



Physico chemical and phytochemical analysis of *Vidathari chooranam*, a Siddha herbo-mineral formulation

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Abstract

Background: Standardization of Siddha drugs is necessary one in this scientific word '*Vidathri chooranam*' is a herbo- mineral drug indicated for skin diseases particularly for '*Kaanakadi* (Urticaria)'.

Aim and Objective: To do the physico chemical and phytochemical analysis for the drug '*Vidathari chooranam*'

Material and Methods: The drug prepared as per the method mentioned in the classical Siddha literature. The drug is subjected to physico chemical analysis such as total ash, loss on drying, microbial load, heavy metal analysis, pesticide residues, aflatoxins, thin layer chromatography and high performance thin layer chromatography as per the pharmacopeial laboratory standards of Indian medicine.

Results and Conclusion: The drug had the microbial contamination with in permissible limit and shows positive for the presence of tannins, phenols, glycosides, saponins, etc. The ash value is 10.82.0%. The heavy metals such as lead and mercury is below detectable limit.

Keywords: Herbo-mineral, Siddha, Physico chemical analysis, High performance thin layer chromatography.

I. Introduction

Siddha system of medicine is one of ancient system of medicine which is practiced among the Tamil speaking people. Sage Siddhars are the founder of this system. The medicine in this system is prepared from raw material of herbs, metals, minerals and animal products. '*Vidathari chooranam*' is one of the Siddha drug chosen from the text Siddha Maruthuvam Sirappu¹. It is indicated for '*Kaanakadi*' (Urticaria). The use of scientific tools is essential to validate the traditional claim. Though Siddha drugs are considered to be safe and effective, it is the utmost duty of the

physicians to standardizing the Siddha prepared medicine before trying out in human being. The drug is a herbo-mineral drug which contains only one mineral Silazet and the others are herbal ingredients.

II. Aim and Objective:

The aim of this study is to do Physico chemical analysis, phyto chemical analysis and HPTLC finger printing for the drug '*Vidathari chooranam*'.

III. Materials and Methods

Collection and identification of raw drug:

The mineral drug Silazet was identified and authenticated by Dept. of Geology, Madras University,

Preparation of the drug *Vidathari chooranam*²

Ingredients of the drug *Vidathari chooranam* as given below

Manjitti (<i>Rubia cordifolia</i> .Linn.)	-20 gm
Kukkil (<i>Canarium strictum</i> .Roxb.)	-30 gm
Vengai pattai (<i>Pterocarpus marsupium</i> .Roxb.)	-10 gm
Vasambu (<i>Acorus calamus</i> .Linn)	-10 gm
Moongil uppu (<i>Bambusa arundinacea</i> .Retz Willd)	-10 gm
Komoothira Silazet (<i>Asphalt mineral</i> Pitch)	-10 gm
Maramanjil (<i>Coscinium fenestratum</i> . Coleber)	-5 gm

After the purification each ingredients are made in to fine powder separately and then it is mixed together well.

Purification of raw drug³:

The raw drugs are purified as per the method mentioned in the Siddha literature.

Analytical specification of chooranam⁴:

1. Description: microscopic and macroscopic, 2.Loss on drying at 105° C, 3. Total ash, 4.Acid-insoluble ash, 5.Water soluble extractive, 6.Alcohol soluble extractive, 7. pH, 8. Particle size (80- 100 mesh for chooranam: bulky density and tap density), 9. Test for heavy / toxic metals: lead, cadmium, mercury, arsenic,

Chennai. The herbal ingredients were identified and authenticated by Dept. of Botany, National Institute of Siddha, Chennai. The raw drugs were purified as per the method mentioned in the literature.

10. Microbial contamination: total viable aerobic count, Enterobacteriaceae, total fungal count, 11. Test for specific pathogen: *E.coli*, *Salmonella* spp., *S.aureus*, *Pseudomonas aeruginosa*, 12. Test for aflatoxins: B1, B2, G1, G2), 13. TLC/HPTLC with marker, 14. Pesticide residue: organochlorine pesticides, organophosphorus pesticide, pyrethroids.

Thin layer chromatography/HPTLC condition:

Preparation of spray reagent – vanillin-sulphuric acid reagent

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

Instrument	: CAMAG (Switzerland).
Sample Applicator	: Camag Linomat - IV applicator with N ₂ gas flow.
Photo documentation System	: Digi store - 2 documentation system with Win Cats & Video scan software.
Scanner	: Camag HPTLC scanner - 3 (030618), Win Cats - IV.
Development Chamber	: Camag HPTLC 10X10, 10 X 20 twin trough linear development chamber.
Quantity applied	: 5, 10 µl for extracts and 5 µl for standards
Stationary phase (E. Merck)	: Aluminium plate pre-coated with silica gel 60 F ₂₅₄
Plate thickness	: 0.2 mm.
Mobile Phase	: For Chloroform extract - Toluene: Ethyl acetate (9:1) and ethanol extract – Toluene: Ethyl acetate (1:1).
Scanning wavelength	: 254 nm
Laboratory condition	: 26 ± 5°C and 53 % relative humidity

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. All the physicochemical parameters were carried out as per WHO guidelines.

Physico chemical analysis⁵:

The sample is tested for the following parameters as per the guidelines followed by WHO. Loss on drying at 105° C, Total ash, Acid-insoluble ash, Water soluble extractive, Alcohol soluble extractive, pH, Particle size (80- 100 mesh for chooranam): bulky density and tap density , Test for heavy / toxic metals: lead, cadmium, mercury, arsenic, Microbial contamination: Total viable aerobic count, Enterobacteriaceae, Total fungal count, Test for specific pathogen: *E.coli*, *Salmonella* spp., *S.aureus*, *Pseudomonas aeruginosa*, test for aflatoxins, Pesticide residue: organochlorine pesticides, organophosphorus pesticide, pyrethroids

Preliminary Phytochemical test^{6,7}:

Preliminary Phytochemical tests such as test for Protein, Flavonoids, Quinine, Phenol, Tannin, Alkaloids, Glycosides, Cardiac glycosides, Reducing sugar, Coumarin, Anthroquinone and saponins were carried out.

IV. Results and Discussion

Organoleptic characters⁸:

The ‘*Vidathari chooranam*’ was light brown coloured moderately fine powder with characteristic odour, bitter taste. The organoleptic characters of the formulation were evaluated based on the method described Siddique et al.

Physicochemical parameters:

The results of the physicochemical parameters are given in table -1.

Table -1 Result of physicochemical parameters:

Sl. no	Parameter	Results		
		I	II	Mean
1.	Loss on drying at 105°C	7.33%	7.46%	7.59%
2	Total ash	10.58%	10.63%	10.82%
3.	Acid insoluble ash	8.465%	8.50%	8.68%
4.	Alcohol extract values	18.12%	18.16%	18.36%
5.	Water extract values	17.20%	17.61%	17.84%

Loss of drying indicates the moisture content. The total ash content is the measure of inorganic constituents present in the drug. High ash content gives the details of its unsuitable nature to be used as drug.

Heavy metal analysis:

The observed results of heavy metal analysis is tabulated below in table 2.

Table 2: Results of heavy metal analysis

Sl.no	Heavy metals	Results	Permissible limit
1	Lead	1.0801ppm	10ppm(WHO)
2	Cadmium	Not detected	0.3ppm(WHO)
3.	Arsenic	Not detected	3ppm(API)
4.	Mercury	0.1062ppm	1ppm(API)

The heavy metals such as lead, cadmium, arsenic and mercury are found below detectable limit.

Test for Aflatoxin and Pesticide residues:

The observed results of Aflatoxin and Pesticide residues is tabulated below in table 3.

Table 3: Results of test for Aflatoxins and pesticide residues:

Sl.no	Test parameters	Units of measurement	Results
1.	Aflatoxin B1	µg/kg	BLQ(LOQ:0.5)
2.	Aflatoxin B2	µg/kg	BLQ(LOQ:0.5)
3.	Aflatoxin G1	µg/kg	BLQ(LOQ:0.5)
4.	AflatoxinG2	µg/kg	BLQ(LOQ:0.5)
5.	Organophosphorus	Mg/kg	BLQ(LOQ:0.01)
6.	Organochloride	Mg/kg	BLQ(LOQ:0.01)
7.	Pyrethroids	Mg/kg	BLQ(LOQ:0.01)

BLQ-Below limit of Quantification, LOQ-Limit of Quantification

Analysis of microbial load:

The observed results of microbial load analysis is tabulated below in table 4.

Table 4: Results of microbial load

Sl.no	Parameter	Results	Permissible limit for internal use
1	Total bacterial count	1x10 ² cfu/g	10 ⁵ cfu/g
2	Total fungal count	Less than10 cfu/g	10 ³ cfu/g
3.	Enterobacteriaceae	Absent	10 ³ cfu/g
4.	<i>Escherichia coli</i>	Absent	10 cfu/g
5.	<i>Salmonella</i> spp.	Absent	Absent
6.	<i>Staphylococcus aureus</i>	Absent	Absent
7.	<i>Pseudomonas aeruginosa</i>	Absent	Absent

The bacterial and fungal loads are within permissible limits. The above results indicate that the drug 'Vidathari chooranam' is on standard quality.

Phytochemical tests:

Table 5: Results of phytochemical analysis of 'Vidathari chooranam'

Sl.no	Phytochemical test	Inference
1.	Phenol	Present
2.	Tannin	Present
3.	Flavonoids	Absent
4	Proteins	Absent
5.	Reducing sugar	Present
6.	Quinine	Absent
7.	Saponins	Present
8	Coumarin	Absent
9.	Alkaloids	Absent
10	Acid	Absent
11	Glycoside	Present
12	Cardiac glycoside	Absent
13	Anthroquinone	Absent

The above table shows the presence of some secondary metabolites such as phenol, tannin, saponin, glycosides and reducing sugar. Presence of this phytochemicals indicates their clinical validation.

TLC/HPTLC finger print profile is done for the drug 'Vidathari chooranam'

TLC image of chloroform extract is shown in figure 1 and their Rf value is given in table 6. HTLC image of chloroform extract is shown in figure 2. and their Rf value is given in table 7.

TLC image of alcohol extract is shown in figure 3, and their Rf value is given in table 8. HTLC image of alcohol extract is shown in figure 4 and their Rf value is given in table 9.

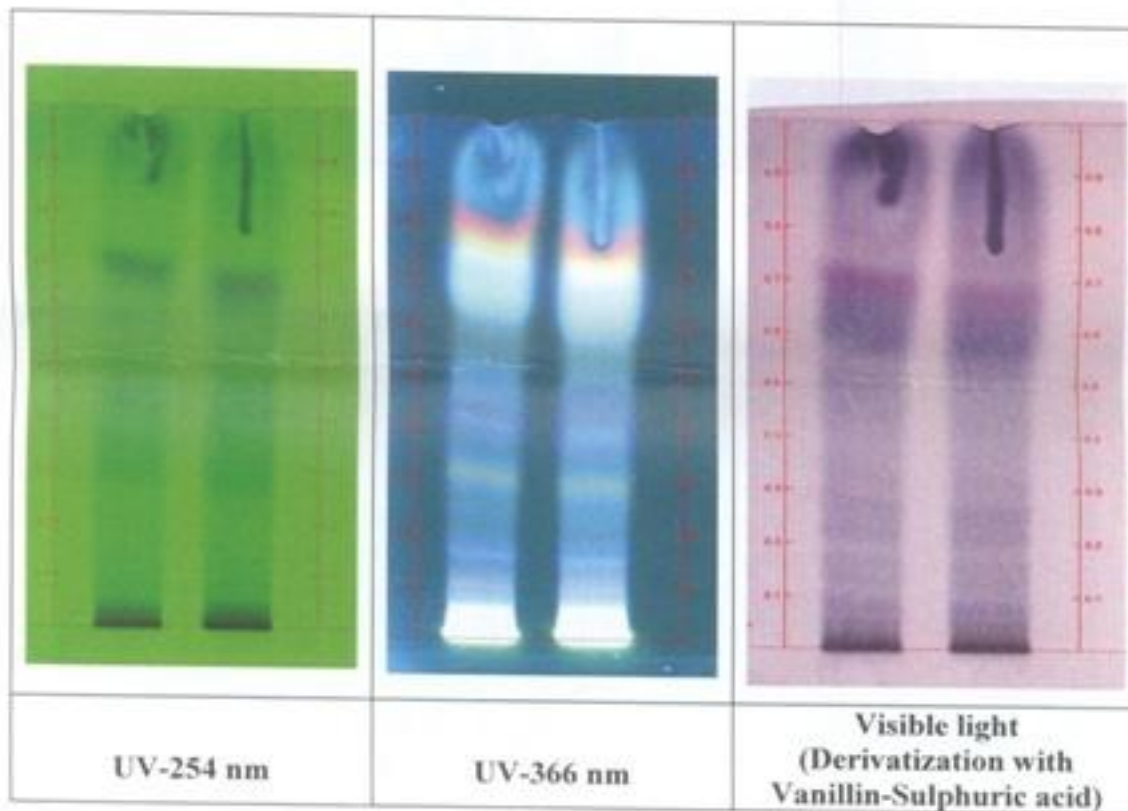


Figure 1: TLC profile of chloroform extract of 'Vidathari chooranam'

Table 6: Rf. value of TLC finger print for the chloroform extract of 'Vidathari chooranam':

Solvent system	UV-254nm	UV-366nm	Visible light(vanillin-sulphuric acid reagent)
Toluene: Ethyl acetate: Formic acid (8:2:0:2)	0.70 green	0.79 red	0.79 grey
	0.43 green	0.75 yellow	0.69 violet
	0.34 green	0.67 fluorescent blue	0.62 grey
	0.29 green	0.49 blue	0.45 grey
	0.16green	0.41 violet	0.27 grey
		0.34 blue	0.22 grey
		0.32 yellow	0.15 grey
		0.16 violet	0.11 grey
	0.10 brown		

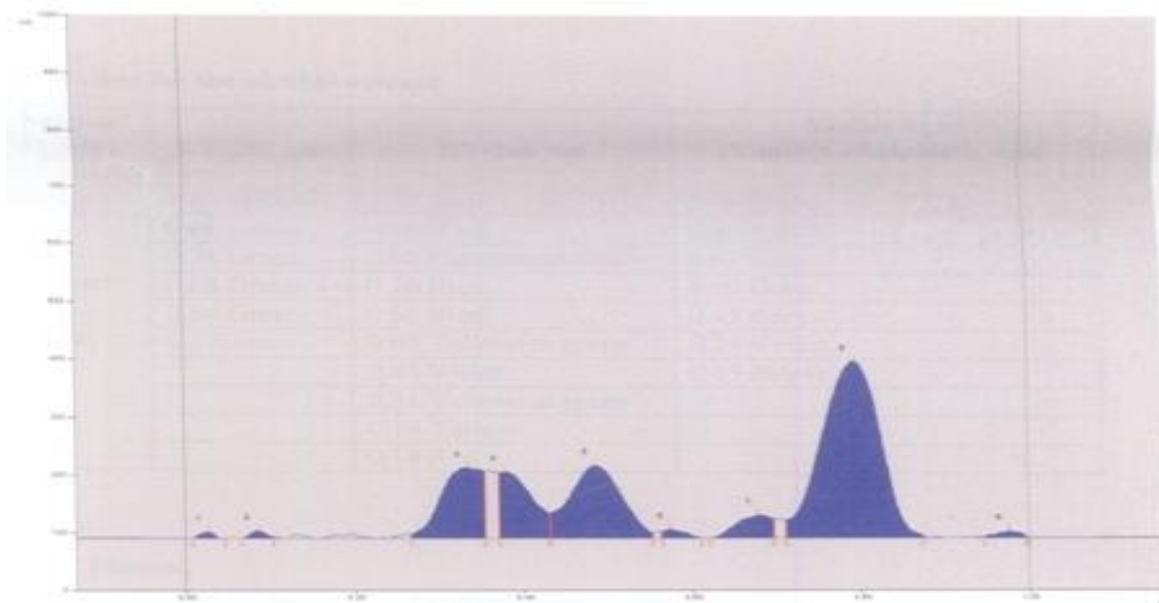


Figure 2: HPTLC finger print profile of chloroform extract of 'Vidathari chooranam':

Table 7: Rf value of HPTLC finger print for the chloroform extract of 'Vidathari chooranam'

Peak	Start Position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.01 Rf	0.0AU	0.03Rf	10.7AU	1.42%	0.05Rf	0.0AU	123.1AU	0.41%
2	0.07 Rf	0.1AU	0.09Rf	13.2AU	1.74%	0.10Rf	2.4AU	169.5AU	0.56%
3	0.27 Rf	7.0AU	0.34Rf	121.7AU	16.12%	0.36Rf	15.3AU	4386.6AU	14.47%
4	0.37 Rf	113.4AU	0.38Rf	114.6AU	15.8%	0.43Rf	44.0AU	3231.7AU	10.66%
5	0.43 Rf	44.3AU	0.49Rf	125.2AU	16.58%	0.55Rf	8.9AU	5621.1AU	18.54%
6	0.56 Rf	11.7AU	0.58Rf	14.9AU	1.97%	0.61Rf	0.1AU	275.4AU	0.91%
7	0.62 Rf	0.0AU	0.68Rf	39.5AU	5.23%	0.70Rf	32.9AU	1169.5AU	3.86%
8	0.71 Rf	31.5AU	0.80Rf	304.1AU	40.27%	0.88Rf	3.2AU	15125.7AU	49.88%
9	0.95 Rf	1.7AU	0.98Rf	11.2AU	1.49%	1.00Rf	1.2AU	220.4AU	0.73%

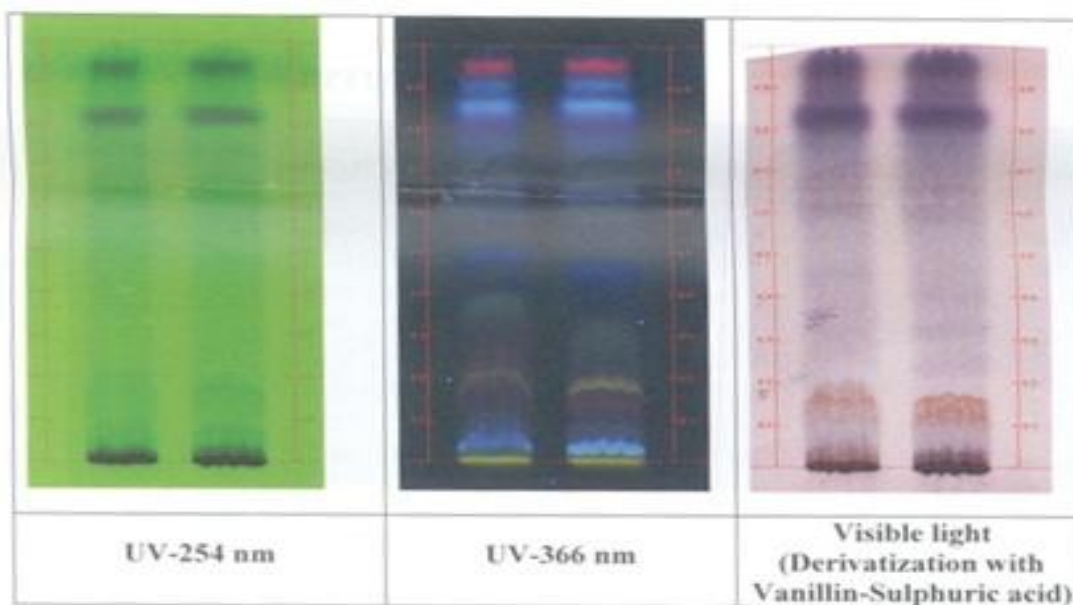


Figure 3: TLC profile of Alcoholic extract of 'Vidathari chooranam'

Table 8: Rf. value of TLC finger print for the alcoholic extract of ‘*Vidathari chooranam*’:

Solvent system	UV-254nm	UV-366nm	Visible light(vanillin-sulphuric acid reagent)
Toluene: Ethyl acetate: Formic acid (8:2:0:2)	0.93 green	0.95 red	0.95 violet
	0.82 green	0.90 blue	0.83 violet
	0.74 green	0.86 fluorescent blue	0.67 grey
	0.68 green	0.76 blue	0.60 grey
	0.64green	0.65 blue	0.45 grey
	0.17 green	0.60 yellowish green	0.24 violet
		0.45 violet	0.15 brown
		0.31 yellowish green	
		0.19 yellow	
	0.14 red		

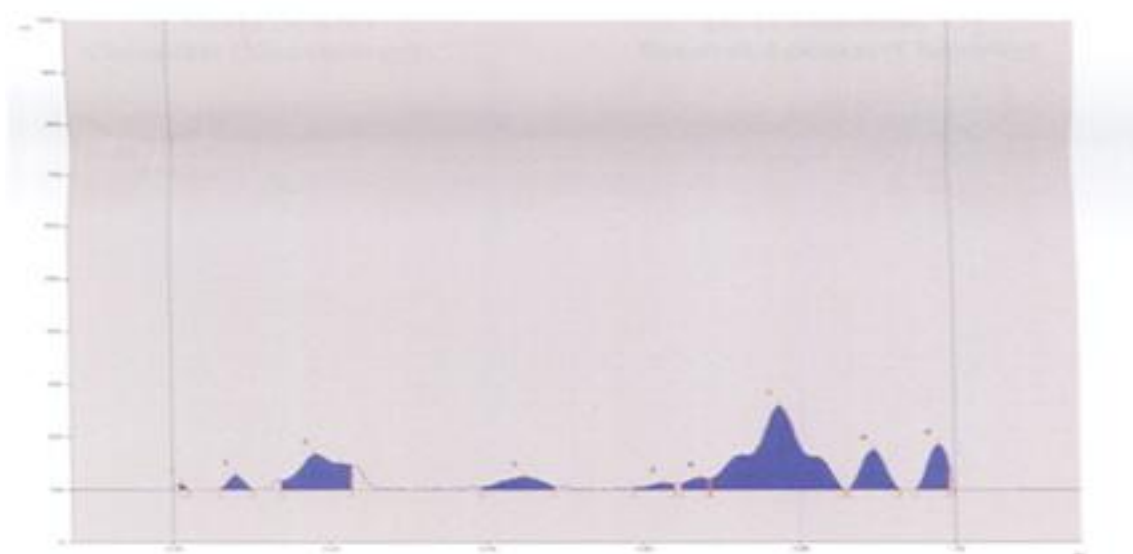


Figure 4: HPTLC finger print profile of alcoholic extract of ‘*Vidathari chooranam*’:

Table 9: Rf value of HPTLC finger print for the alcoholic extract of *Vidathari chooranam*


Peak	Start Position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.01 Rf	10.8AU	0.01Rf	11.4AU	2.33%	0.02Rf	0.1AU	62.0AU	0.39%
2	0.06 Rf	0.4AU	0.08Rf	28.2AU	5.73%	0.10Rf	0.2AU	394.2AU	2.48%
3	0.14 Rf	17.3AU	0.18Rf	68.4AU	13.91%	0.23Rf	47.2AU	2679.6AU	16.88%
4	0.39 Rf	4.8AU	0.45Rf	25.1AU	5.11%	0.49Rf	6.4AU	961.4AU	6.06%
5	0.59 Rf	3.2AU	0.63Rf	13.7AU	2.79%	0.64Rf	11.0AU	310.1AU	1.95%
6	0.65 Rf	11.7AU	0.68Rf	24.3AU	4.94%	0.69Rf	21.3AU	446.5AU	2.81%
7	0.69 Rf	21.4AU	0.78Rf	159.1AU	32.34%	0.86Rf	1.1AU	7916.4AU	49.87%
8	0.86 Rf	0.6AU	0.90Rf	76.0AU	15.45%	0.93Rf	0.5AU	1633.3AU	10.29%
9	0.95 Rf	0.5AU	0.98Rf	85.6AU	17.40%	0.99Rf	46.3AU	1469.8AU	9.26%

V. Conclusion

Based on the above results, it can be assumed that the drug '*Vidathari chooranam*' has validated the traditional claim.

References

1. Dr.T.Thiyagarajan, Siddha Maruthuvam Sirappu, directorate of Indian Medicine and Homoeopathy, Arumbakkam, Chennai-106, second edition-1995, page 303.
2. Dr.T.Thiyagarajan, Siddha Maruthuvam Sirappu, directorate of Indian Medicine and Homoeopathy, Arumbakkam, Chennai-106, second edition-1995, page 303.
3. Dr.T.Thiyagarajan, Gunapadam thathu jeeva vaguppu, directorate of Indian Medicine and Homoeopathy, Arumbakkam, Chennai-106, edition.
4. Dr.D.R.Lohar, Pharmacopoeial laboratory for Indian medicine, department of Ayurveda, Yoga and Naturopathy, Siddha, Unani and Homoeopathy (AYUSH), Ministry of Health and family welfare, 2011, page 20.
- 5.13 Anonymous, (Quality control methods for medicinal plants materials, WHO: Geneva, 1998.)
6. JB (Phytochemical methods, Chapman and Hill, London 1973)
7. Harbone Trease G.E and W.C.Evans, (Pharmacognosy, Brailliar Tiridel and MacMillian Publishers, London Ed-11)
8. Siddique, Hakim MA, Formet for the Pharmacopoeial standards of compound formulation, Workshop on standardization of Unani drug, (appendix), New Delhi, Central Council for research in Unani Medicine (CCRUM) 1995 Jan 24-25.

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