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GC MS Analysis of Thulasi ennai – A Siddha polyherbal formulation

S. Yavana Rani[1*], P. Mirunaleni[2], M. Sathiyabama[3], K. Suresh[4], M. Meenakshi Sundaram[5], V. Banumathi[6]

[1]Siddha Consultant, Yavanarani Siddha Clinic, Kanchipuram, Tamil Nadu, India
[2]Siddha Consultant, AYUSH Wellness Clinic, President Estate, New Delhi, India
[3]Siddha Physician, Panruti, Tamil Nadu, India.
[4]Lecturer, Department of Kuzhandhai maruthuvam, National Institute of Siddha, Chennai, India
[5]HOD, Department of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai, India
[6]Director, National Institute of Siddha, Chennai, India
*Corresponding Author: S. Yavana Rani

Keywords

Thulasi ennai,
Childhood asthma,
Siddha Medicine,
Gas Chromatography – Mass spectrometry

Abstract

The present study deals with the Gas Chromatography-Mass Spectrometry (GC MS) analysis of Thulasi Ennai (THULASI OIL - TO), a polyherbal siddha formulation. Thulasi Ennai said to cure soolikanam which is mentioned in classical Siddha textbook balavagadam. The symptoms of soolikanam are continuous cough, wheezing, tightness of chest, Shortness of breath, abdominal bloating, loss of appetite. These symptoms are correlated with childhood asthma. Though the individual herbs used in this formulation have the previous record of standardization, there is no evidence of the molecules present in the physical form of the study drug TO and hence this study was aimed. TO is a oil form of medicine (Ennai) and it is a combination of 11 types of different drugs. All the ingredients were produced from reputed raw drug store and botanically authenticated by the medicinal botanist of National institute of Siddha, Chennai. Purification was done individually as per the Siddha classical literature and the formation was prepared as per the procedure mentioned in Sastric Siddha text Balavagadam. The prepared drug was subjected to analysis. The derived GC MS analysis result were indicated that the presence of eighteen bio molecules in the formulation.

1. Introduction

Siddha System of medicine is considered to be the ancient documented medical system of the world. Kuzhandhai Maruthuvam is one among the glorious branches of Siddha system inside which is hidden an enormous treasure for a healthy society. Kuzhandhai Maruthuvam in Siddha literature is unique in itself, eminently illustrates childhood illness and their ailments. Thulasi Ennai was mentioned in Balavagadam. The formulations of Siddha medicines are well documented and time- tested standard preparations, it is the need of the hour to document standardization procedures based on current analytic techniques to prevent adulteration and to maintain quality control on per with contemporary medical world.

Thulasi Ennai is a polyherbal formulation prescribed in the management of childhood asthma. Literature Review of the ingredients of TO revealed that the drugs are having good expectorant, anti-allergic, anti-inflammatory, antidote, anti-pyritic and carminative but the compound form of the medicine Thulasi Ennai

(TO) has not been established by through standardization procedure for to identify the bio molecules present in the medicine for itglobal acceptance. So the author interested to go Gas Chromatography Mass Spectrometry (GC MS) to get the complete molecular picture of the study drug TO.

2. Materials and Methods

2.1 Plant materials

The required drug of Thulasi Ennai would be purchased from a well reputed country shop, Chennai, and raw drugs were authenticated by the medicinal botanist of National Institute of Siddha. The medicine will be prepared in Gunapadam lab of National Institute of Siddha after proper purification. The prepared medicine would also be authenticated by the concerned head of the department for its completeness.

2.2 Preparation of Thulasi Oil (TO)

Take 352ml of Castor oil, And 160 ml of Thulasi, Nilathulasi juice, Kanchakorai juice, Thalisapathiri juice, Vilvam fruit leaves juice, Onion juice. Mix the juices with oil in a mud vessel. Take 8gms of dried ginger, Long pepper, Black pepper fry it and make it fine powder mix 160ml of honey and heat it till muster form appears and filter it.

2.3 GC MS analysis of Thulasi Oil (TO)

Thulasi oil (TO) was extracted with ethanol and the extract was subjected to the GC -MS analysis.
Agilent 7890B GC connected to 5977A MSD, NIST Ver.2.1 MS data library Specification:

Column Name

- HP_5MS 5% Phenyl Methyl Silox -60°C-325°C (325°C) 30m×250µm×0.25µm
- Split less mode injection
- 1µL injection volume

Oven program

- 50°C for 2min then ramp 5°C per minute till 270°C, then 270°C maintained for 2min,total run time 42 min
- Detector temperature 275°C
- Injector temperature 250°C
- Solvent delay 2min
- m/z Scan range 50-600amu

Start Time (min)	End Time(min)	Start m/z	End m/z Scan	Speed
2.50	18.00	50.00	650.00	2000

GC-MS Plays a key role in the analysis of unknown components of plant 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 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.

3. Results and Discussion

Raw material for this polyherbal formalation Thulasi Oil (TO) were listed in table-1.Gas Chromatography-Mass Spectroscopy (GCMS) is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitrogen compound of a polyherbal formulation. Ethnolic extract of Thulasi Oil (TO) was prepared as test sample. In the present study 18 chemical compounds have been identified. Depicts the retention time, names of the compounds, molecular formula, molecular weights and % peak values of the identified molecules were given in table 2 and fig-1. GCMS analysis of the sample TO reveals the presence of most significant compound like Eugenol, Naphthalene, Oleic acid and Ricinoleic acid derivatives

Table 1: Ingredients Thulasi Ennai

Sl.No	Tamil name	*Botanical name*	Quantity
1	Thulasi	*Ocimum sanctum*	160 ml
2	Nilathuasi	*Ocimum prostratum*	160 ml
3	Kanchakorai	*Ocimum canum*	160 ml
4	Thalisapathiri	*Taxus buccata*	160 ml
5	Vilvam	*Aegle marmelos*	160 ml
6	Eerulli	*Allium cepa*	160 ml
7	Chukku	*Zingiber officinale*	8 gm
8	Thippili	*Piper longum*	8 gm
9	Milagu	*Piper nigrum*	8 gm
10	Then	-	160 ml
11	Chitramanakku nei	*Ricimus communis*	352 ml

Table. 2: GC MS Analysis results of Thulasi Ennai

Sl.No	Peak Name	Retention time	Peak area	%Peak area
1	Name: Furan, 2-hexyl- Formula: $C_{10}H_{16}O$ MW: 152	11.2	7216832	5.423
2	Name: 2-Ethylnon-en-3-ol Formula: $C_{11}H_{22}O$ MW: 170	18.5	1604098	1.205
3	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	18.8	2968710	2.231
4	Name: Cyclohexane, 1- ethenyl-1-methyl-2,4-bis(bis(1- methylethenyl)-,[1S(1.alpha., 2.beta, 4.beta.)]- Formula: $C_{15}H_{24}$ MW: 204	19.7	1513729	1.137
5	Name: Benzene, 1,2-dimethoxy- 4(2-propenyl)- Formula: $C_{11}H_{14}O_2$ MW: 178	20	4966194	3.731
6	Name: Caryophyllene Formula: $C_{15}H_{24}$ MW: 204	20.4	5617864	4.221
7	Name: Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)- Formula: $C_{15}H_{24}$ MW: 204	23	1359441	1.021

8	Name: Pentadecanoic acid, 14-methyl-, methyl ester Formula: $C_{17}H_{34}O_2$ MW: 270	31.8	1332421	1.001
9	Name: 9-Acetamido-1mathyl-3,6-diazahomoadamantane Formula: $C_{12}H_{21}N_3O$ MW: 223	32.1	1930526	1.451
10	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	32.8	12094831	9.088
11	Name: 9,12-Octadecadienoic acid (Z, Z)-, methyl ester Formula: $C_{19}H_{34}O_2$ MW: 294	35	2009342	1.509
12	Name: 8-Octadecenoic acid, methyl ester Formula: $C_{19}H_{36}O_2$ MW: 296	35.1	1846165	1.387
13	Name: Oleic acid Formula: $C_{18}H_{34}O_2$ MW: 282	36.2	42060281	31.606
14	Name: Octadecanoic acid Formula: $C_{18}H_{36}O_2$ MW: 284	36.5	3775941	2.387
15	Name: Methyl ricinoleate Formula: $C_{19}H_{36}O_3$ MW: 312	38.5	9606146	7.218
16	Name: 17-Hydroxy-3-methoxy- estra-2,5(10)-diene Formula: $C_{19}H_{28}O_2$ MW: 288	39.2	2432395	1.827
17	Name: Ricinoleic acid Formula: $C_{18}H_{34}O_3$ MW: 298	39.5	28691809	21.561
18	Name: 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all- E)- Formula: $C_{30}H_{50}$ MW: 410	46.7	2049766	1.541

GC-MS Chromatogram of TO

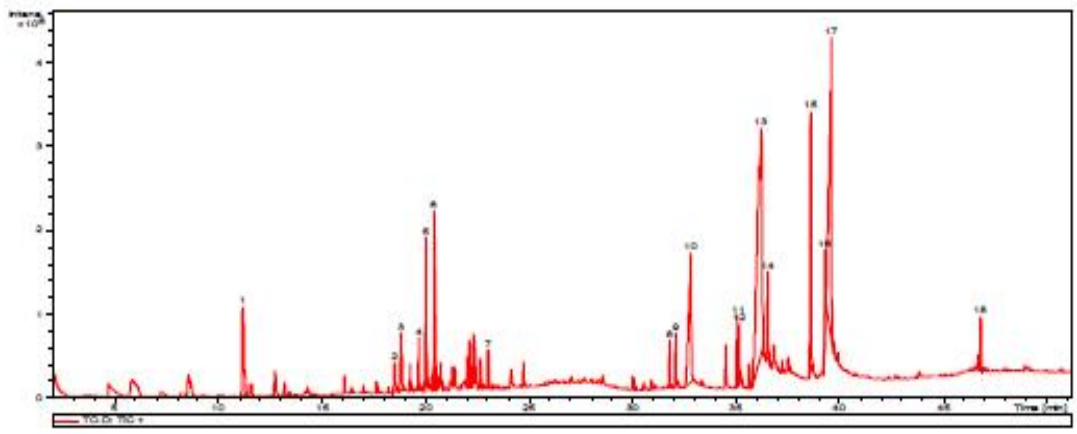

Figure 1: GC MS Chromatogram of Thulasi Oil (TO)

4. Conclusion

Thulasi Oil (TO), is a special medicine for childhood asthma. The encouraging results showed the present of 18 active molecules and it would be adding evidence for the curative effect of prescribing this medicine since ancient years. This study outcome will be a boon for the young researchers of indigenous medicine and herbal scientists.

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6. Conflicts of Interest

There are no conflicts of interest from the author

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