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Physicochemical Analysis, Piperine Quantification and GC- MS Analytical Profiling of *Thippili Chooranam* – A Siddha Poly Herbal Formulation

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ABSTRACT

Background: The modern instrumentation era originated at the beginning of the 19th century, and newer inventions of modern analytical instruments caused a stormy revolution in the scientific world. These instruments are useful in analytical studies up to the atomic level. Instruments like SEM, TEM, GC-MS, XRD and ICP-MS switched the analytical study into a new pinnacle. With the aid of these instruments, both organic and inorganic materials can be completely analysed. Since herbal medicines contain many organic matters, it is highly expected to analyse these medicines with the modern instruments. Now days the land pollution increased into a remarkable level causing contamination of heavy metals in herbals. Hence herbal medicines are also to be screened for heavy metals. **Aim and Objectives:** Instrumental analysis for physio chemical characters, heavy metal contamination, gas chromatographic analysis and piperine quantification study had been executed for *Thippili chooranam* a poly herbal formulation used in Siddha medicine for respiratory ailments. **Results:** The analytical study reveals that this formulation fulfils the universal analytical standards and safe to use. **Conclusion**: Many of Siddha formulations are not screened through modern instrumentation and hence they are inadmissible in the modern medical world. Analytical profiling is the only way to standardize the herbal formulations and also for accreditation. Thippili chooranam a poly herbal formulation was analyzed and the standards of this chooranam has been substantiated.

Keywords: *Thippili chooranam, Physicochemical analysis, Piperine quantification, Siddha poly herbal formulation.*

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INTRODUCTION

The herbal world is a treasure trove of phytochemicals and presents enormous opportunities for analytical research, as it contains millions of naturally occurring organic compounds. New compounds continue to emerge through ongoing analytical investigations. Although this chemical diversity offers significant therapeutic potential, it also poses challenges in identifying the precise pharmacologically active constituents of herbal medicines. Furthermore, the complex chemical composition of herbal formulations may influence the pharmacokinetic properties of their constituents and potentially result in herb–drug interactions. The Indian AYUSH systems of medicine rely extensively on herbal ingredients for the preparation of medicines. Siddha, one of the ancient traditional medical systems of India, originated in Tamil Nadu and has a rich heritage. According to Siddha principles, treatment should initially be initiated with herbal medicines, followed by higher-order medicines when necessary [1]. Siddha pharmacology is traditionally based on three major categories of medicinal substances: herbal materials, metals and minerals, and animal-derived products [2].

In recent years, the use of animal-derived ingredients in medicinal preparations has declined, primarily due to concerns regarding the extinction and conservation of certain animal species. Consequently, ingredients such as musk, turtle shells, rhinoceros horns, and deer

antlers are restricted or prohibited in medicinal preparations under applicable conservation regulations.

Herbal medicines form the foundation of several traditional systems of medicine worldwide, including Indian, Chinese, and Tibetan medicine. However, environmental contamination, particularly soil contamination, poses a significant challenge to the safety and quality of herbal medicines. Medicinal plants may accumulate toxic heavy metals from contaminated soil, water, and environmental sources. Modern analytical techniques provide reliable methods for detecting and quantifying heavy metal contamination in herbal formulations. The global acceptance of herbal medicines requires comprehensive scientific evaluation, including physicochemical characterization, phytochemical profiling, identification and quantification of active constituents, and assessment of toxic contaminants using modern analytical instrumentation. Thippili Chooranam (T.C.), a Siddha polyherbal formulation traditionally used for respiratory ailments such as bronchial asthma, chronic cough, and dyspnoea, was selected for the present study. The formulation was subjected to physicochemical evaluation, identification of phytochemical constituents using gas chromatography–mass spectrometry (GC–MS), assessment of heavy metal contamination using atomic absorption spectroscopy (AAS), and quantification of piperine.

MATERIALS AND METHODS

Selection and preparation of the formulation

Thippili Chooranam (T.C.) [3], was selected for the present study based on the classical Siddha text *Kannusamy Pillai Parambarai Vaithiyam*. The formulation comprises 19 herbal ingredients. Each ingredient was purified according to the procedures described in the Siddha literature *Sarakkugalin Suththi Sey Muraikal*. The ingredients were incorporated into the formulation in the proportions specified in the classical reference. The composition and quantities of the individual ingredients are presented below. Composition of Thippili Chooranam

Table 01:

S. No.	Ingredient (Tamil name)	Quantity
1	Thippili	10 palam (350 g)
2	Chukku	5 palam (175 g)
3	Milagu	1 palam (35 g)
4	Val Milagu	1 palam (35 g)
5	Kadukkai	1 palam (35 g)
6	Nellikkai (dried)	1 palam (35 g)
7	Thandrikkai	1 palam (35 g)
8	Araththai	1 palam (35 g)
9	Thalisapathri	1 palam (35 g)
10	Kodiveli Ver Pattai	1 palam (35 g)
11	Seeragam	1 palam (35 g)
12	Sombu	1 palam (35 g)
13	Karunjeerakam	1 palam (35 g)
14	Omum	1 palam (35 g)
15	Kurosani Omum	1 palam (35 g)
16	Lavangam	1 palam (35 g)
17	Lavanga Pattai	1 palam (35 g)
18	Lavanga Pathri	1 palam (35 g)
19	Elam	1 palam (35 g)

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Note: Palam is a traditional unit of measurement used in Siddha medicine. In the present formulation, one palam is considered equivalent to approximately 35 g.

Table 02: Botanical details and pharmacological actions of the ingredients of Thippili Chooranam

S. No.	Vernacular name (Tamil)	English name	Botanical name	Family	Part used	Pharmacological actions
1	Thippili	Long pepper	*Piper longum*	Piperaceae	Fruit	Antiasthmatic [4]
2	Chukku	Dried ginger	*Zingiber officinale*	Zingiberaceae	Rhizome	Antitussive [5].
3	Milagu	Black pepper	*Piper nigrum*	Piperaceae	Fruit	Antiasthmatic, antiplatelet [6].
4	Val Milagu	Tailed pepper	*Piper cubeba*	Piperaceae	Fruit	Antiallergic [7].
5	Kadukkai	Chebulic myrobalan	*Terminalia chebula*	Combretaceae	Fruit	Immunomodulatory, adaptogenic [8].
6	Nellikkai (dried)	Indian gooseberry	*Phyllanthus emblica*	Phyllanthaceae	Fruit	Antitussive, anti-inflammatory [9].
7	Thandrikkaai	Belleric myrobalan	*Terminalia bellirica*	Combretaceae	Fruit	Beta-lactamase inhibitory, antiulcer [10].
8	Araththai	Galangal	*Alpinia officinarum*	Zingiberaceae	Rhizome	Bronchodilatory, antiallergic [11, 12].
9	Thalisapathri	English yew	*Taxus baccata*	Taxaceae	Leaves	Antiasthmatic [13].
10	Kodiveli Ver Pattai	Ceylon leadwort	*Plumbago zeylanica*	Plumbaginaceae	Root bark	Antiviral, anti-inflammatory [14].
11	Seeragam	Cumin	*Cuminum cyminum*	Apiaceae	Seeds	Anti-inflammatory [15].
12	Sombu	Anise	*Pimpinella anisum*	Apiaceae	Seeds	Antiviral, antibacterial [16,17].
13	Karunjeeragam	Black cumin (black seed)	*Nigella sativa*	Ranunculaceae	Seeds	Antihistaminic, immunomodulatory [18].
14	Omum	Ajwain (carom seeds)	*Trachyspermum ammi*	Apiaceae	Seeds	Anti-inflammatory, antihistaminic [19].
15	Kurosani Omum	Henbane	*Hyoscyamus niger*	Solanaceae	Seeds	Analgesic, spasmolytic [20].
16	Lavangam	Clove	*Syzygium aromaticum*	Myrtaceae	Flower buds	Antimicrobial, antiviral [21, 22].
17	Lavanga Pattai	Cinnamon bark	*Cinnamomum verum*	Lauraceae	Bark	Antibacterial, immunomodulatory [23, 24].
18	Lavanga Pathri	Indian bay leaf	*Cinnamomum tamala*	Lauraceae	Leaves	Anti-inflammatory [25].
19	Elam	Cardamom	*Elettaria cardamomum*	Zingiberaceae	Fruits (capsules)	Antimicrobial [26].

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Collection and Authentication of Herbal Ingredients

The ingredients of this formulation were purchased from a reputed country drug store located at Broadway, Chennai. They were authenticated by a botanist from D.G. Vaishnav College of Arts and Science, Chennai, and Siddha experts from the Department of Gunapadam (Siddha Pharmacology), Government Siddha Medical College, Chennai. Authentication numbers were assigned to the ingredients as per the records maintained in the department. Specimen samples were preserved in the Department of Gunapadam for future reference.

All the herbal ingredients were thoroughly dried after purification and pounded into a fine powder using an iron mortar and pestle. The powder was sieved, tightly packed in a food-grade plastic container, and used for the study.

The study was undertaken at the Government Laboratory, namely, the Research and Development Wing and the State Drug Testing Laboratory (Indian Medicine), Directorate of Indian Medicine and Homoeopathy, Government of Tamil Nadu, Chennai – 600 106. The analytical study laboratory number was 10/QA/G/DTL (I.M.)/24-25.

Physicochemical Analysis of Thippili Chooranam

Table 03:

Parameter	Observation
Colour	Brown
Odour	Pungent
Taste	Acrid
Consistency	Fine

Table 00: 04

S. No.	Parameters	Result of Analysis	SPI Specification
1.	Colour	Brown-coloured powder	Brown colour
2.	Microscopic Examination	Answers the identification test for *Piper longum* Linn.	Answers the identification test for *Piper longum* Linn.
3.	Foreign Matter	Nil	Not more than 2%
4.	Total Ash	6.05%	Not more than 7%
5.	Total Insoluble Ash	0.06%	Not more than 0.5%
6.	pH at 5% aqueous solution	4.51	---
7.	Loss on Drying	11.10%	---
8.	Water-Soluble Extractive	18.50%	Not less than 7%
9.	Alcohol-Soluble Extractive	7.11%	Not less than 5%

Microbiological Assay of Thippili Chooranam

As per the PLIM guidelines, a sample should be free from microbial contamination. The Thippili Chooranam sample was assayed for the presence of microorganisms. The results showed that the sample was within the prescribed limits for microbial contamination.

Table 05:

Parameter	Result of Analysis	SPI Specification
Total Bacterial Count	2×10^4 CFU/g	Maximum 10^5/g
Total Fungal Count	1×10^2 CFU/g	Maximum 10^3/g
E. coli	Absent	Should be absent
Salmonella sp./g	Absent	Should be absent
Staphylococcus aureus/g	Absent	Should be absent
Pseudomonas aeruginosa	Absent	Should be absent

Heavy Metal Analysis of Thippili Chooranam by AAS (Atomic Absorption Spectrophotometer)

An atomic absorption spectrophotometer is an instrument used to identify and quantify metallic elements present in a sample. The sample is vaporised at a high temperature, and if metallic elements are present, they are converted into free ground-state atoms. These atoms absorb light at characteristic wavelengths based on the Beer–Lambert law. The monochromator isolates the wavelengths to be analysed by filtering out background light. The detector measures the intensity of the absorbed radiation and quantifies the metallic elements.

This analysis was carried out at the Research and Development Wing, Directorate of Indian Medicine and Homoeopathy, Government of Tamil Nadu, Chennai. The reference number was 117/R&D/2025.

Table 06:

S. No.	Name of the Heavy Metal	Result of Analysis	SPI Specification
1.	Lead	BOQ < 5 ppm	Not more than 10 ppm
2.	Arsenic	BOQ < 1 ppm	Not more than 3 ppm
3.	Mercury	BOQ < 0.5 ppm	Not more than 1 ppm
4.	Cadmium	BOQ < 0.1 ppm	Not more than 0.3 ppm

The analysis of the Thippili Chooranam (T.C.) sample showed that the heavy metals were below the observable quantity.

Phytoconstituent Analysis of Thippili Chooranam: Approximately 25 g of the sample was taken and boiled with 100 mL of water for 20–30 minutes. When the volume was reduced to 50 mL, the extract was filtered through a Büchner funnel fitted with Whatman No. 1 filter paper. The filtrate was collected and used for analysis. The following tests were performed to detect the phytoconstituents present in the sample.

Detection of Alkaloids: This was carried out using Dragendorff's test.
Detection of Glycosides: Bornträger's test.
Detection of Saponins: Foam test.
Detection of Phenols: Ferric chloride test.
Detection of Tannins: Gelatin test.
Detection of Flavonoids: Alkaline reagent test.
Detection of Terpenoids: Copper acetate test.
Detection of Amino Acids: Ninhydrin test.
Detection of Carbohydrates: Molisch's test.
Detection of Proteins: Xanthoproteic test.

The Thippili Chooranam sample showed the presence of the following phytoconstituents.

Table 07:

S. No.	Name of the Phytoconstituents	Aqueous Extract of T.C.
1.	Alkaloids	Present
2.	Flavonoids	Present
3.	Glycosides	Present
4.	Lignin	Absent
5.	Saponins	Absent
6.	Steroids	Present
7.	Terpenoids	Absent
8.	Tannins	Present
9.	Phenolic Compounds	Present
10.	Proteins and Amino Acids	Absent
11.	Carbohydrates	Present

GC–MS Analysis of Thippili Chooranam

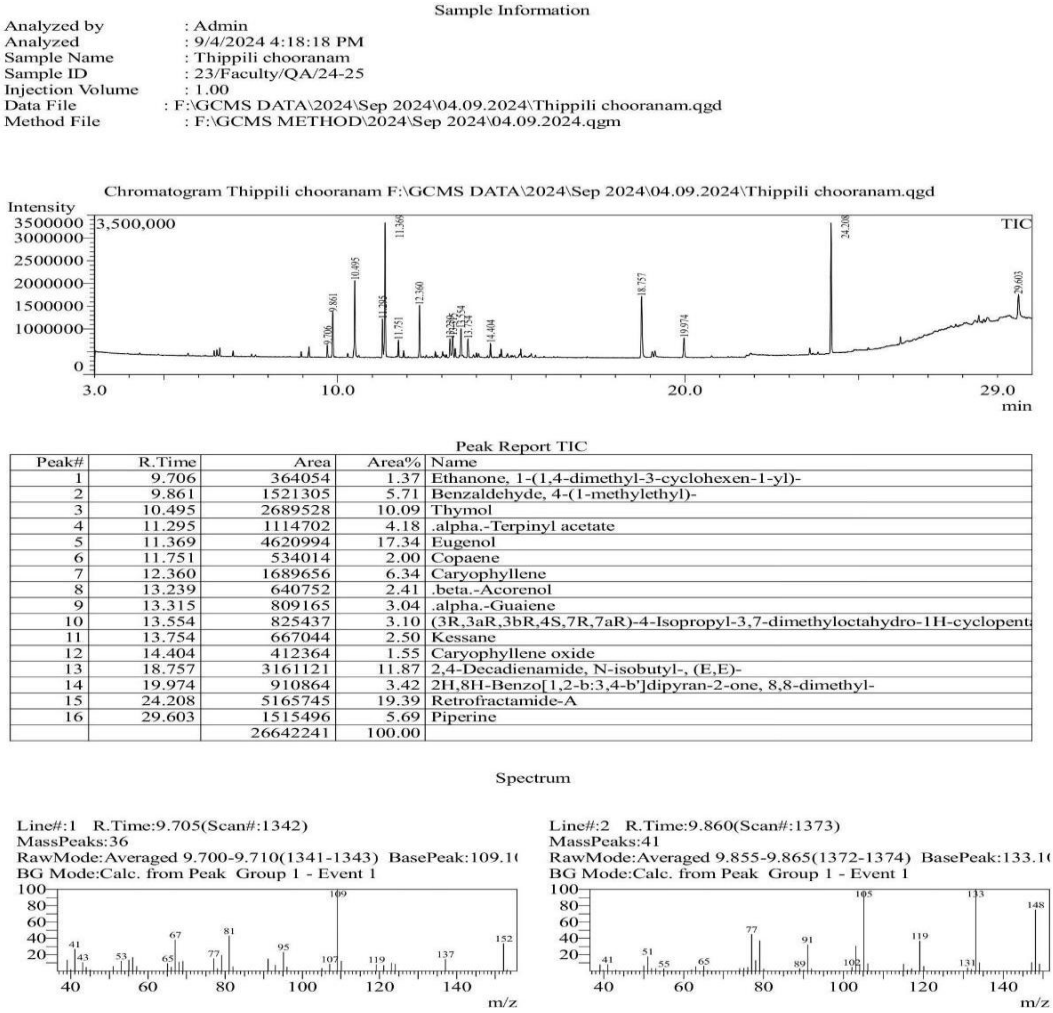
GC–MS is a combined analytical technique that integrates gas chromatography with mass spectrometry. It is useful for the identification and quantification of phytoconstituents in various mixtures. Initially, the sample is liquefied and injected into the inlet of the gas chromatograph. It is immediately vaporised and mixed with an inert carrier gas, such as helium or hydrogen. The vapour then passes through a column containing the stationary phase of the chromatographic system. The individual constituents interact with the stationary phase based on their physicochemical properties, including polarity and boiling point. In mass spectrometry, the separated compounds are ionised using high-energy electrons, resulting in the formation of characteristic fragments. These ionised fragments enter the mass analyser and are separated according to their mass-to-charge ratio. This analysis was carried out at the Research and

Development Wing, Directorate of Indian Medicine and Homoeopathy, Government of Tamil Nadu, Chennai. The reference number was 256/R&D/2024.

This analysis revealed the presence of 16 compounds in the Thippili Chooranam (T.C.) sample.

Table 08:

S. No.	Name of the Compound
1.	Ethanone, 1-(1,4-dimethyl-3-cyclohexen-1-yl)
2.	Benzaldehyde, 4-(methyl ethyl)
3.	Thymol
4.	Alpha-terpinyl acetate
5.	Eugenol
6.	Copaene
7.	Caryophyllene
8.	Beta-acorenol
9.	Alpha-guaiene
10.	(3R,3aR,3bR,4S,7R,7aR)-4-isopropyl-3,7-dimethyloctahydro-1



Peak#	R.Time	Area	Area%	Name
1	9.706	364054	1.37	Ethanone, 1-(1,4-dimethyl-3-cyclohexen-1-yl)-
2	9.861	1521305	5.71	Benzaldehyde, 4-(1-methylethyl)-
3	10.495	2689528	10.09	Thymol
4	11.295	1114702	4.18	.alpha.-Terpinyl acetate
5	11.369	4620994	17.34	Eugenol
6	11.751	534014	2.00	Copaene
7	12.360	1689656	6.34	Caryophyllene
8	13.239	640752	2.41	.beta.-Acorenol
9	13.315	809165	3.04	.alpha.-Guaiene
10	13.554	825437	3.10	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopent
11	13.754	667044	2.50	Kessane
12	14.404	412364	1.55	Caryophyllene oxide
13	18.757	3161121	11.87	2,4-Decadienamide, N-isobutyl-, (E,E)-
14	19.974	910864	3.42	2H,8H-Benzo[1,2-b:3,4-b']dipyran-2-one, 8,8-dimethyl-
15	24.208	5165745	19.39	Retrofractamide-A
16	29.603	1515496	5.69	Piperine
		26642241	100.00	

Figure 01:

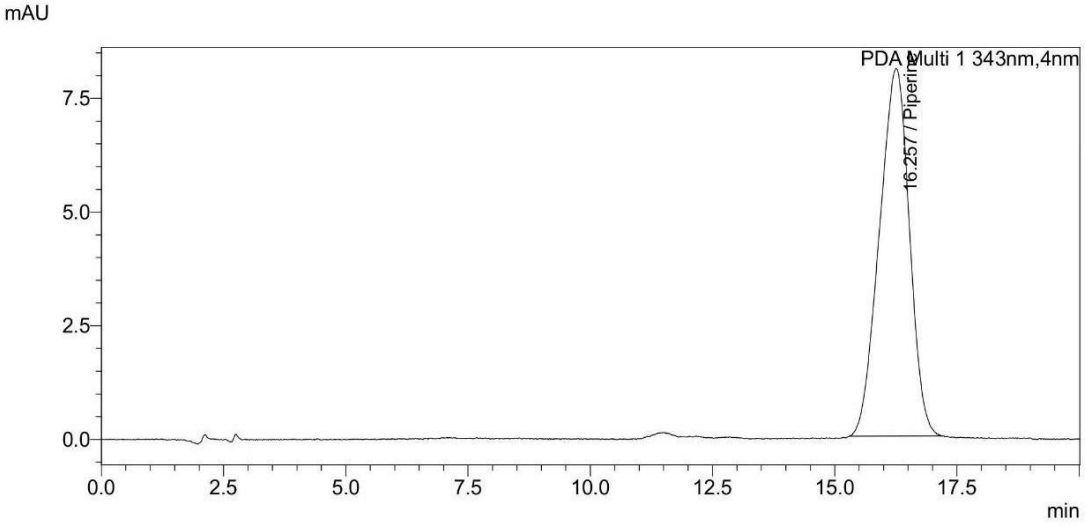
Piperine Quantification of Thippili Chooranam by High performance Liquid Chromatography (HPLC)

High-performance liquid chromatography is previously known as high-pressure liquid chromatography. It is used to separate, identify, and also quantify the active ingredients in a pharmaceutical sample. It is a pressurised form of column chromatography. Multiple solvents are injected through high pressure, which take up the different molecules of the sample and deliver them into a cylinder (column). The column is filled with an absorbent substance like silica. Each component in the sample reacts differently with the stationary phase. When the components are separated, they are shown by peaks in the analyser. This test was done at Drug Testing Laboratory (Indian Medicine), Directorate of Indian Medicine and homeopathy, Government of Tamil Nadu, Chennai 106. The sample Thippili Chooranam (T.C) showed a remarkable quantity of piperine.

<Sample Information>

Sample Name	: Piperine Std		
Sample ID	:		
Data Filename	: Piperine std.lcd		
Method Filename	: Piperine.lcm		
Batch Filename	: 08.07.2025.lcb		
Vial #	: 1-2	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 7/8/2025 1:08:47 PM	Acquired by	: System Administrator
Date Processed	: 7/8/2025 2:31:01 PM	Processed by	: System Administrator

<Chromatogram>



<Peak Table>

PDA Ch1 343nm

Peak#	Ret. Time	Name	Area	Area%
1	16.257	Piperine	345727	100.000
Total			345727	100.000

Figure 02:

Table 09:

Thippili Chooranam	Piperine 0.3457%	Specification Value 0.29 to 0.38%

RESULTS AND DISCUSSION

Thippili Chooranam, a polyherbal formulation used in the Siddha system of medicine for the treatment of respiratory ailments, was prepared according to the reference text Kannusamy Pillai Parambarai Vaithiyam. The prepared formulation was sent to the Drug Testing Laboratory for qualitative analysis of various parameters specified in the PLIM guidelines.

Initially, the sample was evaluated for its organoleptic characteristics. The odour and taste of the sample were found to be pungent and acrid, respectively. It was a fine powder with a brown colour.

The sample was subsequently screened for various physicochemical parameters. The total ash value of the sample (6.05%) indicates the presence of inorganic constituents within the formulation. The loss on drying value of 11.10% indicates the moisture content of the sample. No foreign matter was detected in the sample, indicating that the formulation was prepared with proper care and adherence to the prescribed procedures. The acid-insoluble ash value of 0.06% indicates a low level of acid-insoluble inorganic matter, suggesting minimal silica contamination.

The water-soluble extractive and alcohol-soluble extractive values indicate the presence of extractable phytoconstituents in the formulation. These extractives may include various phytochemical constituents such as phenols, alkaloids, flavonoids, and terpenoids. These parameters also provide useful information regarding the quality and purity of the formulation.

The microbiological assay revealed that the microbial counts were within the prescribed limits. No common pathogenic microorganisms were detected in the sample. The sample was also analysed for heavy metal contamination, and the results indicated that lead, mercury, arsenic, and cadmium were below the observable quantity.

Thippili Chooranam was further analysed for the identification of phytoconstituents. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, steroids, tannins, and carbohydrates, while other tested constituents were absent.

The gas chromatography–mass spectrometry (GC–MS) analysis revealed the presence of 16 different compounds in Thippili Chooranam. Among these, piperine, caryophyllene, eugenol, thymol, and copaene are reported to possess various pharmacological activities. Piperine quantification by high-performance liquid chromatography (HPLC) revealed that the formulation contained piperine at a concentration higher than the specified value.

Considering the results of this analytical study, Thippili Chooranam, a Siddha polyherbal formulation, may possess significant potential in the management of respiratory ailments. Moreover, the findings of this study provide preliminary scientific support for its traditional indications as mentioned in the classical Siddha reference text.

CONCLUSION

GC-MS analysis is the gold standard screening process for organic and herbal formulations. It reveals not only the active phyto constituents but also the total phyto principles present in a herbal drug. Atomic Absorption Spectroscopy is useful to detect the contaminations present in a sample, thereby it assures the safety of the drug. Thippili Chooranam had been screened to establish the phyto constituents to substantiate its pharmacological activity ie., anti asthmatic and anti histaminic activity. Piperine quantification reveals that the chooranam contains piperine, a major phyto principle of the drug. The results of the modern instrumentation techniques assert the pharmacological activities of the drug and also it validates the effficacy of this chooranam in the treatment of respiratory ailments.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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