.35 | 3383940 | 65.12 | 538-24-9   | Dodecanoic acid, 1,2,3-propanetriyl ester         |
| 25 | 43.336 | 15409313  | 6.24  | 499619  | 9.61  | 91925-73-4 | 1-Dodecanoyl-3-myristoylglycerol                  |

Table 3: Biological activities of phytoconstituents of Rasnadi Ghrita found using PubChem database.

| Sl No | Retention Time | Compound Name                                      | Compound Formula | Peak Height | Functional Group                           | Biological Activity                                          |
|-------|----------------|----------------------------------------------------|------------------|-------------|--------------------------------------------|--------------------------------------------------------------|
| 1     | 4.133          | 2-Pentanone, 4-hydroxy-4-methyl-                   | C6H12O2          | 54730       | Alcohol, Ketone                            | Antioxidant, anti-inflammatory                               |
| 2     | 7.410          | Maltol                                             | C6H6O3           | 27186       | Alcohol, Ketone (Pyrone ring)              | Neuroprotective, antioxidant, Anti-inflammatory              |
| 3     | 7.715          | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl | C6H8O4           | 23616       | Alcohol, Ketone (Lactone-like)             | Antioxidant                                                  |
| 4     | 8.152          | Decanal                                            | C10H20O          | 11875       | Aldehyde                                   | Anti-inflammatory                                            |
| 5     | 9.786          | Dodecanal                                          | C12H24O          | 20739       | Aldehyde                                   | Anti-inflammatory, antimicrobial                             |
| 6     | 10.605         | 2-Heptadecanone                                    | C17H34O          | 19618       | Ketone (Fatty ketone)                      | Anti-inflammatory potential                                  |
| 7     | 12.085         | Tetradecanal                                       | C14H28O          | 42526       | Aldehyde                                   | Anti-inflammatory                                            |
| 8     | 13.525         | 2-Pentadecanone                                    | C15H30O          | 23123       | Ketone                                     | Analgesic potential (fatty ketone)                           |
| 9     | 20.092         | 2H-Pyran-2-one, tetrahydro-6-nonyl-                | C14H26O2         | 16144       | Lactone (Cyclic ester)                     | Anti-inflammatory (lactone group)                            |
| 10    | 22.358         | Glycidyl palmitate                                 | C19H36O3         | 175969      | Ester, Epoxide                             | Preparation of isophosphatidic acid which inhibits apoptosis |
| 11    | 24.982         | Tetracosane, 1-bromo-                              | C24H49Br         | 21705       | Alkyl halide                               | Anti-inflammatory activity                                   |
| 12    | 25.072         | Sulfurous acid, 2-pentyl tridecyl ester            | C18H38O3S        | 39409       | Sulfonic ester                             | Potential anti-inflammatory                                  |
| 13    | 26.175         | Glycidyl (Z)-9-Heptadecenoate                      | C20H36O3         | 52572       | Ester, Epoxide, Alkene (Unsaturated ester) | Anti-inflammatory                                            |

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|    |        |                                                  |          |         |                               |                         |
|----|--------|--------------------------------------------------|----------|---------|-------------------------------|-------------------------|
| 14 | 27.453 | Myristin, 2,3-diaceto-1-                         | C21H38O6 | 48332   | Ester (Diacetate of glycerol) | Anti-inflammatory       |
| 15 | 27.590 | Butyric acid, 3-pentadecyl ester                 | C19H38O2 | 136967  | Ester (Fatty ester)           | Anti-inflammatory       |
| 16 | 28.023 | Eicosanoic acid, 2,3bis(acetyloxy)propyl ester   | C27H50O6 | 27980   | Triester (Fatty ester)        | Anti-inflammatory       |
| 17 | 31.043 | Hexadecanoic acid, 2,3bis(acetyloxy)propyl ester | C23H42O6 | 169646  | Triester (Fatty ester)        | Anti-inflammatory       |
| 18 | 31.262 | Butyric acid, 3-tetradecyl ester                 | C18H36O2 | 290410  | Ester (Fatty ester)           | Anti-inflammatory       |
| 19 | 36.181 | 9-Octadecene, 1,1-dimethoxy-, (Z)-               | C20H40O2 | 61010   | Ether, Alkene                 | Anti-inflammatory       |
| 20 | 36.895 | Oxalic acid, decyl neopentyl ester               | C17H32O4 | 72097   | Diester                       | Anti-inflammatory       |
| 21 | 42.166 | Dodecanoic acid, 1,2,3-propanetriyl ester        | C39H74O6 | 3383940 | Triester (Triglyceride)       | Acidifier, acidulant,   |
| 22 | 43.336 | 1-Dodecanoyl-3-myristoylglycerol                 | C29H56O5 | 499619  | Ester(Diacylglycerol)         | Analgesic-related lipid |

**Probable Mode of Action of Rasnadi Ghrita:**

The GC-MS profile of Rasnadi Ghrita indicated important biomolecules like Dodecanoic acid, 1,2,3-propanetriyl ester, Hexadecanoic acid, Butyric acid, Maltol, Heptadecane, and Eicosanoic acid. It revealed the presence of 25 chemical constituents with retention times ranging from 4.133 minutes to 43.336 minutes.

**Dodecanoic acid and 1,2,3-Propanetriyl Ester:**

Oleic acid, 1,2,3-propanetriyl ester, dodecanoic acid ethenyl ester, and dodecanoic acid 1,2,3-propanetriyl ester are examples of bioactive compounds that have therapeutic qualities such as acidifying effects, modulation of amino acid decarboxylase activity, inhibition of uric acid production, and suppression of arachidonic acid pathways. In particular, they inhibit the production of prostaglandins by suppressing arachidonic acid, which leads to the downregulation of cyclooxygenase enzymes (COX-1 and COX-2). Since non-steroidal anti-inflammatory medications (NSAIDs) have this mode of action, these ingredients may help the formulation's analgesic and anti-inflammatory effects (6).

**N- Hexa decanoic acid (palmitic acid):**

Phospholipase A2 (PLA2), a key enzyme involved in inflammation, is competitively inhibited by n-hexadecanoic acid (palmitic acid), suggesting its potential anti-inflammatory properties. Studies have demonstrated that n-hexadecanoic acid exhibits strong enzyme inhibition, high binding affinity confirmed by isothermal titration calorimetry (ITC), and direct active site binding as shown in crystal structure analyses. Its strong entropy-driven binding to PLA2, formation of a 1:1 molar binary complex in solution, and consistent inhibitory activity in enzyme kinetics further support its bioactivity. These findings highlight its dual role—not only as a drug carrier but also as an active therapeutic component in topical anti-inflammatory treatments, aligning with its traditional use in Ayurvedic medicine for rheumatic conditions (7).

**Butyric acid:**

Butyric acid, a short-chain fatty acid produced when the gut bacteria ferments dietary fiber, has garnered attention due to its potential to affect inflammation and immunological function. It can affect the behaviour of immune cells, lower the synthesis of pro-inflammatory cytokines, and promote the growth of regulatory T cells, according to research(8).

These immunomodulatory properties indicate that butyric acid could offer therapeutic advantages for autoimmune and inflammatory diseases, such as ankylosing spondylitis (AS). Recent preclinical research has explored the effects of butyric acid supplementation in experimental models of Ankylosing Spondylitis, demonstrating promising anti-inflammatory and disease-modifying potential (9).

Butyrate was found to promote bone formation by increasing the expression and activity of osteoblastic markers, especially alkaline phosphatase (ALP), while also reducing bone resorption by inhibiting osteoclastic factors like RANKL. Furthermore, it assisted in reducing pro-inflammatory cytokines that are connected to inflammatory bone loss, including IL-1 $\beta$  and IL-18(10).

**Maltol :**

Inflammation plays a key role in osteoarthritis (OA), leading to interest in safer treatments than NSAIDs. The study highlights maltol, a plant-derived flavoring agent with anti-inflammatory and antioxidant effects. Maltol reduced inflammation and extracellular matrix components like collagen II and aggrecan in human chondrocytes. Moreover, maltol significantly suppresses the PI3K/AKT signaling pathway, which controls the NF-κB pathway. In animal models, it also reduced cartilage degradation and slowed the course of OA(11).

**Heptadecane:**

A second study was carried out to assess the anti-inflammatory and antioxidant qualities of heptadecane and to clarify its biological role. An endothelial cell line was used for supplementary in vitro investigations after heptadecane was given to elderly rats. According to the results, heptadecane inhibits NF-κB activity, which reduces age-associated elevation of pro-inflammatory gene expression. This impact seems to be mediated by reactive species (RS)-induced activation of the NIK/IKK and MAPK signaling pathways, offering mechanistic insight into heptadecane's anti-inflammatory properties(12).

**Eicosanoic acid:**

Eicosanoic acid is a constituent of membranes and the precursor to a class of hormones that are crucial for the control of several physiological processes, including prostacyclins, thromboxanes, and prostaglandins. It has been reported that the eicosanoids have anti-inflammatory qualities (13).

**2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) :**

The compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) exhibits strong antioxidant activity primarily due to its hydroxyl groups at the C-3 and C-5 positions, with the enol hydroxyl at C-5 playing a particularly crucial role. Blocking these groups significantly reduces its radical-scavenging ability, indicating that its antioxidant effect stems from its enol structure and redox potential. DDMP can neutralize up to two free radicals per molecule, but it is relatively unstable and prone to oxidation, leading to the formation of other active compounds like hydroxymaltol (14).

**Glycidyl palmitate:**

Glycidol and palmitic acid, a common fatty acid, combine to generate the glycidyl ester known as glycidyl palmitate. The molecular formula for the fatty acid glycidyl palmitate is  $C_{19}H_{36}O_3$ (15)(16) .

**Glycidyl (Z)-9-heptadecenoate:**

Glycidol and (Z)-9-heptadecenoic acid combine to generate the glycidyl ester known as glycidyl (Z)-9-heptadecenoate. Compounds containing glycidyl (Z)-9-hexadecenoate are known to have antibacterial properties (17).

**CONCLUSION:**

The current GC-MS analysis of Rasnadi Ghrita identified 25 phytocompounds, several of which have notable pharmacological activities beneficial in managing *Sandhigatavata*—a Vata disorder comparable to knee osteoarthritis. Key bioactive constituents like Dodecanoic acid and 1,2,3-Propanetriyl ester, N-Hexadecanoic acid (palmitic acid), Butyric, Eicosanoic, Maltol, and Tetradecanoic acid demonstrate strong anti-inflammatory, analgesic, antioxidant, antimicrobial, and neuroprotective properties. Rasnadi Ghrita is widely used in various Ayurvedic treatments, Such as *Basti*, *Pana*, and *Nasya*. Its use across these therapies highlights its broad applicability and effectiveness in enhancing overall health and well-being. In summary, the significance of Rasnadi ghrita lies in its deep-rooted traditional background, wide therapeutic scope, and support from both classical Ayurvedic literature and modern scientific research.

**CONFLICT OF INTEREST:**

The authors have no conflicts of interest regarding this investigation.

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