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Physico-Chemical and Instrumental Standardization of the Siddha Herbal Drug Aavaraivithaadhi Chooranam

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ABSTRACT

Aavaraivithaadhi Chooranam is the classical Siddha medicine used in the treatment of Diabetes mellitus. The aim of the present study was to standardize the Siddha herbal drug formulation Aavaraivithaadhi chooranam by subjecting the drug to various studies like analyzing its organoleptic character, Physicochemical and Instrumental analytical studies like Scanning Electron Microscope (SEM), Energy Dispersing X-Ray Analyser (EDAX) and Fourier Transform Infrared Spectroscopy (FTIR) and the results were noted. The Total Ash value was10.2 %±0.25, Acid Insoluble Ash value was 07.9 %±0.74, Water soluble ash value was 3.4 %±0.08, Alcohol soluble Extractive was 5.78% ±0.39, water soluble ash value was8.18% ±0.58; Loss on drying at 105°c was 0.0566 %±0.007. The SEM analysis shows the presence of micro particles and its morphology and topography is revealed, EDAX results shows the presence of compounds such as Carbon, Oxygen, Zinc, Magnesium, Calcium, Iron, Potassium, Silica which is responsible for its activity. FTIR analysis revealed the functional groups present through the bonds and stretches which may be the reason for its therapeutic effect. Standardization is an important analytical tool for the identification of the drug. This study brings the effective standardization of the Siddha Herbal formulation through the standard modern analytical studies thus bringing the siddha drug to the next level.

Keywords: Standardization, ash values, SEM, EDAX, FTIR spectroscopy

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INTRODUCTION

Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Siddha medicines revitalize and rejuvenate the body. Though Siddha system has its own value it has been latent due to the modern medicine which has its way of immediate healing and treatment. In recent years the Siddha system has its dawn among worldwide for its natural inheritance, holistic approach, healthy lifestyle and preventive treatment. Especially of non-communicable and chronic illness where modern medicine has their own limits. The Siddha medicine requires standardization though it has been practiced for many years. Standardization of the drug derives the efficacy and potency of the drug that can be documented and exposed to the world thus bringing the Siddha medicine to limelight. Standardization of the drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics, phytochemical properties, instrumental analysis etc.,so standardization brings the qualitative analysis and potency of the drug. According to Siddha classical textthe medicines are obtained from herbs, metals, minerals and animals products[1]. Siddha system of medicine has abundant number of formulations for various diseases. The study drug Aavaraivithaadhi Chooranam taken from the Siddha classical literature. Aavarai (*Cassia auriculata)* has been in use for many years in treating diabetes mellitus. Aavarai seed has taken as the key ingredient in this drug. The Siddha herbal formulation Aavaraivithaadhi Chooranam was subjected to various studies for standardization according to guidelines[2]. Standardization is an important analytical tool for the identification of the drug. The drug mainly has a therapeutic effect in treating Diabetes mellitus and also its complications. Thus by standardizing this drug its potency and efficacy is revealed to the world scientifically.

MATERIALS AND METHODS

The herbal preparation Aavaraivithaadhi Chooranamwas selected from the classical Siddha literature[3]. The formulation was listed in the Table 1

Table 1 Herbal formulation Aavaraivithaadhi Chooranam

S.No	INGREDIENTS	
1	Seeds of Aavarai(tanner's cassia)	Seeds of *Cassia auriculata*
2	Juice of *Atthippattai*	Bark juice of *Ficus racemosa*
3	Juice of *Maruthampattai*	Bark juice of *Terminalia arjuna*
4	Juice of *Nellippazham*	Fruit juice of *Phyllanthus emblica*
5	Juice of *Thanneervittankizhangu*	Tuber juice of *Asparagus racemosus*

6	Juice of *Vazhaikkizhangu*	Tuber juice of *Musa paradisiaca*
7	Juice of *Nerunjiver*	Root juice *of Tribulus terestris*
8	Juice of *Seendhirkodi*	Juice of *Tinospora cordifolia*
9	Juice of *Sanbagapoo*	Flower juice of *Michelia champaca*
10	Juice of *Kattrazhai*	Sap juice of *Aloe barbadensis*

Ingredients

Seeds of *Cassia auriculata*, Bark juice of *Ficus racemosa*, Bark juice of *Terminalia arjuna*, Fruit juice of *Phyllanthus emblica*, Tuber juice of *Asparagus racemosu.*, Tuber juice of *Musa paradisiaca*, Root juice *of Tribulus terestris*, Juice of *Tinospora cordifolia*, Flower juice of *Michelia champaca* and Sap juice of *Aloe barbadensis).*

Collection, Identification and Authentication of the drug

All plant materials were freshly collected from in and around Trichy, Tamilnadu. All the plant materials were identified and authenticated by the Botanist and *Gunapadam* experts in Government Siddha Medical College, Arumbakkam, and Chennai – 106. The specimen sample of all the herbs have been preserved in PG *Gunapadam* department individually for future reference.

Purification of the drugs

All the drugs mentioned here were purified as per the Siddha literature. Seeds of *Cassia auriculata* were cleaned well without any dust and impurities, Bark of *Ficus racemosa* and *Terminalia arjuna* were first dusted with a clean cloth and then purified by gently removing the outer skin with a knife[4]. Fruits of *Phyllanthus emblica* were purified by removing the seed and the flesh is obtained, Roots of *Tribulus terrestris* were washed in the running tap water to remove the soil and impurities, Tuber of *Asparagus racemosus* was washed thoroughly and the outer skin is peeled off and also the center part is removed, Flowers of *Michelia champaca* were cleaned by removing its stalk, sepals and stamens. Only the flowers were used. The sap of *Aloe vera* was purified by cleaning the sap in running water for seven times. Then the sap is mixed with an astringent (powder of *Terminalia chebula*) to extract the juice[5]

Preparation of the Drug

Procedure

The seeds of *Cassia auriculata* were soaked and dried each day in the above mentioned juices respectively. Then the seeds were dried in the shade until complete evaporation of the moisture content. It was finely powdered and kept in an air tight container. It was labeled as Aavaraivithaadhi Chooranam (AVC). Then the Chooranam was purified by steam boiling process according to the Siddha classical text [6].

Standardization of the drug

Organoleptic character

The organoleptic characters of the sample drug were evaluated as per the standard procedure[7].1gm of the test drug was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted.

Physicochemical analysis

Physicochemical studies of the trial drug had been done according to the text 8. Determination of Ash values –Total ash, Water insoluble ash, Acid soluble ash. Determination of Extractive Value - Alcohol soluble extractive, Water soluble extractive. Loss on drying were studied by the standard methods and recorded.

Instrumental analysis

Scanning Electron Microscope Analysis (SEM)

To evaluate the size of the particle in the test drug, surface topography SEM analysis was carried out using S-3400n SEM-Hitachi at a magnification range of 12 X to 1,00,000X at Anna University, Chennai[9].The sample was placed over the specimen stub, it was then placed inside the microscope's vacuum column evaporator. High-energy electron beam was focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This result in the emission of electrons or photons was collected by an appropriate detector. The electrons were counted by the detector and the signals were sent to the amplifier. The resultant image was the number of electrons dispersed from each spot of the sample. The micrographs obtained from this analysis gave enough data about the topography of the sample.

Energy Dispersive X-ray Analysis (EDAX)

The SEM instrument equipped with EDAX enabled the instrument to perform compositional analysis of the sample Aavaraivithaadhi Chooranam. The data produced by the EDX analysis consists of the spectra containing the elements present in the given sample which was being analyzed.

Fourier Transform – Infra Red Spectroscopy Study (FTIR)

IR data acquired with Spectrum one FT-IR Spectrometer by means of KBr Pellet was carried out at Anna University**,** Chennai[10].In FT-IR the sample was made into pellet by KBr pellet method. Infrared was passed from a source through a sample. This infrared was absorbed by the sample according to the chemical properties and some were transmitted. The computer display showed spectrum of graphs with peaks and the results were printed. The spectrum that appears denotes the molecular absorption and transmission.

RESULTS AND DISCUSSION

The AVC was taken from a classical Siddha literature and was prepared as per the procedure. The drug had a good therapeutic effect on Diabetes mellitus and also its complications. Though there are modern medicines available they have their own limits in curing chronic diseases. So this drug AVC is potent and standardization is very essential as scientific approach is highly required in exploring the drugs. Thus this drug AVC is subjected to various analysis in order to standardize and make it useful to mankind.

Organoleptic characters

The Aavaraivithaadhi Chooranam appeared to be brown in colour with characteristic taste (slightly bitter with astringent properties) and had a pleasant odour. The Chooranam was a fine powder and completely passed through sieve no. 88. The results were tabulated in the Table 2

Table 2 Organoleptic Characters

S.No	Parameter	Results
1	Colour	Brown
2	Odour	Pleasant
3	Taste	Characteristic taste
4	Texture	Fine powder
5	Particle size	Completely pass through sieve no 88

Physicochemical analysis

Physicochemical analysis of Aavaraivithaadhi Chooranam was done and the results were presented in Table 3. The Total ash value was(10.2 %±0.25), Acid insoluble ash value (7.9 %±0.74),Water soluble ash value (3.4 %±0.08)Alcohol soluble Extractive (5.78% ±0.39), Water soluble ash value (8.18%±0.58), Loss of drying at 105°c (0.0566 %±0.007)

Table 3Physico-chemical analysis

S.No	Parameters	Value %w/w
1.	Total Ash	10.2
2.	Acid insoluble ash	7.9
3.	Water Soluble Ash	3.4
4.	Water Soluble Extractive	8.18
5.	Alcohol soluble Extractive	5.78
6.	Loss on drying at 105^0 c	0.0566

Ash values

Herbal drug on complete combustion gives out the inorganic residues which forms the ash value. Thus Ash value may be a effective parameter to assess the degree of purity of a given drug. Total ash value of plant material denotes the amount of minerals and other earthy materials present. The total inorganic content (Ammonium, Potassium, Calcium, Chloride, Iron, etc.,) present in the drug

is measured through the Total ash value and it is of 10.2 % for AVC. The Acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. It is 7.9 for AVC. Water-soluble ash is the part of the total ash content, which is soluble in water. It is 3.4 for AVC

Extractive Values

They are the approximate amount of the chemical constituents present in the raw drug. The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive. Based on the extractive value suitable solvent can be selected. It also gives the percentage of drug which will correlate with the metabolism reactions. Water-soluble extractive value plays a major role in evaluation of crude drugs**.** The alcohol-soluble extractive value was also the same use as the water-soluble extractive value.

Loss on Drying

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. It may bring the early contamination of the drug. Thus low moisture content could get maximum stability and better shelf life.

Instrumental Analysis

SEM: (Scanning Electron Microscope)

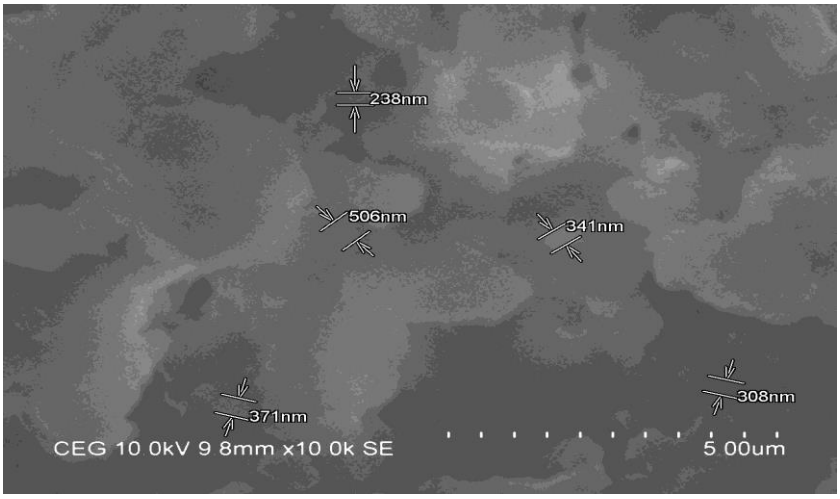

Figure 1: SEM picture showing micro particles

The SEM picture (Figure 1) of microscopic resolution of 1.00kx and examining surface area of 800x800µm^2, showed objects of sizes ranging from 238nm to 506nm.The surface of the sample grains were arranged uniformly in agglomerates. They are micro particles ranging from 238nm, 506nm, 308nm, 341nm, 371nm. The SEM analysis of the drug showed the presence of micro

particles which are solid particles with a size in the range of 100-1000nm in diameter. Size and surface of micro particles can be easily manipulated. It was mainly to achieve both passive and active drug targeting. They control and sustain the release of drug during the transportation and alter drug distribution in the body. It was also essential for the subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby increasing the bio-availability and reduced side effects.

Energy Dispersive X-ray Analysis EDAX:

The EDAX results were summarized in the Table 4 and Figure 2 showed the amount of Carbon which constitutes the organic part (herbal) of the Chooranam. Some of the other elements present were Magnesium, Aluminum, Silica, Potassium, Calcium, Iron and Zinc. The EDAX analysis showed the elements present in the drug and they may be responsible for the therapeutic effect of the drug. Zinc is concentrated in the islet cells of the pancreas and is interconnected to the synthesis, secretion and storage of insulin[11].It is essential in insulin action and carbohydrate metabolism. Zinc is the structural part of the vital Anti-Oxidant enzymes such as superoxide dismutase. Therefore deficiency of zinc impairs their synthesis; leading to increased oxidative stress[12].It improves the glycemic control and lipid parameter with probable movement in Anti-Oxidant status. It has a beneficial effect on diabetic neuropathy and nephropathy. Magnesium is the necessary cofactor for many enzymes that play a vital role in glucose metabolism. Magnesium has a beneficial effect on insulin action and glucose metabolism. Mg repletion may play a role in delaying the onset of Diabetes and potentially warding off its complications [13]. Calcium is necessary in normalizing the glucose tolerance[14].

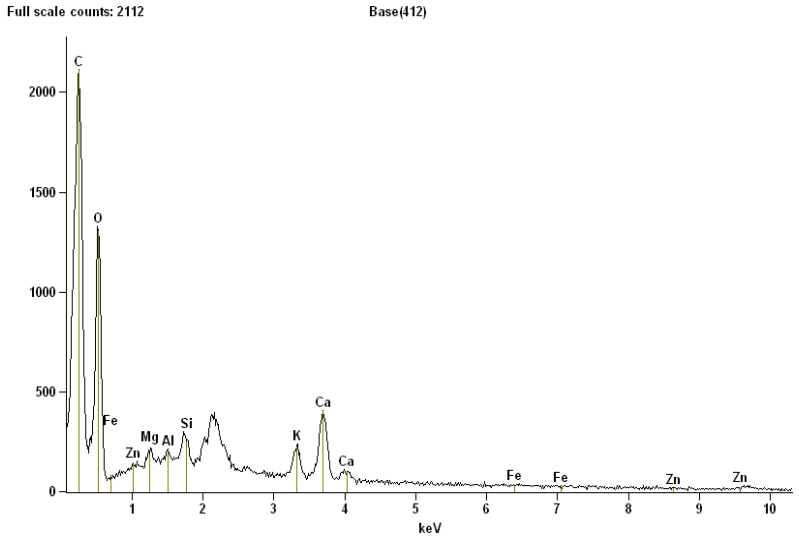

Figure 2: Showing the graph of Edax

Table 4 Showing the Quantitative results of AVC by EDAX analysis

Element	Net Counts	Weight %	Atom %
C	16744	59.03	68.93
O	10247	31.46	27.58
Mg	693	0.37	0.21
Al	318	0.18	0.09
Si	1706	0.93	0.47
K	1877	1.82	0.65
K	0		
Ca	4679	5.38	1.88
Ca	0		
Fe	59	0.19	0.05
Fe	0		
Zn	65	0.64	0.14
Zn	210		
Total		100.00	100.00

FT-IR (Fourier Transform Infra Red spectroscopy)

FTIR instrumental analysis was done. The test drug was identified to have 15 peaks. They were the functional groups present in the trial drug Aavaraivithaadhi Chooranam. The Table 5 and Figure 3 shows the presence of Amide, Phenols, Alkanes, Alkynes, Alkenes, Ester, Ether and Alcohol groups which represents the peak value. The FTIR analysis of AVC shows the spectrum that appears which denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. It is the functional group and determines the amount of compounds present in the sample. These functional groups may be responsible for the therapeutic effect of the drug.

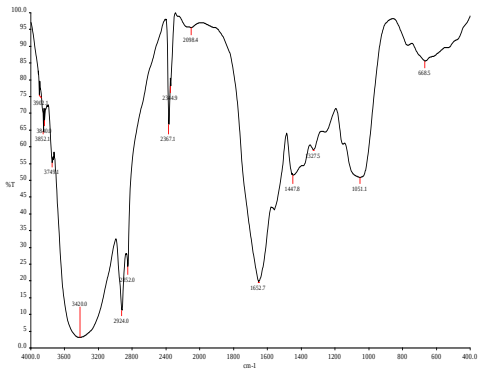

Figure 3. Showing the graph of FTIR spectrum

The name of each spectra was mentioned in the Table 5.

Table 5. Results of FTIR analysis of AVC

Absorption peak cm^{-1}	Stretch	Functional group
3852.1	N-H stretch	Amide
3420.0	O-H stretch	Phenols and alcohols

2924.0	H-C-H stretch	Alkanes
2852.0	C=O	Aldehyde
2367.1	N-H	Amine
2344.9	N-H	Amine
1652.7	C-C=C symmetric stretch	Alkenes
1447.8	H-C-H bend	Alkanes
1327.5	C-O stretch	Ester , ether
1051.1	C-O stretch	Ester, ether
668.5	C-H bend, C-C stretch	Alkyne

CONCLUSION

The above study includes organoleptic character and physicochemical analysis such as total ash value (10.2 %±0.25), acid insoluble ash value (7.9 %±0.74),Water soluble ash value (3.4 %±0.08)Alcohol soluble Extractive (5.78% ±0.39), water soluble ash value (8.18%±0.58), loss of drying at 105°c (0.0566 %±0.007), the pH value (7.4). The sophisticated instrumental analysis like FTIR shows the presence of functional groups through their stretch and bends which responsible for its functional activity. SEM analysis reveals the morphology and partial size of the drug which is essential for its bio-availability. The EDAX results gives the active elements present in the drug like Carbon, Oxygen, Zinc, Magnesium, Calcium, Potassium, Iron which is necessary for its therapeutic effect against the diseases. On scrutinizing all the above studies it is concluded that the Siddha herbal formulation Aavaraivithaadhi Chooranam was subjected to many studies to validate its efficacy and safety through proper standardization procedure and the results revealed its potency and efficacy. Thus this drug can be taken to the next level of preclinical and clinical studies to validate the pharmacological activities and therapeutic effect.

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