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DEVELOPMENT OF QUALITY CONTROL PARAMETERS OF CLASSICALLY DRIED AND LYOPHILIZED LEAF POWDER OF *VASA* (*JUSTICIA ADHATODA* L.)

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ABSTRACT

Introduction: *Vasa* (*Justicia adhatoda* L.) is a very common growing plant in the surroundings and used to treat the diseases of human kinds since thousands of years. Quality standard parameters are an essential tool for accurate identification, authentication and standardization of Ayurvedic formulations. In present study, powder of *Justicia adhatoda* L. leaves was prepared by classical shade drying and lyophilization. The study is an attempt to develop and compare the quality control parameters of classically dried and lyophilized leaf powder of *Vasa* by using scientific approaches and also assess the effect of both drying methods on quality parameters. **Materials & Methods:** Fresh *Vasa* leaves were manually collected for preparation of shade drying and lyophilization. Both the samples were packed in LDPE (Low Density Polyethylene) container and stored under controlled temperature. Pharmacognostic, Physico-chemical parameters, Assay of Vasicine, Limit test for heavy metals and microbial limit test were evaluated for both the samples. **Results:** Microscopic evaluation of fresh leaf of *Vasa* and both the powders revealed the similarity of characters. Values of Physico-chemical parameters of both the powders were within the limit mentioned in Ayurvedic Pharmacopeia of India but better in lyophilized powder. Assay of Vasicine shows that no significant differences are found in both samples, while it was decreased up to 60% from at six months in both samples. **Conclusion:** Present study was help to asses a standard for the identity of *Vasa* leaves and the obtained data for both the powders can be considered the developed quality standard.

Keywords: *Justicia adhatoda* L., Microscopic study, Classical shade drying, Lyophilization, HPLC

INTRODUCTION: *Justicia adhatoda* L is a shrub belongs to Acanthaceae family. The leaves are simple, opposite and 7–19 cm long and 4–7 cm wide. The frequent use of *Vasa* has resulted in its inclusion in the WHO manual[1].Due to easy availability and resourceful biological activities, the different dosage forms such as *Svarasa* (Expressed Juice), *Kwatha* (Hot Infusion), *Churna* (Powder) and *Ghrita* (Medicated Ghee) of *Vasa* have been found in classical texts. Here, two different drying processes i.e. classical shade drying and lyophilization were used for preparing powder of *Vasa* leaves to assess the effect of drying process on their quality parameters.

Particular therapeutic effect of the drugs always depends on the genuineness raw material. So, proper identification of medicinal plants is quite necessary and can be possible by Pharmacognosy of the plants.

The main limitation of powder is the loss of active ingredient and chances of contamination during drying and powdering process. This dosage form also has short shelf life. Lyophilization is one of the accepted drying techniques by modern pharma industry nowadays. It takes very less time for drying in compare to classical drying. It has been effectively practiced by many disciplines to overcome

the limitations of the traditional method of drying.

The present study is aimed to develop quality control parameters of classically dried and lyophilized leaf powder of *Vasa* by using scientific approaches which ascertained the genuineness and quality of both the powders and also assess the effect of both drying methods on quality parameters.

MATERIALS AND METHODS:

Procurement of raw material and identification:

Fresh *Vasa* leaves were collected from the botanical garden of Rajpipla, Gujarat. The correct identity of the *Vasa* species and its family was confirmed by studying its morphological characters and authenticated the characters mentioned in various floras and text book of botany by subject expert in Pharmacognosy Division of Food and Drugs Laboratory, Vadodara.

Preparation of both the samples

There are classical shade drying (CP) powder was prepared by reference of Sharangdhara Samhita[2] and lyophilized powder (LP) was prepared as per pharmaceutical engineering[3].

Procedure

Fresh *Vasa* leaves were collected and thoroughly washed with water. Then the quantity of leaves was equally divided for making powder. For shade drying leaves were dried in shade in a S.S.Tray. For lyophilization, the paste of leaves were prepared and subjected to freeze drying machine. Then both the powders were sieved (85 #). Dried powders were stored in LDPE containers in the quantity of 50g each samples and stored in controlled temperature. Precautions were taken while preparation of powder and packaging these samples.

Pharmacognostical evaluation

Pharmacognostical analysis of fresh *Vasa* leaf and both powders comprises of organoleptic characters i.e., colour, odour, taste and texture, were recorded and microscopic studies were carried out. Section of leaf was placed for two minutes in the saffranin solution in a petri dish and washed in other petri dish containing distilled water. Then the section were mounted on clean glass side with help of Glycerine water and covered by cover slip avoiding air bubbles. The section was focused under microscope and arrangement of cells was studied.

A pinch of powder was taken and kept on slide covered the section with cover slip avoiding air bubbles Slide was visualized under microscope**.** The microphotographs were taken under Carl Zeiss Binocular microscope attached with camera[4,5].

Physico-chemical evaluation

Both the powders were analysed through Physico-chemical parameters such as pH[6], Loss on Drying (LOD)[7], Water Soluble Extractive (WSE)[8], Alcohol Soluble Extractive (ASE)[9], Total Ash (TA)[10] and Acid Insoluble Ash (AIA)[11].

Assay of Vasicine by HPLC[12]

- **Preparation of Sample Solutions :**

Accurately weighed 1.0 g of sample (classical /lyophilized powder) in a 100 ml iodine flask, add 50 ml Methanol HPLC Grade and Sonicate for 5 minutes. Filter it using 0.22 microns syringe filter and it for HPLC analysis.

Table No.1: Chromatographic system

HPLC System	Shimadzu UFLC Modular
Stationery Phase	C18
Column Temperature	27^{o}C
Filtering System	0.22 µ syringe filter
Run time	25 minutes
Sample Application Volume	20 µL
Mobile Phase (MP)	Hexane sulphonic Acid : Water : Acetonitrile : Glacia acetic acid (0.5 g : 375 ml: 125 ml : 10 ml)
Flow Rate	1.0 ml/min
Wavelength	280 nm
Mode of Separation	Isocratic

Toxic contaminants evaluation

Heavy metal contamination

Heavy metals contamination for both the samples were analysed by Atomic Absorption Spectrometer (AAS)[13] .

Microbial Limit test

Microbial limit test was carried out as per the standard methods described in Ayurvedic Pharmacopoeia of India[14].

OBSERVATIONS AND RESULTS

Pharmacognostical analysis

Macroscopically Evaluation of *Vasa* leaf

Fresh leaves of *Vasa* are green in colour, characteristic odour and bitter in taste. The shape of leaves is ovate-lanceolate, apex is acuminate, margin slightly crenate to entire, base is symmetric, venation is pinnate and texture is leathery.

Microscopic Evaluation of *Vasa* leaf

Transverse section of fresh leaf showed, dorsoventrally surface with 2 layer of palisade cells, epidermal cells sinous with anomocytic stomata on both surfaces, more numerous on the lower, few clothing trichomes, 1-3 celled, thin walled, uniseriate, and grandular trichomes with unicellular stalk and 4 celled head cystoliths in mesophyll layers, elongated and cigar shaped, acicular and prismatic forms of calcium oxalate crystals present in mesophyll.

The mesophyll tissue was differentiated into palisade tissue towards upper epidermis and it contain double layered columnar cell compactly arranged with chloroplast. Spongy tissue towards lower epidermis cells were polygonal loosely arranged with numerous intercellular spaces. Each vascular bundle was conjoint, collateral and closed. Xylem present towards upper epidermis and phloem toward lower epidermis. The vascular bundle was enclosed by a parenchymatous bundle sheath (Figure No.1).

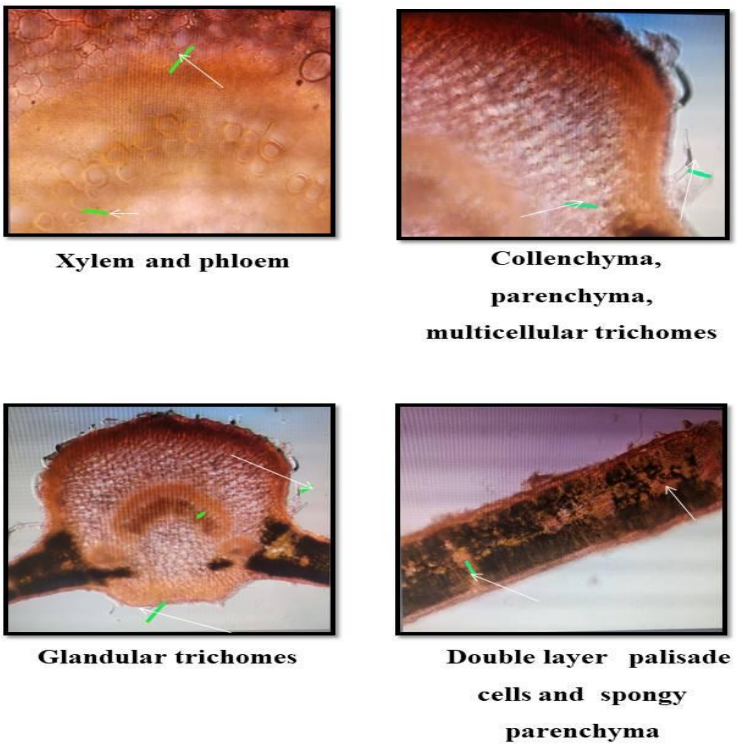

Figure No.1: Microscopic characters of *Vasa* Leaf (*Justicia adhatoda* L.)

Powder Microscopy

Organoleptic characters of both the powders like colour, odour, texture and taste were reported in table no.2.

Table No.2: Organoleptic characters of Classical and Lyophilized powders

Sr. No.	Sample	Organoleptic Characters			
		Colour	Odour	Texture	Taste
1	CP	Green	Characteristic	Slightly rough	Bitter
2	LP	Dark Green	Characteristic	Slightly rough	Bitter

Microscopically Characteristics:

Green colour and bitter in test showed the fragments of epidermal cells in surface view, thick walled covering and glandular trichomes, palisade and spongy parenchyma, epidermal cells with stomata and cystoliths were present (Figure No.2).

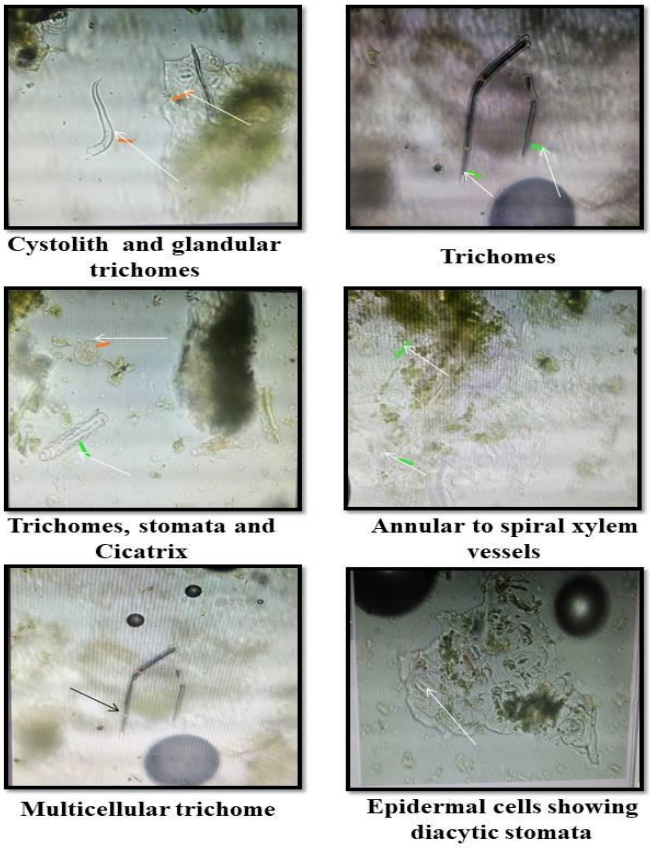

Figure No.2: Powder microscopy of classical dried and lyophilized *Vasa* leaf powder (*Justicia adhatoda* L.)

Physico-chemical analysis

Table No.3: Physico-Chemical Parameters of both of the samples

Physico-Chemical Parameters	Limits According to API*	CP	LP
pH	-	8.06	8.69
LOD (% w/w)	Not More Than 12 %(As per IP*)	6.36 %	0.891%
WSE(% w/w)	Not less than 13 %	22.81 %	23.67 %
ASE (% w/w)	Not Less Than 6 %	11.99 %	11.91 %
TA (% w/w)	Not More Than 19 %	12.92 %	14.06 %
AIA (% w/w)	Not More Than 3 %	0.50 %	0.44 %

API* Ayurvedic Pharmacopeia of India, IP* Indian Pharmacopeia

Table No.4: Assay of Vasicine by HPLC (% w/w)

Sr. No.	Sample	0 Month	6 Months	IP* Value
1	CP	1.69 %	0.55 %	NLT* 0.6%
2	LP	1.73 %	0.56 %	

IP* Indian Pharmacopeia, NLT* Not less than

Table No. 5: Heavy Metal Analysis of Classical and Lyophilized powders

Sr. No	Heavy Metal Content	CP	LP	API Value
1	Lead	0.888 ppm	2.675 ppm	10 ppm*
2	Cadmium	N.D.*	N.D.	0.5 ppm
3	Arsenic	0.173 ppm	0.234 ppm	3 ppm
4	Mercury	N.D.	N.D.	1 ppm

N.D.* Not detected, ppm* Parts per million

Table No. 6: Microbial Limit Test of Both Samples

Sr. No.	Microbial Test	CP		LP		Permissible limit
		0 Month	6 Months	0 Month	6 Months	
1	Total Plate Count (cfu/g*)	2115 cfu/g	655 cfu/g	1701 cfu/g	345 cfu/g	10^5cfu/g
2	Total Yeast & Mould Count (cfu/g)	538 cfu/g	296 cfu/g	95 cfu/g	Nil	10^3cfu/g
3	Escherichia Coli	Absent	Absent	Absent	Absent	Should be Absent
4	Salmonella Spp	Absent	Absent	Absent	Absent	Should be Absent
5	Staphylococcus aureus	Absent	Absent	Absent	Absent	Should be Absent
6	Pseudomonas aeruginosa	Absent	Absent	Absent	Absent	Should be Absent

cfu/g* Colony forming unite per gram

DISCUSSION

Every plant which is used therapeutically requires study before to its use. Pharmacognostical study provides identification and morphological characteristic of plant, which helps to differentiate species of the same genus of the same family. It is also the first step to standardize a drug which is the need of the hour.

Pharmacognostical study

Microscopic evaluation of fresh leaf of *Vasa* and both the powders revealed the similarity of characters as per the references of API. The appearance of similar characters among the drugs obtained from Government Ayurvedic Pharmacy attached garden, Rajpipla and those with the characters mentioned in API, reflects the botanically genuine selection of the drugs (Table No.2).

Physicochemical parameters

According to IP, LOD should not more than 12% w/w. So, both the samples of *Vasa* powders fulfill the quality parameters. LOD values were found more in CP than LP sample which indicates the presence of certain extent of moisture in CP sample while freeze drying technique dehydrate the sample by mechanical process applied in it but due to hygroscopic nature, instead of controlled conditions of temperature and humidity, LP sample absorbed the moisture (Table No. 3).Water Soluble Extractive and Alcohol Soluble Extractive were per API limit. The result of Total ash and Acid insoluble ash on both samples (Table No.3) suggest that there were no more inorganic impurities in samples.

Assay of Vasicine by HPLC

Assay of Vasicine is as per IP limit while after six months these values were decreased. The data showed that the quantity of Vasicine was slightly more in LP than CP sample at initial month and at 6 months no differences were observed in the quantity of marker component in both

the samples (Table No.4). Both the samples fulfil the criteria for limit of Vasicine mentioned in IP at initial stage but it was slightly less at 6 months.

The fungal deterioration, temperature given during process and also nature of plant are affects the chemical composition of the material and thereby decreases the medicinal potency of herbal drugs[15]. The data revealed that marker compound of *Vasa* i.e. Vasicine in both the samples are decreased quantitatively after 6 months in controlled environment. It may be considered as natural process of deterioration of the active constituents in the plant. There are many alkaloids in *Vasa*, among them Vasicine is present in the concentration of 90 % by HPLC analysis[16].

Heavy metal determination

On heavy metal analysis lead and arsenic found very less in quantity, while these values were below detectable limit (Table No.5). It may be due to absorption of lead from the water resources while cleaning process because Pb has poor solubility in water[17]. The growing condition of plant, chemical treatments and processing steps are affected to plants by heavy metals[18].So, it can be said that these two metals were present in both the samples from aforementioned conditions.

Microbial Contamination

The microbial study reveal that total plate count and total yeast &mould count were found in both the samples, while these values are below permissible limit (Table No.6). There may be possibility of the microbial growth in both of the samples at initial stage due to the extrinsic factors such as moisture content and humidity but after in controlled environment or the anti - bacterial activity of the drug, the inhibition of growth might be materialized. On overall observation of all parameters, LP found more stable than CP.

CONCLUSION

Drying methods (classical drying and lyophilization) does not affect on the macroscopic and microscopic characteristics of *Vasa* powder. The Pharmacognostical study serves as a microscopic reference standard for leaf identification and no any significant differences found in the powder characters and no adulterants were found. Classical shade drying method was consumed more time than lyophilization for the preparation of powder. Assay of Vasicine by HPLC shows that no significant differences are found in both samples, while it was decreased up to 60% from its initial stage to six months in both samples.

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