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Nigella (*Nigella sativa*): a high value seed spice with immense medicinal potential

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ABSTRACT

Nigella sativa L.*,* a common herbaceous seed spice of India and Middle East, was identified by many researchers to have a spectrum of pharmacological and nutraceutical potentials. The seeds of nigella and its oil were widely used for centuries in the treatment of various ailments throughout the world. It is an important drug in the Indian traditional system of medicine. It is established that most of the potential and fruitful activities are inbuilt in its volatile oil and protein components. Most of the therapeutic properties of this herb are due to the presence of thymoquinone and its isomers which are the major active chemical component of the essential oil. Nigella seeds are also used in food as flavouring, additive in the breads and pickles because it has very low level of toxicity. The significant content of fats, proteins and minerals in the seeds, makes nigella suitable to be investigated as a source of these nutrients. This review incorporates a deeper view into crop cultivation along with various quality parameters of *N. sativa* and its constituents responsible for its pharmaceutical and nutraceutical attributes.

Key words: Essential oil, Nigella, *Nigella sativa*, Nigellone, Pharmacological profile, Thymoquinone

Nigella (*Nigella sativa* L.) is a very important minor seed spice crop. It is an annual and seasonal (winter) cross pollinated crop belonging to the family Ranunculaceae, commonly known as black cumin in English and *kalonji* in Hindi. It is an elite, herbaceous plant, distributed widely in the Mediterranean countries, Southern Europe, North Africa, South and south west Asia. In India, nigella is cultivated in the northern and eastern parts covering states of Punjab, Himachal Pradesh, Madhya Pradesh, Gangetic plains of Bihar and Bengal, Asom and Maharashtra. As far as the research related to development of improved varieties, production and plant protection technologies and post-harvest management is concerned, little work has been done on *Nigella* crop (Singh and Solanki 2015). The last three decades have witnessed enormous research activities to identify and establish the omnipotential qualities of *Nigella sativa* in terms of its pharmacological profile and drug development, using active plant compounds, condiments and culinary uses, yield and quality improvement, etc. Herbal medicines are the main source for achieving primary health care in many nations. About 80% of the world's population depends on traditional medicines in one or the other way (Ali and Blunden 2003). Since ancient time, plants are the richest source of effective and safe medicines. The ancient science of *Ayurveda* and *Unani* medical practices has successfully exploited herbs for their therapeutic uses (Kurion 2003). Nigella seeds were considered as most revered in the Middle East (Yarnell and Abascal 2011). Islamic prophet Mohammad had quoted that it "can heal every disease except death". *The Canon of Medicine, Avicenna*; included it in the list of natural drugs of "*Tibb-e-Nabavi*" (Unani-Tibbetian system of medicine) as a valuable remedy for number of diseases. The use of plant based drugs is increasing in many of the developing countries because modern life saving drugs are beyond the reach of three quarters of the third world's population (pharmabiz.com). Many of such developing countries spend 40 to 50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future. The World Health Organisation is also promoting the use of traditional medicines and phytotherapy. The latest export statistics of India's Ayush and herbal products have revealed that India stands as second leading exporter in the world accounting for ₹ 1 318.69 crore for the year 2010-11 after China (http://pharmabiz.com).

Since time immemorial medicinal plants have been a prominent source of therapeutic agents to promote health and fight diseases. In the recent past, naturally derived drugs have gained much more importance as compared to

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the synthetic drugs due to their no post remedial effects on the human system. Spices and herbs maximize nutrient density. Herbs and spices contain antioxidants, minerals and multivitamins. As spices are nutrient concentrates, they are thermogenic, which means they naturally increase metabolism. Spices have real medicinal properties. Keeping in mind the importance of *Nigella sativa* as one of the important seed spice crops of India which has immense medicinal properties and being grown in many parts of the world with a substantial area in India, it was studied in detail by several researchers (Agarwala *et al.* 1971, Aboutabl *et al.* 1986, Datta *et al*. 2012 and Aftab *et al.* 2013).

Customary uses

Nigella sativa is a valuable spice, having distinctive aroma and taste; its seeds were used in pickles, bread preparations and condiments, culinary and savoury dishes. *N. sativa* is regarded as a valuable remedy for various ailments, the seeds, oil and extracts have played an important role over the years in ancient Islamic system of herbal medicine. The dried seeds are used as astringent, bitter, diuretic, emmenagogue; stimulant and anthelmintic. It's decoction is useful in paralysis, jaundice, intermittent fever, dyspepsia, piles and skin diseases (Paarakh 2010 and Ali *et al.* 2003). Black seed has also been used externally where it is applied directly to abscesses, nasal ulcers, orchitis, eczema, and swollen joints Rathore *et al.* 2013 and Kurion 2003. have also reported the potential health benefits of the major seed spices. Many uses of the folk medicinal claims of nigella have been tested scientifically.

Plant and floral morphology

Nigella sativa is a hermaphrodite, erect, annual herb, with a more or less branched stem, pinnately dissected leaves; plant height 35-45 cm with determinate flowering patterns. The flower terminates the main shoot and ends with the flowers on the lowermost branches. The leaves are 2.5-5.0 cm in length, linear to lanceolate in shape; flowers are delicate, usually pale blue and white in colour, 2.0-2.5 cm across, solitary and peduncular. The seed bearing capsule is 1-1.5 cm long. The perianth is differentiated into an outer whorl of five, 15–20 mm long, whitish, petaloid sepals and an inner whorl of eight, 7–8 mm long, nectariferous petals. The flowers are protandrous with 5 to 10 petals and characterized by the presence of nectaries. The androecium comprises a large number of stamens, which shed their pollens as the filament curves outward during the male phase.

The male phase is initiated a few days before the stigmas become receptive; however, the maturing styles often become twisted around the last dehiscing anthers, which results in self-pollination; delayed selfing. The gynaecium consists of up to five completely united follicles, each with a long, indehiscent style and composed of a variable number of multi ovule carpels. Fertilized flowers develop into 2- to 3 cm/long capsules with numerous seeds, developing into a follicle after pollination with a single fruit partially connected to form a capsule like structure. Due to slow sepal development, all perianth parts are exposed during the early bud stage. Nigella plant flowers once and then dies; therefore, all resources accumulated prior to

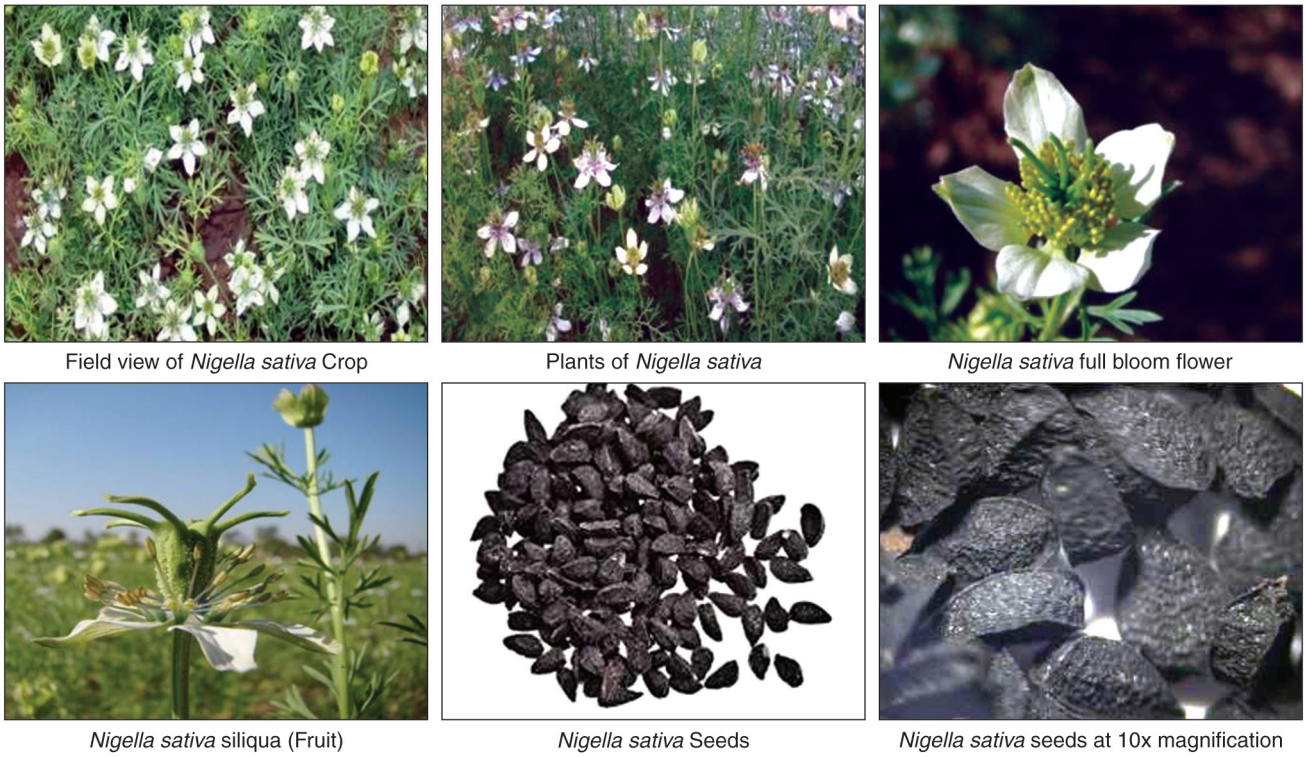

Fig 1 Morphological features of *Nigella sativa*, vegetative and reproductive

flowering are devoted to reproduction. Being entomophilous, cross pollination occurs through insects. Seeds are flat, oblong, angular, funnel shaped, size 0.20-0.25 cm long and 0.1-0.15 cm wide, dark black in colour, generally small (1-5 mg) dark grey or black. The fruit is large and its inflated capsule contains numerous seeds. It is cultivated as a winter crop with flowering and fruiting from January to April (Lloyd 1987).

Crop husbandry

Climate: It is cultivated in the northern plains, central and Peninsular region of India with temperature range of 20-25^{0}C during sowing and warm sunny climate during seed formation stage. In other parts of the world it grows well in cool-dry regions. Nigella is frost sensitive at any of the growth stages, hence its cultivation in Europe and tropical highlands are rarely feasible. The sowing period of Nigella depends upon its location, therefore in the Northern Hemisphere, it is sown during spring or early summer, but in the regions under monsoon climate, during the early rainy period. Cool and humid weather favours flowering and seed setting. It cannot grow in shade also.

Edaphology: Nigella can be grown on well-drained, light (sandy), medium (loamy) and heavy soils. Soils with slightly acidic, neutral and slightly basic (alkaline) pH are suitable, sandy/loamy soils rich in microbial activity are the most suitable soils for cultivation. Soil pH 7.0 to 7.5 is most favourable for cultivation.

Varieties: Nearly 22 varieties of nigella are reported from India out of which 20 are from Himachal Pradesh, two varieties from National Research Centre on Seed Spices, namely AN-1 and AN-20 suitable for different agro-techniques in India and two varieties namely Tunisian and Iranian also reported by Cheikh-Rouhoua *et al.* (2007).

Cultivation and crop management: Nigella is mainly propagated through seeds. The farmers cultivate local varieties which were developed through selection from local races. Land preparation for nigella cultivation broadly includes incorporation of 10-15 tonnes/ha of well decomposed manure, 30 kg N, 60 kg P_2O_5 and 20 kg K_2O/ha into the soil with proper mixing. Seeds are sown at 30 cm row spacing, plant spacing varies between 15-25 cm and the seed rate varies between 8-10 kg/ha. In Indian sub-continent the sowing conditions for nigella has been found to be optimal during October. The mature seeds germinate mostly within 12-15 days. Depending upon the soil moisture conditions, irrigation should be provided after sowing to facilitate germination. Post-germination irrigation schedules include one or two early (after 6-7 days) watering and again after 10-15 days intervals. 30 kg of nitrogen in two split doses should be applied after 40 and 60 days of sowing. Frequent weeding reduces weed competition and produces good environmental condition for growth and development. About 3-5 weedings at an interval of 20 to 25 days is recommended by hand hoe or suitable devices. Nigella grown as rabi crop (winter crop) in India is generally harvested during late March to first week of April (Malhotra 2004a, b, Malhotra and Vashishtha 2008). Area of cultivation and annual production respectively were reported to be– India: 6 234 600 ha and 254 000 tonnes; Turkey: 8 122010 ha and 689 350 tonnes; USA: 16 420 ha and 11 200 tonnes; UK: 500 ha and 10 to 20 tonnes (Datta *et al.* 2012).

*Harvest and post-harvest***:** The collection and harvest of *N. sativa* crop needs extensive labour as the capsules tends to dehisce at the maturity. Post-harvest management of the fruits usually involves their harvest, one by one, by hand and dry storage till natural dehiscence. The crop is harvested before shedding at a little green stage which gives high aromatic oil contents thus providing good market. Nigella retains seed viability longer when it is fully ripe. The seeds are enclosed inside the capsules with five compartments, each topped by a spike. The compartments open when dried to disperse the seeds**.** It is rather essential to harvest before shedding (shattering of fruits is a major problem) and therefore 2 to 3 or more pickings should be done to avoid loss of seeds due to shattering of the capsules. The harvested crop is dried under sun and threshed by beating with stick. The mature fruits do not require much attention as they are self-preserving and their essential oil is a great deterrent to fungal attack, insect attack as well as rodent infestation (Antuono *et al*. 2002).

Disease, pest and pesticide application: The damages are generally caused by a number of polyphagous garden insects. In the field, larvae of *Spodoptera litura* may cause 40% loss in a particular season or crop. Empty carpels often occur on plants probably due to the larvae of borer beetles. Most record of diseases is from the ornamental varieties. A leaf spot caused by *Cereospora nigellae* was recorded to cause minor damages in Ethiopia. Root rot was also reported due to *Macrophomina phaseolina* and *Rhizoctonia-Fusarium* complex in some cases in India (Weiss 2002). The visible symptoms of this include yellowing and drying of leaves, premature drying of plants resulting into low yield. As a control measure the seeds should be treated before sowing, deep summer ploughing and crop rotation should be used. Prolonged survival of *Fusarium udum* for up to 8 years was reported in roots. Pests like caterpillars make holes in the bulbs and cut down the seedlings. Army worm and semi-loopers feed on the flowers, seeds, and damage the crop. Treating with 0.05% methyl parathion – 1 ml/l of water or Thiodian or Endosal 35EC @ 1 ml/l of water at 15 days interval can be used as a control measure against the pests. Leaf spot of nigella root rot and caterpillar infestation can be eradicated by treatment with Mancozeb @ 0.2%, Carbendazim 50WP @ 0.1% and Chlorpyriphos 20EC @ 0.04% or Quinolphos 50 EC @ 0.03%, respectively.

Storage shelf life: The seeds of *N. sativa* can be stored in air tight packages as planting material as well as spices without loss in aroma. Due to its over bearing flavour and aroma, it should be stored away from other spices, else it may disturb the flavour of other spices.

Market prices: The rate of *N. sativa* seeds as well as oil products varies within the country as well as

International markets. The rates of the seeds vary from ₹ 250-300/kg in Indian markets. In Pakistan, the rate varies between ₹ 275-500/kg and in International market it is sold for ₹ 850-1 000/kg.

Uses and value added products: As such nigella seeds are used as spice, condiments, pepper substitute, pickles, baked goods, confectionary, pharmaceuticals and perfume industries. The dried seeds are used in Asian countries as a spice and condiment. The major processed products from nigella seeds is essential oil and fixed oil. These are used as adjuncts for flavouring foods, preservatives in confectionaries, pharmaceutical industries and stabilizing agents for edible fats. As a preservative the seeds are used in pickles and as an insect repellent. Nigella has been used as a folklore medicine in India and Mediterranean countries.

Nigella plant biochemistry

Nutritional ingredients: Proximate analysis of nigella *sativa* seeds from different countries have shown to have a high nutritional potential, viz. proteins (13.5-22%), arginine, glutamic acid, leucine, lysine, methionine, tyrosine, proline and threonine; fats (38-40%), moisture (5.5%), ash (3.7%) and carbohydrates (17-32%) (Abdel-Al and Attia 1993, A1-Jassir 1992, Takruri and Dameh 1998 and Babayan *et al.* 1978). The mineral and vitamin content per kg seeds was found to be; iron (10-105 mg) (Siong *et al.* 1989b), copper (12-18 mg), zinc (45-60 mg), phosphorus (325-527 mg), calcium (500-1860 mg) (Siong *et al.*1989a), thiamine (2.5-15.4 mg), niacin (9.5-57 mg), pyridoxine (0.7-5.0 mg), folic acid (95-160 pg) and tocopherol (~34 mg) (Takruri and Dameh 1998). Amino acid analysis of the seed protein hydrolysate by gas chromatography of the n-propyl, N-acetyl derivatives showed the presence of 15 amino acids including 9 essential amino acids. In the dormant seeds amino acids such as cystine, lysine, aspartic acid, glutamic acid, alanine and tryptophan were present. The other chemical constituents isolated in nigella seeds are glucosides, melanthin and melanthingenin. Viewing the significant content of fats, proteins and minerals in the seeds, it should be investigated as a source of these nutrients and its potential applications in flavoring various types of foods (Cheikh-Rouhoua *et al*. 2007 and Abdel-Ghany *et al.* 1998).

Saponin: The major saponin in the defatted seeds of *N. sativa* is the glycoside a-hederin or Helixinor melanthin, which on acid hydrolysis releases its sugar rhamnose/ arabinose and gives the glycone hederagenin (Ansari *et al.* 1988, Khan 1999).

Alkaloids: Three types of alkaloids were isolated from the defatted seeds of *N. sativa* identified as indazole nigelicine and its N-oxide, the indazole alkaloid nigellidine and the isoquinoline nigellimine (Atta-ur-Rahaman *et al.* 1985a, Atta-ur-Rahaman 1995).

Biochemical constituents

A qualitative examination of *Nigella sativa* seeds and plant extracts used in folk medicine has revealed the presence of sterols, sesquiterpenes, diterpenes, triterpenes, tannins, flavanoids, cardiac glycosides, alkaloids, saponins, volatile oils, coumarins, volatile bases, glucosinolates, oxygenated hydrocarbons such as alcohols, esters, aldehydes, ethers, ketones and anthraquinones (Khan 1999). Qualitative analysis of the nigella seed oil by capillary GC-MS technique has enabled the identification of >100 compounds which are classified into various functional groups viz. monoterpenes (~46%); carbonyl compounds (~25%); phenols (~1.7%); alcohols (~0.9%) and esters (~16%) (Rathee *et al.*1982 and Ustun *et al.*1990).

The major phytochemical compounds present in *Nigella sativa* seeds are nigellone, nigellicine, nigellimine, nigellimine-N-oxide, avenasterol-5-ene, avenasterol-7-ene, campesterol, cholesterol, citrostadienol, cycloeucalenol, 2-4-ethyl-lophenol, gramisterol, lophenol, 2-4-3-methylophenol, obtusifoliol, sitosterol, stigmastanol, stigmasterol, stigmasterol-7-ene, β-amyrin, butyrospermol, cycloartenol, 2-4-methylo-cycloartanol, taraxerol, tirucallol, 3-O-[b-D-xylopyranosyl (1-3)-a-L-rhamnopyranosyl (1-2)-a-Larabinipyranosyl], 2-8-O-[a-lrhamnopyranosyl (1-4)-b-D-glucopyranosyl (1-6)-b-D-glucopyranosyl, hederagenin, volatile oil (0.5-1.6%), fatty oil (35.6-41.6%), oleic acid, esters of unsaturated fatty acids with C15and higher, terpenoids, esters of dehydrostearic and linoleic acids, aliphatic alcohols, nigellidine, carvone, d-limonene, cymene, a, b-unsaturated hydroxyl ketone, steroids, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, protein, reducing sugars, glycosidal saponins, 3-O-[b-D-xylopyranosyl (1-2)-a-L-rhamnopyranosyl (1-2)-b-D-glucopyranosyl]-1-1-methoxy-1-6,2-3-dihydroxy-2-8-methyl- olean-1-2-enoate, stigma-5-2-2-dien-3-b-D-glucopyranoside, nigellidine-4-O-sulphite, nigellaminesa1, A2, A3, A4, A5, B1 and B2 (Paarakh 2010).

Essential oil extraction: Different procedures, solvents and suitable parameters have been utilised for the extraction of essential oils, viz. Conventional method – extraction with hexane in soxhlet apparatus; enzymatic extraction; ultrasound assisted extraction; microwaves assisted extraction; supercritical solvent extraction and surfactant assisted method; based on the use of aqueous solution of polyethylene glycol sorbitan monolaurate. (Datta *et al*. 2012 and Aitzetmuller 1997).

Fractions of N. sativa seed oil

The volatile oil: It is a pale yellow liquid with a characteristic unpleasant aromatic odour and taste which is readily soluble in organic solvents such as ether, chloroform and ethanol but only sparingly soluble in water (Al-Jassir 1995, Nergiz and Otles 1993 and Atta 2003). The volatile oil content of *N. sativa* was determined to be 0.4%-0.5% w/w. However, an improved method for isolating the volatile oil involves soxlet extraction from the seeds with petroleum ether (b.p.~ 60-80^{0}C) to give an oil (35%) which on steam distillation gives the volatile oil in a much higher yield (1.5%). The active ingredients of volatile oil fraction consists of p-cymene (isopropyl toluene) (31%), thymoquinone (2-isopropyl-5-methyl-benzoquinone) (25%), ethyl linoleate

(9%), α-pinene (9%), ethyl hexadecanoate (3%), ethyl oleate (3%), β- pinene (2, 6, 6-trimethylbicyclo [3.1–1]-hepta-2-ene) (2%), limonene (4.3%), carvone (4.0%) (Nickavara *et al*. 2003) and 2-(2-methoxypropyl)-5-methyl-1, 4-benzenediol, thymine, thymohydroquinone, dithymoquinone, thymol, d-limonene, d-citronellol and carvacrol (2-methyl-5-(1-methyl ethyl) phenol) which is also known as 2-hydroxy-p-cymene or isothymol) in minute quantities (Paarakh 2010 and Ali *et al.* 2003).

The fixed oil: It was determined by TLC and GLC chromatographic techniques (Ansari *et al.* 1988, Bhakare *et al.* 1992). The total stable oil or fixed oil or fatty oils content of the seeds has been found to be more than 30%. Isolation of the fixed oil by extraction of the crushed seeds of *N. sativa* with hot petroleum ether (boiling range 60-80~) is a more efficient procedure, that gives oil in a higher yield from 35 to 42% (Rathee *et al*. 1982, Ebrahiem 1998 and Houghton *et al.* 1995). The fatty acid composition of the triglyceride fraction in the fixed oil contains the saturated fatty acids-palmitic acid (12.5%), stearic acid (<1%), myristic acid and unsaturated fatty acids-linoleic acid (55.6%), oleic acid (23.4%), arachidonic, eicosadienoic (3%) and dihomolinoleic fatty acids (10%) (Zeitoun and Neff 1995). Fatty acid analysis of the extracted oil from *Nigella sativa* seeds determined using gas-liquid chromatography by Babayan *et al.* (1978); showed 56% linoleic acid, 24.6% oleic acid, 12% palmitic acid, 3% stearic acid, 2.5% eicosadienoic acid, 0.7% linolenic acid and 0.16% myristic acid. Traces of few unidentified fatty acids were also found. Fatty acid compositions (% of total fatty acids) of *N. sativa* L. seed extract from Morocco have slight variation in terms of Myristic (C14:0)- 1 ± 0.1, Palmitic (C16:0)- 13.1 ± 0.2, Stearic (C18:0)- 2.3 ± 0.1, Oleic (C18:1)- 23.8 ± 0.1, Linoleic (C18:2)- 58.5 ± 0.1, Linolenic (C18:3)- 0.4 ± 0.1, saturated fatty acids-16.8 ± 0.5 and unsaturated fatty acids-82.9 ± 0.5, (Gharby *et al.* 2015).

The oil obtained by pressing the seeds was found to be quite rich in fatty acids (Turkay *et al.* 1996 and Al-Jasass *et al*. 2012). The acidity in the oil arises as a result of the enzyme lipase which is present in the raw seeds, hydrolysing the fatty acid esters *in-situ* (Ustun *et al.* 1990, Dandik and Aksoy 1996).

The *Nigella sativa* seed oil contains 2% w/w foreign matter; 6% w/w total ash; 0.2% w/w acid insoluble ash; 20 % w/w alcohol soluble extractive; 15 % w/w water soluble extractive; 3.91 % w/w organic matter and 4 % w/w loss on drying (Nickavara *et al.* 2003. Ramadan and Morsel 2003) reported that seeds contain carotene, which is converted to vitamin A in liver. Acetylated triterpene, saponin (penta hydroxyl pentocyclic triterpene) has been isolated from the species.

A deacidification process was devised for the *Nigella sativa* oils that involve extraction with supercritical carbon dioxide. Using this method of extraction the amount of neutral oil that co-extracts with the fatty acids was increased to 94% (Turkay *et al.* 1996). The fatty acid composition of *Nigella sativa* seeds of Turkish origin has been investigated by Aitzetmuller *et al.* (1997) and found to contain specific pattern of certain fatty acids. In particular the considerable amounts of dihomo-linoleic acid in nigella seeds is in contrast to the edible oils.

The major triacylglycerols found were trillinolein (24.6%), oleodilinolein (19.6%), palmito-di-linolein (17.5%), palmito-oleo-linolein (12.9%), dioleolinolein (9.6%) and stearo-oleolinolein (Abdel-Aal and Attia 1993a and Merfort *et al*. 1997). Since, the peroxidation of polyunsaturated fatty acids is believed to be biochemically inhibited by tocopherols, a study examining the link between the degree of unsaturation and tocopherol content of the oil has been carried out (Kamal-Eldin and Anderson 1997). The autoxidative stability and peroxide value (76 mg/kg after 5 days) of crude *N. sativa* oil of Egyptian origin was determined (Zeitoun and Neff 1995). Traces of few unidentified fatty acids were also found.

Glycerides of some volatile acids are also present in the oil in small quantities along with tannins, resins, proteins, reducing sugars, cystine, lysine, aspartic acid, leucine but asparagines could not be identified (Prajapati *et al.* 2003).

The total lipid content was reported to be 31.8% in which neutral lipids predominate. The minor lipids consisted of glycolipids (monogalactosyl diglyceride, digalactosyl diglyceride, acylated-sterylgalactoside and sterylgalactoside) and phospholipids (phosphatidylcholine, phosphatidyl ethanolamine, phosphatidyl inositol, candiolipin and phosphatidyl glycerol) (Abdel-Ghany *et al.* 1998).

Pharmacological attributes

Phytochemical studies of Nigella seed showed the presence of >100 constituents. The constituents, viz. fatty acids, volatile oils and trace elements contribute to the pharmacological activity of *Nigella sativa* seed oil. The results of exhaustive pharmacological studies justify the traditional therapeutic value of nigella seeds. The studies found nigella seeds to have diuretic and antihypertensive, bronchodilator, analgesic, antilipemic, postcoital contraceptive and calcium antagonist, histamine release inhibitor, hepatoprotective, anthelmintic, antifungal, antimicrobial (against organisms), anticancer, and anti inflammatory properties. *Nigella sativa* seeds can be used in various forms for therapeutical uses.

Generally, there were no significant variations in the chemical composition of the fixed oils of nigella seeds grown in Egypt, Sudan, Ethiopia, India, Turkey and Syria. However, Al-Jassir (1995) noted that the seeds grown in Qassim, Saudi Arabia, contained, in addition to the fatty acids two more acids which were lignoceric acid about (1%) and myristoleic acid (0.18%) without the presence of eicosadienoic acid (C20:2). Lignoceric acid is not found in many other edible vegetable oils. The medicinal values of different fractions of nigella seeds extracts is presented in Table 1.

Specific chemical analyses of the volatile oil started during the years 1960–1966 by Mahfouz and El-Dakhakhny (1960) and Canonica *et al*. (1963). These studies

Table 1 Medicinal values of different fractions of nigella seeds extracts

Different fractions of nigella seeds	Medicinal values	References
Whole seeds	Whole seeds powder at doses of 1g twice daily for 4 weeks increased the ratio of T-lymphocytes helper cells to T-suppressor cells by 72% and enhanced T-killer cells function and number.	Sogut *et al.* (2008)
	Administration of the powdered seeds orally at 40mg/kg of body weight to those infected with *Ascaris lumbricoides*, *Taeni asaginata* or *Hymenolepsis nana* decreased the parasites' fecal eggs count by up to 93%,	Akhtar and Riffat (1991)
	Effects of the whole seeds in animals, significant phagocytic activity against *Candida albicans* was noted in mice at the doses of 2g/kg/day for 5 days and significant enhancement of milk production was observed in goats at doses of 0.1g/kg/day.	Agarwala *et. al.* (1971)
	The crushed seeds can be used against *Schistosoma mansoni* at 2-5 µg/ml.	El-Din Hussein *et al.* (2006)
Seed aqueous extract	*In vitro* and *in vivo* administration of the aqueous extract in doses of 2g/kg orally to rats induced significant protection against aspirin-induced increase in the volume of the gastric juice, the acid output and the gastric ulcers.	Haq *et al.* (1999)
	Exposure of the the isolated rat pancreatic *Islets of Langerhan* cells to aqueous extracts or their basic aqueous sub-fractions in concentrations up to 5 mg/ml induced significant increase in insulin release.	Benhaddou-Andaloussi *et al*. (2008)
Seed ethanolic extract	Studies revealed the successful effectiveness of the alcoholic extract of *N-sativa* seeds in doses of 150 mg/kg daily for 8 days in inducing significant reductions of the volume of the gastric secretion, the free acid, the total acid content and the gastric ulcers induced by pyloric ligation in rats.	Rajkapoor *et al.* (2002)
	The extract in single doses equivalent to 40 mg powdered seeds/kg also decreased the *Ascaris lumbricoidis, Taenia saginata* or *Hymenolepsis nana-cestodal* fecal egg count by 93% in children infected with some cestodes.	Akhtar and Riffat (1991)
Seed methanolic extract	Treatment of mice with the extract at doses of 10 mg/kg/day (i.p) for 10 days significantly suppressed Ehrlich ascites carcinoma cells development via inhibition of DNA synthesis in the cells.	Salomi *et al.* (1991)
	In vitro studies demonstrated the potent inhibitory effect of the methanolic extract in suppressing the growth of *Bacillus subtilis, Escherichia coli*, *Streptococcus foecalis*, *Staphylococcus aureus, Pseudomonas aeroginosa,* and *Candida albicans*.	Nair *et al*. (1991)
Seed ether extract	Administration of the dried petroleum ether extract of *N. sativa* seeds to rats orally for four weeks resulted in significant decreases in blood triglycerides, increased high density lipoprotein and potentiated insulin-induced activation of protein kinase enzyme K.	El-Din Hussein *et al.* (2006)
	In vitro studies revealed that exposure of the gram-positive bacteria *Staphylococcus aureus*, the gram negative *Pseudomonas aerugenosa* and *Escherichia coli* and the yeast *Candida albicans* in concentrations of 250-400 µg/disc eradicated the microorganisms.	Hanafy and Hatem (1991)

were complemented by most recent ones (Paarakh 2010) which revealed various pharmacologically active constituents that included Thymoquinone (2-isopropyl-5-methyl-benzoquinone) that may attain up to 27.8% of the volatile oil (w/w). Carvacrol (2-methyl-5-(1-methyl- ethyl phenol) which is also known as 2- hydroxy-p-cymene or isothymol) (5.8–11.6% (w/w)) , p-cymene (isopropyl toluene) in the range of 15.5–31.7% (w/w), α-pinene (2,6,6-trimethyl-bicyclo [3,1–1]-hepta-2-ene (9.3%) , 4-terpineol (or α-terpineol or α, α, 4-trimethyl-3-cyclo-hexene-1-methanol or p-menth-1-en- 8-ol) 2–6.6%; longifolene (or Junipene or Kuromatsuene or decahyro-4,8,8-trimethyl-9- methylene-1,4-methanoazulene) 1–8% (w/w)), t-anethole (p-propenyl anisole or 1-methoxy- 4-(1- propenyl)benzene 0.25–2.3% w/w and the reduction product of thymoquinone and thymohydroquinone together with some esters about 16% (Table 2). Some of the compounds have been shown in Fig 2.

Therapeutical attributes

Thymoquinone is one of the major components of *N. sativa* volatile oil. For this reason, the influence of this

Table 2 Medicinal uses of some active compounds of *Nigella sativa* oil

Name of active ingredient	Medicinal properties	References
Thymoquinone	Cardiovascular system- Administration of thymoquinone i.v. into rats in the dose-range 0.2-1.6 mg/kg induced cardiovascular depressant actions; it also protected mice against doxorubicin-induced cardiotoxicity. Thymoquinone improves respiration, decreases serum levels of glucose, cholesterol and reduces blood pressure. Thymoquinone is reported to possess hepatoprotective activity. An *invitro* study showed the protective effect against tert-butyl hydroperoxide (TBHP) induced oxidative damage to hepatocytes.	Rooney and Ryan (2005), Houghton *et al.* (1995), Khader and Bresgen (2009), Abou Basha *et al.* (1965), Chaieb *et al.* (2011), Harzallah *et al.* (2012), Mutabagani *et al*. (1997) and Marozzi *et al.* (1970).
p-cymene	Cardiovascular system-Administration of p-cymene in doses of 2–32 µl/kg (i.v.) into rats induced dose-dependent decreases in the arterial blood pressure and the heart rate. Unlike α-pinene, it did not affect body temperature in mice. Its other actions included local anaesthetic effect and a mild antibacterial action.	El -Tahir *et al.* (1993) and Ashour *et al*. (2006).
α-Hederin	Anti cancerous-treatment of human cancers namely lung, larynx, colon and pancreas with á-hederin *in vitro* resulted in reduction of cytotoxicity, necrosis and apoptosis. At doses of 5–10 mg/kg orally to mice, for 8 or 15 days it produced significant inhibition of murine P388 leukemia and Lewis lung carcinoma cells. Its protective action seemed to be due to depletion of intracellular glutathione and production of reactive oxygen species together with activation of caspase-3.	Rooney and Ryan (2005), Jeong (1998), Kanter *et al*. (2005) and Vihan and Panwar (1987)
	Hepato-protective-studies in mice revealed that administration of α-hederin decreased the hepatic content of Cytochrome P450 and the activities of the subtypes CYP 1A1, 1A2 and 2E1. The treatment decreased the levels of mRNA except that of CYP 2E1. Treatment of mice with doses of 30 µmol/kg/day S.C for 3 days protected mice from paracetamol, bromobenzene, CCl_4, furosemide and thioacetamide-induced hepatotoxicity.	Kanter *et al.* (2005) and Turkdogan *et al.(*2003)
Nigellone	Nigellone exerted some pharmacological actions that included protection of guinea-pigs against histamine-induced broncho-constriction and suppression of bronchial asthma in children. It was devoid of any hypotensive activity.	El-Dakhakhny (1965), Atta-ur-Rahaman and Hasan (1995).
Melanin	Melanin has been recently isolated and purified from the outer coats of the seeds of *N. sativa.* It showed its ability to protect against alcohol- aspirinindomethacin and stress- induced ulcers in presence or absence of commensal gastric bacteria.	El Obeid *et al.* (2006).

substance (whether natural or synthetic) was explored in most of the systems investigated for the volatile oil. Based upon the available literature the various therapeutical uses of *N. sativa* seed oil and its various fractions have been grouped under the following sub heads.

Antimicrobial activity: The essential oil shown to have activity against gram-positive and gram-negative bacteria. It exhibited strong antimicrobial activity against *Salmonella typhi, Pseudomonas aeruginosa* and others. Comparatively higher sensitivity against gram-positive bacteria *Staphylococcus aureus* and *Vibrio cholera* was found to be stronger than gram negative bacteria. *Staphylococcus aureus, Staphylococcus pyogenes* and *Staphylococcus viridans* are more susceptible to *Nigella sativa*. The dried seeds of *N. sativa* exhibited bactericidal activity against *Pseudomonas aeruginosa* (Hanafy and Hatem 1991, Kahsai 2002). The activity of the volatile oil against drug-resistant strains of *Shigella spp., Vibrio cholera* and *Escherichia coli* was found to have a synergistic action with streptomycin and gentamycin. A clear inhibition of the growth of *Staphylococcus aureus* was observed by concentration of 300 mg/ml. The positive inhibition may be attributed to the two important active ingredients of *N. sativa*, Thymoquinone (TQ) and melanin. TQ exhibited a significant bactericidal activity against various human pathogenic bacteria especially gram positive cocci, i. e. *Staphylococcus aureus* and *Staphylococcus epidermidis* (Chaieb *et al.* 2011, Morsi 2000, Bakathir and Abbas 2011, Salem *et al.* 2010 and Hannan *et al.* 2008).

Hepato-protective activity: The active constituents Thymoquinone is reported to possess hepatoprotective activity. An *invitro* study showed the protective effect against tert-butyl hydro peroxide (TBHP) induced oxidative damage to hepatocytes (Kanter *et al.* 2005 and Vihan and Panwar 1987). The activity was demonstrated by decrease of alanine trans-aminase (ALT), aspartic trans-aminase (AST) and decreased trypan blue uptake (Kanter *et al.* 2005 and Turkdogan *et al.* 2003).

Anti diabetic activity: Significant hypoglycaemic activity was reported. Antihyperglycemic effects of *N. sativa* seed extract are attributed to a combination of therapeutically relevant insulinotropic and insulin-like properties (Benhaddou-Andaloussi *et al.* 2008, Benhaddou-Andaloussi *et al.* 2011 and Salama 2011). Polyherbal formulation

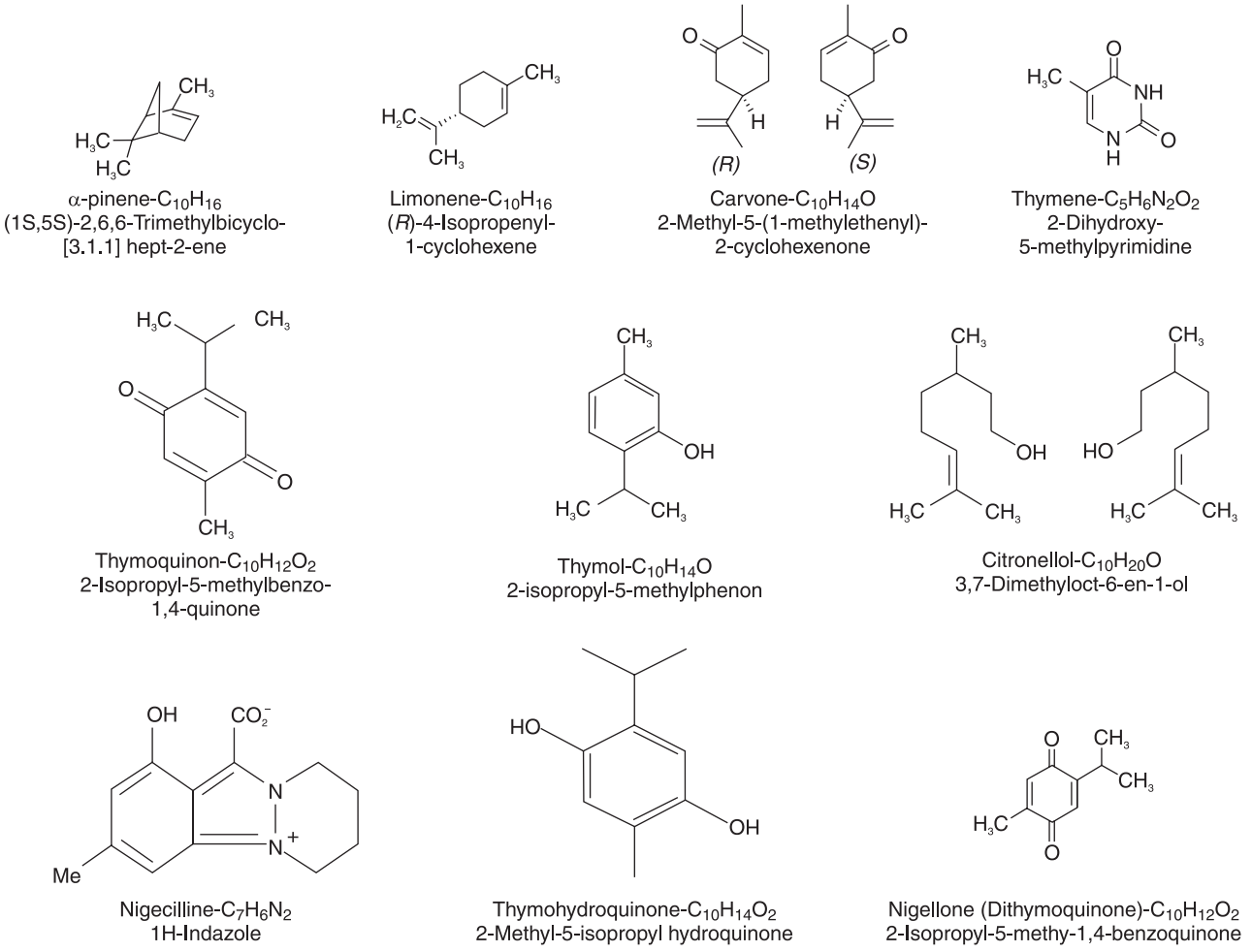

Fig 2 Structures and molecular formulae of some *Nigella sativa* active compounds

(kalonji sugar powder) has antidiabetic effect. It reverses the abnormal lipid profile observed in diabetic animals. Therefore the water extract of this formulation is useful in maintaining healthy glucose levels and cholesterol levels (Alam *et al.* 2013). Combination of α-lipoic acid, L-carnitine and *N. sativa* may contribute significantly in improvement of the carbohydrate metabolism in diabetic rats, thus increasing the rate of success in management of diabetics. It was also demonstrated that *in vivo* treatment with *Nigella sativa* seed extract exerts an insulin-sensitizing action by enhancing ACC phosphorylation, a major component of the insulin-independent AMPK signalling pathway, and by enhancing muscle Glut4 content (Kapoor 1999, Bamosa *et al.* 2010, Rchid *et al.* 2004 and Najmi *et al.* 2008).

Anti inflammatory activity: The chronic inflammatory disorders, asthma and arthritis involve a variety of inflammatory mediators and pathways. The fixed oil and thymoquinone were found to inhibit eicosanoid generation in leucocytes and membrane lipid peroxidation and a significant reduction in rat paw oedema and a reduction ingranuloma pouch weight. Nigellone in low concentration is effective in inhibiting the histamine release from the mast cells, which supports an anti asthmatic role for the plant (Mutabagani *et al.* 1997).

Antifertility characteristics: The antifertility activity of *N. sativa* in male rats was established by inhibition of spermatogenesis and significant reduction in sialic acid content of the testis, epididymis, seminal vesicles and prostate (Agarwal *et al.* 1990 and Keshri *et al.* 1995).

Anthelmintic properties: N. sativa was found to have an anthelmintic activity against tapeworm comparable to that of piperazine (Ri Doux *et al.* 2001).

Analgesic qualities: The essential oil produced significant analgesic activity using chemical and thermal noxious stimuli methods such as acetic acid-induced writhing, hot plate and tail flick tests. The results show that *N. sativa* could reduce the presence of the nasal mucosal congestion, nasal itching, runny nose, sneezing attacks, turbinate hypertrophy and mucosal pallor during the first 2 weeks (day 15). The anti-allergic effects of *N. sativa* components could be attributed to allergic rhinitis. Moreover, *N. sativa* should be considered for treating allergic rhinitis when the effects of other anti-allergic drugs need to be avoided (Khanna *et al.* 1993 and Gilani *et al.* 2001).

Anticancerous qualities: Treatment of hamsters with *N. sativa* volatile oil did not induce any dysplastic alterations or carcinomas in the cheek pouches of the animals (Salomi *et al.* 1991). Furthermore, chemical treatment of rats with 1,

2-dimethyl-hydrazine-induced colon cancer in the post initiation stage but daily treatment with *N. sativa* volatile oil for 14 weeks induced significant reductions in the colonic lesions via suppression of the cell proliferation in the colonic mucosa (Kumara and Huat 2001). The treatment did not induce any harmful effects in the blood or urine parameters and no pathological changes in the various vital body organs. Furthermore, exposure of various human cancer cell lines to the volatile oil in concentrations of 120-380 µg/ml induced significant death of the cells showing clear cytotoxic effect (Nazrul *et al.* 2004, Rooney and Ryan 2005a, Harzallah *et al.* 2012, Jeong 1998, Salim and Fukushima 2003, El-Obeid *et al.* 2006 and Worthen *et al.* 1998).

Antioxidant activity: Treating broiler chicks with black seeds for 6 weeks prevented the liver from oxidative stress by increasing the activities of enzymes such as myeloperoxidase, glutathione-S-transferase, catalase, adenosine deaminase, myeloperoxidaseand by decreasing hepatic lipid peroxidation. The crude methanolic extract of black cumin seed cake was found to show significant antioxidant properties under *in vitro* systems. The thymoquinone pre-treatment restored the increased level of malonyl dialdehyde and conjugated diene levels (Mariod *et al.* 2009, Sogut *et al.* 2008, Baldioli *et al.* 1996, Burits and Bucar 2000), Ramadan and Morsel 2004 and Turkdogan *et al.* 2001).

Nephroprotective activity: The nephroprotective effect of vitamin C and *N. sativa* oil was observed against gentamicin (GM) associated nephrotoxicity in rabbits. Serum creatinine, blood urea nitrogen, and antioxidant activity were measured as indicators of nephrotoxicity for all the groups of rabbits. It was revealed that vitamin C and *N. sativa* oil both had nephroprotective effect as they lowered the values of serum creatinine, blood urea nitrogen, and antioxidant activity as compared to GM control group values. When these two antioxidants were given as combination, they proved to have synergistic nephro protective effect (Swamy and Huat 2003, Al-Naggar *et al.* 2003 and Saleem *et al.* 2012).

Neuropharmacolgical activities: The aqueous and methanol extracts of defatted *N. sativa* seeds were shown to possess a potent central nervous system and analgesic activities, especially depressant action in the case of the methanolic extract. The neuroprotective effects of both the extracts of *N. sativa* in cerebral ischemia were observed. The neuroprotective effects could be due to its antioxidant, free radical scavenging, and anti-inflammatory properties (Akhtar *et al.* 2012, Aqel and Shaheen 1996 and El-Naggar *et al.* 2010).

Contraceptive and antifertility activity: Oral administration of hexane extract of *N. sativa* seeds prevented pregnancy in Sprague-Dawley rats at a dose of 2 g/kg daily,while column fractions and sub-fractions of hexane extract of *N. sativa* seeds also showed significant antifertility activity. At contraceptive dose, the active hexane extract exhibited only mild uterotrophic activity comparable almost to 0.002 mg/kg dose which is directly proportional to ethinylestradiol, but was devoid of any estrogenicity in the immature rat bioassay. 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 (*Agarwal *et al.* 1990 and Keshri *et al.* 1995).

Activity of Dethymoquinonated volatile oil: The de-thymoquinnated oil retained its tachypnic effect and lost its bronchoconstricting action (Malhotra 2004a). Thus, these experiments paved the way for the use of *N. sativa* volatile oil without any fear from thymoquinone-induced bronchoconstriction or its potential ulcerogenic action, if any. Abou Basha *et al.* (1965) managed to remove thymoquinone of the whole volatile oil of *N. sativa* and re-examined the cardiovascular effects of the dethymoquinonated oil. The results revealed that intravenous administration of this oil into rats in doses of (2–16 µl/kg) produced dose-dependent decreases in both the arterial blood pressure and heart rate to an extent twice that of the whole volatile oil especially with regard to the induced bradycardia. At a dose of 16 µl/kg (i.v.) the induced decreases in the arterial blood pressure were 18.6 ± 4.6 and 33.5 ± 8.3 mm Hg and the decreases in the heart rate were 27.6 ± 5.9 and 53.6 ± 3%, following administration of the whole volatile oil and the de-thymoquinnated oil, respectively.

Qualitative specifications for nigella seeds and oils

*Seed specifications***:** Colour of the seeds- matt black; Shape- semi triangulate, 2-2.5 mm; long, uniform in size shape and texture; Seed moisture >11% w/w; Ash content >=6% w/w; Acid in soluble ash >=1% w/w; Organic matter (extraneous) >=3% w/w; Inorganic matter(extraneous) >= 2% w/w; Volatile oil >=1% v/w; Ether extract >=35% v/w and Alcoholic acidity >=7% v/w.

Specifications for volatile and fixed oil: Specific gravity at 15^0C= 0.875 to 0.886; Refractive index at 20^0C= 1.4836 to 1.4844; Optical rotation at 20^0C = +1.43 to 2.86; Acid value ≈ 1.9; Ester value ≈ 1.0 to 21.6; Ester value (after acetylation) ≈ 15 to 73 and solubility ≈ 2 to 4.5 or more volumes of alcohol.

Specifications for fatty oil

The fatty oil obtained from nigella seeds is also used for edible purpose. Extraction with benzene and subsequent steam distillation of extract to remove volatile oil gives about 31% of reddish-brown, semi-drying oil with specific gravity at 25^0C ≈ 0.91, refractve index at 21^0C ≈1.46, acid value ≈ 42.83, saponification value ≈ 199.6, iodine value ≈ 17.6, Reichert-Meissl value ≈ 3.9% and unsaponifiable matter ≈ 0.03% (Malhotra 2004b**).**

Adulteration

Nigella seeds are available as whole seeds as well as powder form. The whole seeds are generally adulterated with onion seeds which have same texture and colour and difficult to segregate. The exhausted seeds or residual seeds after oil extraction is also adulterated in whole seed or powder form. The nigella oil is generally adulterated by

mixing with chaff oil. Genuine oil can be identified through chromatographic techniques using the specifications, i.e. the high ratio of eicosadienoic acid to eicosamonoenoic acid, combined with a high level of carboxyfatty acids, which is a characteristic of nigella seed oils (Weiss 2002). (The quality standards have been laid down under the Prevention of Food Adulteration (PFA) Act and rules summed up to 1997 by Ministry of Health, Government of India for whole nigella seeds and powder (Anon. 1998).

Toxicological studies

Many toxicological studies have been carried out on *N. sativa* seeds. It has been shown that no toxic effects were reported when *N. sativa* fixed oil was given to mice via the stomach in an acute study. In a chronic toxicity study rats treated daily with an oral dose for 3 months caused no changes in key hepatic enzyme levels particularly aspartate-amino transferase, alanine-amino tranferase, and gamma glutamyl-transferase. Moreover, the histopathological results also showed to be normal for the tissues of heart, liver, kidneys and pancreas LD_{50} values of fixed oil of *N. sativa* obtained by single doses orally and intra peritoneally in mice, were reported to be 26.2-31.6 and 1.86-2.26, respectively (Khader and Bresgen 2009). In some studies it was suggested that the seeds should be used in moderate doses as the volatile oil contains melanthin, nigelline, damascene and tannin which are toxic in higher doses. Chronic toxicity of nigella fixed oil has been reported by Zaoui *et al*. (2002). The methanol extract of some species show increased oestrogenic activity (Chevallier 2001).

Future perspectives

Further studies are required to consolidate the good agricultural practices for obtaining high essential oil and fixed oil yield in *N. sativa* seeds so as to improve its medicinal and other properties. Mechanism of actions of *N. sativa* seed extracts and its constituents by which they exert their therapeutic effects needs to be further investigated and tested. Chemical modifications in the molecular structure of Thymoquinone, α-Hederin and other constituents of *N. sativa* seeds could lead to prepare more effective and safer drugs for the treatment of wide variety of diseases in the future in suitable combinations. Moreover, further researches should focus and explore the specific cellular and molecular targets of various constituents of *N. sativa*, particularly Thymoquinone. This review article is dedicated to all those researchers who are interested in focussing their research on this miracle herb and hope, this review article would help them in investigating and conducting further preclinical and clinical studies on the use of *N. sativa* for the treatment of variety of diseases.

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