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RESEARCH ARTICLE

Validation of Quality Parameters of Siddha Formulation "*Vatharatchasan Mathirai*"

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ABSTRACT

Vatha Ratchasan Mathirai (VRM) Vatha Ratchasan Mathirai is a commonly prescribed herbo-metallic compound drug mainly composed of mercurial compounds ground with herbal juices. The qualities of stability over an extended period, decreased dosage requirements, ease of storage, and prolonged availability are the benefits of herbo-metallic preparation over herbal medications. During the purifying and preparing processes, treating metals and minerals with herbs transforms them into forms that are substantially more bio-compatible, even though they are thought to have less bioavailability. Assessment of quality parameters is necessary to confirm the identity and evaluate the purity of the medicine. We must investigate the medicine quality for human use using data supporting their efficacy and safety. For this reason, quality assessment is crucial to future preclinical and clinical research. So quality of VRM is analyzed. The Study results conclude Vatha Ratchasan Mathirai is solid dark black in colour, uniformity in weight, disintegrate within 20 minutes, free from pestiside residues, aflatoxins, microbial contamination and heavy metals.

Keywords: Osteoarthritis, Physico-chemical analysis, Qualitative assessment, Siddha Medicine, *Vatha ratchasan Mathirai.*

INTRODUCTION

Osteoarthritis (OA) is a chronic joint bone disease characterized by inflammatory destruction and hyperplasia of bone [1]. The prevalence of OA in India according to 2019 is 62.35 million [2]. There is currently no known treatment for Osteoarthritis (OA) because the pathological process behind the onset of the disease and its progression is not fully understood. To create therapeutic targets and develop new drugs, a deeper comprehension of the pathogenic signaling pathways and important molecules implicated in the pathogenesis of OA is essential.[1]The application of herbs, metals, and minerals among the wealth of therapeutic knowledge that the Siddha system has amassed. The practitioners of the Siddha medical system referred to as *Siddhars, are* exceptional entities possessing advanced cultural and intellectual capacities.[3]To change the vitiated humor, *Siddhars* blends formulas based on complementary and antagonistic tastes, due to which they produce higher efficacy with less toxicity. When it comes to treating non-communicable diseases in clinical settings, Siddha formulations are incredibly advantageous.[4] Research on Siddha formulations is often conducted using a reverse pharmacology approach. *Vatha Ratchasan Mathirai*[*5*] is a Herbo-metallic medicine that has been used for millennia to cure a variety of conditions, including polio, chronic arthritis, delirium, and diseases resulting from vitiated humour.[6] Due to a clear grasp of the mechanism through research, rising popularity and long-term stability have raised the current demand for Siddha formulations globally.[7] Recently, there has been interest both domestically and globally in the commercialization of Siddha medicine manufacturing. Siddha formulations now require quality control, safety, and crucial standardization. To support globalization, it is imperative that these ideas be presented, comprehended, and incorporated more precisely into Siddha formulations. As a result, the manufacturing of high-quality, safe, effective, and standard medications must be the top priority for Siddha drug processing units.[8] A quality analysis of *Vatha Ratchasan Mathirai* is necessary to demonstrate the purity and excellence of the drug. Physiochemical, microbiological, pesticide residue, aflatoxin, and heavy metal analysis are the main methods used to accomplish this under the Protocol for Testing of Parameters for Quality Assessment of Ayurvedic and Siddha Medicines.[9]

MATERIALS AND METHODS

The medicine was purchased from IMPCOPS, Chennai. Heavy metals and physiochemical analysis were performed at SCRI, Arumbakkam, Chennai 600 106. On the premises of the Asthagiri Herbal Research Foundation, Perungudi, Chennai 600 096, microbiological contamination was conducted. Furthermore, tests for aflatoxin and pesticide residue were conducted at Noble Research Solutions, an ISO 9001-2015 accredited business located in Chennai.

PHYSICOCHEMICAL ANALYSIS

Determination of Moisture Content (Loss on Drying)

Without prior drying, 10 gm of the medicine was correctly weighed in a tared evaporating dish, dried at 105°C for 5 hours, chilled in desiccators, and weighed. Subsequently, the drying and weighing procedure was repeated every hour until the discrepancy between the initial and two subsequent weightings did not exceed 0.25 percent. The percentage of moisture content was computed using the air-dried drug as a reference once the constant weight was determined.[10]

Uniformity of weight

Calculate the average sample weight by weighing each of the twenty randomly chosen sample units separately.[11]

Disintegration Test

Five tablets (80–100 mm/μg) were placed in the glass tube and the guided disc was used to raise and lower the tube 30 times in a minute. When there are no more particles above the gauze that are difficult to pass through, the tablets have broken down. Then the time for disintegration of the tablet was calculated.[11]

Hardness Test

Two tests were used to perform the hardness test: the point bend test and the compression test. The tablet was squeezed between a stationary and moving jaw during the compression test. The original machines used a spring and screw threads to apply force continuously until the tablet began to shatter. A sliding scale was used to determine the hardness when the tablet broke.[11]

Determination of Total Ash

In a tared platinum or silica dish, 2-3g precisely weighted drug was incinerated at not more than 450ºC till it was carbon-free, then cooled and weighed. Calculate the percentage of ash reference to the air-dried drug.[10]

Determination of Acid Insoluble Ash

The insoluble materials were collected on ash-less filter paper, boil the ash acquired in the total ash technique for five minutes with 25 milliliters of diluted HCL, washed with hot water, and ignited to constant weight. The percentage of acid insoluble ash was calculated.[10]

Determination of Alcohol and soluble extractive

Five grams of drug powder were macerated in 100 milliliters of water for six hours, shaking constantly, and then placed in a conical flask with a glass stopper and allowed to stand for the next eighteen hours. After that, the filtrate was rapidly collected using a dry filter. A flat-bottom Petri plate that had been weighed, tar-coated, and water-bathed was used to hold 25 milliliters of the filtrate. The remaining material was dried for six hours at 105°C and desiccated for thirty minutes before being weighed. The percentage of water-soluble components was calculated in relation to the medicine dosage. As previously noted, alcohol was substituted for water to determine the proportion of alcohol-soluble material.[10]

Determination of pH

Using a standard glass electrode set at 240 degrees Celsius, the pH of various formulations in 1% w/V and 10% w/V of water-soluble fractions was measured in conformity with the recommended standard procedure mentioned in Indian pharmacopeia.[11]

MICROBIAL CONTAMINATION ANALYSIS

Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated/unsterile sample (formulation) when encounter the nutrition-rich medium it promotes the growth of the organism and after a stipulated period of incubation the growth of the organism was identified by a characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).[12]

Methodology

The test sample was admixed with sterile distilled water and the mixture has been used for the sterility evaluation. About 1 ml of the test sample was inoculated in a sterile petri dish to which about 15 ml of molten agar 45ºC was 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 37º C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.[12]

TEST FOR AFLATOXINS

Procedure

Standard Aflatoxin was applied onto the surface to 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.[13]

PESTICIDE RESIDUE

Determination of pesticide residues for analysis reagents without any external components was chosen and the samples were analyzed using Gas chromatographic methods. Later the number of different components such as organophosphorus, organochlorine and pyrethroid contents was recorded.[14]

HEAVY METAL ANALYSIS BY ICP-OES METHOD

Standard

Hg, As, Pb, and Cd

Methodology

100 mg of sample was taken in the Teflon microwave digestion vessel and 1.0 mL of ultrapure nitric acid was added and digested for 45 minutes in a closed vessel microwave digestion unit. Then the sample was made up to 50 ml in a standard measuring flask. The calibration standard was prepared to elucidate the linearity of the analytic ranging from 0.25µg/ml to 10.0 µg/ml. Agilent 5100 VDV ICP-OES instrument was used with the following operation conditions: View: Axial view, RF powder: 1.2 kW, Plasma gas flow rate: 12L min^{-1}, and nebulizer gas flow rate: 0.70 L min^{-1}. The samples are introduced into the plasma using a nebulizer and spray chamber.[15]

RESULTS

Standardization of the drug is essential to derive the efficacy and potency of the drug which was analyzed by various methods. The results of organoleptic characters (Table No: 1), physicochemical analysis (Table No: 2), Microbial contamination (Table No: 3),Aflatoxin (Table No: 4), Pesticide residue(Table No: 5), and Heavy metal analysis (Table No: 6) of VR is tabulated below.

DISCUSSION

The loss on drying at 105ºC was found to be 0.75% which indicated less moisture content in ***VRM*** thus preventing it from early spoilage. Loss on drying assesses both moisture and volatile matter in ***VRM***. Their low moisture and volatile matter levels curb microbial growth, fungal or insect presence, and hydrolysis-related degradation. Thus, the drug has a higher shelf-life. pH is 5.35 determining the nature of the drug to be acidic. The acidic nature of the drug enables its absorption in the stomach. It demonstrates that VRM is appropriate and readily absorbed when taken orally. The higher total ash value compared to the acid insoluble ash values could be attributed to naturally occurring adhered inorganic salts. The total ash (43.52%w/w) shows the total inorganic components in the drug. Acid insoluble ash (13.15%w/w) is more than 1% indicating siliceous material i.e., inorganic components such as Si, Bo. The extractive values indicate the presence of both polar and non-polar components. Water soluble extractive & Alcohol soluble Extractive were found to be 10.75%w/w and 9.35%w/w The value of water-soluble extractive is higher showing the presence of carboxylic acids tannins, sugars, and phytoconstituents. The value of Alcohol soluble extractive is 9.35% indicating the presence of alkaloids. [16,17] A collection of substances used to eradicate pests that pose a threat to domesticated plants is known as a pesticide. The pesticides leave behind residue that is harmful for consumption. The presence of pesticide residue is essential to be evaluated for safe consumption in humans. The results of ***VRM*** showed that it is free from pesticide residue. [18] Aflatoxin isa naturally occurring mycotoxin produced by *Aspergillus parasitics* and *AspergillusFlavus*. In medicines composed of herbs, it is necessary to assess the presence of aflatoxin to prevent toxicity. Thus,reliable, and sensitive aflatoxin detection typically requires elaborate

procedures and a powerful detection device. By using TLC plates the formulation VRM is studied for aflatoxin and found tobe free from Aflatoxin.[19] One of the most significant safety issues associated with herbo-mineral medicines is contamination by bacteria of various types that may be adherent to the herbs from which medicine is manufactured. VRM is free from microbial contamination. [20] The heavy metal analysis showed **pb**, **cd**, and **As** were below the detection level but **Hg** is 0.53ppmwhich is under the AYUSH permissible limit. The detectable level of Hg is due to the presence of mercury as one of the ingredients in the formulation. [20]

CONCLUSION

From the results, it is concluded Vatha Ratchasan Mathira is safe for consumption since it is free from aflatoxin, heavy metals, and microbial contamination. Also, the Physicochemical evaluation shows the purity, integrity, and safety of the drug. Furthermore, research studies must be carried out to assess the toxicity and efficacy of *VathaRatchasan Mathirai*.

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Table: 1-Organoleptic Characters of *Vatha Ratchasan Mathirai*

PARAMETERS	RESULTS
State	Solid
Appearance	Dark black
Odour	Mild Aromatic
Nature	Soft tablet

Table: 2-Physicochemical properties of *Vatha Ratchasan Mathirai*

PARAMETER	RESULT
Loss on drying	0.75 %
Uniformity of weight	0.102g (Average weight) none deviates beyond permissible limit
Disintegration Time	All disintegrated with 20 minutes
Hardness	Withstands up to 2.3kg
Total Ash content	43.52%
Acid insoluble Ash	13.15%
Water soluble Extraction	10.75%
Alcohol soluble Extraction	9.35%
pH (10% of solution)	5.35%

Table: 3-Microbial Contamination Test of *Vatha Ratchasan Mathirai*

S. No	Parameters	Results (CFU)
1.	Total Bacterial Content	**Nil (10^5)**
2.	Total Fungal Content	**Nil (10^3)**

Table: 4-Aflatoxin Analysis of *Vatha Ratchasan Mathirai*

Aflatoxin	Sample	AYUSH Specification Limit
B_1	Not Detected	0.5 ppm
B_2	Not Detected	0.1 ppm
G_1	Not Detected	0.5 ppm
G_2	Not Detected	0.1 ppm

Table: 5- Pesticide Residue Analysis of *Vatha Ratchasan Mathirai*

S. No	Parameters	Sample	AYUSH Limit mg/kg
	Organo Chlorine Pesticides		
1.	Alpha BHC	BQL	0.1mg/kg
2.	Beta BHC	BQL	0.1mg/kg
3.	Gamma BHC	BQL	0.1mg/kg
4.	Delta BHC	BQL	0.1mg/kg
5.	DDT	BQL	1mg/kg
6.	Endosulfan	BQL	3mg/kg
	Organo Phosphorus pesticides		
7.	Malathion	BQL	1mg/kg
8.	Chlorpyrifos	BQL	0.2mg/kg
9.	Dichlorovos	BQL	1mg/kg
	Organo carbamates		
10.	Carbofuran	BQL	0.1mg/kg
	Pyrethroid		
11.	Cypermethrin	BQL	1mg/kg

BQL - Below Quantifiable Limit, DL -Detection Limit.

Table: 6-Heavy Metal Analysis of *Vatha Ratchasan Mathirai*

Elements	Max. Absorption	Result	Max. Limit
Arsenic (As)	193.7 nm	**BDL**	3 ppm
Mercury (Hg)	253.7 nm	**0.53**	1 ppm
Cadmium (Cd)	228.8 nm	**BDL**	0.3 ppm
Lead (Pb)	217 nm	**BDL**	10 ppm

BDL – Below Detection Limit

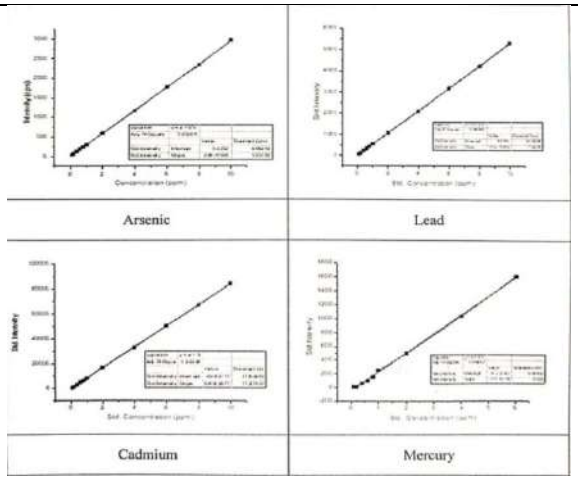

Fig – 1: Standard Linearity

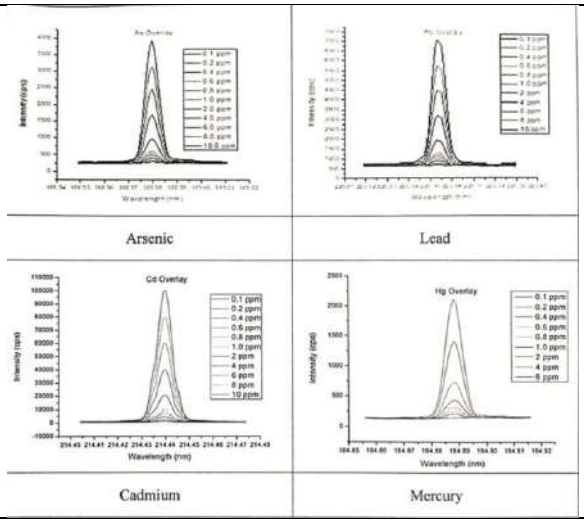

Fig – 2: Standard Overlay