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Standardization of the drug Parangipattai chooranam – A Siddha herbal formulation.

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Abstract

Siddha system of medicine is the system that distinctly explains the complete integrated relation of body, sense, mind and soul with the universe to attain immortality. “Siddha system of medicine is an evolution and not an overnight invention”. In Siddha medicine there are 32 types of Internal medicine and 32 types of external medicine. Chooranam is one among the 32 types of internal medicine. Parangipattai chooranam is a familiar polyherbal formulation in Siddha system of medicine which is selected by the author for standardisation. Aim of this study was to assess the quality of the drug Parangipattai chooranam by conducting physicochemical analysis, preliminary phytochemical analysis and other analytical techniques. Physicochemical analysis and preliminary phytochemical analysis was done based PLIM reference. Other analytical technique like High Performance thin layer chromatography (HPTLC) finger printing was done using CAMAG. The physicochemical analysis of the drug Parangipattai choornam showed that the pH value of 4.8 indicates the drug is slightly acidic. The preliminary phytochemical analysis showed the presence of Flavonoids, steroids, triterpenoids, coumarin, phenol, tannin, saponin and sugar. The drug was free of microbial contamination; heavy metals and pesticide were below deductible limit. The results obtained indicate that the drug is of standard quality and can be used as reference standard in laying pharmacopoeia standard.

Keywords: Parangipattai chooranam, Physicochemical analysis, Standardization, Siddha medicine.

1. Introduction

Siddha system of medicine remains the one which I admired more. Siddhars, are the creators of this delightful system. Carvings of our Siddhars are still an astonishing one. Every country has its own system of medicine. Likewise in our country siddha system of medicine is most popular. Due to recent scientific research methods Siddha system of medicine is not fully accepted by other system of medicine and moreover not recognized by other countries. Accordingly Siddha research scholars are in need to prove the efficacy of each and every formulations carved by our great Siddhars.

Standardisation method for all AYUSH drugs of Indian system of Medicine may contain a single herb or combination of different herbs believed to have complementary and or synergistic effects. Both the raw and finished products contain complex mixtures of fatty acids, sterols, alkaloids, flavonoids, glycosides etc. In the modern era due to commercialization and bulk production of herbal drugs there is chance of adulteration and imperfect processing of drugs. Hence there is need for standardization of all herbal drugs to sustain their quality. Therefore it is extremely desirable that these drugs should be characterized with modern

instruments, based on which the specifications of such drugs can be well standardized on a scientific basis. [1]

The selected drug Parangipattai chooranam is a classical siddha formulation, simple and cost effective, has diverse medicinal properties and used in the treatment of various diseases like Megam, Karunkuttam, Venkuttam and diseases of Vatham and Pitham. [2]

2. Materials and Methods

The drug Parangipattai chooranam is prepared in Gunapadam laboratory, National Institute of Siddha, Chennai. The standardization methods are carried out on standard research laboratory in Chennai. Ingredients of Parangipattai chooranam are given in the Table 1.

Table 1: Ingredients of Parangipattai chooranam:

Sl.no	Ingredients	Botanical name	Parts used
1	Parangipattai	*Smilax china* Linn.	Tuber
2	Karunthulasi	*Ocimum tenuiflorum* Linn.	Whole plant juice

2.1. Identification of raw drugs:

Parangipattai was purchased from local raw drug store and Karunthulasi was collected from herbal garden, National Institute of Siddha, Chennai. The ingredients were authenticated by Department of Medicinal botany, National Institute of Siddha, Chennai. All the ingredients mentioned in the formulation are purified as per the direction described in the Siddha literature.[3]

2.2. Preparation of the drug:

Parangipattai was soaked in equal amount of Karunthulasi juice and kept in sunlight until it was dried. Then it was grinded and filtered by Vasthirakaayam (filtration method with the help of cloth) to get the texture offine powder.[2]

2.3. Organoleptic characters:

Colour, odour, taste and consistency of the drug were noted.

2.4. Physico-chemical parameters:

All the physico-chemical parameters were carried out as per the methods mentioned in standard books. The parameters are as follows.

2.4.1. Particle Size Determination by Microscopic Method:

Particle size determination was carried out by optical microscopic method in which the samples were dissolved in the sterile distilled water (app 1/100th dilution). Diluted sample was mounted on the slide and fixed with stage of appropriate location. Light microscopic image were drawn with scale micrometer to arrive at the average particle size. Minimum 30 observations were made to ascertain the mean average particle size of the sample.[4]

2.4.2. Percentage Loss on Drying:

10gm of test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed. [5, 6]

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

2.4.3. Determination of Total Ash:

3 g of test drug was accurately weighed in silica dish and incinerated at the furnace at a temperature of 400 ºC until it turns white in colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug. [5,6]

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

2.4.4. Determination of Acid Insoluble Ash:

The ash obtained by total ash test was boiled with 25 ml of dilute hydrochloric acid for 6 mins. 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. [5,6]

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

2.4.5. Determination of Water Soluble Ash:

The ash obtained by total ash test was 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 15 mins 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.[5,6]

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

2.4.6. Determination of Alcohol Soluble Extractive:

About 5 g of test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing tostand 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 airdried drug. [5,6]

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

2.4.7. Determination of Water Soluble Extractive:

About 5 g of the test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing 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. [5,6]

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

2.4.8. Determination of pH:

About 5 g of test sample was dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the subjected to pH evaluation. [5,6]

2.5. Preliminary phytochemical tests:

The preliminary phytochemical tests were carried out as per the methods mentioned in standard books.

2.5.1. Test for alkaloids:

Mayer's Test: To the test sample, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.[7]

2.5.2. Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.[7]

2.5.3. Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.[7]

2.5.4. Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.[7]

2.5.5. Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.[7]

2.5.6. Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow colour indicates the presence of Flavonoids.[7]

2.5.7. Test for phenols:

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.[7]

2.5.8. Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.[7]

2.5.9. Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.[7]

2.5.10. Test for Cyanins

A. Anthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100ºC. Formation of bluish green colour indicates the presence of anthocyanin.[7]

2.5.11. Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.[7]

2.5.12. Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.[7]

2.6. High Performance Thin Layer Chromatography:

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition it is a reliable method for the quantitation of Nano grams level of samples. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials.[8,9]

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. [9]

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 phyto constituents present in each extract and Rf values were tabulated.[9]

2.7. Heavy/Toxic Metal Analysis By AAS:

Methodology:

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample PPC was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series in orders to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test sample PPC.

Sample Digestion:

Test sample PPC digested with 1mol/L HCl for determination of arsenic and mercury. Similarly for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard preparation:

As & Hg- 100 ppm sample in 1mol/L HCl
Cd & Pb- 100 ppm sample in 1mol/L HNO30

2.8. Microbial contamination by pour plate method:

Objective:

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

Methodology:

About 1ml of the test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{o}C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (About 10 minutes). Plates were then inverted and incubated at 37^{o}C for 24 - 48 hours. Grown colonies of organism was then counted and calculated for CFU.

2.9. Test for specific pathogen:

Methodology:

One part of the test sample was dissolved in 9 mL of sterile distilled water and the test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37^{o}C for 24 – 72 hours for observation. Presence of specific pathogen was identified by their characteristic colour with respect to pattern of colony formation in each differential media.

2.10. Analysis of pesticides organochlorine, organophosphorus and pyrethroids:

Extraction:

About 10 g of test substance were extracted with 100 ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few millilitres of toluene R and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.[10] [11]

2.11. Aflotoxin Assay By TLC (B1,B2,G1,G2):

Solvent:

Standard samples was dissolved in a mixture of chloroform and acetonitrile (9.8 : 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Test solution: Concentration 1 µg per ml.

Procedure:

Standard aflatoxin was applied on to the surface of pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly the test sample was placed and allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85:10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.[12]

3. Results

3.1. Organoleptic characters:

The drug Parangipattai choornam was a fine powder pale milky white in colour with mild odour, bitter and slightly sweet in taste (Figure 1). The inferences are tabulated in Table 2.

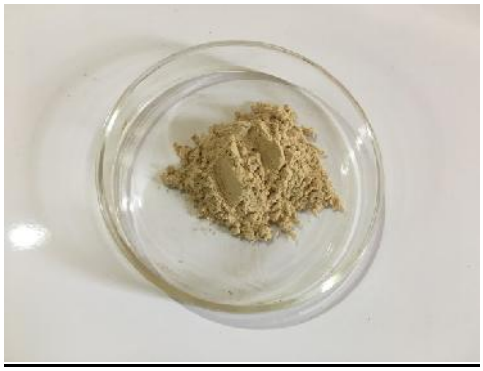

Figure 1: Prepared drug

Table 2: Organoleptic characters:

Sl.no	Specification	Character
1	Colour	Milky white
2	Odour	Mild
3	Taste	Bitter and sweet
4	Consistency	Very fine powder

3.2. Physico-chemical parameters:

The loss on drying which indicates the moisture content of the drug was determined as 8.5 ± 2.8%. The total ash was found to be 0.55 ± 0.05% which indicates the inorganic content of the drug. The water soluble ash was calculated as 7.46 ± 0.72% and the value of acid insoluble ash was found to be 0.32 ± 0.15% which indicates that the drug contains negligible amount of siliceous matter. The water soluble extractive value and alcohol soluble extractive value were found to be 25.86 ± 0.81% and 34.44 ± 2.12%. The pH value is calculated as 4.8 which indicate that the drug is acidic. The observed results were tabulated in Table 3.

Table 3: Physicochemical characters of Parangipattai chooranam:

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	8.5 ± 2.80
2.	Total Ash (%)	0.55 ± 0.05
3.	Acid insoluble Ash (%)	0.32 ± 0.15
4.	Water Soluble Ash (%)	7.46 ± 0.72
5.	Alcohol Soluble Extractive (%)	34.44 ± 2.12
6.	Water soluble Extractive (%)	28.86 ± 0.81
7.	pH	4.8
8.	Particle size	121.9± 45.24

3.3. Particle size determination by microscopic method:

Microscopic observation of the particle size analysis reveals that the average particle size of the sample PPC was found to be 121.9± 45.24 µm further the sample PPC has particle with the size range of lowest 41.1 µm to highest 211.341.1 µm (Figure 2)

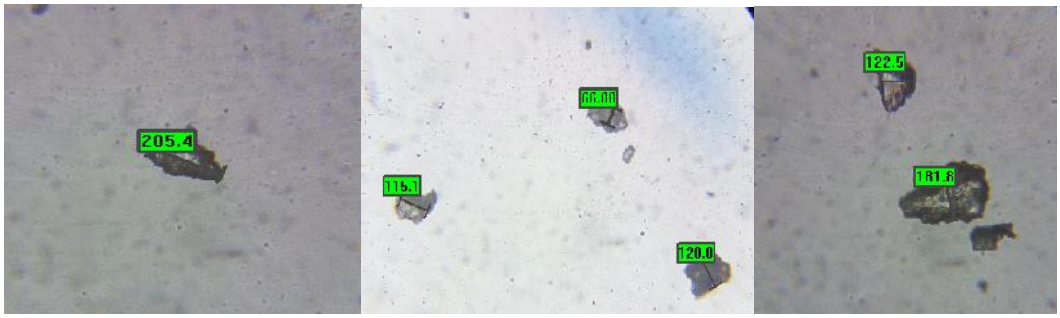

Figure 2: Microscopic Observation of Particle Size for the sample PPC

3.4. Preliminary phytochemical tests:

The drug has high polar secondary metabolites like flavonoids, steroids, triterrpenoids coumarin, phenol, tannin, saponins, sugar are shown in table 4.

Table 4: Preliminary Phytochemical Analysis of Parangipattai Chooranam:

Sl.no	Name of the test	Inference
1	Test for Alkaloids (Mayer's test)	-
2	Test for Flavonoids	+
3	Test for Glycosides (Borntrager's test)	-
4	Test for Steroids	+
5	Test for Triterpenoids (Liebermann–Burchard test)	+
6	Test for Coumarin	+
7	Test for Phenols (Lead acetate test)	+
8	Test for Tannin	+
9	Test for Proteins (Biuret test)	-
10	Test for Saponins	+
11	Test for Sugar (Benedict's test)	+
12	Test for Anthrocyanin	-
13	Test for Betacyanin	-

3.5. High performance thin layer chromatography:

HPTLC finger printing analysis of the sample PPC reveals the presence of four prominent peaks corresponds to presence of four versatile phytocomponents present within it. Rf value of the peaks ranges from 0.30 to 0.89. Further the peak 2 occupies the major percentage of area of 38.76 % which denotes the abundant existence of such compound. Followed by this peak 3 and 1 occupies the percentage area of 31.34 and 16.90%. Peak 4 occupies the percentage area of 13%. (Table 5) (Fig 3)

Table 5: HPTLC peak

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	0.30	12.0	0.31	17.2	16.90	0.33	5.0	179.5	13.42
2	0.45	1.5	0.46	39.4	38.78	0.48	0.5	428.3	32.02
3	0.49	0.3	0.50	31.9	31.34	0.53	0.1	413.9	30.95
4	0.89	0.7	0.92	13.2	13.00	0.94	2.9	315.8	23.61

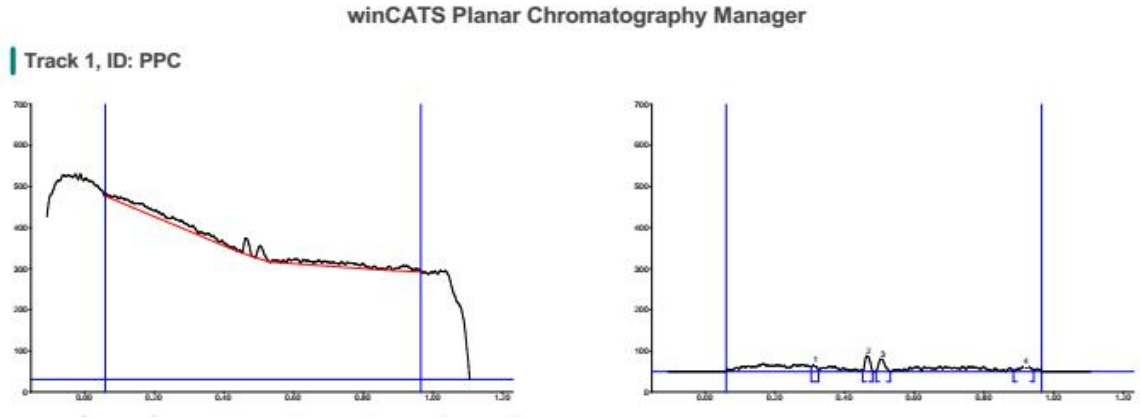

Figure 2: HPTLC Finger printing.

3.6. Heavy/Toxic Metal Analysis By AAS:

Results of the present investigation has clearly showed that the sample PPC has no traces of Mercury and hence it was considered that the heavy metal mercury was absent in the sample PPC. The level of arsenic, lead and cadmium was found to be 0.009 ppm, 0.150 ppm and 0.009 ppm respectively. It was observed that all three reported heavy metals (arsenic, lead and cadmium) seem very less when compared to the allowed recommended limit. (Table 6).

Table 6: Heavy metal analysis report:

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Mercury	253.7nm	BDL	1 ppm
Lead	217.0 nm	0.150 ppm	10 ppm
Arsenic	193.7 nm	0.009 ppm	3 ppm
Cadmium	228.8 nm	0.009 ppm	0.3 ppm

3.7. Microbial contamination by pour plate method:

No growth / colonies were observed in any of the plates inoculated with the test sample shown in Table 7.

Table 7: Sterility report:

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

3.8. Test for specific pathogen:

No growth / colonies were observed in any of the plates inoculated with the test sample shown in Table 8.

Table 8: Specific pathogen report:

Organism	Specification	Result	Method
E. coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

3.9. Analysis of pesticides organochlorine, organophosphorus and pyrethroids:

The results showed that there were no traces of pesticides residues such as Organo chlorine and Organo phosphorus Pesticides in the sample PPC. Further sample shows the presence of Cypermethrin belongs to pyrethroid type of pesticide at the concentration of 0.1 mg/kg which was low when compared the AYUSH prescribed limit of 1mg/kg. (Table 9)

Table 9: Pesticide residue report:

Pesticide Residue	Sample PPC	AYUSH Limit (mg/kg)
I.Organo Chlorine Pesticides:		
Alpha BHC	BQL	0.1
Beta BHC	BQL	0.1
Gamma BHC	BQL	0.1
Delta BHC	BQL	0.1
DDT	BQL	1
Endosulphan	BQL	3
II.Organo Phosphorus Pesticides:		
Malathion	BQL	1
Chlorpyriphos	BQL	0.2
Dichlorovos	BQL	1
III.Pyrethroid:		
Cypermethrin	0.1 mg/kg	1

BQL – Below quantification limit

3.10. Aflotoxin Assay By TLC (B1,B2,G1,G2):

The results shown that there was a presence of aflatoxins B1 in the sample PPC under AYUSH specification limit and further there is no detection of other aflatoxins like aflatoxins B2, G1 and G2 when compare to that of the respective standards.(Table 10).

Table 10: Aflatoxin report:

Aflatoxin	Sample PPC	AYUSH Specification limit
B1	Present – 0.01 ppm level	0.5 ppm
B2	Not Detected – Absent	0.1 ppm
G1	Not Detected – Absent	0.5 ppm
G2	Not Detected – Absent	0.1 ppm

4. Conclusion

From this current study of preclinical standardization of Parangipattai chooranam which is mentioned in Siddha texts shows that the drug Parangipattai choornam was a fine powder pale milky white in colour with mild odour, bitter and slightly sweet in taste. The drug size has a particle size with the range of lowest 41.1 µm to highest 211.341.1 µm. The loss on drying indicates the moisture content of the drug was determined as 8.5 ± 2.8%. The total ash was found to be 0.55 ± 0.05% which indicates the inorganic content of the drug. The water soluble ash was calculated as 7.46 ± 0.72% and the value of acid insoluble ash was found to be 0.32 ± 0.15% which indicates that the drug contains negligible amount of siliceous matter. The water soluble extractive value and alcohol soluble extractive value were found to be 25.86 ± 0.81% and 34.44 ± 2.12% which indicates that there is considerable quantity of chemical constituents in the formulation. The pH value is measured as 4.8 which indicate that the drug is slightly acidic. The phytochemical analysis shows the drug has high polar secondary metabolites like flavonoids, steroids, triterpenoids, coumarin, phenol, tannin, saponins and sugar. HPTLC analysis shows that presence of four prominent peaks corresponds to presence of four versatile phytocomponents with in it and their Rf values were calculated. This study also reveals that the chooranam is sterile and free of bacteria, fungi and specific pathogens like *Salmonella, Staphylococcus aureus, E-coli, Pseudomonas aeruginosa* and pesticide residues. In heavy metals analysis mercury was not detected and lead, arsenic, cadmium were present within the permissible limit there by ensures its safe usage. Aflatoxin like B2, G1, G2 were not detected except B1 which was within the permissible limit. As a result, Parangipattai chooranam was proved its safety over the defined standardization method. The results obtained could be utilized as reference for developing standard formulation of great efficacy.

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