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Standardization of Fennel (*Foeniculum vulgare*), Its Oleoresin and Marketed Ayurvedic Dosage Forms

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ABSTRACT

Fennel, *Foeniculum vulgare*, one of the most common use in India kitchen as a spice and as well as in traditional medicine for its estrogenic, lactagouge, diuretic, antioxidant, immune booster and its usefulness in dyspepsia. The use of medicinal plants by the general population is an old and still widespread practice that makes studies of their correct identification are very essential. In the present study the fruit of the plant, raw materials, their oleo resins and marketed formulations was subjected to Standardization parameter viz macro-microscopic, physico-chemical, microbial, heavy metals and Thin Layer Chromatography to fix the quality standards of this drug. Anethole, an active compound of *Foeniculum vulgare,* was also analyzed in different samples of *Foeniculum vulgare.* The result shown that physicochemical parameters evaluated are useful in standardization, Heavy metals and microbial load for raw materials, oleoresins and finished formulations are found to be within the limit of WHO guidelines, indicating that they are free from pathogens and they are safe to be used in Indian System of medicine. The data obtained from the study would be useful in the identification of the fruits of Fennel and serve as standards. The above parameters studied, may be used as a tool for the correct identification and standardization of the plant also to test the adulterants if any.

Keywords: *Foeniculum vulgare,* Standardization, Quality Evaluation, HPTLC Fingerprint.

INTRODUCTION

Foeniculum vulgare Mill. is a small group of annual, biennial or perennial herb. [1] It is widely cultivated throughout India up to 1830 m and sometimes found wild. [2-3] Fennel is used as a spice and also as an important ingredient in various folklore medicines throughout the world. Moreover, this plant has been investigated extensively for several medicinal and therapeutic activities and has been reported for possessing carminative, flavouring, antioxidant, antibacterial, antifungal and mosquito repellent properties. [4-6]

An analysis of fennel shows it to consist of moisture 6.3%, protein 9.5%, fat 10%, minerals 13.4%, fiber 18.5% and carbohydrates 42.3%. Its mineral and vitamin contents are calcium, phosphorous, iron, sodium, potassium, thiamine, riboflavin, niacin and vitamin C. Its calorific value is 370. [7] Fennel volatile oil is a mixture of at least a dozen of different chemicals and the main ingredients are: anethole (40 - 70%), fenchone (1 - 20%) and estragole (2 - 9%). [8-10]

Need of today is to develop more prominent methods for the standardization and quantification of the biological active compounds for better efficacy, quality and safety of herbal drugs. The present study provides the pharmacognostic evaluation i.e. anatomical and microscopic characteristics, physico-chemical properties, preliminary phytochemical screening, and thin layer chromatography (TLC) fingerprinting profiles for this plant. Anethole, an active compound of *Foeniculum vulgare,* was also analyzed in different samples of *Foeniculum vulgare***.**

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MATERIALS AND METHODS

Collection and identification of Raw Material

The three batches of the fruits of *Foeniculum vulgare* Mill. were collected and identified by Dr. Gyanesh Shukla, Taxonomist, Ranbaxy Research Laboratories, Gurgaon. The Samples were coded as FR-1 Fennel Raw (Uttar Pradesh); FR-2 Fennel Raw (Gujarat); FR-3 Fennel Raw (West-Bengal) FOR-1 Oleo-resin (Uttar Pradesh); FOR-2 Oleo-resin (Gujarat); FOR-3 Oleo-resin (West-Bengal); FF-1; FF-2; FF-3 Fennel marketed Formulations.

Preparation of Oleoresin

The dried fruits of *Foeniculum vulgare* Mill. was coarsely powdered. The methanolic extract was prepared by hot extraction using the soxhlet apparatus for 3 days. The extract was filtered through muslin cloth and concentrated to dryness

using rotary vacuum evaporator. The oleo-resins thus prepared were stored at the temperature 4°C till further use.

Marketed Formulations

Three different formulations were used for the standardization of herbal drugs. Following were the composition is mentioned in Table 1.

Table 1: Marketed Formulations used for study

Formulation A Each powder contains	Formulation B Each capsule contains	Formulation C Each 10 ml contains
Aegel marvelos-10% *Salmalia malabarica*-10% *Zingiber officinale*-10% *Woodfordia fructicosa*-10% *Coriandrum sativum*-20% ***Foeniculum vulgare*-40%**	**Extracts derived from:** Daruharidra-30 mg; Kasani -30 mg; Bhrngaraja-30 mg; Kutki- 40 mg; Sarpunkha-30 mg; Nagarmotha-30 mg; Bhumi 40 mg; Punarnava-20 mg; Shunthi- 20 mg; Haritaki- 20 mg; Nisoth- 20 mg; Chitrak-20 mg; Pippali- 20 mg; Kalmegh- 20 mg; Dhaniya- 10 mg; Vidang- 10 mg; Ajwayan- 10 mg; Ghrith kumari- 10 mg **Saunf-10 mg;** Makoya- 10 mg; Shuddha shilajit- 10 mg; Mandoor Bhasma- 60 mg	**Extracts of:** Punarnava-500 mg; Kakamaci-250 mg; Kasni- 125 mg; **Saunf- 125 mg;** Jehu- 125 mg; Sowa-62.5 mg Dhanyak-62.5 mg; Kasundi-62.5 mg; Svetcandana- 31.2 mg; Arjuna -125 mg **Decoctions of:** Pudina- 50 mg; Indrayava- 50 mg; Gajpippal- 50 mg; Shahtra- 50 mg; Atis-50 mg; Gaduchi- 25 mg; Yashtimadhu-25 mg; Kakoli- 25 mg; Triphala- 25 mg; Ajwain- 12.5 mg; Katuka- 2.5 mg; Rose syrup- q.s

Organoleptic Studies: The organoleptic features such as colour, odour and taste of the fruits were observed with sensory organs. With the help of magnifying lens the external features such as shape, size, texture etc. were observed.

Section Cutting: Healthy and suitable pieces of fruits of *Foeniculum vulgare* Mill. were taken and soaked in distilled water overnight. With the help of a razor blade, free hand transverse sections were cut. The clear and thin sections were selected and mounted on a clean glass slide, covered with cover slip by using glycerin. Routine double staining with safranin and fast green was done. Some sections were also stained with phloroglucinol and hydrochloric acid to observe the presence of lignified cells.

Powder Analysis: Fruits were powdered and then passed through sieve no. 60 and examined for its microscopic characters. [11] The powder of the drug was boiled with chloral hydrate and then mounted on the glass slides using glycerin, covered with a cover slip and viewed under microscope. Powder of the fruits was studied for chemical analysis. [12] The powders were also stained with staining reagent like safranin (1%), fast green (0.2%), phloroglucinol and examined under electron microscope. Drawings were made with the help of camera Lucida. [13]

Physico-chemical studies: Physico-chemical studies like total ash, water soluble ash, acid insoluble ash water and alcohol solubility, loss on drying at 105°C, volatile oil, microbial count, heavy metals were carried out as per the WHO guide lines. [14]

Identification of Anethole in different samples of *Foeniculum vulgare* Mill.

(a) Sample Preparation

(i) Crude raw materials: 1 g of crude drug materials of *Foeniculum vulgare* Mill. were weighed in 25 ml of volumetric flasks. 15 ml of dichloromethane was added in each volumetric flask and then sonicated in ultrasonic water bath for about 15 min. Filtered and then the filtrate was evaporated to dryness. The residue was dissolved in 5 ml of toluene. The solution of each sample was used as test solution.

(ii) Oleoresins: Around 500 mg of oleo-resin of *Foeniculum vulgare* Mill. was dissolved in little quantity of methanol by sonication, and then made up the volume up to 10 ml with methanol.

(iii) Formulations

(a) Powder/Capsule: Accurately weighed 1 g of the powder and filled material in capsule respectively were sonicated with 5 ml of methanol for 15 min. and then volume was made up to 10 ml with methanol.

(b) Syrup: Accurately weighed (25 ml) syrup was extracted with 100 ml of extraction media (mixture of 250 ml chloroform, 50 ml butanol and 10 ml methanol) for three times, collected the combined extracts, filtered, evaporated to dryness using vacuum evaporator. Finally the volume was made up to 5 ml with methanol.

(b) Standard Preparation: Accurately weighed about 49.661 mg of standard anethole (purity 99.5% w/w) was dissolved in 10 ml of methanol by sonication.

(c) Stationary Phase: Precoated silica gel $60F_{254}$ TLC aluminum sheet

(d) Solvent System: Toluene: Ethyl Acetate (93:07)

(e) Preparation of Spraying Reagent (Vanillin Sulphuric acid): The freshly prepared solution of vanillin sulphuric acid was prepared by adding 0.5% of vanillin to the solution of sulphuric acid-ethanol prepared in the ration of 4:1.

(f) Procedure: Applied specific quantity of the each sample and standard solution with the help of Linomat V (Camag's) as bands on the TLC plate and developed with solvent system up to 90mm. The developed chromatoplate was dried through hot air. The spots were found to be visible under UV 256 nm. Then the plate was sprayed with vanillin spraying reagent and dried in hot oven at 105ºC for 5-10 min under observation. Then photo documented with the help of photo-documentation system (Camag's). R_f value of each sample was then calculated.

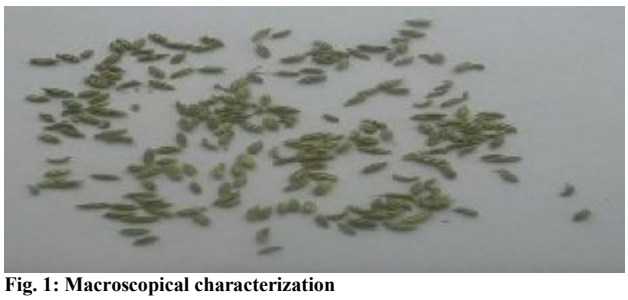
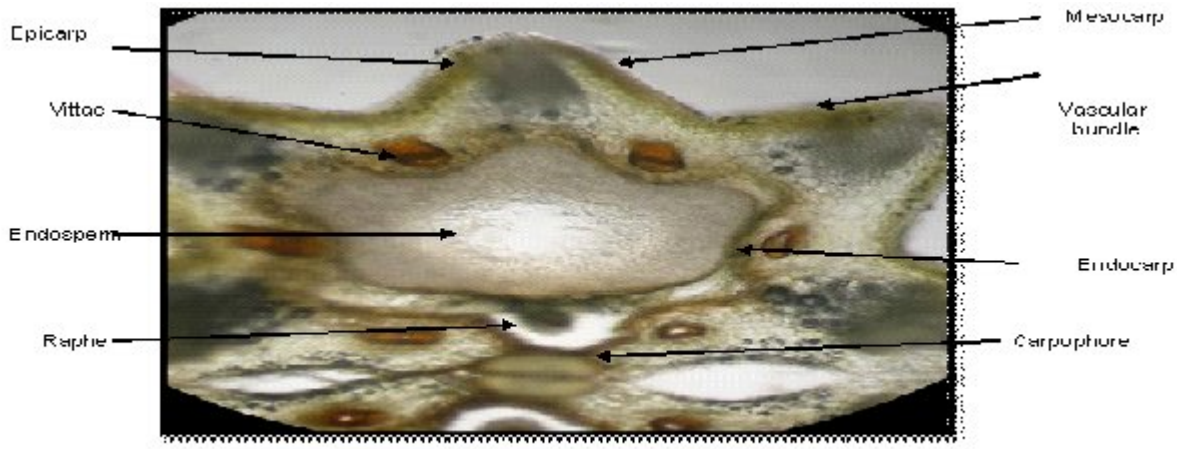


Fig. 1: Macroscopical characterization

RESULT AND DISCUSSION

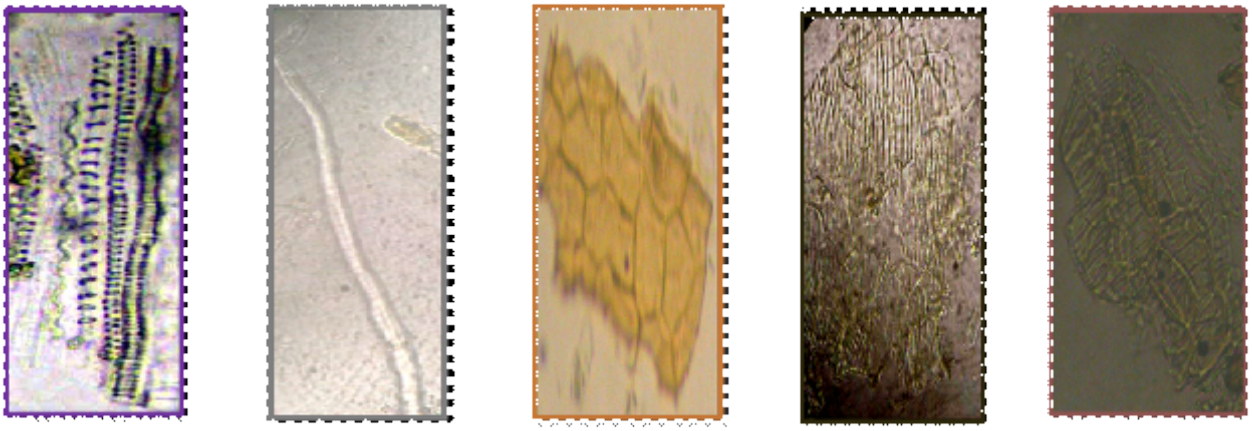
Morphological studies

Description: The fruit shown as entire mericarp with attached pedicels and stylopod at the apex (Fig. 1). Five prominent primary ridges on the dorsal surface carpophore present on ventral surface which holds the two mericarps (Fig. 2). The colour of the fruit was Yellowish green-yellowish brown. Odour and Taste was Aromatic. In Powder characteristics Tracheids, Vittae, Endosperm, Endocarp, Mesocarp were also seen during the microscope study (Fig. 3).



Transverse section of fruit

Fig. 2: Microscopical characterization



Tracheids **Vittae** **Endosperm** **Endocarp** **Mesocarp**

Fig. 3: Powder characteristics

Table 2: Organoleptic studies of *Foeniculum vulgare* Mill. raw materials, their oleo resins and marketed formulations

S. No.	Sample	Size	Shape	Surface	Colour	Odour	Taste	Texture
1.	FR-1	2.5-5×2-4 mm	Oval, curved	Prominent ridges present	light green	Slight	Sweet & Aromatic	-
2.	FR-2	3.6-7×1-3 mm	Oval, oblong	Prominent ridges present	Light green	Slight	Sweet & Aromatic	-
3.	FR-3	4-6.4×2-3 mm	Oval, curved	Prominent ridges present	Green	Slight	Sweet & Aromatic	-
4.	FOR-1	-	-	-	Dark green	Strongly aromatic	Aromatic	Oleo-resin
5.	FOR-2	-	-	-	Dark green	Strongly aromatic	Aromatic	Oleo-resin
6.	FOR-3	-	-	-	Dark green	Strongly aromatic	Aromatic	Oleo-resin
7.	FF-1	-	-	-	Light brown	Slight	Characteristic	Powder
8.	FF-2	-	-	-	Shiny green	-	Faint	Capsule
9.	FF-3	-	-	-	reddish brown	Sweet	Sweetish	Liquid

Table 3: Physicochemical Studies

Sample name	Water soluble extractive value (%w/w)	Alcohol soluble extractive value (%w/w)	Total ash (% w/w)	Acid insoluble ash (% w/w)	Loss on drying (% w/w)	pH of 1% w/v solution	Volatile Oil Content (%w/v)
Limits for crude raw material (API)*	**NLT 14**	**NLT 4**	**NMT 12**	**NMT 1.5**	**-**	**-**	**NLT 1.4**
FR-1	20.87	7.00	8.48	0.98	9.63	5.89	1.45
FR-2	16.31	5.59	8.68	0.86	10.10	6.00	1.50
FR-3	20.21	6.23	7.54	0.70	9.64	5.94	1.40

3). Organoleptic studies of *Foeniculum vulgare* raw materials, their oleo resins and marketed formulations (Table 2).

Table 3 summarizes the various physico-chemical constants observed for the fruits of *Foeniculum vulgare* from three different sources. The physico-chemical analysis indicated the ash content between 7.54-8.68% which is due to the presence of inorganic matter. Acid-insoluble ash indicates the presence of more siliceous matter in the drug. It was found to be between 0.7-0.98%. The alcohol soluble extractive reveals the presence of polar compounds like anthraquinones, alkaloids, glycoside of flavonoids, steroids and triterpenoids present in the plant materials. It was seen to be between 5.59-7.00%. The water soluble extractive reveals the presence of water soluble matters such as sugars, carboxylic acids, vitamins and amino acids and it was found to be between 16.31-20.87%. Loss on drying at 105°C is determined since the presence of excess moisture is conclusive to the promotion of mould and bacterial growth, and subsequently to deterioration and spoilage of the drug. The loss on drying

content was calculated to be between 9.63-10.10%. Volatile oils are mixtures of hydrocarbons and oxygenated compounds derived from. As the oxygenated form is more soluble in both water and alcohol, it is this form that on the whole determines the taste and smell of the mixture. The most common hydrocarbon is the terpene, built up by the successive accumulation of isoprene molecules (C_5H_8). The volatile content was found to be between 1.4-1.5%.

The contents of heavy metals namely lead; mercury, cadmium and arsenic are found to be within the permissible limit for all the raw materials, oleoresins and finished formulations (Table 4).

Table 4: Heavy Metal Analysis

Sample Name	Arsenic (ppm)	Mercury (ppm)	Lead (ppm)	Cadmium (ppm)
Limits (WHO 1992)	**NMT 3**	**NMT 1**	**NMT 10**	**NMT 0.3**
Fennel Raw 1	0.698	NIL	NIL	NIL
Fennel Raw 2	0.834	NIL	NIL	NIL
Fennel Raw 3	0.425	NIL	NIL	NIL
Fennel Oleo Resin 1	0.218	NIL	1.264	NIL
Fennel Oleo Resin 2	2.245	NIL	0.732	0.072
Fennel Oleo Resin 3	0.228	NIL	NIL	0.022
Formulation 1	2.518	NIL	1.464	0.253
Formulation 2	2.217	NIL	2.935	0.163
Formulation 3	2.713	NIL	3.46	0.217

FR-1 Fennel Raw (Uttar Pradesh); FR-2 Fennel Raw (Gujarat); FR-3 Fennel Raw (West-Bengal) FOR-1 Oleo-resin (Uttar Pradesh); FOR-2 Oleo-resin (Gujarat); FOR-3 Oleo-resin (West-Bengal); FF-1; FF-2; FF-3 Fennel marketed Formulations

Table 5: Microbial Limit

Sample name	Total Bacterial Count (cfu/g)	Total Yeast and Mould Count (cfu/g)	Total *Enterobacter* Count (cfu/g)	*E. coli* (cfu/g)	*Salmonella* sp. (cfu/g)	*P. aeruginosa* (cfu/g)	*S aurens* (cfu/g)
Limits for crude raw material (WHO 1992)	**1×10^7**	**1×10^5**	**1×10^4**	**100**	**Absent**	**Absent**	**Absent**
Fennel Raw 1	20	Nil	<10	Absent	Absent	Absent	Absent
Fennel Raw 2	80	Nil	<10	Absent	Absent	Absent	Absent
Fennel Raw 3	<10	Nil	<10	Absent	Absent	Absent	Absent
Limits for extract*	**1000**	**100**	**100**	**Absent**	**Absent**	**Absent**	**Absent**
Fennel Oleo Resin 1		Nil	<	Absent	Absent	Absent	Absent
Fennel Oleo Resin 2		Nil	<	Absent	Absent	Absent	Absent
Fennel Oleo Resin 3		Nil	<	Absent	Absent	Absent	Absent
Limits For Formulations*	**5000**	**100**	**100**	**Absent**	**Absent**	**Absent**	**Absent**
Formulation 1	132	20	<100	Absent	Absent	Absent	Absent
Formulation 2	50	Nil	<10	Absent	Absent	Absent	Absent
Formulation 3	10	Nil	<10	Absent	Absent	Absent	Absent

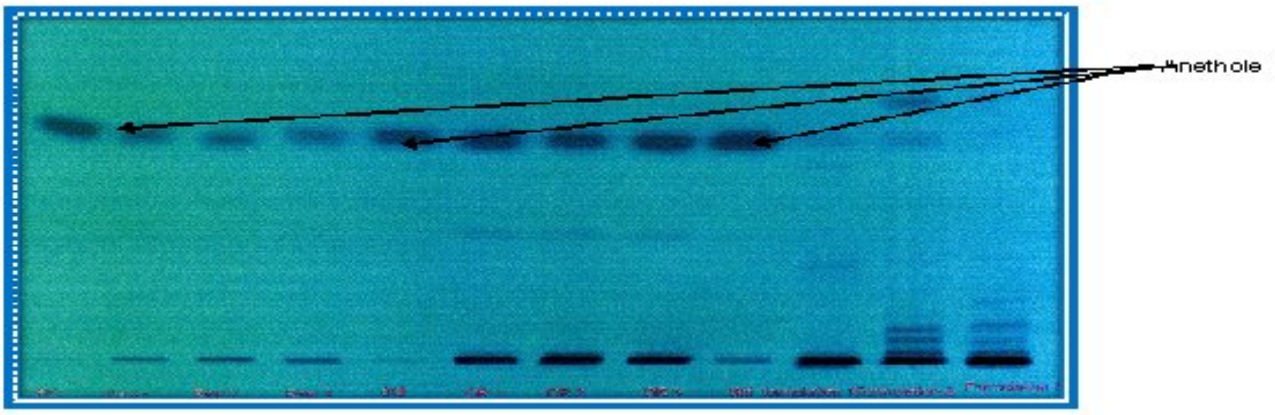


Fig. 4: HPTLC Fingerprint of *Foeniculum vulgare* Mill. Samples under UV (256 nm)

Analysis for the microbial load for the three raw materials, three oleoresins and three formulations is found to be within the limit of WHO guidelines, indicating that they are free from pathogens and can be used as drugs (Table 5)

TLC studies revealed that the solvent system toluene:ethyl acetate (93:07) was ideal and gave a single spot with Rf 0.74 for anethole and well resolved spots for the test samples.

Identification of Anethole in *Foeniculum vulgare* Mill. In various samples has been observed in Fig. 4 and 5. Table 6 shown that the Rf value of Anethole in *Foeniculum vulgare* in various samples.

Currently, there is an emphasis on the standardization of medicinal plant materials for their therapeutic potentials. The modern techniques available make the identification and evaluation of crude drugs by pharmacognostic studies reliable, accurate and inexpensive. According to the WHO, determining the macroscopic and microscopic characteristics are the first steps towards establishing the identity and the purity of such materials, and these steps should be carried out before any further tests are undertaken. [15] Thus we reported the anatomical and microscopic characteristics of the given plant.

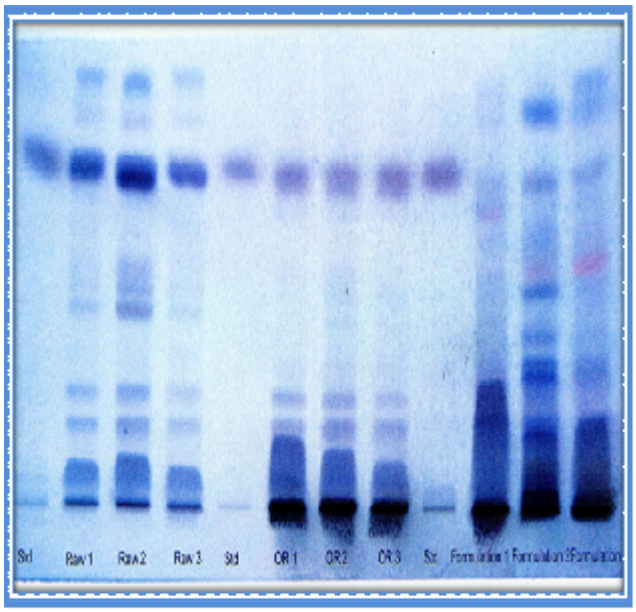


Fig. 5: HPTLC Fingerprint of *Foeniculum vulgare* Mill. Samples after spraying with vanillin Sulphuric acid reagent

Table 6: R_f value of Anethole in *Foeniculum vulgare* Mill. samples

Track No.	Sample	Distance trvelled by Anethole	R_f value 0f Anethole
1	Standard	64mm	0.74
2	FR-1	63mm	0.74
3	FR-2	63mm	0.74
4	FR-3	63mm	0.74
5	Standard	63mm	0.74
6	FOR-1	63mm	0.74
7	FOR-2	63mm	0.74
8	FOR-3	63mm	0.74
9	Standard	63mm	0.74
10	FF-1	63mm	0.74
11	FF-2	63mm	0.74
12	FF-3	63mm	0.74

FR-1 Fennel Raw (Uttar Pradesh); FR-2 Fennel Raw (Gujarat); FR-3 Fennel Raw (West-Bengal) FOR-1 Oleo-resin (Uttar Pradesh); FOR-2 Oleo-resin (Gujarat); FOR-3 Oleo-resin (West-Bengal); FF-1; FF-2; FF-3 Fennel marketed Formulations

In recent years, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics. [16] There are around 6000 herbal manufacturers in India. More than 4000 units are producing Ayurveda medicines. In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process and rationalizing the combination in case of polyherbal drugs. [17] In herbal formulation in general can be standardize schematically as to formulate the medicament using raw materials collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed. [18-19]

The revealed the standardization parameters were studied and these may be used as a tool for the correct identification and standardization of the plant also to test the adulterants if any. The physicochemical parameters evaluated are useful in standardization of the fruits of Fennel. Heavy metals and microbial load for raw materials, oleoresins and finished formulations are found to be within the limit of WHO guidelines, indicating that they are free from pathogens and they are safe to be used in Indian System of medicine. The data obtained from the study would be useful in the identification of the fruits of Fennel and serve as standards.

By this India can emerge as the major country and play the lead role in the production of standardized, therapeutically effective ayurvedic formulation. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques.

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