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Standardization of Siddha Polyherbal Formulation Vaeppampoovathy Mathirai

D.Sandhiya[*1], M. Pitchiah Kumar[1], V.Velpandian[1], P.Thenmozhi[1], V.Banumathi[1]
1. Post graduate departmentof Gunapadam (Pharmacology), Government Siddha Medical College, Chennai, Tamilnadu. Pin: 600 106.

ABSTRACT

Standardization and quality control are essential analytical tools to guarantee the correct identifications of drugs. Adulteration and misidentification of herbal drug can cause dreadful health problems to the public and legal headaches for the pharmaceutical industry. The VPM was prepared according to the Siddha classical literature to treat the anti-dyslipidemic activity. The present study was carried out with specific aim to establish standards for Vaeppampoovathy Mathirai based on organoleptic characters, physico-chemical properties (The total -ash value 9.5%, Acid insoluble ash 1.7%, Water soluble ash 2.8% and the loss on drying at 105^0 c was found to be 0.043%), phytochemical analysis showed the presence of rich source of alkaloids, carbohydrates, saponins, tannins, phenols, phytosterol, diterpenes, flavanoids, proteins& aminoacids. SEM analysis showed that VPM was arranged in agglomerates of size of micro particles. EDAX analysis showed the presence of minerals like carbon, oxygen, magnesium, aluminum, silica, sulphur, chloride, potassium, calcium. FTIR showed the peak values which are the fuctional groups present. The sophisticated analysis of instruments were also an essential criteria in studying the surface morphology, topography and the chemical bonds present in them which indirectly correlates with the activity and also an important factor in the standardization of the drugs.

Keywords: Standardization, SEM, EDAX, FTIR, physico-chemical, phytochemical

*Corresponding Author Email dhiya_shalu@yahoo.com
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INTRODUCTION

Herbs are considered to be of great importance in curing the disease of mankind. The herbs are used as a whole or processed in traditional medicines or isolated active principles are used inmodern medicine. Herbal drug technology includes all the important steps for the transformation of botanical materials into medicines. Standardization and quality control are essential analytical tools to guarantee the correct identifications of drugs. Adulteration and misidentification of herbal drug can cause dreadful health problems to the public and legal headaches for the pharmaceutical industry. The past decade has witnessed the introduction of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished product of botanical origin [1]. Poly herbal formulation standardization is a system that ensures a predefined amount of quantity, quality & therapeutic effect of ingredients in each dose[2]. Central Council for Research in Ayurveda and Siddha (CCRAS) has given preliminary guidelines for standardization of herbs. Standardization of the drugs means confirmation of its identity and determination of its quality and purity. Standardization is necessary to make sure of availability of a uniform product in all parts of the world [3]. Throughout the world dyslipidemia is a grave health problem due to irrational food habits. Most of the hypolipidemic drugs used in modern medicine are reported to have side effects on prolonged use. Hence a necessity for an effective and safe drug arises. One of the potent polyherbal formulations Vaeppampoovathy Mathirai mentioned in Siddha classical text for its anti-dyslipidemic potential. Based on the above rationale, the present study was carried out with specific aim to establish standards for Vaeppampoovathy Mathirai based on organoleptic characters, physico-chemical properties, phytochemicals and instrumental analysis. Phytochemical profile is of distinct significance since it has a direct bearing on the activity of herbal drugs. Microscopic examinations are highly important to ensure quality and purity of herbal medicines in order to maximize the efficacy and minimize adverse side effects. The sophisticated analysis of instruments is also an essential criteria in studying the surface morphology, topography and the chemical bonds present in them which indirectly correlates with the activity and also an important factor in the standardization of the drugs.

MATERIALS AND METHOD

The polyherbal preparation Vaeppampoovathy Mathirai was selected from the siddha literature [4].

Ingredients

Neem flowers (*Azadirachta indica*), dried Ginger (*Zingiber officinalis*), Pepper (*Piper longum*),

long pepper (*Piper longum*), Chebulic myrobalan (*Terminalia chebula*), Indian gooseberry (*Embelica officinalis*), Bellaric myrobalan (*Terminalia bellaric*), Cloves (*Syzigium aromaticum*), Cinnamomum (*Cinnamomumverum*), Cardamom (*Elaterria cardamom*), cuscus grass (*Vetiveria zizanoides*), climbing brinjal (*Solanum trilobatum*), Indian Phyllanthus (*Phyllanthus niruri*), Wedeliachinensis (*Chinensis wedelia*) and lemon (*Citrus limon*)

Collection of Plant & raw material

Drugs were procured from Nagercoil, they were identified and authenticated by botanist of Central Research Institute Chennai and faculties of the department of Gunapadam, Govt Siddha Medical College Chennai, Tamilnadu. Specimens of each sample have been kept in the Post graduate department of Gunapadam for future reference.

Preparation of polyherbal formulation

All the ingredients were cleaned well and dried in sun shade. After complete drying each ingredient was taken separately and purified as per Siddha classical literature [5]. After purification the ingredients were grounded separately to powder. The powder was sieved through a white cloth and then 64gms of Neem flower powder, 32gms of powder of Indian Phyllanthus, climbing brinjal, chinens is wedelia and 8gms powder of remaining ingredients were mixed thoroughly in stone mortar and rubbed with sufficient quantity of lemon juice for 3 days and rolled into size of 130 mg. The pills were stored in airtight container and labeled as VPM.

Standardization parameters

Study of organoleptic characters

The organoleptic characters of VPM such as colour, odour, taste was carried out based on the standard methods described in the texts[6].

Physico-chemical analysis

VPM was evaluated for total ash value, acid insoluble ash, water soluble ash, moisture content, alcohol and water soluble extractives, solubility test, specific gravity, pHand disintegration time according to the methods described in Ayurvedic pharmacopoeia of India.

Phytochemical Screening

Phytochemical screening of the extract gives the nature of chemical constituents present in the crude drug. The phytochemical analysis was adopted as per the methods and procedure [7].

Fourier Transform Infrared Spectroscopy (FTIR)

KBr pellet method

This method changes alkali halides to plastic when applied to pressure and transparent sheet formed in IR region. Commonest alkali halides used in pellets is kBr.0.1-1% of sample mixed

into 250 mg KBr to prepare 13 mm diameter pellets. Under a vacuum of 20 mm Hg, a force of 8 tons is applied to form transparent pellets. The KBr powder was pulverized to 200 mesh max and allowed to dry at 110 ^{0} c for 2 to 3 hours before forming into pellets. The above pellet was inserted into the spectrometer to obtain a spectrum[8 9].

Scanning electron microscope (SEM)

The study was conducted in a very fine powder of a sample and the sample was quick frozen in liquid nitrogen .The sample was mounted firmly on a specimen holder called specimen stub. The mounted sample was placed inside the microscope's vacuum column evaporator through an air tight door. On expelling air from the air pump, a beam of electrons passed from an electron gun. This beam travelled through a series of magnetic lenses designed to focus the electrons. The focused beam moved back across the mounted sample row by row by a set of scanning coils. As the electron beam hit each spot on the sample, secondary electrons are backscattered from its surface. A detector counts these electrons and sends the signals to an amplifier. The final image was built up from the no of electrons emitted from each spot on the sample. The micrographs obtained give sufficient data about the topography of the subjected sample [10].

EDAX (Energy Dispersive X-Ray Analysis)

The sample was kept under vacuum and excited to a higher energy state with an electron beam. Each element while falling down to its original energy state X-rays are emitted at different wavelengths for each element. Results are plotted with x-ray wavelength on the x-axis and y-axis intensity with each peak marked with its corresponding element.

RESULTS AND DISCUSSION

Standardization of poly herbal preparation (VPM)

The VPM was prepared according to the Siddha classical literature to treat the anti-dyslipidemic activity. Standardization of herbal formulation requires implementation of Good Manufacturing Practices (GMP) -WHO guideline. Thus this drug VPM was subjected to various standardization techniques and the results and discussion were established below.

Organoleptic characters

Organoleptic characters revealed that VPM was black in colour, characteristic lemon like odour, acrid and slightly bitter in taste, solid and hard in nature. Bitter taste might be due to presence of alkaloids, glycosides and flavonoids. Hardness was due to the trituration with binding agent and pressure applied during compression. The result of organoleptic characters like colour, odour, taste, state of matter and consistency were summarized in Table 1

Table: 1 organoleptic characters of VPM

S.NO	Parameter	Results
1	Colour	Black
2	Odour	Characteristic lemon like Odour
3	Taste	Acrid and Slightly bitter
4	State of matter	Solid
5	Consistency	Hard

Physico- chemical characters

The drug was soluble in distilled water, methanol, petroleum, benzene, toluene, chloroform, and xylene and sparingly soluble in Propylene glycol, Ethyl alcohol, Carbon tetra chloride. So the bio availability was enhanced and action of the drug was increased. The total -ash value 9.5% represents the presence of inorganic part of the plant. It is the amount and composition of ash remaining after combustion of plant material to identify the purity of crude drug. Acid insoluble ash 1.7% indicates the presence of siliceous present in drug. Water soluble ash 2.8% indicates the presence of sugar, acid and inorganic compounds.

Table: 2 Physico - chemical characterization of VPM

1	**Solubility** • Distilled water • Ethanol • Petroleum ether • Propylene glycol • Benzene • Toluene • Chloroform • Ethyl alcohol • Xylene • Carbon tetrachloride	**Soluble in water** • Soluble • Soluble • soluble • Sparingly Soluble • Soluble • Soluble • Soluble • Sparingly Soluble • soluble • Sparingly soluble
2	Ph	7.4
3	Specific gravity	0.925
4	Loss on drying at 105^0 c	0.043%w/w
5	Total Ash	9.5%w/w
6	Acid insoluble ash	1.7%w/w
7	Water Soluble Ash	2.8%w/w
8	Water Soluble Extractive	7.83%w/w
9	Alcohol soluble Extractive	5.24%w/w
10	Particle Size	Completely passes through sieve no:88
11	Disintegration Time	35 min

The loss on drying at 105^0 c in formulation was found to be 0.043%.The loss on drying value obtained is an indicative of amount of moisture content present in the drug to prevent degradation so low or high moisture content compromises the quality of drug and it affects its

efficacy. The less value of moisture content could prevent bacterial, fungal or yeast growth. The drug passed through the sieve no.88 indicating the consistency of fine powder. It indicates easy absorption and increased bioavailability which are shown below in the Table 2.

Phytochemical screening test

The phytochemical screening tests of polyherbal medicine VPM showed the presence of rich source of alkaloids, carbohydrates, saponins, tannins, phenols, phytosterol, diterpenes, flavanoids, proteins& aminoacids which are shown below in the Table.3. The presence of phytochemicals has biological significance.

Table: 3. Phytochemicals screening test -VPM

Phytochemicals	Test	Result
1. Alkaloids	a. Mayer's test	+
	b. Dragendroff's test	+
	c. Hager's test	+++
2. Carbohydrates	a. Molisch's test	++
3. Saponins	a. Froth test	+
4. Tannins	a. Gelatin test	++
5. Phenols	a. Alcoholic Ferric chloride test	+++
6. Phytosterols	a. Ferric chloride acetic acid test	+
7. Diterpenes	a. Copper acetate test	++
8. Flavanoids	a. Alkaline reagent test	+
	b. Lead acetate test	+++
	c. Ferric chloride test	++
9. Proteins and amino acids	a. Xanthoproteic test	++
	b. Biuret's test	++

Instrumental analysis

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis of VPM showed the presence of characteristic functional groups like amide, phenols, carboxylic acids, ketones, aldehydes, tertiary alcohol, aliphatic fluoro compounds, aliphatic chloro compounds, alkyne and aliphatic bromo compounds as functional groups which are represented in the Table.4 and Figure 1

Table: 4 FTIR results of VPM

Absorption peak cm^{-1}	Type of vibration	Functional group
3852.6	**N**-H Stretch	Amide
3446.5	Hydrogen- bonded 0-H Stretch	Phenols
2925.0	Hydrogen- bonded 0-H Stretch	Carboxylic acids
1718.4	**C=O** stretch	Ketones
1653.0	C=O stretch	Aldehydes
1386.9	OH bend	Tertiary alcohol
1037.7	C-F stretch	Aliphatic fluoro compounds

776.0	C-Cl stretch	Aliphatic chloro compounds
668.6	C-H stretch	Alkyne
602.6	C-Br stretch	Aliphatic bromo compounds

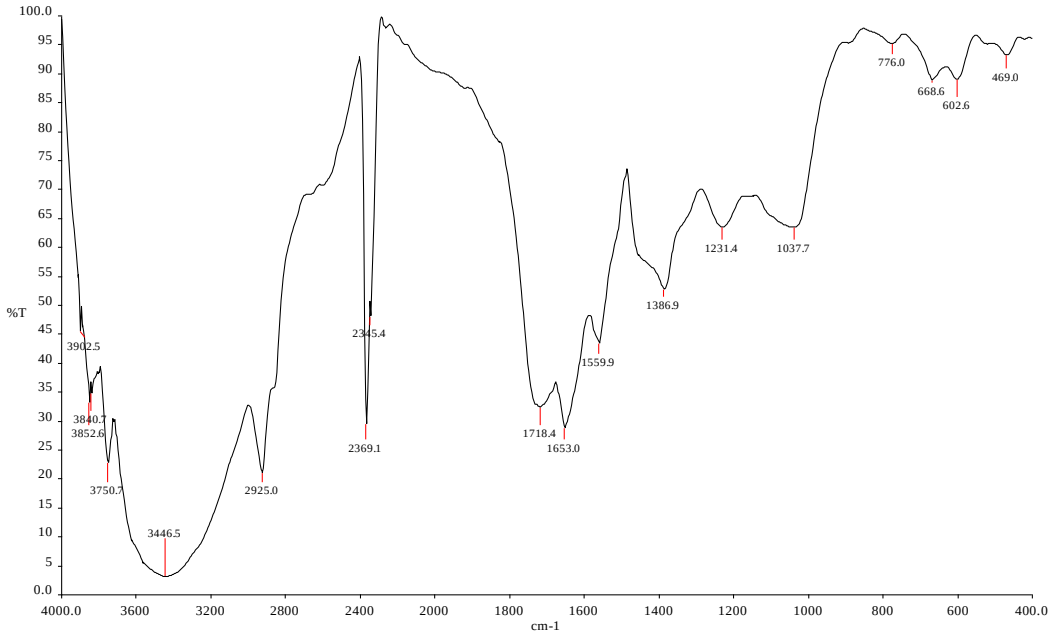

Figure.1. Graph of VPM results on FTIR analysis

Scanning Electron Microscope (SEM)

The SEM studies of microscopic resolution of 1.00kx and examining surface area of 800x800µm^2, showed objects of sizes ranging from 179nm to 304nm. The surface of the sample grains was uniformly arranged in agglomerates. These are micro particles presenting as 179nm, 215nm, 231nm, 298nm and 304nm. The difference in morphology as evident from the micrograph is due to presence of various chemicals in the samples.

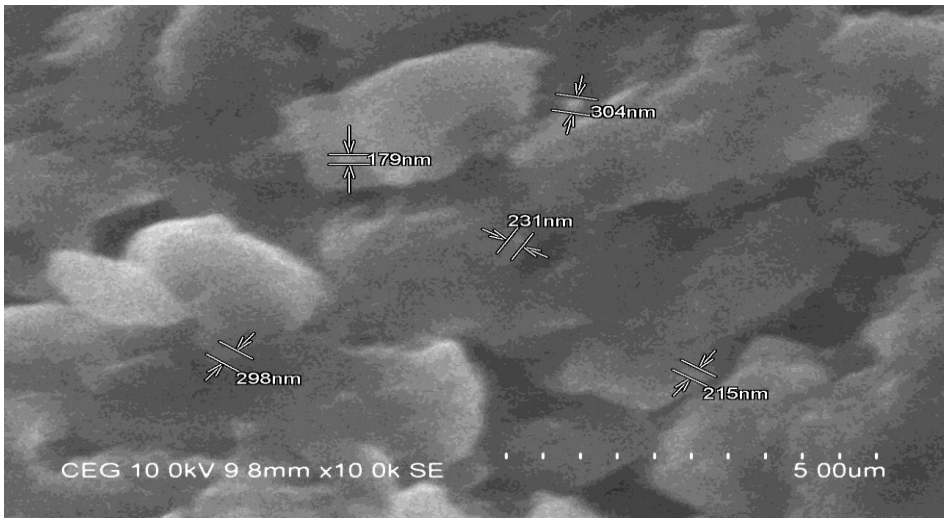

Figure.2. SEM images showing microparticles of VPM

Energy Dispersive X-Ray Analysis (EDAX)

EDAX graph detected the elements present in VPM was carbon, oxygen, silica, potassium, calcium, chlorine, sodium, aluminium, magnesium, iron and manganese. Minerals play and important role in the functioning of various enzymes in biological system and have immune-modulatory functions and thus influence the susceptibility to the variety of lifestyle disorders including obesity [11]. Magnesium regulates HMG-CoA reductase enzyme so as to maintain only a proper amount of cholesterol in the body [12].

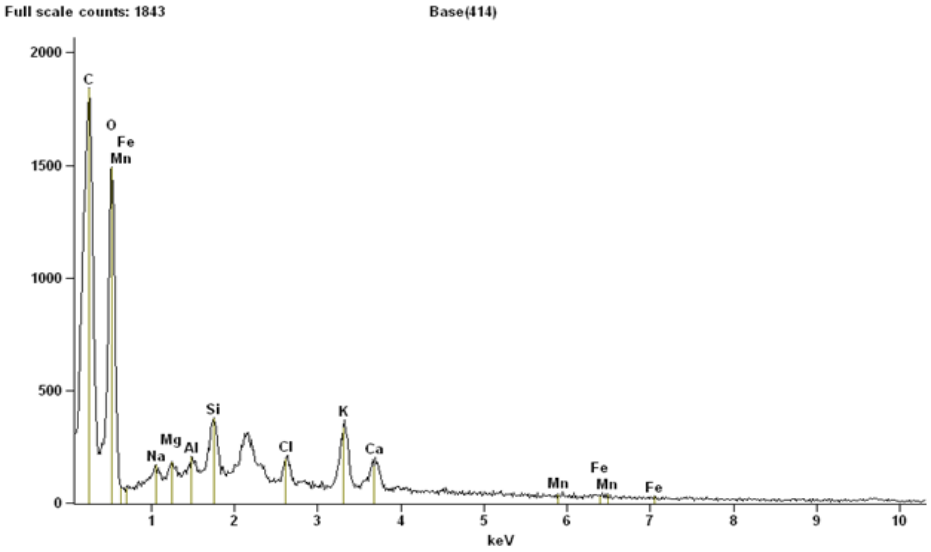

Figure.3. Edax image of VPM

Table 5 :EDAX results of VPM

Element	Net Counts	Weight %	Atom %
C	15786	57.06	66.63
O	12839	33.91	29.73
Na	509	0.43	0.26
Mg	514	0.25	0.14
Al	628	0.32	0.16
Si	3155	1.57	0.78
Cl	1477	1.12	0.44
Cl	5515	---	---
K	3577	3.22	1.15
K	0	---	---
Ca	1540	1.62	0.57
Ca	0	---	---
Mn	63	0.16	0.04
Mn	109	---	---
Fe	113	0.34	0.09
Fe	0	---	---
Total		100.00	100.00

CONCLUSION

Standardization of VPM concluded that the physico-chemical parameters such as the total ash value (9.5%w/w), acid insoluble ash value (1.7%w/w), water soluble ash value (2.8%w/w), water soluble extractive (7.83%w/w), alcohol soluble extractive (5.24%w/w), loss of drying at 105°c (0.048%w/w), pH value (7.4) and specific gravity (0.925). The phytochemicals results showed the presence of alkaloids, flavonoids, carbohydrates, phenols, saponins, phytosterols, diterpenes, tannins, proteins & aminoacids. SEM analysis showed that VPM was arranged in agglomerates of size of micro particles. EDAX analysis showed the presence of minerals like carbon, oxygen, magnesium, aluminium, silica, sulphur, chloride, potassium, calcium which may be responsible for the anti-dyslipidemic activity. FTIR spectroscopy was used to analyse the presence of organic substances in the VPM which are primarily responsible for the anti-dyslipidemic activity. Thus from all these standardization results it would be concluded that the VPM has better quality assessment and its safety and efficacy was also revealed. So it can be taken to next level for its preclinical and clinical studies.

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