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Morphological, physicochemical and preliminary phytochemical evaluation of *Nigella sativa* L. seeds

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Abstract
The present study deals with morphological characteristics, physicochemical factors and preliminary phytochemical screening of *Nigella sativa* (NS) seeds collected from Bhopal region of Madhya Pradesh, India. NS is an annual herb of Rannunculaceae family which grows up to height of 20-60 cm in height. The plant seeds show diverse chemical composition which impart its high therapeutic value against various kinds of diseases and ailments. The NS seeds at macroscopic levels were found compressed, oval, angular, rugose tubercular, small cone shaped, 0.19- 0.2 cm long and 0.08-0.1 cm wide. Organoleptic characters observed by sensory organs revealed black colour, slightly aromatic odor and bitter taste. Microscopic observation of seeds showed the presence of lignified fibers, oil globules, starch grains and endosperm. Physicochemical parameters such as total ash, water soluble, acid soluble and sulphated ash yield percentage w/w were found as 4.94±0.03%, 2.12±0.90%, 3.03±0.04% and 1.21±0.34%, respectively. Preliminary phytochemical screening of extracts showed presence of glycosides, saponins, proteins, flavonoids, terpenoids, fixed oil, steroids and alkaloids.

Keywords: *Nigella sativa*, macroscopic, microscopic, physicochemical factors, organoleptic characters

Introduction
Medicinal plants plays important role in curing various kinds of diseases and ailments. The world health organisation report in 2001 reported that 60% of the world's population rely on traditional medicine among which 80% of the population in developing countries depend extremely on traditional medical practices like herbal medicines for their primary health care practices [1]. *Nigella sativa* is an annual flowering herb of Rannunculaceae family grows up to 20-60cm in height with coarsely divided tongue shaped leaves. The plant bears flowers of different colours with light blue and white as predominant ones. The fruit is of capsular type bearing black trigonal seeds. It is commonly known as kalongi, (English: black cumin /black caraway, Arabic: Habba-al-Barakah, Farsi: Sia danah, Hebrew: Ketzah, French: Cheveux de Venus, Turkish: Siya susam, Tibetan: Zira nagpo and Italian: Nigekka). N. sativa is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia [2]. In India it is found widely cultivated in states of Jammu and Kashmir, Punjab, Himachal Pradesh, Assam and Bihar [3]. *N. sativa* seeds has been found with diverse chemical composition with high nutritional value and pharmacological properties [4]. The proximate chemical composition of *N. sativa* seeds has been reported as protein 21%, Fat 32%, carbohydrates 37%, Ash 4%, moisture 6% and crude fibres 6.6%. [5]. The major active chemical constituents found in NS seeds is mono-oxygenated terpene thymoquinone followed by other compound such as dithymoquinone, thymohydroquinone, thymol, p-cymene, carvacol terpineol, longifolene, α-pinene, α-hederin, Sabinene, α-Thujene, Myrcene, α-Phellandrene, Limonene, γ-Terpinene, Fenchone, Dihydrocarvone, Carvone, sesquiterpenoid α-Longipinene. It has been also found with saturated and unsaturated fatty acids like plamitic acid, linoleic acid, oleic acid and eicodadienoic acid. Other components reported are sterols like campesterol, Stigmasterol, βSitosterol, vitamins tocopherol, thiamin, riboflavin, pyridoxine, niacin, Folic acid and inorganic elements P, Ca, Fe, Cu and Zn [6-8]. Seeds oil also contain alkaloids isoquinoline type Nigellimine, Nigellimine- N-oxide [9], indazole ring type nigellicine, nigellidine[10] and dollabene type Nigellimines (A1-A5) and Nigellimines (B1- B2) [11].

N. sativa is also referred as miracle herb of century because it holds so many health curing properties. Avicenna (Ibn Sina) referred N. sativa in his book ''The Canon of Medicine'', as seeds stimulate the body's energy and helps recovery from fatigue and dispiritedness. The black seeds are traditionally used to treat the ailments like asthma, arthritis, backache, diabetes, diarrhea, dry cough, rhinitis, hair greying, hair loss, dry cough, hypertension, fatigue, memory improvement, muscle aches, anxiety, sexual impotency, Insomnia, toothache, gum disease , amenorrhea and dysmenorrhea, anthelminthic and skin eruptions [12]. However, extensive work by researchers has found large number of pharmacological properties of N.sativa such as anti-diabetic activity [13], anticancer activity [14], anti-microbial activity [15], anti-hyperlipidemic activity [16], anti-inflammatory [17], Cardio-protective activity [18], Anti-schistosomiasis [19], Anti-oxytocic potential [20], Neuro-protective activity [21], Nephro-protective activity [22], Anxiolytic activity [23], Anti- nociceptive activity [24], Anticonvulsant[25] and many other medicinal properties.
The present study is based on the macro, microscopic and some other pharmacognostic characters and physico-chemical standards of seeds of N. sativa which could be used to find any type of adulterant in plant material so that sample safety and efficacy can be increased.

Material and Methods

Plant material: the seed samples of the plant were purchased from local herbal stores of Bhopal (Madhya Pradesh, India).The samples collected were positively identified and authenticated by botanist Dr. Tayyab Saifi, ret. principal, Safia science college Bhopal (M.P). The Seed samples were washed comprehensively washed with water to remove the dust and other adulterants followed by drying in an oven in presence of air at 30 ^{0}C.

Macroscopic characterization: the sample shape, size and colour which were seen to naked eye were observed.

Organoleptic characterization: the taste, smell and odor of the sample were determined by sensory organs.

Microscopic characterization: The seed samples were soaked in distilled water for about 10-12 hours followed by storage in FAA (formalin (5ml), Acetic acid (5ml) 70% Ethyl alcohol (90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of 30 - butyl alcohol[26]. The specimens were infiltrated with paraffin wax at temperature of 65 – 75 ^{0}C by standing container in hot water bath until tertiary butyl alcohol gets saturated with wax. The specimens were casted into paraffin blocks and Senior Rotary Microtome (RMT-30) was used make sections which were 10- 15 µm in thickness. The dewaxing of the sections was carried out as per the procedure described by Johanson [27]. The section was stained with phloroglucinol-hydrochloric acid (1:1) and mounted in glycerin [28].

Photomicrographs: Microscopic images of selected tissues were enhanced by micrographs. Photographs of different magnification (10x x 10x, 10x x 40x) were taken (Olympus 'CH20I' Trinocular) Microscopic unit. For common microscopic analysis, normal unpolarised light was used however, for observation of starch grains, oil globules plane polarized light was used due to which they appear bright against dark background [29].

Physico-chemical parameters: The Physiochemical evaluation was performed according to Ayurvedic Pharmacopoeia of India [30] and were reported as total ash, acid insoluble ash, water soluble ash, Sulphated ash, acid and water soluble extractive value, moisture content, pH and loss on drying. The values were calculated as yield percentage (w/w) whereas, loss on drying was calculated by formula:

$$\text{Percentage Loss} = \frac{\text{Weight of Plant Material after drying (Y)}}{\text{Weight of Plant Material Collected (X)}} \text{x100}$$

Preliminary phytochemical screening: The dried seed samples were coarsely grinded to powder with particle size of 0.5 to 1.0-nm by Wiley Mill grinder (Mill model 4) to ensure homogeneity of sample. The powered sample of about 20 g were subjected to soxhlet for successive solvent extraction. The sample were extracted with petroleum ether, ethyl acetate, methanol and water for 36-48 hours. The extraction process was followed by filtration of extract with whatman grade-1 filter paper. The filtered extracts were concentrated by removal of solvents from extracts with the help of vaccum rotary evaporator (Buchi rotavapor RE-111and water bath model B-46) at 40 ℃. the condensed extracts were subjected to chemical tets to detect the phytochemicals present in the extracts. The presence of a different phytochemical groups were detected by occurrence of particular color [31-32].

Test for Alkaloids

***Hager's test*:** The extracts of 1 ml quantity were separately treated with 3ml Hager's reagent. Yellow precipitate formation indicates presence of alkaloids.

Wagner's test: All the extracts in 2 ml quantity were treated with 1.5 ml of 1% of HCl followed by addition of few drops of Wagner reagent (solution I_2 and KI). Formation of orange colored precipitate indicates presence of alkaloids.

Dragendorff's test: the extracts were separately taken as 1 ml to which 0.2ml of reagent (potassium bismuth iodide solution) was added. The presence of alkaloids is perceived by formation of orange-red precipitate.

Mayer's test: All extracts were taken in quantities of 1 ml and were treated with 1 ml of mayer's reagent (Potassium mercuric iodide solution). Presence of alkaloids was detected by Whitish yellow or cream coloured precipitate.

Test for flavonoids

***NaOH test*:** the extracts in minimal amount and were treated with NaOH $_{(aq)}$ and HCL, formation of orange colour indicates presence of the flavonoids.

Zinc Hydrochloride Test: the extracts were added to a mixture of Zn dust and conc. HCl. The flavonoids are confirmed by emergence of red color.

Shinoda test: the individual extracts of about 0.5 ml were dissolved in ethanol, warmed and then filtered. The filtrates were further treated with conc. Hcl and Mg ribbons. Formation of cherry red colour indicates the presence of

flavonones or orange red colour indicates the presence of flavonols.

Test for terpenoids

Libermann-Burchard test: The individual extracts of 1 ml were treated with chloroform, acetic anhydride and few drops of H_2SO_4. The presence of terpenoids is confirmed by dark green color.

Salkowski test: The discrete extracts about 0.5gm were treated with 2ml of $CHCl_3$ and a few drops conc. H_2SO_4. The interface were observed for reddish brown which indicates the presence of terpenoids.

Test for glycosides

Legal test: The extracts were dissolved in pyridine and nitroprusside reagent was added to the solution. The presence of glycosides is confirmed by formation of pink red to red color.

***Borntrager's test*:** the extracts were hydrolyzed by conc. HCl on a water bath for 2 hours followed by filtration of extracts. The filtrate was treated with chloroform and 10% ammonia, the formation of red color confirms the presence of glycosides.

Test for Saponins

Foam test: The solution was prepared by mixing 0.5gm extracts separately, 5ml of distilled water and few drop of olive oil in a test tube. The solution was shaken strongly, formation of an emulsion confirms saponins.

Heamolytic Test: Sample was added to one drop of blood placed on glass slide. Hemolytic zone confirms the presence of saponins.

Test for fixed oils and fats

***Spot test*:** The extracts in minimal quantities were separately pushed through filter paper. Oil stains on paper indicates the presence of fixed oils.

***Saponification test*:** All extracts taken in 1ml quantity and were mixed with a few drops of 0.5N KOH and phenolphthalein. The mixtures were heated on water bath for 1-2 hours. The fixed oils and fats were detected by formation of soap or partial neutralization of KOH.

Test for Protein and Amino Acids

***Biuret test*:** Add 1 ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution till a blue color is produced, and then add to the 1 ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

***Ninhydrin test*:** Add two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides, or amino acids.

Millon's test: 1ml of test solution was made acidify with sulphuric acid and added Millon's reagent and boiled this solution. A yellow precipitate was formed indicated the presence of protein

Test for Steriods & Triterpenes

Libermann-Buchard test: Extract is treated with few drops of acetic anhydride followed by addition of con. Sulfuric from the sides of the test tube. The formation of two layers appears with brown colored ring at the junction of two layers. Formation of blue green upper layer confirms the presence of Steroids whereas, formation of deep red color indicates the presence of triterpenoids.

Salkowski test: Treat extract in Chloroform with few drops of cone. Sulfuric acid, shake well and allow standing for some time. Formation of red color at the lower layer indicates the presence of Steroids whereas, yellow colored lower layer indicates the presence of triterpenoids

Test for tannins

Ferric chloride test: little amount of extract was dissolved in distilled water to this solution 2 mL of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins

Test for phenol

Ferric chloride test: About 0.2 g of extracts were treated with 5% ferric chloride and observed for the formation of deep blue color which indicates the presence of phenol.

Results and Discussion

Macroscopic evaluation: The seeds observed were trigonus, angular, regulose-tubercular, cone shapped, outside black and white inside, length 2-3.2 mm and width 1-2 mm (fig. 1 and 2).

Organoleptic characters: the odor of seeds was found was slightly aromatic with bitter taste.

Fig 1: capsule containing *Nigella sativa* L. seeds

Fig 2: External morphology of *Nigella sativa* L.

Microscopic study: the transerve sections of seeds showed presence of Edipermis and endosperm (fig. 3, 4 and 5).

Fig 3: microscopic view of seed

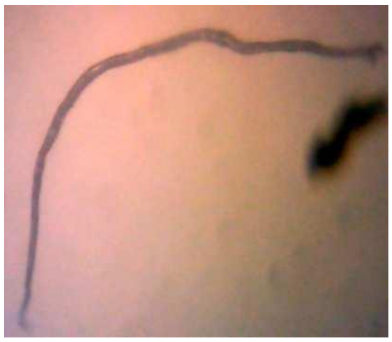

Fig 4: fibers present in seed

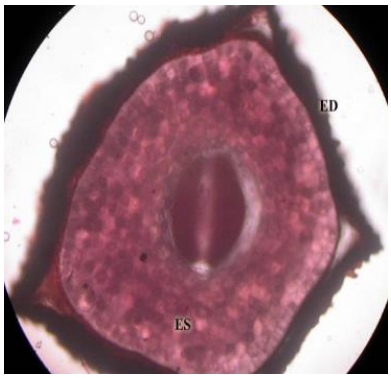

Fig 5: transverse section showing Epidermis and endoderms of *Nigella sativa* L. seeds

Epidermis: it is consisted of elliptical thick walled cells which are covered by thick papillose cuticle from outside. Cuticle is composed of dark brown substances. Inner to epidermis there occurs 2-4 layers parenchyma cells which are laterally elongated. Parenchyma cell layers are followed by rectangularly elongated thick walled layer of cells containing dark brown pigments. Pigmented layer is followed by thick walled rectangular elongated or nearly columnar, elongated cells (fig. 6).

Endosperm: it is consisted of thin walled, rectangular or polygonal cells mostly filled with oil globules and starch grains. The powder microscopy of seed powder shows brownish black, parenchymatous cells, starch grains and oil globules (Fig.7 and 8).

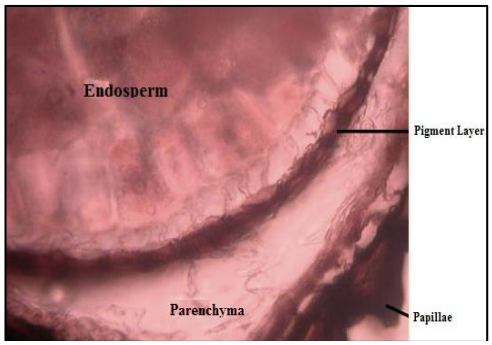

Fig 6: *Nigella sativa* seed Parenchyma, Papilla, Pigment Layer and Endosperm

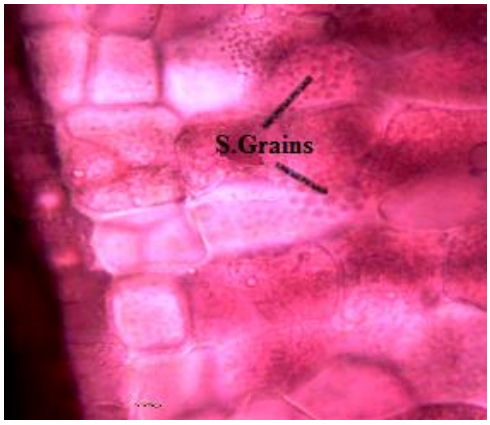

Fig 7: starch grain present in *Nigella sativa* seeds.

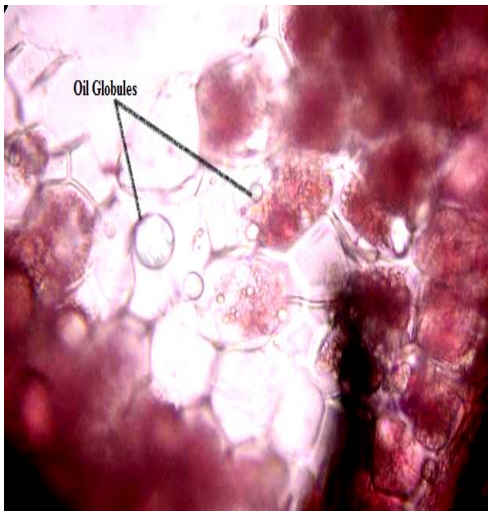

Fig 8: Oil globule present *Nigella sativa* seeds

Physicochemical evaluation: Physicochemical parameters such as total ash, water soluble, acid soluble and sulphated ash yield percentage w/w were found as 4.94±0.03%, 2.12±0.90%, 3.03±0.04% and 1.21±0.34%, respectively. The moisture content and pH were also determined which were found 2.35±0.13 and 6.4, respectively (Table 1).

Table 1: Physico-chemical evaluation of Nigella sativa seeds

Physicochemical parameter	Yield percentage (w/w) ± SE
Total ash	4.94±0.03
Water Soluble ash	2.14±0.91
Acid Soluble ash	3.05±0.02
Sulphated ash	1.23±0.32
Water Soluble Extractive Value	16.28±0.59
Alcohol Soluble Extractive Value	10.47±0.15
Loss on Drying	7.22±0.27
Percentage Loss	30.0±0.07
Moisture content	2.35±0.13
pH	6.4

The physicochemical parameters plays important role in determining purity and efficiency of a particular drug. These parameters simply determine adulteration or improper handling of a particular drugs. Moreover, the high moisture content of a drug increases growth of fungi and bacteria thereby reduces quality of particular drug. *Nigella sativa* has low moisture content 2.5 which is even lower than the normal required moisture content of 14% w/w for a crude drug. Since *Nigella sativa* seeds has numerous pharmacological properties so it becomes necessary to remove adulterants from sample so that drug quality can be improved to high extent.

Preliminary Phytochemical screening: Yield percentage (w/w) of pet ether extract was found highest about 34± 0.15% followed by methanol extract 34.25 ± 0.49%, ethyl acetate extract 30.76±0.64% and water extract 25.20±0.36% (Table 2). Physical parametrs of extracts such as color, odor, consistency and smell are shown in (Table 3). Phytochemical screening of extracts showed the presence of bioactive compounds which have high therapeutic value. Steroids were present in all extracts. Pet. Ether and Ethyl acetate extracts were found to contain terpenoids and tannins in higher quantities. Methanolic and water extracts were found to contain alkaloids. Positive reports of glycosides, phenols and flavonoids were also seen in extracts (Table 4).

Table 2: Extraction parameters and yield percentage of extracts

Solvent used	Temperature	Extraction time	Yield (%) (w/w)
Petroleum ether	40-60˚C	36 hrs.	34.30 ± 0.15
Ethyl acetate	30-40 °C	36 hrs.	30.76 ± 0.64
Methanol	45-50˚C	48 hrs.	34.25 ± 0.49
Water extract	25-30 ºC	32 hours	25.20 ± 0.36

Table 3: Physical parameters of *Nigella sativa* extracts

Parameter	Pet. ether extract	Ethyl acet. extract	Methanolic extract	Water extract
Color	Dark-Brown	Light- brown	Reddish brown	Non specific
Odor	Non-specific	Pungent	Fruity	--
Consistency	Sticky	Sticky	Sticky	Non sticky
Taste	Bitter	Spicy	Sweet	Tasteless

Table 4: phytochemical screening results of *Nigella sativa* extracts

Phytochemical type	Pet. Ether extract	Ethyl acetate extract	Methanol extract	Water extract
Alkaloids	-	-	+	+
Flavonoids	-	-	+	-
Terpenoids	+	+	-	-
Glycosides	-	-	+	-
Saponins	-	-	-	-
Fixed oil	+	+	+	-
Proteins	-	-	-	-
Steroids	+	+	+	+
tannins	+	+	-	-
Phenol	-	-	+	-

*+ = présent *- = absent

Conclusion

The study conducted on morphological characters, physicochemical and preliminary phytochemical screening of *Nigella sativa* L. seeds was purely based on different parameters which are used to identify adulterants and phytochemicals present in seeds. The information obtained from the preliminary phytochemical screening will reveal the useful findings about chemical nature of the drugs. The seeds are rich in secondary metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. The study might provide useful information about such parameters which are used to identify different phytochemicals and impurities in a drug so that its quality can be improved

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