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SYSTEMATIC STANDARDIZATION AND PHYSIOCHEMICAL EVALUATION OF NOVEL SIDDHA FORMULATION PUNGAMPOO CHOORANAM AS PER AYUSH GUIDELINES BY MODERN ANALYTICAL TECHNIQUES

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Keywords: *Siddha system, Global market, Pungampoo Chooranam, Standardization, Physiochemical, AYUSH, TLC, HPTLC*

Abstract

Siddha system of medicine is one of the oldest Indian system of medicine known to the mankind. Several indigenous siddha formulations formulated and hypothesized by ancient siddha practitioners are still used for clinical management of several dreadful metabolic disorders in humans like diabetes mellitus. Global market for siddha formulations seems remarkable but the need of the hour is to ensure the standard of the preparation. Starting from raw material to finished product the quality and genuinity of the product as to be ascertained. Considering the global need the modern standardization method adopted for identity, purity and shelf life of the preparation. Different techniques has been followed to analyze the purity of the raw materials it includes microscopic, macroscopic, Physical, chemical and biological method of analysis. Hence the main aim of the present investigation is to standardize the novel formulation *Pungampoo Chooranam* (PPC) as per AYUSH guideline and to reveal the property of the formulation to the scientific community for better understanding about the standards of the formulation. The results obtained from the physicochemical evaluation revels that the total ash value of PPCwas found to 8.01 %. In which the water soluble ash was 4.75% and acid insoluble ash was 0.69%. Similarly loss on drying value at 105^{o}C was fond to be 9.24% respectively. The water soluble extractive value of PPC was found to be 26.46 % whereas alcohol soluble extractive value is 29.35 %. n-hexane soluble extractive value of the test drug PPC was found to be 19.26%. Preliminary TLC analysis of the sample PPC emits fluorescence indicates the presence of fluorescent emitting compound. The results of HPTLC analysis of the sample PPC reveals the presence of 14 prominent peaks corresponds to 14 different compound's with Rf value ranging from 0.01 to 0.93 with percentage area of 0.82 to 18.52%. From the results of the present investigation it was concluded that the formulation PPC is highly stable and also possess biologically significant phytocomponents which is may be used as an ailment for treating various disease

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Introduction

Physicochemical evaluation of the preparation plays vital role in establishing the monograph of the formulation, as it becomes the documentary evidence to substantiate the standards of the preparation. It renders the useful information like genuinity, stability, selective characteristic feature and nature of the compound's present in the drug. WHO and other regulatory authorities in collaboration with government agency setup a bench mark for proper standardization of the raw drug as well the finished formulations. According to these guidelines parameters such as loss on drying,
In the present era, market of all commodities has become global. Health has been of utmost importance since ancient times for the mankind. Market of health-related products has been active and these products are manufactured at different parts of the world and sold all over. Standardization is necessary to make sure the availability of a uniform product in all parts of the world. Standardization assures a consistently stronger product with guaranteed constituents [1].

According to the global survey it has been identified that nearly 80% of individuals from developed and developing countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to understand their properties, safety and efficacy [2].

In ASU systems plants, minerals, and animal products are used as main drugs to cure various ailments [3] . Herbal medicine also called botanical medicine or phytomedicine refers to the use of plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease [4,5].

Through systematic standardization a formulator can profile the preparation with respect to the following (i) Physiochemical parameters (ii) Category of phytocomponents (iii) Nature of individual chemical component (iv) Structural and functional group analysis (v) Correlation of mechanism with respect to the functional group present in bioactive phytocomponents (vi) Drug stability (vii) Pharmacokinetic profiling (viii) Receptors on which the drug acts (ix) Chances of possible interaction. Because of the emerging knowledge in the field of drug standardization. Now siddha preparations which satisfy the quality and standard are being exported and it is in practice by other countries like Srilanka, USA and Indonesia.

Bioactive phytocomponents present in siddha preparations like legium, chooranam and other oils have unique advantage of multiple mode of action. Synergy of using combined phytotherapeutics and adjuvants like ghee has well established mechanism in living biological system. Development of monograph for indigenous and novel preparations has been considered a right platform for future researcher to select their drug of choice for their research work. As a measure of focusing upon the need of drug standardization the present investigation work undertaken to standardize the traditional polyherbal siddha formulation *Pungampoo Chooranam* (PPC) which has been used for the treatment of life threatening metabolic disorders liker diabetes mellitus. Still now there is no literature evidence available on standardization and phytochemical investigation aspect of this formulation PPC this prompted the investigator to peruse the systematic standardization of PPC by physiochemical evaluation as per AYUSH guidelines.

Materials And Methods

1. Source of raw drugs

The herb is collected from southern zone of Tamil Nadu, and other required ingredient is procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India .Herb were authenticated by the Pharmacognosist, SCRI Chennai, Tamil Nadu, India

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2. Ingredients

The siddha formulation *Pungampoo Chooranam* (PPC) comprises of two main ingredients as listed below
1. Pungam flowers (*Pongamiapinnata*)
2.Cow's Ghee

3. Preparation [6]

The shade dried flowers of *Pongamiapinnata* were roasted slowly by adding little bit of cow's ghee. Then it is powdered and sieved using cloth.

Dosage : 2 gm twice a day
Adjuvant : Warm water
Duration : 48 Days

4. Physicochemical Evaluation [7,8]

Percentage Loss on Drying

10gm of test drug was accurately weighed in evaporating dish .The sample was dried at 105^{o}C for 5 hours and then weighed.

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

Determination of Total Ash

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

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

Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. 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.

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

Determination of Water Soluble Ash

The ash obtained by total ash test will be boiled with 25 ml of water for 5 mins. The insoluble matter is collected in crucible and will be washed with hot water, and ignite for 15mins 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.

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

Determination of Alcohol Soluble Extractive

About 5 g of test sample will be macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared 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.

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

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Determination of Water Soluble Extractive

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

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

Determination of n-Hexane Soluble Extractive

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

n-Hexane soluble extract = Weight of Extract/ Wt of the Sample taken X 100

Determination of pH

About 5 g of test sample will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the subjected to pH evaluation

5. TLC Analysis [9]

Test sample PPC was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Ethyl Acetate : Acetic Acid (5: 1.5: 0.25 v/v/v). After the run plates are dried and were observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm Sample Spotting.

6. HPTLC Analysis [10]

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.

HPTLC Chromatographic condition

Sample	:	PPC
Der Solvent	:	Anisaldehyde
Stationary phase	:	Silica gel GF_{254}
Mobile phase	:	Chloroform: n-butanol: methanol: water: Acetic acid (4:1:1:0.5:0.5)
Scanning wavelength	:	366 nm
Sample concentration	:	10mg/ml

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Applied volume : 5 µl
Application mode : CAMAG HPTLC

Results

1. Physico-chemical Evaluation and standardization of PPC

The results obtained from the physicochemical evaluation revels that the total ash value of PPCwas found to 8.01 %. In which the water soluble ash was 4.75% and acid insoluble ash was 0.69%. Similarly loss on drying value at 105^{o}C was fond to be 9.24% respectively.

Water-soluble and alcohol soluble extractive values plays an important role in evaluation of crude and finished drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. The water soluble extractive value of PPC was found to be 26.46 % whereas alcohol soluble extractive value is 29.35 %.The hexane soluble extractive value signifies the presence of amounts of fats, lipids, and some steroids in the drug. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying, or storage or formulating. The results of the studies shows that hexane soluble extractive value of the test drug PPC was found to be 19.26%. All the results were tabulated in Table 01.

Table 1: Physicochemical Evaluation of Pungampoo Chooranam

S.No	Parameter	Mean (n=3) SD
i.	***Loss on Drying at 105 °C (%)***	9.24
ii.	***Total Ash (%)***	8.01
iii.	***Acid insoluble Ash (%)***	0.69
iv.	***Water Soluble Ash (%)***	4.75
v.	***Alcohol Soluble Extractive (%)***	29.35
vi.	***Water soluble Extractive (%)***	26.46
vii.	***n-Hexane soluble Extractive (%)***	19.26
viii.	***pH***	7.5

2. TLC and HPTLC analysis of PPC

Preliminary TLC analysis of the sample PPC emits fluorescence indicates the presence of fluorescent emitting compound as illustrated in Figure 1 and Table 2. The results of HPTLC analysis of the sample PPC reveals the presence of 14 prominent peaks corresponds to 14 different compound's with Rf value ranging from 0.01 to 0.93 with percentage area of 0.82 to 18.52%. The results were tabulated in Table 3 and illustrated in Figure 2 and 3.

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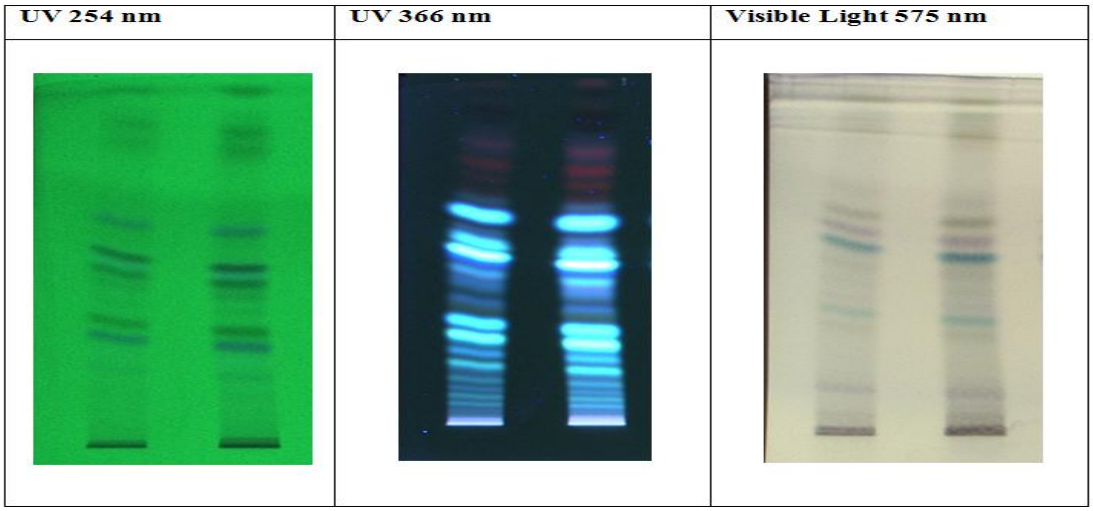

Figure 1: TLC Chromatogram of Pungampoo Chooranam

Table 2: Rf Value of TLC Chromatogram of Pungampoo Chooranam Short UV, Long UV and in Visible Light

UV 254 nm		UV 366 nm		Visible Light 575 nm	
Colour	**R_f value(s)**	**Colour**	**R_f value(s)**	**Colour**	**R_f value(s)**
Grey	0.12	Light Blue	0.06	Grey	0.06
Grey	0.18	Light Blue	0. 09	Pink	0.13
Dark blue	0.27	Blue	0. 11	Yellow	0. 27
Green	0.32	Light Blue	0.13	Blue	0.31
Grey	0.36	Bright Blue	0.18	Pink	0.34
Grey	0.42	Violet	0.21	Light blue	0.39
Green	0.48	Bright Blue	0.26	Light green	0. 44
Dark green	0.49	Bright Blue	0.27	Blue	0.48
Blue	0. 59	Violet	0.33	Pink	0.53
Grey	0.81	Violet	0.37	Yellowish green	0.58
Grey	0.87	Blue	0.41	Violet	0. 64
Grey	0.97	Bright Blue	0.46	Yellowish green	0. 79
		Bright Blue	0.50	Yellowish green	0.86
		Violet	0.53	Yellowish green	0.90
		Bright Blue	0.58		
		Blue	0.62		
		Dark Red	0.65		
		Dark Red	0.68		
		Bright Red	0.73		
		Pink	0.80		
		Violet	0.86		
		Violet	0.91		
		Pink	0.98		

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Table 3: HPTLC Peak Table analysis of Pungampoo Chooranam

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	10.5 AU	0.00 Rf	549.4 AU	19.55 %	0.04 Rf	60.7 AU	8883.2 AU	9.54 %
2	0.04 Rf	60.8 AU	0.04 Rf	61.5 AU	2.19 %	0.09 Rf	30.9 AU	1849.8 AU	1.99 %
3	0.09 Rf	30.9 AU	0.10 Rf	39.8 AU	1.41 %	0.12 Rf	25.9 AU	764.3 AU	0.82 %
4	0.12 Rf	26.0 AU	0.16 Rf	44.5 AU	1.58 %	0.18 Rf	30.9 AU	1858.8 AU	2.00 %
5	0.18 Rf	31.2 AU	0.20 Rf	74.4 AU	2.65 %	0.23 Rf	37.3 AU	1916.9 AU	2.06 %
6	0.23 Rf	36.5 AU	0.29 Rf	258.5 AU	9.20 %	0.31 Rf	20.5 AU	7457.8 AU	8.01 %
7	0.31 Rf	122.0 AU	0.33 Rf	246.6 AU	8.77 %	0.37 Rf	50.1 AU	6497.1 AU	6.98 %
8	0.37 Rf	50.3 AU	0.39 Rf	78.4 AU	2.79 %	0.40 Rf	69.5 AU	2061.0 AU	2.21 %
9	0.40 Rf	69.5 AU	0.47 Rf	257.6 AU	9.17 %	0.49 Rf	70.2 AU	10589.2 AU	11.38 %
10	0.49 Rf	171.3 AU	0.51 Rf	367.7 AU	13.08 %	0.55 Rf	12.3 AU	10921.7 AU	11.74 %
11	0.57 Rf	128.8 AU	0.60 Rf	264.8 AU	9.42 %	0.73 Rf	59.9 AU	17233.8 AU	18.52 %
12	0.76 Rf	69.8 AU	0.82 Rf	150.3 AU	5.35 %	0.84 Rf	35.6 AU	7183.7 AU	7.72 %
13	0.84 Rf	135.9 AU	0.87 Rf	177.9 AU	6.33 %	0.93 Rf	89.9 AU	9027.8 AU	9.70 %
14	0.93 Rf	90.5 AU	0.95 Rf	238.7 AU	8.50 %	1.01 Rf	4.0 AU	6823.9 AU	7.33 %

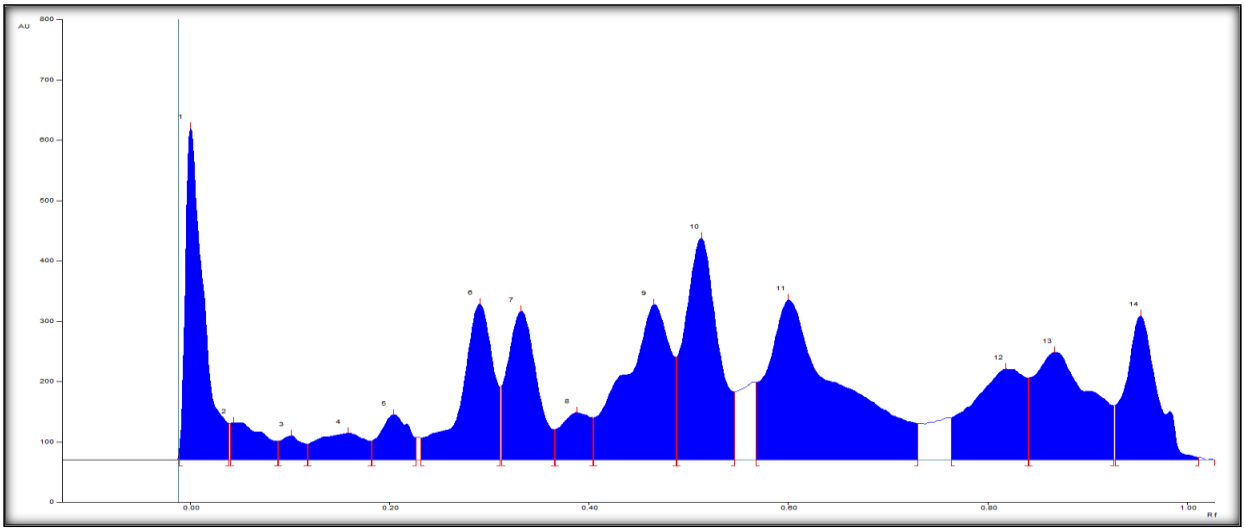

Figure 2: HPTLC Chromatogram of Pungampoo Chooranam

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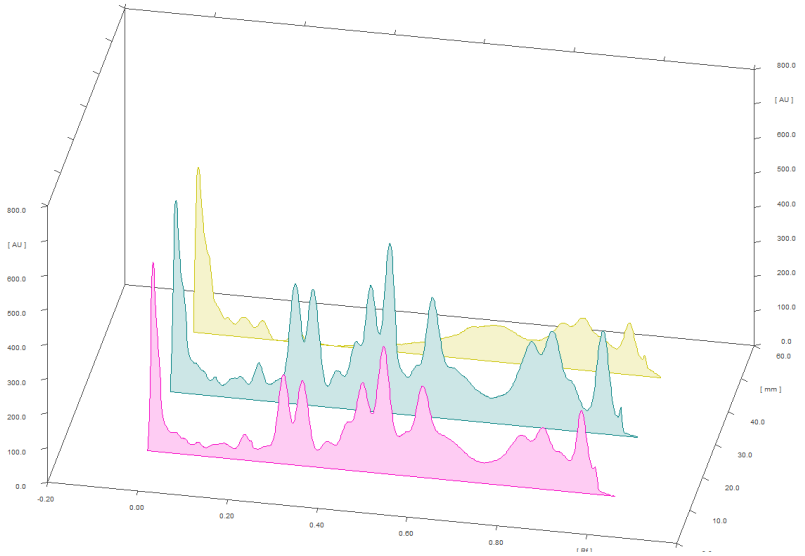

Figure 3: HPTLC 3D Chromatogram of Pungampoo Chooranam

Discussion

The value of herbal market in India is about 1 billion and the export of poly herbal preparations is around 80 million. Polyherbal formulations being a multi componential dosage form often prone to higher chance of contaminants, adulterants, pesticides residue and toxins infestations. Hence now a day it's become mandatory to establish the safety, sterility and standard of the each batch before dispensing the same to the consumer usage [11].

World Health Organization (WHO) has individual herbal drugs as whole, labeled medicinal products that have robust ingredients, aerial or secret parts of the whole plant or other plant material or mixture of them. World Health Organization (WHO) has a set of specific Guidelines for the evaluation of the safety, efficacy and Quality of herbal drugs or herbal medicines [12,13].

In developing countries, 70-95% of the population relies on herbal medicines for primary care mainly due to cost imperatives or unavailability of conventional drugs. In India, in spite of over 80% of the population dependent upon herbal drugs; it occupies less than 2.5% of the global market share. On the other hand, > 60% market share is being controlled by European Union and North America while 16% being shared by Japan and rest 19% by ASEAN countries [14,15].

Standardization requires a natural plant product to be authenticated at origin itself by adoption of good agricultural practices [16] collection strategies from wild and good manufacturing practices for extraction modes and related parameters [17-20]. The acceptance of lead as a future drug candidate requires correct identification, authentication and concentration of active principle [21,22] defined quantities of active components in poly herbal formulations [23,24]. The regulatory approvals to ascertain consistent chemical profile and biological activity of future drug candidate [25] includes a) quality assurance by determining adulterants, pesticides residue, aflatoxin content, bacterial/fungal growth and heavy metals contamination etc. b) prevention of adverse reactions by evaluating pharmacodynamics, pharmacokinetics, dosage, stability, self-life and toxicity (acute/ chronic) etc [26] ; c) reproducibility by repetitive testing using different batches to control batch-to-batch variation and development of standard assay markers and; d) chemiinformatic approaches to ensure that pharmacological profiles matches with the activity profiles of active constituents of drug itself. The results obtained from the physicochemical evaluation revels that the total ash value of PPCwas found to 8.01 %. In which the water soluble ash was 4.75% and acid insoluble ash was 0.69%. Similarly loss on drying value at 105^{o}C was fond to be 9.24% respectively.

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There are some criteria's needs to be optimized in siddha medicines includes time of collection of the herbs, method of processing, preparation techniques, method of identification and authentication, stability verses temperature, detoxification procedures. There are some hurdles in maintain the quality and standards in siddha preparation which includes failure identification of gentility, adulterations and non-availability of monograph with respect to individual herbs. The water soluble extractive value of PPC was found to be 26.46 % whereas alcohol soluble extractive value is 29.35 %. The results of the studies show that hexane soluble extractive value of the test drug PPC was found to be 19.26%.

According to AYUSH regulation standardization means the preparation means the product that ensured the quality and safety. Further the standards of the prepared formulations have been rightly identified by some parameters which include ash value, extract value, moisture content, total foreign materials, pesticide residue etc.

Sophisticated instruments had played a greater significant role in siddha drug standardization some of these includes HPTLC analysis for identifying total number of compounds present, AAS and ICPMS for heavy metal analysis, GCMS for identification of volatile and plant phyto sterols the greater advantage of using GCS is the chromatogram reveals the compound structure and mol wt. SEM and TEM analysis for particle size and surface morphology which is unique for nano sized particle containing formulations. FTIR for functional group identification [27]. Preliminary TLC analysis of the sample PPC emits fluorescence indicates the presence of fluorescent emitting compound. The results of HPTLC analysis of the sample PPC reveals the presence of 14 prominent peaks corresponds to 14 different compound's with Rf value ranging from 0.01 to 0.93 with percentage area of 0.82 to 18.52%.

Conclusion

From the results of the present investigation it was concluded that the siddha formulation *Pungampoo Chooranam* (PPC) prepared according to PLIM standard was stable and also complies with the standard of the AYUSH regulations and further this investigation render some biologically significant information's. TLC analysis of PPC emits fluorescence indicates the presence of bio active compound. . The results of HPTLC analysis of the sample PPC reveals the presence of 14 prominent peaks corresponds to 14 different compound's with Rf value ranging from 0.01 to 0.93 with percentage area of 0.82 to 18.52%.Hence it was concluded that the formulation PPC is highly stable and also possess biologically significant phytocomponents which is may be used as an ailment for treating various chronic disorders, further investigation has to be carried out in depth about the structure and functional group present in the formulation and its relevance in clinical management of diabetes mellitus.

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