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Gas chromatography-Mass Spectromerty (GC-MS) Profiling and Standardization of Punarnavashtak Ghana prepared with Bhavna (Trituration) of Gomutra : Exploring its Therapeutic Potential in Metabolic Dysfunction -Associated Fatty Liver Disease

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Abstract

Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) is a prevalent disorder characterized by hepatic steatosis with metabolic dysregulation such as obesity ,insulin resistance, and metabolic syndrome. Ayurvedic formulation with hepatoprotective potential require scientific validation for broader acceptance. Punarnavashtak Ghana, a classical polyherbal formulation, is traditionally indicated in Yakrit Vikar (liver disorder). Bhavna(trituration) with Gomutra is described in Ayurveda to enhance bioavailability and therapeutic potency.

To standardize Gomutra processed Punarnavashtak Ghana and characterize its volatile and semi-volatile phytoconstituets using Gas chromatography-Mass Spectrometry (GC-MS) to explore its therapeutic relevance in MAFLD.

Punarnavashtak Ghana was prepared according to classical Ayurvedic procedures and subjected to three Gomutra Bhavana cycles. GC–MS analysis was performed in Total Ion Chromatogram mode with spectral matching using the NIST23 library.

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GC-MS profiling identified six major compounds, predominantly phenolic esters (69.59%), along with benzoate derivatives, α-turmerone, and medium-chain fatty acids, known for antioxidant, anti-inflammatory, hepatoprotective, and lipid-modulating activities. This bioactive phytochemical spectrum supports the hepatoprotective and metabolic regulatory potential of Gomutra-processed Punarnavashtak Ghana, providing scientific evidence for its traditional use in MAFLD and aiding its standardization.

Key words : MAFLD, Punarnashtak Ghana, Gomutra Bhavna, GC-MS profiling, Phytochemical standardization, Yajrit Vikar.

A Metabolic Dysfunction -Associated Fatty Liver Disease(MAFLD) is a chronic liver disorder defined by hepatic fat accumulation in the presence of metabolic dysfuction. The term MAFLD was replaced by Non-Alcohol Fatty Liver Disease (NAFLD) to underline the significance of metabolic abnormalities and to establish positive diagnostic criteria rather than a diagnosis of exclusion[3]. MAFLD encompasses a many range of conditions from simple steatosis to it will progresses upto steatohepatitis, fibrosis, cirrhosis and further lead to hepatocellular carcinoma. And it is the one of the most prevalent and rising liver disorder worldwide, affecting nearly 25 % of the adult population globally, with prevalence increasing alongside obesity, type 2 diebetes and metabolic syndrome.[4,9]

The effectiveness of Ayurvedic drugs in treating wide range of diseases is widely proven and given in Ayurvedic literature. However, because due to a lack of scientific support, these drugs are frequently not properly recognized therefore it is critical to examine this drugs with advanced technologies such as GC-MS.[5]

The GC-MS analysis of the Punarvashtak Ghana prepared with Bhavna (Trituration) of Gomutra is the main focus of this Article.

According to the Ayurvedic literature Bhaishajya Ratnawali (Vidhyotiny), Punarnavashtak kwath (PNK) is a polyherbal remedy used to treat asthma and hepatic disorders. It contains eight ingrediensts *Boerhaavia diffusa* Linn. (Nyctaginaceae), *Azadirachta indica* A. Juss. (Meliaceae), *Tinospora cordifolia* (Willd.) Miers (Menispermaceae), *Zingiber officinalie* Rosc. (Zingiberaceae), *Berberis* (Berberidaceae), *Picrorhiza kurroa* Royle ex Benth. (Plantaginaceae), and *Trichosanthes dioica* Roxb. (Cucurbitaceae).[6]

The analytical techniques like Gas chromatography mass spectrometry are frequently employed to find herbal components in complicated fractions. Mass spectrometry finds and examines the compounds identified by chromatography separately, while gas chromatography assists in separating the chemicals from the provided sample combination. These two precise techniques are employed in this investigation. When a chemical is chemically stable and unaltered within the system, GC MS is suitable for substances that evaporate without decomposing further at moderate temperatures.

GC-MS is extremely helpful in isolating and identifying analytes that exist only in the gas phase and at temperatures below 100°C, which is not possible with other methods. Furthermore, it can increase the number of potential analytes by producing stable, volatile derivatives of chemicals that are inappropriate for GC MS in their original condition[8]. Taking these variables into mind, the present study seeks to assess the quality control of Punarnashtak Ghana and investigate its bioactive herbal components by GC MS to determine their therapeutic effects on MAFLD.

While HPTLC fingerprinting demonstrated the presence of phenolics, flavonoids, terpenoids, and glycosides across multiple Rf zones, GC–MS analysis was undertaken to identify volatile and semi-volatile constituents contributing to pharmacological activity and formulation standardization.

➢ *Collection and Authentication of ingredients:*

All the raw materials of Punarnavashtak Ghana were purchased from an GMP Certified pharmacy and All the herbal drugs were collected and authenticated at the GMP Certified pharmacy Panchamrut Herbal Surat (Gujarat) as per the standards mentioned in the *Ayurveda* Pharmacopeia of India (API).

➢ *Gas Chromatography–Mass Spectrometry (GC–MS) :*

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of *Punarnavashtak Ghana* was performed at the Research & Development Cell, Parul University.

Table-1. A Pharmaceutical preparation of Punarnavashtak Ghana are outlined in table-1.[2]

Botanical name	*Family*	*Drug name*	*Part used*	*Part*
Boerhaavia diffusa Linn	*Nyctaginaceae*	*Punarnava*	*Whole plant*	1
Azadirachta indica A. Juss.	*Meliaceae*	*Nimb*	*Leaf*	1
Trichosaanthes dioca Roxb.	*Cucurbitaceae*	*Patol*	*Leaf*	1
Zingiber officinale Roxb.	*Zingiberaceae*	Shunthi	*Dried Rhizome*	1
Picrorhiza kurrroa Royle	*Scrophulariaceae*	*Kutaki*	*Dried Rhizome*	1
Tinospora cordifolia Willd.	*Menispermaceae*	*Guduchi*	*Stem*	1
Berberis aristata DC	*Berberidaceae*	*Daruharidra*	*Dried stem*	1
Terminalia chebula Retz	*Combretaceae*	*Haritaki*	*Fruit*	1

➢ *Preparation :*

- The *Punarnavashtak Kwath* is prepared using the coarse powder of the following herbs: *Punarnava, Nimb, Patol, Sunthi, Kutaki, Guduchi, Daruharidra* and *Haritaki* (Table-1).
- The useful parts of each herb are collected, cleaned thoroughly with water, and dried. All ingredients are crushed to obtain a coarse powder. This powder is then mixed with water in a 1:16 ratio (1 part herb to 16 parts water) and simmered on a low flame until the liquid is reduced to 1/8th of

its original volume to obtain a *kwath*.

- This *kwath* is filtered and evaporated to dryness to yield a *Ghana*. The resulting *Ghana* is further subjected to the *Bhavana* process three times using fresh *Gomutra* to enhance its therapeutic potency.
- Finally, the prepared Ghana is encapsulated to form *Punarnavashtak* Capsules, which offer the benefits of classical *Punarnavashtak Kwath* in a convenient dosage form.

➢ *Rational for Gomutra Bhavna (Trituration) in enhancing potency and Palatability of Punarnavashtak Ghana:*

In the classical preparation of *Punarnavashtak Kwath, Gomutra* is administered as *Anuapana* (adjuvant substance) immediately after a medicine to inhance its absorption, assimilation, and therapeutic efficacy. However due to practical unavailability of fresh *Gomutra* and to make it palatable the *Bhavna* (Trituration) was adopted, thereby increasing the formulations efficacy.

Gas Chromatography Mass Spectrometry (GC–MS) analysis :
Preparation of Test Solution :

The sample of Punarnavashtak Ghana was analyzed in Total Ion Chromatogram (TIC) mode using a GC–MS system operated under the method file *141024_Scan_60Min.qgm*. An injection volume of 1.0 µL of the test sample was introduced into the system, and chromatographic separation was achieved over a 60-minute run. Mass spectral acquisition was performed in scan mode, and compound identification was carried out by matching the obtained mass spectra with the NIST23 mass spectral library database. Retention time (RT), peak area, area percentage, molecular ion fragments, and base peak ions were recorded for each resolved component. Data processing and peak integration were performed using the instrument's dedicated software.

The Total Ion Chromatogram (TIC) displayed six major peaks between retention time (RT) 12.448–27.829 minutes.

Table-2. Identified compounds

Peak No.	RT (min)	Area %	Identified Compound
1	12.448	17.92%	2-Chloroethyl benzoate
2	16.927	69.59%	Methylparaben
3	20.351	4.21%	α-Turmerone
4	22.533	3.22%	n-Decanoic acid
5	24.381	2.88%	Isopropyl myristate
6	27.829	2.18%	n-Decanoic acid

Spectral Interpretation
1. *Chloroethyl Benzoate (RT 12.448 min; 17.92%) :*

Mass spectrum showed base peak at m/z 105 and molecular ion at 184 consistent with benzoate ester structure. This compound represents a benzoate ester derivative, structurally related to phenolic acids. Its presence correlates with HPTLC Rf 0.26–0.44

region attributed to phenolic acids and benzoic derivatives. The compound possesses antimicrobial activity, antioxidant potential and lipid-modulating effect. Benzoate derivatives are known to possess hepatoprotective and antioxidant effects by reducing lipid peroxidation.[6]

2. *Methylparaben (RT 16.927 min; 69.59%):*

This was the predominant peak, showing molecular ion at m/z 152 and base peak at m/z 121, characteristic of p-hydroxybenzoic acid methyl ester. Structurally, methylparaben is a phenolic ester derived from p-hydroxybenzoic acid. Its dominance suggests substantial phenolic ester content in the *Ghana* preparation. This peak in GCMS correlates with strong UV absorption at 254 nm and matches with phenolic-rich zones identified in mid-Rf region in the HPTLC report of the formulation. This phytoconstituent is known for its antioxidant, anti-inflammatory and free radical scavenging properties. Phenolic esters are documented to inhibit oxidative stress-mediated hepatocellular injury.[1]

3. α-*Turmerone (RT 20.351 min; 4.21%):*

Identified with molecular ion m/z 216 and characteristic fragment at m/z 83 α-Turmerone is a sesquiterpene ketone known from *Curcuma* species and other aromatic plants. α-Turmerone has anti-inflammatory (NF-κB inhibition), hepatoprotective, anti-steatotic and antioxidant activities. Turmerones suppress inflammatory cytokines and oxidative stress markers in hepatic injury models.[1] α-Turmerone matches high-Rf terpenoid zone (0.83–0.94) in the HPTLC analysis of the formulation.

4. *n-Decanoic Acid (RT 22.533 & 27.829 min; total 5.4%) :*

Identified with molecular ion m/z 172 and base peak m/z 73, n-Decanoic Acid is a medium-chain fatty acid (MCFA). It is known to improve mitochondrial β-oxidation, enhance lipid metabolism, has anti-inflammatory effect and modulates hepatic lipid accumulation. Medium-chain fatty acids are reported to be improve the insulin sensitivity and reduces hepatic steatosis[10]. This component corresponds likely solvent-front high Rf nonpolar band in HPTLC analysis.

Integrated Interpretation with HPTLC :

The GC–MS profile supports the earlier HPTLC interpretation (Table-3).

HPTLC Zone	**GC–MS Confirmation**
Low-Rf phenolic region	Benzoate derivatives, methylparaben
Mid-Rf terpenoid zone	α-Turmerone
High-Rf nonpolar zone	Fatty acids (decanoic acid), esters

The predominance of phenolic ester (69.59%) suggests strong antioxidant and hepatoprotective potential, consistent with the formulation's use in *Yakrit Vikara* and MAFLD. A multi-target mechanism can be proposed for the action of the formulation which can be substantiated with the presence of terpenoids (anti-inflammatory action), medium-chain fatty acids (lipid-modifying action) and phenolic esters (antioxidant protection). Reduction of oxidative stress, suppression of inflammatory cytokines, improvement of hepatic lipid

metabolism and protection against hepatocellular degeneration collectively aids in the fomulation's therapeutic activity in *Yakrit Vikara* and MAFLD. The GC–MS chromatogram demonstrates a chemically diverse profile dominated by phenolic esters and supported by terpenoids and fatty acids. The high abundance of methylparaben-like phenolic ester (69.59%) indicates substantial p-hydroxybenzoate-type compounds in the formulation, which are known to exert antioxidant and cytoprotective effects in hepatic tissues. The detection of α-turmerone corresponds with the formulation's known anti-inflammatory effects. Turmerones are reported to suppress NF-κB signaling and decrease the expression of TNF-α and IL-6, which are key mediators in the progression of MAFLD. Additionally, medium-chain fatty acids such as decanoic acid support enhanced mitochondrial fatty acid oxidation, thereby potentially limiting hepatic lipid accumulation. Collectively, the GC–MS results corroborate the HPTLC fingerprint profile and confirm the presence of bioactive phytochemical groups contributing to hepatoprotective activity.

Through GC-MS analysis, the current study provides a scientific evaluation of Gomutra-processed Punarnavashtak Ghana with the aim of providing scientific validation for its traditional use in hepatic and metabolic disorders. MAFLD has a multifactorial etiology that includes insulin resistance, oxidative stress, chronic inflammation, and changes in lipid metabolism. Therefore, therapeutic techniques that target numerous disease pathways are necessary for optimal management.

About 70% of the volatile components found were methylparaben-like compounds, according to GC–MS profiling done, which showed that phenolic esters are the most common class of ingredients. The antioxidant activity, membrane-protective properties, and ability to reduce lipid peroxidation in hepatic tissue are all well-known attributes of phenolic compounds. Their high frequency in the formulation suggests a significant potential to reduce hepatocellular damage brought on by oxidative stress, which is a major factor in the development of desease.

Because of its well-established hepatoprotective and anti-inflammatory qualities, the discovery of α-turmerone is significant. The development of steatohepatitis and hepatic fibrosis is significantly influenced by pro-inflammatory cytokines, such as TNF-α and IL-6, which are downregulated by turmerones and have been demonstrated to block NF-κB activation. These results corroborate Punarnavashtak's traditional use in the treatment of inflammatory liver diseases.

It is well known that medium-chain fatty acids, including n-decanoic acid, improve mitochondrial β-oxidation and encourage effective lipid use. By improving insulin sensitivity and reducing hepatic lipid buildup, these actions may help address the primary metabolic abnormalities in MAFLD. Their discovery aligns with the lipid-regulating qualities ascribed to a number of Punarnavashtak Ghana components.

The phytochemical characterization's credibility is increased and efforts to standardize formulations are strengthened when GC-MS results and HPTLC fingerprinting are consistent. It's possible that the Gomutra-based Bhavana procedure enhanced the extraction or biotransformation of active ingredients,

which is consistent with Ayurvedic pharmaceutic principles that ascribe Bhavana's increased bioavailability and therapeutic efficacy.

The formulation as a whole has a synergistic phytochemical composition that targets oxidative stress, inflammation, and lipid imbalance—three key pathways implicated in the pathophysiology of MAFLD. Even though GC-MS provides valuable chemical characterisation, more clinical and pharmacological research is required to validate therapeutic efficacy and elucidate dose-response interactions.

This work strengthens *Punarnavashtak Ghana's* potential as a supplemental strategy in the management of MAFLD by fusing traditional Ayurvedic knowledge with modern scientific techniques.

References :

1. Ali, B. H., G. Blunden, M. O. Tanira, and A. Nemmar, (2008). *Food and Chemical Toxicology*, *46*(2): 409–420. p. 413–416.
2. Critical review on Punarnavashtak Kwatha: An Ayurvedic polyherbal formulation. Available from: Https://www.researchgate.net/publication/384354489_critical_review_on_punarnavashtak_kwatha_an_ayurvedic_Polyherbal_Formulation
3. Eslam, M., P. N. Newsome, S. K. Sarin, Q. M. Anstee, G. Targher, M. Romero-Gomez, S. Zelber-Sagi, Wai-Sun Wong, V., J. F. Dufour, J. M. Schattenberg, T. Kawaguchi, M. Arrese, L. Valenti, G. Shiha, C. Tiribelli, H. Yki-Järvinen, J. G. Fan, H. Grønbæk, Y. Yilmaz, H. Cortez-Pinto, … and J. George, (2020). *Journal of Hepatology*, *73*(1): 202–209. https://doi.org/10.1016/j.jhep.2020.03.039
4. Eslam, M., A. J. Sanyal, J. George, & International Consensus Panel. (2020). *Gastroenterology*, *158*(7): 1999–2014.e1. https://doi.org/10.1053/j.gastro.2019.11.312
5. Gomathi, D., M. Kalaiselvi, G. Ravikumar, K. Devaki, and C. Uma, (2015). *Journal of Food Science and Technology*, *52*(2): 1212–1217. https://doi.org/10.1007/s13197-013-1105-9
6. Rawat, A. K., S. Mehrotra, S. C. Tripathi, and U. Shome, (1997). *Journal of Ethnopharmacology*, *56*(1): 61–66. https://doi.org/10.1016/s0378-8741(96)01507-3
7. Saleem, A., M. Husheem, P. Harkonen, and K. Pihlaja, (2002). *Journal of Ethnopharmacology*, *81*(3): 327–336.
8. Sudhan, A., K. Prabhu, S. Jones, C. S. Janaki, D. Sheriff, M. R. Rao, M. H. Kumar, D. Balakrishnan, and M. Shresht, (2024). *Journal of Pharmacy & Bioallied Sciences*, 16(Suppl 5): S4712–S4716. https://doi.org/10.4103/jpbs.jpbs_876_24
9. Tilg, H., A. R. Moschen, and M. Roden, (2017). *Nature Reviews Gastroenterology & Hepatology*, *14*(1): 32–42. https://doi.org/10.1038/nrgastro.2016.147
10. Xie, W., M. Wang, C. Chen, X. Zhang, and M. F. Melzig, (2022). *Phytomedicine*, *100:* 154016. p. 5–11.