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Research Article

DEVELOPMENT OF ANALYTICAL PROFILE OF *OCIMUM SANCTUM* L. LEAVES POWDER PREPARED BY CLASSICAL AND LYOPHILIZATION METHOD THROUGH ACCELERATED STABILITY STUDIES

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ABSTRACT

Shelf life means the time period during which the potency of a drug remains unaffected from environmental factors or microbial contamination. Nowadays diverse procedures of making powder are available which affect on shelf life of them. *Ocimum sanctum* L. is most common household herb in Indian subcontinent. Hence, the present study is aimed to develop analytical profile of *Ocimum sanctum* L. leaves powder by classical and lyophilized method and compare the stability of both the samples. **Materials and Methods:** *Ocimum sanctum* L. leaves powder was prepared by two different drying methods and accelerated stability study was conducted as per ICH guideline Q1A (R2). Physicochemical analysis was repeated at interval of 0, 1, 3 and 6 months. Organoleptic parameters, microbial limits and heavy-metal analysis were observed at specific intervals. **Result and Discussion**: No significant changes were observed in organoleptic characters of both the samples up to storage of 6 months at accelerated condition. The values of physicochemical parameters of both the samples were within the prescribed limit specified in Ayurvedic Pharmacopeia of India. Results of Microbiological limit test were also below the limit and considerably decreased at specific intervals. Heavy metals namely arsenic, cadmium and mercury were not detected and lead was present below detectable limits. **Conclusion:** Analysis revealed that the classically prepared powder has better shelf life (3.08 years) than lyophilized powder (2.70 years) and also provides the modest and effective ways for optimal Eugenol extraction in accelerated conditions.

INTRODUCTION

Shelf life means the time period during which the potency of a drug remains unaffected from environmental factors or microbial contamination. The information regarding the concept of Shelf life for different dosage forms are found scattered in *Brihatrayi*. According to Acharya Charaka, a drug can be utilized for therapeutic purposes until it retains its fragrance, color, taste etc[1]. During the medieval period authors attempted to compile the information regarding the concept of *Saviryata avadhi* (shelf life) of different dosage forms are available in texts like Vangasena Samhita[2], Sharangadhara Samhita[3], Yogaratnakara[4] etc. The uses of modern packaging technology and preservatives have increased the shelf life period of Ayurvedic medicines.

Churna Kalpana (Powder) is widely accepted dosage form for therapeutic purposes. The limitation of this dosage form is stability and the loss of volatile content during drying and powdering. Lyophilization also known as Freeze drying technique is used nowadays by pharmaceutical industry to increase the shelf life of products, such as live vaccines and other injectable. In classics, the shelf life of *Churna* (powder) is 2 months [5] and according to Rule 161-B, Drugs and Cosmetics Act is 2 years.

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Ocimum sanctum Linn. is most common annual herb used in Indian subcontinent. This plant is *Vata-kaphanashaka* and *Pittavardhaka, Hikkanashaka, Kasanashaka, Vishnashaka, Swasnashaka, Parshwa shoolahara, Durgandhnashaka*,[6] and *Vranashodhaka*[7]. Stability is necessary for development of a pharmaceutical product. Hence, the present study is aimed to develop analytical profile of *Ocimum sanctum* L. leaves powder by classical and lyophilized method and compare the stability of both the samples through accelerated stability study.

MATERIALS AND METHODS

Procurement of raw drug

Fresh leaves of *Ocimum sanctum* L. were collected from the Government Ayurvedic Pharmacy attached garden, Rajpipla, Gujarat. The material was authenticated for its botanical identity in Pharmacognosy Laboratory, Food and Drug Laboratory, Vadodara.

Pharmaceutical preparation of both the powders

First collection and manual sorting of *Ocimum sanctum* L. fresh leaves was done and divided in equal sizes of two batches for making classical powder (OCP) and Lyophilized Powder (OLP). For classical preparation, the material was dried in shade and Lyophilized preparation, paste of the material was subjected to drying in Freeze Dry Machine. After that, both the material were powdered in mixer grinder and packed in airtight LDPE containers. Then container was stored in control chamber for stability studies.

Storage conditions

Accelerated stability study was conducted as per International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines Q1A (R2)[8]. Samples were stored at 40°C±2°C, and 75±5% relative humidity.

Packing

The final products both *Ocimum sanctum* L. leaves powder in required quantities were packed in low-density polyethylene containers.

Sample quantity

Both the powder were packed in 50gm of weight in ten containers and stored in accelerated stability study chamber.

Frequency of withdrawal

Both the samples were withdrawn from the container and analyzed initially and at a gap of 0, 1, 3 and 6 months. To evaluate intercept and slope, Extrapolated shelf life of both the powders were calculated with 10% degradation rate from physico chemical parameters at accelerated condition 40°C±2°C and 75%±5 RH.

Number of months when 10% degradation was occurred was calculated using following formula:

Number of month when 10% degradation occurs =

$$\frac{[0 \text{ month assay value} - \{(0 \text{ month assay value} * 10/100\}] - \text{intercept}}{\text{Slope}}$$

Analytical parameters

Following analytical parameters were carried out at Vasu research center, Vadodara, Gujarat.

i. Organoleptic characters such as color, odor, texture and taste
ii. Physicochemical parameters such as pH[9], Loss On Drying (LOD)[10], Water Soluble Extractive (WSE)[11], Alcohol Soluble Extractive (ASE)[12], Total Ash (TA)[13], Acid Insoluble Ash (AIA)[14]
iii. Microbial limit test[15]
iv. Heavy metal[16]
v. Assay of Eugenol by Gas Chromatography[17]

RESULTS

Organoleptic characters and physico-chemical profiles were evaluated initially and at the end of 0, 1, 3 and 6 months. Results obtained in both the samples are shown in Table No. 1. Observation of Physico-chemical analysis of both powders at 0, 1, 3, 6 moths are shown in Table No. 2. Microbial limit, Heavy Metal test were estimated initially and at the end of 6th months [Table No. 3, 4]. Based on the values obtained at different stage; intercept, slope, expected time (in months) for 10 % of degradation [Table No.6] were calculated for individual parameters of both the leaves powder. As India falls in Zone III; the mean obtained of these months was multiplied with 3.3 to extrapolate shelf-life [Table No.7].

Table1: Organoleptic parameter of OCP and OLP

Sr.No	Sample	Organoleptic Characters at 0, 1, 3 and 6 months interval			
		Colour	Odour	Texture	Taste
1.	OCP	Greenish brown	Characteristic	Slightly rough	Pungent
2.	OLP	Light brown	Characteristic	Slightly rough	Pungent

Table 2: Physico-chemical parameter of OCP and OLP

Parameters		0 month		1 month		3 months		6 months	
Physico-Chemical Parameters	**Limits According to API**	**OCP**	**OLP**	**OCP**	**OLP**	**OCP**	**OLP**	**OCP**	**OLP**
LOD (% w/w)	-	5.19	1.99	5.82	4.55	5.90	4.67	7.90	4.86
WSE(% w/w)	NLT 13 %	24.92	20.14	23.31	18.31	23.27	17.65	23.15	17.31
ASE (% w/w)	NLT 6 %	16.95	14.45	16.83	14.29	15.10	12.97	14.49	12.00
TA (% w/w)	NMT19 %	10.90	11.33	10.91	11.44	11.05	11.51	11.06	15.28
AIA (% w/w)	NMT 3 %	0.15	0.48	0.18	0.80	0.20	0.84	1.19	1.43

NLT – Not Less Than, **NMT** – Not More Than

Table 3: Microbial limit of OCP and OLP

Microbial	Month	OCP	OLP
Total plate count	0 month	1980 cfu/gm	1085 cfu/gm
	6 months	325 cfu/gm	312 cfu/gm
Total Yeast & Mould Count	0 month	429 cfu/gm	155 cfu/gm
	6 months	51 cfu/gm	Absent
E.coli	0 month	Absent	Absent
	6 months	Absent	Absent
Salmonella	0 month	Absent	Absent
	6 months	Absent	Absent
S.aureus	0 month	Absent	Absent
	6 months	Absent	Absent
P.aeruginosa	0 month	Absent	Absent
	6 months	Absent	Absent

Table 4: Heavy Metal Content in OCP and OLP

S.No	Heavy Metal Content	OCP	OLP	Permissible Limits As per API[18]
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

Table 5: Assay of Eugenol by GC in OCP and OLP

Month	OCP	OLP	Limits According to IP[19]
0 month	1.09%	0.27%	NLT 0.40 percent (% w/w)
6 months	0.83%	0.25%	

NLT* - Not Less Than

Table 6: Results of intercept and slope for Shelf life evaluation of OCP and OLP

Parameters	Intercept		Slope	
	OCP	**OLP**	**OCP**	**OLP**
pH	6.156	6.11	-0.037	-0.018
LOD (% w/w)	5.14	1.835	0.423	0.873
WSE(% w/w)	24.211	20.64	-0.220	-0.915
ASE (% w/w)	16.95	15.685	-0.44	-0.894
TA (% w/w)	10.9	9.41	0.030	1.192
AIA (% w/w)	0.158	0.165	0.016	0.289

Table 7: Results of approximate period for 10% degradation for Shelf life evaluation of OCP and OLP

Parameters	Initial		10 % Degradation		Month required for 10 % Degradation	
pH	6.17	6.08	5.55	5.472	16.13	35.44
LOD (% w/w)	5.19	1.99	4.67	1.791	1.11	0.050
WSE (% w/w)	24.92	20.14	22.42	18.126	8.10	2.747
ASE (% w/w)	16.95	14.54	15.255	13.086	3.85	2.857
TA (% w/w)	10.90	11.33	9.87	10.197	36.33	16.44
AIA (% w/w)	0.15	0.48	0.135	0.432	1.31	0.923

Table 8: Results of Extrapolation of Shelf life of OCP and OLP

Sample	Mean Months for 10% degradation	Multiplication Factor	Shelf life	
			Months	Years
OCP	11.12	3.33	37.02	3.08
OLP	9.74	3.33	32.43	2.70

Preparation of Simple *Tulasi Churna*

Collection of *Tulasi Patra*

***Tulasi Patra* kept for shade dry**

Shade dried *Tulasi Patra*

Pulverising method

Tulasi Patra Churna

Finished simple *Tulasi Patra Churna* Air tight packing

Preparations of Lyophilizatio *Tulasi* powder

Collection of *Tulasi Patra*

Wash

***Tulasi Patra* paste (Grinding method)**

Prepare *Tulasi Patra* paste for Lyophilization)

Freeze drying machine After Lyophilization *Tulasi Patra* paste

After Lyophilization *Tulasi Patra* paste

Finished Lyophilized *Tulasi Patra Churna* (Air tight packing)

DISCUSSION

The main factors affecting the shelf life are derivation of the drug, dosage forms, environmental factors (humidity, temperature, and light), microbial contamination, storage conditions and packaging system. The stability data on any dosage form include selected parameters that together form the stability profile. This stability profile is the basis for assigning the storage conditions and shelf life to pharmaceutical products. The main purpose of conducting stability testing of pharmaceutical products is to ensure the efficacy and quality of active compounds in product and to establish shelf life or expiry period.

No changes were observed in organoleptic characters of both the powders at different intervals (Table No.1). Both the samples were found similar organoleptic characteristic like pungent taste and slightly rough texture except colour. The colour of OCP was greenish brown colour while OLP was light brown in colour. The pH conventionally represents the acidity and alkalinity, pH of the both *Ocimum sanctum* L. leaves powder were weak acidic in nature (Table No-2). The data of LOD of both the samples revealed that moisture content was gradually increased in the period of 0, 1, 3 and 6 months and higher in OCP than OLP. According to IP, LOD should not more than 12% w/w. So, both the powders fulfill this quality parameter up to 6 months. WSE and ASE were found more in OCP than OLP (Table No.2). The result of total ash and Acid insoluble ash showed no more inorganic impurities in both the samples. On comparing the assay of Eugenol by GC, it was concluded that both the samples have the major active constituent, Eugenol but this was in higher concentration in OCP sample than the OLP. Even the Eugenol concentration in OLP was found lesser than the standard limit specified in IP (Table No.5). Results of microbiological limit test were also below the permissible limit specified in Ayurvedic Pharmacopeia of India which considerably decreased at specific intervals (Table No.3). AAS showed heavy metals like cadmium, mercury and arsenic were absent in both the samples. But lead was present within the permissible limit (Table No.4).

Extrapolated shelf life of both the powder was calculated with 10% degradation rate (Table No.7) from physicochemical parameters at accelerated condition 40°C±2°C and 75%±5% RH. On the basis of available data from accelerated stability study, it can be extrapolated that shelf life of OCP is found to be 37.02 months (3.08 years) while shelf life of OLP is 32.43 (2.70 years) for countries which come under climatic zone III & IV. It is matched with the implemented rule namely 161 B to display the date of expiry of the ASU drugs and propose shelf life of Ayurvedic formulations i.e. shelf life of *Churna* (fine/ coarse powder drugs) as 2 years.

CONCLUSION

Shelf-life defined for *Churna* at Rule 161-B, Drugs and Cosmetics Act is 2 years. Analysis revealed that *Ocimum sanctum* L. leaves powder prepared by classically has longer shelf life (3.08 years) than lyophilized powder (2.70 years). *Ocimum sanctum* L. powder prepared by classical method is provided the modest and effective ways for optimal Eugenol extraction in accelerated conditions. Results of microbiological limit test were also below the limit and considerably decreased at specific intervals. Heavy metals namely arsenic, cadmium and mercury were not detected and lead was present below detectable limits.

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