Research Article

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STANDARDIZATION OF SIDDHA POLYHERBAL FORMULATION PARANGIPATTAI CHOORNAM

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ABSTRACT

Aim and Objective of this study was to assess the quality of the drug Parangipattai chooranam by conducting physicochemical analysis, preliminary phytochemical analysis and other analytical techniques. Physicochemical analysis was carried out as per the WHO guidelines and preliminary phytochemical analysis was done based on the standard text book. Other analytical techniques such as Thin Layer Chromatography (TLC) photo documentation and High Performance thin layer chromatography (HPTLC) finger printing was done using, Linomat IV (CAMAG, Muttenz, Switzerland). Thermogravimetric analysis was done using TG instrument (TG Q500 V20.10 Build 36). The physicochemical analysis of the drug Parangipattai choornam showed 12.49% of loss on drying at 105oC, 2.822% of total ash, 0.999% of water soluble ash etc which indicates that the drug has remarkable content of inorganic matter. And pH 6.08 indicates the drug is slightly acidic. The preliminary phytochemical analysis showed the presence of steroids, tannins, triterpenoids, flavonoids, phenol, amino acids, glycosides, saponins. Cardiac glycosides and alkaloids were absent. The drug was free of microbial contamination, heavy metals and pesticide were below deductible limit. The results obtained indicate that the drug is of standard quality and can be used as reference standard in laying pharmacopoeial standard.

Keywords: Parangipattai chooranam, Physicochemical analysis, Phytochemistry, TLC, HPTLC.

INTRODUCTION

Herbal traditional medicines have gained considerable momentum worldwide during the past decade and play paramount role in health care programs especially in developing countries[1]. According to W.H.O nearly 80% of population of developing countries rely on traditional medicines for most of their ailments[2]. Being holistic and natural our herbal medicines are devoid of serious adverse effects commonly associated with synthetic drugs. 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[3]. Drugs of Indian system of Medicine may contain a single herb or combination of different herbs believed to have complementary and or synergistic effects. Both the raw and finished products contain complex mixtures of fatty acids, sterols, alkaloids, flavonoids, glycosides etc. In the modern era due to commercialization and bulk production of herbal drugs there is chance of adulteration and incomplete processing of drugs. Hence there is need for standardization of all herbal drugs to maintain their quality. Therefore it is highly desirable that these drugs should be characterized with modern instruments, based on which the specifications of such drugs can be well standardized on a scientific basis[4]. The selected drug Parangipattai chooranam[5] though simple and cost effective, has diverse medicinal properties and used in the treatment of various diseases like Granthi (Abscess), Soolai (Pain), Megam (Sexually transmitted disease), Vettai (Leucorrhea), Vandukadi (Insect bite), Padaigal (Fungal skin infection), Viranangal (Wounds), Kandamalai (Cervical adenitis).

MATERIALS AND METHODS

The drug Parangipattai choornam was prepared in the Gunapadam laboratory of National Institute of Siddha, Chennai, Tamilnadu, India. The analysis was conducted at Siddha central research institute, Chennai and M/s Sargam Laboratory Pvt Ltd, Chennai, India. The ingredients of the drug Parangipattai choornam are given in the Table 1.

Identification of Raw drugs

Parangipattai was purchased from Gopal Aasan herbal drug shop Nagercoil, India. Sivanarvembu and Vellarugu were collected from Kanyakumari district, India. Sangam verpattai, Sathuracalli, Tirugucalli were collected from Tirunelveli district, India. Sugar purchased from local market. Herbal drugs were authenticated by Prof. S. Jeyaraman, Director, Plant Anatomy research centre, Chennai, India. Voucher specimen has been handed over. All drugs were purified as mentioned in the Siddha Classical texts[6].

Preparation of the drug

Sathuracalli and tirucalli are cut into pieces and transferred into earthenware. Water is poured into the

earthenware the mouth of which is then closed using white cotton cloth Parangipattai is made into pieces and spread over the cotton cloth. Another suitable earthenware is placed above the earthenware and sealed using clay. This setup is placed in stove and heated for 5 hours. The seal is removed carefully after one hour. Parangipattai and all other drugs are shade dried and made into powder individually. Then the above powders are mixed well and sugar is added to this mixture. This mixture is made into a new earthenware and kept in dhaniyapudam for 10 days. [5]

Organoleptic characters

Colour, odour, taste and consistency of the drug were noted.

Physico-chemical parameters

All the physico-chemical parameters were carried out as per the methods mentioned in standard books[7-8].

Preliminary Phytochemical Tests

All the preliminary phytochemical tests were carried out as per the methods mentioned in standard books[9-10].

ICP-OES Analysis

Heavy metals like lead, cadmium, mercury & arsenic and nutritional elements like ferrous, copper, zinc, potassium, calcium were studied.

Thin layer Chromatography and High Performance Thin Layer Chromatography
Preparation of extract for TLC/HPTLC

4 g of the drug was first refluxed with 100 ml of hexane and filtered to remove the fatty material. The process was repeated with another 100 ml of hexane. Then the residue was soaked overnight in chloroform. It was boiled on a water bath for 10 minutes, filtered and concentrated to 10 ml.

Solvent system

Suitable solvent system was achieved by trial and error method. The solvent system of Toluene : Ethyl acetate (4:1.5, v/v) showed a better resolution than the other solvent systems attempted. This solvent system was used for developing the extract on the TLC plate.

Visualizing reagent

The vanillin-sulphuric acid reagent was chosen as visualizing reagent (one gram vanillin dissolved in the mixture of ethanol: sulphuric acid in the ratio 95:5) since it gives colour with most of the categories of secondary metabolites.

Instrument

The twin trough chamber (CAMAG) was used for developing the TLC plate. For applying the extract, Linomat IV (CAMAG, Muttenz, Switzerland) applicator was used. The TLC plate is made up aluminum sheet pre-coated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used. The extract was applied as bands of 8 mm width and 6 mm distance in between tracks on a 6 x10 cm TLC plate. TLC scanner 030618 (CAMAG) attached with WINCATS software were used for finger print development under UV 254 nm and after derivatization at 540 nm. CAMAG visualizer was used for photo documentation at UV 254 nm, 366 nm and in visible light after derivatization with vanillin-sulphuric acid reagent.

Procedure

The volumes of chloroform extract applied on the TLC plate were 5µl, 10µl, 15µl. The extract was applied as 8 mm bands with 6 mm distance in between tracks and developed in the selected solvent system. The developed TLC plate was air dried and photographs were taken under UV 254 and 366 nm. The plate was scanned under UV 254 nm using the scanner. The finger print was recorded. Then the plate was dipped in vanillin-sulphuric acid reagent, heated in an oven at 105°C till the appearance of colored spots. Immediately the photograph was taken and scanned for finger print profile at 540 nm.

RESULTS AND DISCUSSION

Organoleptic Character

The drug Parangipattai choornam was a fine powder mud brown in colour with pungent odour, bitter and slightly sweet in taste. (Figure 1) The inferences are tabulated in Table 2.

Physicochemical Analysis

The drug passes through 80 size mesh. The loss on drying which indicates the moisture content of the drug was determined as 12.49%. The total ash was found to be 2.822%.which indicates the inorganic content of the drug. The water soluble ash was calculated as 0.999%.and the value of acid insoluble ash was found to be 0.299% which indicates that the drug contains negligible amount of siliceous matter. The water soluble extractive value and alcohol soluble extractive value were found to be 38.3% and 34.55%.The PH value is measured as 6.08 which indicates that the drug is acidic. The observed results were tabulated in Table 3.

Preliminary Phytochemical Analysis

The drug has high polar secondary metabolites like glycosides, steroids, flavonoids, triterpenoids, phenols, tannin and saponins as shown in Table 4.

Table 1: Ingredients of Parangipattai choornam

S. No	Ingredients	Botanical Name	Parts Used	Quantity
1.	Parangipattai	*Smilax chinensis*	Root tuber	280 grams
2.	Sivanarvembu	*Indigofera aspalathoides*	Whole plant	140 grams
3.	Sangam	*Azima tetracantha*	Root bark	70 grams
4.	Vellarugu	*Enicostemma axillare*	Whole plant	70 grams
5.	Sathuracalli	*Euphorbia antiquorum*	Leaf	1.7 kilograms
6.	Tirugucalli	*Euphorbia tirucalli*	Leaf	1.7kilograms
7.	Sugar	*Saccharum officinarum*		350 grams
8.	Water			32 litres

Table 2: Organoleptic Characters

S.I No	Specification	Inference
1.	Colour	Muddish brown
2.	Odour	Pungent
3.	Taste	Bitter, slightly sweet
4.	Consistency	Very fine powder

Table 3: Physicochemical parameters of Parangipattai Choornam

S. No	Parameter	I	II	Mean
1.	Loss on Drying at 105°C	12.38%,	12.59%,	12.49%,
2.	Total Ash	2.647%,	2.997%,	2.822%
3.	Water soluble Ash	1.199%,	0.799%,	0.999%
4.	Acid insoluble Ash	0.299%	0. 299%	0. 299%
5.	Water Soluble Extractive	38.2%,	38.4%,	38.3%
6.	Alcohol Soluble Extractive	34.7%,	34.4%,	34.55%
7.	PH	6.08		
8.	**Test for heavy/Toxic metals** Lead Cadmium Mercury Arsenic	BDL		
9.	**Microbial contamination** Total bacterial count Total fungal count	19202 CFU/g 175 CFU/g		
10.	**Test for specific Pathogen** *E. coli* *Salmonella spp.* *S. aureus* *Pseudomonas aeruginosa*	Absent		
11.	**Pesticide residue** Organochlorine pesticides Organophosphorus pesticides	Not Detected		
12.	**Test for Aflatoxins** B_1 B_2 G_1 G_2	BDL		
13.	Particle size	Completely passes through 80 mesh		

Table 4: Preliminary Phytochemical Analysis of Parangipattai Choornam

S. No	Name of the Test	Inference
1.	Test for Amino acids (Biurette test)	+
2.	Test for Steroid (Lieberman Burchard Test)	+
3.	Test for Flavonoids (Shinoda test)	+
4.	Test for Triterpenoids (Noller's Test)	+
5.	Test for Phenol	+
6.	Test for Tannin	+
7.	Test for Alkaloids (Dragendorff's Test)	-
8.	Test for Glycosides	+
9.	Test for Saponins	+
10.	Test for Cardiac Glycosides	-

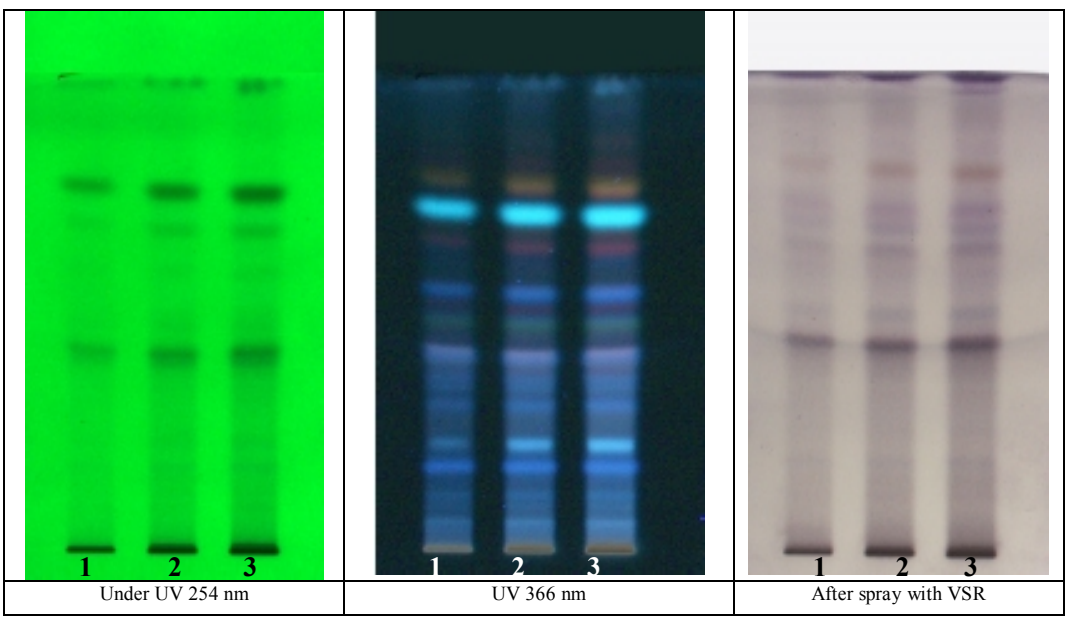
Table 5: R_f and colour details of TLC of chloroform extract of Parangipattai Choornam

S. No	UV 254 nm		UV 366 nm		After spray with VSR	
	R_f value	Colour of the spot	R_f value	Colour of the spot	R_f value	Colour of the spot
1	0.10	Green	0.13	Blue	0.28	Violet
2	0.20	Green	0.20	Blue	0.37	Violet
3	0.26	Green	0.26	Blue	0.47	Violet
4	0.36	Green	0.35	Blue	0.53	Violet
5	0.47	Green	0.42	Blue	0.65	Violet
6	0.54	Green	0.48	Blue	0.69	Violet
7	0.57	Green	0.56	Blue	0.75	Violet
8	0.67	Green	0.62	Blue	0.78	Violet
9	0.78	Green	0.74	Pink	0.88	Brown
10	0.87	Green	0.81	Blue	-	-
11	-	-	0.87	Orange	-	-

Table 6: R_f and percentage peak area of chloroform extract of Parangipattai choornam

Peak	Start Rf	Start Height	Maximum Rf	Maximum Height	End Rf	End Height	Area	Area%
1	0.09	0.5	0.11	36.7	0.12	0.2	471.8	1.20
2	0.13	0.3	0.14	17.4	0.16	0.5	227.9	0.59
3	0.19	0.1	0.21	37.5	37.5	1.2	578.2	1.47
4	0.24	0.5	0.26	42.9	42.9	2.3	1029.6	2.62
5	0.32	2.4	0.36	30.0	30.0	28.5	546.9	1.39
6	0.41	48.6	0.48	328.3	328.3	52.8	14697.6	37.36
7	0.53	53.1	0.55	66.5	66.5	0.7	2323.0	5.91
8	0.64	5.6	0.68	56.7	56.7	0.3	1645.4	4.18
9	0.74	6.7	0.78	118.8	118.8	33.8	3354.4	8.53
10	0.81	33.8	0.87	447.2	447.2	5.3	14461.4	36.76

Figure 1: Prepared Drug



Under UV 254 nm | UV 366 nm | After spray with VSR

Figure 2: TLC profile of chloroform extract of Parangipattai Choornam
Track 1 - 5µl; Track 2 - 10 µl; Track 3 - 15 µl.

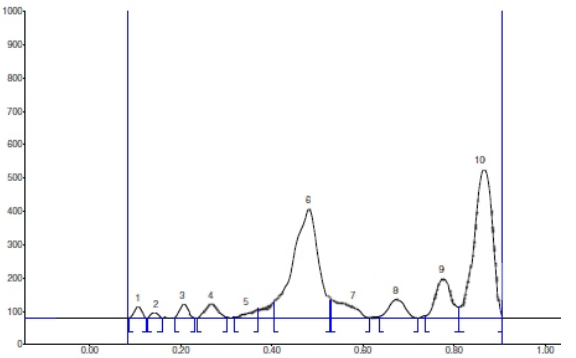


Figure 3: HPTLC finger print profile of chloroform extract of Parangipattai choornam

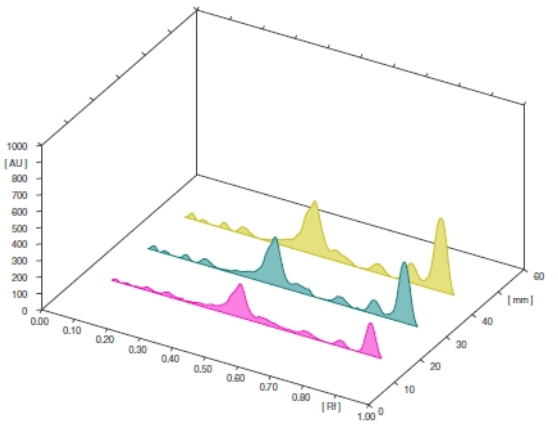


Figure 4: 3D chromatogram of all tracks of chloroform extract Parangipattai Choornam

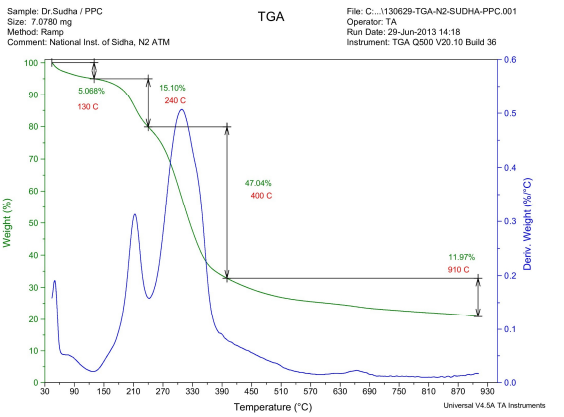


Figure 5: Thermogravimetric analysis Spectra

Thin layer chromatography photo documentation

The TLC photo documentation of the drug under UV 254, UV 366 and after derivatization are shown in Figure 1A-C. Under UV 254 nm, the TLC plate showed ten visible spots at R_f values 0.10, 0.20, 0.26, 036, 0.47, 0.54, 0.57, 0.67, 0.78 and 0.87 (all green). Under UV 366 nm, the TLC plate showed eleven spots at R_f values 0.13, 0.20, 0.26, 0.35, 0.42, 0.48, 0.56, 0.62, 0.74, 0.81 and 0.87. After derivatization with vanillin-sulphuric acid reagent, the plate showed nine spots at R_f values 0.28, 0.37, 0.47, 0.53, 0.65, 0.69, 0.75, 0.78 and 0.88. All the spots with colour under pre and post derivation conditions are listed in the Table 5 and shown in the Figure 2.

High Performance Thin Layer Chromatographic Finger Print Profiling

The HPTLC finger print profile at UV 254 nm is shown in Figure 3 and the R_f of peaks and their respective peak areas are shown in Table 6. From the Figure 3 and Table 6, it is inferred that the major peak appeared at R_f 0.87 with an area of 36.76 % followed by the peak at R_f 0.48with an area of 37.36 % and the peak at R_f 0.78 was found to be the third major peak with an area of 8.53 %. The other peaks at R_f0.55 (5.91%), 0.68 (4.18 %) 0.26 (2.62 %), 0.21 (1.47%), 0.11 (1.20%), 0.36 (1.39%) and 0.14(0.58%) were found to be minor peaks. The Figure 4 shows that all three concentrations are showing increasing order of peak heights.

ICP-OES Analysis

The drug Parangipattai choornam was subjected to ICP-OES analysis. The amount of Iron present was 529mg/kg, Calcium present was 1.07%, and Potassium present was 6383mg/kg. Copper and zinc were below detectable limit shown in Table 7. Iron deficiency leads to decreased resistance to infection[11]. Since the drug has remarkable iron content may prevent recurrence of infection. Calcium is a key secondary messenger that is involved in several signaling cascades critical to wound healing[12]. Calcium influx into cells is known to regulate inflammatory cell infiltration, fibroblast proliferation and keratinocyte migration[13,14]. So Parangipattai choornam can be used in the treatment of wounds as mentioned in the ancient literature. Potassium supplements appear to decrease the pain intensity[15]. The drug can be used in the management of soolai (pain).Heavy metals were below detectable limit shown in Table 2.

Thermogravimetric analysis

The thermogravimetric analysis is used to determine the total weight change in the sample formulations when they are subjected to thermal treatments. The TGA spectra of *Parangipattai choornam* showed peaks at 130^{o}C, 240^{0}C, 400^{0}C, 910^{0}C. At 130^{0}C, 5.068% of the drug is disintegrated which may be due to lose of moisture content present in the drug. Similarly 15.10%, 47.04%, 11.97% of the drug gets disintegrated at temperatures 240^{0}C, 400^{0}C, 910^{0}C respectively. Figure 5 shows various peaks obtained during Thermogravimetric analysis.

CONCLUSION

The obtained results of physico-chemical parameters, preliminary phytochemicals analysis, TLC photo documentation, HPTLC finger print profiling at UV 254 nm provide valuable information. The set parameters are sufficient to authenticate and standardize Parangipattai chooranam. The results obtained could be utilized as reference for developing standard formulation of great efficacy

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