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Pharmacognostic evaluation and establishment of quality parameters of seeds of *Cuminum cyminum* L.

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Cuminum cyminum L. (Family Apiaceae), is a medium sized annual herb whose all parts are useful. Herbal drug standardization is an essential step in order to assess the quality of drugs, based on the concentration of their active principle, physical and chemical standards. An attempt has been made to highlight this herbal medicine through present study which may assist in standardization for quality, purity and sample identification. Various standardization parameters like morphological features, microscopic evaluation, physico-chemical evaluations (foreign matter, loss on drying, ash values, extractive values), preliminary phytochemical screening, thin-layer chromatography, and fluorescence analysis of powdered seeds were carried out and the qualitative parameters were reported. These studies provided referential information for correct identification and standardization of this plant material.

Keywords: *Cuminum cyminum*, Apiaceae, Physico-chemical evaluations, Quality specifications, Standardization.

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Introduction

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. In the western world, as the people are becoming aware of the potency and side effect of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature[1]. Herbal medicine is the mainstay of health care in several developing countries. Traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as "traditional herbal medicines". In many developing countries, a large proportion of the population rely on traditional practioners and their armamentarium of medicinal plants in order to meet health care needs. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available[2]. According to the World Health Organization, the use of herbal remedies exceeds that of the conventional drugs by two to three times throughout the world. The use of plants for healing purposes predates human history and forms the origin of much modern medicine[3]. Many conventional drugs have originated from plant source, one such plant is *Cuminum cyminum* L. belonging to Family Apiaceae.

C. cyminum is commonly known as 'jeera' in Hindi and 'cumin' in English. It is a thin herbaceous annual plant growing to a height of 1 m. It is found in dry, temperate climate and is cultivated in Central Asia, Europe and many other countries. In India, it is found wild in the North Himalayan region and is cultivated in Kashmir, Garhwal and Chamba at an altitude of 3000 to 4000 m(Ref. 4). It is used as drug in Ayurvedic and Siddha System of medicine and it is the second most popular spice in the world after black pepper. The seeds contain a volatile oil mainly composed of monoterpene hydrocarbons, oxygenated mono- and sesquiterpenes, fatty acids, aldehydes, ketones and esters[5]. The flowers are small, white or pink and borne in umbels. Dried seeds have carminative, stimulant and analgesic effect[6], hence is used as a carminative for stomach disorders, diarrhoea and colic. It is therapeutically supportive in promoting digestion and it is a superb addition to any formula when there is a compromised digestive system so it has long history of use in well established systems of medicines, viz. Ayurveda and Siddha[7]. It possesses antibacterial activity against *Pseudomonas aeruginosa*[8],

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antioxidant[9], antiinflammatory[10] and antidiabetic[11] activity. The present work deals with detailed pharmacognostic and standardization parameters on powdered seeds of *C. cyminum*.

Materials and Methods

Collection and authentication

The seeds of *C. cyminum* were purchased from the shop of Matabadal pansari, Aminabad, Lucknow, in March 2013. The taxonomic authentication was done by National Botanical Research Institute (NBRI), Lucknow, India and the voucher specimen (NBRI-SOP-202) was deposited at NBRI, Lucknow. Seeds were used to study macroscopic and microscopic characters. Purchased plant material was coarsely powdered and used for the determination of ash values, extractive values, and preliminary phyto-chemical investigation as per standard methods.

Extraction of plant material

100 g coarsely powdered seeds of *C. cyminum* was packed in muslin cloth and subjected to Soxhlet extractor for continuous hot extraction with petroleum ether, chloroform, ethanol and distilled water for 8 h separately. Then each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of petroleum ether, chloroform, ethanol and the aqueous extracts was calculated.

Macroscopic and microscopic studies

The macromorphology of the seeds were studied according to standard methods[12-14]. Transverse section of the seed was taken, stained and mounted following usual microtechniques[15] and representative diagrams were taken with the help of inverted microscope for photodocumentation (Leitz, Japan).

Physico-chemical analysis

Physico-chemical analysis i e, extractive values of petroleum ether, chloroform, ethanol and aqueous, fluorescent analysis[16], total ash, acid-insoluble ash, water-soluble ash, foreign matter and moisture content were carried out[17]. Calibrated digital pH meter was used to measure the pH of 1 and 10 % aqueous extracts.

Preliminary phytochemical screening

Preliminary phytochemical screening of petroleum ether, chloroform, ethanol and aqueous extract was carried out for the detection of various compounds by using standard procedures described by Harborne[18] and Khandelwal[19].

TLC and HPTLC

Slurry of silica gel G was prepared in distilled water and poured over a glass plates to form a thin layer. The prepared plates were air dried for setting and then kept in an oven at 100-120 °C (30 min) for activation. The extracts were dissolved in respective solvents and spotted over an activated plate (1 cm above from the bottom). The spotted plates were kept in a previously saturated developing chamber containing mobile phase and allowed to run 3/4th of the height of the prepared plate[20]. The plates were air dried and number of spots were noted and retention factor (Rf) value were calculated. Spots were visualized by respective spraying agents. A number of solvent systems were tried, the maximum resolution showed in Toluene: ethyl acetate for the ethanolic and aqueous extract. The same mobile phase was used for the HPTLC profiles of ethanolic extract.

Results

Macroscopical characteristics

Seed colour is brown, ridges are light in colour and it has a characteristic odour and aromatic taste. It is about 4-6 mm in length and about 2 mm wide in size. Its shape is elongated and tapering at both ends. Cremocarp generally separated. Each mericarp is having fine longitudinal ridges. They contain 5 yellowish, straight primary ridges. Altering with these are secondary ridges which are flat and bear conspicuous emergences. Whole cremocarp and isolated mericarps are attached to short pedicels (Plate 1).

Microscopic characters

The transverse section of the *C. cyminum* seed consists of five primary ridges. Epicarp consists of single layer of elongated cells. The middle layer was mesocarp made up of thin walled parenchymatous cells. Small dark reddish brown cells were present in the mesocarp region, known as vittae. Two types of

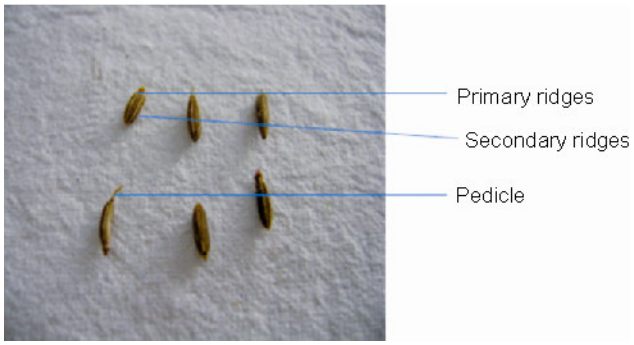

Plate 1—Morphology of *Cuminum cyminum* seeds

the sclereids were found in the mesocarp region, one was single layer, thick walled, longitudinally elongated cells and other was small groups of cells, composed of considerably elongated cells; these sclereids have thickened walls and few pits. Innermost dark coloured endocarp layer and inner to endocarp testa were present; it consists of single layered compact cells. Endosperm contains microspheroidal calcium oxalate crystals (Plate 2).

Physico-chemical parameters

The physico-chemical characters of powdered seeds of *C. cyminum* such as petroleum ether, chloroform, alcohol, and water soluble extractive, ash value, acid insoluble ash, water-soluble ash, loss on drying and foreign matter are presented in Table 1.

The fluorescence analysis in various solvents was performed under normal and Ultra Violet (254 and 366 nm) light (Table 2). The pH of 1 and 10 % aqueous solution were found to be 5.90 and 5.45, respectively.

Preliminary phyto-chemical screening

The preliminary phyto-chemical investigation of the petroleum ether, chloroform, ethanol and aqueous extracts showed the presence of tannins, flavonoids, proteins, amino acids, and glycosides (Table 3).

TLC and HPTLC

TLC of the aqueous and ethanolic extracts were carried out separately using toluene : ethyl acetate (7.5 : 2.5) for the ethanolic extract and toluene : ethyl acetate (8 : 2) for the aqueous extract as mobile phase, respectively and the Rf values were recorded and depicted in Table 4. The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots. The preliminary HPTLC studies revealed that the solvent system

Table 1—Physico chemical parameters of seeds of *Cuminum cyminum*

Quantitative parameters	Values obtained % (w/w)
Petroleum ether extractive	2.86
Chloroform extractive	4.20
Ethanol extractive	4.43
Water soluble ash	5.91
Total ash	7.00
Acid insoluble ash	0.83
Water soluble extractive	2.50
Loss on drying	10.9
Foreign matter	0.233

Table 2—Fluorescence analysis of powdered seeds of *Cuminum cyminum*

Solvents Used	Observation		
	Day Light	UV 254nm	UV 366nm
Drug powder as such	Light brown	Brown	Dark brown
Petroleum ether	Dull yellow	Dark brown	Dark brown
Chloroform	Pale yellow	Yellow	Yellowish brown
Ethyl acetate	Pale yellow	Brownish yellow	Yellowish brown
Toluene	Yellow	Intense yellow	Brownish black
Acetone	Light yellow	Yellow	Dark yellow
Ethanol	Yellowish brown	Dark yellow	Black
Distilled Water	Light brown	Dark brown	Dark brown
Conc. H_2SO_4	Dark brown	Light yellow	Dark brown
Conc. HNO_3	Yellow	Dark yellow	Dark brown

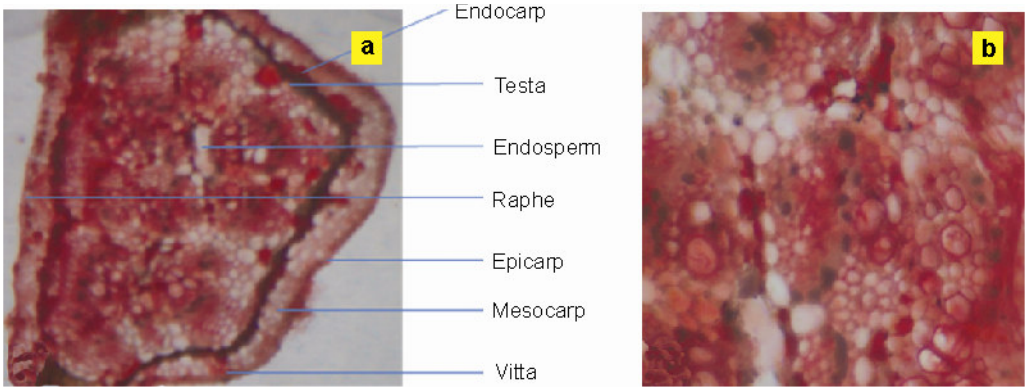

Plate 2—(a) T.S. of *Cuminum cyminum* seed (Mericarp) (b) Enlarged view of endosperm

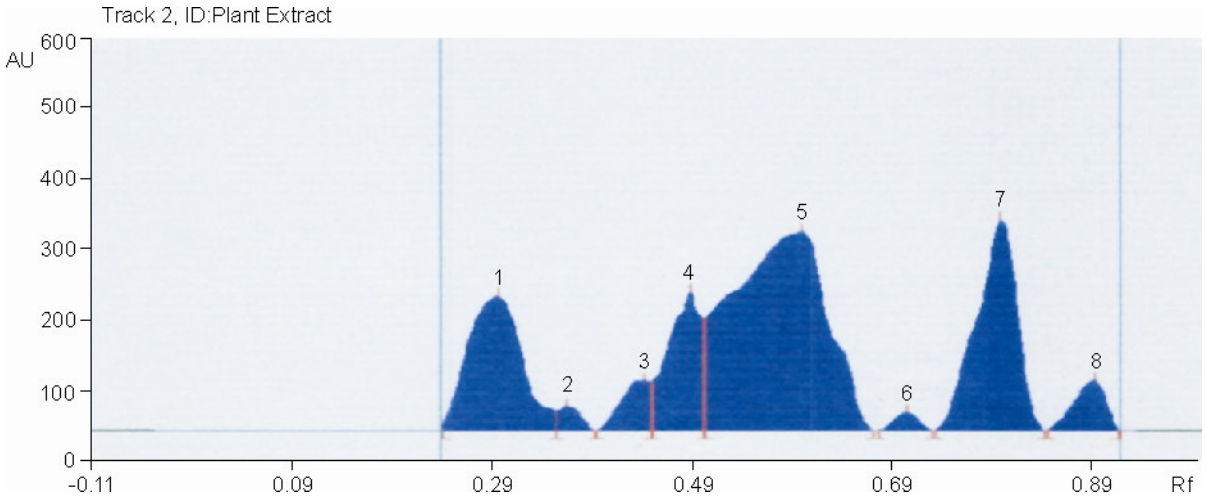

Fig. 1—HPTLC Finger printing of ethanolic extract of seeds of *Cuminum cyminum* L. scanned at wavelength 366nm

Table 3—Qualitative analysis of photochemical in seeds of *Cuminum cyminum*

	Petroleum ether	Chloroform	Ethanol	Aqueous
Sterols	+	+	+	-
Tannins	-	+	+	-
Flavonoids	-	-	+	+
Proteins	-	+	+	+
Amino acids	-	+	+	+
Glycosides	-	-	+	+
Phenolic compounds	-	-	+	-
Carbohydrates	-	-	+	+
Saponins	-	-	-	-
Alkaloids	-	+	+	-

+ = Present, - = Absent

Table 4—Thin layer chromatography of *Cuminum cyminum*

Test extracts	Solvent system	Number of spots	Rf value
Ethanolic extract	toluene:ethyl acetate (7.5:2.5)	4	0.29, 0.60, 0.75, 0.87
Aqueous extract	Toluene:ethyl acetate (8:2)	3	0.34, 0.42, 0.70

toluene: ethyl acetate (7.5: 2.5) was ideal and gave well resolved sample peaks. The spots of the chromatogram were visualized at 366 nm (Fig. 1).

Discussion

Seeds of *C. cyminum* were subjected to various analytical techniques. The macroscopic examination of seeds of *C. cyminum* was done. Macroscopic evaluation is a technique of qualitative evaluation based on the study of morphological and organoleptic characters of drugs. The microscopic characters determine the histological profile of the seed and can serve as a diagnostic parameters[21]. The extractive values, ash value, loss on drying and fluorescent analysis of the seed extract have been carried out. Percentages of the extractive values were calculated with reference to air-dried powdered seeds. The extractive values in different solvents indicate the amount and nature of constituents in the extracts. The value is also helpful in estimation of specific constituents soluble in particular solvent. The fluorescence analysis of the powdered seeds of *C. cyminum* in various solvents was performed under normal and UV light to detect the fluorescent compounds. TLC is particularly valuable for the preliminary separation and determination of plant constituents.

Conclusion

Phytochemical standardization of a plant material is essential to study about its pharmacological activities, therefore present work focuses on the pharmacognostical and phytochemical investigation of *C. cyminum* seeds. The evaluation of these parameters is an essential criteria to ensure that the consumers get the medication, which guarantee purity, safety, potency and efficacy, before proceeding for its toxicological and pharmacological evaluation. The result indicates that the standardization and preliminary phytochemical screening of *C. cyminum* L. seed yielded a set of

standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. This study is a substantial step and further long term study is required to evaluate therapeutic efficacy and toxicity of seed, to establish as the drug.

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