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Research Article

PHYTOCHEMICAL PROFILING OF THE CLASSICAL SIDDHA AYUSH FORMULATION *POOSANI NEI* USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC–MS)

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ABSTRACT

Poosani Nei is a classical Siddha herbal formulation prescribed for the management of respiratory disorders such as *Kaasam* (cough) and *Suvasakasam* (bronchial asthma). Gas Chromatography–Mass Spectrometry (GC–MS) is a sensitive analytical technique widely employed for identifying volatile and semi-volatile bioactive compounds in herbal formulations. **Objective:** The present study was undertaken to characterise the phytochemical constituents of *Poosani Nei* using GC–MS analysis and to correlate the identified compounds with their reported pharmacological activities. **Methods:** *Poosani Nei* was prepared in accordance with the classical Siddha literature using authenticated raw materials. The formulation was subjected to GC–MS analysis under standardised instrumental conditions. Identification of phytoconstituents was performed by comparing the obtained mass spectra with the National Institute of Standards and Technology (NIST) spectral library. **Results:** GC–MS analysis revealed the presence of nine major phytochemical constituents, including pentadecanoic acid, cyclopropane decanoic acid, trioxolane, 1-hexadecanol-2-methyl, tetradecane, 1-hexadecanol, heptadecane, hexadecenoic acid, and 3-methyl pentadec-1-ol. Pentadecanoic acid constituted the highest peak area (32.40%), followed by cyclopropane decanoic acid (11.50%), hexadecenoic acid (10.30%), and tetradecane (11.20%). Several identified compounds have been reported to possess documented anti-inflammatory, anti-microbial, anti-oxidant, and immunomodulatory properties that may contribute to the therapeutic efficacy of *Poosani Nei* in respiratory diseases. **Conclusion:** The GC–MS profile demonstrated that *Poosani Nei* contains multiple pharmacologically active phytoconstituents with potential synergistic effects against inflammatory and infectious respiratory disorders.

INTRODUCTION

The Siddha System of Medicine, one of the oldest traditional healthcare systems practiced in South India, emphasizes the prevention and treatment of diseases through maintaining the equilibrium of the three fundamental humours (*Vali*, *Azhal*, and *Iyyam*). Numerous classical Siddha formulations prepared from medicinal plants, minerals, and animal-derived substances have been traditionally employed for treating chronic respiratory disorders. Among these, *Poosani Nei* is a well-documented herbo-lipid formulation indicated for *Kaasam* (cough), *Suvasakasam* (bronchial asthma), wheezing, and other respiratory ailments described in classical Siddha literature namely *Sarabenthira Vaithiya Muraigal*[1]. Despite its long history of therapeutic use, comprehensive phytochemical characterisation using modern analytical techniques remains limited. Respiratory diseases remain a major global health concern. According to the World Health Organisation (WHO), chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) affect hundreds of millions of people worldwide and are associated with significant morbidity and mortality. Persistent airway inflammation, oxidative stress, microbial infections, and immune deregulation contribute substantially to disease progression. Although corticosteroids and bronchodilators remain

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the mainstay of treatment, prolonged use is associated with adverse effects, creating an increase in demand for safer complementary therapies derived from traditional medicine. Medicinal formulations rich in naturally occurring phytochemicals have attracted increasing scientific interest because of their anti-inflammatory, antioxidant, antimicrobial, bronchodilator, and immunomodulatory properties. Herbal lipid formulations, particularly those prepared with ghee or medicated oils, improve the extraction and bioavailability of lipophilic phytoconstituents, thereby enhancing therapeutic efficacy. Previous studies have reported that fatty acids, long-chain alcohols, hydrocarbons, terpenoids, and oxygenated organic compounds contribute significantly to the pharmacological activities of traditional herbal formulations.

Gas Chromatography–Mass Spectrometry (GC–MS) has become one of the most reliable analytical tools for qualitative and semi-quantitative phytochemical profiling of herbal medicines. The technique combines the excellent separation capability of gas chromatography with the molecular identification power of mass spectrometry, allowing accurate characterisation of volatile and semi-volatile constituents. Identification of compounds is commonly achieved through comparison with the National Institute of Standards and Technology (NIST) spectral database, providing high confidence in compound annotation. GC–MS has therefore become an indispensable technique for herbal drug standardisation, quality assurance, authenticity evaluation, and biomarker identification. Standardisation of Siddha formulations has gained increasing importance following the guidelines issued by the Pharmacopoeial Laboratory for Indian Medicine (PLIM), Ministry of AYUSH. Scientific characterisation not only ensures batch-to-batch consistency and safety but also facilitates the global acceptance of Siddha medicines through evidence-based validation. Analytical profiling using chromatographic techniques provides valuable information regarding marker compounds that may contribute to therapeutic efficacy and can serve as quality control parameters for future pharmaceutical development. In the present investigation, GC–MS analysis of *Poosani Nei* revealed the presence of several bioactive compounds, suggesting their potential contribution to the therapeutic action of the formulation in respiratory disorders. The predominance of fatty acids and long-chain alcohols further supports the traditional rationale of using lipid-based Siddha formulations for inflammatory diseases. Therefore, the present study aimed to perform comprehensive phytochemical profiling of the classical Siddha formulation *Poosani Nei* using Gas Chromatography–Mass Spectrometry (GC–MS), identify its major bioactive constituents through NIST library matching, and correlate the detected phytochemicals with their reported pharmacological activities. The findings are expected to contribute toward the scientific validation, quality standardisation, and future pharmacological evaluation of this traditional Siddha formulation.

MATERIALS AND METHODS

Study Design

The present investigation was designed as an Analytical laboratory-based study to characterise the phytochemical constituents of the classical Siddha formulation *Poosani Nei* using Gas Chromatography–Mass Spectrometry (GC–MS)**.** The study was conducted in accordance with the analytical procedures recommended by the Pharmacopoeial Laboratory for Indian Medicine (PLIM), Ministry of AYUSH**,** for the standardisation of traditional herbal formulations.

Study Area

The preparation and preliminary quality assessment of *Poosani Nei* were carried out at the Postgraduate Department of Gunapadam (Siddha pharmacology) Laboratory, Government Siddha Medical College, Palayamkottai, Tamil Nadu, India. GC–MS analysis was performed at a Noble Research Solutions (NABL) accredited analytical laboratory equipped with advanced chromatographic instrumentation. The study design and laboratory locations are consistent with those described in the source manuscript.

Authentication of Raw Materials

All herbal ingredients used for the preparation of *Poosani Nei* were procured from an authenticated herbal raw drug supplier. The identity and quality of each ingredient were verified by experts from the Department of PG Gunapadam, Government Siddha Medical College, Palayamkottai, based on macroscopic and organoleptic characteristics in accordance with Siddha Pharmacopoeia and PLIM guidelines.[2,3] Foreign matter and deteriorated materials were removed before processing.

Preparation of *Poosani Nei*

Poosani Nei was prepared strictly according to the classical Siddha text *Sarabenthira Vaithiya Muraigal* using the prescribed ingredients and proportions. According to the Siddha Pharmacopoeia of India, the collection of raw drugs focuses on monographs of single raw materials derived from plants, minerals, and animal sources has been mentioned in it[4]. The herbal drugs were cleaned thoroughly, shade-dried where necessary, and processed individually according to classical methods. Fresh juice, decoction, or paste of the respective herbal ingredients was prepared as described in the classical literature. The ingredients were mixed with purified cow's ghee (*Ghrita*) and heated under controlled

temperature with continuous stirring until the characteristic signs (*Nei Pakam*) described in Siddha literature were attained. The completion of preparation was confirmed by the absence of frothing, characteristic aroma, and attainment of the desired consistency. The prepared medicated ghee was filtered through sterile muslin cloth while warm to remove coarse particles and stored in amber-coloured airtight glass containers at room temperature until further analysis. Retention indices and mass spectral interpretation were essential in oil analysis for accurate identification of plant secondary metabolites[5].

Sample Preparation of *Poosani Nei*

Approximately 1ml of the prepared *Poosani Nei* sample was dissolved in HPLC-grade n-hexane to obtain complete extraction of volatile and semi-volatile phytoconstituents. The solution was vortex-mixed for 5 minutes and centrifuged at 3000 rpm for 10 minutes. The clear supernatant was filtered through a 0.22 µm PTFE syringe filter before injection into the GC–MS system.

Instrumentation And Analytical Conditions

GC–MS analysis was performed using a Gas Chromatography–Mass Spectrometry system equipped with an electron ionisation (EI) source operating at 70 eV. The chromatographic separation was achieved using a DB-5MS fused silica capillary column (30m × 0.25 mm internal diameter × 0.25µm film thickness.

Table 1: The GC-MS instrumentation and analytical conditions

Parameter	Condition
Instrument	Gas Chromatography–Mass Spectrometry (GC–MS)
Column	DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm)
Carrier gas	Helium (99.999% purity)
Flow rate	1.0 mL/min
Injection mode	Split
Injection volume	1 µL
Injector temperature	250°C
Ionization mode	Electron Impact (EI)
Ionization energy	70 eV
Ion source temperature	230°C
Interface temperature	280°C
Mass scan range	m/z 40–650
Solvent delay	3 min
Total run time	Approximately 35 min

The oven temperature program was optimised as follows:

Initial temperature: 60°C (2 min hold), increased at 10°C/min to 280°C, Final temperature maintained for 10 minutes.

These analytical conditions ensured adequate separation of volatile phytoconstituents present in the formulation[6].

RESULTS

The chromatographic peaks obtained from GC–MS analysis were identified by comparing the acquired mass spectra with those available in the National Institute of Standards and Technology (NIST) Mass Spectral Library. Compound identification was based on Retention time (RT), Molecular ion pattern, Fragmentation profile, and library matching score.

Table 2: Identified compounds of *Poosani nei* under GC-MS

S.No.	Retention Time (min)	Peak Area (%)	Identified Compound	Chemical Class	Reported Pharmacological Activity
1	16.12	32.40	Pentadecanoic acid	Saturated fatty acid	Anti-inflammatory, antimicrobial
2	18.40	5.80	Cyclopropane decanoic acid	Fatty acid derivative	Anti-inflammatory

3	20.02	6.40	Trioxolane	Oxygenated organic compound	Antioxidant, antimicrobial
4	20.87	10.30	1-Hexadecanol-2-methyl	Long-chain fatty alcohol	Anti-inflammatory, antimicrobial
5	21.75	11.20	Tetradecane	Hydrocarbon	Antimicrobial
6	22.77	11.50	1-Hexadecanol	Fatty alcohol	Antioxidant, antimicrobial
7	24.03	10.30	Heptadecane	Hydrocarbon	Antioxidant
8	25.58	7.30	Hexadecenoic acid	Unsaturated fatty acid	Anti-inflammatory, antimicrobial
9	32.68	4.40	3-Methyl pentadec-1-ol	Long-chain alcohol	Antioxidant

Only compounds exhibiting high spectral similarity with the NIST database were considered for further interpretation[7]. It is an internationally accepted spectral database for compound identification using GC–MS, and it enables accurate matching of unknown compounds based on mass spectra. It improves reliability and reproducibility of phytochemical profiling studies. In the Ayush system of medicine, it is important to do scientific quality control for safety, efficacy, and global acceptance of medicines[8]. In recent research papers, the instrumental analytical studies correlated identified compounds with pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects[9]. Demonstrated the importance of GC–MS in herbal drug discovery and validation. The WHO Benchmarks for Training in Traditional, Complementary and Integrative Medicine: Siddha (2022) established international standards for Siddha education, professional competency, evidence-based practice, quality assurance, and the standardisation of Siddha medicines[10], thereby promoting their global recognition and integration into healthcare systems.

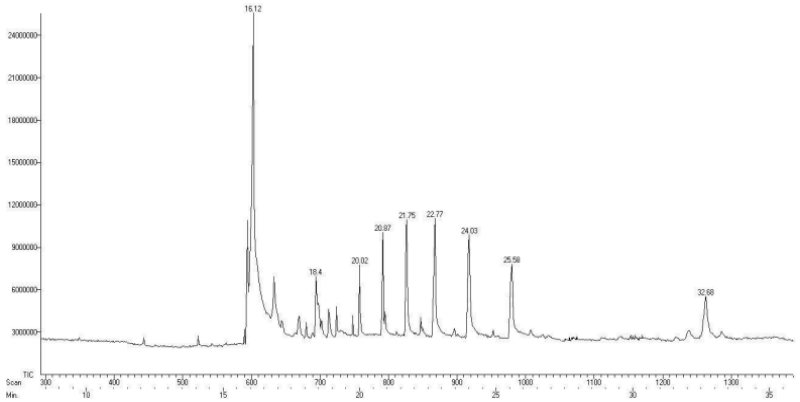

Fig 1: Chromatographic peaks obtained for *Poosani Nei* under GC-MS

DISCUSSION

The chromatographic data generated from the GC–MS analysis were processed using the manufacturer's integrated analytical software. Relative abundance of each phytochemical constituent was calculated as the percentage peak area with respect to the total ion chromatogram (TIC). The identified compounds were categorised by their reported biological activities based on published scientific literature. The chromatographic analysis identified nine major phytochemical constituents, including pentadecanoic acid, cyclopropane decanoic acid, trioxolane, 1-hexadecanol-2-methyl, tetradecane, 1-hexadecanol, heptadecane, hexadecenoic acid, and 3-methyl pentadec-1-ol. The predominance of pentadecanoic acid and other bioactive fatty acids, long-chain alcohols, and hydrocarbons demonstrates the chemical richness of the formulation and provides a characteristic phytochemical fingerprint for quality assessment. The identified compounds have been reported to possess anti-inflammatory, antimicrobial, antioxidant, and immunomodulatory properties, which scientifically support the traditional use of *Poosani Nei* in the management of *Kaasam* (cough) and *Suvasakasam* (bronchial asthma). Particular emphasis was placed on anti-inflammatory, antimicrobial, antioxidant, bronchodilatory, immunomodulatory, and respiratory-protective activities because these pharmacological properties are directly relevant to the traditional Siddha indications of *Poosani Nei* for *Kaasam* and *Suvasakasam*. The chromatographic profile, retention times, peak area percentages, and identified compounds were tabulated and interpreted

to establish the phytochemical profile of the formulation and to provide scientific evidence supporting its traditional therapeutic application. The identified compounds and their retention times correspond to those reported in the uploaded GC–MS results.

CONCLUSION

The present study provides a comprehensive GC–MS-based phytochemical profile of this classical formulation and correlates the identified constituents with their reported pharmacological activities, thereby contributing to its scientific validation and quality standardisation. The GC–MS profile generated in this study can serve as a valuable reference for authentication, batch-to-batch consistency, and the establishment of chemical markers for future pharmacopoeia standards. Furthermore, the presence of multiple pharmacologically active constituents suggests that the therapeutic efficacy of *Poosani Nei* is likely attributable to the synergistic interaction of its phytochemicals rather than to a single active compound.

Overall, the present study provides scientific evidence supporting the traditional claims of *Poosani Nei* and contributes to its evidence-based validation, quality assurance, and wider acceptance in integrative healthcare systems.

Future Scope of the Study

Future investigations should focus on comprehensive phytochemical characterisation using complementary analytical techniques such as LC–MS/MS, HPLC, FTIR, Raman spectroscopy, and NMR spectroscopy. In addition, in vitro, in vivo, and clinical studies are warranted to validate its pharmacological activities and elucidate the molecular mechanisms underlying its therapeutic efficacy.

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