



**STANDARDIZATION AND PHYSIO CHEMICAL ANALYSIS OF SIDDHA HERBO
MINERAL DISTILLATE “SANJEEVI THEENEER”**

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ABSTRACT

Background: Distillates are extracts of raw materials obtained by distillation methods. *Sanjeevi theeneer* is a classical formulation in the form of distillate. The drug specifies so many indications like jaundice, ascites, respiratory diseases, cardiac diseases and skin diseases. **Objectives:** By foreseeing the outcome of the drug in research and clinical practice, the drug has undergone preliminary analytical procedures as per AYUSH guidelines like basic standardization, Heavy metal analysis, Microbial load analysis, Physio chemical screening, Preliminary phyto chemical screening and Gas chromatography (GCMS) studies. **Results and Discussion:** All the physio chemical parameters especially the organo leptic characters meets the traditional standards. The heavy metal and microbial load presence were within the permissible limits. Preliminary phyto chemical analysis reveals the presence of phenolic compounds in the distillates. GC-MS screening confirmed the presence of biologically active compounds like Isothymol, oleic acid, dasycarpidan and linalool in the drug '*Sanjeevi Theeneer*'. **Conclusion:** The study reported fruitful results, which supports the purity, safety and efficacy of the drug.

KEYWORDS: *Sanjeevi theeneer*, Physio chemical analysis, Heavy metal analysis, Microbial load, GC-MS.

I. INTRODUCTION

The special formulations attributed to *siddha* system of medicine (*Deva maruthvam*) is still unexplored for its application in various fields of health sector.^[1] *Theeneer*, one among the special dosage forms is termed as unique extracts of natural compounds.^[2] These distillates have an expected outcome like a neutraceutical, as a drug of choice in specific ailments or in supportive care. Easy palatability, absorption and faster efficacy are the other advantages of a distillate. Out of hundreds of Single or compound distillate formulations in *siddha*, a very few are put into practice. Majority of the formulations lack standardization, preclinical or clinical validation.

Standardization of the drug complies with confirmation of entire feature of a drug starting from identity, purity and quality through all phases of cycle either it means its shelf life, storage and use by its various parameters.^[3] This is very essential to assess the quality of the distillate and for the scientific justification.^[4] As a preliminary step in the standardization of *theeneer* formulations, *Sanjeevi theeneer*, which is a poly herbo mineral distillate, mentioned in the classical text *Chikitsa*

ratnadeepam and *Siddha Formulary of India* (SFI) has been selected.^[5] Methods like Heavy metal and microbial load analysis, Physio chemical and preliminary phyto chemical analysis were performed in the distillate sample. Gas chromatography Mass spectrometry studies (GC-MS) has been executed to screen the biologically active compounds of the distillate. All the Analytical specifications of *Theeneer* are as per the AYUSH protocol for standardization. (Table. 1).

Table 1: Analytical Specifications of Theeneer.^[6]

S.no	TESTS
1.	Organoleptic characters
	<ul style="list-style-type: none"> • Color • Odor • Taste
2.	pH
3.	Volatile matter
4.	Specific gravity at 25 ⁰ C
5.	Clarity Test
6.	Microbial Contamination
7.	Test for Specific Pathogen
8.	Test for Heavy metals
9.	Assays
	<ul style="list-style-type: none"> • Preliminary Phyto chemical Assays
10.	Identification
	<ul style="list-style-type: none"> • Gas Chromatography Mass Spectrometry (GC-MS)

II. MATERIALS AND METHODS

a. Ingredients of Sanjeevi theeneer (Table no: 2 & Fig 1)

All the ingredients were purchased locally from a reputed raw drug shop. The herbal ingredients were

identified and authenticated from the branch of botany (Authentication No: NISMB2472016), the mineral sample were identified from Department of Geology, Anna university, Guindy, Tami Nadu.

Table 2: Ingredients of Sanjeevi Theeneer.

S.NO	Ingredient	Botanical Name	Part Used	Quantity
1	Chukku	<i>Zingiber officinale</i>	Dry Rhizome (Outer skin removed)	60 g
2	Milagu	<i>Piper nigrum</i>	Dry fruit	60 g
3	Thippili	<i>Piper longum</i>	Dry Berry	10g
4	Kadukkai	<i>Terminalia chebula</i>	Dry fruit (seed removed)	25g
5	Nellikkai	<i>Phyllanthus emblica</i>	Dry fruit (seed removed)	50g
6	Tantrikkai	<i>Terminalia bellerica</i>	Dry fruit (seed removed)	25g
7	Omam	<i>Trachyspermum ammi</i>	Dry fruit	25g
8	Vaividangam	<i>Embelia ribes</i>	Dry fruit	25g
9	Chithramoolam	<i>Plumbago zeylanica</i>	Dry Root Bark	30g
10	Korai kizhangu	<i>Cyperus rotundus</i>	Dry Tuber	25g
11	Panam karkandu	<i>Borassus flabellifer</i>	Palm Candy	20g
12	Irumbu Podi	Purified Ferrum powder	--	60 g
13	Water			6 Litres





Fig. 1: Ingredients of Sanjeevi Theeneer.

Table 3: Purification (Suddhi) Process of Individual Raw Drugs.^[7]

S.no	Raw Drug	Method of Purification
1	Ayam	Powdered, finely soaked in lemon juice for three days. In addition, grinded well, washed and dried (Fig: 2)
2.	Chukku	Outer skin removed.
3.	Milagu	Cleaned, sorted, and dried.
4.	Thippili	Cleaned and dried.
5.	Kadukkai	Inner seed removed, washed and dried.
6.	Nelli Vatral	Inner seed removed, washed and dried.
7.	Thantrikai	Inner seed removed, washed and dried.
8.	Omam	Cleaned, sorted and dried.
9.	Vaividangam	Cleaned, sorted and dried.
10	Kodiveli Ver pattai	Washed thoroughly, cut into pieces, and then soaked in <i>chunna neer</i> (Limewater) for 1 <i>samam</i> (3 hours) Finally washed and dried again.
11	Korai kizhangu	Cleaned, sorted, washed and dried.
12	Panam Karkandu	Cleaned and sorted.



Fig. 2: Traditional Purification of Iron Powder.

b. Preparation of distillate sample

All the dried raw drugs were purified (Table 3) with reference to the *siddha* classical texts, powdered and mixed with prescribed quantity of water and kept for fermentation upto a period of 7 days. On the 8th day, the

whole mixture is distilled in *Valai iyanthram* (Traditional mud still) (Fig. 3). The distillates were collected and preserved well.



Fig. 3: Preparation of Distillate sample.

C. Study Methodology

C.1. Organoleptic characters^[8]

Physio chemical analysis starts with the observation of organoleptic characters. This provides first step information regarding the identity, purity and quality of the drug. Traditional quality parameters of a *siddha* distillate formulation are mostly expressed in terms of organoleptic characters which include color, consistency and nature, odor and taste of the distillate.

C.2. Volatile oil content

Volatile oils or essential oils are considered as the 'essence' of the herb material obtained during the first phases of hydro distillation. They are biologically active and are characterized by its peculiar odor or aroma, oil nature, volatility and pungency on taste. They are termed as *Theeneer ennai* that floats as a separate supernatant layer in the distillate collected.^[9] So determination of volatile content of the drug is an important step in the standardization of theeneer.

C.3. pH Values

pH of the drug is a crucial factor affecting the stability of the product. Stability denotes the product retainability within the specified limits or throughout its usage and storage period. pH determination values specifies the acidity or alkalinity of the solution particularly distillates as the alterations have a strong influence on health.

The procedures recommended for analysis of Clarity test, Volatile oil, pH values and Specific gravity are as per the guidelines of WHO.^[8]

C.4. Detection of specific toxic metals (Heavy metal Analysis)^[10,11]

Toxic materials that are hazardous to human health have been categorized widely under chemical contaminants. Out of this, Heavy metals Arsenic, Lead, Cadmium and Mercury are the most common contaminant occurring in

herbal resources. Contaminated herbal materials (due to environment pollution or pesticide usage) and its usage in various medications is a serious threat to the authenticity of the classical drugs in terms of its safety. Heavy metal analysis of any given drug sample is as important as drug standardization and safety assessment. The procedures recommended for analysis of Heavy metals are as per the guidelines WHO (1998).

Instrument details

Thermo Fisher M Series, 650902 V1.27 model Atomic Absorption Spectrometer (AAS) was used for the Heavy metal analysis. The Hollow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined.

C.5. Tests for specific microorganisms (Microbial Load Analysis)^[8]

Herbal materials are most viable for microbiological contamination due to large number of bacteria and fungi often sourced from the soil in which it grows or cultivated. Unscientific approaches of harvesting, collection, handling, transportation and storage may dispose to further contamination and microbial growth. These contaminants can be transferred to the finished goods in its various stages. These should not be present in the herbal formulations intended for internal usage.

Distillates are free from most of the impurities and basic contaminants but it is mandatory as per WHO to undergo microbial analysis as one of the steps to validate the purity of the drug sample. The screening for biological contaminants (Esp bacteria and fungi) in the drug is a very crucial need as it indicates whether the microbial level comply with the limits set in regional or international pharmacopoeias and in assessing its purity. Thereby the sample-Sanjeevi Theeneer has been screened for microbial loads with reference to the standards of WHO.

C.6. Preliminary Phyto chemical screening

Plants are the potent reservoir of high class biological compounds with wide range of pharmacological properties and some of it may include alkaloids, Flavanoids, tannins and phenolic compounds. With importance to herbal distillation, *Theeneer* selectively avails the bio active principles of the drug (Volatile and organic). An initial step of research will be very fruitful in finding the presence of active chemical components in the distillate. Thereby it may define the therapeutic outcome of the formulation in a surface level. The Classical poly herbo mineral distillate-*Sanjeevi theeneer* has been selected for phyto chemical screening to support the view.

Phyto chemical Assays^[12]

Preliminary phyto chemical studies for all the 12 compounds were carried out on the distillate *Sanjeevi theeneer* as per the standards.

1. Test for the presence of Alkaloid- Mayer's reagent

Principle: Most of the alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Potassio mercuric iodide solution (HgI_4K_2)). The presence of Alkaloids is indicated by a cream or dull white precipitate. To the test drug, about 2ml of Mayer's reagent was added and was observed for the presence of Alkaloids.

2. Test for the presence of flavonoids

Principle: First addition of dilute ammonia to the test sample then followed by the addition of Concentrated sulphuric acid (few drops) will indicate a yellowish coloration that disappears on standing confirms the presence of Flavonoids. To 0.1ml of the test sample about 5 ml of dilute ammonia solution were added followed by addition of few drops of conc. Sulphuric acid.

3. Test for the presence of Glycosides -Borntrager's Test

Principle: Test drug is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink color indicates presence of Glycosides.

4. Test for the presence of Triterpenoids - Salkowski test

Principle: To 2ml of the test solution 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml) through the side of the test tube. An interface with a reddish brown coloration is formed if Terpenoids constituent is present.

5. Test for the presence of Steroids - Salkowski test

Principle: To the test solution, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube turns into

red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of Steroids. (Joseph et al., 2013).

6. Test for the presence of Carbohydrates - Benedict's test

Principle: The Benedict's test allows us to detect the presence of reducing sugars (sugars with a free aldehyde or ketone group). The final color of the solution depends on how much of this precipitate was formed, and therefore the color (Green, Orange, Red, and Brown) gives an indication of how much reducing sugar was present. To 0.5 ml of test drug, about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of Sugar.

7. Test for the presence of Phenol- Lead acetate test

Principle: The test sample is dissolved in distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of Phenolic compounds.

8. Test for the presence of tannins (Ferric Chloride Test)

Principle: About 0.5ml of test sample is boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. 0.1% $FeCl_3$ is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of Tannins.

9. Test for the presence of Saponins (foaming Test)

Principle: Demonstration of Frothing: Herbal material containing Saponins can cause persistent foam when an aqueous decoction or distillate is shaken. The test drug were shaken vigorously for 10 mins, and observed for lather formation.

10. Test for the presence of Proteins (Biuret Test)

Principle: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple color indicates the presence of proteins and free amino acids (Boxi et al., 2010).

11. Test for the presence of Coumarins

Principle: 1 ml of test drug, 1 ml of 10% sodium hydroxide was added. The presence of Coumarins is indicated by the formation of yellow color.

12. Test for the presence of Anthocyanin (Sodium Hydroxide Test)

Principle: About 0.2 ml of the extract was weighed in separate test tube; 1ml of 2N Sodium hydroxide was added, and heated for 5 minutes at $100 \pm 2^\circ C$. Observed for the formation of bluish green color, which indicates the presence of Anthocyanin.

C.7 GC-MS Screening for Biologically active compounds^[13,14]

Gas Chromatography- Mass Spectroscopy Analysis (GC-MS) is a well reputed analytical tool for the screening of unknown compounds of the liquid drug sample. Selective active compound of herbal distillate that are responsible for the significant pharmacological properties of the drug can be identified.

III. RESULTS AND DISCUSSION

Sanjeevi Theeneer is a clear aqueous distillate, light lemon yellow colored with pleasant aroma and mild pungent taste. The analytical part reports the percentage of volatile content and the pH specifying alkaline nature of the distillate (Table no. 4). The standards are promising as per the traditional quality parameters. The heavy metal and microbial load presence were within the permissible limits (Table. 5 & 6). Both the results indicate the nontoxicity and purity of the distillate-*Sanjeevi theeneer*.

Qualitative analysis of phytochemical variables in *Sanjeevi theeneer* reports the presence of Phenolic compounds (Table 7). Phenolic compounds are considered as the most active and predominant group of metabolites derived from the herbal resources (Singh 2007). Natural compounds having Anti-oxidant properties are mainly derived from the plants as phenolic compounds, phenolic acids, tocopherols and Flavanoids ((Ali *et al.*, 2008). Phenols exhibit wide range of pharmacological and biological functions especially considering the cardiovascular health. Their role in prevention of atherosclerosis (Anti-atherosclerotic) and inflammation (Anti-inflammatory) and also in improving

endothelial function has been well established. Various studies prove it as an Anti-carcinogenic and Anti-ageing compound.

GCMS analysis reported the presence of major compound Oleic acid (% Peak area: 40.85), Dasycarpidan (% Peak area: 1.98), Thymol (% Peak area: 34.32) and other secondary metabolites like linalool which may have significant biological properties (Fig. 4) & (Table 8, 9, 10). Oleic acid is a mono unsaturated fat with proven cardio vascular effects. So many studies validates oleic acid as a Cardiac tonic ((Lahey *et al.* 2014), Hypo cholestaremic, Anti- atherosclerotic, High density lipoprotein (HDL) enhancer (Ruiz-Gutiérrez *et al.* 1996) and Vaso dilator. The role of oleic acid in inhibiting the functions of platelets and platelet aggregation makes it as an important component for the prevention of Ischemic Heart diseases.^[15,16]

Thymol, are naturally occurring monocyclic phenolic compounds with widest range of biological activities has been proven for its Anti-inflammatory, Anti-allergic properties,^[17] Anti- Oxidant, and Hepato protectivity^[18] The compound Dasycarpidan is an active Anti- Oxidant and Anti- Inflammatory agent.^[19] The compound 3,7-dimethyl-1,6-octadien-3-ol or linalool is a naturally occurring terpene alcohol present in essential oils of spices and also in numerous fruits having significant Anti -Inflammatory and Hypolipidemic effects.^[20] *Sanjeevi Theeneer*, the distillate medicine with its therapeutic potential in Respiratory diseases, Liver diseases, Heart diseases and moreover as an Anti-oxidant is may be due to the activity of above screened valuable compounds.

Table 4: Analytical Reports of *Sanjeevi Theeneer*.

S.no	Parameters		Results
a.	Description		
	1	Color	Light lemon yellow.
	2	Odor	Pleasant and highly aromatic.
	3	Taste	Pleasant and mild pungent.
b.	Clarity test		Confirmed / Complies
c.	Volatile Oil		1.320%, 1.332%, 1.324%
d.	Specific gravity at 25°C		0.1622, 0.1624, 0.1626
e.	pH values		7.6

Table 5: Heavy Metal Analysis (HMA) - Reports of *Sanjeevi Theeneer*.

S. no	Heavy metal	Reference Limits as per API- Vol.-I	Results	Remarks
1.	Lead	Not more than 10ppm	Not detected	Within permissible limits
2.	Arsenic	Not more than 3.0ppm	3.1408ppb	Within permissible limits
3.	Cadmium	Not more than 0.3ppm	0.0073ppm	Within permissible limits
4.	Mercury	Not more than 1.0ppm	14.2894ppb	Within permissible limits

Table 6: Microbial Load Analysis - Reports of Sanjeevi Theeneer.

S.no	Parameters	Reference Limits as per WHO (2007)	Results	Remarks
1.	Total Bacterial Count (TBC)	10 ⁵ CFU/gm	Less than 10 cfu/ml	Within permissible limits
2.	Total Fungal Count (TFC)	10 ³ CFU/gm	Absent	
3.	Enterobacteriaceae	10 ³	Absent	
4.	<i>Escherichia coli</i>	10	Absent	
5.	Salmonella Spp	Absent	Absent	
6.	<i>Staphylococcus aureus</i>	Absent	Absent	

Table 7: Results of Phytochemical Analysis of Sanjeevi Theeneer.

S.no	Test	Observation	Result
1	Test for the presence of Alkaloid- Mayer's reagent	(-) No dull white precipitate	Absence of alkaloids
2	Test for the presence of Flavanoids	(-) No yellowish Discoloration	Absence of Flavanoids.
3	Test for the presence of Glycosides -Borntrager's Test	(-) No Pink color	Absence of Glycosides.
4	Test for the presence of Triterpenoids - Salkowski test	(-) No Reddish or yellowish Color formation	Absence of Steroids.
5	Test for the presence of Steroids - Salkowski test	(-) No colored precipitate.	Absence of Sugars
6	Test for the presence of Carbohydrates - Benedict's test	(-) No reddish Brown discoloration	Absence of Terpenoids
7	Test the presence of Phenol-Lead acetate test	(-) No Yellow color formation	Absence of Coumarins.
8	Test for the presence of tannins (Ferric Chloride Test)	(+) A bulky white Precipitate Present	Presence of Phenols
9	Test for the presence of Saponins (foaming Test)	(-) No Brownish green or Blue Black coloration	Absence of Tannins
10	Test for the presence of Proteins (Biuret Test)	(-) No Lather formation	Absence of Saponins
11	Test the presence of Coumarins	(-) No Pink or Purple color	Absence of Proteins
12	Test for the presence of Anthocyanin (Sodium Hydroxide Test)	(-) No formation of Bluish green color	Absence of Anthocyanin

ST = Sanjeevi Theeneer. (+) = Positive, (-) = Absence

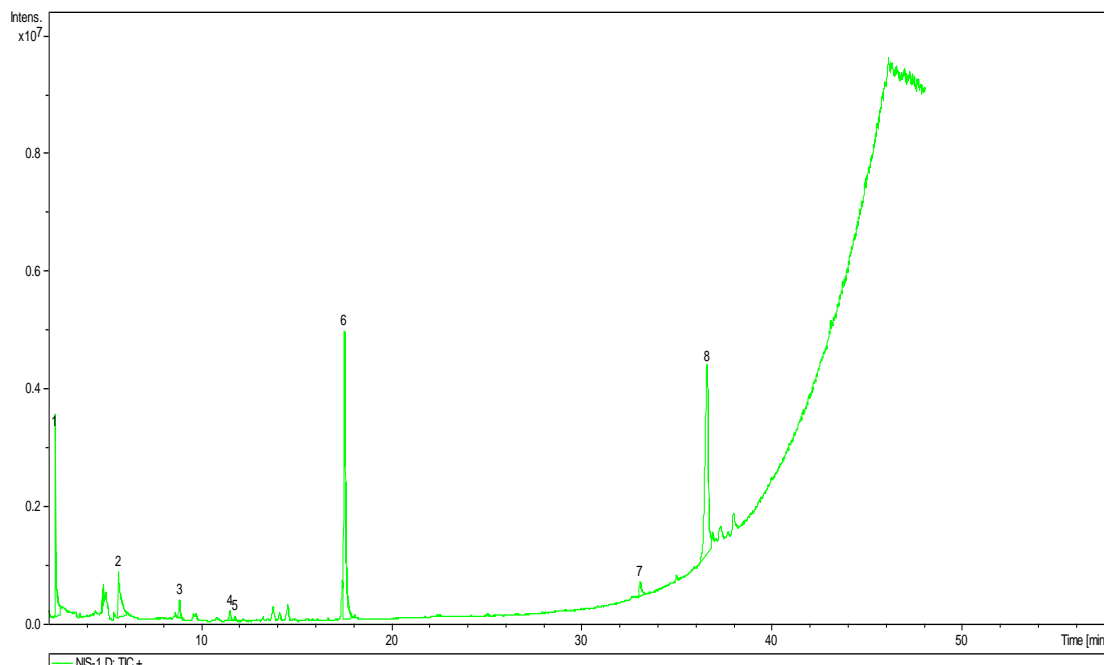


Fig. 4: GC-MS chromatograph of Sanjeevi Theeneer.

Table 8: Gas Chromatograph Report of Sanjeevi Theeneer.

Peak no	Retention Time	%Peak Area	Peak Intensity Rank	Mol. Wt	Name of the Compound	Chemical Formula
1	2.4	8.44	3	78	Dimethyl Sulfoxide (Solvent Peak)	C ₂ H ₆ OS
2	5.7	7.46	4	78	Dimethyl Sulfoxide (Solvent Peak)	C ₂ H ₆ OS
3	8.9	1.53	5	296	Benzoic acid, 5-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	C ₁₄ H ₂₄ O ₃ Si ₂
4	11.6	0.91	7	132	Benzene, 1-methyl-4-(1-methylethenyl)-	C ₁₀ H ₁₂
5	11.8	4.8	8	154	1,6-Octadien-3-ol, 3,7-dimethyl	C ₁₀ H ₁₈ O
6	17.6	34.32	1	150	Thymol	C ₁₀ H ₁₄ O
7	33.0	1.98	6	326	Dasympidan-1-methanol, acetate (ester)	C ₂₀ H ₂₆ N ₂ O ₂
8	36.5	40.85	2	282	Oleic Acid	C ₁₈ H ₃₄ O ₂

Table 9: Bioactive compounds of *Sanjeevi Theeneer*.

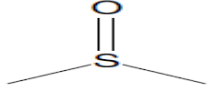
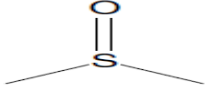
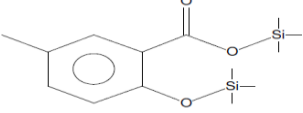
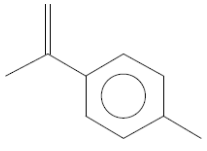
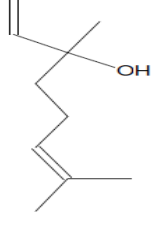
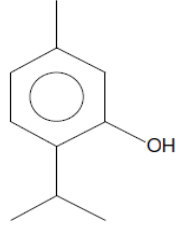
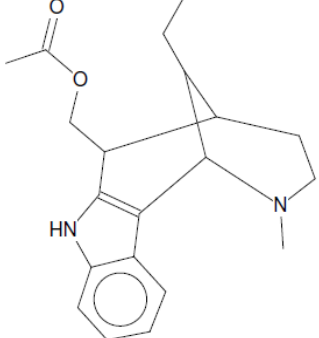

<i>Dimethyl Sulfoxide</i>	<i>Dimethyl Sulfoxide</i>	<i>Benzoic acid, trimethylsilyloxy-, 5-methyl-2-trimethylsilyl ester</i>
		
<i>Benzene, methylethenyl)-1-methyl-4-(1-</i>	<i>1,6-Octadien-3-ol, 3,7-dimethyl-</i>	<i>Thymol</i>
		
<i>Dasycarpidan-1-methanol, acetate (ester)</i>		<i>Oleic Acid</i>
		

Table 10: Compounds reported from GC- MS Analysis -*Sanjeevi Theeneer* and its pharmacology.

S.no	Compounds	Pharmacological importance/Uses.
1.	Oleic Acid ^(15, 16)	<p>* Cardio vascular Effects</p> <ol style="list-style-type: none"> 1. Prevention of Ischemic heart diseases/ Cardio protective. 2. Inhibition of platelet function and aggregation. 3. Hypocholesteremic 4. High density lipoprotein (HDL) enhancer. (Ruiz-Gutiérrez et al. 1996). 5. Vaso dilator effects 6. Cardiac Tonic (Lahey et al. 2014). 7. Anti-atherosclerotic. <p>* Effects on body fat (Lim et al. (2013)³)</p> <ol style="list-style-type: none"> 1. Increases fat oxidation in muscle cells. 2. Increases expression of genes responsible for fat oxidation. <p>* Effects in GIT (de Silva et al. 2014).</p> <p>Prevention of Ulcerative colitis</p> <p>* Effects in Brain</p> <p>Reduce age related changes in brains mitochondria (Ochoa et al. 2011)</p>
2.	Thymol ^(17, 18)	<ol style="list-style-type: none"> 1. Anti-inflammatory 2. Anti-asthmatic 3. Anti-microbial 4. Neuro protective 5. Anti-fungal 6. Anti-bacterial

		7. Hepato protective 8. Gastro protective 9. Cardio protective 10. Anti-hyperglycemic 11. Anti hyperlipidemic 12. Anti-oxidant
3.	Dasycarpidan-1-methanol, acetate (ester) ⁽¹⁹⁾	1. Anti-oxidant 2. Anti-microbial 3. Anti-inflammatory
4.	1,6-Octadien-3-ol, 3,7-dimethyl-(Linalool) ⁽²⁰⁾	1. Anti-inflammatory 2. Anti-bacterial 3. Anti-fungal 4. Spasmolytic 5. Anti-oxidant 6. Hypolipidemic

IV. CONCLUSION

Sanjeevi theeneer prepared as per the classical literature were meeting the quality standards with reference to both traditional and scientific parameters. The qualitative phyto chemical analysis part supports the therapeutic value of the drug and GC-MS studies revealed its wide range of Pharmacological activity. Qualitative assays vary with the distillate samples. The percentage of each phytochemicals in a sample distillate depends on the quality of the raw drug used, the major active compounds present it and the methods used to extract it. By developing standards in *Theeneer* preparation, the quality and efficacy of the distillates can be further improved by maximum extraction of volatile and organic compounds. Further in-depth researches should be carried out to set the common standards of this *Theeneer* preparation, and for promoting it as a safe and potent drug for wide range of diseases preclinical (Toxicological and pharmacological) evaluations should be carried out.

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