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Research Article

GC-MS ANALYSIS OF *ANDA THAILAM* - A CLASSICAL SIDDHA MEDICATED OIL

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ABSTRACT

Anda Thailam is a traditional Siddha medicated oil mentioned in The Siddha Formulary of India Part I. In practice, it is used for neurological and digestive problems in children and infants, such as *Pakkavatham, Mantham*, and *Sanni*. It is also applied on the tongue for *Naakku vatham*. Even though it has been used for a long time, there hasn't been much scientific study on what exactly is in this oil. The goal of this study was to find out the main chemical constituents present in *Anda Thailam* using gas chromatography–mass spectrometry (GC-MS) analysis. A sample of the oil was first extracted with ethanol. Then it was treated with a derivatization agent (BSTFA+TMCS) to make the compounds easier to detect. The prepared sample was run on an Agilent 6890N GC system connected to a JEOL GC-Mate II high-resolution mass spectrometer. An HP-5 MS capillary column (30m length, 0.25mm inner diameter, 0.25µm film thickness) was used for separation. The total ion chromatogram showed five major peaks at retention times of 11.77, 16.08, 16.78, 17.53, and 18.18 minutes. Library matching (NIST/WILEY) identified these as: pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester (11.77 min), oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans- (16.08 min), pentadecanoic acid, methyl ester (16.78 min) – this was the largest peak, 9-Octadecenoic acid (Z)-, hexyl ester (17.53 min), 10-Octadecenoic acid, methyl ester (18.18 min). Almost all of the identified compounds are fatty acid methyl esters (FAMEs), which fits well with the fact that *Anda Thailam* is a lipid-rich, animal-based oil.

INTRODUCTION

In the traditional system of Siddha medicine there are several types of medicated oils where used.[4] *Anda thailam* is one that differ from many other Siddha medicated oils as because of its preparation method. Unlike conventional Siddha medicated oils prepared through herbal infusions or decoctions, *Anda Thailam* is differed by the incorporation of pyrolyzed egg yolk, which substantially modifies its chemical profile and therapeutic properties. This distinctive characteristic necessitates a thorough investigation, particularly through Gas Chromatography-Mass Spectrometry, to elucidate the specific lipid derivatives and volatile organic compounds generated during the pyrolysis of egg yolk and their potential pharmacological contributions. The present study therefore, aims to comprehensively characterize the molecular profile of *Anda Thailam* using GC-MS, focusing on identifying and quantifying the thermolytic products of egg yolk and their potential roles in the traditional therapeutic applications of this unique formulation. [6]

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Background of *Anda Thailam*

Anda Thailam is a traditional Siddha medicated oil, primarily employed for its purported analgesic and anti-inflammatory properties, often used in conditions like musculoskeletal pain and inflammatory disorders. *Anda thailam* is employed for various conditions like *Pakkavatham, Sanni, Mantham* in children and infant. But it is widely used clinically for *Naakuvatham* by topical application of the oil on the tongue.[1,2,3] However, a comprehensive understanding of its molecular constituents, particularly those derived from the pyrolytic transformation of egg yolk, is crucial for validating its ethnomedicinal claims and discerning its pharmacological basis.

MATERIALS AND METHODS

Collection and Preparation of *Anda Thailam*

Anda Thailam was prepared in accordance with the formulation method described in "The Siddha Formulary of India Part-I". For the present study, fertile chicken eggs were sourced from a local poultry farm and the eggs got authenticated from department of Gunapadam, Government Siddha Medical College, Tirunelveli. To make this oil, take hard-boiled egg yolk and heat it in a dry pan until it breaks down (that's called pyrolysis). First, boil whole eggs until they're fully cooked. Then, peel off the egg white and take out just the yolk. Next, put that yolk in a dry pan or wok and keep heating it over high heat without any oil or water. Keep going until the yolk turns into a dark brown or black charred lump, and you see oil starting to come out of it.[1] Finally, collect that dark oil that oozes from the burnt residue- that's the oil people use for therapeutic purposes.

GC-MS Instrumentation

The gas chromatography-mass spectrometry analysis was done using an Agilent 6890N gas chromatograph connected to a JEOL GC MATE-II HR mass spectrometer. The GC had a photon multiplier tube detector and a front injector (type 1079). For separation, we used an HP-5 MS capillary column – that's a common non-polar column, 30 meters long, with an inner diameter of 0.25mm and a film thickness of 0.25µm. The mass spectrometer worked in electron impact (EI) mode at 70 eV, which is standard for breaking molecules into fragments that give us a fingerprint spectrum. The ion source and the transfer line (the heated tube connecting GC to MS) were both kept at 250°C. The use of GC-MS as a hyphenated technique allows reliable compound identification.[5]

The Gas chromatography mass spectrometer (GC-MS) analysis

The chemical constituents of *Anda Thailam* were studied using Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The sample was allowed to ethanol extraction followed by silylation derivatization with N,O-Bis (trimethylsilyl) trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS), and analysed on an Agilent 6890N GC system coupled to a JEOL GC-Mate II High-Resolution Mass Spectrometer (HR-MS) equipped with an HP-5 MS capillary column (30m×0.25mm i.e., film thickness 0.25µm) [8].

Interpretation of GC-MS Data

The library matches were all above 90%, so we are confident about the identifications.[7] The chromatogram doesn't show many small peaks– meaning the sample is not extremely complex. This fits with a thermally degraded oil where many original components (like proteins and phospholipids) have broken down into a simpler set of fatty acid derivatives.

The absence of large peaks for free cholesterol (which is abundant in egg yolk) is interesting. Possibly cholesterol was converted into other products during pyrolysis or was not efficiently derivatized under our conditions.

Data Acquisition and Analysis

The total ion chromatogram (TIC) was recorded for the whole run. Each peak in the TIC was integrated automatically. To identify the compounds, we compared the mass spectra of each peak with two reference libraries: WILEY and NIST. We also looked at the retention times (RT) and calculated retention indices where possible. A match score above 90% was considered reliable.

RESULTS AND OBSERVATION

Individual mass spectra of all five identified peaks, each showing the experimental spectrum (upper panel) alongside the respective NIST library reference spectrum (lower panel), are presented in Figs. 2–6.

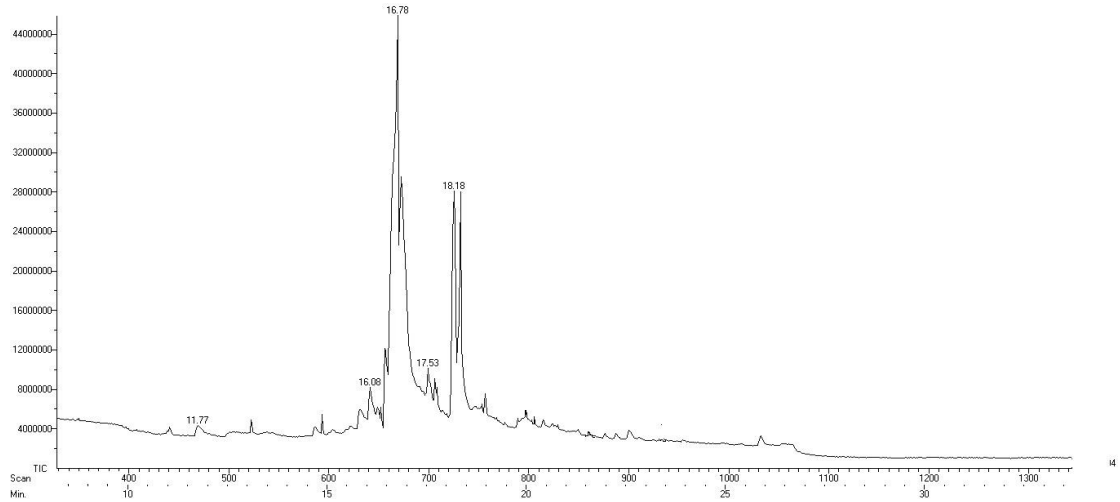

Fig. 1: Total Ion Chromatogram (TIC) of *Anda Thailam* (Ethanol extract, BSTFA+TMCS derivatised; HP-5 MS column, 70–200°C)

Table 1: GC-MS Analysis of *Anda Thailam* – Identified Compounds

S.No.	RT (min)	Compound Identified (NIST Match)	Molecular Formula	Mol. Wt.	TIC
1	11.77	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester	C19H30O3	306	4,336,192
2	16.08	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	C19H36O3	312	8,251,568
3 **	16.78	Pentadecanoic acid, methyl ester	C16H32O2	256	45,895,104
4	17.53	9-Octadecenoic acid (Z)-, hexyl ester	C24H44O2	364	10,158,976
5 **	18.18	10-Octadecenoic acid, methyl ester	C19H36O2	296	28,114,272

** Major peaks (TIC > 20,000,000); RT = Retention Time; TIC = Total Ion Count; Mol. Wt. = Approximate Molecular Weight (Daltons).

The dominant peak was observed at RT 16.78 min, identified as pentadecanoic acid, methyl ester (methyl pentadecanoate; C15:0 FAME), with characteristic fragment ions at m/z 85.80, 123.68, 153.42, 194.59, 212.57, and 255.44 (Fig. 4). The second major peak at RT 18.18 min was identified as 10-Octadecenoic acid, methyl ester (C18:1 FAME), with a putative molecular ion at m/z 294.42 (Fig. 6). A moderate peak at RT 17.53 min was assigned to 9-Octadecenoic acid (Z)-, hexyl ester (hexyl oleate; Fig. 5), and a peak at RT 16.08 min (Scan 641; TIC = 8,251,568) was identified as Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans- (Fig. 3). A minor peak at RT 11.77 min was tentatively identified as Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester (Fig. 2).

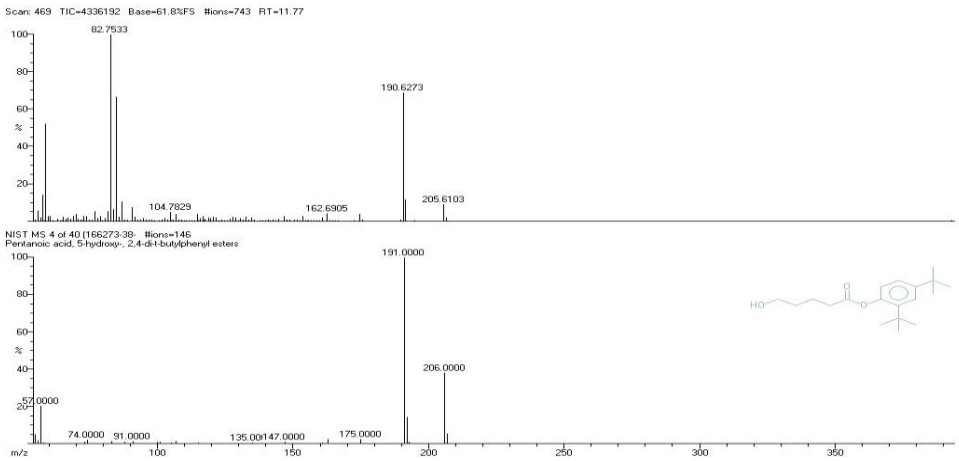

Fig. 2: Mass Spectrum of peak at RT 11.77 min – Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester (upper: experimental; lower: NIST reference)

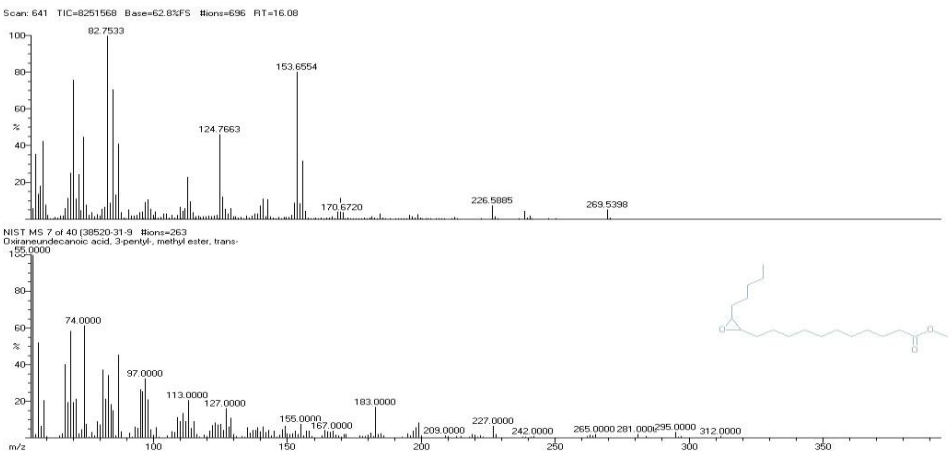

Fig. 3: Mass Spectrum of peak at RT 16.08 min – Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans- (upper: experimental; lower: NIST reference)

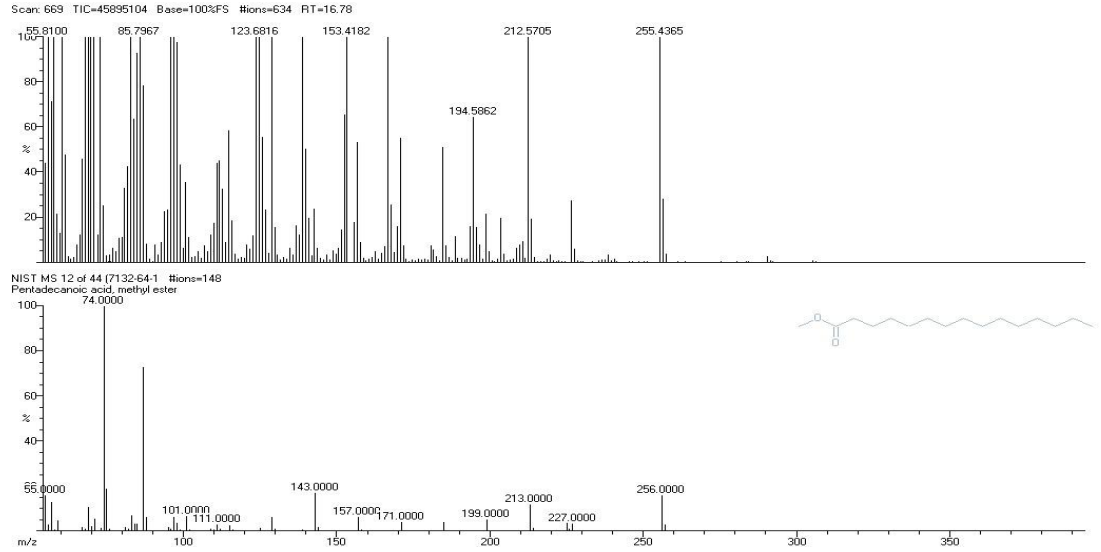

Fig. 4: Mass Spectrum of peak at RT 16.78 min – Pentadecanoic acid, methyl ester [Major Peak] (upper: experimental; lower: NIST reference)

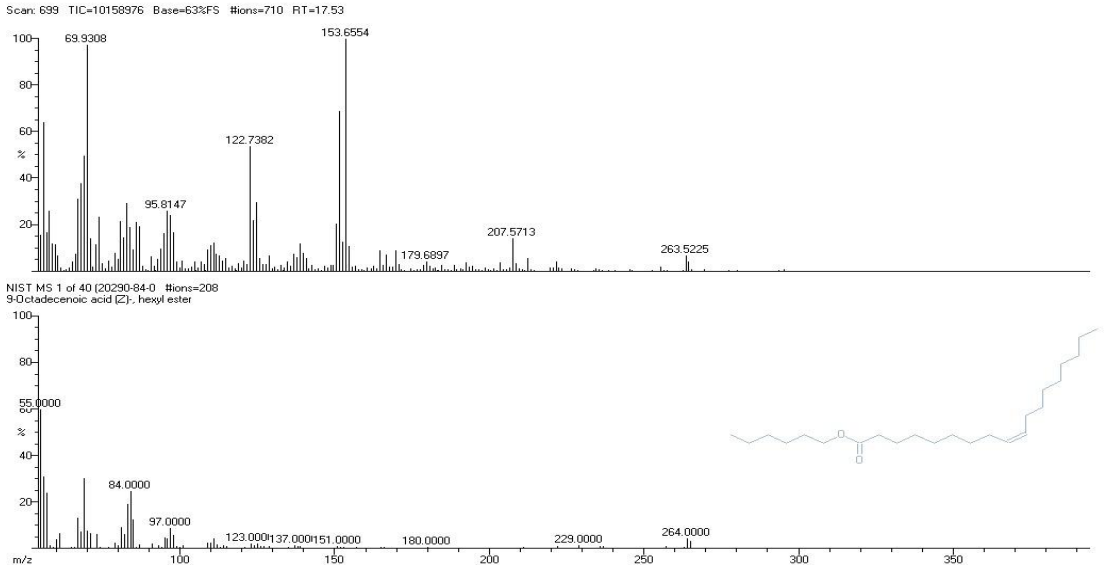

Fig. 5: Mass Spectrum of peak at RT 17.53 min – 9-Octadecenoic acid (Z)-, hexyl ester (upper: experimental; lower: NIST reference)

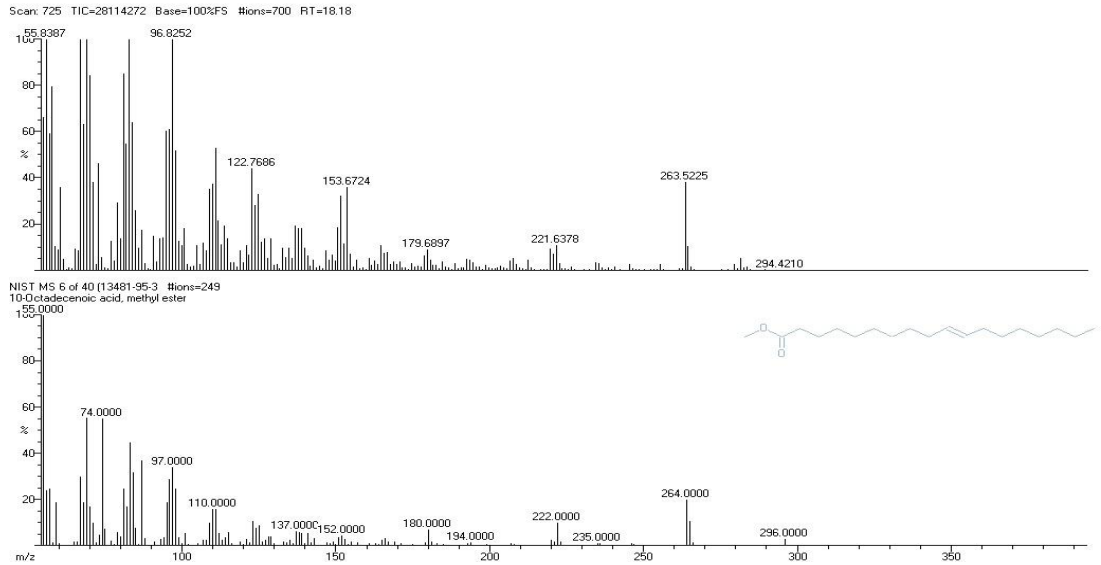

Fig. 6: Mass Spectrum of peak at RT 18.18 min – 10-Octadecenoic acid, methyl ester [Second Major Peak] (upper: experimental; lower: NIST reference)

DISCUSSION

The most abundant compound, Pentadecanoic acid, methyl ester (RT 16.78 min; TIC = 45,895,104), is the methyl ester of pentadecanoic acid – a C15:0 odd-chain saturated fatty acid (OCFA). They are predominantly found in animal-derived lipids such as egg yolk and dairy fats, and are present only in trace amounts in plant oils[9]. The dominance of pentadecanoic acid methyl ester in the GC-MS profile of *Anda Thailam* directly reflects the egg *(Anda)* content of the formulation and may serve as a chemotaxonomic marker for this preparation. Biologically, pentadecanoic acid has been reported to exhibit anti-inflammatory, antidiabetic, and cardioprotective properties, and has demonstrated regulatory effects on inflammatory cytokine networks[10].

The second major compound, 10-Octadecenoic acid, methyl ester (RT 18.18 min; TIC = 28,114,272), is a C18:1 monounsaturated fatty acid methyl ester (MUFA). Monounsaturated fatty acids and their esters are well-established modulators of neuroinflammation, membrane fluidity, and myelin synthesis. They exert neuroprotective effects through maintenance of neuronal membrane phospholipid composition, modulation of NMDA receptor activity, and attenuation of oxidative neuronal damage. Oleic acid isomers, including vaccenic acid (18:1n-7), are reported to support axonal integrity and nerve conduction, directly relevant to the management of *Pakkavatham* and peripheral neuropathies in Siddha medicine [11,12].

Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans- (RT 16.08 min) is an epoxy fatty acid (EFA) derivative. Epoxy fatty acids are potent lipid mediators with documented anti-inflammatory and antinociceptive (analgesic) properties, mediated through inhibition of cyclooxygenase (COX), lipoxygenase (LOX), and soluble epoxide hydrolase (sEH) pathways. EFAs have been shown to attenuate neuroinflammation and reduce neuropathic pain in experimental models[13], providing a compelling mechanistic basis for the analgesic efficacy of *Anda Thailam* in *Vatham*-related neurological pain syndromes.

9-Octadecenoic acid (Z)-, hexyl ester (hexyl oleate; RT 17.53 min), the ester of oleic acid with hexanol, is a documented skin-penetration enhancer and emollient[14]. As *Anda Thailam* is a topically administered on tongue, the presence of hexyl oleate is pharmacologically significant: its likely functions as a permeation promoter that facilitates the absorption of bioactive lipid constituents and act deeper neuromuscular target tissues, thereby potentiating the overall therapeutic efficacy of the formulation when applied topically.

The minor compound at RT 11.77 min, tentatively identified as pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester, is a phenolic ester derivative. The 2,4-di-tert-butylphenol (2,4-DTBP) moiety is a well-characterised lipophilic free radical scavenger and antioxidant, frequently identified in natural product studies.[15] Oxidative stress and reactive oxygen species (ROS) generation are central to neuronal degeneration pathophysiology in conditions such as *Pakkavatham*, and the antioxidant constituents in *Anda Thailam* may thus contribute additional neuroprotective benefits against oxidative damage.

Collectively, the identified constituents of *Anda Thailam* demonstrate a multi-mechanistic pharmacological profile encompassing anti-inflammatory (epoxy fatty acids, MUFAs), neuroprotective (C15:0 OCFA, C18:1 MUFA), analgesic (epoxy fatty acids), antioxidant (phenolic ester), and percutaneous permeation-enhancing (hexyl oleate) activities. This profile provides a coherent molecular basis for the traditional Siddha therapeutic use of this formulation in *Vatham* disorders, particularly *Pakkavatham* and peripheral sensory-motor impairments. These findings also underscore the value of GC-MS-based profiling as an evidence-generating tool for the scientific validation of classical Siddha formulations.

Limitations of the Study

1. No absolute quantification– We only have relative peak areas. We don't know the exact amount (mg/g) of each compound.
2. Derivatisation bias– BSTFA + TMCS works well for fatty acids but may not capture all non-polar compounds like hydrocarbons.
3. Single batch analysis– We analysed only one preparation of *Anda Thailam*. Batch-to-batch variation in traditional pyrolysis can be high.
4. No bioassay– We identified compounds, but we didn't test them for actual therapeutic activity.

CONCLUSION

The GC-MS analysis of *Anda Thailam*, a classical Siddha medicated oil indicated for neurological *Vatham* disorders, revealed five major chemical constituents: Pentadecanoic acid, methyl ester (dominant; TIC=45,895,104); 10-Octadecenoic acid, methyl ester (TIC=28,114,272); 9-Octadecenoic acid (Z)-, hexyl ester; Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-; and pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester. The phytochemical profile is dominated by fatty acid methyl esters with well-documented anti-inflammatory, neuroprotective, analgesic, antioxidant, and skin-penetration enhancing properties, providing a comprehensive scientific basis for the traditional therapeutic claims of this

formulation in the management of *Pakkavatham* (paralysis).

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