



Identification of Phytocomponents from Novel Siddha formulation *Oma Theeneer* by GCMS Analysis

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Abstract

Background: *Theeneer* or medicinal herbal distillates are simple and effective formulations used in *Siddha* medicine which are promising both as a health promoter and as a medicine for wide range of diseased conditions. *Oma Theeneer* is a very common and popular drug of choice in *Siddha* medicine used in respiratory and gastro intestinal ailments. **Objectives:** Present study aimed at evaluating the biological active compounds of *Oma Theeneer* through Gas Chromatography Mass Spectrometry (GC-MS) and to elaborate its therapeutic efficiency. **Methods:** The Distillate *Oma Theeneer* is prepared in accordance with *Siddha* classical references as per the standard operating procedures evident in the literature and the sample is screened through GC-MS analysis. **Results:** The distillate of *Oma Theeneer* found colorless, with peculiar thymolic odor and pungent taste this complies the good traditional quality parameters. GC-MS analysis reported the presence of 7 compounds of volatile and organic nature, predominant active principle being Thymol then Phenol, 2-methyl-5-(1-methylethyl) and Cyclopropanebutanoic acid. Other 4 includes the derivatives of Linoleic acid such as n-Hexadecanoic acid, 10-Octadecenoic acid, 9, 12-Octadecadienoic acid, and 7, 10-Octadecadienoic acid. The phenolic compounds and organic acids thus screened from *Oma Theeneer* may have a leading role in establishing its indicated effectiveness.

Keywords: *Siddha* medicine, *Theeneer*, *Oma Theeneer*, Traditional quality parameters, GCMS, Phenolic compounds.

1. Introduction

Theeneer is a kind of novel *siddha* formulation actually derived from the herbal distillates [1], the herbal distillates is considered as the first drug of choice in *VinnavarUrai* [2](Twelve supreme formulations of *Siddha* medicine) which has numerous advantages over other medicines in terms of palatability, efficacy or potency. The Principle and preparation of *Theeneer* involves the efficient distillation of water soaked coarse powders of the herbal raw drug parts. The Volatile principles which are evolved admixture with water vapor from the still are condensed and taken [3,4]. Specific herbal parts yield high quality distillates.

Omam (*Carum copticum*) or ajwain belonging to Umbelliferae family is highly regarded in Traditional *Siddha* medicine[5]. It is an annual erect herb up to 90cm tall, which is cultivated almost throughout India. The plant is uprooted and threshed for collecting the fruits. Its usage in various forms has been under practice for thousands of years.

The fruit is a cremocarp consisting of two portions each called mericarp and connected by central stalk (carpophore), A single seed is seen in each mericarp. Fruit surface is, greyish brown, ovoid, compressed about 2 mm long and 1 mm wide with pale colored protuberances and 5 primary ridges pale in color, each of the 2 mericarps show 5 ridges [6].

Table. 1 General Description of *Omam*[7-8]

Table. 1 General Description of <i>Omam</i> [7-8]	
Odor	Thymolic
Taste	Pungent
Potency	Hot
Division	Pungent
Chemical Constituents	Volatile essential oil (5-6 %), cumene, terpene, thymene. The seeds of <i>Carum copticum</i> contain the antiseptic thymol and they yield 2-3% of an essential oil (ajowan oil) which contains no less than 40-50% of thymol, Carvacrol, alpha and beta pinene, camphene.
Major Alkaloids	
Minor Alkaloids	Carvone, limonene, dillapiol, quercetin, kaemferol, fatty acids (oleic and Linoleic acid)
Pharmacological Actions	Stimulant, Stomachic, Carminative, Sialogogue, Tonic, Anti septic, Anti spasmodic, Anti Diarrhoeal, Anti Oxidant, Anti Peptic Ulcer.
Indications in <i>Siddha</i> Medicine	<i>Kapham</i> (Phlegmatic diseases), <i>Kaphasuram</i> (Phlegmatic fever), <i>Kazhichil</i> (Diarrhoeal diseases), <i>Oozhinoi</i> (cholera), <i>Seriyamantham</i> (Dyspepsia's), <i>Vayu</i> (Gaseous disturbances), <i>Iraippu</i> (Bronchial asthma), <i>kasam</i> (cough), <i>peruvayiru</i> (Ascitis).

Oma Theeneer

Distillates of ajwain seeds commonly called by its name *Oma Theeneer* [9-10] is a very common and popular drug of choice in *Siddha* medicine which is used as a regular appetizer and indicated extensively in conditions like *Ajeernam* (Dyspepsia's), *Seriyakazhichil* (Diarrhea due to indigestion), *Vatha* and *Kapha Grahani* (Dysenteries and other diarrheal diseases), *Oozhinoi* (Cholera), *Vayitru Soolai* (colic and intestinal spasms), *Vayitrumantham*, *Vayu Kolaru* (Gaseous disturbances & flatulence), *Pitham* (Biliousness). It is also used in traditional *Siddha* practice for respiratory ailments especially *Eraippunoi*

(Bronchial asthma), *Vanthi*(emesis), *Suram* & *Veppanoikal* (febrile conditions), *Neerkaduppu* (Urinary stranguary), *peenisam*(Sinus affections including sinusitis) etc.

In the present investigation, *OmaTheeneer* will be prepared in *Valaiyanthram*[11] (Traditional still) which consist of 2 components, lower still for charging the materials along with water and upper still the condenser part for cooling the apparatus. Provisions are made in the upper still for water inlet and outlet to maintain regular water current and a distill outlet for collecting the condensed distillates.

Both the still parts will be connected and sealed properly with traditional binding agents like clay soil, wheat flour or lime smeared cloth roll. After drying of the seal the entire apparatus is vertically installed and by using conventional fuels, the distillation is initiated. The distillates which are collected in the collecting vessel are properly stored in stoppered glass bottles kept away from sunlight or moisture[12].

Standardization of *Siddha* distillate formulations is an important step to assess the quality also the efficacy of the drug for its scientific justification. GC-MS is a mandatory tool as per the *Ayush* analytical specifications of *Theeneer* [13]. GC-MS traces out both volatile and organic active biological compounds within the distillates including its peak percentage. The study fills up the gap in understanding the biological compounds of *Oma Theeneer* and its Pharmacological role associated with the indications.

2. Materials and Methods

For the GC-MS studies *Oma Theeneer*, a single herbal distillate mentioned in the *Siddha* classical text *Siddha Vaidhya Thirattu* has been selected.



Fig A



Fig B



Fig C

D. Gas Chromatography- Mass Spectroscopy Analysis (GC-MS)

GC-MS Plays a key role in the analysis of phyto components of herbal origin. GC-MS ionizes compound and measures their mass numbers. Ionization method includes EI (Electron Ionization). The EI method produces ions by colliding thermal electrons emitted from a filament with sample gas molecules. This method provides high stability in

A. Ingredient Details

Omam (Carum copticum)

B. Raw Drug Collection and Authentication

The raw drugs were purchased from a reputed raw drug store in *Chennai*. After it has been identified and authenticated from the Department of Botany, National Institute of *Siddha* (NISMB2472016)

C. Method of Preparation of Distillate Sample

The raw drug (250 g) were cleaned and sorted, coarsely powdered, taken in a porcelain jar (Fig B) and soaked in suitable proportion of pure water (4 liters) for a period of 2 days. On the 3rd day the whole mixture is charged in a *Valaiyanthram* (traditional still) and distilled (Fig c). The distillates were collected and preserved well for GC-MS studies.

ionization and obtained mass spectra show good reproducibility. The EI method provides good result for quantitative analysis as well. Quantitative analysis with GC-MS, in which only ions specific to the compounds are measured, is highly selective method without interfering components. Gas chromatography Technique involves the separation of volatile components in a test sample using suitable capillary column coated with polar or non-polar or intermediate polar chemicals.

Elite-1 column (100% Dimethyl polysiloxane) is a non-polar column used for analysis of phyto-components. Elite -5 column (5% phenyl and 95% methyl polysiloxane) is an intermediate column and also used for the estimation of Phytochemical. An inert gas such as hydrogen or nitrogen or helium is used as a carrier gas. The compounds of test sample is evaporated in the injection port of the GC equipment and segregated in the column by absorption and adsorption technique with suitable GC programme [14-15].

3. Results

Initial phases of distillation are marked by the presence of thymol colored fumes then with slow collection of distillates. The distillates were clear and with peculiar thymol aroma and pungent taste. A

tinge of yellow colored volatile oil in the supernatant layer. The quality of the *Oma Theeneer* greatly satisfies the Traditional parameters of a good grade Distillate. The volatile tinge was highest at the initial phases of Traditional distillation processes.

3.1. GC-MS Result Analysis of *Oma Theeneer*

GCMS analysis of *Oma Theeneer* reveals the presence of total 7 compounds of volatile and organic nature. Predominant active principle being Thymol, Phenol, 2-methyl-5-(1-methylethyl) and Cyclopropanebutanoic acid. Other 4 includes the derivatives of Linoleic acid such as n-Hexadecanoic acid, 10-Octadecenoic acid, 9, 12-Octadecadienoic acid, and 7, 10-Octadecadienoic acid. As shown in Fig 3 and listed Table 2.

Fig. 1 GC-MS Chromatogram of *Oma Theeneer*

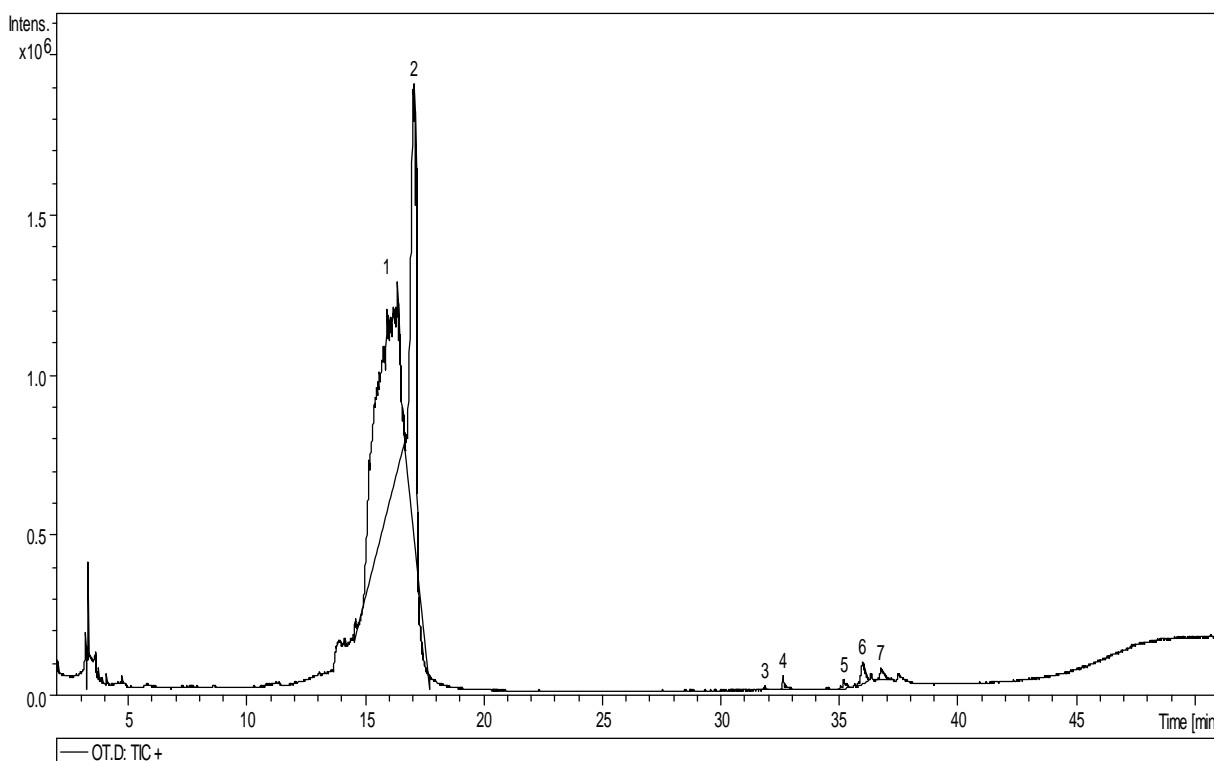
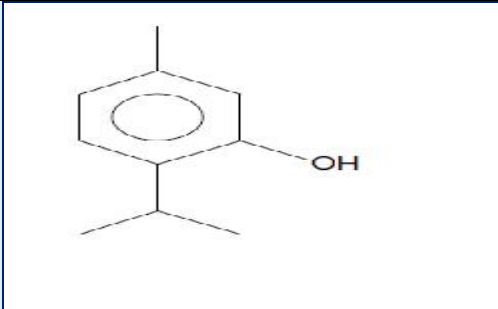
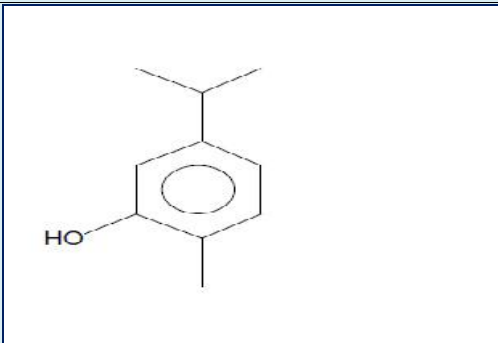
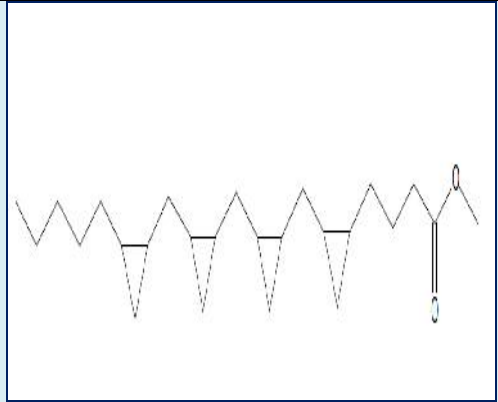
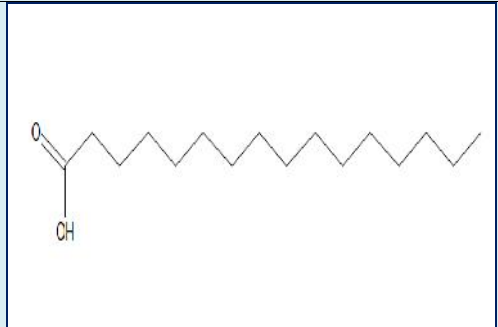
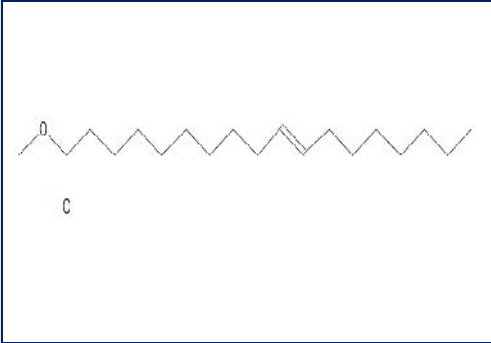
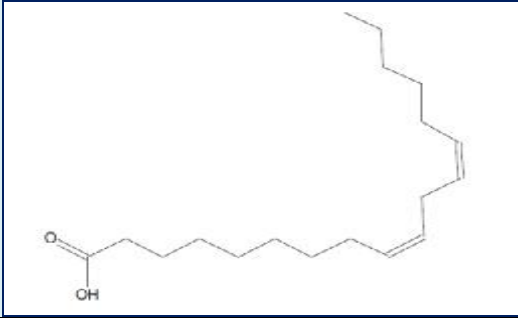
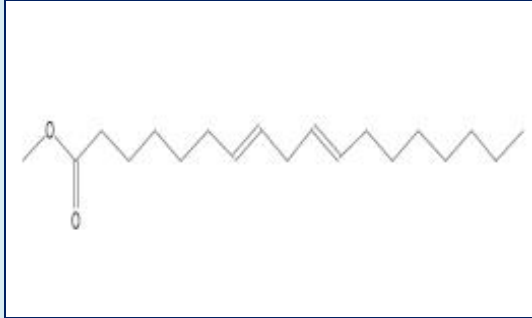


Table 1: Peak Table analysis of *Oma Theeneer*

Peak No	Retention Time	% Peak Area	Peak Intensity Rank
1	16	42.40	1
2	17.1	20.90	2
3	31.8	1.50	4
4	32.6	20.50	7
5	35.1	2.50	6
6	36	8.00	3
7	36.7	4.20	5

Table. 2GCMS Interpretation Report (<i>OmaTheeneer</i>)		
1		
Peak No	1	
Retention Time	16	
% Peak Area	42433546	
Peak Intensity Rank	1	
Mol. wt	150	
Name	Thymol	
Mol. Formula	C ₁₀ H ₁₄ O	
2		
Peak No	2	
Retention Time	17.1	
% Peak Area	22929699	
Peak Intensity Rank	2	
Mol. wt	150	
Name	Phenol, 2-methyl-5-(1-methylethyl)-	
Mol. Formula	C ₁₀ H ₁₄ O	
3		
Peak No	3	
Retention Time	31.8	
% Peak Area	58997	
Peak Intensity Rank	4	
Mol. wt	374	
Name	Cyclopropanebutanoic acid	
Mol. Formula	C ₂₅ H ₄₂ O ₂	
4		
Peak No	4	
Retention Time	32.6	
% Peak Area	250619	
Peak Intensity Rank	7	
Mol. wt	256	
Name	n-Hexadecanoic acid	
Mol. Formula	C ₁₆ H ₃₂ O ₂	

5		
Peak No	5	
Retention Time	35.1	
% Peak Area	254580	
Peak Intensity Rank	6	
Mol. wt	296	
Name	10-Octadecenoic acid, methyl ester	
Mol. Formula	C ₁₈ H ₃₂ O ₂	
6		
Peak No	6	
Retention Time	36	
% Peak Area	829576	
Peak Intensity Rank	3	
Mol. wt	280	
Name	9,12-Octadecadienoic acid (Z,Z)-	
Mol. Formula	C ₁₈ H ₃₂ O ₂	
7		
Peak No	7	
Retention Time	36.7	
% Peak Area	429324	
Peak Intensity Rank	5	
Mol. wt	294	
Name	7,10-Octadecadienoic acid, methyl ester	
Mol. Formula	C ₁₉ H ₃₄ O ₂	

4. Discussion

The distillation selectively extracts the Phenolic volatile compounds along with organic acids from the seeds of *Carumcopticum* which may be responsible for the pharmacological role of *OmaTheeneer* in wide range of diseased conditions. One of the main Phenolic volatile compounds such as Thymol exhibits broad spectrum activities which justifies the effect of the drug (Table:3). The minor alkaloids like derivatives of Linoleic acid have considerable role in Cardiovascular health. And it can be ascertained that if

the distillate is up to the good grade standards of traditional quality parameters like Color, Aroma and taste, such quality reflects the good percentage extraction of bioactive compounds by the process. Thymol considered being a vital compound of interest in the field of phytochemistry since several years. Several researches has evident the pharmacological and therapeutic activity of the thymol in treating respiratory, cardiovascular and obesity related disorders. The detailed review of the same was listed in table 3.

Table 3 Pharmacological significance of Thymol

Activity	Type of Studies & Results
Respiratory Effects Antispasmodic	Thymol has been studied for its effect on Tracheal and ileum smooth muscles in rats, Which demonstrated a dose dependant considerable Anti spasmodic activity and in addition to that an increased mucosal clearance also has been observed [16].
Cardio Vascular Effects Anti hypertensive	Fall in Blood pressure was demonstrated in mice by administering 1-10mg/kg Thymol. Further it was also concluded that the compound render anti-hypertensive activity by blocking calcium channel [17].
Anti Obesity	Studies on high fat diet rats with thymol administration of thymol 14mg/kg shown increased Anti-oxidant enzyme levels with prevention of obesity [18].
Anti Microbial Anti Biotic	Anti microbial activity of essential oil from Iranian <i>C.copticum</i> (including 72% thymol) has been studied by broth micro dilution method. Results of the investigation reveals that the compound inhibited the growth of both gram –positive & negative organism in the medium [19].
Anti Oxidant	Essential oil of <i>C.copticum</i> including Thymol reveals significant anti-oxidant activity in ABTS Radical scavenging assay [20]. Similar type of activity was observed in DPPH radical scavenging assay with IC50 value of 10±0.0 µg/ml [21].
Anti proliferative effects	Anti proliferative effects of thymol has been reported against human acute promyelotic leukemia cell line HL-60 [22].

5. Conclusion

From the results obtained from the present investigation it was concluded that the distillate type of preparations derived from the herbal components through traditional distillation techniques considerably have high concentration of very potent therapeutic compounds along with secondary derivatives. GCMS type of sophisticated instrumentation techniques is highly helpful in identifying the nature and structure of novel components like thymol. In the present investigation the presence of compound thymol in the *siddha* formulation *Oma Theeneer* was well identified and documented for the need of future researcher.

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