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PHARMACOGNOSTIC STANDARDIZATION, PHYSICO AND PHYTOCHEMICAL EVALUATION OF *NIGELLA SATIVA* LINN. SEED

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Keywords:

Nigella sativa Linn., Pharmacognostic standardization, Physicochemical evaluations, Phytoconstituents, TLC (Thin Layer Chromatography), Rf (Retention Factors), Alkaloids, Steroids, Glycosides, Sugars

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ABSTRACT

Context: *Nigella sativa* Linn. (Ranunculaceae) is native to southwest Asia. This tree species has been of interest to researchers because it has a long history of folklore usage in various systems of medicines.

Objective: Pharmacognostic Standardization, Physico and Phytochemical Evaluation of the seeds of *N. sativa* were carried out to determine its macro-and microscopical characters and also some of its quantitative standards.

Materials and Methods: Microscopical studies were done by using trinocular microscope. Total ash, water-soluble ash and acid-insoluble ash values; Alcohol-and water-soluble extractive values were determined for physico-chemical evaluations. Preliminary phytochemical screening was also done to detect different phytoconstituents.

Results and Discussion: Microscopically, seed showed epidermis with parenchymatous cells, papillae and reddish brown pigmented layer of cells; endosperm with starch grains, oil globules and embryo. Total ash was approximately, 32 (thirty two) and 3 (three) times more than acid insoluble and water soluble ash, respectively. Water soluble extractive was approximately 1 times more than Ethanol soluble extractive. T.L.C. of Petroleum-ether extract using Benzene: Ethyl acetate (6:1), showed five spots. In the chloroform extract, using Benzene: Ethyl acetate (4:1), five spots and in ethanol extract, using Chloroform: Methanol (93:7), six spots were observed using Iodine vapor as a viewing medium. Phytochemically, seed exhibited alkaloids, glycosides, steroids and sugars.

Conclusion: These findings might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

INTRODUCTION: *Nigella sativa* commonly known as karayal (English: Small Fennel, Black Cumin; Sanskrit: Kalonji, Kalajira, Kalajaji, Mugrela, Upakuncika) is an annual flowering plant, native to southwest Asia. The plant is indigenous to the Mediterranean region but now found widely in India (Jammu, Kashmir, Himachal Pradesh, Bihar, Assam and Punjab). The herb is also cultivated in Bengal and north-east India [1]. It is active as an aromatic, respiratory stimulant, diuretic, hypoglycemic, anti- tumor and an analgesic [2]. Seeds have anti-inflammatory, analgesic, antipyretic, antimicrobial and antineoplastic activity and used in folk (herbal) medicine all over the world for the treatment and prevention of asthma, diarrhoea and dyslipidaemia. Its oil decreases blood pressure and increases respiration [3].

The main aim of the present work is to study the macro, microscopic and some other pharmacognostic characters and physico-chemical standards of seeds of *N. sativa* Linn. which could be used to explore this plant.

MATERIALS AND METHODS:

Plant Material: The plant specimens for the study were collected from the sour of the Arpa river, Bilaspur (Chhattisgarh, India) 22°06'35.83"N and 82°08'06.23"E and were positively identified and authenticated by the Botanist Dr. Shiddhamallaya N, Regional Research Institute (Ay.), Central council for research in Ayurveda and Siddha, Ashoka pillar, Jayanagar, Bangalore. A voucher specimen no. (RRCBI/mus.5-27), Reference no. (RRI/BNG/SMP/ Drug Authentication/2008-09/959), dated 28/02/2009. Care was taken to select healthy fully grown plant with normal organs. The samples of different organs were cut suitably and removed from the plant and thoroughly washed with water to remove the adherent impurities and dried in sunlight.

Macroscopical characterization: Macroscopical studies of seed were done by naked eye and shape, color, taste and odor of seed were determined and reported.

Microscopical characterization:

Sectioning: Selected samples of the dried seed were stored in a solution containing formalin (5 ml), acetic acid (5 ml) and 70% v/v ethyl alcohol (FAA) (90 ml). After 24 (twenty four) hours of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the method [4]. Infiltration of the specimens was carried by gradual addition of paraffin wax (50-60˚C, m.pt.) until tertiary- Butyl alcohol solution attained supersaturation. The specimens were casted into paraffin blocks. The paraffin-embedded specimens were sectioned with the help of Senior Rotary Microtome, RMT-30 (Radical Instruments, India). The thickness of the sections was kept between 10 and 12µm. The dewaxing of the sections was carried out as per the procedure described by Johanson [5]. The section was stained with phloroglucinol-hydrochloric acid (1:1) and mounted in glycerin [6].

Photomicrograph: Microscopic descriptions of selected tissues were supplemented with micrographs. Photographs of different magnifications were taken with Nikon Lab Photo 2 (Two) Microscopic unit. For normal observations, bright field was used. For the study of starch grains, polarized light was employed. Since this structure has birefringent property under polarized light they appear bright against dark background [7].

Physico-chemical evaluations: Physicochemical parameters of *N. sativa* seed powder were determined [8] and reported as total ash, water-soluble ash and acid-insoluble ash. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol

soluble components. The moisture content and pH was also determined.

Preliminary phytochemical Screening: The coarse seed powder of *N. sativa* (25g) was subjected to soxhlet for successive solvent extraction. Extract were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents [9, 10].

RESULTS:

Macroscopical Study: Seeds were flattened, oblong, angular, rugose tubercular, small, funnel shaped, 0.2 cm long and 0.1 cm wide. It had black color, slightly aromatic odor and bitter taste (**Fig. 1**).

FIG. 1: EXTERNAL MORPHOLOGY OF *N. SATIVA* SEED

Microscopical Study: Transverse section of seed showed epidermis and endosperm (**Fig. 2**).

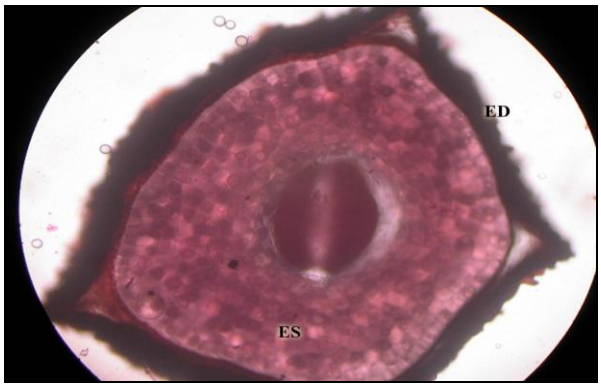

FIG. 2: MICROSCOPICAL VIEW OF T. S. OF *N. SATIVA* SEED AT 10X X 10X. [ED: Epidermis, ES: Endosperm]

Epidermis: It was single layered consisting of elliptical, thick walled cells covered externally by a papilla's cuticle, filled with reddish-brown content; epidermis followed by 2-4 (two to four) layers of thick walled, tangentially elongated; parenchymatous cells, followed by a pigmented layer composed of tangentially elongated, cylindrical thick walled cells filled with reddish brown pigment. Below pigmented layer, parenchyma composed of thick walled, rectangular, radially elongated cells, present in a layer (**Fig. 3**).

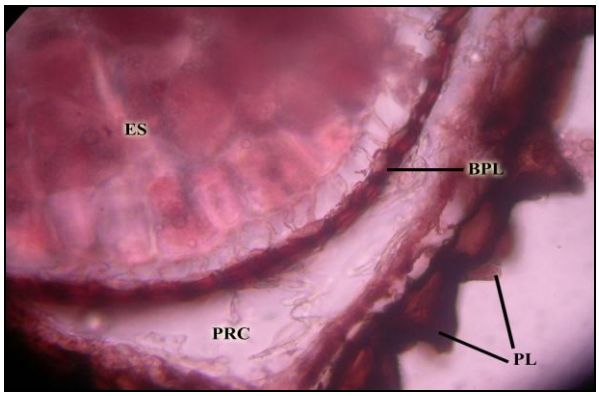

FIGURE 3: MICROSCOPICAL VIEW OF T. S. OF *N. SATIVA* SEED AT 10X X 40X. [PL: Papillae, PRC: Parenchyma, BPL: Brown Pigment Layer, ES: Endosperm]

Endosperm: It consists of moderately thick walled, rectangular to polygonal cells, a few filled with oil globules and starch grains; embryo embedded in endosperm (**Fig. 4 & 5**).

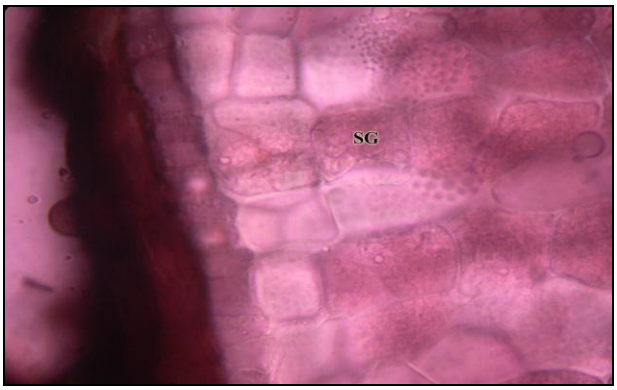

FIG. 4: MICROSCOPICAL VIEW OF T. S. OF *N. SATIVA* SEED AT 10XX40X. [SG: Starch Grains]

FIG. 5: MICROSCOPICAL VIEW OF T. S. OF *N. SATIVA* SEED AT 10X X 40X. [OG: Oil Globules]

Physicochemical Parameters: *P. zeylanica* seed powder showed the presence of total ash- 4.82 % w/w, acid-insoluble ash- 0.15 % w/w, water-soluble ash- 1.71 % w/w, water-soluble extractive- 11.59 % w/w, alcohol-soluble extractive - 9.16 % w/w, moisture content- 2.91 % and pH- 6.6 (**Table 1**).

Preliminary Phytochemical Studies: Phytochemical analysis showed the presence of steroid in chloroform extract. Alcohol extract showed positive report for alkaloids, glycosides and sugars (**Table 2**). T.L.C. of Petroleum-ether (60-80°C) extract of drug on Silica gel 60 F_{254} pre coated sheets using Benzene: Ethyl acetate (6:1) showed five spots in Iodine vapor. In the chloroform extract, using Benzene: Ethyl acetate (4:1), five spots and in ethanol extract, using Chloroform: Methanol (93:7) solvent system, six spots were observed using same viewing medium (**Table 3**).

TABLE 1: PHYSICOCHEMICAL ANALYSIS OF *NIGELLA SATIVA* LINN. SEED

Physicochemical parameters	Value Mean±SE.
Total Ash	4.82 % w/w
Acid insoluble ash	0.15 % w/w
Water soluble ash	1.71 % w/w
Water soluble extract	11.59 % w/w
Ethyl alcohol soluble extract	9.16 % w/w
Moisture content	2.91 %
pH	6.6

* w/w: weight/weight

TABLE 2: PHYTOCHEMICAL ANALYSIS OF *NIGELLA SATIVA* LINN. SEED

Test for constituents	Petroleum ether extract	Chloroform extract	Ethyl alcohol extract
Alkaloid	Negative	Negative	Positive
Steroid	Negative	Positive	Negative
Terpene	Negative	Negative	Negative
Flavonoid	Negative	Negative	Negative
Glycoside	Negative	Negative	Positive
Sugars	Negative	Negative	Positive
Saponin	Negative	Negative	Negative
Tannin	Negative	Negative	Negative
Color &Consistency	Colorless oily	Very light yellow oil	Yellow gum

* Positive: present, Negative: absent

TABLE 3: TLC PATTERN OF VARIOUS EXTRACT OF *NIGELLA SATIVA* LINN. SEED

Extractives	Adsorbent	Solvent system	Viewing medium	R_f Values
Petroleum-either 60-80°C	Silica gel 60 F_{254} pre coated sheets	Benzene: Ethyl acetate (6:1)	Iodine vapor	0.11, 0.38, 0.65 0.78, 0.85
Chloroform	Silica gel 60 F_{254} pre coated sheets	Benzene: Ethyl acetate (4:1)	Iodine vapor	0.15, 0.50, 0.56 0.65, 0.81
Ethanol	Silica gel 60 F_{254} pre coated sheets	Chloroform: Methanol (93:7)	Iodine vapor	0.06, 0.09, 0.13 0.54, 0.75, 0.85

DISCUSSION: The macroscopic study of seed indicated that its color, odor and taste may be an important characteristic feature for identifying the plant. The anatomy of the seed was studied by taking transverse section. Transverse section of the seed showed epidermis with parenchymatous cells, papillae and reddish brown pigmented layer of cells. Endosperm consists of moderately thick walled, rectangular to polygonal cells, a few filled with oil globules and starch grains; embryo embedded in endosperm (Figure 1-5).

Total ash was approximately, 32 (thirty two) times and 3 (three) times more than acid insoluble ash and water soluble ash respectively. Water soluble extractive was approximately 1 times more than Ethanol soluble extractive (Table 1).

Phytochemically, seed was found to contain alkaloids, glycosides, steroids and sugars (Table 2). T.L.C. of Petroleum-ether extract using Benzene: Ethyl acetate (6:1), showed five spots. In the chloroform extract, using Benzene: Ethyl acetate (4:1), five spots and in ethanol extract, using Chloroform: Methanol (93:7), six spots were observed using Iodine vapor as a viewing medium (Table 3).

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14 % w/w [11]. The ash values, extractive values and moisture content of seeds were determined. The results are depicted in Table 2**.** Pharmacognostic standardization including physico-chemical evaluation in Table 1 and 2 is meant for identification, authentication, and detection of adulteration and also compilation of quality control standards of crude drugs [12]. Since the plant, *Nigella sativa* Linn. is useful in traditional medicine for the treatment of various ailments, it is important to standardize it for use as a drug.

The Pharmacognostic constants for the seeds of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

CONCLUSION: The present study on pharmacognostic standardization, physico and phytochemical evaluation of *Nigella sativa* Linn. seed might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

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