ejpmr, 2015,2(7), 156-160

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

STANDARDIZATION OF CHUNTAIVATRAL CHOORANAM – A POLYHERBAL SIDDHA FORMULATION

Anitha John[1*], Sasikala E[2], Jayachandran R[3], Shakila R[4] and Sathiyarajeswaran P[5]

[1*,4]Research Officer (Chemistry), Siddha Central Research Institute, Arumbakkam, Chennai -106
[2]Consultant (Pharmacognosy), Siddha Central Research Institute, Arumbakkam, Chennai -106
[3]Research Assistant (Chemistry) Siddha Central Research Institute, Arumbakkam, Chennai -106
[5]Research Officer (Siddha)- Scientist 2 Siddha Central Research Institute, Arumbakkam, Chennai -106

***Correspondence for Author Anitha John**
Research Officer (Chemistry), Siddha Central Research Institute, Arumbakkam, Chennai -106.

Article Received on 25/09/2015 **Article Revised on 14/10/09/2015** **Article Accepted on 04/11/2015**

ABSTRACT

The aim of the present study is to lay down pharmacopoeial standards for an important Siddha polyherbal formulation, Chuntaivatral chooranam using parameters such as organoleptic characters, microscopical identification of the ingredients, physico-chemical analysis, preliminary phytochemical analysis, TLC photo documentation and HPTLC finger print profile. The physico-chemical parameters such as loss on drying at 105°C, total ash, water soluble ash, acid insoluble ash, water & alcohol soluble extractives and pH of 10 % water extract and preliminary phytochemicals were analysed.

KEYWORDS: Chuntaivatral chooranam, identification, physico-chemical parameters, phytochemical analysis, TLC, HPTLC.

INTRODUCTION

World Health Organization (WHO) based on expensive survey showed that a great majority of people are dependent on traditional medicines.[1] As a result, the need arose for the standardization of traditional drugs & medicines.Science has developed tremendously, it is therefore essential now to gear up research to testify and verify the old traditions attributed to Siddha medicine. Chuntaivatral chooranam, a Siddha polyherbal formulation is useful in the treatment of flatulence, indigestion, borborygmi, haemorrhoid and chronic diarrhea.[2,3] Clinical studies showed that the drug was found to be more effective in diarrhoea and even in associated symptoms like borborygmus, tenasmus and bloating of abdomen.[4]

Hence in this paper an attempt was made to evaluate & standardize Chuntaivatral chooranam by identifying the ingredients microscopically & using chemical parameters such as physico-chemical parameters, preliminary phytochemical analysis, TLC photo documentation & HPTLC finger printing profile.

MATERIALS AND METHODS

The name & quantity of the ingredients of Chuntaivatral chooranam are presented in Table 1. The drugs Kariveppilai, Mamparuppu & Matulampazhathol were collected and dried in shade. Chuntaivatral, Omum, Nellivatral and Vendayam were procured from Chennai raw drug market. All the drugs were identified & authenticated by the Botanist, Siddha Central Research Institute (SCRI), Arumbakkam, Chennai-106.

Method of preparation of Chuntaivatral chooranam

Chuntaivatral chooranam was prepared in the pharmacy of Siddha Central Research Institute (SCRI), Chennai as per the procedure given in 'The Siddha Formulary of India' – Part I.[2] Equal quantity of all the drugs (Fig 1) were taken, shade dried, powdered individually and mixed well.

Organoleptic characters

Organoleptic characters such as colour, odour & taste were noted.

Identification of Ingredients

For the identification of ingredients different mounts were prepared. A few mg of chooranam was cleared in chloral hydrate. Different stains such as safranine, sudan III, ruthenium red, phloroglucinol & a drop of hydrochloric acid and IKI solution, ferric chloride solution, picric acid & 2% potassium hydroxide were employed.[5] Glycerin was used as final mountant to study the identification characters.

Physico-chemical analysis

Analysis of physico-chemical constants of Chuntaivatral chooranam has been done to evaluate the quality and purity of the drug. Physico-chemical parameters such as

total ash, water soluble ash, acid insoluble ash, solubility in alcohol and water, pH of 10 % water extract and loss on drying at 105°C were determined by standard methods.[6] The chooranam was subjected to successive soxhlet extraction using hexane, chloroform and alcohol.

Preliminary phytochemical analysis

Preliminary phytochemical analysis for terpenoids, flavonoids, phenols, steroids, alkaloids, tannins, quinones, acids, glycosides, sugars, saponins and coumarins were performed by following standard procedures.[7,8]

High performance thin layer chromatographic (HPTLC) studies

Sample preparation

The extract for the HPTLC study was prepared by soaking 4 g of the chooranam in 40 ml of chloroform and kept overnight. The solution was boiled for 10 minutes and filtered. The filtrate was concentrated and made up to 10 ml in a standard flask. This chloroform extract was used for chromatographic studies.[9,10]

Developing solvent system

A number of solvents were tried, but satisfactory resolution was obtained in the developing solvent system, Toluene: Ethyl acetate: Formic acid (6:1:4 drops).[9,10]

Sample application

The chloroform extract was applied as bands of width 10 mm on silica gel 60 F_{254} pre-coated aluminium sheets through CAMAG microlitre syringe using Automatic TLC Sampler 4 (ATS4). Sample was applied in two bands using 5µl and 10 µl of the extract.

Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase, Toluene: Ethyl acetate:Formic acid (6:1:4 drops).

Documentation

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG visualizer and the images were captured under UV light at 254 nm and 366 nm.

Densitometry

The plate was scanned under 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with winCATS software associated with the scanner.

Post chromatographic derivatisation

The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105^0 C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. Documentation is necessary to record the results in an auditable manner to comply with current good manufacturing practices.[11]

RESULTS AND DISCUSSION

Organoleptic characters

Chuntaivatral chooranam is a powder, greenish in colour, with a characteristic odour and astringent taste.

Table1. Ingredients of Chuntaivatral chooranam

S.No	Ingredients	Botanical Name	Parts used	Quantity
1	Chuntaivatral	Solanum torvum Sw.	Dried fruit	1 part
2	Kariveppilai	Murraya koenigii (L.) Spreng.	Leaf	1 part
3	Mamparuppu	Mangifera indica L.	Kernel	1 part
4	Matulaipazhathol	Punica granatum L.	Fruit rind	1 part
5	Omum	Trachyspermum ammi(L.) Sprague	Fruit	1 part
6	Nellivatral	Phyllanthus emblica L.	Dried Pericarp	1 part
7	Vendayam	Trigonella foenum graecum L.	Seed	1 part

Identification of ingredients Microscopy

Chuntaivatral chooranam of in house preparation was subjected to microscopical studies (Fig 2). Sinuous thick walled cells of the testa in surface view, black epidermal cells with prismatic calcium oxalate crystals, parenchyma cells containing crystal sands observed are from the fruits of Solanum torvum; unicellular thick walled, sickle shaped or straight trichomes gradually tapering towards the tip & anomocytic stomata are from the leaf of Murraya koenigii; fragment of resin duct, needle shaped crystals, plenty of starch grains round to oval measuring 2 – 28 mm in diam. are from the kernel of Mangifera indica; single or groups of large oval shape stone cells, having heavily thickened striated walls with minute central lumen, epicarp cells with pigments, vessels with scalariform thickenings must be from the fruit rind of Punica granatum; parquetry tissue, fragments of epidermis with papillae, brownish septate vittae in surface view, conical warty hairsare observed from the fruits of Trachyspermum ammi; short pitted vascular fibre, tracheids, isodiametric parenchyma cells with corner thickenings, brachysclereids with pitted wide lumen must be from the dried pericarp of Phyllanthus emblica; groups of thick walled palisade epidermis of testa in surface view, bearer cells having radial rib like thickenings, mucilaginous cells of the endosperm are from the seeds of Trigonella foenum graecum.

Fig 1: Chuntaivatral chooranamand its ingredients

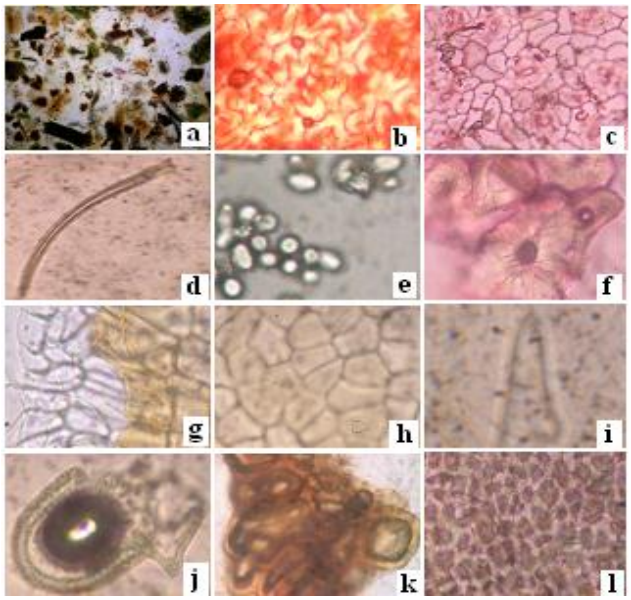


Fig 2: Microscopy of Chuntaivatral chooranam

a) Powder microscopy of Chuntaivatral chooranam
b) sinuous thick walled cells of the testa in surface view
c) leaf fragment showing anomocytic stomata
d) unicellular trichome e) starch grains f) stone cells with heavily thickened striated walls g) parquetry tissue h) epidermis in surface view i) epidermis with papillae j) conical warty hair
k) brachysclereids l) thick walled palisade epidermis of testa in surface view

Physico-chemical analysis

The physico-chemical parameters of Chuntaivatral chooranam were determined and the results are given in Table 2. Physico-chemical parameters help to a great extent for the purpose of standardization. Ash values are helpful to determine the quality and purity of a drug. Acid- insoluble ash which was less than 1% suggesting that less content of silicious matter in the chooranam. The water soluble ash was found to be 2.55 %. The test for loss on drying determines both water and volatile matter. Alcohol soluble extractive value (14.8%) revealed the presence of polar chemical constituents in the ingredients. Water soluble extractive value (27.8%) was high due to the presence of sugars, carboxylic acids and tannins present in the ingredients of chooranam.The pH value (5.45) of drug suggests its little acidic nature. The chooranam was when subjected to successive soxhlet extraction, the hexane soluble extractive value was 2.78 %. The chloroform soluble extractive value was observed as 1% where as the ethanol soluble extractive was 11.12 %.

Table 2. Physico-chemical parameters of Chuntaivatral chooranam

Sl.No.	Parameter	I	II	Mean
1.	Loss on drying at 105^{o}C %	5.85	5.90	5.87
2.	Total ash content %	5.65	5.65	5.65
3.	Acid insoluble ash %	0.25	0.25	0.25
4.	Water soluble ash %	2.59	2.52	2.55
5.	Water soluble extractive %	27.90	27.70	27.80
6.	Alcohol soluble extractive %	14.90	14.70	14.80
7.	pH (10 % solution)	5.50	5.40	5.45
8.	Successive Extraction % Hexane Chloroform Alcohol		2.78 1.00 11.12	

Preliminary phytochemical analysis

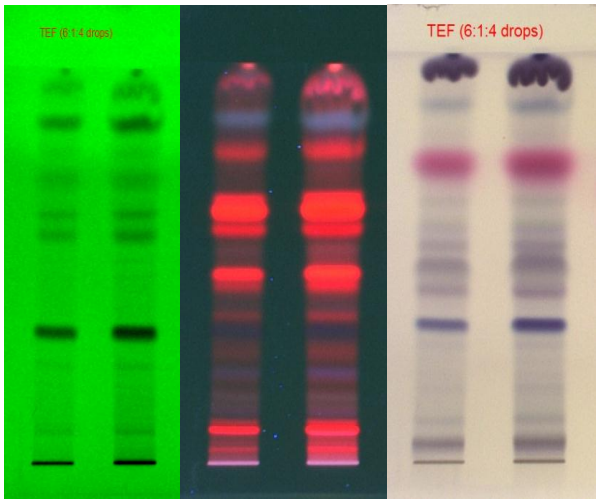
The preliminary phytochemical study revealed that the chooranam contains almost all types of secondary metabolites which are responsible for their potent therapeutic activity. Presence of steroids and flavonoids leads to an assumption of antimicrobial property of Chuntaivatral chooranam and tannins in the formulation definitely help in preventing diarrhoea. Tannin will also helpful in haemorrhoid[12]. The results of the screening are expressed in Table 3.

Table 3: Preliminary Phytochemical studies of Chuntaivatral chooranam

Sl. No.	Phytochemicals	Observation
1	Terpenoids	+ve
2	Phenols	+ve
3	Steroids	+ve
4	Flavonoids	+ve
5	Alkaloids	-ve
6	Tannins	+ve
7	Glycosides	+ve
8	Quinones	+ve
9	Acids	+ve
10	Coumarins	-ve
11	Sugars	+ve
12	Saponins	-ve

High performance thin layer chromatographic (HPTLC) analysis

TLC photo documentation profiles of the chloroform extract of Chuntaivatral chooranam at 254 nm, 366 nm and after derivatisation are given in Fig 3. The solvent system, Toluene: Ethyl acetate: Formic acid (6:1:4drops) efficiently resolved the components present in the crude extract. The TLC profile showed six major bands under UV 254 nm, six major bands under UV 366 nm and seven clear bands after derivatisation and the observed R_f values and their colours are given in Table 4. The 3D densitometric chromatogram and the HPTLC fingerprinting pattern of the chloroform extract of the chooranam at 366 nm are shown in Fig 4 and Fig 5 respectively. The R_f values and their relative peak areas are tabulated in Table 5. It is evident from Table 5 that in the HPTLC finger printing pattern at 366 nm there are 15 spots at R_f values 0.04, 0.08, 0.14, 0.17, 0.22, 0.28, 0.36, 0.47, 0.53, 0.57, 0.63, 0.77, 0.85, 0.93 and 0.97 indicating the occurrence of at least 15 different components in chloroform extract. It is also clear, (Table 5 and Fig 5) that out of 15 components, the components with R_f values 0.08, 0.47, 0.63, 0.77, 0.85 and 0.93 were found to be more predominant as the percentage area is more with 6.38%, 20.83%, 28.94%, 11.10%, 8.11% and 7.95% respectively. The other peaks were found to be minor as the percentage area for the peaks were less.



Under UV254 | Under UV366 | **Derivatised in vanillin – sulphuric acid and viewed under white light**

Fig 3: HPTLC photo documentation profile of the chloroform extract of Chuntaivatral chooranam

Table 4: R_f values and colour of major bands of chloroform extract of Chuntaivatral chooranam

Under UV 254 nm		Under 366 nm		After derivatisation under white light	
R_f values	**Colour**	**R_f values**	**Colour**	**R_f values**	**Colour**
0.33	Dark Green	0.08	Red	0.05	Light Brown
0.56	Green	0.47	Red	0.34	Blue
0.62	Green	0.63	Red	0.42	Grayish Purple
0.71	Green	0.77	Red	0.49	Grayish Purple
0.84	Dark Green	0.85	Blue	0.72	Reddish Violet
0.93	Green	0.93	Red	0.86	Light Blue
				0.94	Brown

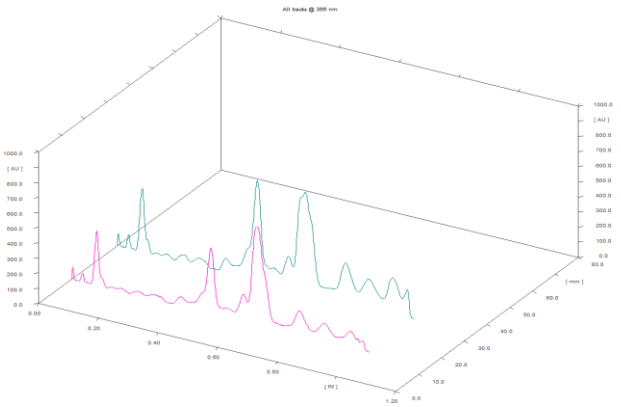


Fig 4: 3D densitometric chromatogram of 5µl and 10 µl of chloroform extract of Chuntaivatral Chooranam at 366 nm

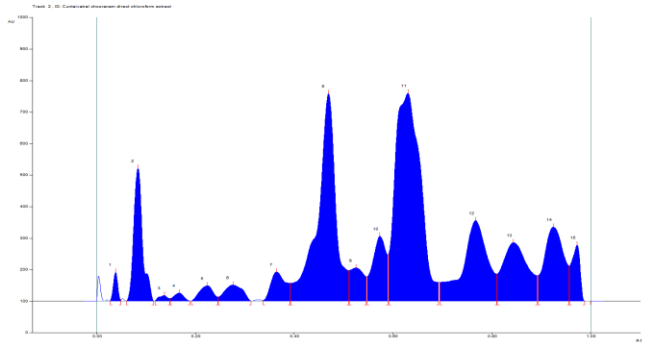


Fig 5: HPTLC finger print profile of chloroform extract of Chuntaivatral chooranam at 366 nm

Table 5: R_f table of chloroform extract of Chuntaivatral chooranam at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	1.4 AU	0.04 Rf	92.0 AU	2.85 %	0.05 Rf	4.8 AU	691.4 AU	0.70 %
2	0.06 Rf	0.0 AU	0.08 Rf	421.9 AU	13.05 %	0.12 Rf	0.5 AU	6273.1 AU	6.38 %
3	0.12 Rf	0.3 AU	0.14 Rf	17.8 AU	0.55 %	0.15 Rf	8.0 AU	268.1 AU	0.27 %
4	0.15 Rf	8.2 AU	0.17 Rf	25.4 AU	0.79 %	0.19 Rf	0.1 AU	432.0 AU	0.44 %
5	0.19 Rf	0.3 AU	0.22 Rf	48.8 AU	1.51 %	0.25 Rf	12.8 AU	1138.3 AU	1.16 %
6	0.25 Rf	12.9 AU	0.28 Rf	51.1 AU	1.58 %	0.31 Rf	0.4 AU	1602.4 AU	1.63 %
7	0.34 Rf	2.3 AU	0.36 Rf	92.5 AU	2.86 %	0.39 Rf	56.9 AU	2355.1 AU	2.39 %
8	0.39 Rf	56.8 AU	0.47 Rf	658.2 AU	20.36 %	0.51 Rf	97.0 AU	20480.4 AU	20.83 %
9	0.51 Rf	97.3 AU	0.53 Rf	105.1 AU	3.25 %	0.55 Rf	77.0 AU	2503.4 AU	2.55 %
10	0.55 Rf	77.1 AU	0.57 Rf	205.9 AU	6.37 %	0.59 Rf	46.5 AU	4857.4 AU	4.94 %
11	0.59 Rf	149.1 AU	0.63 Rf	659.3 AU	20.40 %	0.69 Rf	59.6 AU	28463.8 AU	28.94 %
12	0.70 Rf	59.4 AU	0.77 Rf	255.3 AU	7.90 %	0.81 Rf	85.5 AU	10916.5 AU	11.10 %
13	0.81 Rf	86.1 AU	0.85 Rf	185.7 AU	5.74 %	0.89 Rf	80.6 AU	7972.9 AU	8.11 %
14	0.89 Rf	80.9 AU	0.93 Rf	234.7 AU	7.26 %	0.96 Rf	11.7 AU	7818.5 AU	7.95 %
15	0.96 Rf	111.9 AU	0.97 Rf	178.5 AU	5.52 %	0.99 Rf	0.3 AU	2567.0 AU	2.61 %

CONCLUSION

From the above observations, it is concluded that the results of detecting single drugs in the compound formulation, physico-chemical analysis, preliminary phytochemical studies, TLC photo documentation & HPTLC finger print studies can be used as a diagnostic tool to determine the quality & purity of the drug and to lay down pharmacopoeal standards for Chuntaivatral chooranam.

ACKNOWLEDGEMENT

The authors are thankful to the Director General, Dr. (Prof.) R.S. Ramaswamy, Central Council for Research in Siddha, Chennai for providing necessary facilities to carry out this work.

REFERENCES

1. Kimmatkar N, Thawani V, Hingorani L, Khiyani R. Phytomed, 2003; 10: 3-7.
2. Anonymous, The Siddha Formulary of India, Part I (English),Ministry of Health and Family Welfare, Govt of India, 1992; 153.
3. Anonymous,Teraiyarkarical–300,SundaramVR.(publishing author)Shanmuga Mudaliyar Sachitananda press, Madras, 1915; 88-89
4. Saroja PR, Sivaprakasam K,Veluchamy G. Role of Chuntaivatralchuranam in the Management of (Non-Specific Diarrhoea) Athisaram, J.R.A.S.,1998; 3-4: 128-131.
5. Johansen DA, Plant Microtechnique, New York: McGraw Hill Book Co. Ltd.; 1940.
6. World Health Organization (WHO), Quality control Methods of Medicinal Plant Materials. Geneva, 1998; 8: 28–34: 45-46.
7. Arther I Vogel, Vogel's Text Book of Practical Organic Chemistry, Longman Group Limited London, 4th edition; 1978.
8. Raman N, Phytochemical Techniques, New India Publishing Agency, New Delhi, 2006.
9. Harbone JB, Phytochemical Methods, Aguide to modern techniques of plant analysis, London: Chapman and Hall Ltd., 1984.
10. Wagner H and Bladt S, Plant drug analysis - A Thin Layer Chromatography Atlas, Springer - Verlag, Berlin., 1996.
11. Camag, Application Notes on instrumental thin layer chromatography, 2015.
12. Harbone JB & Williams CA, Advances in flavonoid research since 1992, Phytochemsitry Oxford, 2000; 55: 481-504.