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STANDARDIZATION OF A SIDDHA FORMULATION WITH MODERN TECHNIQUES

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ABSTRACT

Standardization of Siddha formulation is essential in order to assess the quality, purity, safety and efficacy of drugs based on the amounts of their active principles. The objective of the present study was to evaluate the Siddha formulation containing four active ingredients for asthma. In this study an attempt has been made to develop pharmacognostical and pharmaceutical standards for Siddha formulation. For evaluation of Siddha formulation containing many herbs various parameters were tested. Parameters for finished product include macroscopic, microscopic, loss of drying, total ash and extractive values. HPTLC study, heavy metal analysis, microbial analysis and pesticide residue analysis were carried out as a part of evaluation. Results indicate that Siddha formulation has passed through all organoleptic and physicochemical parameters.

KEY WORDS: Standardization, Siddha formulation, Extractive value, HPTLC, Pharmacognostical and Pharmaceutical standards.

INTRODUCTIONS

Siddha, the Indian traditional system of medicine has been curing the several ailments of living beings since ages. Lots of formulations have been mentioned in Siddha texts with its specific use for ailments like respiratory diseases, gastro intestinal disorders, fever and vomiting. The development of these Siddha traditional systems of medicines with the perspectives of safety, efficacy, and quality will helps not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare. So evaluation is necessary to ensure quality and purity of the herbal product. It is very important to establish a system of evaluation for every

plant medicine in the market, since the scope for variation in different batches of medicine is enormous (Soni Hardik et al., 2010). The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy (WHO., 2002b). Based on the above rationale the present study was undertaken to standardize Siddha formulation based on their Pharmacognostical and Pharmaceutical characteristics. The present paper reports the standardization of Siddha formulation based on organoleptic characters, physical characteristics, and physicochemical properties. HPTLC study, heavy metal analysis, microbial analysis and pesticide residue analysis were also carried out as a part of evaluation.

2. MATERIAL AND METHODS

Following four herbal drugs were chosen *Adhatoda vasica* (Acanthaceae) Leaf powder, *Solanum xanthocarpum* (Solanaceae) Whole plant powder, *Tylophora asthamatica* (Asclepiadaceae) Leaf powder, *Ocimum tenuiflorum* (Lamiaceae) Leaf powder. All four herbs were procured from kolli hills, Salem and were authenticated by Dr. P. Jayaraman, Director Plant Anatomy Research Centre (PARC), Tambaram, Chennai. A voucher specimen has been deposited for further reference.

2.1. Preparation of Siddha formulation

The selected plant organs were thoroughly washed with the clean running water and shade dried. After drying, the herbals grounded separately as per process in a stone mortar and passed through a clean white muslin cloth and filtered. They were mixed in the equal proportion (1:1:1:1) and stored in well closed container.

2.2. Macroscopic study of Siddha formulation

The Organoleptic evaluation refers to evaluation of the formulation by Appearance, colour, odour and taste.

2.3. Microscopic study of Siddha formulation

The study was carried out at Pharmacognosy dept., Vel's university, Chennai. Small quantity was mixed with distilled water and was mounted on slides for the study. The characters were studied with and without staining. Staining was done with (Khandelwal., 2007)

phloroglucinol and conc. HCl. Microphotographs was taken using Nikon lab photo 2 microscopic units (Esau.,1964).

2.4. Physicochemical properties

Physio-chemical studies like Loss of drying at 105˚, total ash, acid insoluble ash, water soluble ash, water soluble extract, alcohol soluble extract were carried out as per the standard guide lines (Anonymous., 2008).

3. HPTLC

High Performance Thin layer chromatography (HPTLC) (Ashworth & Stahl 1969) studies were carried out; following procedure was adopted in chromatography.

Chromatographic conditions

The following Chromatographic Conditions were used.

1. Stationary phase : TLC Silica gel 60 F
2. Mobile phase : Toluene:Ethyl acetate:Formic acid :Methanol (70:30:7.5:2.5)
3. Sample volume : 50mg/ml
4. Sample solvent for HPTLC : Ethanol
5. Spray reagent : N_2

Instrumental Conditions

1. Application mode : Camag Automatic TLC Sampler 4 (ATS4)
2. Plates : HPTLC plates silica gel 60 F **254**
3. Chamber Saturation : 30 min.
4. Development Time : 30 min.
5. Development distance : 7 cm.
6. Scanner : Camag TLC Scanner III.
7. Scanning mode : Linear at 254 nm and 366 nm
8. Photo documentation : CAMAG reprostar
9. Detection : Hg
10. Data System : Win cats software
11. Drying dev : Oven

4. Heavy Metal Analysis

To 3 ml of the sample, 10 ml water, 2 ml Hydrochloric acid and 2 ml Nitric acid were added and boiled for 10 minutes. The mixture was cooled down and volume

made up to 100 ml with water. 0.1N Nitric acid was used as blank. The samples were detected for presence of heavy metals like lead, cadmium, arsenic and mercury (WHO., 2002a).

5. Microbial Analysis

Microbial analysis was carried for Siddha formulation as per Standard guideline. The test included total bacterial count, total yeast and mould count, Identification of specified organisms such as *Escherichia coli, Salmonella, spp, S.aureus, Pseudomonas aeruginosa* (Anonymous., 2008).

6. Determination Of Pesticidal Residue By Tlc

6.1 Extraction of common pesticide from material

10 g of sample were taken in a round bottom flask and added Sodium sulfide with 100ml n-Hexane. It was refluxed for 1 hour and filtered. The filtrate extracted with 50ml and 25ml of Acetonitrile. The Acetonitrile layer was mixed with 500ml Demineralised water with 2.5ml saturated sodium sulfide and then extracted with an n-Hexane layer and evaporated on a water bath. This residue was used for the analysis of organochloro, organophosphate and Pyrethroids pesticides by Thin layer chromatography using standard reference standards (Accu standards, USA).

6.2 TLC details

Sample solution : Residue in methanol

Development system : Benzene: Methanol (60: 40)

Stationary Phase : Silica gel 60 F254 TLC plate of 0.2mm thickness.

Detection : By UV Absorption Range from 200 to 300nm.

The Extracts were spotted along with reference standards and chromatogram was developed and analyzed under UV from 200 to 300 nm (Smith, 1991).

7. RESULTS

Pharmacognostical study of organoleptic evaluation

The sample was brown in colour with characteristic odour and predominant Bitter taste. (**Table 1**)

Table 1: Organoleptic characters of Siddha formulation

Colour	Brown
Odour	characteristic
Taste	Bitter
Appearance	Fine powdered

Microscopic characters

Powder microscopy of Siddha formulation shows the constituents which is summarized in (**Table 2**).

Table 2: Observation of various microscopic characters of Siddha formulation

Siddha Formulation	Characters
Adhatoda vasica	Fig.1.1. Adaxial epidermis showing stomata Fig.1.2. Stomata enlarged Fig 1.3. One cystolith enlarged.
Ocimum tenuiflorum	Fig.2.1. Non glandular epidermal trichome Fig.2.2. Trichome enlarged Fig.3.1. Glandular trichome in surface view. Fig.3.2. Epidermal peeling showing wavy anticlinal walls and diacytic stomata.
Solanum xanthocarpum	Fig.4.1. Starch grains stained with IKI. Fig.4.2.Stellatetype of trichome with spreading arms.
Tylophora asthamtica	Fig.5.1. Laticifer Fig.5.2. Bundle of tracheids. Fig.6.1. Fibre. Fig.6.2. Epidermal trichome

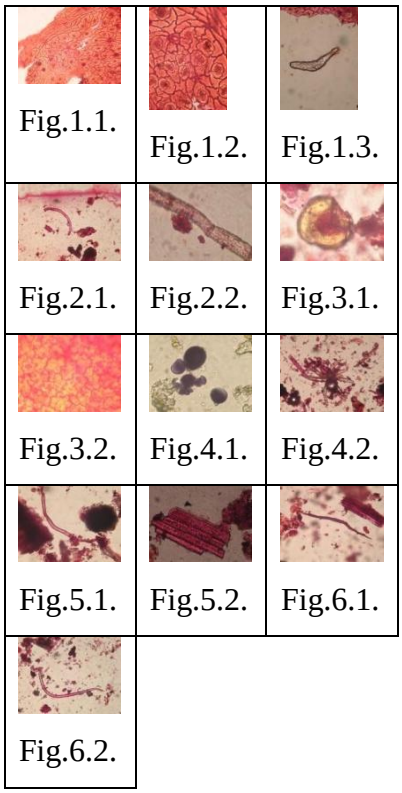

Fig .1

Table 3: Quality tests of Siddha formulation All the values are expressed as mean ± SD, n=3

PARAMETER	VALUES
Loss of Drying (% w/w)	3.74±0.32
Total Ash(% w/w)	12.38±0.54
Acid soluble ash(% w/w)	0.85±0.04
Water soluble ash (% w/w)	11 ± 0.26
Water soluble extractive (% w/w)	21±0.36
Alcohol soluble extractive(% w/w)	12±0.31

Table 4: Phytochemical screening of Siddha formulation

Sr. No.	Chemical Constituents	Siddha formulation
1	Triterpenoids	+
2	Flavones	+
3	Alkaloids	+
4	Carbohydrates	+
5	Glycosides	+
6	Phenols	+
7	Proteins	+
8	Amino acids	-
9	Saponins	+
10	Tannins	+
11	Steroids	+

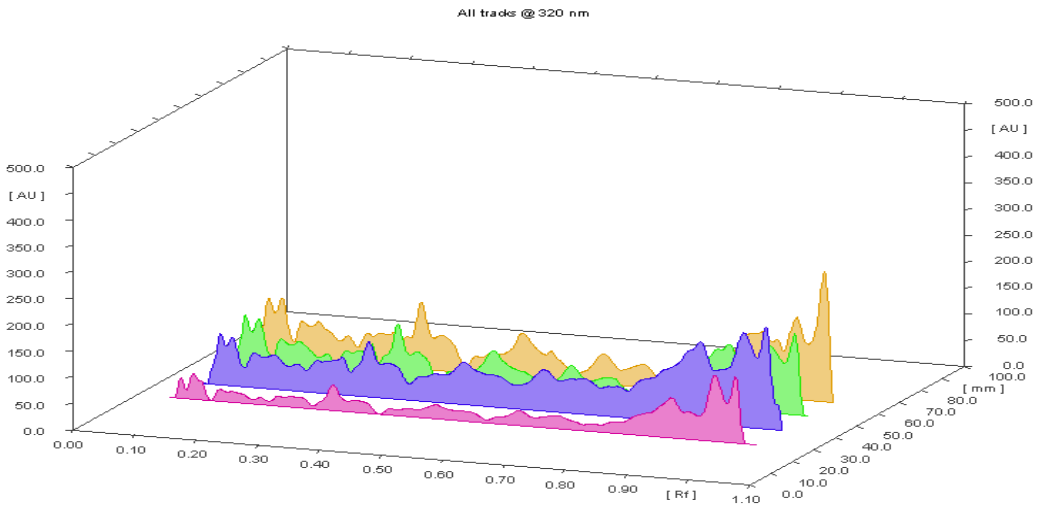

Figure 2: HPTLC Finger printing analysis for the Siddha formulation

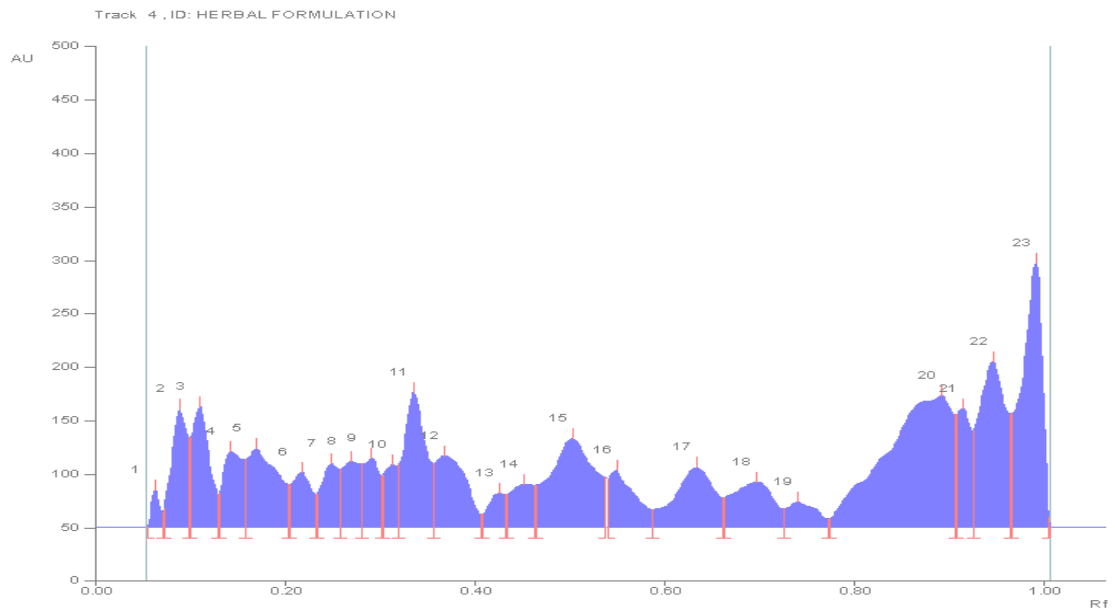

Table 4: Densitometry Results

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	0.0	0.06	35.0	1.89	0.07	13.8	263.8	0.61
2	0.07	17.1	0.09	110.2	5.93	0.10	83.6	1579.1	3.65
3	0.10	84.5	0.11	112.5	6.06	0.13	31.0	1779.8	4.11
4	0.13	32.2	0.14	71.1	3.83	0.16	63.3	1282.3	2.96
5	0.16	63.6	0.17	73.6	3.96	0.20	40.1	2046.3	4.73
6	0.21	40.4	0.22	51.5	2.77	0.23	30.9	905.0	2.09
7	0.23	31.1	0.25	59.2	3.19	0.26	53.9	941.1	2.18
8	0.26	54.2	0.27	61.5	3.31	0.28	59.3	1004.7	2.32
9	0.28	59.2	0.29	64.5	3.47	0.30	47.7	938.9	2.17
10	0.30	47.9	0.31	58.5	3.15	0.32	57.6	718.9	1.66
11	0.32	58.3	0.34	126.3	6.80	0.36	59.5	2472.8	5.72
12	0.36	59.5	0.37	66.8	3.60	0.41	11.9	1859.4	4.30
13	0.41	12.1	0.43	31.9	1.72	0.43	30.7	478.9	1.11
14	0.43	30.7	0.45	40.3	2.17	0.46	39.3	859.0	1.99
15	0.46	39.4	0.50	82.8	4.46	0.54	46.6	3352.6	7.75
16	0.54	44.8	0.55	53.6	2.89	0.59	16.6	1264.5	2.92
17	0.59	16.6	0.63	56.0	3.01	0.66	27.8	2004.5	4.63
18	0.66	27.8	0.70	42.5	2.29	0.73	17.1	1592.7	3.68
19	0.73	17.1	0.74	23.6	1.27	0.77	7.6	635.5	1.47
20	0.77	8.1	0.89	122.9	6.62	0.91	105.3	7546.9	17.44
21	0.91	105.6	0.91	110.9	5.97	0.93	88.9	1439.5	3.33
22	0.93	90.4	0.95	155.2	8.35	0.97	106.0	3762.2	8.70
23	0.97	106.4	0.99	247.2	13.31	1.01	8.9	4533.8	10.48

Table 5: Heavy Metal Analysis of Siddha Formulation

S.No.	Heavy Metal	Limit	Siddha Formulation
1.	Lead (Pb)	10ppm	0.90ppm
2.	Cadimum(Cd)	0.3ppm	0.2ppm
3.	Arsenic (As)	10ppm	1.05ppm
4.	Mercury (Hg)	1ppm	Absent

Table 6: Microbial Analysis of Siddha Formulation

S.No.	Microbial Analysis	Limit	Siddha Formulation
1.	Total aerobic viable count	10^5/gm	140cfu/gm
2.	Total yeast and mould	10^3/gm	Nil
3.	E.coli	Absent	Absent
4.	Salmonella spp	Absent	Absent
5.	S.aureus	Absent	Absent
6.	Psendomonas aeruginosa	Absent	Absent

Table 7: Pesticide Residue Analysis of Siddha Formulation

S.No	Parameters analysed	Results	Permissible limits as per WHO
1	Chlorpyriphos	Nil	Not more than 0.20mg/kg
2	DDT	Nil	Not more than 1.00mg/kg
3	Endosulfan	Nil	Not more than 3.00mg/kg
4	Malathion	Nil	Not more than 1.00mg/kg
5	Parathion	Nil	Not more than 0.50mg/kg

ND – No spots were detected

Table 8: Aflatoxin Contamination of Siddha Formulation

S.No	Parameters analysed	Results	Permissible limits as per WHO
1	B1	Nil	Not more than 0.50mg/kg
2	B2	Nil	Not more than 0.10mg/kg
3	G1	Nil	Not more than 0.50mg/kg
4	G2	Nil	Not more than 0.10mg/kg

8. CONCLUSION

The current study was subjected to physio chemical analysis and other parameters such as microscopic study, organoleptic characters. Heavy metal analysis, Pesticide residue and aflatoxins contamination was analyzed and found absent as per WHO guidelines. Microbial load was found within the permissible limits as per standard guidelines. HPTLC fingerprinting analysis reveals the components having 23 spots. Solvent system shows good separation of compounds which can be used for further analysis. The parameters of this study can be used for the authentication and further research which helps in quality assurance of drug used in siddha system of medicine and its development.

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