Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 3, https://carcinogenesis.com/

Evaluation of Phytochemical, Physiochemical, And Microbial Safety of The Siddha Formulation Venpoosani Legiyam: A Scientific Validation of Its Traditional Application

AnavarathanVallipuram[1], Rajappan Chandra Satish Kumar[2,*3], Bhuvaneshwaran Mothishwaran[2], Chittaranjan Das[3], Patana Teng-umnuay[4], Balasubramanian Swetha[2], Anoop A S[3]

[1]Faculty of Siddha Medicine, Trincomalee Campus, Eastern University, Sri Lanka.

[2]Clinical Trial and Research Unit, Interdisciplinary Institute of Indian System of Medicine, SRM Medical College Hospital and Research Centre, SRM Institute of Science and Technology, Chennai, Tamilnadu, India.

[3]Sri Jayendra Saraswathi Ayurveda College and Hospital, Sri Chandrashekarendra Viswa Vidyalaya, Kanchipuram, 600 123, Tamil Nadu, India.

[4]Anti-aging and regenerative program of Dhurakij Pundit University, Thailand.

ABSTRACT

Background: Traditional Siddha Medicine Venpoosani Legiyam is used for its supposed health benefits, especially in dealing with oxidative stress, inflammation, and other connected conditions. Through in-depth biochemical, phytochemical, physiochemical, and microbiological tests, this study aims to give medicine a scientific base for its possible use.

Methodology: Alkaloids, flavonoids, and glycosides were found in phytochemical screening, whereas biochemical analysis tested for important components and chemicals. Stability was determined by physiochemical study of moisture, total ash, and extractive values. Microbial load and aflatoxin testing followed WHO requirements for safety, testing for Escherichia coli, Salmonella spp., and Staphylococcus aureus.

Results: Biochemical and phytochemical analyses showed high quantities of antioxidant and anti-inflammatory flavonoids and phenols. Physiochemical tests showed a stable composition with appropriate moisture and ash levels. Microbial investigation showed no infections or aflatoxins, and bacterial and fungal levels met WHO guidelines.

Conclusion: Venpoosani Legiyam appears to be safe and stable, having bioactive components supporting its traditional medicinal use. Lack of pollutants and quality requirements proves its suitability for ingestion. This study supports Venpoosani Legiyam's complementary therapeutic potential, with future clinical trials required to assess its efficacy in modern healthcare.

Keywords: *Venpoosani Legiyam, Siddha Medicine, Polyherbal formulation, biochemical, phytochemical, physiochemical, analysis analysis*

How to Cite: AnavarathanVallipuram, Rajappan Chandra Satish Kumar, Bhuvaneshwaran Mothishwaran, Chittaranjan Das, Patana Teng-umnuay, Balasubramanian Swetha, Anoop A S, (2025) Evaluation of Phytochemical, Physiochemical, And Microbial Safety of The Siddha Formulation Venpoosani Legiyam: A Scientific Validation of Its Traditional Application, *Journal of Carcinogenesis*, *Vol.24, No.3*, 264-272

1. INTRODUCTION

Siddha comes from Siddhi, meaning to achieve celestial delight. The method has long worked in India. [1] This system has declined due to changing lifestyles and modern medicine. [2] It stays popular with most people because it has unique qualities that make it appealing. Siddha medicine can treat all kinds of illnesses, especially long-term ones that even the most powerful modern medicine can't figure out.[3] The body's physiological processes are mediated and maintained by three forces. The physiological processes of the body are supported and maintained by three forces. Azhal (Pitham), Vali (Vatham), and Aiyam (Kapam) are the beings. The terms enclosed in parenthesis are used in both Ayurveda and Siddha.[4] Eighteen prominent Siddhars developed this medicinal methodology in the past to enhance public health. Consequently, it is referred to as the Siddha medical system. Siddhars argued that a robust physique was essential for nurturing a sound

spirit.[5] They developed drugs and procedures to improve their physical bodies and, so, their souls. Venpoosani Legiyam is a classic Siddha dish comprising various medicinal herbs. These herbs—which have possible anti-inflammatory, detoxifying, and antioxidant qualities—include Cocos nucifera (coconut), Pandanus odoratissimus (screw pine), and Benincasa hispida (winter melons).[6] Herbal drugs, which are prone to contamination from microbial and aflatoxin sources, must be guaranteed in safety by standardized testing approaches. We look at Venpoosani Legiyam's elemental composition, microbial load, and aflatoxin levels using a thorough biochemical, phytochemical, and physiochemical study.[7] The objective of this investigation is to verify the medicinal properties of Venpoosani Legiyam by integrating contemporary scientific standards with traditional knowledge. This validation substantiates its potential for safe, standardized clinical applications.

2. PROPERTIES OF VENPOOSANI LEGIYAM

S.NO.	INGREDIENTS	QUANTITY
	VenpoosanikaaiChaaru – (Benincasa hispida)	5200 ml
	ThaalaivizhuthuChaaru – (Pandanus odoratissimus)	1300 ml
	Thennam poo Chaaru – (Cocos nucifera)	1300 ml
	PazhaChaaru – (Citrus limon)	1300 ml
	PasuPaal (Milk)	2600 ml
	Sarkarai (Sugar) - (Saccharum officinarum)	2600 ml
	Seerakam - (Cumiuscyminum)	350 g
	Koththumalli - (Coriandrumsativum)	35 g
	Kostam - (Costus speciosus)	35 g
	Milaku - (Piper nigram)	35 g
	Maasikkaai - (Quercus infectoriaolivier)	35 g
	Elam - (Elettaria cardamomum)	35 g
	Saathikkai	35 g
	Saathipathiri	35 g
	Athimathuram	35 g
	Thaalisam	35 g
	Nei	650 ml
	Thenn	325 ml

Table 1. Ingredients of Venpoosani Legiyam

3. METHODOLOGY

Objectives:

- To conduct a biochemical examination of the Investigational product.
- Physiochemical and phytochemical investigation of Investigational product.
- To conduct Aflotoxin analysis and microbial upload investigations for investigational product.

A. Biochemical Evaluation:

Experimental procedure: A 250 ml clean beaker was filled with 5 grams of Venpoosani Legiyam with 50ml of pure water. After that, it boiled well for ten minutes. Following that, it is cooled and filtered in a 100 ml volumetric flask. Distilled water is added to make it up to 100 ml. Mixture is used to look at the acidic and basic radicals and biological parts in it in a qualitative way. 5gm Venpoosani Legiyam was carefully weighed and placed in a 250ml clean beaker with 50ml distilled water. Then it boiled well for 10 minutes. Let it cool, filter it in a 100ml volumetric flask, and add 100ml distilled water. The National Institute of Siddha Biochemistry Lab in Chennai-47 analyzed Venpoosani Legiyam.

Preliminary test for Copper, Sodium, Silicate, and Carbonate: Test for Silicate, Action of Heat, Flame Test, and Ash Test.

- Test For Acid Radicals: Test for Sulphate, Test for Chloride, Test for Phosphate, Test for Carbonate, Test for Nitrate, Test for Sulphide, Test for Fluoride & Oxalate, Test for Nitrite.
- Test For Basic Radicals: Test for Lead, Test for Copper, Test for Aluminium, Test for Iron, Test for Zinc, Test for Calcium, Test for Magnesium, Test for Ammonium, Test for Potassium, Test for Sodium, Test for Mercury, Test for Arsenic.
- Miscellaneous: Test for Starch, Test for Reducing Sugar, Test for the Alkaloids, Test for Tannic Acid, Test for Unsaturated Compound, Test for Amino Acid.

B. Preliminary Phytochemical Screening Venpoosani Legiyam:

Following the standard method, the first phytochemical screening test was done for detecting alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins, amino acids, diterpenes, fixed oils, fats, and quinones in each extract of Venpoosani Legiyam.

C. Physiochemical Analysis of -Venpoosani Legiyam:

- Loss On Drying
- Determination of total ash
- Determination of acid-insoluble ash
- Determination of water-soluble ash
- Determination of alcohol soluble extractive

D. Examination of Microbial Load:

The Regional Research Institute of Unani Medicine, RRIUM, Rayapuram, Chennai 13, determined the microbial load on the sample in accordance with WHO criteria (Anonymous 2007).

Procedure:

- Plate Count: For bacteria, add 1 ml of pre-treated herbal material to 15 ml casein-soybean digest agar at 45°C max. Incubate at 30-35°C for 48-72 hours and count colonies, using plates with up to 300 colonies. For fungi, use Sabouraud dextrose agar with antibiotics, incubate at 20-25°C for 5 days, and count up to 100 colonies.
- Coli: Transfer 1 g/ml of homogenized material into lactose broth, then to MacConkey agar, and incubate at 43-45°C for 18-24 hours. Red colonies indicate possible E. coli, confirmed with indole or biochemical tests. The material passes if no colonies or negative reactions occur.
- Salmonella: Incubate pre-treated material at 35-37°C for 5-24 hours for enrichment. For the primary test, culture in tetrathionate broth and subculture on selective agar, then incubate at 35-37°C for 24-48 hours. The presence of Salmonella is indicated by gas and color change in triple sugar iron agar, confirmed with biochemical and serological tests.

- Staphylococcus aureus: Enrich culture as for *Pseudomonas aeruginosa* and subculture on Baird-Parker agar. Incubate at 35-37°C for 24-48 hours. Black colonies with clear zones suggest *S. aureus*, confirmed with catalase, coagulase, or DNase tests.

E. Test for Aflatoxin:

The procedures recommended for the detection of Aflatoxin as per WHO guidelines (WHO 2007). The instrument used is a CAMAG Automatic TLC Sampler, Scanner, and Visualizer, utilizing nitrogen (N_2) as the spray gas and equipped with both deuterium and tungsten lamps. Thin Layer Chromatography and High-Performance Thin Layer Chromatography were performed on the samples using acceptable solvent systems. The plate was air-dried and inspected under UV 366nm after development. The sample (8µl) and Standard (20µl) were placed to a TLC aluminum sheet silica gel 60 F 254 (E. MERCK) and developed using the solvent system Chloroform: Acetone: Water (14: 2: 0.2). The plate was air-dried and inspected under UV 366 nm after development.

4. RESULTS

I. BIO-CHEMICAL AND ELEMENTAL ANALYSIS OF VENPOOSANI LEGIYAM

Venpoosani Legiyam's biochemical study revealed chloride, phosphate, reducing sugar, iron, tannic acid, oxyquinolone, epinephrine, pyrocatechol, unsaturated molecules, and alkaloids. The analyses indicate that the sample is devoid of numerous common acidic and basic radicals, demonstrating its chemical stability and purity. It is important to highlight that silicate, carbonate, copper, sodium, lead, aluminium, zinc, magnesium, ammonium, potassium, mercury, and arsenic are absent. Identified trace elements include chloride and iron, alongside mild levels of alkaloids and tannic acid, indicating potential bioactive properties. The sample demonstrates the presence of unsaturated compounds, which could play a role in its therapeutic effects. The lack of starch, reducing sugars, and amino acids, combined with the presence of oxyquinoline, epinephrine, and pyrocatechol, underscores a distinctive biochemical profile that supports traditional medicinal applications. The results indicate a stable, pure sample containing selective bioactive compounds, consistent with its intended therapeutic application.

PHYTOCHEMICAL ANALYSIS:

The phytochemical analysis reveals a diverse composition in the sample, including carbohydrates, glycosides, phytosterols, phenols, flavonoids, proteins, diterpenes, and fixed oils. The presence of carbohydrates and proteins suggests nutritional value, while glycosides, flavonoids, and phenols offer antioxidant and anti-inflammatory properties. Phytosterols and diterpenes add potential for cholesterol-lowering and pharmacological effects, respectively. The absence of alkaloids and tannins suggests a mild phytochemical profile, with quinones, gums, and mucilage further enhancing the sample's therapeutic potential. This composition indicates the sample's suitability for various health-promoting applications.[8]

S. No	Phytochemicals	Test Name	H_2O ext.
1.	Alkaloids	Mayer's Test	**Negative**
		Wagner's Test	**Negative**
		Dragendroff's Test	**Negative**
		Hager's Test	**Negative**
2.	Carbohydrates	Molisch's Test:	**Positive**
		Benedict's Test	**Positive**
		Fehling's Test	**Negative**
3.	Glycoside	Modified Borntrager's Test	**Positive**
		Cardiac glycoside (Keller-Killiani test)	**Positive**
		Legal's Test	**Positive**

4.	Saponin	Froth Test	**Negative**
		Foam Test	**Positive**
5.	Phytosterol	Salkowski's Test	**Positive**
6.	Phenol	Ferric Chloride Test	**Positive**
7.	Tannins	Gelatin Test	**Negative**
8.	Flavonoids	Alkaline Reagent Test	**Positive**
		Lead acetate Test	**Positive**
9.	Proteins and amino acids	Xanthoproteic Test	**Positive**
		Ninhydrin Test	**Negative**
		Biuret test	**Positive**
10.	Diterpenes	Copper Acetate Test	**Positive**
11.	Gum and Mucilage	Extract + alcohol	**Positive**
12.	Fixed oils and Fats	Spot test	**Positive**
13.	**Quinones**	**NAOH + Extract**	**Positive**

Table 2. Test for Phytochemical analysis

II. PHYSIOCHEMICAL ANALYSIS OF -VENPOOSANI LEGHEYAM

The sample's analysis indicates a loss on drying of 11.09%, reflecting its moisture content and suggesting moderate stability. The total ash value of 1.49% shows the inorganic residue remaining after incineration, with acid-insoluble ash and water-soluble ash both under 1%, suggesting minimal non-dissolvable and water-dissolvable minerals. High water-soluble extraction at 54.84% suggests significant water-soluble components, likely contributing to therapeutic effects. The alcohol-soluble extraction at 35.08% indicates the presence of alcohol-soluble compounds, which may include bioactive phytochemicals beneficial for health applications. This profile suggests a stable sample with a rich composition of water and alcohol-soluble constituents.[9]

S.No.	**Parameters**	**Percentage**
1	Loss on drying	**11.09%**
2	Total ash value	**1.49%**
3	Acid insoluble ash	**Less than 1%**
4	Water soluble ash	**Less than 1%**
5	Water soluble extraction	**54.84%**
6	**Alcohol soluble extraction**	**35.08%**

Table 3. Test for Physiochemical Analysis

III. DETERMINATION OF MICROBIAL LOAD

S. No.	Parameters	Reference Limits as per WHO (2007)	Results	Remarks
1	**Total Bacterial Count (TBC)**	10^5CFU/gm	3x10^3cfu/ml	**Within permissible limits**
2	**Total Fungal Count (TFC)**	10^3CFU/gm	Less than 10 cfu/ml	
3	**Enterobacteriaceae**	10^3	Absent	
4	***Escherichia coli***	10	Absent	
5	**Salmonella Spp**	Absent	Absent	
6	***Staphylococcus aureus***	**Absent**	**Absent**	

Table 4. Determination of Microbial Load

IV. AFLATOXIN ANALYSIS TEST:

Similar Rf values were seen in the standard and test sample, upon derivatization with isopropyl alcohol and conc. H_2SO_4 (9:1), the band colour changed from bluish green to yellow in Track 1 (standard), whereas no colour change was seen in Track 2 (TS), which indicated the absence of aflatoxins in the Test sample.

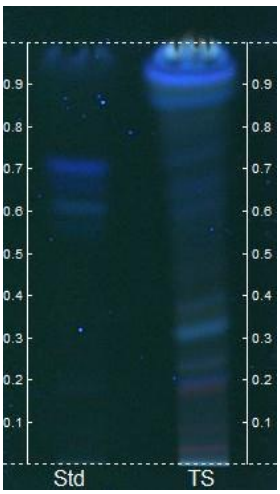

Figure.1 UV-366nm

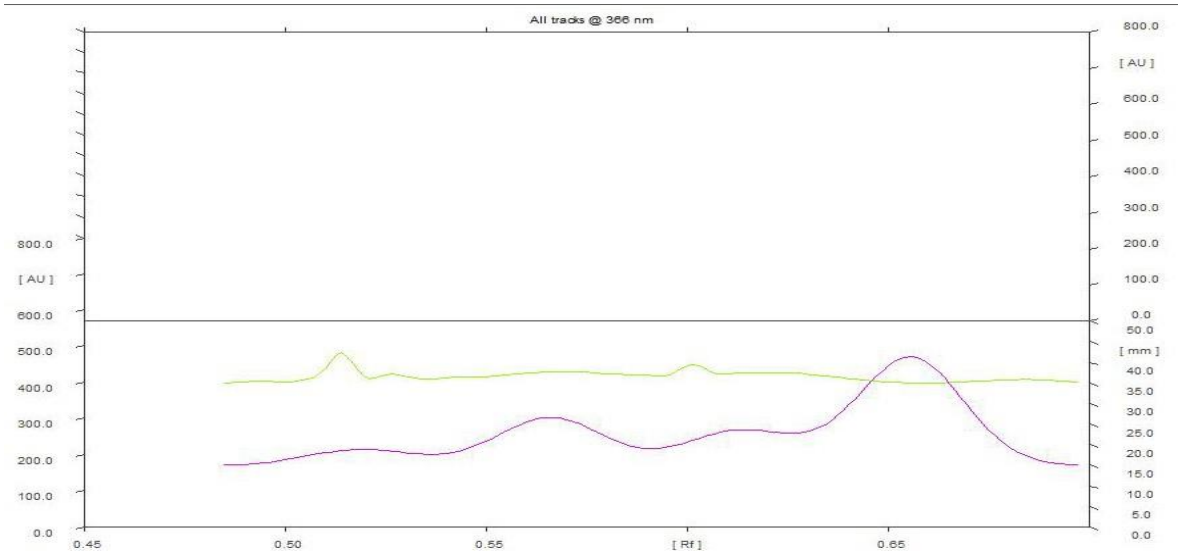

Figure.2 Densitometric chromatogram at UV-366nm Standard – G2, G1, B2 & B1; Test sample

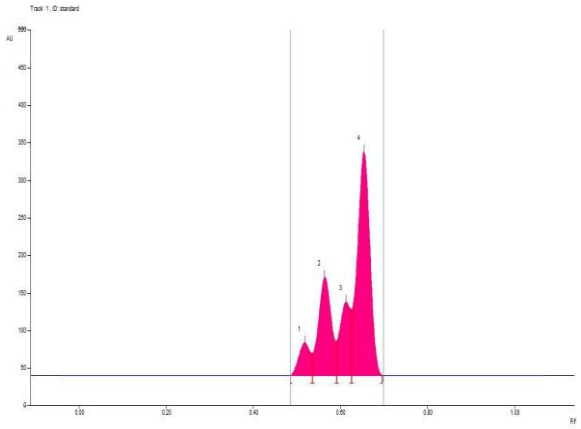

Figure.3 HPTLC fingerprint of Standard at 366nm

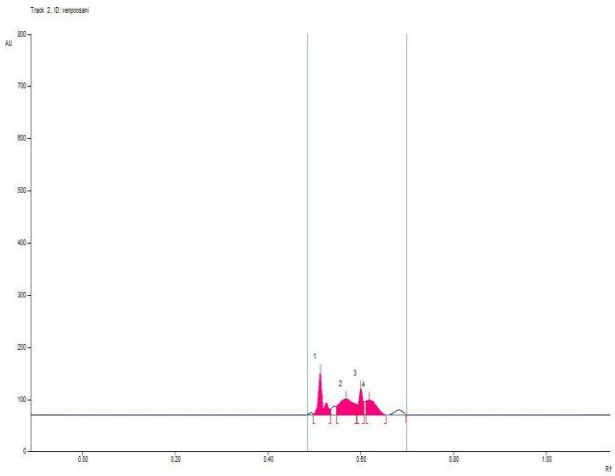

Figure.5 HPTLC fingerprint of Test at 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.50 Rf	2.1 AU	0.51 Rf	83.1 AU	43.08 %	0.54 Rf	10.9 AU	702.9 AU	28.90 %
2	0.55 Rf	16.5 AU	0.57 Rf	30.3 AU	15.69 %	0.59 Rf	20.1 AU	796.4 AU	32.75 %
3	0.59 Rf	19.7 AU	0.60 Rf	51.3 AU	26.59 %	0.61 Rf	23.9 AU	399.7 AU	16.44 %
4	0.61 Rf	26.3 AU	0.62 Rf	28.2 AU	14.64 %	0.66 Rf	0.0 AU	533.0 AU	21.92 %

Figure.6 Rf value of Test at 366nm

5. DISCUSSION

This study investigates the traditional Siddha formulation, Venpoosani Legiyam, to determine its detailed biochemical composition, physiochemical stability, and microbiological safety. Biochemical and elemental analyses reveal the presence of active components such as alkaloids, iron, tannic acid, and unsaturated compounds, all of which contribute to the formulation's therapeutic properties. Iron is a crucial element in cellular respiration and may improve hematological activity, in line with the traditional applications of herbal remedies to promote blood health.[10] As alkaloids and tannic acid are present, it is possible that the plant has astringent and anti-inflammatory properties that could help treat metabolic and gastrointestinal problems. The phytochemical study shows that Venpoosani Legiyam has many secondary metabolites,

such as sugars, glycosides, phenols, flavonoids, phytosterols, and diterpenes. The fact that Venpoosani Legiyam contains flavonoids and glycosides, two substances that have been shown to be antioxidants, suggests that it may help lower oxidative stress, which is a factor in many chronic diseases like metabolic and cardiovascular conditions. Phytosterols and diterpenes are known for their ability to lower cholesterol and reduce inflammation, so Venpoosani Legiyam may also be helpful as an extra treatment for dyslipidemia and the heart problems that come with it.

The potential stability and solubility of the formulation are underscored by its physiochemical characteristics, which include a moisture content of 11.09% (which is lost during drying) and 54.84% water-soluble extractives. The shelf-life of a product is contingent upon its low moisture content, which reduces the risk of microbial development and destruction. Furthermore, high water solubility may enhance the bioavailability of water-soluble phytochemicals, thereby facilitating the formulation's absorption upon use. His total ash and acid-insoluble ash values, which are less than 2%, indicate low inorganic matter levels. This indicates that the manufacturing process was effective in reducing soil contamination from extraneous plant materials. Microbial load testing guarantees that the formulation adheres to the WHO safety criteria by assessing the presence of pathogenic organisms, including Escherichia coli, Salmonella spp., and Staphylococcus aureus, in conjunction with appropriate bacterial and fungal counts. These findings underscore the quality and safety of Venpoosani Legiyam for internal use, as microbial contamination is a significant concern for immunocompromised individuals and can lead to gastrointestinal and systemic maladies. The absence of aflatoxins serves as confirmation of the formulation's safety, as aflatoxins are highly carcinogenic mycotoxins that are frequently encountered in botanical products when stored improperly.

Venpoosani Legiyam presents promise as an adjuvant in controlling oxidative stress, inflammation, and maybe metabolic dysregulation, given its rich phytochemical makeup and adherence to safety criteria.[11] The therapeutic assertions made in ancient Siddha medicine are supported by the presence of bioactive substances, including alkaloids and phenolic compounds. Future research is required, nevertheless, to clarify the exact processes by which these drugs work, as well as clinical trials to prove efficacy and safety in different populations. This study usually endorses Venpoosani Legiyam's potential as a traditional herbal medication with several pharmacologically advantageous ingredients.[12] By combining conventional formulations with thorough scientific validation, Siddha medicine and evidence-based practice can come closer together and provide healthcare professionals with uniform choices for supplemental therapy. Standardized dose and formulation changes to enhance bioavailability and therapeutic efficacy for clinical application should potentially be investigated further.

6. CONCLUSION

This study supports the Siddha formulation, Venpoosani Legiyam, as a possible complementary therapy by conducting extensive biochemical, phytochemical, physiochemical, and microbiological investigations. The formulation was discovered to include beneficial bioactive chemicals such as flavonoids and glycosides, which support its traditional use for reducing oxidative stress and inflammation. Physiochemical examinations suggest stability, and microbiological testing demonstrates safety, with results that match WHO requirements. The lack of aflatoxins and pathogens enhances its quality and acceptability for food. These findings pave the way for future clinical trials to demonstrate its efficacy, connecting Siddha therapy and evidence-based healthcare.

REFERENCES

[1] Subbarayappa BV. Siddha medicine: an overview. Lancet. 1997 Dec 20-27;350(9094):1841-4. doi: 10.1016/s0140-6736(97)04223-2. PMID: 9428267.

[2] Thas JJ. Siddha medicine--background and principles, and the application for skin diseases. Clin Dermatol. 2008 Jan-Feb;26(1):62-78. doi: 10.1016/j.clindermatol.2007.11.010. PMID: 18280906.

[3] Piet J.H.A.: In (eds): Logical Presentation of the Saiva Siddhanta Philosophy. Madras: Christian Literature Society for India, 1952.

[4] Narayansami V.: In Pandit S.S (eds): Introduction to the Siddha System of Medicine. T. Nagar, Madras: Anandam Research Institute of Siddha Medicine, 1975.

[5] Stephen E.S.: Complementary and alternative medicine. In Dennis L.K., Eugene B., Anthony S.F., Stephen L.H.D., and Llarry J.L. (eds): Harrisons Principles of Internal Medicine, 16th ed. pp. 67.

[6] Yadav M, Jain S, Tomar R, Prasad GB, Yadav H. Medicinal and biological potential of pumpkin: an updated review. Nutr Res Rev. 2010 Dec; 23(2):184-90. doi: 10.1017/S0954422410000107. PMID: 21110905.

[7] G. Ramakrishnan et al, Physiochemical and phytochemical standardization of Thraatchathi Chooranam - A poly herbal siddha formulation. j.pharm.sci. & Res. Vol.7 (6), 2015, p.305-13

[8] Raaman N, (2006), Phytochemical Techniques, New India Publishing Agency, New Delhi, p.19-23.

[9] S. Iyswarya, Physicochemical Evaluation and Phytochemical Screening of Siddha formulation, Narasinga Rasayanam, International Journal of Current Research in Biology and Medicine, 22192/ijcrbm Volume 3,

Issue 1 – 2018, p.100-108.

[10] Caili, F, Huan, S, & Quanhong, L. (2006). A review on the pharmacological activities and utilization technologies of pumpkin. Plant Foods Hum Nutr 61, 73–80.

[11] Antimicrobial Screening of Venpoosani Nei (Benincasa Hispida Medicated Ghee) - A Siddha Herbal Formulation

[12] Khan IJ, Nadeem M, Imran M, et al. Antioxidant properties of Milk& dairy products: a comprehensive review of the current knowledge. Lipids Health Dis, 2019; 18: 41.