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Standardization of commercial lavangathi chooranams – A comparative study

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Abstract
Lavangathi chooranam is a well-known herbal remedy that is included in the official Indian herbal formulary. It is a multi-herbal remedy that is useful in treating all phases of whooping cough and chronic bronchitis and many respiratory disorders. The aim of study is to compare and standardise the three commercial Lavangathi chooranams. Quality and safety were evaluated using physicochemical, chromatographic, and heavy metal analysis methods. The results of the investigation showed that the quantities and physicochemical properties of the market compounds varied amongst copies of the same formulation made by different manufacturers. The study's findings show that trace amounts of heavy metal residues in the drug of Lavangathi chooranam were within WHO standards. GC-MS method is used to estimate thymol and Eugenol which are the major herbal ingredients. Using HPLC technology, eugenol and piperine is quantified from commercially available formulations of lavangathi chooranam. These parameters may be useful finger prints for standardisation of the drug.

Keywords: Lavangathi chooranam, siddha medicine, poly herbals, standardization

Introduction
The South Indian saviours known as Siddhars founded the world's most ancient medical systems. Siddhars knowledge of the universe's natural resources is incredible. This includes formulations that are minerals, poly-herbals, and mono-herbals. Because they have fewer side effects and may have more therapeutic benefits, siddha medicines are gaining increased attention. Standardisation is crucial to guarantee the effectiveness, safety, and quality of herbal medicine and integrate it into the current healthcare system [1,2]. Lavngathi Chooranam is a commonly used drug which is mentioned in Schedule 1 of Siddha pharmaceutics – *Siddha Vaithya Thirattu*. The main ingredients in Lavangathi chooranam (Kiraambu Chooranam) are clove – Kiraambu (*Syzygium aromaticum (L.)*, Cinnamon – Lavangapattai (*Cinnamomum aromaticum*), long pepper – Thippili (*Piper longum L.*), Ajwain - Omam (*Trachyspermum ammi*), and Ginger - Chukku (Zingiber officinale) and Cumin Jeeragam (*Cuminum cyminum L.*) Major component Eugenol has various properties like antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer agent. The United States Food and Drug Administration (USFDA) has approved eugenol as safe, and it is being studied as a potential biomarker [6,7]. Thymol is a major component of the naturally occurring compounds called biocides. Thymol has antibacterial properties that can lower bacterial resistance to some common medications [8]. Piper nigrum is one of the spices commonly used. Its fruits contain pro-phenyl phenols, lignans, amides, terpenes, and flavonoids which have diverse chemical makeup [9-11]. Ginger has bioactive substances shown in several modules for its antioxidant properties [12-15]. Significant Indian spice like cumin, used as effective anti-carcinogenic agents and to include carminative, stimulant, antioxidant, and anti-cancer properties [16,17].

Materials & methods
1. Purchased Drugs of Lavangathi Chooranam
The three branded commercial Lavangathi Chooranam drugs were purchased from local retail pharmacy in Arumbakkam chennai. They are LCA (Aravindh Herbal Labs private Ltd), LCE (Earth India Naturals) and LCP (Anna Hospital Pharmacy).

2. Organoleptic Characters
The Different Marketed lavangathi chooranam Colour, odour, taste and consistency of the drugs were noted.

3. Physicochemical Analysis [18-19]
3.1 Determination of Loss on Drying
Approximately 2gms of sample was Weighted and kept in a Hot air oven for about 4 to 5hrs at a temperature of 105ºC then it was allowed to cool and weighed to calculate the loss on drying present in the sample.

3.2 Determination of Ash Values
Approximately 2gms of sample and incinerated in a Muffle Furnace at 450ºC temperature for about 7hrs until ash free from carbon was obtained. Then it was cooled in a desiccator and weighed.

3.3 Determination of Acid - Insoluble Ash
In a Silica Crucible (containing sample collected from Total Ash) about 25ml of dil. HCl was added and gently heated in a Mantle for about 5mins and then cooled. The insoluble matters filtered in a whatmann filter paper of 40 Size. The filter paper was kept into the silica crucible and incinerated in a Muffle Furnace for about 6hrs at a temperature of 650ºC. After cooling in a desiccator, the material was weighed.

3.4 Determination of Water - Soluble Extractive
Approximately 5gms of sample was weighed in Iodine Flask and 100ml of distilled water was added to it and kept in a shaker for about 6hrs for constant shaking. Then it was allowed to rest overnight and was filtered in a whatmann filter paper of 4 Size. Then the filtrate is dried in a Hot air oven at a temperature of 110ºC and it was allowed to cool and then weighed**.**

3.5 Determination of Alcohol - Soluble Extractive
Approximately 2.5gms of sample was weighed in an Iodine Flask and 50ml of Ethanol was added to it and kept in a shaker for about 6hrs for constant shaking. Then it was allowed to rest overnight and was filtered in a whatmann filter paper of 4 Sized. Then the filtrate is dried in a Hot air oven at a temperature of 110ºC and it was allowed to cool and then weighed**.**

3.6 pH (1% Aqueous solution)
Approximately 1gm of sample was weighed in an Iodine Flask and 100ml of deionized water was added to it and occasional shaking for 30mins. It was filtered and then subjected to pH evaluation.

4. AAS instruments and conditions
Thermo Fisher Scientific Company and model is iCE 3000 Series Atomic Absorption Spectrometers. The SOLAAR Series Data Station Software provides a data station software manual. Hollow Cathode Lamps (HCLs) is known as High intensity, steady light sourcesemit the element specific spectral lines needed for atomic absorption spectrometry. The fuel gas is Acetylene and supporting gases are compressed Air and Nitrous-oxide.

Standard preparation: The standard solutions for all of the heavy metals under investigation were created in three different concentrations to obtain a calibration curve by diluting a stock standard solution of 1000ppm.

Sample preparation: The different marketed Lavangathi chooranam was weighed approximately 0.5gms in 250ml beaker and added 25 ml of 1N HNO_3 and 25ml of 1N HCl and kept in waterbath until 50ml of solution approximately reduced to 25ml. Then filtered and make up to 100ml with distilled water.

5. GC-MS instruments and conditions
GC-MS data were obtained on a Shimadzu single quadrupole QP-2020NX Mass spectrometer. The GC Instrument Shimadzu Nexis GC-2030, fused silica capillary column SH Rxi-5Sil MS (0.25 mm ID × 30 m, film thickness 0.25 µm). The column oven initial temperature was 60°C for 2 mins, and programmed at 10°C/min to 200°C hold for 3 mins then continued at 10°C/min to 280°C hold for 3 mins. For the Carrier gas helium was used with a flow rate of 1.2 ml/min. ion source temperature and interface temperature were 200°C and 290°C. Split ratio is 1:10. Mass spectra were taken at 70eV. Mass range was from 35m/z to 700m/z.

GC-MS Sample Preparation: Approximately 1gm of different marketed Lavangathi chooranam was weighed and added 50 ml of HPLC grade Methanol and it was soaked overnight.

6. HPLC instruments and conditions
The HPLC system (Shimadzu), consisting LC-10AT pump, UV detector (Shimadzu SPD-M10A), system controller (SCL-10A) manual injector with 20 µl loop and syncronis C18 column (250 × 4.6 µm ID, 5 µm) was used. The mobile phase consisted Methanol and water (75:25 v/v). The samples were eluted from the column at room temperature with flow rate of 1 ml/min. Eugenol detected at 221nm and piperine detected at 324nm. Lab solution software was used for the study of HPLC chromatogram. Identification and quantification of eugenol and piperine was verified by peak areas obtained in HPLC analysis.

HPLC Sample Preparation: The various brands of Lavangathi chooranam were weighed to be about 1 gram, then 50 ml of HPLC-grade methanol were added, and it was let to soak overnight.

Results
1. Organoleptic characters
Appearance of LCP and LCE is light brown in colour whereas LCA is brown in colour. The result was shown below in the table 1.

Table 1: Organoleptic characters

S. No	State	Brands		
		LCP	LCE	LCA
1.	Nature	Fine powder	Fine powder	Fine powder
2.	Odour	Aromatic	Aromatic	Aromatic
3.	Appearance	Light brown	Light brown	Brown
4.	Taste	Pungent	Pungent	Pungent

Physicochemical evaluation
In the analysis of loss on dryingof the different marketed drugs revealed that LCA had the highest loss of moisture content, followed by LCE, and LCP in descending order. The total ash content analysis was also conducted on the different formulations. In this LCA contains highest amount of carbon, followed by LCP and LCE. Similarly, the acid insoluble ash analysis showed that LCA had the highest inorganic compound, followed by LCP and LCE. The water soluble extractive analysis was carried out, and LCA had the highest, followed by LCE and LCP. The alcoholic extractive analysis was performed, and LCA had the highest, followed by LCP and LCE. Detailed results showed in Table 2.

Table 2: Comparative study of Physicochemical Parameters for different brands:

S. No	Parameters	Brands [Mean Value (n=3) ± S.D]		
		LCP	LCE	LCA
1.	Loss on Drying at 105ºC	5.19% ± 1.00	7.21% ± 1.05	7.53% ± 0.20
2.	Total Ash	3.12% ± 0.23	2.61% ± 0.91	4.46% ± 0.11
3.	Acid Insoluble Ash	0.40% ± 0.11	0.41% ± 0.13	0.48% ± 0.04
4.	Water Soluble Extractive	53.44% ± 4.18	54.28% ± 5.12	57.26% ± 5.00
5.	Alcohol Soluble Extractive	6.92% ± 1.17	5.75% ± 2.22	10.16% ± 2.33
6.	pH (1% Aqueous solution)	5.82	5.76	5.61

AAS Results

Atomic absorption spectrophotometer (AAS) was used to detect heavy metal analysis in lavangathi chooranam. When we consume internally, heavy metal contamination can have harmful effects that go beyond what is safe for humans. Lead (Pb), Cadmium (Cd), Mercury (Hg), and Arsenic (Ar) were found in lavangathi chooranam within the limits given in the current study. Hence there is no heavy metal toxicity and it is safe for use. Results showed in Table 3.

Table 3: Comparative study of Heavy metal analysis for different brands

S. No	Heavy Metals	Absorption wavelength (^ max)	Brands			Maximum Limit
			LCP	LCE	LCA	
1.	Lead	217.0nm	BOQ < 5ppm	BOQ < 5ppm	BOQ < 5ppm	10ppm
2.	Arsenic	193.7nm	BOQ < 1ppm	BOQ < 1ppm	BOQ < 1ppm	3ppm
3.	Cadmium	228.8nm	BOQ < 0.1ppm	BOQ < 0.1ppm	BOQ < 0.1ppm	0.3ppm
4.	Mercury	253.7nm	BOQ < 0.5ppm	BOQ < 0.5ppm	BOQ < 0.5ppm	1ppm

*BOQ = Below of Quantification

GC-MS Quantification

By comparing the different marketed lavangathi chooranam chromatograms of each sample solution to those of standard eugenol and thymol, the identities of the eugenol and thymol peaks were quantified. The GCMS chromatogram revealed distinct peaks for the samples, namely LCA, LCE, and LCP. Notably, the highest concentration of thymol was detected in the LCP followed by LCA and LCE.The highest concentration of eugenol was detected in the LCE followed by LCA and LCP. Detailed chromatograms can be showed in figures no [1-4]. Quantification of thymol and eugenol shown in figures no [5-6]. Chemical constituents present in the various companies formulated lavangathi chooranam tabulated in table [4-7] and of its uses tabulated in table 8.

Comparative study of GCMS Analysis results

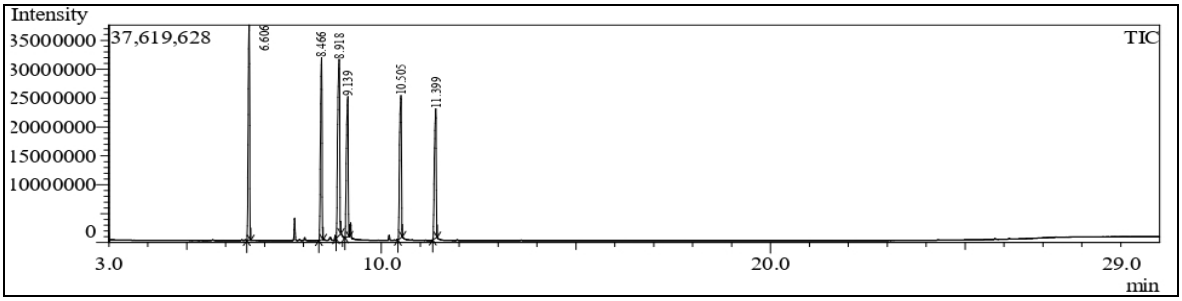

Fig 1: GCMS Chromatogram of Thymol and Eugenol

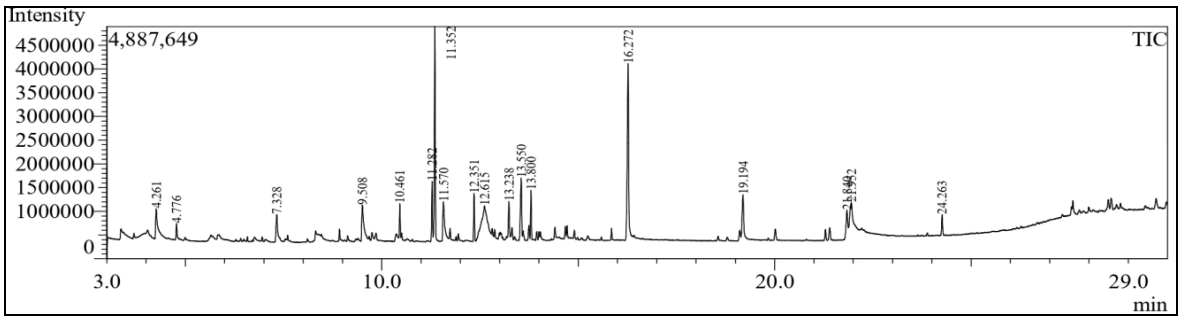

Fig 2: GCMS Chromatogram of LCA

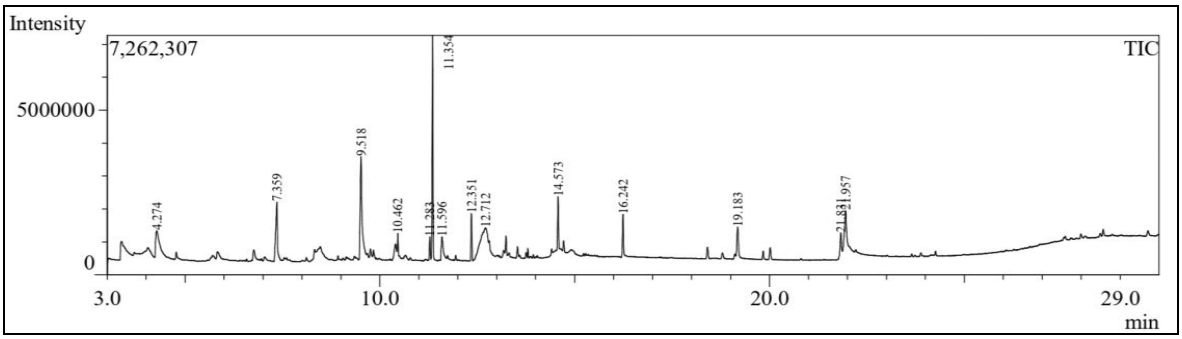

Fig 3: GCMS Chromatogram of LCE

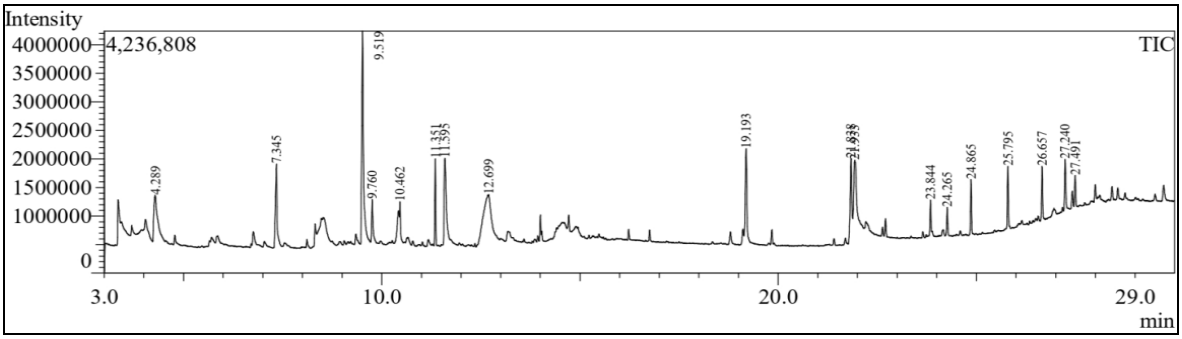

Fig 4: GCMS Chromatogram of LCP

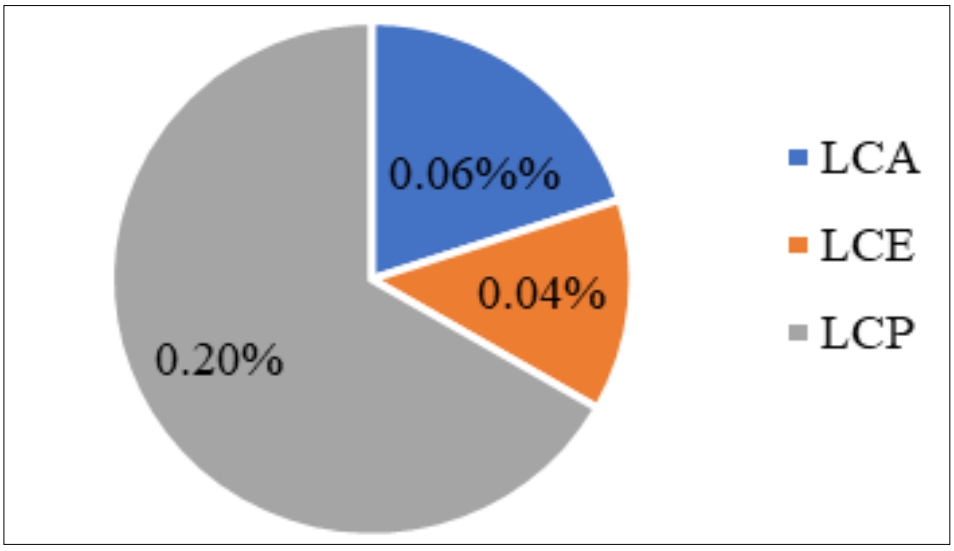

Fig 5: Quantification of Thymol by GC-MS from different marketed lavangathi chooranam

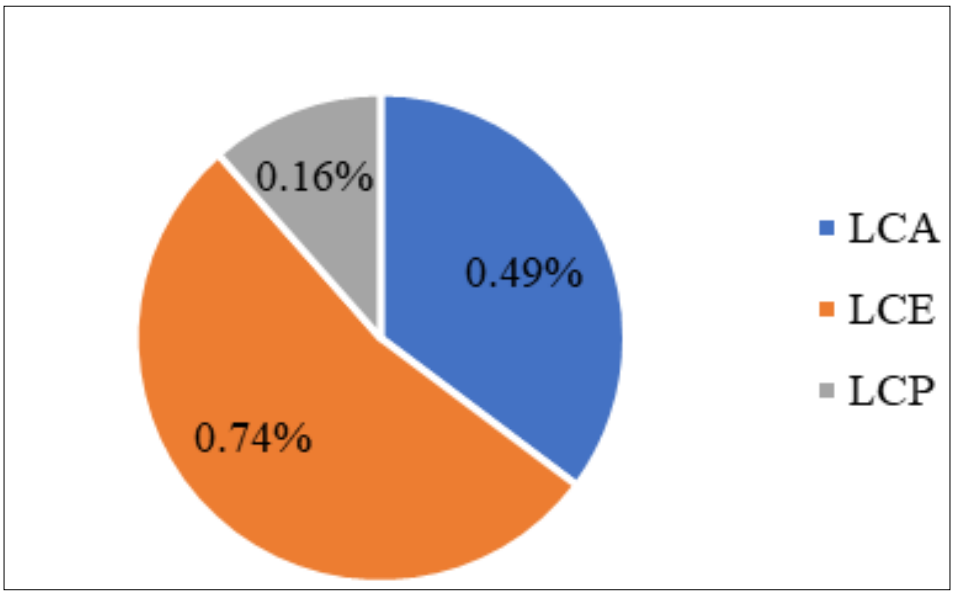

Fig 6: Quantification of Eugenol by GC-MS from different marketed lavangathi chooranam

Table 4: GCMS Standards

S. No	Peak	Area%	Name
1	6.606	18.40	Eucalyptol
2	8.466	16.53	Camphor
3	8.918	20.82	Menthol
4	9.139	15.00	Methylsalicylate
5	10.505	15.65	Thymol
6	11.399	13.60	Eugenol

Table 5: Chemical constituents of LCA

S. No	R. Time	Area%	Name
1	4.261	3.17	Dihydroxyacetone
2	4.776	1.20	1,2-Cyclopentanedione
3	7.328	2.90	Thymine
4	9.508	4.82	5-Hydroxymethylfurfural
5	10.461	2.24	Thymol
6	11.282	4.11	alpha.-Terpinylacetate
7	11.352	15.64	Eugenol
8	11.570	4.75	1,2,3-Benzenetriol
9	12.351	3.35	Caryophyllene
10	12.615	12.12	Sucrose
11	13.238	2.72	beta.-Humulene
12	13.550	6.00	Isoledene
13	13.800	3.21	Asarone
14	16.272	20.45	Tetradecanoicacid
15	19.194	5.07	n-Hexadecanoicacid
16	21.840	1.73	9,12-Octadecadienoicacid
17	21.952	4.71	9-Octadecenoicacid
18	24.263	1.79	Retrofractamide-A

Table 6: Chemical constituents of LCE

S. No	R. Time	Area%	Name
1	4.274	7.56	Dihydroxyacetone
2	7.359	8.95	Thymine
3	9.518	16.33	5-Hydroxymethylfurfural
4	10.462	1.18	Thymol
5	11.283	1.73	.alpha.-Terpinylacetate
6	11.354	17.80	Eugenol
7	11.596	3.63	1,2,3-Benzenetriol
8	12.351	3.72	Caryophyllene
9	12.712	18.19	Sucrose
10	14.573	4.02	.beta.-Asarone
11	16.242	3.88	Tetradecanoicacid
12	19.183	3.69	n-Hexadecanoicacid
13	21.831	2.56	9,12-Octadecadienoicacid
14	21.957	6.78	6-Octadecenoicacid

Table 7: Chemical constituents of LCP

S. No	R. Time	Area%	Name
1	4.274	7.56	Dihydroxyacetone
2	7.359	8.95	Thymine
3	9.518	16.33	5-Hydroxymethylfurfural
4	10.462	1.18	Thymol
5	11.283	1.73	alpha.-Terpinylacetate
6	11.354	17.80	Eugenol
7	11.596	3.63	1,2,3-Benzenetriol
8	12.351	3.72	Caryophyllene
9	12.712	18.19	Sucrose
10	14.573	4.02	beta.-Asarone
11	16.242	3.88	Tetradecanoicacid
12	19.183	3.69	n-Hexadecanoicacid
13	21.831	2.56	9,12-Octadecadienoicacid
14	21.957	6.78	6-Octadecenoicacid

Table 8: Uses of major chemical constituents of lavangathi chooranams obtained from GCMS

S. No	Chemical name	Uses
1.	Dihydroxyacetone	DHA is involved in brain and retinal development and it is helpful for the proper functioning of neuron system.
2.	Thymine	Its helps to stabilize the nucleic acid structures.
3.	5-hydroxy methyl furfural	Antioxidant properties, blocks immune mediated allergic reactions, downregulates xanthine oxidase and aids in preventing sickle haemoglobin.
4.	Thymol	Anti-inflammatory, local anaesthetic, antinociceptive, cicatrizing, antiseptic especially antibacterial and antifungal properties.
5.	alpha.-Terpinyl acetate	It is a p-menthane monoterpenoid that naturally found in cardamom and used as an important flavouring agent.
6.	Eugenol	It has demonstrated antibacterial properties against many species, such as Staphylococcus

		aureus, Pseudomonas aeruginosa, and Escherichia coli.
7.	1,2,3-Benzenetriol	It is used as antiseptic properties.
8.	Caryophyllene	It can support the immune system, relieve pain and reduce inflammation. It can help to soothing and relax the patient.
9.	Sucrose	It stimulates energy
10.	beta. Asarone	It has the pharmacological properties including anti-apoptotic, anti- cancer, and neuroprotective effects.
11.	Decanoic acid	It is a Saturated fatty acid with a straight chain. It has a role as metabolites of human, plant and algae and it plays anti-inflammatory and anti-bacterial agent.

HPLC Quantification

Comparing the different marketed lavangathi chooranam chromatograms of each sample solution to those of standard eugenol and piperine, the identities of the eugenol and thymol peaks were quantified. The HPLC chromatogram revealed distinct peaks for the samples, namely LCA, LCE and LCP. Notably, the highest concentration of piperine was detected in the LCP followed by LCE and LCA. The highest concentration of eugenol was detected in the LCE followed by LCA and LCP. Detailed chromatogram was showed in figures no [7-11]. Quantification of eugenol and piperine was shown in figures no [12-13].

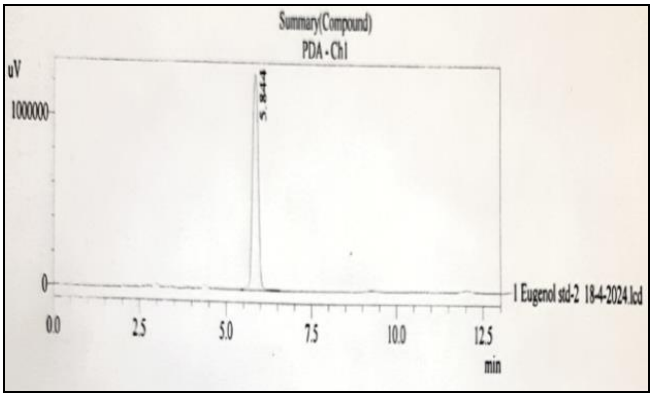

Fig 7: HPLC chromatogram of standard Eugenol

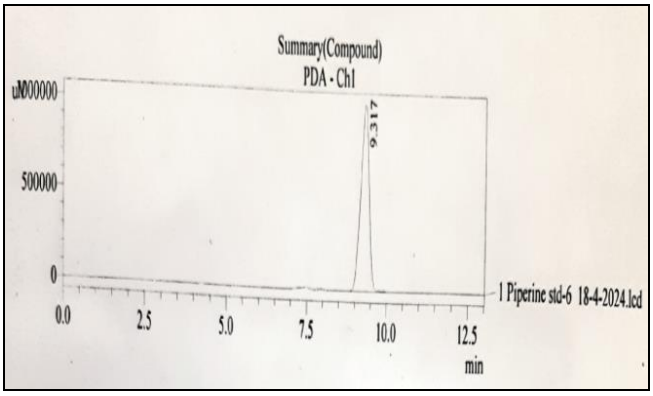

Fig 8: HPLC chromatogram of standard Piperine

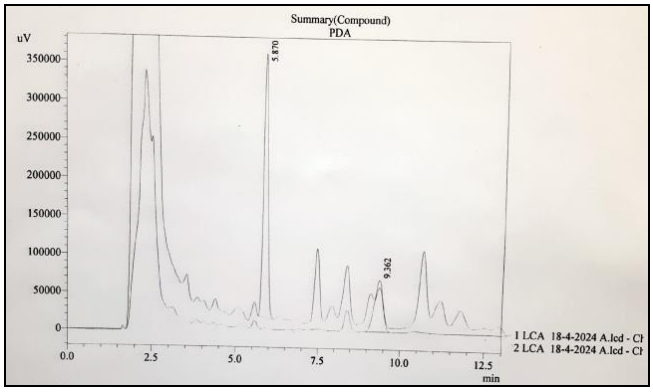

Fig 9: HPLC chromatogram of LCA

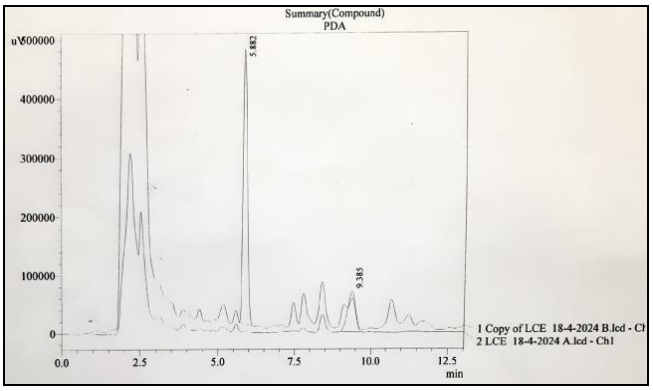

Fig 10: HPLC chromatogram of LCE

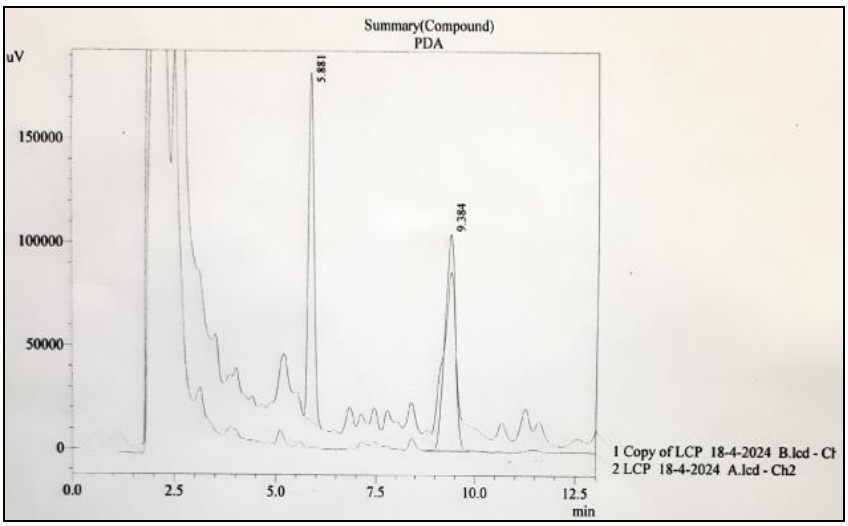

Fig 11: HPLC chromatogram of LCP

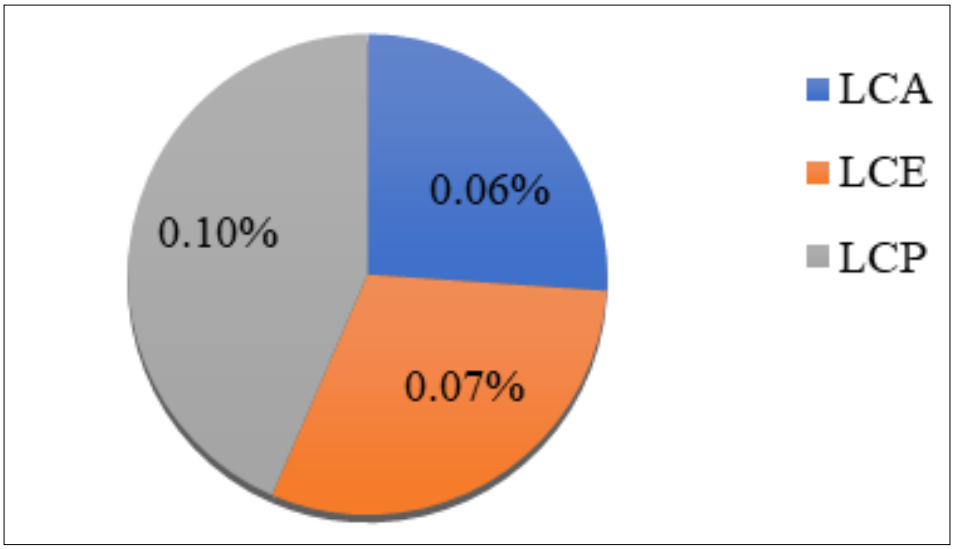

Fig 12: Quantification of Piperine by HPLC from different marketed lavangathi chooranam

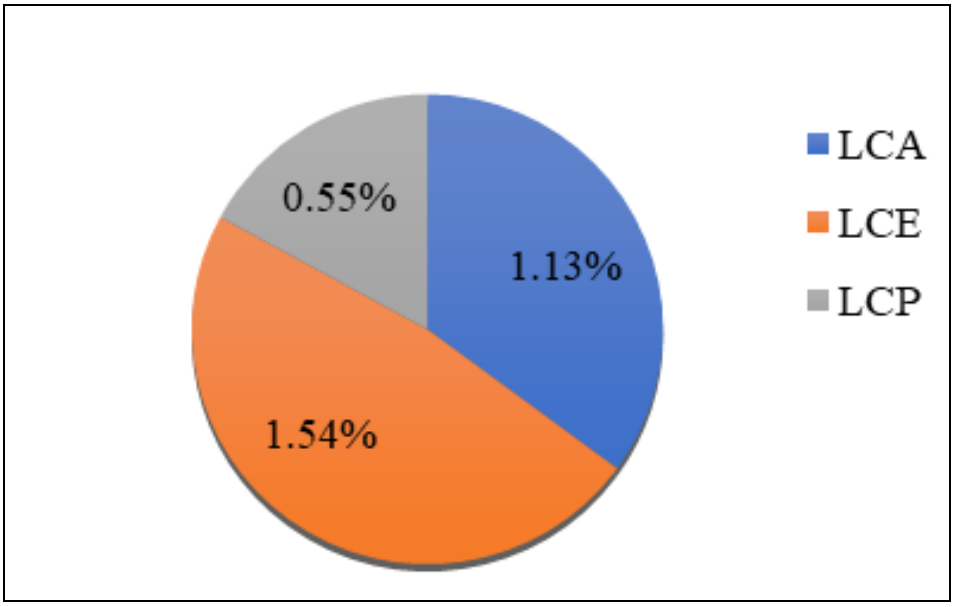

Fig 13: Quantification of Eugenol by HPLC from different marketed lavangathi chooranam

Discussion

The Lavangathi chooranam was purchased from different brands and tests were carried out. Based on the physicochemical characteristics and constituent composition of the lavanagthi chooranam, minor variations were observed in the experiments. If the drying process is not done properly, there is a risk of microbial contamination in the sample and reduce the sample's capacity. Total ash is that obtained after a sample has burned completely. The two parts of the ash are the usable component the part that is soluble in diluted acids and includes all necessary minerals and the ash itself. The remaining part is mostly made up of silica and sand and is insoluble in diluted acids. These physicochemical studies were carried out

Internal consumption of contaminated heavy metals can have adverse effects in human. From the AAS study it is found that lavangathi chooranam is safe to use and does not contain any heavy metal toxicity. In HPLC, the mobile phase consists of various combinations of water and methanol, and separation was satisfactory at 75% methanol. Following which comparison was made and discovered that 1ml/min was the most appropriate flow rate. The detection wavelength was measured at 221nm in accordance with the maximum UV absorption of the eugenol. This method is suitable for piperine but detection wavelength was measured at 324nm in accordance with the maximum UV absorption. HPLC results showed Quantification of Piperine from LCA, LCE and LCP respectively 0.06%, 0.07% and 0.10% and quantification of Eugenol from LCA, LCE and LCP respectively 1.13%, 1.54% and 0.55%. GC-MS results showed all molecules were almost identical. Only a few molecules are different and some molecules differ quantitatively. Quantification of thymol from LCA, LCE and LCP respectively 0.06%, 0.04% and 0.20% and quantification of Eugenol from LCA, LCE and LCP respectively 0.49%, 0.74% and 0.16%.

The variations in different dugs in these parameters may be attributed to adulteration or varied qualities of raw drugs. Most of the raw drugs doesn't have monograph.

Conclusion

Lavangathi chooranam is a common siddha drug available in market which is useful in many clinical conditions. In this study, standard parameters were followed in the

standardisation and evaluation of the various commercial Lavangathi chooranams for their organoleptic, physicochemical, heavy metal analysis and quantification. The qualitative and quantitative HPLC results of different marketed lavangathi chooranam revealed that marker eugenol and piperine were the major components. GCMS indicates the presence of major compounds including 5-Hydroxymethylfurfural, Eugenol, Sucrose and Decanoicacid. Lavangathi chooranam had Lead(Pb), Cadmium(Cd), Mercury(Hg), and Arsenic(Ar) within the ranges determined by this investigation. It is therefore safe to use and does not contain any heavy metal toxicity. These phytochemical bioactive compounds found in the Lavangathi Chooranam herbal formulations, may serve as a valuable bioactive biomarker for evaluating the efficacy of commercial drugs and their formulations.

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