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Standardization of polyherbal Siddha formulation *aavarai poovarisi chooranam* – anti-diabetic drug by pharmaceutical analytical techniques

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Abstract

*Aavarai Poovarisi Chooranam*is a Siddha polyherbal formulation that plays an important role in treating diabetes alone or in combination. *Chooranam* is named for a powder made by a single or a blend of herbal ingredients.The drugs required for making *Aavarai Poovarisi Chooranam*were collected and authenticated by experts andprepared as per Siddha literature. It was then subjected to preliminary phytochemical analysis, Physico-chemical, biochemical analysis, heavy metal analysis, pesticide residue, aflatoxin and microbial load analysis prior to toxicology and pharmacology testing. The obtained results were within normal limits in this study, which is briefly described in thispaper.

Keywords:*Aavarai Poovarisi Chooranam*, Polyherbal formulation, Phyto-chemical, Physico-chemical

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Introduction

Diabetes Mellitus is described as *Neerizhivu Noi* in the Siddha system.Diabetes is a metabolic disorder characterised by high levels of blood sugar. Approximately 463 million adults (20-79 years) were living with diabetes mellitus; by 2045 this will rise to 700 million [1]. 1 in 6 adults with diabetes in the world comes from India.The prevalence of diabetes in Tamil Nadu was 5.99% as per a survey conducted among 1936 participants [2]. There are many plant derived drugs for treatment of diabetes like *Tinospora cordifolia, Syzygium cumini, Strychnos potatorum etc*. Aavarai Panchangam (whole plant) mentioned in the book Pathartha Guna Chindamani is said to cure diabetes. *Aavarai* (*cassia auriculata*) plays an enormous role in the treatment of diabetes [3]. May however the efficacy of *"AAVARAI POOVARISI CHOORANAM''*isunparalled in view of its pleiotropic effects and multimodality of its action such as Anti-diabetic, Anti-oxidant property (reduce free radical induced damage to endothelium), Anti-dyslipidemic property. It may act as a safer drug in adjunct to allopathic preparations. It is advantageous that it mitigates the side effects of allopathic preparations like metformin such as hypoglycemia, dyspepsia and kidney damage.Standardization is an important factor for herbal preparation in order to estimate the quality of the drugs based on the concentration of their active

principle. Thus the present study deals with the standardization of Siddha poly-herbal preparation *Aavarai Poovarisi Chooranam*. *Aavarai Poovarisi Chooranam* is mentioned in the *Mega Nivarana Bodini Ennum Neerizhivu Maruthuvam* (Pg.no:164-165) by Hakkim P.M Abdulla Sayabu for the treatment of *Neerizhivu*(Diabetes mellitus). *Aavarai Poovarisi Chooranam* is a compound preparation of four ingredients used for diabetes mellitus. It consists of *Aavarai poovarisi* (cassia auricluata's flower buds), *Nellivatral (Phyllanthus emblica), Elavangam (Syzygium aromaticum), Jathikai (Myristica fragrans).* The aim of this paper is to validate the standardization of *Aavarai Poovarisi Chooranam* through qualitative analysis according to PLIM guidelines.

Materials and Methods

Drug Selection

Aavarai Poovarisi Chooranam was acquired as a test medicine for its activity against diabetes as described in *Mega Nivarana Bodini Ennum Neerizhivu Maruthuvam*(Pg.no:164-165)

Ingredients of *"Aavarai Poovarisi Chooranam"* [4]

Figure 01. Ingredients of APC

Table no.1. Composition of APC

S.No	Name	Botanical Name	Amount
1.	*Aavaram poovarisi*	*Cassia auriculata*	10 *palam* (350g)
2.	*Nellivatral*	*Phyllanthus emblica*	5 *palam* (175g)
3.	*Elavangam*	*Syzygium aromaticum*	¼ *palam* (8.75g)
4.	*Jathikai*	*Myristica fragrans*	¼ *palam* (8.75g)

Collection of the Drugs

Cassia auriculata's flower budswere freshly collected from Yelagiri hill, Tirupattur district. *Phyllanthus emblica, Syzygium aromaticum, Myristica fragrans* werepurchased from authentic stores.

Recognition and verification of Drugs

The plant and raw ingredients were recognised then verified by a botanist of the department. Samples of each raw material have been labelled as 1007-1010/PGG/321912102/GSMC-CH/2019-2022 respectively.

Purification Process

This was conducted as per *Sarakugalin suthisei Muraigal*. [5] Flower buds of *Cassia auriculata* were cleaned well devoid of impurities and then dried in shadow and powdered. Dried fruit of Indian gooseberry was boiled in milk and the seed was removed and then slightly roasted and powdered. Impurities such as dust were removed from clove, nutmeg and then mild roasted and powdered.

Method of Preparation

The flower buds taken from the middle of *Cassia auriculata* were dried in the shadow. Then dried flower buds were pounded in iron motor and filtered through mesh size 88 as a fine powder. Dried and pure Indian gooseberry powder was prepared. Purified and slightly roasted clove and nutmeg were also separately pounded, completely sieved through mesh size 88 as a fine powder. And then all the above mentioned powders were mixed well together. This mixture of *Aavarai Poovarisi Chooranam* was preserved in a tight container for further testing.

Adjuvant: Buffalo Butter Milk

Dosage: *1\4 palam* (8.75g) & twice a day

Indication: *Neerizhivu* (Diabetes mellitus)

OorganolepticNature

The state, nature, odour, feel, flow property, physical appearance, and taste were noted from the drug.

Qualitative Analysis Investigation

Qualitative analysis of APC was performed as per PLIM Guidelines. Analysis of Physico-chemical, Phyto-chemical, Biochemical and Heavy metals were done. Sterility testing, High-performance thin layer chromatography, Specific pathogen testing, Pesticide residue analysis, Aflatoxins assay were done at, Noble research institute, Perambur, Chennai.

Physicochemical Evaluation: [6-8]

For the various physicochemical evaluation, the following tests were done for calculation of the

percentage loss on drying, total ash, acid insoluble ash, alcohol soluble extractive, and water-soluble extractive. pH was determined. The particle size was determined by microscopic method.

Phytochemical Evaluation [9]

For the various phytochemical evaluation, tests were done to evaluate alkaloids, coumarins, saponins, tannins, glycosides, flavonoids, phenols, steroids, triterpenoids, cyanins and carbohydrates.

Theevaluation of botanical materials and quality control analysis was done through chromatographic analysis **[10,11]**

Biochemical Analysis of Basic and Acidic Radicals [12]

For detection of carbonates, sulphides, phosphates, zinc and magnesium

Heavy Metal Analysis by Atomic Absorption Spectroscopy [AAS] [13]

Cadmium, Lead, Mercury, and Arsenic were tested

Sterility Test [14]

The pour plate technique was the send for identification of the organism. The CFU has counted accordingly.

Individual Pathogen Testing [15]

A specific medium like EMB agar, Deoxycholate agar, Mannitol salt agar, and cetrimide agar was usedfor precise identificationof *E.Coli*, *Salmonella*, *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* respectively.

Finally, Pesticide Residue Analysis[16, 17] and AFLATOXIN ASSAY [18] for done

Results

Organoleptic Character

The drug *Aavarai Poovarisi Chooranam* is a moderately fine powder brownish in colour with a rich aroma and non-free-flowing nature (Figure 2). The results are shown in Table 3.

Figure 2. APC drug – Prepared form

Table no. 2 Organo-leptic Character

S. No	Specifications	Nature
1.	State	Solid
2.	Nature	Fine
3.	Odour	Strongly Aromatic
4.	Tactile nature	Soft
5.	Flow	Non-free flowing
6.	Colour	Brownish
7.	Taste	Astringent

Physicochemical Characteristics

The dryness related loss was determined as0.4667 ± 0.1155. This indicated the moisture content. The overall ash content was found to be 10.1 ± 1.277%. This is the inorganic matter of the drug. The acid-insoluble ash was 0 ± 0%. The extractive (water-soluble and alcohol) values were observed to be 17.43 ± 2.065% and 8.333 ± 0.6429%. The pH value was 6.5 (acidic in nature). The results are shown in table no.4.

Table no.3 Results of Physico-chemical Analysis of APC

No	Parameters	Mean (n=3) SD
1.	Loss at 105 °C (%)	0.4667 ± 0.1155
2.	Ash - Overall (%)	10.1 ± 1.277
3.	Acid insoluble Ash (%)	0 ± 0
4.	Water-soluble Extractive (%)	17.43 ± 2.065
5.	Alcohol Soluble Extractive (%)	8.333 ± 0.6429
6.	pH	6.5
7.	Particle Size	22.71 ± 6.13

Particle Size Determination

The particle (average) size was observed to be 22.71 ± 6.13 µm. The size range was the lowest from 13 µm to the highest at 31 µm.

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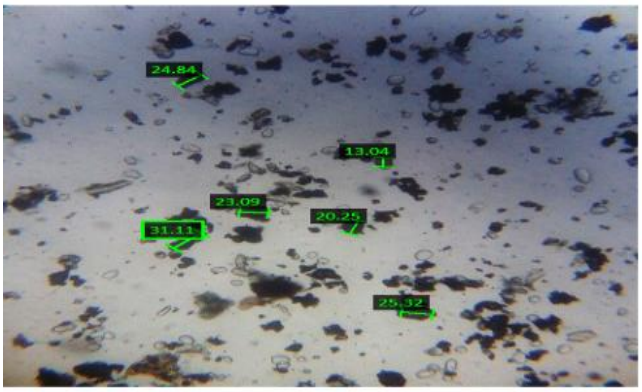


Figure no.3 Particle Size microscopic observation for the sample APC

Table no.4 Results of Solubility Profile of APC

S.No	Solvent	Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

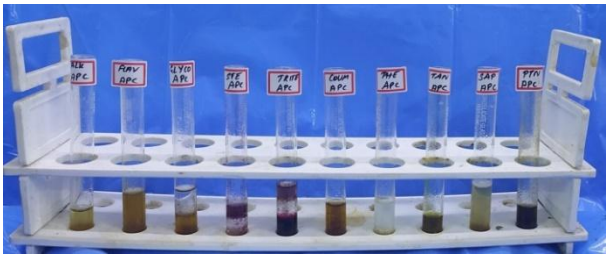
Qualitative Phyto-Chemical Analysis of *APC*

The result of the qualitative phytochemical analysis indicates that the formulation of APC shows the presence of biologically significant phytochemicals. Alkaloids, flavonoids, glycosides, steroids, triterpenoids, coumarin, phenol, tannins, saponins, sugar, anthocyanin and betacyanin. Results are shown in table no.6

Table no. 5 Phytochemical Analysis of APC

S.No	Assessment	Inference
1	Alkaloid	Positive
2	Flavanoid	Positive
3	Glycoside	Positive
4	Steroids	Positive
5	Triterpenoid	Positive
6	Coumarins	Positive
7	Phenols	Positive
8	Tanins	Positive
9	Saponins	Positive
10	Sugar	Positive
11	Anthocyanin	Positive
12	Betacyanin	Positive

Figure 4. Results of phytochemical analysis of APC



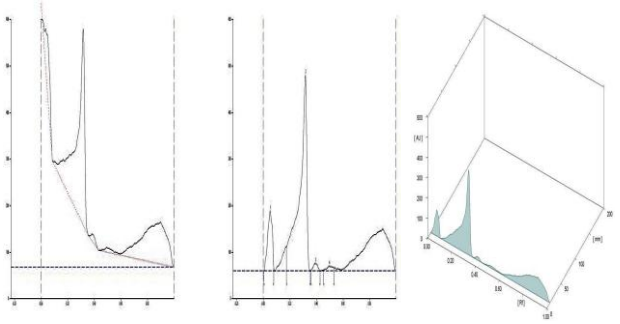
High performance thin layer chromatography analysis reveals four major peaks correlating with four variable phytocomponents present. The Retention frequency value of the peaks were from 0.00 to 0.45.

Figure.5 TLC Chromatogram of *Aavarai poovarisi chooranam*

TLC Visualization of APC at 366 nm

3D – Chromatogram

Figure.6 Fingerprinting of Sample APC-HPTLC



Peak Table no. 6

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	0.1	0.05	129.6	22.44	0.08	7.1	2107.7	15.91
2	0.17	51.9	0.32	420.8	72.87	0.35	0.3	10650.6	80.39

3	0.36	0.5	0.39	16.6	2.87	0.43	0.0	259.8	1.96
4	0.45	2.3	0.50	10.5	1.82	0.53	6.7	231.0	1.74

Biochemical Analysis

The results of the biochemical analysis of the test sample APC show the presence of carbonates, sulphides, phosphates, zinc, and magnesium. Refer to Tables no.8 & 9.

Table no.7 Test for acid radicles

N	Test	Inference	Result
i	Carbonates test	Creation of brisk effervescence	Present
ii	Sulfides test	Creation of colourless gas with the smell of rotten egg	Present
iii	Phosphates test	Creation of yellow precipitate	Present

Table no.8 Test for basic radicles

S.No.	Test	Inference	Result
1.	Zinc Test	Creation of White precipitate	Present
2.	Magnesium Test	Creation of white precipitate	Present

Heavy Metal Analysis by Atomic Absorption Spectroscopy[AAS]

There were nil heavy metal traces of Lead, Arsenic, Mercury and Cadmium

Table no.9 Results of Heavy Metal Analysis of APC

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

BDL – Below detection limit

Pour Plating Method – For Sterility

Figure.7 Sterility Testing - Pour Plate Method

Result

No growth or colonies were noticed on inoculated plates. They were tabulated in table 11.

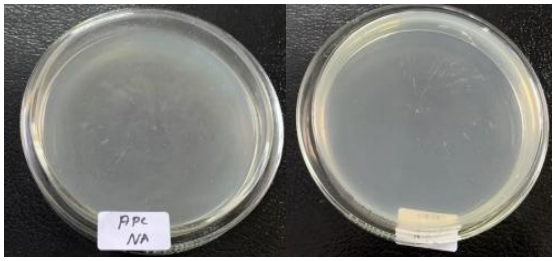


Table no.10 Results of Sterility Testing by Pour Plating

Test	Observation	Specification
Total Count – Bacteria	Negative	NMT 10^5CFU/g
Total Count - Fungus	Negative	NMT 10^3CFU/g

Pathogen (Specific) Testing

Result

The sample did not reveal any growth or colonies in the inoculated plates. Table no.12 & figures 8, 9, 10, 11.

Table no. 11 Results of Pathogen (Specific) of APC

Organism	Result	Method
E-coli	Absent	Specification by AYUSH
Salmonella	Absent	
Staphylococcus Aureus	Absent	
Pseudomonas Aeruginosa	Absent	

Figure.8 -Staphylococcus Aureus (ST) specific medium

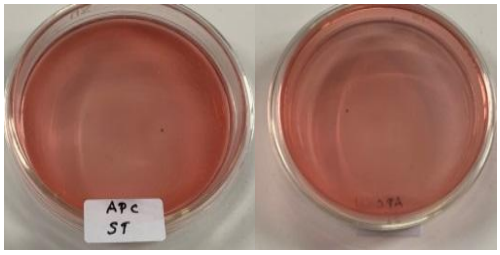


Figure.9 Pseudomonas Aeruginosa (PS) specific medium

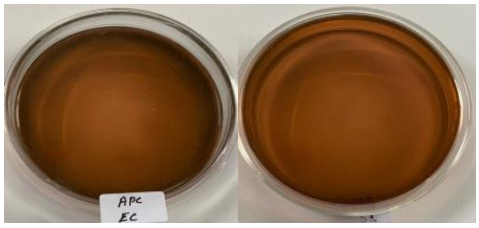


Figure 10. E.coli (EC) specific medium

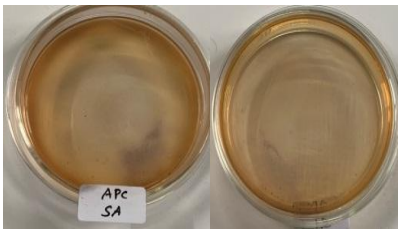


Figure 11. Salmonella (SA) specific medium

Pesticide Residue Analysis - Organochlorine, Organophosphorus, Organo Carbamates, Pyrethroids

Pesticide residues like Organochlorine, Organophosphorus, Organocarbamates and Pyrethroids were below the quantification limit. Results are tabulated in table 13.

Table no.12 Results of Pesticide Residue

Residue of pesticide	Sample	Limit (mg/kg)
I. Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulfan	BQL	3mg/kg
II. Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
IV.Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

AFLATOXINS

The results revealed that there were no spots were identified in the test sampleloaded on TLC plates when compared to the standard which denotes that the sample APC were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. The results were tabulated in table 14.

Table no.13 Results of Aflatoxins

Aflatoxin	Sample APC	Specification Limit - AYUSH
B1	Not Detected	0.5 ppm
B2	Not Detected	0.1 ppm
G1	Not Detected	0.5 ppm
G2	Not Detected	0.1 ppm

Discussion

Aavarai Poovarisi Chooranam is a delicate powder. It is brown in colour with a strong aroma and is non-free-flowing. Moisture content was indirectly denoted by loss on drying was determined as0.4667% suggesting a good shelf life and stability. The total ash value represents the minerals present which is 10.1% in *Aavarai Poovarisi Chooranam* indicating its purity. The amounts of siliceous matter in the drug are indicated by the acid insoluble ash value highlights the siliceous matter which is 0% in this drug indicating good quality. The water-soluble extractive indirectly indicates the diffusion capacity which was determined to be 17.43%. Alcohol soluble extractive is useful for quality as well as purity. Here, the alcohol-soluble extractive value of APC is 8.333%. The result revealed that the drug has good quality and purity and it indicates no adulteration in the raw drug *APC. The* pH of *Aavarai Poovarisi Chooranam*is 6.5 (Acidic). This drug has very good oral bioavailability.

"Solubility is a big challenge for formulation scientists. Any drug to be absorbed must be present in the form of solution at the site of absorption"[19]. The test drug *Aavarai Poovarisi chooranam* was soluble in ethanol, water and DMSO (Dimethyl sulfoxide). The test drug APC was insoluble in chloroform, and ethyl acetate. Soluble in water and ethanol directly related to enhancing the bioavailability of the test drug.

The natural bioactive compound of phytochemicals in plants and fibresacts against conditions like diabetes. The presence of phytochemicals in the drug *Aavarai Poovarisi Chooranam* such as alkaloids, flavonoids, glycosides, steroids, phenol, triterpenoids, coumarin,

tanins, saponins, sugar, anthocyanin and betacyanin will enhance the therapeutic efficacy of the drug. Plants are natural source of antioxidants and effective herbal medicines, in part due to their anti-diabetic compounds, such as alkaloids, flavonoids, tannins, and phenols that enhance the performance of pancreatic tissues by raising the insulin secretion or reducing the intestinal absorption of glucose[20].Gallic Acid(phenolic compound) and its derivates function as potent antioxidants and free radical scavengers, with the potential to modulate inflammation, apoptosis or oxidative stress in various pathophysiological situations. Gallic acid has been revealed to possess antihyperglycemic potential through its antioxidant and anti-inflammatory properties [21]. Gallic Acid from *Emblica Officinalis* fruit juice promoted insulin sensitivity and glucose homeostasis in adipocytes[22].

There were four significant peaks in the HPTLC fingerprint scan in the chloroform extract correlating with four variable photo componentscontained within it. The range of Rf values was from 0.00to0.45.

The acid radicle test reveals the presence of carbonates, sulfides,andphosphates. The action of carbonates on metabolic and inflammatory abnormalities in type 2 diabetes mellitusmay affect the progression of early diabeticCKD. It significantly reduces HBA1c, fibroblast growth factor 23, lipid and markers of inflammation and oxidative stress, and markedly increases antioxidant markers. **[23]** Sulphide plays an enormous role in the pathogenesis of Beta-cell dysfunction that occurs in response to type 1 and types 2diabetes. [24] The lack of intracellular phosphate complementary to the raised intracellular glucose takes place in the insulin-insensitive cells andtissues. The phosphates imbalance may lead to affinity hypoxia & impaired formation of high energyphosphates[25].

Basic radical testing reveals that Zinc,Magnesium is present and other heavy metals were absent. Zinc plays an enormous role in β-cell function, insulin action, glucose homeostasis and the pathogenesis of diabetes mellitus and its complications [26].Magnesium is necessary as a cofactor of various enzymes that play important roles in glucose metabolism involved in multiple levels, such as in insulin secretion, binding and activity [27].

Heavy metals, viz., lead, arsenic, cadmium and mercury were well under the WHO prescribed limits 10, 3, 0.3 and 1 ppm respectively. The drug was free from *Escheria coli, Salmonella, Pseudomonas aeruginosa,Staphylococcus aureus* and the total bacterial count and total fungal count were within the safety limit for internal medicine. The organochloride pesticides, organophosphorus pesticides and organo carbamates and pyrethroid were also found to be below the limit of quantification indicating the collection of wild plants as per good collection practices. Since aflatoxin B1, G1 (less than 0.5 ppm), B2 and G2 (less than 0.1 ppm) were found to be below the limit of the drug could be administered internally.

Conclusion

Theachievedresultsof physicochemical, phytochemical, biochemical Screening, sterility test, and test for specific pathogen, aflatoxin and pesticideresiduewillbe a very usefultoolforstandardizationand qualitycontrolassessmentofthe polyherbal formulation *Aavarai poovarisi chooranam.*The observed pH value was 6.5 indicating the weekly acidic nature of the drug. The phytochemical analysis discovered the increased polar secondary metabolites such as alkaloids, glycosides, flavonoids, steroids, phenol, triterpenoids, coumarin, tannins, sugar, saponins, anthocyanin and betacyanin. This establishes that the above trial drug *Aavarai Poovarisi Chooranam* was safe to use as internal medicine. Furthermore, pharmacological studies accomplish the medicinal value of the drug.

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Author Contribution

All authors contributed equally.

Conflicts of interest

The authors state that there are no conflicts of interest.

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