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A comparative pharmacognostical outlook and estimation of Eugenol in *Ocimum Sanctum* Linn. Leaves powder prepared from shade drying and lyophilized method

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Abstract
Introduction: *Ocimum Sanctum* Linn. is an annual herb commonly known as basil in Indian subcontinent belonging to the family Liliacae. It is widely used in the ailments of respiratory tract. The impact of drying methods on volatile matter of aromatic plants play major role in efficiency of the plant. The present study mainly deals with the effect of classical and lyophilized drying method on pharmacognostical characteristic and the content of volatiles i.e. Eugenol by GC method.
Material and Method: Fresh leaves of *Ocimum Sanctum* Linn. were collected and equally divided for shade drying and freeze drying method. After complete drying, both the samples were sieved through 85 no. mesh and packed in LDPE container. Then both the samples were analysed separately for organoleptic characters, powder microscopy, physico-chemical parameters, Assay of Eugenol by Gas chromatography, Limit test for heavy metals and microbial limit test.
Result and Conclusion: Microscopic photographs of transverse section (T.S) of fresh leaf had presentation of epidermal trichomes, calcium collenchymatous, xylem, phloem and stomata. On seeing the values of physicochemical parameters, classical shade dried powder contains more moisture content than lyophilized powder. Considering the obtained quantitative data of Eugenol estimation in both samples, it can be said that classical shade drying method is deliberated as appropriate drying method for *Ocimum sanctum* L. leaves in comparison to lyophilisation.

Keywords: *Ocimum Sanctum* L., shade drying, lyophilization, pharmacognosy, eugenol, *Tulasi*, *Churna Kalpana*, accelerated stability studies

Introduction
Ocimum Sanctum L. (O.S.) is considered to be a ubiquitous plant in India. It is used in many cough syrups as an ingredient. The juice of this plant is used as an adjuvant in the management of many diseases [1] and also used as liquid media for levigation in medicine preparations, e.g.- *Tribhuvanakirti Rasa*. It is extensively used by Ayurvedic physicians and Ayurvedic Pharma Companies as single drug or in formulations.
Volatile matters are considered as the most delicate constituents during the process of drying. Drying of plant material can be achieved by several processes, including shade drying, hot-air and freeze-drying. Freeze-drying or Lyophilisation is an effective way of drying materials without harming them. The loss of volatile contents during drying and powdering, decreased shelf life, the increased bulk compared with other pharmaceutical preparations which reduced the acceptability of powder could effectively be overcome through lyophilization. In shade drying, drug takes more time to dry and can be affected by dust, insects etc. In lyophilization water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapour without passing through a liquid phase [2]. The method has been proved the effective in minimizing the colossal wastage of volatile forms.
This study has been planned to evaluate the influence of two different drying methods (Shade drying and freeze-drying) on pharmacognostical characteristic and the volatile compound i.e Eugenol of *Ocimum sanctum* L.

Materials and Methods

Procurement of drug

Fresh leaves of *Ocimum sanctum* L. were collected from the Government Ayurvedic Pharmacy attached garden, Rajpipla Vadodara, Gujarat. The samples were preserved and placing high quality air tight containers avoiding any kind of infectivity. The raw material was authenticated in Pharmacognosy Laboratory, Food and Drug Laboratory, Vadodara, Gujarat.

Preparation of classical and lyophilized powders

First collection and manual sorting of *Ocimum sanctum* L. fresh leaves were done and separated foreign unwanted matters from the samples. Fresh leaves were divided in equal size of two batches for making both the samples.

Shade dried Powder (OSP) preparation

Fresh leaves were dried in shade for 12 days and powdered in mixer grinder after a complete drying. Powder was sieved with 85 no. mesh and packed in airtight LDPE containers of 50 g with proper labelling.

Lyophilized Powder (OLP) preparation

Fresh leaves were grinded to make a paste and kept in freeze drier at Freeze Dry Systems Private Ltd, Savli, Vadodara, Gujarat. After completion of freeze drying process, the material was powdered in mixer grinder. Sieved the lyophilized powder with 85 no. mess and packed in airtight LDPE containers.

Pharmacognostical evaluation

Pharmacognostical analysis of fresh O.S. leaf and O.S. powder comprised of organoleptic characteristic [i.e., color, odor, taste, and texture] and microscopic studies.

Fresh leaf microscopy

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.

Powder microscopy of both the powders

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.

Physicochemical evaluation

Both the powders were analyzed through relevant physicochemical parameters such as pH [3], loss on drying (LOD) [4], Water soluble extractive (WSE) [5], Alcohol soluble extractive (ASE) [6], Total Ash (TA) [7] and Acid insoluble Ash (AIA) [8]. Microbial limit test [9] and Heavy metal [10] were computed according to the methods described in Indian Pharmacopoeia. Assay of Eugenol by Gas Chromatography [11] was also evaluated.

Preparation of Sample Solution (T)

Accurate weighed 0.500 mg of both the samples in a 100 ml volumetric flask, add Menthol gC Grade to dissolve it up to mark. Sonicate for 5 minutes. Filter it using 0.22 microns syringe filter and it for GC analysis.

GC System: Shimadzu GC-2014

Stationery Phase: Crossbond 5% diphenyl / 95% dimethyl polysiloxane

Column Temperature: Initial 50 ^{0}C, hold for 1 minute, increase up to 220 ^{0}C at the rate of hold for 13 minutes.

Observations and Results

Macroscopical / Organoleptic evaluation of fresh leaf

O.S. Leaves are found to be elliptic to oblong in shape with slender and hairy. It is upper surface green and lower pale green having pungent and mucilaginous taste.

Microscopy of O.S. fresh leaf

Transverse section of leaf showed cordate outline, consisting of single layered epidermis composed of thin walled, oval cells having a number of covering and glandular trichomes; covering trichomes multicellular, uniseriate 1-7 celled long, rarely slightly reflexed at tip; glandular trichomes short, sessile or with 1-2 celled stalk and 2-8 celled, balloon-shaped head, upper epidermis, followed by 3-4 layers of collenchymatous and 1-2 layers of parenchymatous cells; lower epidermis followed by 1-3 layers of collenchymatous and 2-3 layers of parenchymatous cells; three vascular bundles situated centrally, middle one larger than the other two, consisting of xylem and phloem.

Powder Microscopy

Organoleptic characters

Both the sample powders were evaluated on the basis of organoleptic characters i.e. Colour, odour, taste and illustrated in table no. 1.

Table 1: Organoleptic parameters of OSP and OLP

S. N.	Organoleptic Parameters	OSP	OLP
1	Colour	Greenish brown	Light Brown
2	Odour	Characteristics	Characteristics
3	Taste	Pungent	Pungent

Microscopical Characteristics:

Light-green; showed fragments of polygonal, less wavy walled epidermal cells in surface view, covering and glandular trichomes as a whole or in pieces showing warty wall, palisade and spongy parenchyma, anomocytic and diacytic stomata.

Physicochemical analysis

The observations of physicochemical parameters of both the samples are presented in Table No.2.

Table 2: Physico-Chemical Parameters of OSP and OLP

Physico-Chemical Parameters	Limits According to API	OSP	OLP
pH	-	6.17	6.08
LOD (% w/w)	-	5.19	1.99
WSE(% w/w)	NLT 13%	24.92	20.14
ASE (% w/w)	NLT 6%	16.95	14.45
TA (% w/w)	NMT 19%	10.90	11.33
AIA (% w/w)	NMT 3%	0.15	0.48

NLT – Note Less Than, NMT – Note More Than

Table 3: Result of Assay (%) of OSP and OLP

Name of Sample	Assay of Eugenol by GC	Limits According to IP [12]
OSP	1.09%	NLT 0.40 percent (% w/w)
OLP	0.27%	

Table 4: Microbial limit test of OSP and OLP

Microbial	OSP	OLP
Total plate count	1980 cfu/gm	1085 cfu/gm
Total Yeast & Mould Count	429 cfu/gm	155 cfu/gm
E. coli	Absent	Absent
Salmonella	Absent	Absent
S. aureus	Absent	Absent
P. aeruginosa	Absent	Absent

Table 5: Heavy Metal Analysis of OSP and OLP

Sr. No	Heavy Metal Content	OSP	OLP	Permissible Limits As per API [13]
1	Lead	0.881 ppm	0.447ppm	10 ppm
2	Cadmium	Not Detected	Not Detected	0.3 ppm
3	Arsenic	Not Detected	Not Detected	3 ppm
4	Mercury	Not Detected	Not Detected	1ppm

Discussion

Drying is the most common and effective method that increase the shelf life of herbs by inhibiting the growth of microorganisms and preventing the onset of some biochemical reactions that may alter the organoleptic and nutritional characteristics of the dried leaf.

Microscopic evaluation of powders of O.S. revealed the similarity of characters as per the references of API. In microscopic study transverse section of leaf shows oval cells having a number of covering and glandular trichomes; covering trichomes multicellular (Plate - 1.1.3, 1.1.4), upper epidermis, followed by 3-4 layers of collenchymatous and 1-2 layers of parenchymatous cells; lower epidermis followed by 1-3 layers of collenchymatous and 2-3 layers of parenchymatous cells (Plate - 1.1.2, 1.1.3). No major differences were observed between OSP and OLP in Powder microscopy.

Organoleptic evaluation of the respective samples is depicted in Table No. 1. There is significantly difference observed in organoleptic characters between OSP and OLP. pH of both the samples were weak acidic in nature (Table No.2).

The data of moisture content of both the samples was higher in OSP than OLP. Though, the values of both the samples were within the IP acceptable range (NMT 12% w/w). So, both the samples fulfill this quality parameter. (Table No.2)

Water soluble and alcohol soluble extractive values are indicative of the solubility of active principles of the material in water and alcohol respectively Findings are suggestive that ASE and WSE values were fulfill the API criteria in both the samples. Quantity of WSE and ASE was more in OSP as compare to OLP (Table No. 2). The WSE value found to be higher than ASE which shows that the constituents of the drug are more extracted and soluble in water as compared to alcohol.

The result of Total Ash and Acid Insoluble Ash of both the samples suggest that there were no more inorganic impurities in samples (Table No.2). Assay of Eugenol by GC shows that OSP had good amount of Eugenol in compare to IP standard. In OLP Eugenol couldn't meet the official limit. With these findings, it is said that OSP retained more active principle than OLP (Table No.3). In Lyophilized method volatile compounds may be removed by high vacuum. *Ocimum sanctum* leaves contain 70% of Eugenol volatile content [14]. This volatile content may be removed when dried *Ocimum sanctum* leaves by Lyophilized method and for that reason OLP sample couldn't meet the official limit of Eugenol.

Most drying methods are known to affect volatiles but freeze-drying generally has the most pronounced effect and consistently fails to preserve fully the volatile profile of the fresh plant. On occasion, total volatiles were not changed but, as a rule, freeze-drying changed the relative concentrations of volatile compounds, usually failing to preserve the volatiles that give the studied plant its unique aroma characteristics [15].

In this test heavy metals like Cadmium, Mercury and Arsenic were not detected in both OSP and OLP (Table No.4). But lead was found in both OSP (0.881 ppm) and OLP (0.447 ppm) but it is under the permitted levels (10 ppm). The microbial profile revealed that the total microbial count, yeast and mould count of Both *Ocimum sanctum* powder were found to be below API specified limits. Moreover, the pathogenic bacteria, i.e. E. coli, Salmonella, S. aureus, P. aeruginosa were found to be absent (Table No.5). Temperature and humidity, macro and micro nutrients, oxygen levels low water activity and extreme pH levels etc. factors are affect the microbial growth. Herbal medicinal products usually contain bacteria and moulds from soil and atmosphere. Raw material, finished dosage forms and the packaging components to maintain appropriate quality and stability of products [16]. Microbial growth in the both sample may have due to any of the above factors.

Conclusion

Powder microscopy of *Ocimum sanctum* L. leaves were complied with the parameters mentioned in API. Pharmaceutical processing (Shade drying and lyophilization) does not affect the macroscopic and microscopic characteristics of the plant. The obtained data of physicochemical parameters and microbial limit showed that classical shade drying method is more stable than Lyophilization associated to small variations found in moisture content in classical method. Considering the obtained quantitative data of Eugenol estimation in both samples, it can be said that classical shade drying method is deliberated as appropriate drying method for *Ocimum sanctum* L. leaves in comparison to lyophilisation.

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