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# Phytochemical Profiling of Thontha Sura Kiyaazham Using GC-MS

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**ABSTRACT**
*Thontha Sura Kiyaazham is a traditional Siddha polyherbal formulation used for managing fever related symptoms such as sore throat, cough, and joint pain. Scientific validation of its phytochemical components is essential for evidence-based application. To identify and evaluate the phytochemical constituents of Thontha Sura Kiyaazham using Gas Chromatography-Mass Spectrometry (GC-MS). The decoction was prepared from Thontha sura Kiyaazham chooranam as per classical Siddha methods. GC-MS analysis was performed using an Agilent G3440A 7890A GC system coupled with a 7000 Triple Quadrupole MS. Compounds were identified by comparing spectral data with NIST and WILEY libraries. A total of 80 phytochemicals were identified. Major constituents included Mequinol (11.32%), trans-Cinnamic acid (11.08%), cis-6-Octadecenoic acid (11.23%), Pentadecanoic acid (5.59%), Linoelaidic acid (4.83%), and β-Sitosterol (3.62%). These belong to categories such as phenolics, fatty acids, diterpenoids, and phytosterols. Many identified compounds possess known anti-inflammatory, antioxidant, and antimicrobial activities. However, the presence of potential contaminants like phthalates and lidocaine was also noted but in a minimal quantity. The GC-MS profile supports the traditional claims of Thontha Sura Kiyaazham for the management of fever. Further studies are needed to assess compound synergy, safety, and clinical efficacy.*
***KEY WORDS:*** *GC-MS analysis, Kiyaazham Chooranam, Siddha Medicine, Thontha Sura Kiyaazham*

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## INTRODUCTION
In Siddha medicine, therapeutic preparations are broadly classified into internal and external medicines. Among the internal formulations, Kiyaazha Chooranam represents a powdered form used to prepare decoctions known as Kiyaazham in classical Siddha texts, this decoction is made with precise concentration to ensure efficacy [1]. Thontha Sura Kiyaazham is one such classical polyherbal formulation described in the Siddha system of medicine, traditionally used for the management of all types of fever, particularly ***thontha suram***—a condition characterized by combination of many symptoms like body pain, respiratory symptoms and eye irritation [2]. This formulation is composed of a combination of herbs known for their anti-inflammatory, antipyretic, antimicrobial, and immunomodulatory properties. The synergistic action of these ingredients is believed to alleviate upper respiratory tract infections, support mucosal immunity, and enhance respiratory health. In recent years, to evaluate the therapeutic potential of a drug, it is essential to analyze its phytochemical constituents, as this helps determine its efficacy. Scientific evaluation of its phytochemical profile using advanced analytical techniques such as GC-MS helps in identifying its bioactive constituents, providing a better understanding of its mechanism of action and supporting its integration into evidence-based complementary therapies [3].
Gas Chromatography-Mass Spectrometry (GC-MS) is an advanced analytical technique that synergistically combines the separation efficiency of gas chromatography (GC) with the precise detection and identification capabilities of mass spectrometry (MS). This integrated approach facilitates both qualitative and quantitative analyses of volatile and semi-volatile compounds within complex mixtures, offering exceptional sensitivity and specificity. In this methodology, GC serves to separate the individual components of a sample based on their volatility and interaction with the stationary phase of the chromatographic column. Following separation, MS detects and identifies these components by ionizing

the molecules and analyzing their mass-to-charge ratios. This dual-process enhances the accuracy of compound identification, as it is improbable for different molecules to exhibit identical behavior in both GC and MS analyses [4]. This study aims to assess the GC-MS profile of ***Thondha Sura Kiyaazham***, a classical Siddha formulation, to identify its bioactive constituents and explore their potential therapeutic relevance.

**MATERIAL AND METHODS**

**INGREDIENTS AND PREPARATION:**

The ingredients are listed in Table – 1 [2]

**TABLE – 1 DRUG PROFILE OF THONTHA SURA KIYAAZHAM**

| S.NO | INGREDIENT | QUANTITY |
|---|---|---|
| 1. | *Andrographis paniculata* (Burm.f.) Nees | 6g |
| 2. | *Mollugo cerviana* Linn | 6g |
| 3. | *Cyperus rotundus* Linn | 6g |
| 4. | *Tinospora cordifolia* (Willd.) Hook. f. & Thoms | 6g |
| 5. | *Picrorhiza kurroa* Royle ex Benth | 6g |

The aforementioned ingredients were undergone purification to eliminate any contaminants and adulterants. Subsequently, the purified ingredients were coarsely powdered and placed in a suitable vessel. A total of 650 mL of water was added, and the mixture was boiled until the volume is reduced to approximately 160 mL.

**AUTHENTICATION AND IDENTIFICATION:**

The raw drugs were procured from local reputed raw drug store on Nov, 2023.The drugs were authenticated by the Department of Botany (Maruthuva Thavaraiyal), National Institute of Siddha, Tambaram Sanatorium. The Authentication ID is NISMB6242023. Further, the GC-MS analysis was made in Sastra Deemed University, Thanjavur with the project ID Thonthasura kasayam 020525.

**METHODOLOGY:**

Gas Chromatography-Mass Spectrometry (GC–MS) analysis was carried out using an Agilent system (GC model G3440A 7890A and MS model 7000 Triple Quadrupole) equipped with a mass spectrometric detector. For sample preparation, 100 µl of the sample was dissolved in 1 ml of a suitable solvent and vigorously stirred for 10 seconds to obtain a clear extract, which was then subjected to GC–MS analysis. The system utilized a DB-5 MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness; 5% phenyl, 95% methylpolysiloxane) with helium (99.999%) as the carrier gas at a constant flow rate of 1 ml/min. The injector and ion source temperatures were maintained at 280°C, and the auxiliary temperature was set at 290°C. The electron impact ionization was performed at 70 eV. The oven temperature program began at 50°C (held for 1 min), ramped up at 40°C/min to 170°C (held for 4 min), followed by an increase of 10°C/min to 310°C (held for 10 min). Mass fragments were detected in the range of 45 to 450 Da, with a total runtime of 32.02 minutes. Compound identification was performed using the NIST and WILEY spectral libraries" [5].

**RESULTS**

**FIGURE – 1 GC-MS ANALYSIS OF THONTHA SURA KIYAAZHAM**

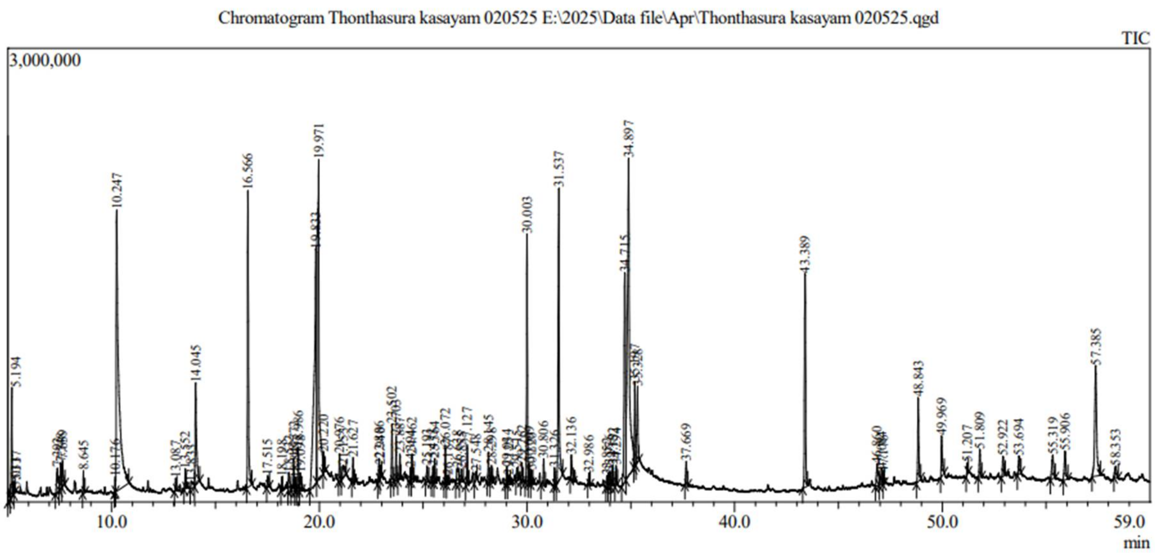


 

**TABLE – 2 GC-MS ANALYSIS OF THONTHA SURA KIYAAZHAM**

| Peak# | R.Time | Area | Area% | Similarity | Name |
|---|---|---|---|---|---|
| 1 | 5.013 | 375582 | 0.28 | 73 | 4-Aminocyclohexanone, N-acetyl- |
| 2 | 5.194 | 1848450 | 1.39 | 97 | o-Xylene |
| 3 | 5.317 | 209679 | 0.16 | 73 | Ethyl glycolate, TMS |
| 4 | 7.392 | 841786 | 0.63 | 85 | Cyclotetrasiloxane, octamethyl- |
| 5 | 7.548 | 913131 | 0.69 | 83 | Sulfurous acid, bis(2-methylpropyl) ester |
| 6 | 7.639 | 613143 | 0.46 | 89 | Benzene, ethoxy- |
| 7 | 8.645 | 326172 | 0.25 | 93 | 2-Propyl-1-pentanol |
| 8 | 10.176 | 204153 | 0.15 | 95 | Benzaldehyde, 2-methyl- |
| 9 | 10.247 | 15053185 | 11.32 | 96 | Mequinol |
| 10 | 13.087 | 213198 | 0.16 | 83 | "4a,8a-(Methaniminomethano)naphthalene-9,11-dione, 10-phenyl-" |
| 11 | 13.552 | 381716 | 0.29 | 94 | Tridecane |
| 12 | 13.833 | 323457 | 0.24 | 84 | "Hexahydrofuro[3,2-b]furan-3,6-diol" |
| 13 | 14.045 | 3051058 | 2.29 | 97 | 4-Vinylphenol |
| 14 | 16.566 | 6254946 | 4.70 | 91 | Phenol, 5-ethenyl-2-methoxy- |
| 15 | 17.515 | 353244 | 0.27 | 87 | 2,3-Dimethoxyphenol |
| 16 | 18.198 | 218678 | 0.16 | 90 | "Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester" |
| 17 | 18.532 | 586769 | 0.44 | 71 | 2,2'-Biphenylene dicinnamate |
| 18 | 18.772 | 684940 | 0.52 | 95 | 1-Tridecene |
| 19 | 18.847 | 285287 | 0.21 | 89 | "2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-" |
| 20 | 18.986 | 734883 | 0.55 | 96 | Pentadecane |
| 21 | 19.098 | 430668 | 0.32 | 86 | '1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-" |
| 22 | 19.833 | 11796389 | 8.87 | 86 | 1H-1,3-Benzodiazole-5-carbaldehyde |
| 23 | 19.971 | 14733800 | 11.08 | 94 | trans-Cinnamic acid |
| 24 | 20.220 | 442646 | 0.33 | 88 | Cyclohexane, octyl- |
| 25 | 20.976 | 517493 | 0.39 | 93 | Apocynin |
| 26 | 21.153 | 659972 | 0.50 | 75 | "Bicyclo[5.3.0]dec-1(7)-ene-2,5-dione" |
| 27 | 21.627 | 412508 | 0.31 | 96 | 2,4-Di-tert-butylphenol |
| 28 | 22.886 | 514113 | 0.39 | 72 | 3',5'-Dimethoxyacetophenone |
| 29 | 22.940 | 267695 | 0.20 | 88 | Dodecanoic acid |
| 30 | 23.502 | 1073294 | 0.81 | 93 | Diethyl Phthalate |
| 31 | 23.703 | 717100 | 0.54 | 95 | 9-Eicosene, (E)- |
| 32 | 23.887 | 377562 | 0.28 | 95 | Hexadecane |
| 33 | 24.394 | 246221 | 0.19 | 80 | 9H-Cycloisolongifolene, 8-oxo- |
| 34 | 24.462 | 606501 | 0.46 | 87 | n-Propyl cinnamate |
| 35 | 25.193 | 334711 | 0.25 | 83 | "Succinic acid, tridec-2-yn-1-yl trans-hex-3-en-1-yl ester" |
| 36 | 25.473 | 357006 | 0.27 | 87 | 8-Pentadecanone |
| 37 | 25.564 | 473533 | 0.36 | 89 | "8-Isopropyl-1,5-dimethyltricyclo[4.4.0.02,7]dec-4-en-3-one" |
| 38 | 26.072 | 649922 | 0.49 | 95 | (3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2H-3a,7-methanoazulen-2- |
| 39 | 26.187 | 220263 | 0.17 | 76 | "7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one" |
| 40 | 26.638 | 596205 | 0.45 | 78 | "cis-Z-.alpha.-Bisabolene epoxide" |
| 41 | 26.855 | 314540 | 0.24 | 77 | "(-)-Isolongifolol, methyl ether" |
| 42 | 27.127 | 747844 | 0.56 | 93 | "7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one" |
| 43 | 27.548 | 242160 | 0.18 | 79 | 1-Heptatriacotanol |
| 44 | 28.145 | 510162 | 0.38 | 95 | 1-Octadecene |
| 45 | 28.298 | 353677 | 0.27 | 93 | Hexadecane |
| 46 | 29.034 | 299148 | 0.22 | 88 | Neophytadiene |
| 47 | 29.125 | 250957 | 0.19 | 74 | 2-Pentadecanone, 6,10,14-trimethyl- |
| 48 | 29.515 | 360888 | 0.27 | 79 | 1-Ethynylcyclododecanol |
| 49 | 29.762 | 328380 | 0.25 | 83 | 8-Octadecanone |
| 50 | 30.003 | 4560142 | 3.43 | 97 | Lidocaine |
| 51 | 30.099 | 465345 | 0.35 | 89 | Corymbolone |
| 52 | 30.220 | 265207 | 0.20 | 75 | Isoaromadendrene epoxide |

| | | | | | |
|---|---|---|---|---|---|
| 53 | 30.806 | 544528 | 0.41 | 92 | Hexadecanoic acid, methyl ester |
| 54 | 31.326 | 302463 | 0.23 | 93 | Dibutyl phthalate |
| 55 | 31.537 | 7438522 | 5.59 | 92 | Pentadecanoic acid |
| 56 | 32.136 | 571785 | 0.43 | 95 | Hexadecanoic acid, ethyl ester |
| 57 | 32.986 | 208026 | 0.16 | 85 | "4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester" |
| 58 | 33.852 | 226540 | 0.17 | 93 | n-Nonadecanol-1 |
| 59 | 33.976 | 300381 | 0.23 | 94 | 9,12-Octadecadienoic acid, methyl ester |
| 60 | 34.132 | 474663 | 0.36 | 93 | 6-Octadecenoic acid, methyl ester, (Z)- |
| 61 | 34.294 | 368131 | 0.28 | 87 | Oxirane, decyl- |
| 62 | 34.715 | 6429213 | 4.83 | 95 | Linoelaidic acid |
| 63 | 34.897 | 14938871 | 11.23 | 94 | 6-Octadecenoic acid, (Z)- |
| 64 | 35.197 | 1775978 | 1.34 | 95 | trans,trans-9,12-Octadecadienoic acid, propyl ester |
| 65 | 35.328 | 1974506 | 1.48 | 91 | (E)-9-Octadecenoic acid ethyl ester |
| 66 | 37.669 | 559887 | 0.42 | 65 | "(2R,3S,4R,4aR,7R,8S)-8-[2-(Furan-3-yl)ethyl]-4a,7,8-trimethylspiro[2,3,5,6,7,8a-he" |
| 67 | 43.389 | 5274205 | 3.97 | 91 | Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate |
| 68 | 46.860 | 852255 | 0.64 | 88 | '9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-" |
| 69 | 47.084 | 584250 | 0.44 | 86 | Flavone, 5-hydroxy-7,8-dimethoxy- |
| 70 | 47.176 | 219025 | 0.16 | 92 | Dotriacontane, 1-iodo- |
| 71 | 48.843 | 1981437 | 1.49 | 93 | Andrograpanin |
| 72 | 49.969 | 1038162 | 0.78 | 95 | Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z,Z)- |
| 73 | 51.207 | 250264 | 0.19 | 63 | 14-Deoxyandrographolide |
| 74 | 51.809 | 698972 | 0.53 | 59 | Pregn-5-en-20-one, 3-(acetyloxy)-16-bromo-, (3.beta.,16.alpha.)- |
| 75 | 52.922 | 602211 | 0.45 | 92 | 14-Deoxyandrographolide |
| 76 | 53.694 | 570273 | 0.43 | 87 | 14-Epiandrographolide |
| 77 | 55.319 | 924446 | 0.70 | 83 | Ergost-5-en-3-ol, (3.beta.)- |
| 78 | 55.906 | 1046072 | 0.79 | 88 | Stigmasterol |
| 79 | 57.385 | 4813601 | 3.62 | 85 | .gamma.-Sitosterol |
| 80 | 58.353 | 428074 | 0.32 | 71 | D:A-Friedoolean-6-ene |
| | | **132996250** | **100.00** | | |

**TABLE – 3 ANTI-INFLAMMATORY AND ANTIPYRETIC PROFILE OF PHYTOCHEMICALS**

| S.NO | COMPOUND | BIOACTIVITY |
|---|---|---|
| 1. | Mequinol (4-methoxyphenol) | Antioxidant, anti-inflammatory [6] |
| 2. | trans-Cinnamic acid | Anti-inflammatory, antimicrobial, analgesic [7] |
| 3. | Apocynin | NADPH oxidase inhibitor; anti-inflammatory [8] |
| 4. | 2,4-Di-tert-butylphenol | Antioxidant and anti-inflammatory activity [9] |
| 5. | Lidocaine | Local anaesthetic with anti-inflammatory effect in topical use [10] |
| 6. | Flavone, 5-hydroxy-7,8-dimethoxy- | Flavonoids often exhibit strong anti-inflammatory action [11] |
| 7. | Stigmasterol | Potent anti-inflammatory, anti-arthritic, antipyretic, and analgesic [12] |
| 8. | γ-Sitosterol | Strong anti-inflammatory, antipyretic, and immunomodulatory [13] |
| 9. | 4-Vinylphenol | Phenolic derivative; shows COX inhibition and anti-inflammatory effects [14] |
| 10. | Hexadecanoic acid, methyl/ethyl esters | Shown to inhibit lipoxygenase and COX pathways [15] |
| 11. | Linoelaidic acid | Unsaturated fatty acid; exhibits anti-inflammatory activity [16] |
| 12. | Corymbolone | Anti-plasmodial [17] |
| 13. | Andrograpanin | Major bioactive from *Andrographis paniculata* with potent anti-inflammatory, antipyretic action [18] |
| 14. | 14-Deoxyandrographolide | Strong anti-inflammatory & immune-modulatory activity [18] |
| 15. | 14-Epiandrographolide | reduces fever, cytokine storm [18] |

**DISCUSSION**

The GC–MS analysis of Thontha Sura Kiyaazham decoction revealed a diverse array of volatile constituents (Figure 1). Identification of the metabolites was performed by comparing the retention times and mass spectral fragmentation patterns with those available in the NIST spectral library integrated into the Shimadzu GC-MS software (version 1.10 beta) [19]. The results revealed the presence of 80 different molecules, providing additional evidence to support the longstanding use of this medicine for its therapeutic benefits. Major peaks include simple phenolics (e.g. 4-methoxyphenol [Mequinol], 11.32% area; 4- vinylphenol, 2.29%), phenolic acids (e.g. trans-cinnamic acid, 11.08%; benzimidazole-5-carbaldehyde, 8.87%), odd-chain and unsaturated fatty acids (e.g. pentadecanoic acid (C15:0), 5.59%; (Z)-6-octadecenoic acid (cis-6-octadecenoate), 11.23%; linoelaidic acid (trans-9,cis-12-octadecadienoic acid), 4.83%), and steroidal phytochemicals (β-sitosterol, 3.62%; stigmasterol, 0.79%). Minor peaks include an andrographolide diterpenoid series (andrograpanin, deoxyandrographolides, 14-epiandrographolide) from Andrographis paniculata. Also, some detected compounds raise safety concerns. Mequinol and 4-vinylphenol are toxic at certain levels, while linoelaidic acid is a harmful transfat. Non-herbal agents like lidocaine and phthalates suggest possible contamination (Table- 2 and Figure - 1).

Many identified plant-derived compounds have known bioactivity. Trans-cinnamic acid has documented antimicrobial, antioxidant and antiinflammatory properties [20]. Pentadecanoic acid (C15:0), an odd-chain saturated fat, has recently been shown to attenuate inflammation and improve metabolic profiles in animal models [21]. The diterpenoids (andrographolides) are well-known immunomodulatory and anti-inflammatory agents, consistent with the use of Andrographis (Nilavembu) in traditional febrile remedies [22]. The phenolic antioxidant 2,4-ditert-butylphenol (peak 21.627, 0.31%) also has reported antimicrobial and anti-inflammatory effects [23]. The phytosterols (β-sitosterol, stigmasterol) are associated with cardioprotective and antiinflammatory effects [24]. The chemical constituents identified in the GC–MS analysis of Nilavembu Kudineer, a traditional Siddha formulation, align with its documented therapeutic properties. Notably, andrographolide derivatives, characteristic of *Andrographis paniculata* (Nilavembu), are present and support the formulation's antiviral and anti-inflammatory effects [22, 25]. Additionally, compounds such as cinnamic acid and phenolic terpenoids like 2,4-di-tert-butylphenol have been detected. Cinnamic acid, commonly found in plants like Cinnamomum and Ocimum, is known for its antioxidant and anti-inflammatory activities. Similarly, 2,4-di-tert-butylphenol exhibits significant antioxidant and anti-inflammatory properties (Table - 3).

## CONCLUSION

The GC–MS analysis of Thontha sura Kiyaazham highlights the presence of several pharmacologically relevant phytochemicals, including cinnamic acid derivatives, andrographolides, and phytosterols, which are known for their anti-inflammatory, anti -pyretic antioxidant, and immunomodulatory properties. These constituents play a vital role in supporting the traditional therapeutic claims of the formulation. Further research is warranted to explore their synergistic effects and to establish a robust scientific basis for the clinical efficacy of Thontha sura Kiyaazham.


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## CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

## ABBREVIATION:

**GC-MS** – Gas Chromatography Mass Spectroscopy; **g** – Gram; **ml** – Milli litres; **µl** – Micro litres; **µm** – Micro metre; **mm** - Millimeter

## AUTHOR CONTRIBUTION:

Conceptualization: UG; Medicine Preparation: UG; Data collection and compilation: UG; Manuscript Writing: UG ; Proofreading and editing: KS

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