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Standardization of Polyherbal Siddha Formulation Vithuvagai Chooranam

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Abstract: Drug standardization is a pivotal tool for the identification of drug, purity, safety, efficacy of drug and to follow good manufacturing practice (GMP) and good laboratory practice (GLP) standards as per varied restrictive authorities. Standardization has been well defined and documented in the Classic Siddha literature. However, these were not written with an industrial or commercial purposes, but rather with an individualistic intent. To evaluate the crude drugs and their finished products, the World Health Organization has established suitable specific standardization parameters. The present study aimed to standardize the Siddha poly-herbal formulation *Vithu vagai chooranam* (VVC), which is mentioned in the Siddha classical literature.

Physiochemical parameters such as Loss on drying, ash values, extractive values, and chemical analysis were estimated as per Pharmacopoeia Laboratory for Indian Medicines (PLIM) guidelines. For standardization, organoleptic characters were done. Physicochemical analysis revealed that ash value is 2.10%, Acid insoluble ash is 0.83%, loss on drying in 105^{0}c is 4.50%, Alcohol and water-soluble extractive values are 10.1% and 63.97%, respectively. The Chemical analysis showed the presence of Carbonate, Ammonium, Potassium, reducing sugar and Tannins. The drug is free of aflatoxins, microbial contamination, and pesticide residues. Hence, the drug is safe for consumption. HPTLC fingerprint analysis of extracts showed the presence of 5 prominent peaks at UV 254 nm in which each peak corresponds to number of versatile components present within it. From the data obtained from the present investigation, it was evident that the *Vithuvagai chooranam* complies with the regulatory standard and for further evidence for its safety and efficacy, toxicity and pharmacological studies have to be carried out.

Keywords: Standardization, Vithu vagai chooranam, Physiochemical, HPTLC fingerprint

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Introduction

The Siddha system of medicine is a most ancient medical systems that uses a scientific and holistic approach to provide preventive, curative, rejuvenating, and rehabilitative health care. This system treats not only the physical body but also the mind and soul. As per Siddha, the medicinal preparations are categorized into 64 types (Shanmugavelu ,1987). Thirty-two types of dosage

forms are mentioned as internal medicines and thirty-two are laid down for external therapies, that are provided based on the form of medicine, method of preparation, or application. The drug formulation in Siddha is based on two principles: (i) used as single drug and (ii) use of more than two drugs. Use of more than one herb is known as poly herbal formulations. They have greater therapeutic efficacy than single herb. The active constituents of individual medicinal plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. This phenomenon of positive herb – herb interaction is known as synergism (Kandaswamy Pillai,1998). *Chooranam* is a fine powder of drugs. The *Chooranam* may be applied to powder of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity (Anonymous, 1993). *Chooranam* retains its potency for 2 months and then gradually deteriorate. If it is packed and stored properly, *Chooranam* can be kept for a year.

Nowadays, Siddha medicines are becoming popular and many Siddha drugs are now clinically tested and accepted for manufacture. Standardization in Siddha formulations ensures the quality and purity of raw drugs, quality control during the drug manufacturing process, production, storage, and distribution to maintain the quality of the final product and analytical study of the drug aids in interpreting pharmacokinetic and pharmacodynamic of the drug in a suitable manner. The most of the traditional formulations lack quality control standards and methods for its evaluation. The World Health Organization (WHO) has emphasized the need to ensure the quality of medicinal plant products by using modern sophisticated techniques and applying the suitable standards (World Health Organization, 1998; Jain and Dashora, 2012). *Vithu vagai chooranam, a* poly herbal formulation is a combination of ten drugs which is mentioned in *Noigaluku Siddha parigaram*- part 1, written by Dr. M. Shanmugavelu. The formulation is indicated for the Oliguria, spermatogenesis, Leucorrhea, Ascites (Shanmugavelu, 1967). But scientific evidence of *Vithu vagai chooranam* (VVC) has not been reported. The prime most requirement of a safe and effective health management programme is the accurate analysis for quality control of all the herbal products. A scientific explanation is needed in this modern era in order to understand the concept of Siddha and its quality and efficacy. In this research work, an attempt was made to analyse the physiochemical screening of Siddha poly herbal formulation *Vithu vagai chooranam as* per PLIM guidelines.

Materials and Methods

Procurement of Raw Drugs: The drug was purchased from authorized country Raw Drug Store in Parry's corner, Chennai, India.

Identification and Authentication of the drug: The collected raw materials were identified and authenticated at Department of Medicinal Botany, National Institute of Siddha, Tambaram Sanatorium (Certificate No: NISMB4812021).

Preparation of Trial drug: Vithu vagai chooranam:

The ingredients (Table 1) after purification were ground separately to powder. The powder was sieved through a white cloth. All these powdered ingredients were mixed thoroughly in a stone mortar. The prepared test drug was stored in a clean, air-tight glass container.

Standardization of Vithu Vagai Chooranam:

Organoleptic characters:

Organoleptic characters such as form, colour, odor, taste, and texture were observed.

Microscopic characters:

The microscopic character of *Vithu vagai chooranam* was observed under microscope.

Physio chemical parameters:

Physico-chemical studies of the plant drugs helps in understanding the significance of physical and chemical properties of the substance being

Table 1: Ingredients of *Vithu vagai chooranam*

S. No.	Name of the drug	Scientific name	Common name	Quantity
1	*Ulunthu Maavu (Fabaceae)*	*Vigna mungo (L.)*	Black gram	*1 palam* (35g)
2	*Ellu (Pedaliaceae)*	*Sesamum indicum* L.	Sesame	*1 palam* (35g)
3	*Poonaikkalivithu (Fabaceae)*	*Mucuna pruriens (L.)*	Velvet bean	*1 palam* (35g)
4	*Neermullivithu (Acanthaceae)*	*Hygrophila auriculata (Schumach.) Heine*	Marsh Barbel	*1palam* (35g)
5	*Nilappanai Kizhangu (Amaryllidaceae)*	*Curculigo orchioides Gaertn.*	*Golden Eye Grass*	*1 palam* (35g)
6	*Thanneervittan Kizhangu (Lilliaceae)*	*Asparagus racemosus Willd.*	Wild asparagus	*1 palam* (35g)
7	*Chukku(Zingiberaceae)*	*Zingiber officinale Rosc.*	Ginger	*½palam* (17.5g)
8	*Milagu(Piperaceae)*	*Piper nigrum L.*	Black Pepper	*½palam* (17.5g)
9	*Thippili (Piperaceae)*	*Piper longum L.*	Long pepper	*½palam* (17.5g)
10	*Vellai sarkkarai (Poaceae)*	*Saccharum officinarum L.*	White Sugar	*½palam* (17.5g)

analysed and especially for the determination of their purity and quality. The analysis includes the determination of Total ash, Loss on drying at 105ºC, water soluble ash, acid-insoluble ash, water soluble extractive, alcohol soluble extractives were determined as per the standard methods (Lohar, 2007).

Qualitative organic and inorganic analysis:

Qualitative test for acid and basic radicals: Test for organic compounds were carried out as per the methods mentioned in standard practical guide (Feigl and Anger, 1972).

Heavy metal analysis:

Tests for heavy metals, viz., lead, cadmium, arsenic, and mercury were carried out in ICP-OES instrument (Perkin Elmer Optima 5300 DV) (Mathew *et al*., 2011).

Pesticide residue analysis:

Various pesticide residues of trial drug were done using Gas Chromatography- Tandem Mass Spectrometry (GC-MS- MS) (WHO, 2007)

Microbial contamination:

Tests for total bacterial /fungal counts Escherichia coli, *Salmonella* spp., *Staphylococcus aureus* and Enterobacteriacea were done (Lohar, 2007).

Test for aflatoxins:

Aflatoxins such as B_1, B_2, G_1 and G_2 present in trial drug was done using HPLC (High Performance Liquid Chromatography) (Lohar, 2007).

HPTLC Finger print profiling:

Chromatographic identification and Fingerprinting analysis of trial drug was done by using HPTLC (High Performance Thin Layer Chromatography) (Sayyada Khatoon *et al*; 2014).

Procedure:

Preparation of spray reagent-vanillin-sulphuric acid reagent:

Vanillin (1 g) was dissolved in ice cold ethanol (95 ml). Add to 5 ml of cooled concentrated sulphuric acid. Ice was added and stirred well. The solution was stored in refrigerator.

Reagents and solvent:

Sample preparation:

The sample was prepared in polar solvent and sonicated/refluxed the solution and filtered with Whatman 41 paper and re-filtered with syringe filter (0.45 µ). Filtered solutions were applied to HPTLC 60 silica gel glass-backed layers (Merck,).

Linomat 5 (*sample applicator*) *conditions*:

Syringe delivery speed, 10 s μl^{-1}; Injection volume, 1-10 µl; Band width, 6 mm; space between bands, 9 mm; start position, 9 mm; Distance from bottom, 8 mm.

The HPTLC plates were developed in a horizontal chamber (Camag 20 x10), after saturation with the same mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature. The length of the chromatogram run was 80 mm. The developed layers were dried in an oven at 100-105 ºC for 15 min and then detected. Initially, the separated components were visually detected. The layers were allowed to dry in air for 30 min and then analysed under the proper detection way. For the fingerprinting, a Camag TLC scanner 3 linked to win CATS software was set at 350 nm, after multi-wavelength scanning between 250 and 400 nm in the absorption mode had first been tried. The sources of radiation were fluorescence, deuterium, and tungsten lamps. The slit dimension was kept at 6.00 × 0.45 mm and the scanning speed used was 20 mm s^{-1}.

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally, the plates were derivatized using vanillin-sulphuric acid reagent heated at 105°C till colour spots appeared.

Results and Discussion

The process of evaluating the quality and purity of herbal drugs by means of various parameters such as physical, chemical, and biological observation is called standardization. Following are the results of the physicochemical and estimation of basic and acidic radicals. Results obtained are depicted in Tables 2-4.

Heavy metal analysis:

ICP-OES results showed that Heavy metals were found below the detection level (Table 5).

Test for aflatoxins (B_1, B_2, G_1, $G2$):

All four aflatoxins were not detected in the drug. As the total fungal count was within the permissible limit, toxins were not promoted in the drug, and is free from these aflatoxins (Table 6).

Microbial contamination:

The total bacterial count and the total fungal count were within the permissible limits as per WHO Standards. The drug was found free from *E. coli*, *Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results are shown in Table 7.

Pesticide residue analysis:

All the tested pesticides were found to be lower than the limit of quantification (Table 8). Hence, it is safe for internal consumption.

HPTLC Fingerprint studies:

HPTLC of VVC was carried out at 254 nm Rf and area % - Track 1 (Fig. 1) and Track 2 (Fig. 2). Figure 3 depicts the TLC plate at 254 nm.

Organoleptic characters of *Vithu vagai chooranam* (VVC) showed that the drug was Light brown in colour which has sweet taste and characteristic odour.

The Ash value of VVC is 2.10%. it is the residue remaining after incineration that determines the inorganic substances present in the drug. Similarly, it can also detect the nature of the material, whether it is adulterated or not. So, determination of the ash value is a criterion for judging the identity and purity of the drug. Acid insoluble ash was 0.83% which clearly indicates the absence of siliceous matters. Alcohol and water-soluble extractive values of VVC are 10.1 % and 63.97%, respectively. This indicates that most

Table 2: Physico-chemical properties of VVC

S. No.	Parameters	Results
1	Loss on drying (at 105°C)	4.50%
2	**Ash value**	
	a. Total ash (%w/w)	2.10%
	b. Acid insoluble ash (%w/w)	0.83%
3	**Extractive values**	
	a. Alcohol soluble extractives(%w/w)	10.1%
	b. Water soluble extractives(%w/w)	63.97%

Table 3: Test for Acid radical studies of VVC

S. No.	Parameters	Results
1	Silicates	Absent
2	Sulphate	Absent
3	Chloride	Absent
4	Phosphate	Absent
5	Carbonate	Present
6	Nitrate	Absent
7	Sulphide	Absent
8	Oxalate	Absent
9	Nitrite	Absent
10	Borate	Absent

Table 4: Chemical Analysis of VVC– basic radicals and Miscellaneous

S. No.	Parameters	Results
1	Lead	Absent
2	Copper	Absent
3	Aluminum	Absent
4	Iron	Absent
5	Zinc	Absent
6	Calcium	Absent
7	Magnesium	Absent
8	Ammonium	Present
9	Potassium	Present
10	Sodium	Absent
11	Mercury	Absent
12	Arsenic	Absent
13	Starch	Absent
14	Reducing sugar	Present
15	Tannins	Present
16	Alkaloids	Absent
17	Unsaturated compounds	Absent
18	Amino acid	Absent

Table 5: ICP-OES analysis of VVC

S. No.	Elements	Detection level
1	Lead (as Pb) (ppm)	BLQ(LOQ:0.5)
2	Arsenic (as As) (ppm)	BLQ(LOQ:0.5)
3	Mercury (as Hg) (ppm)	BLQ(LOQ:0.5)
4	Cadmium (as Cd) (ppm)	BLQ(LOQ:0.25)

Table 6: Aflatoxins assay of VVC

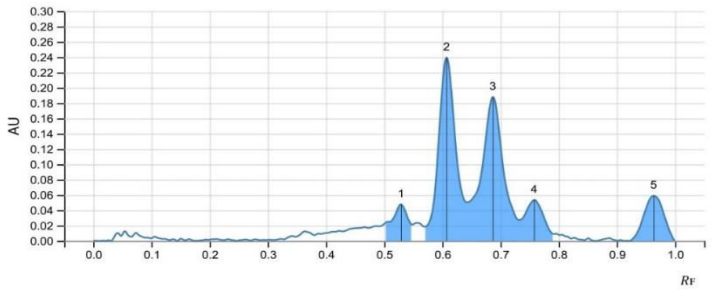
A	Aflatoxins $B_1+B_2+G_1+G_2$ (ppm)	BLQ (LOQ : 0.0005)

Table 7: Test for microbial load and Specific pathogens of VVC

		Reference Limits as per WHO (2007)	Results	Remarks
A	Total viable aerobic count, cfu/g	10^5 CFU/g	165000	Within permissible limits
B	Total fungal count, cfu/gm	10^3 CFU/g	28500	
C	*E. coli*/g	10	Absent	
D	*Salmonella*/g	Absent	Absent	
E	*S. aureus*/g	Absent	Absent	
F	*P. aeruginosa*	Absent	Absent	

Table 8: Pesticide residue analysis of VVC

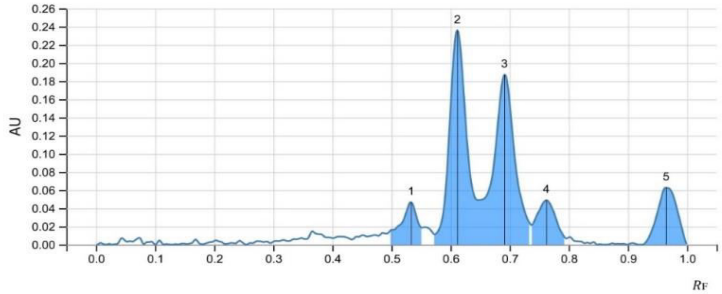
Pesticide residue	Sample VVC	AYUSH limit (mg/kg)
I. Organochlorine pesticides		
Alpha BHC	BQL	0.1 mg/kg
Beta BHC	BQL	0.1 mg/kg
Gamma BHC	BQL	0.1 mg/kg
Delta BHC	BQL	0.1 mg/kg
DDT	BQL	1 mg/kg
Endosulphan	BQL	3 mg/kg
II. **Organophosphorus Pesticides**		
Malathion	BQL	1 mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1 mg/kg
III. Carbamates		
Carbofuran	BQL	0.1 mg/kg
III. Pyrethroid		
Cypermethrin	BQL	1 mg/kg



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.503	0.0243	0.529	0.0478	8.12	0.548	0.0212	0.00151	6.66	No	
2	0.569	0.0185	0.607	0.2395	40.72	0.642	0.0505	0.00827	36.57	No	
3	0.642	0.0505	0.687	0.1881	31.98	0.732	0.0273	0.00845	37.35	No	
4	0.732	0.0273	0.758	0.0536	9.11	0.791	0.0089	0.00207	9.17	No	
5	0.923	0.0000	0.963	0.0592	10.06	0.999	0.0006	0.00232	10.26	No	

Track 2:	
Type	Sample
Vial ID	A01-2202280130
Description	Vitthu Vagai Chooranam
Volume	5.0 µl

Fig. 1: HPTLC of VVC at 254nm Rf and area % - Track 1.



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.496	0.0136	0.533	0.0468	8.04	0.552	0.0188	0.00155	7.09	No	
2	0.572	0.0115	0.611	0.2358	40.56	0.646	0.0490	0.00796	36.37	No	
3	0.646	0.0490	0.691	0.1871	32.18	0.736	0.0219	0.00811	37.07	No	
4	0.737	0.0216	0.762	0.0490	8.43	0.795	0.0068	0.00184	8.40	No	
5	0.924	0.0000	0.965	0.0628	10.79	0.999	0.0006	0.00242	11.07	No	

Fig. 2: HPTLC of VVC at 254 nm Rf and area %- Track 2.

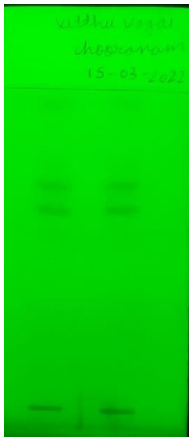


Fig. 3: TLC Plate at 254 nm.

of the compounds present in the drug were water soluble. Solubility of the compounds in water is essential for crossing the water layer adjacent to the lipid layer of the cell wall. Higher water solubility of the compounds in the drug indicates that the compounds can easily cross the water layer of cell wall.

The Chemical analysis shows the presence of Carbonate, Ammonium, Potassium, reducing sugar and Tannins. The Heavy metal analysis by ICP-OES reveals that the drug contains no heavy metals such as mercury, lead, cadmium, and arsenic. Since the drug does not contain any heavy metals, the drug will not cause any cumulative toxicity in chronic administration. The VVC shows no growth of bacteria and fungi and specific pathogens like *E. coli*, *Salmonella* sp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. All four aflatoxins (B_1, B_2, G_1, G_2) were not detected in the drug. As the total fungal count was within the permissible limit, toxins were not promoted in the drug, and is free from these aflatoxins. There were no traces of pesticide residues such as Organochlorine, Organophosphorus, Carbamates and Pyrethroid in the drug VVC. Hence, it is safe for internal consumption. Qualitative fingerprinting technologies have been used for the quality control of herbal materials. HPTLC analysis was performed and the study shed a light on the active constituents present in the *Vithuvagai chooranam* (VVC) which is depicted by the presence of 5 prominent peaks at UV 254nm in which each peak corresponds to number of versatile components present within it. The HPTLC results are interpreted based on the area coverage of the peak, the height of the peak, the number of peaks, and the Rf value of the peaks. HPTLC fingerprint profile of VVC under UV 254 nm, the peak 2,3 occupies the major percentage of area 36.57% (Rf 0.642) and 37.35% (Rf 0.732) which denotes the abundant existence of this compound followed by the peak 5,4,1 occupies the percentage area of 10.26% (Rf 0.999),9.17%(Rf 0.791), 6.66% (Rf 0.548).

Conclusion

It is concluded from this study that the Siddha herbal formulation VVC possess all properties of *Chooranam* as per PLIM Guidelines. The Drug VVC has essential active therapeutics which may help in treating various disorders. In future these evidences can be used as references for the standardization of the drug VVC. Clinical trials have to be carried out to validate the safety and efficacy of this drug in humans after the preclinical studies.

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