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NIGELLA SATIVA – AN ETHNOMEDICAL REVIEW

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INTRODUCTION

Nigella sativa (*N. sativa*) (Family Ranunculaceae) commonly known as black seed, have been used for thousands of years as a spice and food preservative, as well as a protective and curative remedy for several disorders. Traditionally, there is a common Islamic belief that blackseed is a universal remedy for all ailments, but cannot prevent aging or death. Blackseed is also known as the curative black cumin in the Holy Bible and is described as Melanthion by Hippocrates and Dioscorides and as Gith by Pliny. During the last two decades, many studies have been conducted, on the effect of *N. sativa* seed extracts on various body systems in vitro or in vivo. Seed extracts reveal a broad spectrum of pharmacological activities including immunopotentiation and antihistaminic, antidiabetic, anti-hypertensive, anti-inflammatory, and antimicrobial activities. Many of these activities have been due to the quinone constituents of the seed.[1]

Taxonomic classification

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Ranunculales
Family	Ranunculaceae
Genus	Nigella
Species	N. sativa

Common names

Black cumin, Fennel Flower, Nutmeg Flower, Black seed, Black Caraway, Roman Coriander, Damascena, Devil in-the-bush, Wild Onion Seed.

Habitat

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]

Description

N. sativa is an annual flowering plant grows at 20-90 cm tall, with finely divided leaves; the flowers are white, yellow, pink, pale blue or pale purple color, with 5-10 petals. The fruit is a large and inflated capsule consists of 3-7 united follicles, each containing several seeds.[3] Seeds are small dicotyledonous, trigonus, angular, tubercular, black externally and white inside, odor slightly aromatic and taste bitter.[4] Annual herb which grows about 45 cm in height. Leaves: 2.5-5.0 cm long, linear-lanceolate. Flower pale blue, 2.0-2.5 cm across, solitary on long peduncles; capsule 1.2 cm long; seeds flattened, oblong, angular, funnel shaped, small, 0.2 cm long and 0.1 cm wide, black in colour. Flowering and fruiting occur from January to April. It is generally cultivated on dry soil between November to April and seeds take about 10-15 days to germinate. It can also be propagated from the callus culture *in vitro* from leaf, stem and root explants from aseptically grown seedlings. The seed are small dicotyledonous, trigonus, angular, regulose-tubercular, 2-3.5 × 1-2 mm, black externally and white inside; odor slightly aromatic and taste bitter.[5-12]

Fig. 1: *N. sativa* flower.

Fig. 2: *N. sativa* seeds.

Ethnomedicinal uses

The seeds of *N. sativa* are used in the treatment of various diseases like bronchitis, diarrhea, rheumatism, asthma and skin disorders. It acts as a liver tonic, anti- diarrheal, appetite stimulant, emmenagogue. It is used in digestive disorders, to increase milk production in nursing mothers to fight parasitic infections, and to strengthen immune system.[13] Seeds are also used in food like flavoring additive in the breads and pickles because it has very low level of toxicity.[14] Seeds are useful in the treatment of worms and skin eruptions. Oil is used as an antiseptic and local anesthetic externally. Roasted black seeds are given internally tostop the vomiting.[15]

Pharmacological activities

N. sativa has been extensively studied for its biological activities and shown to possess wide spectrum of activities such as diuretic, antihypertensive, bronchodilator, gastroprotective, hepatoprotective, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, analgesics and anti-inflammatory, spasmolytic, renal protective and antioxidantproperties.

Antibacterial activity

Different crude extracts of *N. sativa* exhibited antimicrobial efficacy against different bacterial strains which comprised either gram negative or gram positive bacteria. Crude extracts of *N. sativa* showed a potential effect against some of the test organisms. The most effective extracts of *N. sativa* were the crude alkaloid and water extracts. Gram negative isolates were more susceptible than the gram positive ones.[1] Hannan*etal.* Investigatedin 2008 theantibacterialactivityof *N. sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. All tested strains of methicillin resistant *Staphylococcusaureus*in hisstudyweresensitiveto ethanolic extract of *N. sativa* at a concentration of 4 mg/disc with an MIC range of 0.2-0.5 mg/mL.[16] In another study antibacterial activity of *N. sativa* against and triple therapy in suppression of *Helicobacter Pylori* in patients with non-ulcer dyspepsia was determined. *N. sativa* seeds exhibited clinically useful anti *H. pylori* activity, comparable to triple therapy.[17]

Antifungal activity

The aqueous extract of N. sativaseeds exhibits inhibitory effect against candidiasis in mice.[18] Antidermatophyte activity of ether extract of N. sativaand thymoquinone was tested against eight species of dermatophytes: four species of Trichophytonrubrumand one each of Trichophytoninterdigitale, Trichophytonmentagrophytes, Epidermophytonfloccosumand

Microsporumcanisusing Agar diffusion method. The ether extract of *N. sativa*and thymoquinone show inhibitory activity against fungal strains. The results show the potentiality of *N. sativa*as a source for antidermatophytedrugs.[19] In another study anti yeast activity of the black cumin seed quinines, dithymoquinone, thymohydroquinone, and thymoquinone were evaluated *in vitro* against six dairy spoilage yeast species. Thymohydroquinone and thymoquinone possessed significant anti yeast activity.[20]

Antioxidant and antiarthritic activity

The antioxidant and antiarthritic activity of thymoquinone in Wistar rat by collagen induced arthritis was evaluated. Oral administration of thymoquinone significantly reduced the levels of pro-inflammatory mediators [IL-1β, IL-6, TNF-α, IFN-γ and PGE (2)] and increased level of IL-10.[21]

Cardiovascular activity

The acute effects of diesel exhaust particles on cardiopulmonary parameters in mice and the protective effect of thymoquinone were studied. Diesel exhaust particles were given to mice, intratracheally. Diesel exhaust particles caused systemic inflammation characterized by leucocytosis, increased IL-6 concentrations and reduced systolic blood pressure. Diesel exhaust particles reduced platelet numbers and aggravated in vivo thrombosis in pial arterioles. In vitro, addition of diesel exhausts particles to untreated blood-induced platelet aggregation. Pretreatment of mice with Thymoquinone prevented diesel exhaust particles induced decrease of systolic blood pressure and leucocytosis, increased IL-6 concentration. Thymoquinone also averted the decrease in platelet numbers and the prothrombotic events but not platelet aggregation in vitro.[22]

Gastro-protective activity

Ischaemia/reperfusion (I/R) induced gastric lesion, model was used to assess the antioxidant effects of *N. sativa* oil and thymoquinone on gastric mucosal redox state and gastric lesions, 1 and 24 h after reperfusion. I/R raised the levels of lipid peroxide and lactate dehydrogenase, while diminished glutathione and superoxide dismutase. These biochemical changes were accompanied by an increase in the formation of gastric lesions, which was reduced by both treatments. *N. sativa* oil normalizes the level of lactate dehydrogenase, reduced glutathione and superoxide dismutase. These results indicate that both *N. sativa* oil and thymoquinone possess gastroprotective effect against gastric lesions which may be related to the conservation of the gastric mucosalredoxstate.[23]

Hepatoprotective activity

Aqueous extract of the seeds of *N. sativa* were evaluated for hepatoprotective activity in male Wistar rats against carbon tetrachloride induced hepatotoxicity. Various biochemical parameters were studied to determine the hepatoprotective potential. Aqueous extract showed significant hepatoprotective effect against carbon tetrachloride-induced toxicity on the liver indicating the hepatoprotective activity.[24]

Contraceptive and anti-fertility activity

Hexane extract of *N. sativa* seeds when orally administered prevented pregnancy in experimental rats at a dose of 2 g/kg daily on day's 1-10 postcoitum.[24] In another study The ethanolic extract of *N. sativa* seeds was found to possess an anti-fertility activity in male rats which might be due to inherent estrogenic activity of *N. sativa.*[26]

Antioxytocic activity

N. sativa seeds oil inhibit the uterine smooth muscle contraction induced by oxytocin stimulation in rat and guinea pig uterine smooth muscles suggest the anti-oxytocic potential of *N. sativa* seeds oil.[27]

Antidiabetic activity

The study was conducted to determine the effects of *N. sativa* seed ethanol extract on insulin secretion in INS832/13 and β TC-tet lines of pancreatic β-cells and on glucose disposal by C_2C_{12} skeletal muscle cells and 3T3-L1 adipocytes. Treatment with *N. sativa* amplified glucose-stimulated insulin secretion by more than 35% without affecting sensitivity to glucose. *N. sativa* treatment also accelerated β-cell proliferation. *N. sativa* increased basal glucose uptake by 55% in muscle cells and approximately 400% in adipocytes. Finally, *N. sativa* administration of pre-adipocytes undergoing differentiation accelerated triglyceride accumulation comparably with treatment with 10 μ M rosiglitazone. It is concluded that *in vivo*. Antihyperglycemic effects of *N. sativa* seed extract are attributable to a combination of therapeutically relevant insulinotropic and insulin-like properties.[28]

Anticancer activity

In vitro and *in vivo* anti-cancer effects of *Nigella sativa* L. seed extracts was evaluated in one of the study. In the study the essential oil and ethyl acetate extracts were showed more cytotoxic effects against the P815 cell line than the butanol extract. Extracts showed a comparable cytotoxic effect against the ICO_1cellline, with IC_{50} values ranging from 0.2 to

0.26% (v/v), but tests on the BSR cell line revealed a high cytotoxic effect of the ethyl acetate extract (IC_{50} = 0.2%) compared to the essential oil (IC_{50} = 1.2%).[29]

Character of seed

They are small dicotyledonous, trigonus, angular, regulose- tubercular, 2-3.5 × 1-2 mm, black externally and white inside. Odor slightly aromatic and taste bitter. Transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells, covered externally by a papillose cuticle and filled with dark brown contents. Epidermis is followed by 2-4 layers of thick walled tangentially elongated parenchymatous cells, followed by a reddish brown pigmented layer composed of thick walled, rectangular elongated cells. Inner to the pigment layer, is present a layer composed of thick walled rectangular elongated or nearly columnar, elongated.[30-3]

Phytochemical importance

In view of its wide range of medicinal uses, the plant has under gone extensive phytochemical studies and a variety of compounds isolated. The seeds of *Nige11a sativa* contain a yellowish volatile oil (0.5- 1.6%), a fixed oil (35.6-41 .6%), proteins (22.°%), aminoacids; e.g. albumin, globulin, lys me, leucine, isoleucine, valine, glycine, alanine, phenylalanine, arginine, asparagine, cystine, glutamicacid, asparticacid, isoleucine, proline, serine, threonine, tryptophan and tyrosine, reducingsugars, mucilage, alkaloids, organic acids, tannins, resins, toxic glucos ide, metarbin, bitter principles, glycosidalsaponins, melanthin resembling helleborin, melanthigenin, ash, moisture and arabic acid. The seeds have also been found to contain fats, crude fiber, mineralse. g. Fe, Na, Cu, Zn, P, Caandvitaminslike ascorbicacid, thiamine, niacin, pyridoxineandfolicacid, thus also possessing nutritional value. *Nigella sativa* seeds yield esters of fatty acids; e.g. palmitic acid, oleic acid, linoleie acid and dehydrostearic acid, higher terpenoids, aliphatic alcohols and n-§-unsaturated hydroxy ketones. Free sterols, steryl esters, sterylglucosides and acylatedsterylglucosideswere isolated from the seed oil. A novel alkaloid, nigellicine, an isoquinoline alkaloid, nigellimine and an indazole alkaloid, nigellidine, were also isolated from the seeds of *Nigella sativa!*. The seeds also contain lipase, phytosterols and§-sitosterol.[32]

The active constituents of the seeds include the volatile oil consisting of carv one, anunsaturated ketone, terpene or d-limonene also called carvene, n-pinene and p-

cymene. The crystalline active principle, nigellone, is the only constituent of the carbonyl fraction of the oil. Pharmacologically active constituents of volatile oil are thymoquinone, dithymoquinone, thymohydroquinone and thymol. Water stress influences the yield and composition of essential oil. The content of thymoquinone was highest (5°.°8%) when water was withheld for 12days.

In a recent study, *Nigella sativa* seed oil was extracted with two different solvents; n-Hexane and a mixture of Chloroform/Methanol, the latter was found to contain higher amounts of total lipids. Major fatty acids werelinoleicacid, palmiticacid, oleicacidandstearicacid and major phospholipids as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinosito1. Phosphtidylgly cero1, 1ysophosphatidy1ethano1amine and lysophosphatidylcholine were isolated in smaller quantities.[33]

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.[34] 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.[35] The section was stained with phloroglucinol-hydrochloric acid (1:1) and mounted in glycerine.[36]

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.[37]

Physico-chemical evaluations

Physicochemical parameters of *N. sativa* seed powder were determined[38] 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.[3]

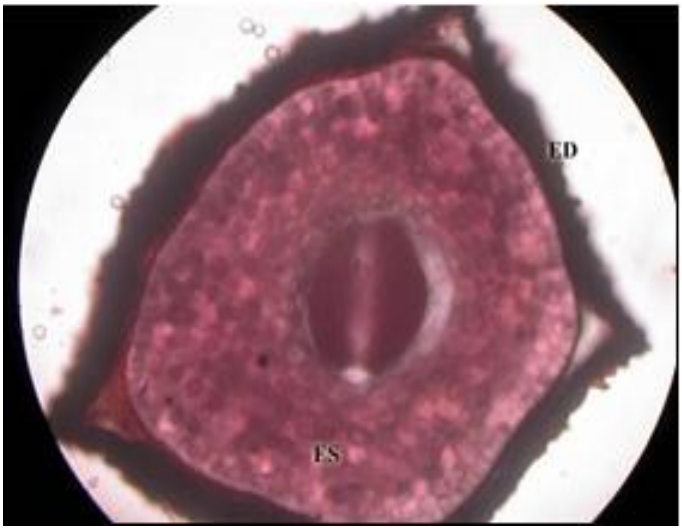
Seed morphology

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: Morphology of *N. sativa* seed.

Microscopical study

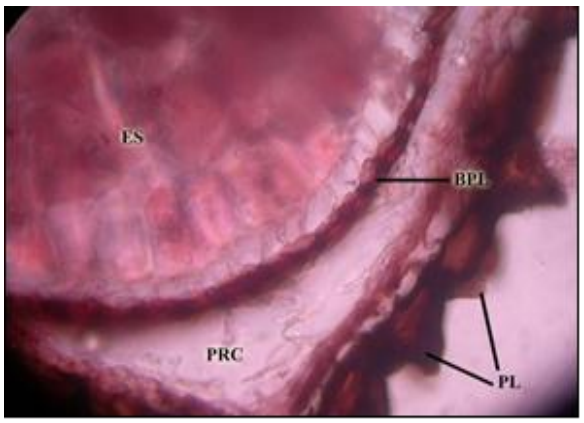
Transverse section of seed showed epidermis and endosperm (Fig. 2).



10X X 10X. [ED: Epidermis, ES: Endosperm]

Fig. 2: Microscopical view of T. S. of *N. sativa* seed at.

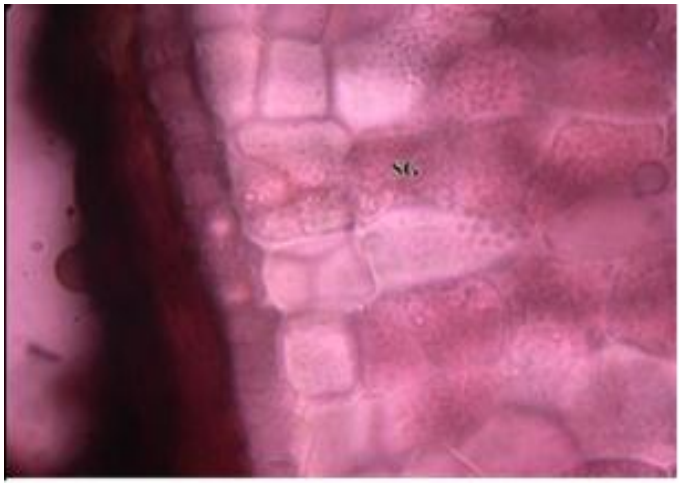
Epidermis: It was single layered consisting of elliptical, thick walled cells covered externally by a papilla'scuticle, filledwithreddish-browncontent; epidermis followed by 2-4 (two to four) layers of thick walled, tangentially elongated; parenchymatous cells, followed by a pigmented layer composed 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).



AT 10X X 40X. [PL: Papillae, PRC: Parenchyma, BPL: Brown Pigment Layer, ES: Endosperm]

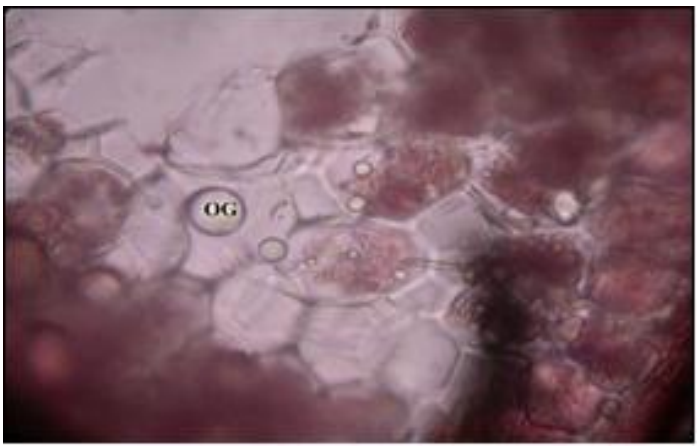
Figure 3: Microscopical view of T. S. of *N. sativa* seed.

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).



10XX40X. [SG: Starch Grains]

Fig. 4: Microscopical view of T. S. of *N. sativa* seed at.



10X X 40X. [OG: Oil Globules]

Fig. 5: Microscopical view of T. S. of *N. sativa* seed at.

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 chloroformextract. 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/weigh

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