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Physiochemical and Phytochemical Standardisation of Thraatchathi Chooranam- A Polyherbal Siddha Formulation

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Abstract:

World Health Organization (WHO) emphasis the necessity of quality and safety of herbal formulations also proposes guidelines for its standardization. Standardization assures the identity, determination of quality, purity of herbal formulation through its active or marker compounds. Thraatchathi chooranam (TC), a Siddha polyherbal formulation which comprises 32 medicinal plants, has the traditional claim for the management and treatment of cardio vascular diseases, diabetes mellitus, cough, asthma, ulcer etc. Scientific standardization of Thraatchathi chooranam is not yet studied. In the present study, we have standardized the TC using standard physio-chemical and phytochemical protocols such as Ash values, Extractive values, chemical profiling and marker quantification such as Gallic acid, Ellagic acid, Naringenin, Quercetin and Galangin using HPTLC fingerprinting. In addition, residue analyses such as heavy metal content, microbial load, pesticide analysis were also examined to strength the standardization process. Qualitative phytochemical screening revealed the presence of total phenols, tannins, flavones, saponins and glycosides. Microbial load and heavy metals were found to be within the AYUSH permissible limits. Pesticide residues and aflatoxins were absent. Quantification of Gallic acid (1.8 mg/g), Ellagic acid (1.9mg/g), Naringenin (6.3 mg/g), Quercetin (21.4 mg/g) and Galangin (3.4 mg/g) confirms the presence of lead molecules. Our results give an idea about the active ingredients responsible for the beneficial effect of Thraatchathi chooranam and thereby evidence the traditional claim.

Key words: Standardization, Thraatchathi chooranam, Gallic acid, Ellagic acid, Naringenin, Quercetin and Galangin

INTRODUCTION

Herbal based traditional remedies are highly recommended by World Health Organization (WHO) because of their safety, easy availability, low cost in the treatment of various diseases. In traditional system, these medicines have a richest bio-resource such as phenols, micro and macronutrients etc. They can act as a nutraceuticals, food supplements, pharmaceutical intermediates etc [1]. An herbal based formulation improves the quality of human life through its potent natural antioxidants [2] and bioactive compounds [3]. They provide remedy for various chronic diseases and metabolic disorders which are multifactorial and therapeutic intervention [4].

World Health Organization (WHO) and National Center for Complementary and Alternative Medicine (NCCAM) accentuates the need to ensure quality and safety of herbal medicine by modern techniques and applying suitable standards and has proposed guidelines for development of standard herbal medicine [5]. Quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine [6]. But we don't have a rigid quality control profiles for standardization of herbs and their formulations. It is mainly due to the lack of inadequate regulatory standards and implementation protocols [7].

Development of standards for plant-based drugs being a challenging task, it needs innovative and creative approaches [8]. At each and every step of standardization viz; identification, organoleptic, pharmacognostic, physiochemical, phytochemical, presence of xenobiotics, microbial load and toxicity needs special attention because of complex nature of plant based medicines and the inherent variability of their constituents [4].

Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs. Multiple marker based standardization can be executed through HPTLC, a sophisticated analytical technique. It is a simple, fast, reproducible and economic method for standardization of herbal formulation which has an ability to determine the quality of drug thereby enhances the beneficial effects of herbal products. In addition to qualitative detection, HPTLC also provides quantitative information on the major active constituents of a drug, thus enabling an assessment of drug quality.

This is the first report on the standardization of Thraatchathi Chooranam, a Siddha polyherbal formulation comprises equal proportion of 32 herbs such as *Vitis vinifera, Phoenix dactylifera, Cyperus rotundus, Piper wallichi, Santalum album, Oryza sativa, Curcuma anguistifolia, Elattaria cardamomum, Cuminum cyminum, Vetiveria zizonoides, Zingiber officinale [dried], Piper nigrum, Piper longum, Terminalia chebula, Terminalia bellarica, Embilica officinalis, Pavonia odorata, Costus speciosus, Glyzhirrizha glabra, Pavonia zeylanica, Tinospora cordifolia, Gmeliana asiatica, Tribulus terrestris, Plectranthus vittiviroides, Coccinium fenestratum, Nymphaea pubaecens, Syzigium aromaticum, Curcuma aromatic, Crocus sativus, Kaempferia galangal, Neliumbo nucifera* and *Sitramalli*. It has been traditionally used for the management and treatment of chronic diseases such as cardio vascular diseases, diabetes mellitus etc. In the present study, we have elucidated the physio-chemical, phytochemical profile of TC using standard and modern techniques.

MATERIALS AND METHODS

Chemicals

Prepared Thraatchathi Chooranam was procured from Arogya Health Care Pvt.Ltd, Chennai. Standards of Gallic acid, Ellagic acid, Quercetin, Naringenin, Galangin were purchased from Sigma Chemicals, USA. All other chemicals and solvents were of analytical grade obtained from SISCO Research Laboratories Pvt Ltd. Mumbai.

Physicochemical testing

Physical identity, loss on drying, ash values, extractive values, moisture content, crude fibre content, pesticide residues, heavy and toxic metals, microbial contamination like total viable count, pathogens like *E. coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus* etc. Physicochemical characters of Thraatchathi chooranam were determined as per standard protocols [9, 10]. Analyses were performed in triplicates.

Identity of TC

Sensory characters such as color, odour, taste, consistency and texture of the formulation was studied.

Loss on drying and moisture content

About 10 g of TC was taken in a dry weight petri dish and the sample was spread to uniform thickness and again weighed. The set up was placed in a drying oven, and heated at 105^{o}C for 3 h and then allowed to cool to room temperature and weighed. Drying and weighing was continued at one hour interval until difference between two successive weighing corresponds to not more than 0.25 percent. The loss on drying was calculated on the basis of the dried weight.

Ash Content

High ash content explains its unsuitable nature to be used as a drug. The residue remaining after incineration is the ash content of drugs, which simply represents inorganic salts, naturally occurring in drugs or adhering added to it as form adulteration [11]. Total ash, water soluble ash and acid insoluble ash of TC was determined by below mentioned protocol:

Total Ash

About 5 g of TC was taken in a silica dish and incinerated at a temperature not exceeding 450°C until it becomes free from carbon. Then it was cooled and weighed to determine total ash content.

Water Soluble Ash

About 500 mg of total ash was boiled for 5 min with 25ml of water. The insoluble matter was collected in an ashless filter paper. It was washed with hot water and ignited for 15min at a temperature not exceeding 450°C. Then it was cooled and weighed to determine water soluble ash content from air dried material.

Acid Insoluble Ash

About 500 mg of total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid.

The insoluble matter was collected in an ashless filter paper and washed with hot water and ignited to 15min at a temperature not exceeding 450°C. Then it was cooled and weighed to determine acid-insoluble ash.

Determination of Crude Fibre Content

About 1 g of TC was weighed and transferred to a porcelain dish. 50 ml of 10% nitric acid was added, boiled for 30 sec with constant stirring and filtered through fine mesh cotton cloth. The residue was washed with 5 ml of boiled water. The material from the cloth was collected in a porcelain dish and boiled with 50 ml of 25% caustic soda. Then the liquid was filtered using a fine mesh cotton cloth. The residue was washed with 100 ml of boiling water. The fibre was then collected in a filter paper by filtering and dried at 105°C and weighed. From the weight of residue, the Crude fibre content was calculated.

Extractive value

Water Soluble Extractive

One gram of the TC was taken and macerated with 100 ml chloroform water of specified strength in a closed flask for 24hrs. It was shook frequently during 6 hrs and allowed to stand for 18hrs. After 24 hrs it was filtered and 25 ml of the filtrate was taken and evaporated to dryness in a tarred flat bottomed shallow dish. Then it was dried at 105ºC to constant weight and weighed to calculate water soluble extractive value.

Alcohol Soluble Extractive

One gram of the TC was macerated with 100ml alcohol of specified strength in a closed flask for 24hrs. It was shook frequently during 6 hrs and allowed to stand for 18hrs.After 24 hrs it was filtered rapidly to prevent the loss of solvent. 25 ml of the filtrate was taken and evaporated to dryness in a tarred flat bottomed shallow dish. Then it was dried at 105ºC to constant weight and weighed to calculate alcohol soluble extractive value.

pH

10% TC solution was taken to determine the pH value using glass electrode pH meter

Pesticide residue

Herbal formulations contain pesticide residues which accumulate from agricultural practices and administration of fumigants during storage. WHO and FAO (Food and Agricultural Organization) set limits of pesticides, which are usually present in the herbs. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene, aldrin cause serious side-effects in human beings if the crude drugs are mixed with these agents [12]. The analytical procedure for the assessment of specific pesticide residues like organochlorine compounds, Organophosphorous compounds and Pyrethroids compounds were using **GC ECD.** The analysis was carried out in TA Labs Private Limited**,** Chennai.

Microbial contamination and Aflatoxin

Medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil. Major microorganisms contaminate the herbal formulations are identified using streak culture analysis of *E. coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus* in crude Thraatchathi chooranam are as follows: 100mg / 100µl of the formulation was taken and dissolved in 100ml of peptone water. Using the standard loop which carried 0.01ml of the extract solution was inoculated in blood agar and McConkey agar plates. The plates were incubated at 37°C overnight and the colonies were counted. The organisms were identified using biochemical tests such as Indole test, Triple sugar

iron (TSI), Urease, Citrate, Mannitol Motility, and Phenyl pyruvic acid. Aflatoxin (B1, B2, G1 G2) were evaluated according to the standard methods using a HPLC (Fluorescent detector). The analysis was carried out in TA Labs Private Limited, Chennai.

Heavy metal content

Heavy metal toxicity and its implications have been deal in a serious manner. Nickel (Ni), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg) and Arsenic (Ar) was quantified by Atomic Absorption Spectrophotometer (AAS model 400 Perkin Elmer) attached to a hydride generator at HIMRL, Sri Ramachandra University.

Phytochemical Analysis

Few grams of crude TC was extracted with 50% ethanol and the extracts were used for the phytochemical analysis.

Qualitative analysis

TC extract was subjected to preliminary phytochemical analysis such as phenolic compounds, Reducing sugars, Flavones, Glycosides, Saponins, Alkaloids, Anthroquinones, Proteins and Tannins [13].

Quantitative analysis

Secondary metabolites like total phenols [14], flavonoids [15], Tannins [16], Vitamin C [17] & Vitamin E [18] were quantified in TC extract using standard protocols.

Quantification of gallic acid, epicatechin, quercetin narginine, galanin

In order to identify the quantity of active principles such as Gallic acid, Ellagic acid, Naringenin, Quercetin and Galangin in the TC formulation, HPTLC technique has been used.

Preparation of Sample and Biomarker compounds

500 mg TC extract was dissolved in 10 ml ethanol in a standard flask. Marker compounds such as gallic acid, ellagic acid, quercetin, naringenin, galangin were weighed and dissolved in methanol to get a concentration of 0.1mg/ml each for analysis.

Instrumentation

Stationary phase: Silica gel 60F 254 HPTLC plates, Camag twin trough glass Chamber (10X20cm), Sample applicator: automatic TLC applicator Linomat V with N2 flow (CAMAG, Switzerland), Scanner: CAMAG TLC scanner III, Photo documentation: CAMAG REPROSTAR 3, Development Mode: Ascending mode.

Chromatographic Condition

Marker Specific Solvent systems and their scanning wave length were selected based on the literature.

i. Gallic acid and Ellagic acid (0.1mg/ml)

Mobile phase: Toluene:ethyl acetate :formic acid :methanol (3:3:0.8:0.2)

Scanning wavelength: 280nm

ii. Naringenin, Quercetin & Galangin (0.1mg/ml)

Mobile phase: Toluene:ethyl acetate :Formic acid (7:5:1)

Scanning wavelength: 350 nm

Calibration curves of Biomarkers

Five concentrations of standard solutions (3-7 µl, 300 – 700 ng) of Gallic acid/ Ellagic acid/ quercetin/ Naringenin / galangin and 6 µl volume of Sample was applied as 6mm wide band 8mm from the bottom in triplicates on a precoated HPTLC plate. Spots were dried in hot air oven at 45-60 ºC for 2 minutes. The plate was developed in the respective mobile phase in a migration distance of 60 mm. After run, solvent front was marked and the TLC plates were dried at 60 º C. Following drying, plates were scanned and documented at specific wavelength and peak areas were recorded. Calibration curves of standards were prepared by plotting areas vs concentration. By using calibration curve, markers were quantified in TC.

Statistics

All experiments were carried out in triplicate and the results are expressed as Mean ± Standard Error Mean (SEM).

RESULTS & DISCUSSION

Nowadays medicinal plants and herbal formulations are predominantly used due to their less side effects and the presence of enormous level of active ingredients. But adulteration and misidentification of the herbs may lead to deleterious effects in pharmaceutical industry which paves the way for the standardization of herbal drug. Standardization imparts an information on chemical, biological, physico-chemical profile and amount of heavy metals consistently. WHO and AYUSH insisted many guidelines to be followed for quality control for a better standardization of the drugs [19]. Siddha system of medicines comprises many numbers of safe and valuable herbal medicines have better therapeutic efficiency either at its raw state or processed form and are clinically used by the Siddha practitioners. One such formulation in clinical practice since ancient times is Thraatchathi Chooranam which comprises equal proportion of 32 herbs and has been used for the management and treatment of cardio vascular diseases, diabetes mellitus etc. There were no scientific claims on the standardization of Thraatchathi chooranam. To fulfill this lacuna, in the present study, we have studied the organoleptic characters, physico-chemical characters as per Ayurvedic pharmacopeia and compared with AYUSH standards. Phytochemical quantification of TC also studied using HPTLC technique.

Commercially procured Thraatchathi chooranam is a fine powder and light brown in colour, possess aroma flavour and astringent taste. The results of physiochemical parameters are presented in table 1. Percentage of loss on drying (7.8 ±0.01) and total ash content (7.4±0.04) were found to be within a limit in TC which provides information on the moisture level and inorganic matters respectively. It should be less than 10% as per regulation which implies that TC is free from impurities. Similarly high extractive value gives an idea of the amount of the phytoconstituents and less extractive value indicates adulteration, substitution of drug. In the present study, it was observed that the water soluble extractive value is found to be higher than alcohol soluble extractive. It confirm the presence of polar compounds like phenol, glycosides, tannins, flavones etc., than non polar components and are corroborated with the phytochemicals (Table 2 and Table 3).

Table 1: Physicochemical evaluation of Thraatchathi chooranam

S.No.	Parameter	Thraatchathi Chooranam (%)
1.	Loss on Drying	7.8± 0.01
2.	Total ash	7.4± 0.04
3.	Water soluble ash	4.9± 0.01
4.	Acid insoluble ash	1.1± 0.01
5.	Water soluble Extractive	28.86± 0.13
6.	Alcohol soluble Extractive	12.80± 0.11
7.	Crude fibre content	21.25± 0.34
8.	pH	3.45± 0.01

Results are expressed Mean ± SEM of triplicate sample.

Table 2: Preliminary Qualitative phytochemical analysis - Thraatchathi chooranam

Chemical test	Method	Results	Inference
Carbohydrates	**Molisch's tests** Alcoholic a-naphthol+sulphuric acid	Purple to violet colour rings	+
Reducing sugars	**Fehling test** Equal volume of Fehling's solution I and II	No red color precipitate	-
Glycosides	**Anthrone test** Anthrone + Conc H2SO4	Dark green coloration	++
Steroids	**Libermann-Burchard Test** Acetic anhydride with Conc H2So4	Bluish green colour	+
Proteins	**Millon Test** Million's reagent	White precipitate warmed turns brick red	+
Phenols	**Ferric Chloride test** Alcoholic ferric chloride solution	Bluish black	+++
Flavones	**Shinoda test** Magnesium turnings + few drops of conc HCl	Red coloration	+
Tannins	Lead acetate Test Basic lead acetate solution	orange red precipitate	+
Quinones	**Alkali test** 10 % sodium hydroxide	Blue, green or red color	+
Saponins	Shake well with water	Copious formation	+
Alkaloids	**Mayer's tests:** Potassium mercuric iodide solution	No Cream precipitate	-
	Wagner's tests Iodine potassium solution	No Brown precipitate	-
Anthroquinones	**Borntrager's Test** Ether+ aqueous ammonia	pink or red or violet color	+
Triterpenoids	**Salkowski test** Chloroform+ Con H2So4	Formation of brown ring	-

Inference: + indicate Low; ++ indicate moderate; +++ indicate high; - indicate absent

Table 3: **Quantification of Secondary metabolites and Vitamins in Thraatchathi chooranam**

Extract	mg/g				
	Total phenols	Flavanoids	Tannins	Vitamin C	Vitamin E
TC	101.45±0.06	10.54±0.08	84.57±0.14	8.9±0.11	0.53±0.00

Results are expressed Mean ± SEM of triplicate sample

The quality of standardization of TC, in the present study few phenolic compounds such as Tannnins - Gallic acid, Ellagic acid, Flavanoids – Quercetin, Naringenin, Galangin were quantified in HPTLC. HPTLC is a convenient tool for finding out the distribution pattern of phyto constituents which is unique to each plant. HPTLC finger-printing profile establishes the identity and purity of the raw drug being used and helps in the authentification of the plant material [20]. The standard calibration curve, peak documentation with area, peak tables and quantification results obtained using HPTLC were presented in Figure 1, 2 and 3 and Table 4a, 4b and 4c respectively.

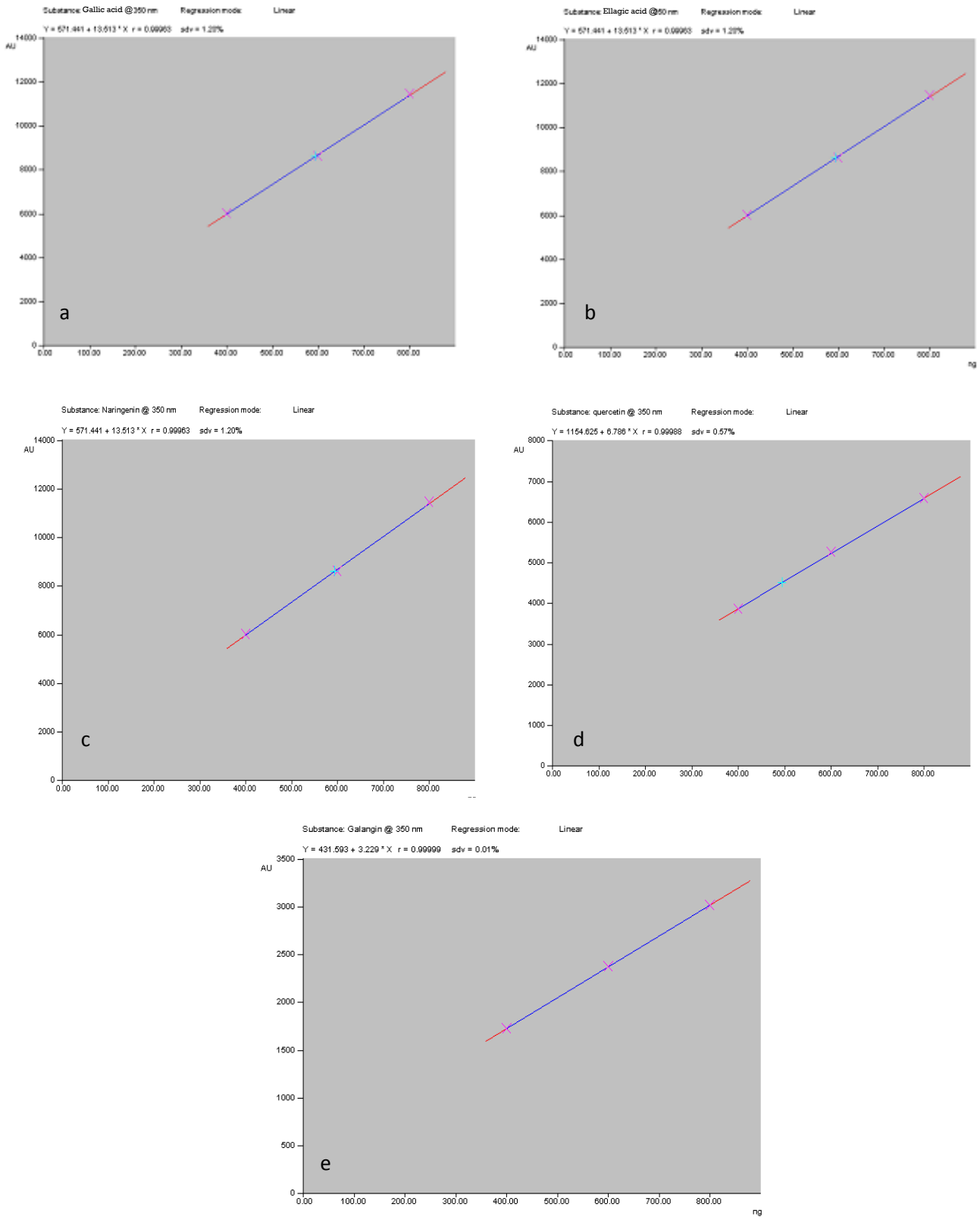

Figure 1: Calibration Curves of Standard Gallic acid, Ellagic acid, Naringenin, Quercetin and Galangin. a) Calibration Curves of Standard Gallic acid (300 – 700 ng);**b)** Calibration Curves of Standard Ellagic acid (300 – 700 ng); c) Calibration Curves of Standard Naringenin (300 – 700 ng);d) Calibration Curves of Standard Quercetin (300 – 700 ng); e) Calibration Curves of Standard Galangin (300 – 700 ng)

Table 4a: Chromatogram Peak Table – Standard Gallic acid and Ellagic acid and its content in Thraatchathi chooranam

Track	Peak	Start Rf	Start Height	Max Rf	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	**1**	**0.41**	**8.2**	**0.47**	**100**	**0.51**	**5.9**	**8513.6**	**100**	**Gallic acid**
2	1	0.1	1.5	0.13	2.07	0.16	0.4	1107.4	1.6	unknown
2	2	0.16	0.3	0.17	0.58	0.20	0	166.5	0.24	unknown
2	**3**	**0.23**	**0.2**	**0.33**	**9.49**	**0.42**	**0.8**	**18179**	**26.29**	**Ellagic acid**
2	**4**	**0.43**	**0.7**	**0.47**	**17.29**	**0.53**	**0.7**	**10693.2**	**15.46**	**Gallic acid**
2	5	0.54	0.2	0.59	7.73	0.61	126.8	5386.3	7.79	unknown
2	6	0.61	127.2	0.64	12.12	0.65	197.9	5656.2	8.18	unknown
2	7	0.65	202.6	0.67	20.71	0.69	185.9	10969.5	15.86	unknown
2	8	0.69	186.5	0.71	12.95	0.75	51.5	7827.6	11.32	unknown
2	9	0.75	52.4	0.76	4.29	0.78	0	1527.4	2.21	unknown
2	10	0.79	0	0.83	12.77	0.86	6.5	7639.1	11.05	unknown
3	**1**	**0.23**	**9.8**	**0.39**	**100**	**0.45**	**10.8**	**15043.3**	**100**	**Ellagic acid**

Peak table represents Rf and area obtained for 6 µl of Gallic acid (Track 1), Thraatchathi chooranam (Track 2) and Ellagic acid (Track 3)

Table 4b: Chromatogram Peak table – Standard Naringenin, Quercitin and Galangin and their content in Thraatchathi chooranam

Track	Peak	Start Rf	Start Height	Max Rf	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	**1**	**0.59**	**15.6**	**0.67**	**100**	**0.71**	**0.9**	**8594**	**100**	**Naringenin**
2	**1**	**0.44**	**13.8**	**0.48**	**100**	**0.52**	**7.2**	**3856.9**	**100**	**Quercetin**
3	**1**	**0.54**	**1.4**	**0.57**	**100**	**0.6**	**1.6**	**2369.4**	**100**	**Galangin**
4	1	0.13	0.2	0.19	10.42	0.25	0.2	6925.6	20.19	unknown
4	2	0.26	0	0.3	7.24	0.34	1.1	2402.3	7	unknown
4	3	0.4	4.8	0.45	5.96	0.47	38.5	2222.8	6.48	unknown
4	**4**	**0.47**	**38.6**	**0.52**	**14.6**	**0.54**	**48.7**	**4506.4**	**13.14**	**Quercetin**
4	**5**	**0.54**	**49.2**	**0.57**	**43.77**	**0.61**	**63.6**	**13988.9**	**40.78**	**Galangin**
4	**6**	**0.61**	**63.6**	**0.63**	**12.12**	**0.68**	**2.1**	**2956**	**8.62**	**Naringenin**
4	7	0.76	0.3	0.79	3.65	0.8	22.9	793	2.31	unknown
4	8	0.8	23.2	0.81	2.25	0.85	0.1	511.1	1.49	unknown

Peak table represents Rf and area obtained for 6 µl of Naringenin (Track 1), Quercitin (Track 2), Galangin (Track 3) and **Thraatchathi chooranam** (Track 4)

Table 4c: Quantification of Gallic acid, Ellagic acid, Naringenin, Quercetin & Galangin in Thraatchathi chooranam

Sample	mg/g				
	Tannins		Flavanoids		
	Gallic acid	Ellagic acid	Naringenin	Quercetin	Galangin
TC	1.8	1.9	6.3	21.4	3.4

Herbal materials are liable to contain heavy metals, microbial load and pesticide residues which accumulate from atmosphere, agricultural practices, such as spraying, treatment of soils during cultivation and administration of fumigants during storage, current practices of harvesting, handling and production which affects the health of the consumer. Especially, aflatoxin desires special attention because it will produce serious side-effects if consumed along with the crude drugs [11]. To overcome this deleterious effect, WHO and FAO recommended many regulations like analytical methodologies for the assessment of specific microbes and pesticide residues. As per regulatory limits aflatoxins should be completely removed or should not be present in herbal medicine by imposing regulatory limits. The determination of heavy metals and pesticides will indicate the quality of production and harvesting practices. The heavy metals such as Pb, Cd, Hg and Ar contents (Table 5) and bacterial count (Table 6) in crude drug TC were found to be within the permissible limits and total fungal count (Table 6), Pesticides residue (Table 7) and Aflatoxins (Table 8) were absent as recommended by the AYUSH guidelines for herbal drugs.

Table 5: Concentration of Heavy metals in Thraatchathi chooranam

Elements	Concentration
Nickel (Ni)	0.197 ± 0.1
Arsenic (As,ppb)	ND
Lead (Pb)	0.11 ± 0.01
Mercury (Hg)	0.03 ± 0.002

Results are expressed Mean ± SEM of triplicate sample. ND - <0.01 ppb

Table 6: Microbial load of Thraatchathi chooranam

Parameters	*Crude TC*	AYUSH Limits
Total bacterial count	10^3/g	**10^5 - 10^7/g**
Total fungal count	Absent	**10^3/g**
Escherichia coli	Absent	**Absent**
Salmonella spp	Absent	**Absent**
Staphylococcus aureus	Absent	**Absent**
Pseudomonas aeruginosa	Absent	**Absent**

TC -Thraatchathi chooranam

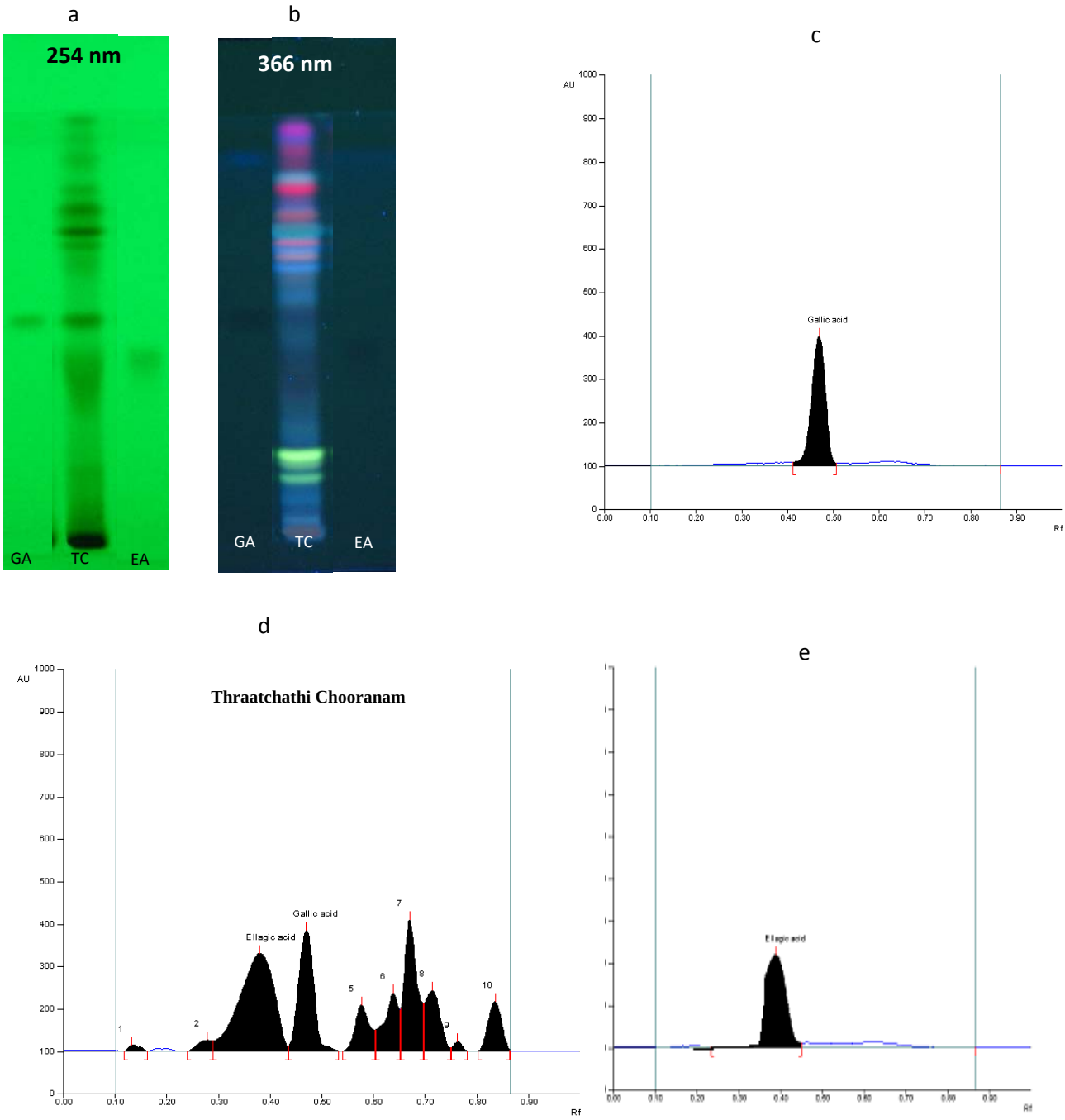

Figure 2: HPTLC Photodocument and Peak Area – Thraatchathi chooranam, Gallic acid and Ellagic acid; a) Peak Documenation showing Standard Gallic acid, TC and Elagic acid at 254 nm; b) Peak Documenation showing Standard Gallic acid, TC and Elagic acid at 366 nm; c) Peak Area of Gallic acid at 600 ng; d) Peak Area of TC at 6 µl; e) Peak Area of Ellagic acid at 600 ng

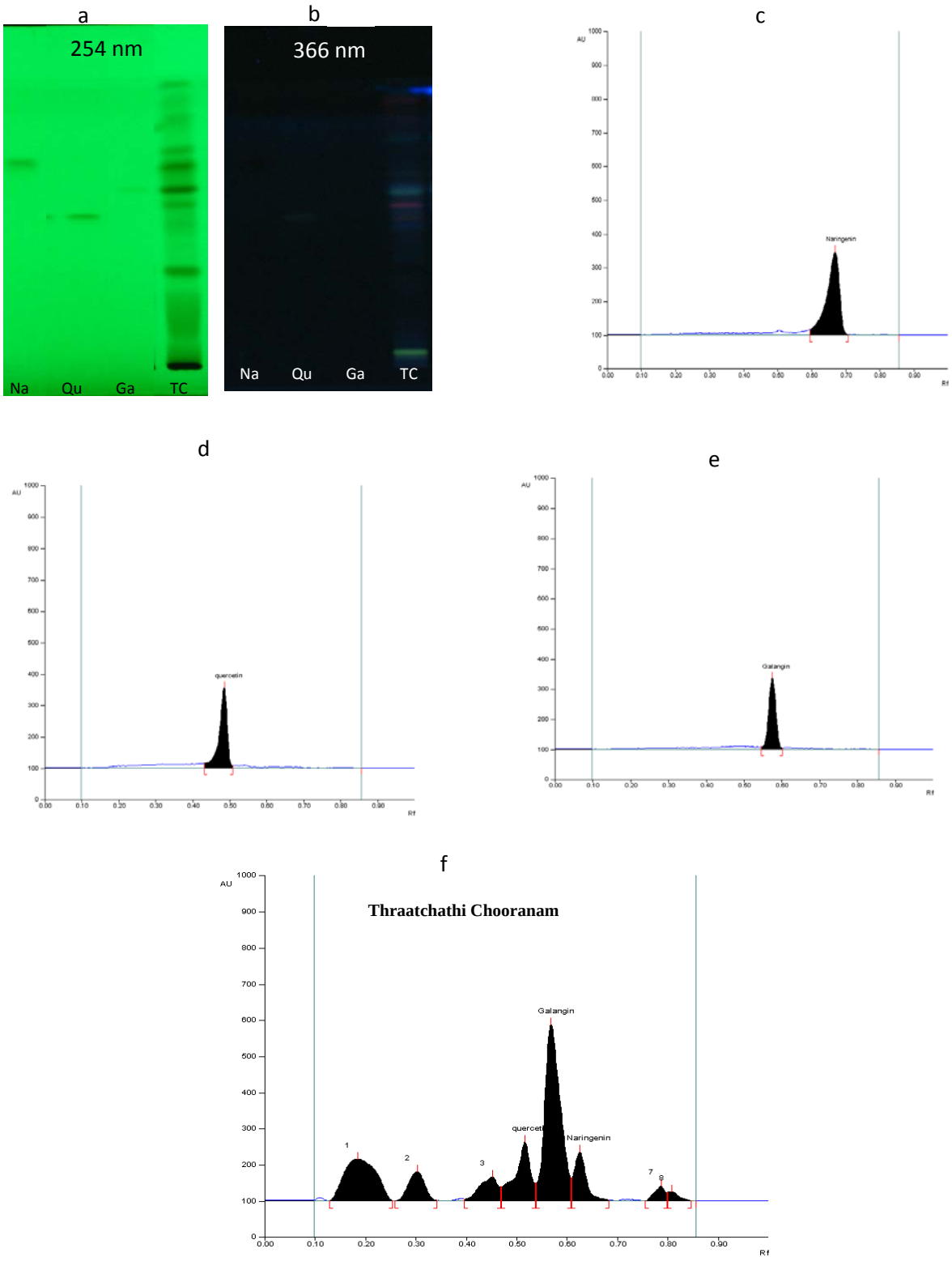

Figure 3: HPTLC Photodocument and Peak Area – Thraatchathi choornam, Naringenin, Quercitin and Galangin a) Peak Documentation showing Standard Thraatchathi choornam, Naringenin, Quercitin and Galangin at 254 nm; b) Peak documenation showing Standard Thraatchathi choornam, Naringenin, Quercitin and Galangin at 366 nm; c) Peak Area of Naringenin at 600 ng; d) Peak Area of Quercitin at 600 ng; e) Peak Area of Galangin at 600 ng f) Peak Area of TC at 6 µl

Table 7: Pesticide residues of Thraatchathi chooranam

Pesticide Residues	*Crude TC*	AYUSH Limits (mg/kg)
Organochlorine compounds		
Alachlor	ND	0.02
α- BHC, β- BHC, γ- BHC, γ- BHC, Butachlor	ND	-
Aldrin and Dieldrin, Chlordane (cis & trans), Endrin, Endrin aldehyde, Endrin ketone,	ND	0.05
Chlorthalonil, Dicofol, Epoxide, Heptachlor, Heptachlor, Methoxychlor	ND	-
, o,p'DDT, p',p'DDT, o,p'-DDE, p',p'-DDE, o,p'-DDD, p,p'-DDD,	ND	1.00
Endosulfan-Alpha, Endosulfan-Beta, Endosulfan-Sulphate	ND	3.0
Organophosphorous compounds		
Acephate, Dimethoate, Etrimphos, Iprobenphos, Malaxon, Methamidaphos, Methyl paraxon, Monocrotophos, Omethoate, Parathion ethyl, Pencanozole, Phorate, Phorate sulfone, Phosalone, Phorate sulphoxide, Phosphamidone, Profenophos	ND	-
Diazinon	ND	0.5
Diclorvos, Malathion	ND	1.0
Ethion	ND	2.0
Parathion methyl, Chlorpyrifos	ND	0.2
Chlorpyrifos-methyl detected	ND	0.1
4-Bromo-2-Chlorophenol, Chlorfenvinphos (cis & trans), Penitrothion	ND	-
Pyrethroids		
Permethrin, Cypermethrin (I, II, III & IV)	ND	1.0
Cyfluthrin (I & II), Etofenprox, Lambda cyhalothrin	ND	-
Deltamethrin	ND	0.5
Fenvelarate	ND	1.5

ND- Not Detected; TC -Thraatchathi chooranam

Table.8. Aflatoxins content of Thraatchathi chooranam

Formulations	Aflatoxins				AYUSH Permissible Limit
	B1	B2	G1	G2	
Thraatchathi chooranam	ND	ND	ND	ND	B1& G1 : 0.5 ppm

ND: Not detected

CONCLUSION

The therapeutic effect of Siddha medicine depends on quality of plant materials and appropriate protocols for development of herbal formulations. Therefore, the necessity of standardization of various herbal formulations will pave way to explore the therapeutic effects as claimed in Siddha literature and thereby improving the scientific credibility of Siddha medicine.

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