ejpmr, 2022,9(6), 290-294

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

STANDARDIZATION OF AAVARAI BHAVANA CHOORANAM - A SIDDHA POLYHERBAL FORMULATION

Harishanbuselvan V.*[1], Rathinam J.[2], Arthi G.[3], Lakshmanakumar V.[4] and Sushil Kumar PN[5]

[1]Resident Medical Officer, National Institute of Siddha, Chennai.
[2]Assistant Medical Officer, Government Primary Health Centre, Thuneri, The Nilgiris.
[3]M.D (Siddha), Sai Siddha Clinic, Salem, Tamilnadu.
[4]Medical Officer Siddha (AYUSH), All India Institute of Medical sciences, Raipur, Chhattisgarh, India.
[5]Research Associate, Siddha Clinical Research Unit, New Delhi.

***Corresponding Author: Harishanbuselvan V.**
Resident Medical Officer, National Institute of Siddha, Chennai.

Article Received on 28/03/2022 **Article Revised on 18/04/2022** **Article Accepted on 08/05/2022**

ABSTRACT

Siddha system of medicine is the system that clearly explains the complete integrated relation of body, sense, mind and soul with the universe to attain immortality. In Siddha medicine there are 32 types of Internal medicine and 32 types of external medicine. Chooranam is one among the 32 types of internal medicine. Aavarai Bhavana Chooranamis a esteemed siddha polyherbal formulation mentioned in the ancient books of Siddha Medicine for the treatment of kalladaipu (Uroliathiasis)and diabetes mellitus .The intention of the study was to standardize the Siddha formulation Aavarai Bhavana Chooranam (ABC).Initially, Organoleptic characters like appearance, colour, taste and odour of ABC was noted. ABC was screened for moisture content, total ash value, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value, to estimate the quality of study drug. All the ingredients were procured and botanically authenticated. Ingredients were purified individually as per the siddha literature after which the formulation was prepared. The prepared ABC was subjected to analyses. The derived physico-chemical parameters, TLC profiling, HPTLC fingerprint profiles serve as diagnostic parameters to identify this polyherbal formulation. The achieved results of physico-chemical, TLC profiling, HPTLC finger print profiling will be useful as tools for documentation and profile of the poly herbal formulation.

KEYWORDS: Aavarai Bhavana Chooranam, Siddha Medicine, Standardization, HPTLC.

1. INTRODUCTION

Many plant products have found to play a main role in various diseases. Plant materials 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].Siddha system is one of the Indian Medicine practiced in South India especially in Tamil Nadu. Siddha system is one of the ancient system in the world, given by the great creators called Siddhars. They differentiated the diseases through three humours Vatha, Pittha, Kabha and found that there are 4448 diseases. They formulated the medicines for diseases on the basis of Suvai (Taste), Gunam (Character), and Veeriyam (Division). Siddha system focuses strongly on both physical and mental health of human being. Standardization method for all AYUSH drugs of Indian system of Medicine may contain a single herb or combination of different herbs believed to have complementary and synergistic effects. In the modern era due to exploitation and bulk production of herbal drugs there is chance of adulteration and imperfect processing of drugs. Hence there is need for standardization of all herbal drugs to sustain their quality. Therefore it is extremely 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. [2]The selected drug Aavarai Bhavana chooranam is a classical siddha formulation, It has various medicinal properties and used in the treatment of various diseases like Kalladaippu and Madhumegam. To ensure the efficacy and safety of this formulation, standardization using scientific methods is indispensable. As per WHO guidelines for standardization of herbal drugs, the organoleptic characters, chemical analysis, chromatographic fingerprinting and microbial screening shall be mandatory for herbal formulations. The prepared drug was investigated for physicochemical, thin layer chromatographic documentation and high performance thin layer chromatographic finger printing to achieve standardization protocol.

2. MATERIALS AND METHOD

2.1 Plant materials

All plant materials were freshly collected from in and around Tanjavur, Tamilnadu. some drugs were procured from raw drug shop in Parys, Chennai. And were authenticated by the Botanist, Department of Gunapadam, National Institute of Siddha. The list of the ingredients is exhibited in Table 1.

Table 1: Details of Ingredients of Aavarai Bavana Chooranam (AVBC).

S.NO	INGREDIENTS	BOTANICAL NAME	PARTS USED
1	Aavarai	*Cassia auriculata*	Seed
2	Atthippattai	*Ficusracemosa*	Bark
3	Maruthampattai	*Terminaliaarjuna*	Bark
4	Nellippazham	*Phyllanthusemblica*	Fruit
5	Thanneervittankizhangu	*Asparagus racemosus*	Tuber
6	Vazhaikkizhangu	*Musa paradisiaca*	Tuber
7	Nerunjiver	*Tribulusterestris*	Root
8	Seendhirkodi	*Tinosporacordifolia*	Stem
9	Sanbagapoo	*Micheliachampaca*	Flower
10	Kattrazhai	*Aloe vera*	Root

2.2. Purification of the raw materials

Purification of the raw material was done in conformance with the Siddha as per the direction mentioned in the Siddha Literature.[3]

2.3 Preparation of the Drug

The seeds of *Cassia auriculata* were soaked and dried each day in the juices of 2 to 10 respectively as mentioned in Table-1. Then the seeds were dried in the shade until loose the moisture content. It was finely powdered and sieved by white cloth which is mentioned as Vasthirakayam in classical Siddha literature then kept in an air tight bottle.[4]

2.4 Organoleptic Character

The organoleptic characters of the sample drug were assessed. 1gm of the test drug was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result is recorded.

2.5 Physicochemical Analysis

2.5.1. Percentage Loss on Drying

10gm of test drug was meticulously weighed in evaporating dish. The sample was dried at 105ºC for 5 hours and then weighed.[5,6]

Percentage loss in drying = Loss of weight of sample/Wt. of the sample X 100

2.5.2. Determination of Total Ash

3 g of test drug was accurately weighed in silica dish and incinerated at the furnace at a temperature of 400 ºC until it turns white in colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.[5,6]

Total Ash = Weight of Ash/Wt. of the Crude drug taken X 100

2.5.3. Determination of Acid Insoluble Ash

The ash obtained by total ash test was boiled with 25 ml of dilute HCL for 6 minutes. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.[5,6]

Acid insoluble Ash = Weight of Ash/Wt. of the Crude drug taken X 100

2.5.4. Determination of Water Soluble Ash

The ash obtained by total ash test was boiled with 25 ml of water for 5 minutes. The insoluble matter is collected in crucible and will be washed with hot water, and ignite for 15 minutes at a temperature not exceeding 450ºC. Weight of the insoluble matter will be subtracted from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.[5,6]

Water Soluble Ash = Weight of Ash/Wt. of the Crude drug taken X 100

2.5.5. Determination of Alcohol Soluble Extractive

About 5 g of test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing toast and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air dried drug.[5,6]

Alcohol sol extract = Weight of Extract/ Wt. of the Sample taken X 100

2.5.6. Determination of Water Soluble Extractive

About 5 g of the test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent,evaporate25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105ºC, to constant weight and Calculate the percentage of water soluble extractive with reference to the air-dried drug.[5,6]

Water soluble extract = Weight of Extract/ Wt. of the Sample taken X 100

2.5.7. Determination of pH

About 5 g of test sample was vanished in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 minutes and the subjected to pH evaluation.[5,6]

3. TLC/HPTLC finger print analysis

2gm 0f sample with 20 ml of alcohol and reflux on water bath for 30min.Filter and concentrate to 5ml and carryout the thin layer chromatography. Apply the alcohol extract on TLC plate. Develop the plate using Toluene: Ethyl acetate(1:1) as mobile phase. After development allow the plate to dry in air and examine under UV (254 nm), 366 nm and Vanillin-sulphuric acid. Thin layer chromatography (TLC) is a technique used to differentiate the components of a mixture using a thin stationary phase supported by an inert backing. It may be executed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound [7].

TLC/HPTLC is an analytical tool widely used because of its plainness, relative low cost, high sensitivity, and speed of separation. TLC/HPTLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC/HPTLC is to obtain well defined, well separated spots.[8]

Retention Factor

After a separation was complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent.

The Rf formula is Rf = distance traveled by sample /distance traveled by solvent

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions. The compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value.

4. RESULTS AND DISCUSSION

For the siddha formulations Aavarai Bhavana Chooranam (ABC), reports of organoleptic, preliminary, physiochemical, screening tests are not available. From this study of preclinical standardization of Aavarai Bhavana Chooranamwhich is specified in Siddha texts shows that the drug Aavarai Bhavana Chooranamwas a fine powder Brown in colour with Pleasant odor, and Characteristic taste. The drug size has a particle size completely pass through sieve no 100.The loss on drying denotes the moisture content of the drug was determined as 10.7%. The total ash was found to be 10.1% which denotes the inorganic content of the drug. The water soluble extractive value and alcohol soluble extractive value were established to be 25.87 ± 0.81% and 33.44 ± 2.12% which denotes that there is considerable quantity of chemical constituents in the formulation. The water soluble ash was resulted as 7.46% and the value of acid insoluble ash was arrived to be 0.1% which denotes that the drug contains in considerable amount of siliceous matter. The pH value is measured as 6.75 which indicate that the drug is slightly acidic.

The TLC photo substantiation of the drug under UV 254 nm shows three major spots at Rf 0.83, 0.71 and 0.63 and other spots are minor. The TLC photo of the drug substantiation under UV 366 nm also shows three major spots at Rf 0.88, 0.40 and 0.82 and the same TLC plate after derivatization with vanillin-sulphuric acid shows the spot at Rf 0.89 which is the only major spot and all other are minor compared to that spot. The HPTLC finger print of the drug at UV 254 nm The finger print chromatogram shows 11 peaks from of which 2 peaks Rf 0.10 and 0.97 were the major peaks and other moderately smaller peaks.

Table 2: Organoleptic characters of Aavarai bhavana Chooranam.

Colour	Brown
Odour	Pleasant
Taste	Characteristic taste
Texture	Fine powder
Particle size	Completely pass through sieve no 100

Table 3: Physicochemical properties of Aavarai bhavanai Chooranam.

S.no	Parameters	Results
1	**Loss on Drying at 105 °C (%)**	**10.7%**
2	**Total Ash (%)**	**10.1%**
3	**Acid insoluble Ash (%)**	**0.11%**
4	**The water soluble ash(%)**	**7.46%**
5	**Water Soluble Extractive(%)**	**25.87 ± 0.81%**
6	**Alcohol Soluble Extractive (%)**	**33.44 ± 2.12%**
7	**PH**	**6.75**

Table 4: TLC analysis.

UV-254nm	UV-366nm	V-S Reagent
0.83 Green	0.88 Pink	0.89 Grey
0.71 Light green	0.82 Light pink	0.79 Violet
0.63 Light green	0.62 Light blue	0.38 Light grey
0.40 Green	0.49 Blue	0.22 Light grey
0.11 Green	0.30 Light blue	0.17 Light grey
	0.13 Fluorescent blue	0.11 Light grey

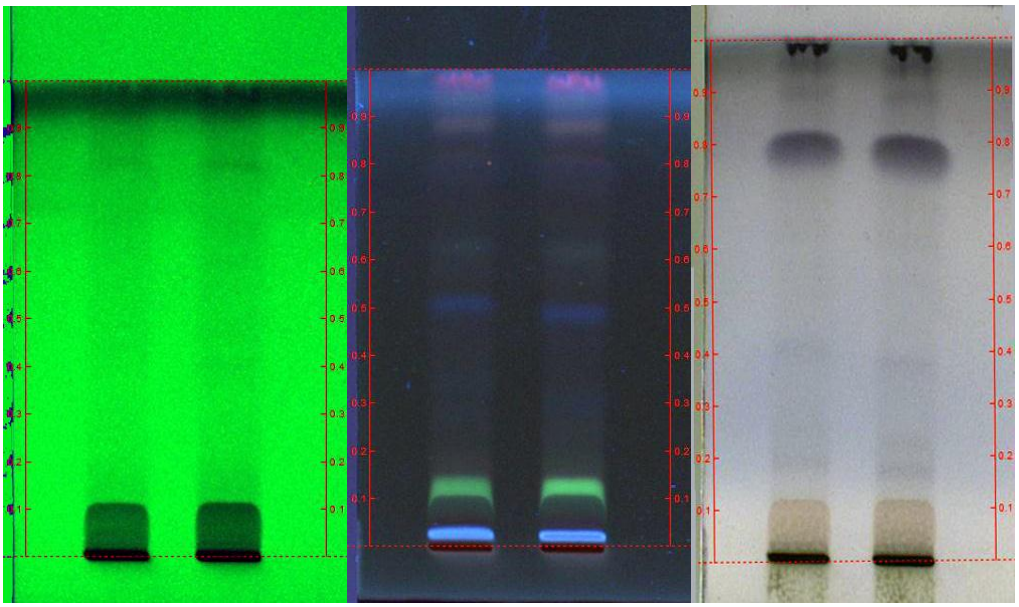

Fig. 1: TLC photo documentation of chloroform extract of Aavarai bhavana Chooranam A.UV 254 nm; B.UV 366 nm; C. Vanillin-sulphuric acid

Table 5: Rf values and the Peak Areas at UV 254 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.02 Rf	23.9 AU	3.15 %	0.03 Rf	10.5 AU	139.7 AU	0.67 %
2	0.03 Rf	11.7 AU	0.10 Rf	365.2 AU	48.20 %	0.11 Rf	0.7 AU	9933.0 AU	47.56 %
3	0.12 Rf	0.8 AU	0.13 Rf	19.0 AU	2.50 %	0.14 Rf	12.5 AU	204.5 AU	0.98 %
4	0.15 Rf	12.3 AU	0.16 Rf	15.1 AU	2.00 %	0.20 Rf	0.5 AU	289.8 AU	1.39 %
5	0.34 Rf	1.7 AU	0.40 Rf	21.6 AU	2.85 %	0.44 Rf	2.6 AU	715.9 AU	3.43 %
6	0.44 Rf	2.9 AU	0.48 Rf	21.6 AU	2.86 %	0.52 Rf	0.8 AU	651.7 AU	3.12 %
7	0.54 Rf	3.4 AU	0.56 Rf	14.5 AU	1.92 %	0.58 Rf	0.1 AU	133.2 AU	0.64 %
8	0.60 Rf	3.0 AU	0.63 Rf	13.5 AU	1.78 %	0.65 Rf	0.8 AU	268.2 AU	1.28 %
9	0.68 Rf	4.8 AU	0.71 Rf	18.5 AU	2.45 %	0.75 Rf	1.9 AU	431.4 AU	2.07 %
10	0.81 Rf	0.2 AU	0.83 Rf	47.6 AU	6.28 %	0.87 Rf	0.4 AU	782.0 AU	3.74 %
11	0.89 Rf	0.2 AU	0.97 Rf	197.0 AU	26.00 %	0.99 Rf	77.2 AU	7336.5 AU	35.13 %

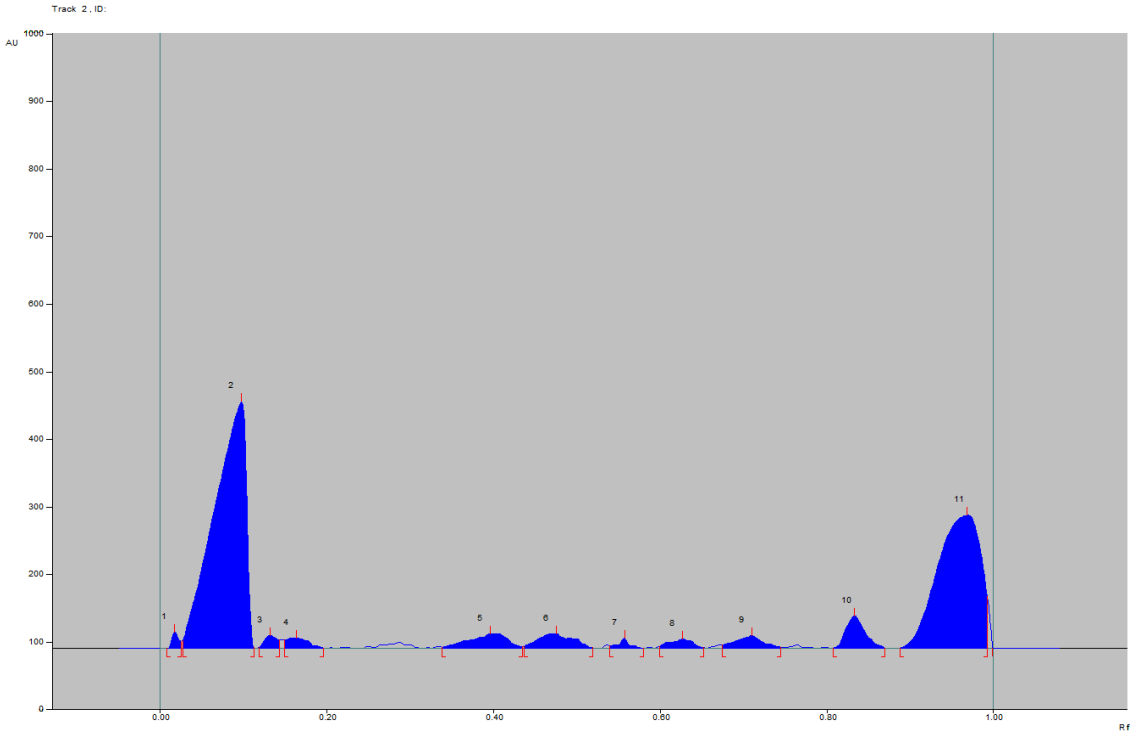

Fig. 2: HPTLC finger print profile of chloroform extract of AVBC at UV 254 nm.

5. CONCLUSIONS

The perceived results of physico-chemical, TLC profiling, HPTLC finger print profiling will be useful as tool for documentation, standardization profile and quality control assessment of the siddha poly herbal formulation Aavarai bhavana Chooranam.

ACKNOWLEDGMENTS

Authors are thankful to Director, National Institute of Siddha for providing infrastructural support and all the faculties of Department of Gunapadam for their valuable suggestions for this study.

Conflict of interest

Authors declare that there is no conflict of interest.

REFERENCES

1. World Health Organization (W.H.O.), Organization Mondiale De La Sante, Quality control methods for plant materials, 559, Rev.1, Original English, 1998; 8 - 67.
2. Madhavi.N, Anil Kumar.E, Maheswar.T, Neha Arya, Standardization of Swasarivati with reference To gas chromatography, scanning electron microscopy And EDAX. Int. J. Res.Ayurveda Pharm, 2014; 5(2): 126-131. http://dx.doi.org/ 10.7897/2277-4343.05226.
3. Sarakkusuthimuraigal, published by Siddha MaruthuvanoolVeliyituPirivu, Indianmedicine and Homoeopathy deptt., First edition, 2008.
4. Hakeem.P.M.Abdullah sahib. Meganivarana Bodhini Ennum Nirizhivu Maruthuvam 3rd Edition. Published by Department of Indian Medicine and Homoeopathy.
5. India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
6. Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems. Department AYUSH Ministry of Health & Family Welfare, Govt. of India.
7. Stahl I. Thin layer chromatography, A Laboratory Hand Book (student edition). Springer-Verlag, Berlin, 1969; 52– 86.
8. Sethi PD. High Performance Thin Layer Chromatography. Edn 1, Vol. 10, CBS Publishers and Distributors, New Delhi, 1996; 1–56.