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STANDARDIZATION OF A SIDDHA POLY HERBAL FORMULATION ADHATHODAI CHOORANAM

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ABSTRACT

Siddha Drugs are natural products obtained from herbs, metals, minerals and animal kingdom. With the growing awareness of health care and safety aspects, people are moving towards herbal products. Proper standardization of drug preparation method as well as chemical analysis of traditional formulation is mandatory to gain support for its use worldwide. Aadhathodai chooranam is a siddha sastric drug for the treatment of Bronchial Asthma. Literature review evidenced no standardization work so far. The present study was carried out to standardize Aadhathodai Chooranam by evaluating its organoleptic properties, preliminary phyto chemical screening, physico chemical analysis, Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC) finger print, heavy metal analysis, Microbial load and Aflatoxin content for further application in clinical trial of bronchial Asthma. The loss on drying at 105°C, Ash, Acid insoluble Ash, Water soluble extractive, Alcohol soluble extractive and pH of Aadathodai Chooranam were 11.16%, 7.90%, 2.5%, 17.25%, 12.29% and 5.79%. Alkaloids, Flavonoids, Glycosides, Terpenes, Saponins, Amino acids, Phenols, Tannins, Quinones, Lignans, Steroids, Chloride, Phosphate, Ammonium iron were detected and the Aadhathodai Chooranam was below the WHO/FDA permissible limits in Heavy metals, Microbial load and Aflatoxin. HPTLC finger print result was responsible for expression of its Biological and clinical actions.

Keywords: Siddha drug, Aadhathodai chooranam, Bronchial Asthma, Standardization, TLC, HPTLC.

INTRODUCTION

Plant parts are used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the global drug market.¹ Herbal medicines as the major remedy in traditional system of medicine have been used in Medical field since antiquity.² Aadhathodai chooranam is a siddha poly herbal drug which comprises 17 ingredients such as *Alpinia galanga* Wild (Rhizome), *Alpinia officinarum*, *Hance* (Rhizome), *Justicia adhatoda* Linn (Root bark), *Tragia involucrata* (Root), *Piper longum* Linn (fruit), *Styrax benzoin* Dryand (Resin), *Curcuma longa* Linn (Finger rhizome), *Costus speciosus* (Koen.) sm. (Root), *Embelia ribes* Burm.f (fruit), *Clerodendrum serratum* (Linn.), *Moon* (Root), *Cyperus rotundus* Linn (Rhizome), *Ficus tsiela* (Stembark), *Woodfordia fruticosa* Kurz (flowers), *Solanum trilobatum* Linn (Leaf), *Solanum surattense* Burm.f (whole Plant) and *Piper nigrum* Linn (fruit). Standardization is mandatory to confirm the quality and reliability of traditional medicines rapidly increases all over the world.³ Drug Standardization is an essential factor for herbal formulation in order to assess the quality of the drugs based on the concentration of their active principle and to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic action.⁴ In this study the parameters for quality assessment have been followed as per CCRAS guidelines for Analytical specifications of Chooranam.⁵

MATERIALS AND METHODS

Procurement of raw drugs

All the raw materials were purchased from The Indian Medical practitioner's co-operative pharmacy and stores Ltd, X-185, Thiruvannmiyur, Chennai. The Aadhathoda inflorescence, root bark and *Solanum trilobatum* leaf are collected from Herbal Garden, National Institute of Siddha, Chennai.

Authentication of herbs

All the herbs were authenticated by Assistant Professor of Botany, National Institute of Siddha, Chennai (Voucher No. NIS/NB/69/2012) were deposited in the Medicinal Botany Laboratory, NIS, Chennai.

Preparation of Aadhathodai Chooranam

The Aadhathodai chooranam was prepared according to the procedure given in Siddha text.⁶

Analytical Study

Organoleptic characteristics (Appearance, color, odour, taste, touch), physico chemical analysis like loss on dry at 105° C, Ash value, acid insoluble ash, pH value, acid insoluble ash, water soluble extractive, Alcohol soluble extractive, TLC and HPTLC were carried out as per Indian Pharmacopeia in Captain Srinivasa Murti Drug Research Institute of Ayurveda and Siddha Drug Development, Arumbakkam, Chennai. Tests for presence of certain heavy metals (mercury, arsenic, lead, and cadmium), microbial contamination and Aflatoxin content

were carried out by using ICP MS and HPLC FLD as per the procedure WHO Q AS/05-131 and AOAC 990.33. Phyto chemical analysis was carried out in Bio chemistry Lab, National Institute of Siddha and Chemistry Lab, Siddha Central Research Institute, Arumbakkam, Chennai.⁷⁻¹¹

Preliminary Phytochemical Tests^{12,13,14}

Test for Chloride

2 ml of the extract is added with dil. HNO₃ till the effervescence cease. Then 2 ml of silver nitrate solution is added. The presence of cloudy appearance indicates the presence of chloride.

Test for Phosphate

2 ml of the extract is treated with 2 ml of ammonium molybdate solution and 2 ml of Con. HNO₃. The presence of cloudy yellow appearance indicates the presence of phosphate.

Test for Ammonium

To 2 ml of extract few ml of Nessler's reagent and excess of Sodium hydroxide Solution are added. Appearance of brown colour indicates the presence of Ammonium.

Test for iron

To the 2 ml of extract add 2 ml of ammonium thiocyanate solution. Appearance of mild red colour indicates the presence of Iron. To the 2 ml of extract 2 ml ammonium thiocyanate solution and 2 ml of Con HNO₃ is added. Appearance of blood red colour indicates the presence of Iron.

Test for Alkaloids (Dragendorff's test)

Few mg of extract in separate test tube was warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendorff's reagent were added. The presence of orange red precipitation indicates the presence of Alkaloids.

Test for Flavonoids (Shinoda Test)

Substance is dissolved in alcohol, added with magnesium bits and concentrated hydrochloric acid. On heating over a water bath, the appearance of magenta colour shows the presence of flavonoids.

Test for Glycosides

Substance is treated with anthrone and concentrated sulphuric acid. On heating over a water bath, the appearance of green colour shows the presence of Glycoside.

Test for triterpenoid (Noller's test)

To few mg of extract add tin and thionyl chloride and heat in water bath purple colour indicates the presence of triterpenoids.

Test for Saponin

To few mg of extract distilled water is added and shaken well. The formation of foam indicates the presence of saponin.

Test for Amino acids (Ninhydrin test)

The Ninhydrin reagent is 0.1% W/u solution of minhydrin in n-butanol. A little of this reagent was added to the test extract. A Violet or purple colour indicates the presence of Amino acids.

Test for Phenol.

Substance in water is added with 5% alcoholic ferric chloride. Dark blue or green colour shows presence of phenol.

Test for tannin

Substance is shaken with water and added with lead acetate solution. White precipitate shows the presence of tannin.

Test for Anthraquinones

1 ml of sample was taken to that aqueous ammonia was added and observed for change in colour. Pink red or violet colour in aqueous layer indicates the presence of Anthraquinones.

Test for Steroids (Lieberman Burchard Test)

To few mg of the extract 2 ml of chloroform is added in a dry test tube. Few drops of acetic acid is added, heated and few drops of acetic anhydride and 2 drops of concentrated sulphuric acid are added. The green colour indicates the presence of steroids.

TLC and HPTLC Methodology

4 gm of the sample was soaked in 40 ml of 90% ethyl alcohol, kept overnight, boiled for ten minutes and filtered. The filtrate was concentrated to 10 ml and made up to the mark in a 10 ml standard flask. 15 µl and 20 µl of this solution was applied on (Merck) Aluminium plate 60F 254 precoated with silica gel of 0.2 mm thickness and the plate was developed in Tolouene:Ethyl acetate (5:2). Then the plate was visualized under UV 254 nm and 366 nm UV and photos were taken. Then the plate was scanned at 254 nm and the plate was dipped in Vanillin sulphuric Acid and kept in the oven at 105° C till the colour of the spots appeared. The photos and finger print were taken.

RESULTS AND DISCUSSION

The field of the herbal drugs and formulations is very vast and there is still lot to explore on the subject of standardization of these. So, while developing an herbal drug formulation it is must to have the related knowledge of that particular drug including all its organoleptic characters to phyto-constituents to pharmacological action to its standardization in respect to various parameters via various techniques.⁴ Standardization need for Global acceptance, documentation, reproducibility, industrial scale production, prevent adulteration and contamination, assess the quality of raw material and finished product, estimate the amount of active principle, achieve batch to batch consistency of finished product.¹⁵

Organoleptic characters

Aadathodai Chooranam was light brown coloured fine powder with characteristic odour, bitter taste and completely pass through Sieve No.100.

Physicochemical Evaluation

The physicochemical parameters of the Aadathodai Chooranam are tabulated as Table 1.

Loss on drying

Loss on drying indicates the total volatile content and moisture content of sample. High Moisture contents may affect the quality of the drug. Low moisture content could get maximum stability and better shelf life.¹⁶ In the present sample the loss on drying at 105 °C is 11.16% which may be due to the presence of volatile component

i.e. resin of *Styrax benzoin* Dryand in the Aadhathodai chooranam. The sublimate of *Styrax benzoin* Dryand in the Adhathodai Chooranam possess antiseptic action, so it may not affect the quality of the drug.

Ash Values

Ash value depends upon the inorganic substances present in the particular drug. Ash value may be a effective parameter to assess the degree of purity of a given drug. The ash value of Aadhathodai chooranam is 7.90% which correlates with the inorganic contents Ammonium, Chloride, Iron and Phosphate of sample drug through the phyto chemical analysis. The acid insoluble ash value of the drug denotes the amount of Siliceous matter present in the plant.¹⁶ The quality of the drug is better if the acid insoluble value is low. It is 2.5% for Aadhathodai chooranam.

Extractive values

They are the approximate amount of the chemical constituents present in the raw drug. The percentage of soluble matters present in the drug is determined by the value of water extractive and ethanol extractive. Based on the extractive value suitable solvent can be selected. It gives the percentage of drug which will correlate with the metabolism reactions. Water soluble extractive value plays a major role in evaluation of crude drugs. The alcohol soluble extractive value was also the same use as the water soluble extractive value.¹⁶

Preliminary Phytochemical Screening

Qualitative tests were done to detect the functional groups. The study reveals the presence of Chloride, Phosphate, ammonium, iron, Alkaloids, flavonoids, Glycosides, terpene, saponins, Amino acids, Phenol, Tannins, Quinones, Lignans, Steroids in the aadhathodai Chooranam. As per Literature review these phytochemicals are presented in all the ingredients of Aadhathoodai Chooranam. The phytochemical analysis results also give additional support to its usage in clinical trial with potent Antioxidant, anti-inflammatory, antihistaminic, anti-spasmodic, immunomodulator and bronchodilator action.¹⁷ The Phytochemical constituents of Aadhathodai Chooranam are tabulated as Table 2.

Heavy Metal Analysis

Heavy Metals may be present in crude drugs through atmospheric pollution and through the soil. Moreover minerals and metals are also used in preparing indigenous formulations. However, heavy metals have been associated with various adverse effects. Hence, Heavy metals need to be detected in such preparations.¹⁹ In Table 3 result of heavy metals (Pb, Cd, As and Hg) in Aadhathodai Chooranam was compared with the permissible limits and acceptable daily intake as set by AYUSH, WHO and Food and Drug Administration¹⁸ and the result revealed that the Aadhathodai Chooranam was below the WHO/FDA permissible limits of Heavy metals and safe for consumption (Pb:1.77 mg/kg, Cd:0.25 mg/kg, As:0.25 mg/kg, Hg:0.25 mg/kg).

Microbial load and Aflatoxin Content of Aadathodai Chooranam

Medicinal Plant matters normally carry bacteria and moulds often originating in soil or after final drug preparation in product.¹⁹ In Aadhathodai Chooranam the total bacterial count was within the permissible limit (79,000 CFU/g). Total fungal count was within the permissible limit (<10CFU/g). Alfatoxin B1, B2, G1, G2 were within the permissible limits BLQ (LOQ:0.50µg/Kg).¹⁸ This indicates the proper hygiene norms followed during the preparation of formulation and packing which are essential to establish the quality standard at finished product level. The microbial load and Aflatoxin content in Aadathodai Chooranam are presented in Table 4.

TLC and HPTLC profile

Table 5 summarizes the Rf values and color of spots visible in the TLC profiles of Aadhathodai Chooranam. Visualization was attempted by spraying vanillin sulphuric acid reagent (Figure 3).

HPTLC FINGER PRINT PROFILE

Chromatographic study (HPTLC) was carried out under 254 nm and 520 nm UV to establish the fingerprinting profile and to reveal the possibly active phyto constituents. Table 6 shows HPTLC finger print of Aadhathodai Chooranam in 254 nm UV (Figure 1). The peak correspond to the Rf values 0.35 has maximum peak area of 12446.9 Au. This 6th peak (area % is 48.98%) could serve as a Marker. Table 7 shows HPTLC finger print of Aadhathodai Chooranam in 520nm UV (Figure 2). The peak correspond to the Rf value 0.35 has maximum peak area of 13576.3 AU. This 9th Peak (area % is 41.27%) could serve as a Marker. Phyto components with Rf values 0.01, 0.35, 0.76, 0.81, 0.89 (1st, 9th, 13th, 14th, 15th peaks) in Aadhathodai Chooranam which may be responsible for expression of its pharmacological and clinical actions.

CONCLUSION

The loss on drying at 105 °C, Ash, Acid insoluble Ash, Water soluble extractive, Alcohol soluble extractive and pH of Aadathodai Chooranam were 11.16%, 7.90%, 2.5%, 17.25%, 12.29%, 5.79%, respectively. The quality of the drug is better if the acid insoluble ash value is low which is 2.5% for Aadhathodai Chooranam. In phyto chemical analysis Alkaloids, Flavonoids, Glycosides, Terpenes, Saponins, Aminoacids, Phenols, Tannins, Quinones, Lignans, Steroids, Chloride, Phosphate, Ammonium iron were detected. The Phytochemical analysis results correlate with the literature survey of all the ingredients of Aadhathodai Chooranam which give additional supports to its usage in clinical trial of Bronchial Asthma with potent antioxidant, anti-inflammatory, antihistaminic, antispasmodic, immunomodulator and bronchodilator action. Heavy metal analysis, Microbial load and Aflatoxin content of Aadhathodai Chooranam revealed that the Aadhathodai Chooranam was below the WHO/FDA permissible limits and safe for consumption.

Table 1: Physicochemical evaluation of Aadhathodai Chooranam

Properties	Value
Loss on drying at 105 °C	11.16%
Ash value	7.90%
Acid insoluble ash	2.5%
Water soluble extractive	17.25%
Alcohol soluble extractive	12.29%
pH	5.79%

Table 2: Phytochemical analysis of Aadhathodai Chooranam

Test	Result
Alkaloids	Positive
Flavonoids	Positive
Glycosides	Positive
Terpenes	Positive
Saponins	Positive
Amino acids	Positive
Phenols	Positive
Tannins	Positive
Quinones	Positive
Lignans	Positive
Anthraquinones	Negative
Steroids	Positive
Organic Acids	Negative
Chloride	Positive
Phosphate	Positive
Iron	Positive
Ammonium	Positive

Table 3: Heavy Metal Contents of Aadhathodai Chooranam

Heavy Metal	Result	Specification as per Ayush /WHO /FDA
Lead (Pb)	1.77 mg/kg	10.0 mg/Kg
Cadmium (Cd)	BLQ (LOQ : 0.25 mg/kg)	0.3 mg/Kg
Arsenic (As)	BLQ (LOQ : 0.25 mg/kg)	3.0 mg/kg
Mercury (Hg)	BLQ (LOQ : 0.25 mg/kg)	1.0 mg/kg

BLQ - Below Limit of Quantification; LOQ - Limit of Quantification

Table 4: Microbial load and Aflatoxin content in Aadhathodai Chooranam

Microbiological analysis	Result	Specification as per Ayush /WHO /FDA
Total Bacterial Count	79,000 CFU/g	NMT 1,00,000 (CFU/g)
<i>E. Coli</i>	Absent / g	Absent / g
<i>Staphylococcus aureus</i>	Absent / g	Absent / g
<i>Salmonella</i>	Absent / g	Absent / g
<i>Pseudomonas Aeruginosa</i>	Absent / g	Absent / g
<i>Enterobacteriaceae</i>	<1CFU/g	NMT 1000 CFU/g
Total Fungal count	<10CFU/g	NMT1000 CFU/g
Aflatoxin:B1,B2,G1,G2	BLQ(LOQ:0.50µg/Kg)	0.5PPb

Table 5: TLC Analysis (Rf value and colour of the Spots) of Aadhathodai Chooranam

UV 254 nm		UV 366 nm		With Vanillin Sulphuric acid Spray Reagent	
Colour	Rf	Colour	Rf	Colour	Rf
Green	0.01	Pink	0.05	Grey	0.42
Green	0.05	Blue	0.20	Grey	0.05
Green	0.08	Blue	0.38	Grey	0.18
Green	0.17	Green	0.42	Grey	0.13
Green	0.27	Blue	0.49	Grey	0.17
Green	0.42	Pink	0.54	Grey	0.21
Green	0.58	Pink	0.63	Grey	0.24
Green	0.65	Pink	0.70	Grey	0.30
Green	0.71	Blue	0.74	Brown	0.43
Green	0.93	Pink	0.81	Grey	0.63
Green	0.99	Pink	0.88	Grey	0.71
				Grey	0.75
				Blue	0.80
				Grey	0.85
				Grey	0.94

Table 6: HPTLC finger print of Aadhathodai Chooranam in 254 nm UV

Peak	Start Position Rf	Start Height Au	Max Position Rf	Max Height AU	Max %	End Position Rf	End Height Au	Area Au	Area %
1	0.01	144.8	0.01	155.7	11.73	0.03	0.0	832.3	1.57
2	0.03	0.0	0.05	12.1	0.91	0.06	0.4	97.9	0.18
3	0.06	0.1	0.06	20.1	1.51	0.10	0.0	243.9	0.46
4	0.12	0.1	0.17	24.5	1.85	0.18	18.6	630.8	1.19
5	0.20	18.3	0.27	118.1	8.90	0.34	0.3	5262.4	9.92
6	0.35	0.3	0.42	408.3	30.76	0.54	79.3	25991.0	48.98
7	0.54	80.1	0.58	307.6	23.17	0.62	36.7	12446.9	23.46
8	0.63	87.3	0.65	141.2	10.64	0.69	4.7	4215.9	7.95
9	0.69	4.9	0.71	21.7	1.64	0.74	2.0	452.2	0.85
10	0.89	0.2	0.93	82.2	6.19	0.97	0.2	2524.4	4.76
11	0.97	0.6	0.99	35.8	2.70	0.99	28.2	363.7	0.69

Table 7: HPTLC finger print of Aadhathodai Chooranam in 520 nm UV

Peak	Start Position Rf	Start Height Au	Max Position Rf	Max Height AU	Max %	End Position Rf	End Height Au	Area Au	Area %
1	0.01	84.6	0.02	171.2	16.55	0.03	1.8	1947.9	5.92
2	0.04	1.0	0.05	9.5	0.91	0.06	0.0	58.3	0.18
3	0.07	1.8	0.08	23.5	2.27	0.10	6.0	350.0	1.06
4	0.11	0.1	0.13	12.4	1.20	0.14	4.1	151.9	0.46
5	0.17	3.3	0.17	13.8	1.33	0.18	0.8	82.0	0.25
6	0.20	0.6	0.21	10.1	0.98	0.22	7.5	147.3	0.45
7	0.23	7.4	0.24	10.4	1.00	0.26	0.0	142.0	0.43
8	0.28	4.6	0.30	25.5	2.47	0.32	9.3	584.4	1.78
9	0.35	5.8	0.43	291.1	28.13	0.52	1.5	13576.3	41.27
10	0.58	0.2	0.63	44.8	4.33	0.67	16.0	1802.8	5.48
11	0.67	16.2	0.71	31.9	3.08	0.72	27.5	1074.4	3.27
12	0.73	27.8	0.75	35.1	3.39	0.76	25.6	747.0	2.27
13	0.76	25.6	0.80	95.5	9.23	0.81	35.8	2281.5	6.94
14	0.81	85.9	0.85	172.7	16.69	0.87	78.7	6625.9	20.14
15	0.89	5.9	0.94	87.1	8.42	0.99	0.1	3321.0	10.10

Figure 1: HPTLC finger print of Aadhathodai chooranam at 254 nm UV

Figure 2: HPTLC finger print of Aadhathodai chooranam at 520 nmUV

Figure 3: TLC Photo documentation of Alcohol Extract of Aadhathodai chooranam
Solvent system Toluene:Ethyl acetate (5:2) Track 1: DTL – 15 µl; Track – 2: DTL – 20 µl

HPTLC finger print of Aadhothodai Chooranam in 254 nm UV revealed that the peak corresponds to the R_f value of 0.35 has maximum area of 12446.9 Au which could serve as a marker. The HPTLC finger print of Aadhothodai Chooranam in 520 nm UV the peak corresponds to the R_f value 0.35 has maximum peak area of 13576.3 AU which could serve as a marker and which is responsible for expression of its Biological and clinical actions.

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