Biological Forum – An International Journal **15(3a): 89-96(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Preliminary Standardization of Siddha Formulation *avuri Karpam*

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(Received: 04 February 2023; Revised: 20 February 2023; Accepted: 14 March 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: Standardization of Siddha medicines is an important one to know the quality of drugs. *Avuri Karpam* (AK) is a poly herbal formulation consists of seven ingredients which have mentioned in classical Siddha literature *"Pathartha Gunavilakkam"* for treating *Keelvatham, Udhirakkattu, Sarpavisham*. This present study was intended to determine the organoleptic characters, Physico chemical testing, preliminary phytochemical analysis, powder microscopy, Thin layer chromatographic (TLC) and High-performance thin layer chromatographic (HPTLC) analysis. Plant materials were identified and authenticated by the experts. Purification and preparation process were done as per classical Siddha literature. All the parameters were analyzed by standard protocols. The results revealed the organoleptic characters, parameters of physico-chemical analysis, and the presence of phyto chemicals such as alkaloids, carbohydrates, saponin, phenols, tannin, flavanoids and diterpenes. The HPTLC fingerprint analysis revealed sixteen bioactive compounds when scanned at 520 nm, seventeen bioactive compounds when scanned at 366 nm, thirteen bioactive compounds when scanned at 254 nm. Powder microscopy documentation shows the authenticity of the formulation *Avuri Karpam.* From the results *Avuri Karpam* was proved that it has various potent medicinal properties over the defined standardization method. This research work will be used for further Pre-clinical and clinical studies.

Keywords: *Avuri Karpam*, Herbal drug standardization, Siddha drug, Phytochemical analysis, Chromatographic studies, Powder microscopy.

INTRODUCTION

The Siddha system has a vast wealth of knowledge which makes use of plants, minerals, metals and living organisms in the universe. It is a prestigious system belonging to South India. According to Siddha system, medicine is a substance that helps to alleviate or eradicate the disease, gives strength to the body, and normalizes the functions of the body (Sambasivampillai, 1931). Ancient siddha literatures are well provided with references on the use of herbs with medicinal properties. Most of the herbal drugs produced, currently lack proper quality specifications and standards (Shirwaikar *et al*., 2013). Standardization of drugs means confirmation of its identity and resoluteness of its standard and purity. Lack of quality control can affect the potency and well- being of drugs that may lead to health problems in the consumers (Sumbul *et al.,* 2012). Standardization of siddha medicines is important to assess the quality of drugs. Combinations of herbal medicines or phytochemical actives are found to be beneficial in certain diseases when given along with modern synthetic drugs (Badole *et al*., 2008). The Selected drug *Avuri Karpam* (AK)is a herbal formulation mentioned in Classical Siddha literature and it contains *Avuriilai (Indigofera tincotria), Kaiyanthagarai (Eclipta alba), Kuppaimeni (Acalypha indica), Kottaik karanthai (Spaeranthus indicus), Vallarai (Centella asiatica), Pottrilai kaiyanthagarai (Wedelia chinensis), Seruppadai (Coldenia procumbens)* indicated for treating *Keelvatham, Udhirakkattu, Sarpavisham (*Kannusamy pillai).The aim of this study is to standardize Siddha polyherbal formulation *Avuri karpam* through organoleptic characters, Physicochemical analysis, (loss on drying, acid insoluble ash, water soluble extractive, alcohol soluble extractive, pH) determination of particle size, qualitative phytochemical analysis, Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), Powder microscopy according to PLIM guidelines.

MATERIALS AND METHODS

Collection of the Plant materials. *Avuriilai* and *Seruppadai* were purchased from Rajendran Herbals, Thuckalay. *Kaiyanthagarai, Kuppaimeni, Vallarai* and *Pottrilai kaiyanthagarai* were bought from my native

place (Tiruppattur) Herbal garden. *Kottaik karanthai* was bought from the Ramasamy Mudhaliyar Store, Parry's corner, Chennai.

Identification and Authentication of the drug. All the plant materials were identified and authenticated by the experts of Siddha Central Research Institute (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India), Govt. Anna Hospital Campus, Arumbakkam, Chennai -106.

Purification of the drugs. Purification process was done as per classical *Siddha* literature (Lohar).

Preparation of *Avuri Karpam*

Procedure. All the above purified ingredients were powdered separately and it was sieved by a cotton cloth. Then these powders were mixed together and it had gone for steaming process (*Pittaviyal murai*) for final purification. After this, the powder was dried and sieved again and stored in a clean air tight glass container. Itwaslabeledas *Avuri Karpam.*(AK)

Administration of the Drug. Formofthemedicine: *Chooranam* Route of Administration:Enteral
Dose: 800-1000 mg (twice a day)
Vehicle: Honey

Analytical methods. The analytical methodology includes determination of organoleptic characters, physico chemical analysis, preliminary phytochemical analysis, Thin layer chromatographic (TLC), High-performance thin layer chromatographic (HPTLC) analysis and Powder microscopy.

Organoleptic characters. The organoleptic characters such as colour, taste and odour were noted.

Physico-chemical parameters (Lohar). The preliminary physicochemical screening test was carried out for *Avuri karpam* as per the standard procedures mentioned hereunder.

Determination of total ash. Weighed accurately 2g of *Avuri karpam* formulation was added in crucible at a temperature 600°C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air-dried drug.

Losson Drying. *Avuri karpam* was dried in theovenat 100-105°C to constant weight.

Determination of acid insoluble ash. Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air-dried drug.

Determination of water-soluble ash. Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

Determination of water-soluble Extractive. 5gm of air-dried drug, coarsely powered *Avuri karpam* was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100°C and weighted. The percentage of water-soluble extractive was calculated with reference to the air-dried drugs.

Determination of alcohol soluble extractive. 1 gm of air-dried drug coarsely powdered *Avuri karpam* was macerated with 20 ml alcohol in closed flask for 24 hrs. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100°C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

Preliminary phytochemical analysis. The preliminary phytochemical screening test was carried out for each extract of *Avuri karpam* as per the standard procedure mentioned hereunder.

Detection of alkaloids. Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

(a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colour precipitate indicates the presence of alkaloids.

(b) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.

(c) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

(a) Molisch's Test: To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

(b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of saponins

Foam Test: 0.5 ml of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols Ferric Chloride Test. Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of tannins Gelatin Test. The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

Detection of Flavonoids. a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Detection of diterpenes Copper Acetate Test. Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

Gum and Mucilage

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

Thin layer chromatographic (TLC) and High-performance thin layer chromatographic (HPTLC) analysis

Sample Preparation. 1g of sample was sonicated in10ml of ethanol solvent. Then filtered and used for TLC.

Thin-layer Chromatography Methodology. Applied 12 µl ethanol extract of sample on TLC plate using Camag's ATS4 applicator and developed by the mobilephase, Toluene: Ethylacetate: Formicacid (8:1:0.5) upto 9cm distance. After development, the plate was photo documented using Camag's TLC Visualizerunder UV254nm and UV366nm. The plate was then scanned using Camag's Scanner 4at UV254 nm (D2l amp, Absorptionmode),UV366nm (Hglamp, fluorescent mode) finger print profiles of the extracts were documented. Then the plate was dipped in 5% vanillin-sulphuric acid reagent followed by heating at 105^{o}C till development of coloured spots. The plate was then photo documented in white light.

Powder microscopy (Wallis, 1965; Chauhan and Pillai 2007). A small amount of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with 0.1% chloral hydrate. Characters were observed under Nikon Eclipse E200 microscope fitted with Axiocam ERc5s color digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.

RESULTS AND DISCUSSION

Organoleptic characters. The texture of *Avuri karpam* was fine powder, dark green in colour, pleasant odour, and bitter in taste and completely passes through sieve no. 44. The inferences are tabulated in Table 1.

Table 1: Organoleptic characters.

Sr. No.	Specification	Character
1.	Texture	Fine Powder
2.	Colour	Dark green
3.	Odour	Pleasant
4.	Taste	Bitter

Physico-chemical parameters. The total ash was found to be 16.61% which indicates the inorganic content of the drug. The loss on drying which indicates the moisture content of the drug was determined as 9.38%. The value of acid insoluble ash was found to be 5.90%. The water soluble ash was calculated as 4.71%. The water soluble extractive value and alcohol soluble extractive value were found to be 12% and 3.43% respectively. The pH value is calculated as 6 which indicate that the drug is slightly acidic**.** The observed values of the physic-chemical properties are listed in Table 2.

Table 2: Physico-chemical parameters

Sr. No	Parameters	Percentage
1.	Total ash value	16.61%
2.	Loss on drying	9.38%
3.	Acid insoluble ash	5.90%
4.	Water soluble ash	4.71%
5.	Water soluble extraction	12.0%
6.	Alcohol soluble extraction	3.43%
7.	pH	6 (Slightly acidic)
8.	Particle size	Completely pass through sieve no.44 mesh

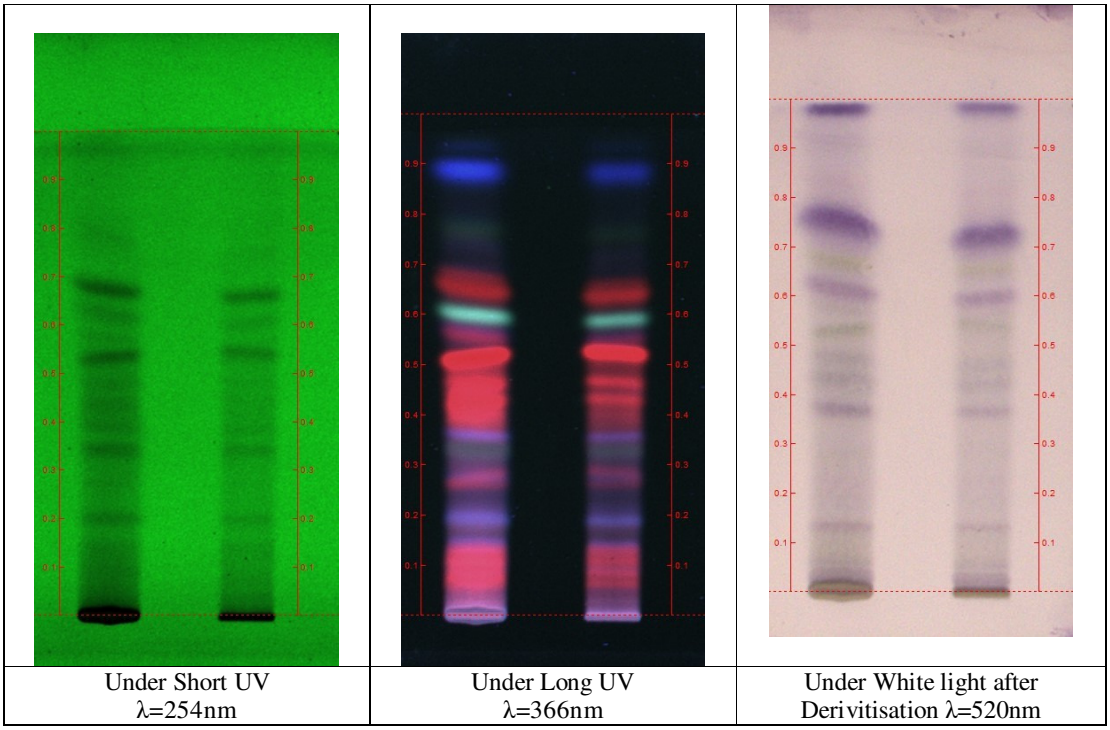
Preliminary phytochemical analysis. The result shows that the qualitative phytochemical analysis indicates that the formulation *Avuri karpam* shows the presence of biologically significant phytochemicals such as alkaloids, carbohydrates, saponins, flavonoids, tannins, phenols, Diterpenes. The results were presented in Table 3.

Table 3: Preliminary phytochemical analysis.

Sr. No.	Phytochemicals	Test Name	H_2O Extract
1.	Alkaloids	Mayer's Test Dragendroff's Test Wagner Test	+ve +ve +ve
2.	Carbohydrates	Molisch's Test Benedict Test	+ve +ve
3.	Saponin	Foam Test	+ve
4.	Phenols	Ferric Chloride Test	+ve
5.	Tannins	Gelatin Test	+ve
6.	Flavonoids	Alkaline Reagent Test Lead acetate	-ve +ve
7.	Diterpenes	Copper Acetate Test	+ve
8.	Quinones	Test for Quinones	-ve
9.	Gum & Mucilage	Test for Gum & Mucilage	-ve

+ve/-ve present or absent if component tested

Thin layer chromatography photo documentation. The TLC photo documentation of the drug under UV 254, UV 366 and after derivatization is shown in Fig. 1. Under UV 254 nm, the TLC plate showed eleven visible spots at Rf values 0.15, 0.20, 0.35, 0.40, 0.45, 0.49, 0.54, 0.59, 0.64, 0.68 and 0.80 (all green). Under UV 366 nm, the TLC plate showed sixteen spots at Rf values 0.07, 0.10, 0.14, 0.21, 0.28, 0.33, 0.36, 0.40, 0.43, 0.46, 0.52, 0.57, 0.60, 0.67, 0.78 and 0.91. After derivatization, the plate showed ten spots at Rf values 0.05, 0.15, 0.36, 0.43, 0.48, 0.54, 0.62, 0.68, 0.77 and 0.99. All the spots with colour under pre and post derivation conditions are listed in the Table 4.



Under Short UV λ=254nm	Under Long UV λ=366nm	Under White light after Derivitisation λ=520nm

Fig. 1. TLC Photo of *Avuri karpam* extract.

Table 4: Rf and color of spots.

λ=254nm		λ=366nm		λ=520nm (Derivitized)	
Rf	**Colour**	**Rf**	**Colour**	**Rf**	**Colour**
0.15	Green	0.07	Fluorescentred	0.05	Purple
0.20	Green	0.10	Fluorescentred	0.15	Purple
0.35	Dark Green	0.14	Fluorescentred	0.36	Purple
0.40	Green	0.21	Fluorescentblue	0.43	Purple
0.45	Green	0.28	Fluorescentred	0.48	purple
0.49	Green	0.33	Green	0.54	Yellowishgreen
0.54	DarkGreen	0.36	Pink	0.62	Purple
0.59	Green	0.40	Fluorescentred	0.68	Yellowishgreen
0.64	Green	0.43	Fluorescentred	0.77	Purple
0.68	DarkGreen	0.46	Fluorescentred	0.99	Purple
0.80	Green	0.52	Fluorescentred		
		0.57	Fluorescentred		
		0.60	Fluorescent green		
		0.67	Fluorescentred		
		0.78	Fluorescent green		
		0.91	Fluorescent blue		

High Performance Thin Layer Chromatographic Finger Print Profiling. Results of HPTLC photo documentation, Rf values, densitometric scan, finger print profiles are presented in respective tables and figures. The HPTLC finger print profile at UV 254 nm is shown in Fig. 2a and 2b and the Rf of peaks and their respective peak areas are shown in Table 5. From the table 5, it is inferred that the presence of 13 prominent peaks with Rf value ranging from 0.03 to 0.99 with percentage area of 0.85 to 17.15%. The HPTLC finger print profile at UV 366 nm is shown in Fig. 3a and 3b and the Rf of peaks and their respective peak areas are shown in Table 6. From the Table 6, it is inferred that the presence of 17 prominent peaks with Rf value

ranging from 0.04 to 0.99 with percentage area of 0.61 to 18.20%. The HPTLC finger print profile at UV 520 nm is shown in Fig. 4a and 4b and the Rf of peaks and their respective peak areas are shown in Table 7. From the table 7, it is inferred that the presence of 16 prominent peaks with Rf value ranging from 0.03 to 1.00 with percentage area of 0.15 to 31.48%.

Table 5: Peaklevel@254 nm.

Track 1, ID: Avuri karpam

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.3 AU	0.02 Rf	238.6 AU	23.91 %	0.03 Rf	3.4 AU	2266.7 AU	10.37 %
2	0.12 Rf	0.0 AU	0.15 Rf	11.1 AU	1.11 %	0.17 Rf	0.0 AU	185.2 AU	0.85 %
3	0.17 Rf	0.2 AU	0.21 Rf	52.2 AU	5.23 %	0.24 Rf	3.1 AU	1128.9 AU	5.17 %
4	0.29 Rf	4.1 AU	0.35 Rf	99.7 AU	9.99 %	0.37 Rf	21.9 AU	2644.0 AU	12.10 %
5	0.37 Rf	22.7 AU	0.39 Rf	47.7 AU	4.78 %	0.41 Rf	40.2 AU	1040.3 AU	4.76 %
6	0.41 Rf	40.5 AU	0.43 Rf	69.4 AU	6.96 %	0.45 Rf	41.8 AU	1979.6 AU	9.06 %
7	0.46 Rf	41.9 AU	0.47 Rf	61.4 AU	6.15 %	0.50 Rf	24.2 AU	1602.8 AU	7.34 %
8	0.50 Rf	24.2 AU	0.53 Rf	123.1 AU	12.34 %	0.56 Rf	25.3 AU	2873.2 AU	13.15 %
9	0.56 Rf	25.4 AU	0.57 Rf	29.8 AU	2.99 %	0.59 Rf	7.7 AU	523.9 AU	2.40 %
10	0.59 Rf	7.9 AU	0.62 Rf	30.5 AU	3.05 %	0.64 Rf	25.1 AU	783.5 AU	3.59 %
11	0.64 Rf	25.2 AU	0.67 Rf	146.9 AU	14.71 %	0.71 Rf	0.2 AU	3746.1 AU	17.15 %
12	0.72 Rf	0.1 AU	0.78 Rf	46.3 AU	4.63 %	0.83 Rf	5.0 AU	1676.0 AU	7.67 %
13	0.92 Rf	6.1 AU	0.96 Rf	41.3 AU	4.14 %	0.99 Rf	8.4 AU	1399.2 AU	6.40 %

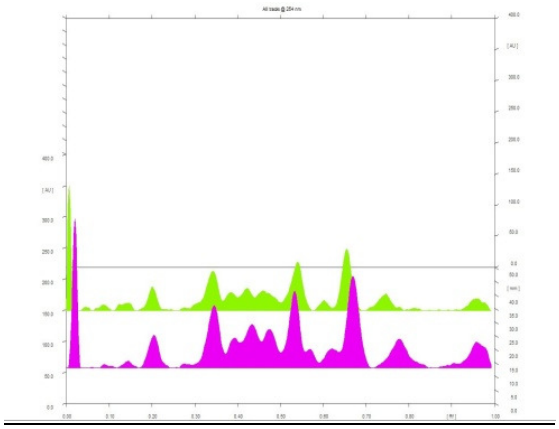


Fig. 2a. 3D Chromatogram of 254 nm

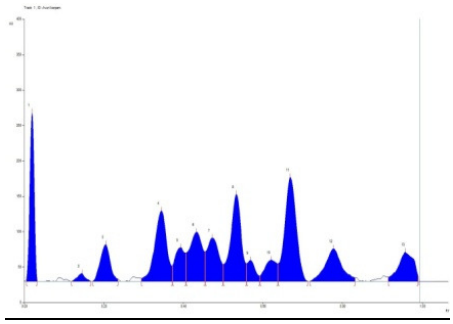


Fig. 2b. HPTLC finger print profile of Ethanol extract scanning 254nm.

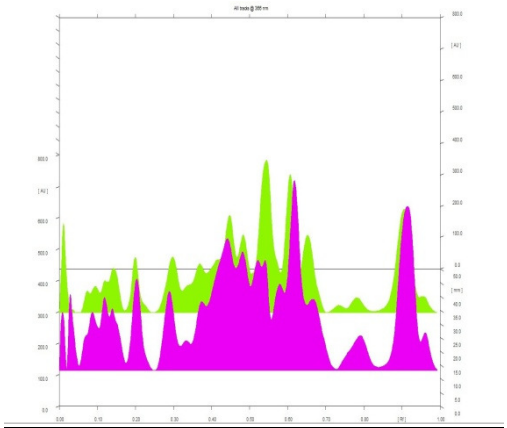


Fig 3a. 3D Chromatogram of 366nm

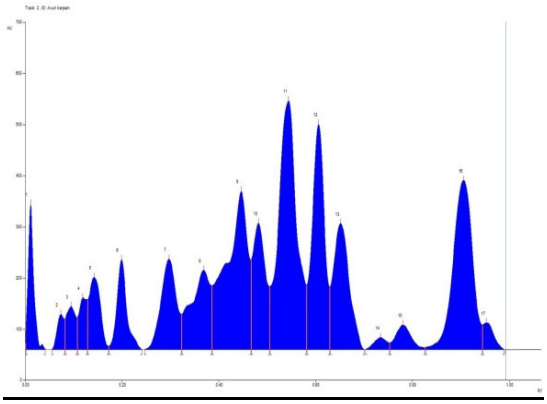


Fig. 3b. HPTLC finger print profile of Ethanol extract scanning 366nm

Table 6: Peaklevel@366nm.

Track 2, ID: Avuri karpam

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	47.0 AU	0.01 Rf	284.5 AU	8.43 %	0.04 Rf	0.6 AU	2886.2 AU	3.29 %
2	0.05 Rf	0.3 AU	0.07 Rf	69.0 AU	2.05 %	0.08 Rf	59.7 AU	868.9 AU	0.99 %
3	0.08 Rf	61.4 AU	0.09 Rf	84.0 AU	2.49 %	0.11 Rf	62.1 AU	1395.5 AU	1.59 %
4	0.11 Rf	62.5 AU	0.12 Rf	101.1 AU	3.00 %	0.13 Rf	97.8 AU	1429.7 AU	1.63 %
5	0.13 Rf	98.5 AU	0.14 Rf	140.9 AU	4.18 %	0.17 Rf	6.7 AU	2866.6 AU	3.27 %
6	0.17 Rf	7.3 AU	0.20 Rf	176.1 AU	5.22 %	0.24 Rf	0.4 AU	3117.2 AU	3.56 %
7	0.25 Rf	0.1 AU	0.30 Rf	177.1 AU	5.25 %	0.32 Rf	69.0 AU	4904.1 AU	5.60 %
8	0.32 Rf	69.6 AU	0.37 Rf	155.3 AU	4.60 %	0.39 Rf	24.8 AU	5302.1 AU	6.05 %
9	0.39 Rf	124.9 AU	0.45 Rf	309.8 AU	9.18 %	0.47 Rf	75.2 AU	12027.4 AU	13.72 %
10	0.47 Rf	177.2 AU	0.48 Rf	247.7 AU	7.34 %	0.51 Rf	23.4 AU	5579.8 AU	6.37 %
11	0.51 Rf	123.5 AU	0.54 Rf	486.5 AU	14.42 %	0.58 Rf	26.3 AU	15947.9 AU	18.20 %
12	0.58 Rf	126.7 AU	0.61 Rf	440.8 AU	13.06 %	0.63 Rf	22.3 AU	9788.1 AU	11.17 %
13	0.63 Rf	122.6 AU	0.65 Rf	246.5 AU	7.31 %	0.70 Rf	0.1 AU	7047.5 AU	8.04 %
14	0.70 Rf	0.3 AU	0.73 Rf	23.5 AU	0.70 %	0.75 Rf	13.0 AU	538.7 AU	0.61 %
15	0.75 Rf	13.0 AU	0.78 Rf	47.8 AU	1.42 %	0.83 Rf	4.1 AU	1348.2 AU	1.54 %
16	0.83 Rf	4.3 AU	0.91 Rf	331.3 AU	9.82 %	0.95 Rf	48.2 AU	11600.8 AU	13.24 %
17	0.95 Rf	48.4 AU	0.95 Rf	52.0 AU	1.54 %	0.99 Rf	0.0 AU	999.5 AU	1.14 %

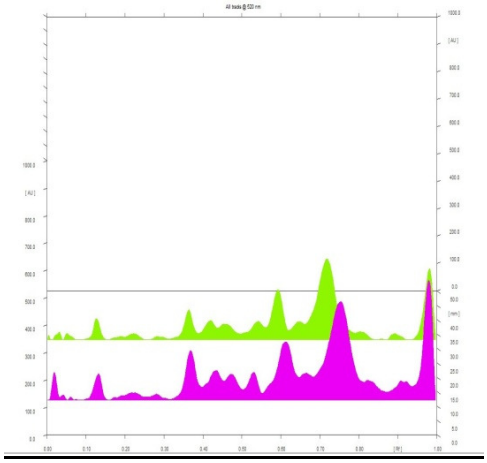


Fig 4a. 3D Chromatogram of 520nm.

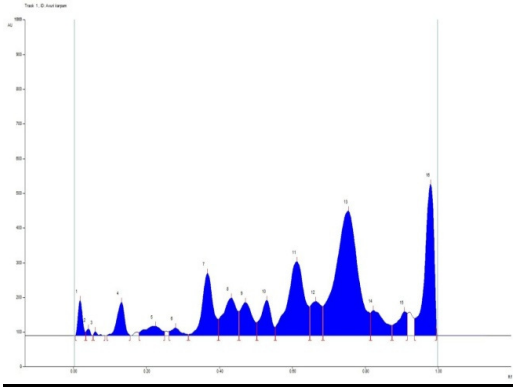


Fig. 4b. HPTLC finger print profile of Ethanol extract scanning 520nm.

Table 7: Peaklevel@520nm.

Track 1, ID: Avuri karpam

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.1 AU	0.02 Rf	101.4 AU	5.06 %	0.03 Rf	9.7 AU	1044.4 AU	1.73 %
2	0.03 Rf	11.0 AU	0.04 Rf	18.6 AU	0.93 %	0.05 Rf	0.2 AU	160.9 AU	0.27 %
3	0.05 Rf	2.3 AU	0.06 Rf	10.9 AU	0.54 %	0.09 Rf	0.1 AU	88.0 AU	0.15 %
4	0.09 Rf	0.2 AU	0.13 Rf	95.8 AU	4.78 %	0.16 Rf	0.4 AU	1679.6 AU	2.78 %
5	0.18 Rf	8.0 AU	0.23 Rf	26.7 AU	1.33 %	0.25 Rf	11.3 AU	1027.1 AU	1.70 %
6	0.26 Rf	11.5 AU	0.28 Rf	21.8 AU	1.09 %	0.31 Rf	3.0 AU	485.2 AU	0.80 %
7	0.32 Rf	3.0 AU	0.37 Rf	179.6 AU	8.96 %	0.40 Rf	46.9 AU	4511.5 AU	7.46 %
8	0.40 Rf	47.2 AU	0.43 Rf	107.8 AU	5.37 %	0.45 Rf	69.2 AU	3382.6 AU	5.59 %
9	0.45 Rf	69.6 AU	0.47 Rf	94.9 AU	4.73 %	0.50 Rf	37.6 AU	2652.4 AU	4.39 %
10	0.50 Rf	38.1 AU	0.53 Rf	101.5 AU	5.06 %	0.55 Rf	24.4 AU	2411.8 AU	3.99 %
11	0.55 Rf	25.3 AU	0.61 Rf	212.5 AU	10.59 %	0.65 Rf	82.9 AU	8368.2 AU	13.84 %
12	0.65 Rf	83.5 AU	0.67 Rf	97.9 AU	4.88 %	0.68 Rf	84.0 AU	2473.4 AU	4.09 %
13	0.69 Rf	84.6 AU	0.75 Rf	359.1 AU	17.90 %	0.81 Rf	65.0 AU	19035.2 AU	31.48 %
14	0.82 Rf	65.7 AU	0.82 Rf	72.3 AU	3.60 %	0.87 Rf	29.3 AU	2256.5 AU	3.73 %
15	0.87 Rf	29.4 AU	0.91 Rf	69.1 AU	3.44 %	0.92 Rf	64.9 AU	1578.8 AU	2.61 %
16	0.94 Rf	49.5 AU	0.98 Rf	436.1 AU	21.74 %	1.00 Rf	27.7 AU	9313.2 AU	15.40 %

Powder Microscopy. The powder is dark green in colour with characteristic taste and odour. The powder shows biarmed trichomes and pitted parenchyma from *Indigofera tinctoria* L. (whole plant) shown in Fig. 5a and 5b, warty trichome, epidermis with papilla and pollen grains from *Eclipta alba* (L.) L. (whole plant) shown in Fig 6a and 6b, sickle shaped trichome and rosette crystals from *Acalypha indica* L. (whole plant) shown in Fig. 7a and 7b, fruit wall trichomes, mesocarp and cotyledon cells from *Sphaeranthus indicus* L. (fruit) shown in Fig. 8a and 8b, multicellular trichome and parquetry layer from *Centella asiatica* (L.) Urb. (Leaf) shown in Fig. 9a and 9b, warty trichome from *Wedelia chinensis* (L.) Pruski (leaf) shown in Fig. 10a, warty trichome, trichome with contents and pitted vessels from *Coldenia procumbens* L. (whole plant) Fig. 11a and 11b. This documentation shows the authenticity of the formulation *Avuri karpam*.

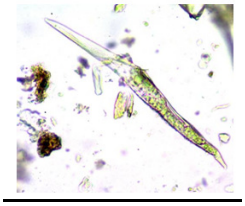


Fig. 5a. *Indigofera tinctoria* L. Biarmed trichome.

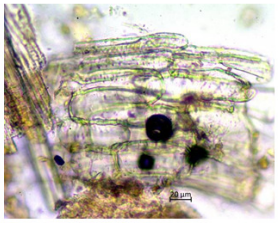


Fig. 5 b. *Indigofera tinctoria* L. Pitted parenchyma from rachis.

Fig. 6a. *Eclipta alba* (L.) Warty trichome.

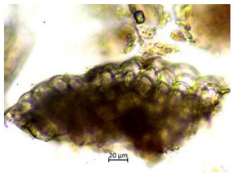


Fig. 6b. *Eclipta alba* (L.) Epidermis with papillary outgrowth

Fig. 7a. *Acalypha indica* L. Sickle shaped trichome

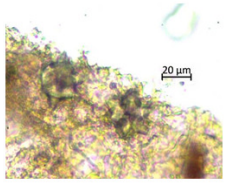


Fig. 7b. *Acalypha indica* L. Rosette crystal.

Characters from *Sphaeranthus indicus* L. (fruit)

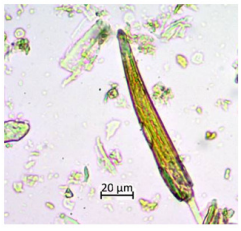


Fig. 8a. Trichome from fruit wall.

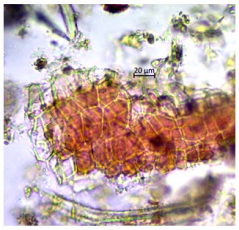


Fig. 8b. Mesocarp in surface view.

Characters from *Centella asiatica* (L.) Urb. (leaf)

Fig. 9a. Multicellular trichome.

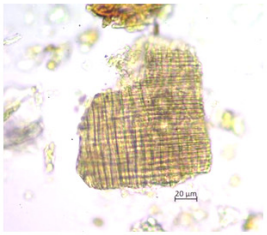


Fig. 9b. Parquetry layer.

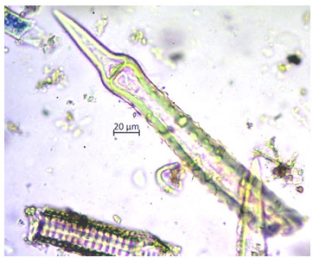


Fig. 10a. Characters from *Wedelia chinensis* (L.) Pruski (leaf).

Characters from *Coldenia procumbens* L. (whole plant)

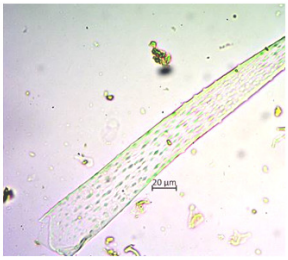


Fig. 11a. Warty trichome

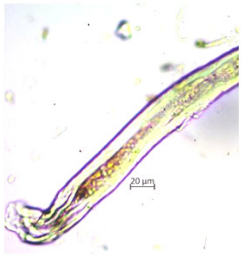


Fig. 11b. Trichome with contents.

CONCLUSIONS

From the results *Avuri karpam* was proved that it has various potent biological active properties over the defined standardization method and it may act therapeutically in treating various diseases. This research work will be used for further pre-clinical and clinical studies.

FUTURE SCOPE

In this era, it is very important for every individual to keep their health on right track. People have started to look back their traditional science which the natural atmosphere dwells. This study drug *Avuri karpam* may have various pharmacological activities and it will be the solution for treating many diseases.

Acknowledgement. The authors are grateful to the Department of Siddha, The Tamil Nadu Dr. M.G.R. Medical University, Chennai -32 and Siddha Central Research Institute (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India), Government Anna Hospital Campus, Arumbakkam, Chennai -106 for providing facilities to carry out this study and also thankful to the Ministry of AYUSH, CCRS for their financial support.
Conflict of Interest. None.

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How to cite this article: T. Giftillda Selva Elsee, E.M. Manikgantan and G. Bharath Kumar (2023). Preliminary Standardization of Siddha Formulation *avuri Karpam. Biological Forum – An International Journal, 15*(3a): 89-96.