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Impact factor 2.958*

ISSN 6299 -0975

Chemical Abstracts Service (www.cas.org)

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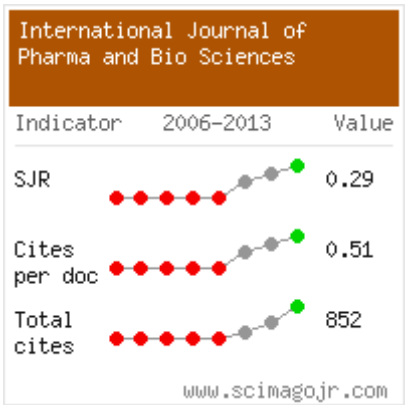

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Int J Pharm Bio Sci 2014 Oct; 5(4): (P) 175 - 185

Research Article **Analytical chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ANALYTICAL STUDY ON SIDDHA HERBOMINERAL FORMULATION: CHANDRAKANTHI CHOORANAM

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ABSTRACT

Chandrakanthi Chooranam, a siddha sastric herbomineral formulation comprises of 25 ingredients. Literature review evidenced no standardization work so far. Authors aims to investigate various analytical standardization parameters such as physicochemical standards, preliminary phytochemical analysis, Thin Layer Chromatography (TLC) photo documentation, High Performance Thin Layer Chromatography (HPTLC) finger print profiles, Thermo Gravimetric Analysis (TGA), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis, heavy metal determination, pesticide residues, mycotoxins, safety evaluation as microbial contamination were evaluated for this herbomineral formulation. Phytochemical analysis showed that it contains amino acids, steroids, triterpenes, flavonoids, phenols, tannins, anthraquinones and saponins. ICP-OES analysis for heavy metals were found to be below detection level and the content of nutritional elements calcium, magnesium, iron, zinc and copper were found to be 6482.9, 1870, 988.6, 21.98, 8.09 ppm respectively. The formulation is free from microbial contamination. Pesticide residues and aflatoxin were found to be absent.

KEY WORDS: **Silasathu parpam, oligospermia, polyurea, TGA, mycotoxins, ICP-OES.**

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INTRODUCTION

Standardization is an important aspect for maintaining, assessing the quality and safety of the polyherbal formulation as these are combinations of more than one herb to attain the desired therapeutic effect[1]. Some of the siddha sastric preparations were reported for pharmacognosy and physicochemical studies[2,3]. Authors selected the siddha sastric preparation Chandrakanthi Chooranam which is a herbomineral formulation comprising of 25 ingredients and indicated in the siddha literature as therapeutically useful in the treatment of oligospermia, poly urea, vaginal disease, veneral disease and all biliousness[4]. Main aim of the present investigation is to study various standardization parameters such as physicochemical standards, preliminary phytochemical analysis, TLC/HPTLC finger printing profiles, safety evaluation as microbial contamination, heavy metal determination, pesticide residues, mycotoxins, TGA analysis, ICP-OES analysis was evaluated in the herbomineral formulation. Other unpublished investigations include toxicity study, pharmacological activity for oligospermia and clinical trial for oligospermia.

MATERIALS AND METHODS

(i) *Identification of Raw Drugs*

Adhatoda vasica seeds were collected from the Research Institute for Indian System of Medicine, Joginder Nagar, Mandi, Himachal Pradesh, India. *Alternanthera sessilis* seeds were collected from the herbal garden, National Institute of Siddha, Chennai, India. Other herbal drugs were procured from the local market, Chennai. The mineral drug Gomutra silasathu was procured from SKM, Tamil Nadu, India. All the herbal drugs and mineral drugs were identified, authenticated and specimen samples were deposited in the Institute.

(ii)*Ingredients Of Chandrakanthi Chooranam*[4]

The ingredients, anatomical parts used and their quantities are presented in the Table 1

.

Table 1
Ingredients of the Chandrakanthi chooranam

S.No	Ingredients	Botanical Name	Parts Used	Quantity in gram
1.	Nilapanai kizhangu	*Curculigo orchioide* Gaertn	Rhizome	35
2.	Iluppai	*Madhuca longifolia* Linn	Flower	35
3.	Lavangap pattai	*Cinnamomum verum* Presl	Stem Bark	35
4.	Lavangap pathiri	*Cinnamomum tamala* Nees	Leaf	35
5.	Kirambu	*Syzygium aromaticum* Linn	Flower bud	35
6.	Maramanjal	*Coscinium fenestratum* Gaertn	Stem bark	17.5
7.	Sirunagappo	*Mesua ferrea* Linn	Flower	35
8.	Bhumi chakkarai	*Maerua arenaria* Hook	Root tuber	35
9.	Aadaathodai	*Adhatoda vasica* Nees	Seed	35
10.	Murungai	*Moringa oleifera* Lam	Seed	35
11.	Maruthani	*Lawsonia inermis* Linn	Seed	35
12.	Drakshai	*Vitis vinifera* Linn	Fruit	35
13.	Elavampisin	*Bombax ceiba* Linn	Gum	35
14.	Moongil uppu	*Bambusa aurundinaceae* Willd	Bamboo salt	35
15.	Perichankai	*Phoenix dactilifera* Linn	Unripe fruit	35
16.	Takkolam	*Ilicium verum* Hook	Fruit	35
17.	Poonaikaali	*Mucuna prurita* Hook	Seed	35
18.	Korai kizhangu	*Cyperus rotundus* Linn	Rhizome	35
19.	Athimathuram	*Glycyrrhiza glabra* Linn	Root	35
20.	Nerunjil	*Tribulus terrestris* Linn	Fruit	35
21.	Seerakam	*Cuminum cyminum* Linn	Fruit	35
22.	Koshtam	*Costus speciosus* Koen	Root	35
23.	Jaathikkai	*Myristica fragrans* Houtt	Seed	35
24.	Ponnakanni	*Alternanthera sessilis* Linn	Seed	35
25.	Gomuthra silasathu	Asphaltum	Parpam [fine ash]	35

(iii) Purification process

Initially all the drugs were purified as per the methods mentioned in the Siddha literature[5-7].

(iv) Preparation of silasathu parpam

Silasathu parpam added in the Chandrakanthi chooranam was prepared as per mentioned in the Siddha literature[8].

(v)Preparation of Chandrakanthi Chooranam[4]

The purified drugs and silasathu parpam were powdered and shifted in 100 size mesh.Then it was subjected to final purification process of chooranam[5]. Finally chooranam was dried and stored in air tight container.

(vi) Organoleptic characters

Colour, Odour, Taste, Consistency were observed.

(vii) Preliminary Phytochemical Tests

All the preliminary phytochemical tests were carried out with the methods mentioned in standard procedure mentioned in literature[9,10].

(viii) Physico-chemical parameter

Loss on drying at 105°C, total ash, water soluble ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, pH and particle size were studied as per the standard guidelines[11,12].

(ix) ICP-OES Analysis

Heavy metals like lead, cadmium, mercury & arsenic and nutritional elements like calcium, copper, iron, magnesium, selenium & zinc were studied.

(x)ThinLayer(TLC) and High Performance ThinLayer Chromatography (HPTLC)

Preparation of extract

4 g of the drug was soaked overnight in chloroform. Boiled over a water bath for 10 min, filtered and concentrated to 10 ml.

Solvent system

Many solvent systems were attempted to get a better resolution. The solvent system Toluene : Ethyl acetate (5:0.5, v/v) showed a better separation than the other solvent systems tried. This solvent system was used for developing the extracts in the TLC plate.

Visualizing reagent

The most commonly used visualizing reagents namely Vanillin-sulphuric acid reagent (one gram vanillin dissolved in the mixture of ethanol: sulphuric acid in the ratio 95:5) was used.

Instrument

The CAMAG's twin chamber was used for developing the TLC plate. Linomat IV (CAMAG, Muttenz, Switzerland) applicator was used for the application of the extract. Aluminium plate precoated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used TLC plate. Bands with a width of 8 mm and 6 mm distance between tracks were applied on 6 x10 cm plate. CAMAG TLC scanner 030618 attached with WINCATS software were used for finger print analysis under UV 254 nm. CAMAG visualizer was used for photo documentation at UV 254 nm, 366 nm; and invisible lights after dipping in vanillin-sulphuric acid reagent followed by heating in an air circulated oven till the development of coloured spots.

Procedure

The extract was applied to the TLC plate as 7 µl, 10 µl, 12 µl bands with 6 mm distance in between tracks using Linomat IV applicator and developed in the above mentioned solvent system. The developed TLC plate was air dried and photographs were taken under UV 254 and 366 nm. The plate was scanned under UV 254 nm, UV 366 nm using the scanner. The finger print was recorded. Then the plate was dipped in vanillin-sulphuric acid reagent, heated in an oven at 105°C till the development of coloured spots and photograph taken and again scanner for finger printing.

(xi)Microbial contamination and specific Pathogens

Microbial contaminations viz., total bacterial count and total fungal count were tested. Other specific pathogens such as *E. coli, Salmonella* spp, *Staphylococcus aureus* and

Pseudomonas aeruginosa were also tested as per standard procedures.

(xii)Test for Aflatoxin and Pesticide residue

The mycotoxins called as aflatoxins viz., B1, B2, G1 & G2 and organo chlorine pesticides and organo phosphorus pesticides were tested as per the standard procedures.

(xiii)Thermo gravimetric analysis

The thermogravimetric analysis of samples was performed using a TG instrument (TGA Q500 V20.10 Build 36). An accurately weighed quantity of sample was heated in high resolution nitrogen atmosphere by maintaining a rate of 20º/min.

RESULTS AND DISCUSSION

(i) Organoleptic characters

Chandrakanthi chooranam is a brown coloured fine powder wih characteristic Spicy odour. It is slight bitter, sweet and astringent to taste. Inferences are presented in the Table 2.

Table 2
Organoleptic characters of Chandrakanthi chooranam

S.No	Specification	Inference
1.	Colour	Brown
2.	Odour	Spicy
3.	Taste	Slight bitter, sweet, astringent
4.	Consistency	Fine powder

(ii) Preliminary phytochemicals

The preliminary phytochemical analysis revealed the presence of amino acids, steroids, triterpenes, flavonoids, phenols, tannins, anthraquinones, saponins and absence of alkaloids. Aminoacids are needed for sperm activity[7]. Glycoside, saponins, sterols, these steroidal constituent increase the steroidogenesis and elevate androgen levels[14,15]. Phenols[15], flavonoids[16,17] and tannis[16] show antioxidative property. These antioxidant defense systems are of major importance because peroxidative damage is currently regarded as the single most important cause of impaired testicular function. Shilajit a mineral rich pitch containing various organic acids and triterpenoids, has been shown in raising sperm count and testosterone level[18] Root decoctions of anthraquinone-containing plants are often taken as a postpartum tonic and aphrodisiac. Anthraquinones has antioxidant and anticancer activity[19]. The phytochemical results are presented in the Table 3.

Table 3
Preliminary phytochemical results of Chandrakanthi chooranam

Sl. No	Name of the Test	Result
1.	Test for Amino acids (Biurette's test)	+ve
2.	Test for Steroid (Lieberman Burchard's Test)	+ve
3.	Test for Flavonoids (Shinoda's test)	+ve
4.	Test for Triterpenoids (Noller's Test)	+ve
5.	Test for Phenol	+ve
6.	Test for Tannin	+ve
7.	Test for Alkaloids (Dragendorff's Test)	-ve
8.	Test for Glycosides	+ve
9.	Test for Saponins	+ve
10.	Test for Anthraquinones	+ve

(iii) Physico-chemical parameters

The drug passes through 100 mesh and showing its fineness. The physico-chemical parameters showed 8.458% of loss on drying at 105°C which indicates that the drug may have better shelf life; 13.043% of total ash which indicates that the drug consists of more

inorganic content; 3.392% of water soluble ash which indicates that the drug consists of remarkable amount of water soluble inorganic content; 5.611% of acid insoluble ash which indicates that the drug consists of acid insoluble ash which may be attributed to one of the ingredient Gomuthra shilajit and remaining 4.04% acid soluble ash; 19.25% of water soluble extractive and 16.85% of alcohol soluble extractive both indicates that the drug consists of more polar compounds like glycosides, tanins, phenols, flavonoids, saponins, etc which is also evident from the results of preliminary phytochemical tests; 6.37 value of pH which indicates that the drug is slightly acidic. The physico-chemical parameters of the drug are presented in Table 4.

Table 4
Physico-chemical parameters of Chandrakanthi chooranam

S.No	Parameter	I	II	Mean
1.	Loss on Drying at 105°C, %	8.591	8.325	8.458
2.	Total Ash, %	13.074	13.011	13.043
3.	Water soluble Ash, %	3.244	3.539	3.392
4.	Acid insoluble Ash, %	5.788	5.434	5.611
5.	Water Soluble Extractive, %	19.20	19.30	19.25
6.	Alcohol Soluble Extractive, %	16.8	16.9	16.85
7.	pH	6.37		
8.	Particle size	Completely passes through 100 mesh		

(iv) ICP-OES

ICP-OES analysis for heavy metals and nutritional elements are presented in Table 5 and Table 6 respectively. Heavy metals were found to be below detection level. The content of nutritional elements calcium, magnesium, iron, zinc and copper were found to be 6482.9, 1870, 988.6, 21.98, 8.09 ppm respectively. Selenium was found to be absent. The content of calcium was found to be high among the trace elements. Magnesium and iron were found to be comparatively in moderate amount. Zinc and copper were found to be in lesser amount. There is substantial evidence that magnesium, calcium and copper are involved in sperm motility[20]. Zinc, selenium, copper and calcium plays important role in spermatogenesis[21]. Iron plays an essential role in spermatogenesis and in normal function of the testis[22]. Concentration of zinc is so high in male sex organs like testicles, prostate and in the spermatozoa itself and hence its important role in reproduction is undeniable. It enhances maturation of spermatozoa, preservation of germinative epithelia, increases sperm activation, regulator of enzyme activity in the semen by mediating metabolic regulation of sperm, highly concentrated in the tail of mature spermatozoa and is involved in sperm motility. Calcium ion is crucial for the initiation of sperm motility, capacitation and its level is found to be proportional to the sperm of the caudal epididymis. Calcium, copper and magnesium are found in ionic form in human semen. Abnormal levels of these elements may affect spermatogenesis with respect of sperm production, maturation, motility and fertilizing capacity. Magnesium in seminal plasma significantly affects sperm concentration, but not motility. Copper concentration within normal physiological range is essential in enzymatic activities[23]. Presence of iron, calcium, magnesium, zinc, copper, in the Chandrakanthi chooranam may be attributing to the spermatogenic activity as claimed in siddha literature.

Table 5
Heavy metal analysis of Chandrakanthi chooranam

Heavy Metals	Specification As per AYUSH/WHO/FDA	Observed Result
Lead	10 ppm	BDL (DL- 0.05 ppm)
Cadmium	0.3 ppm	
Arsenic	3.0 ppm	
Mercury	1 ppm	
BDL: Below Detection level; DL: Detection Limit		

Table 6
ICP-OES analysis of Chandrakanthi chooranam

Nutritional elements	Quantity (in ppm)
Calcium	6482.9
Magnesium	1870.0
Iron	988.6
Zinc	21.98
Copper	8.09
Selenium	BDL (DL:1.0)
BDL: Below Detection level ; DL: Detection Limit	

(v) Thin Layer Chromatography

The TLC photo-documentations at UV 254 nm, UV 366 nm and after derivatization are shown in Fig. 1A-C. The R_f values and colour of the spots under UV 254 nm, UV 366 nm and after derivatization are presented in the Table 7.

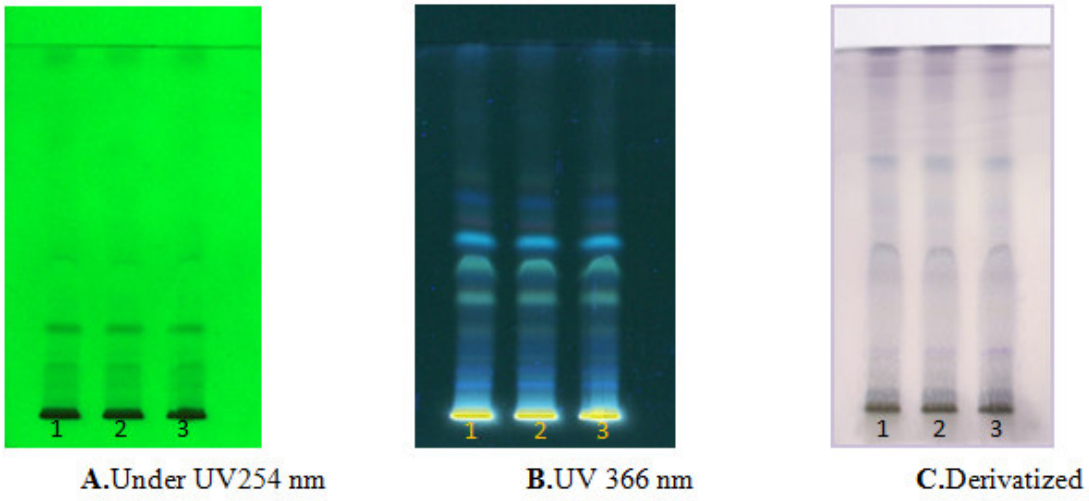

Figure 1
TLC profile of $CHCl_3$ extract of Chandrakanthi chooranam.
Track 1. 7µl; Track 2. 10 µl; Track 3. 12 µl.

Table 7
R_f values and colour of spots of $CHCl_3$ extract of Chandrakanthi chooranam

Under UV 254 nm		Under UV 366 nm		After Derivatization	
R_f value	Colour of the spot	R_f value	Colour of the spot	R_f value	Colour of the spot
0.09	Green	0.09	Pale blue	0.05	Purple
0.14		0.23	Greenish blue	0.17	Purple
0.24		0.32	Greenish blue	0.38	Purple
0.43		0.41	Greenish blue	0.44	Purple
0.75		0.48	Greenish blue	0.58	Purple
-		0.54	Pale blue	0.69	Bluish Purple
-		0.59	Blue	0.96	Purple
-		0.65	Greenish blue	-	-

The TLC photo-documentation of Chandrakanthi chooranam at UV 254 nm showed visible five green spots at R_f value 0.19, 0.14, 0.24 (major), 0.43 and 0.75. Under UV 366 nm, the TLC photo-documentation of Chandrakanthi chooranam showed eight spots at R_f 0.09 (pale blue), 0.23 (greenish blue), 0.32 (greenish blue), 0.41 (greenish blue), 0.48 (greenish blue), 0.54 (pale blue), 0.59 (blue) and 0.65

(greenish blue) in which the spots at R_f 0.41, 0.48 and 0.54 are major. The TLC plate after derivatization with vanillin sulphuric acid and subsequent heating showed 7 spots at R_f 0.05 (purple), 0.17 (purple), 0.38 (purple), 0.44 (purple), 0.58 (purple), 0.69 (bluish purple), 0.96 (purple) in which the spots at R_f 0.17, 0.38, 0.44 and 0.58 are found to be major.

(vi)*HighPerformanceThinLayer Chromatography*

The HPTLC finger printing of chloroform extract at UV 254 (Fig. 2) showed 7 peaks at R_f 0.09, 0.14, 0.24, 0.31, 0.43, 0.54 and 0.75. The percentage area of these peaks are 9.58, 22.11, 39.00, 3.64, 17.43, 1.38, 6.87 respectively. The peak at R_f 0.24 is the major peak followed by peaks at R_f 0.14 and 0.43. Table 8 shows R_f values and percent peak area of all peaks under UV 254 nm. The HPTLC finger printing of chloroform extract at UV 366 (Fig. 3) showed 9 peaks at R_f 0.09, 0.12, 0.23, 0.32, 0.41, 0.48, 0.54, 0.59 and 0.65. The percentage area of these peaks are 2.83, 1.67, 3.00, 19.96, 27.45, 36.22, 4.50, 2.41 and 1.96 respectively. The peak at R_f 0.48 is the major peak followed by peaks at R_f 0. 41 and 0.32. Table 9. shows R_f values and percent peak area of all peaks under UV 366 nm. The HPTLC finger printing of chloroform extract at 540 nm after derivatization (Fig. 4) showed 11 peaks at R_f 0.15, 0.36, 0.44, 0.54, 0.67, 0.72, 0.76, 0.78, 0.81, 0.87 and 0.90. The percentage area of these peaks are 3.37, 21.66, 18.42, 6.16, 16.98, 17.97, 1.73, 1.68, 0.66, 3.74 and 7.63 respectively. The peak at R_f 0.36 is the major peak followed by peaks at R_f 0. 44, 0.72 and 0.67. The Table 10 shows R_f values and percent peak area of all peaks at 540 nm after derivatization.

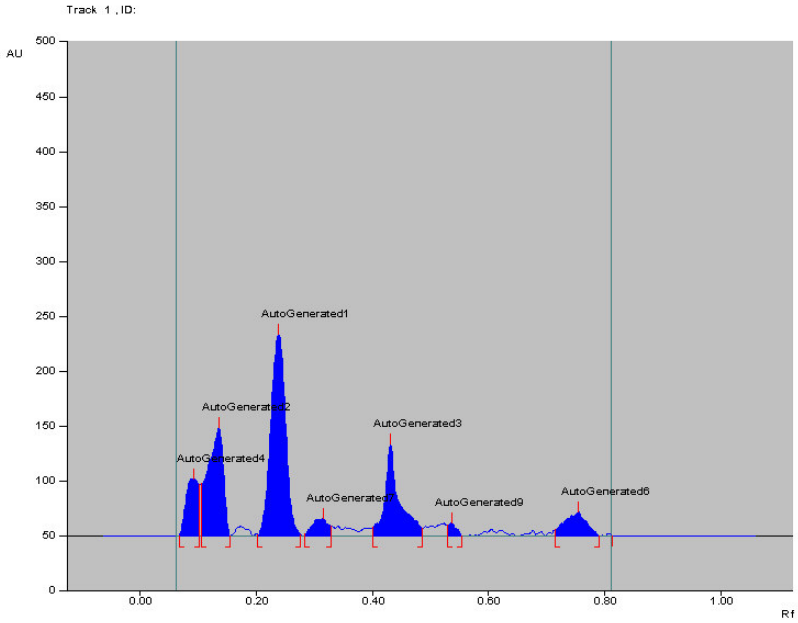

Figure 2
HPTLC finger print of $CHCl_3$ extract at UV 254 nm

Table 8
R_f values and % peak area of all peaks under UV 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.07	0.3	0.09	52.4	11.16	0.10	46.6	1049.8	9.58
2	0.11	47.5	0.14	98.4	20.98	0.16	0.1	2423.7	22.11
3	0.20	1.4	0.24	184.1	39.25	0.28	1.3	4274.7	39.00
4	0.28	1.5	0.31	16.3	3.47	0.33	9.1	398.5	3.64
5	0.40	7.6	0.43	84.3	17.97	0.49	6.5	1910.5	17.43
6	0.53	9.8	0.54	11.9	2.55	0.55	0.4	151.3	1.38
7	0.71	6.2	0.75	21.7	4.63	0.79	0.5	752.9	6.87

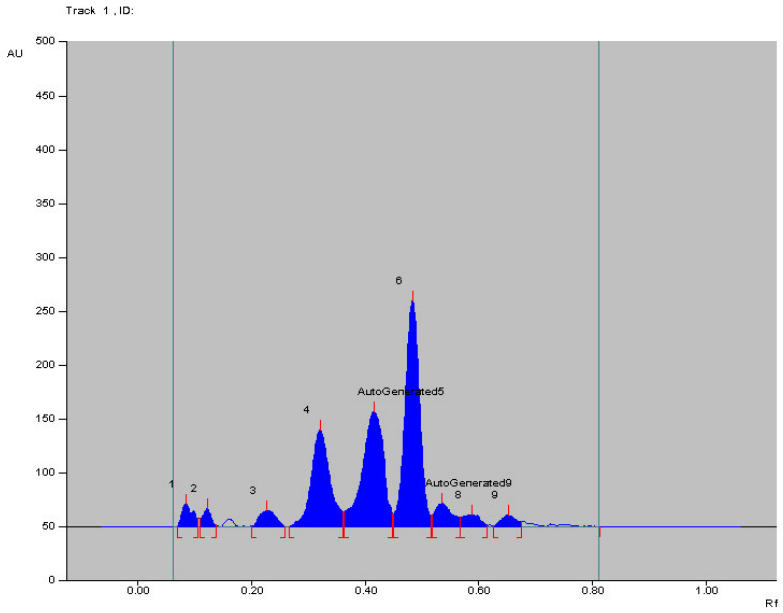

Figure 3
HPTLC finger print of $CHCl_3$ extract at UV 366 nm

Table 9
R_f values and % peak areas of all peaks under UV 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.07	0.7	0.09	21.5	4.26	0.10	8.1	383.1	2.83
2	0.11	7.8	0.12	16.7	3.31	0.14	1.9	225.9	1.67
3	0.20	0.6	0.23	14.6	2.90	0.26	0.2	405.5	3.00
4	0.27	0.1	0.32	90.0	17.89	0.36	14.2	2700.9	19.96
5	0.36	14.3	0.41	106.7	21.20	0.45	11.7	3713.6	27.45
6	0.45	11.8	0.48	210.0	41.73	0.52	10.6	4901.4	36.22
7	0.52	10.9	0.54	21.7	4.31	0.57	8.8	609.2	4.50
8	0.57	8.9	0.59	11.3	2.24	0.62	1.3	325.8	2.41
9	0.63	0.7	0.65	10.8	2.15	0.67	4.9	265.3	1.96

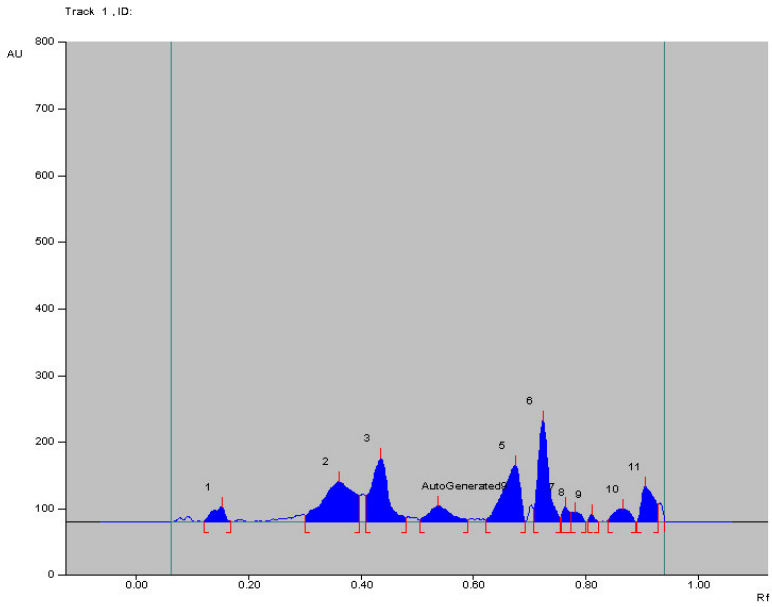

Figure 4
HPTLC finger printing of chloroform extract at 540 nm

Table 10
R_f values and % peak area of all peaks of $CHCl_3$ extract at 540 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.12	0.5	0.15	23.0	4.11	0.17	0.9	470.2	3.37
2	0.30	9.8	0.36	59.9	10.73	0.40	39.5	3023.5	21.66
3	0.41	39.6	0.44	95.1	17.02	0.48	6.7	2571.1	18.42
4	0.50	2.7	0.54	24.1	4.31	0.59	3.2	860.2	6.16
5	0.62	3.3	0.67	84.9	15.19	0.69	1.4	2371.1	16.98
6	0.71	19.7	0.72	151.5	27.10	0.75	6.8	2508.5	17.97
7	0.75	7.6	0.76	21.7	3.88	0.77	13.8	242.1	1.73
8	0.77	14.0	0.78	14.7	2.64	0.80	0.6	234.0	1.68
9	0.80	0.9	0.81	11.6	2.07	0.82	0.2	91.6	0.66
10	0.84	2.5	0.87	19.4	3.47	0.89	0.6	521.8	3.74
11	0.89	0.1	0.90	53.0	9.48	0.93	27.0	1065.7	7.63

(vii) Microbial contamination and specific Pathogens

The bacterial and fungal count was found to be within the prescribed limits. Specific pathogens *E. coli*, *Salmonella* spp, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were found to be absent. The results tabulated in the Table 11suggests that the prepared drug Chandrakanthi chooranam is of standard quality.

Table 11
Results of Microbial contamination and specific Pathogens

Test	Observed Result	Specification as per AYUSH/WHO/FDA
Total bacterial count	17,000 CFU/g	NMT 10^5 CFU/g
Total fungal count	305 CFU/g	NMT 10^3 CFU/g
E. coli	Absent/g	Absent/g
Salmonella		
Pseudomonas aeruginosa		
Staphyloccus aureus		

(viii) Aflatoxins and Pesticide Residues

Aflatoxins B1,B2,G1& G2 were found to be below detection limit. Pesticide residues organochlorine and organo phosphorus were not detected in the sample. Results of test for aflatoxins and pesticide residues are presented in the Table 12.

Table 12
Aflatoxin and Pesticide Residue Test Results

Test	Observed Result
Aflatoxin B1	BDL(DL: 0.3 µg/kg)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Organo phosphorus	Not detected (DL: 0.005 mg/kg)
Organo chlorine	

(ix) Thermo gravimetric analysis

TGA is used to determine total weight change in the sample formulations during thermal treatments. The TGA spectra of Chandrakanthi chooranam (Figure 5) showed peaks at 120^{0}C, 235^{0}C, 390^{0}C and 910^{0}C. At 120^{0}C, 5.148 % of the drug decomposes which may be due to loss of moisture present in the drug. At 235^{0}C, 12.78 % of the drug is decomposed or disintegrated. Similarly at 390^{0}C, 41.29 % of the drug is disintegrated and at 910^{0}C, 13.43 % of the drug is disintegrated.

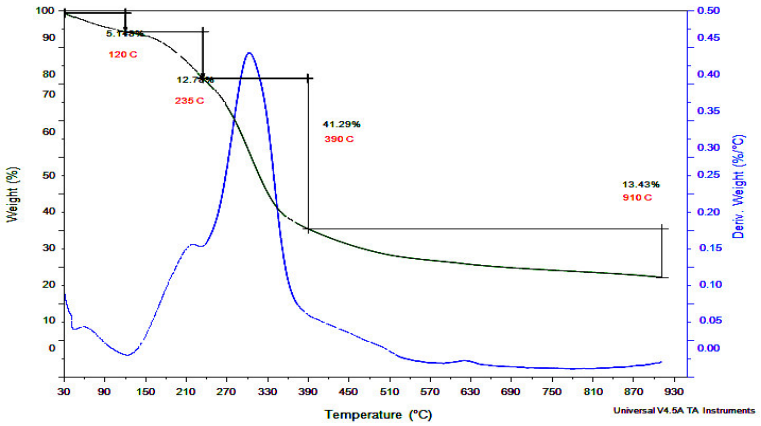

Figure 5
TGA spectra of Chandrakanthi chooranam.

CONCLUSION

Chandrakanthi chooranam showed the presence of amino acids, steroids, triterpenes, flavonoids, phenols, tannins, anthraquinones and saponins. ICP-OES analysis for heavy metals were found to be below detection level 0.05 ppm and showed the presence of nutritional elements calcium, magnesium, iron, zinc and copper. Pesticide residues, aflatoxins were absent and the formulation is free of microbial contamination. Analytical parameters, TLC/HPTLC finger printing profiles were established. The data evolved in this study ensure the quality of the drug and could be used as reference standard in laying the pharmacopoeial standards for this Chandrakanthi chooranam.

ACKNOWLEDGEMENT

Authors are thankful to Dr. D. Aravind, Assistant Professor, NIS for botanical authentication, Prof. V. Ram Mohan, Department of Geology, University of Madras, Chennai for mineral authentication, Department of Chemistry, Indian Institute of Technology and M/s. Sargam Laboratory Pvt. Ltd. for instrument facilities.

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