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Nigella sativa: Properties, processing and food applications

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Abstract

Nigella sativa (black cumin or black seed) is an herb which is having an ample level of preservative and therapeutic effects. Black cumin is applied for its healing effects like bronchodilator, hypotensive, hypoglycaemic, CNS depressant, anti-inflammatory, analgesic and immunopotentiating and is even emphasized about the same in some of the ancient religious literatures. It has been used as powder, whole spice or as essential oil or oleoresins. It has been documented that thymoquinone is the potential compound for all such properties of black cumin. The present review is aimed to deal with the therapeutic and food applications of black cumin (*Nigella sativa*) seeds and their essential oil.

Keywords: *Nigella sativa*, therapeutic effects, bioactive components, food applications

1. Introduction

To enhance the flavour and aroma of food and beverages many spices and condiments can be used. Medicinal plants are being popularly used as one such natural source (Reddy *et al.*, 2018) [67]. Besides using them in their dried or powdered form, the use of essential oils have increased recently. Due to higher level of bioactive constituents such as phenolic acids, flavonoids, and aromatic compounds essential oils act as best antioxidant and antimicrobial agents in foods. These characteristics increased their use in food preparations and classified as generally recognized as safe (GRAS) (Shan *et al.*, 2011) [73].

It is understood that most of the spices possess a wide range of biological and pharmacological activities among which antimicrobial and antioxidant activity are mostly exploited in food formulations. The rich phenolic compounds in *Nigella sativa* (black cumin) seeds make them possible to act as stabilizers, antioxidant and antimicrobial properties, thereby help in increasing the shelf-life of foods and beverages. Though India and Egypt are the highest producers of black cumin, some other countries like Srilanka, Iraq, Nepal and Pakistan also produce them on a small scale basis. Production quantity of brown/black cumin is shown in Fig. 1 (Bajuri, 2015) [12]. Azad Kalongi from Kanpur and AN-1 from the National Research Centre on Seed Spices are the two nigella varieties developed in India (Malhotra, 2012) [48]. Mostly in Egypt and Middle East, black cumin oils are widely applied in treatment of chronic cough and bronchial asthma (Kaskoos, 2011) [36], airway disorders, diabetes, infection, paralysis, eczema, blisters, arthritis (Yimer *et al.*, 2019) [81]. A varieties of compounds are available in the seeds, which contribute to the various potential impacts of black cumin as food as well as medicine. Apart from seeds of black cumin, other plant parts such as roots and shoots also bear various potencies which gave them an inevitable role in the Mediterranean kitchen (Bourgou *et al.*, 2010) [15]. Those effects of this spice including antioxidant effect, antimicrobial effect, anti-inflammatory effect, anti-cancerous effect etc. are even emphasized in the ancient Egyptian, Greek and Roman literatures. In the holy book of Islam, "Quran", the seeds and oils of *Nigella sativa* is claimed as the precious medicine for all kind of health problems other than death (Ramadan, 2016) [62]. The eminent compound of black cumin which provide the bioactive nature is estimated to be thymoquinone, even it is rich in many other active constituents (Amin and Hosseinzadeh, 2016) [8]. The present review is focussed on the major bioactive compounds, therapeutic potentials and food applications of *Nigella sativa* seeds or oil.

2. Major Phytochemicals of seeds

The black seeds or black cumin seeds are highly nutritious and the nutrient content varies based on growing area, cultivars etc.

The nigella seeds usually contain an yellowish volatile oil, a fixed oil, proteins, amino acids such as valine, lysine, leucine, isoleucine, phenylalanine, glycine, alanine, cystine, threonine, glutamic acid, aspartic acid, proline, tryptophan, tyrosine and serine; reducing sugars, mucilage, alkaloids, organic acids, tannins, resins, toxic glucoside, metarbin, bitter principles, glycosidal saponins, melanthin resembling helleborin, melanthigenin, moisture and arabic acid. The seeds also found to possess crude fibre, minerals like Fe, Cu, Zn, Ca, P, and Na and vitamins like ascorbic acid, thiamine, niacin, pyridoxine and folic acid (Kabir *et al.*, 2019; Reza and Moghadam, 2015) [35, 68]. The composition of major nutrients and their concentration is shown in Table 1 and the vitamin and mineral

content is represented in the Table 2 (Kabir *et al.*, 2019; Abd El-Hack *et al.*, 2016; Khoddami *et al.*, 2011; Mamun and Absar, 2018) [1, 35, 38, 49]. It has been identified that these seeds also hold β -carotene which is the precursor of vitamin A (Yessuf, 2015) [52].

Some of the main fatty acids present in black seeds are myristic acid, stearic acid, palmitic acid, oleic acid and linoleic acid (Malhotra, 2012; Ramadan, 2016) [48, 62]. The volatile oil or essential oil extracted out of black cumin possesses a well pronounced antioxidant activity. The volatile oil extracted out of nigella seeds is about 0.5% [4]. Also they possesses many medicinal properties as shown in Fig. 2 (Kaskoos, 2011; Lutterodt *et al.*, 2010) [36, 44].

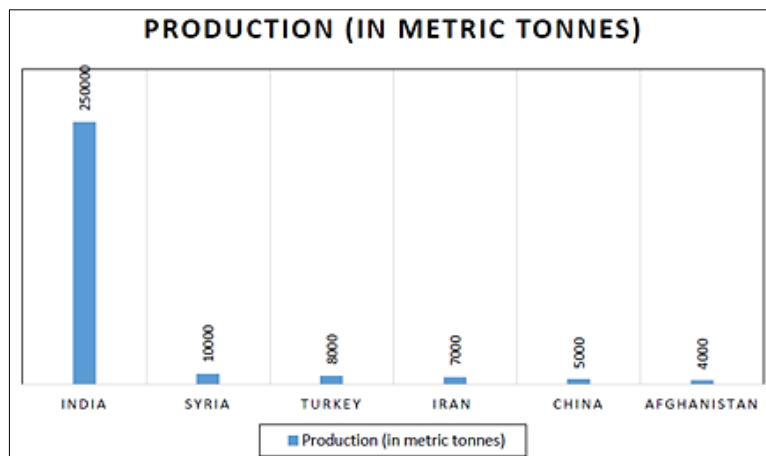


Fig 1: Production quantity data of brown/black cumin (Bajuri, 2015) [12]

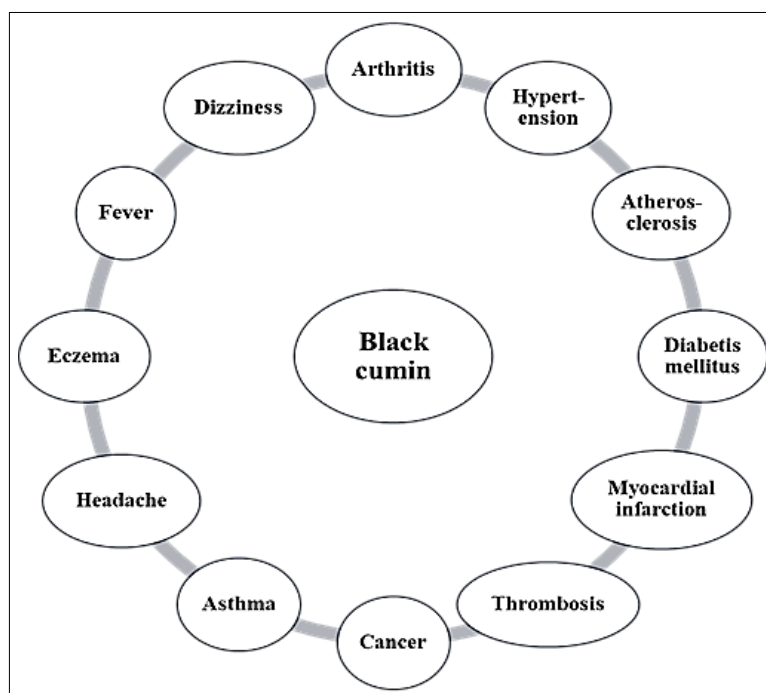


Fig 2: Medicinal potencies of black cumin (Kaskoos, 2011; Lutterodt *et al.*, 2010) [36, 44]

Essential oils are aromatic oily substances recovered from various plant parts like flowers, buds, seeds, leaves, bark, fruits and roots. Essential oils have a strong odour and are the secondary metabolites formed by medicinal or aromatic plants. The main advantage of essential oils is that they can be used in any food and have an antioxidant effect that are generally recognized as safe (Hassanien *et al.*, 2015) [31]. The main components of the volatile oil which are responsible for

their antioxidant activity include thymoquinone, p-Cymene, carvacrol, thymol, thymohydroquinone, nigellimine-N-oxide, nigellidine, nigellin, nigellidine, quercetin, butylhydroxytoluene, ascorbic acid, α -Terpinene, α -Pinene, carvacrol, limonene, β -Phellandrene, α -Terpinolene, 1, 8-Cineole, dithymoquinone, beta-sitosterol, melanthin etc. and the structure of some of them are given in Fig. 3 (Abd El-Hack *et al.*, 2016; Agbaria *et al.*, 2015; Forouzanfar *et al.*,

2014; Hangargekar *et al.*, 2020; Adegbeye *et al.*, 2020) [1, 3, 4, 24]. It is important to understand that the well-known antimicrobial effect of these essential oil derived from spices

are also due to the phenolic compounds (Reddy *et al.*, 2018) [67].

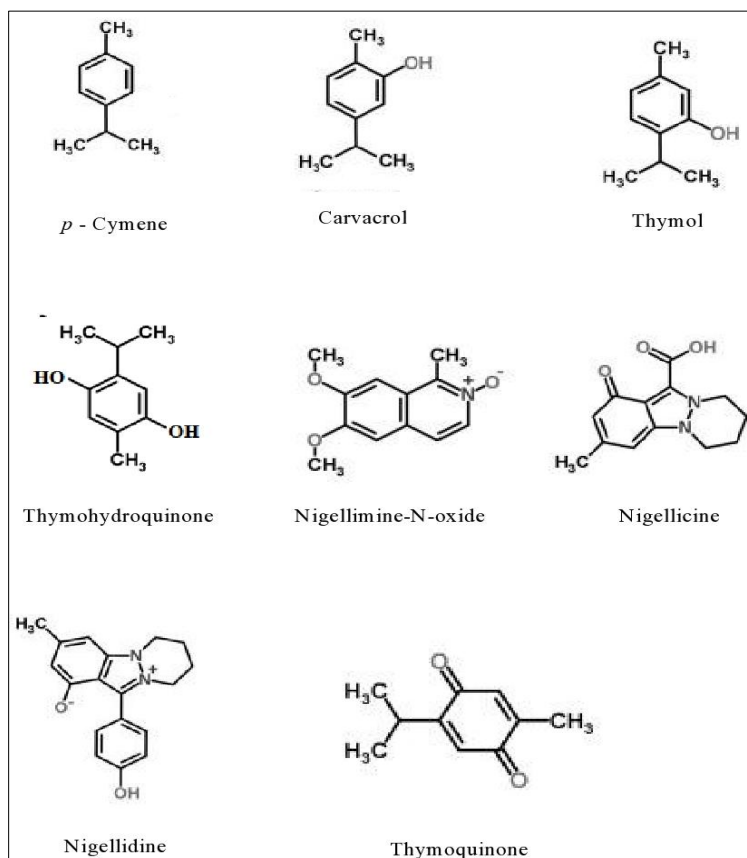


Fig 3: Chemical structure of some of the major phytochemicals in black cumin (Agbaria *et al.*, 2015; Forouzanfar *et al.*, 2014; Hangargekar *et al.*, 2020) [4, 24, 30]

Table 1: Nutritional constitution in black cumin (Kabir *et al.*, 2019; Abd El-Hack *et al.*, 2016; Khoddami *et al.*, 2011) [1, 35, 38]

Nutrient components	Concentration (g kg ⁻¹)
Moisture	38.0 – 73.4
Ash	37.0 – 67.2
Crude fibre	58.3 – 84
Protein	172.7 – 312.0
Lipid	320 – 459.3
Total carbohydrates	192.6 – 340.0

Table 2: Vitamins and minerals composition in black cumin (Kabir *et al.*, 2019; Abd El-Hack *et al.*, 2016; Khoddami *et al.*, 2011) [1, 35, 38]

Vitamins and minerals	Concentration (mg kg ⁻¹)
Thiamine	15.4
Niacin	57
Pyridoxine	5
Folic acid	160
Iron	105 – 451
Copper	14.7 – 18
Phosphorus	527 – 5289
Zinc	60 – 68
Calcium	1860 – 3868

3. Quality specifications for black cumin

In order to provide high quality and wholesome products government has provided certain specifications to be followed in relation to the black cumin seeds as well as its powder. Those products which satisfy the specifications are only

considered to be of good quality and exhibit the benefits carried by them. The specifications for black cumin whole powder and seeds are shown in Table 3 and Table 4 (<http://fsdaup.gov.in>) [32].

Table 3: Specifications for black cumin (Kalonji) powder (<http://fsdaup.gov.in>) [32]

Features	Values (% by weight)
Moisture	≤ 10.0
Total ash on dry basis	≤ 7.0
Ash insoluble in dilute HCl on dry basis	≤ 1.5
Volatile oil content on dry basis	≥ 0.9
Non-volatile ether extract on dry basis (ml/100gm)	≥ 12

Table 4: Specification for black cumin (Kalonji) whole seeds (<http://fsdaup.gov.in>) [32]

Features	Values (% by weight)
Extraneous matter	≤ 1.5
Broken fruits	≤ 5.0
Moisture	≤ 10.0
Total ash on dry basis	≤ 8.0
Ash insoluble in dilute HCl on dry basis	≤ 1.5
Non-volatile ether extract on dry basis	≥ 12.0
Volatile oil content on dry basis	≥ 1.0
Edible seeds other than cumin black	≤ 2.0
Insect damaged matter	≤ 1.0

4. Extraction of seed oil

Extraction is the foremost and the crucial step for studying about natural antioxidants obtained from plant materials. It is this process which helps in separating or deriving essential oils from the plant parts, say leaves, seeds, bark, floral part, fruits, fruit peel etc. The quantity and quality of essential oil extracted out of various plant parts might be different and will absolutely vary in case of different plants, in all aspects. Many extraction factors play important roles in the extraction efficiency, such as extraction temperature, extraction time, extraction pH, type and concentration of extraction solvent (Khoddami *et al.*, 2011) [38]. Along with this, the amount and nature of the raw material that are used for extraction also affect the yield and quality of essential oil Table 5. Several essential oil extraction methods are available and some techniques that can be applied for the extraction of essential oil out of black cumin seed include hydrodistillation (steam distillation), solvent extraction, supercritical fluid (CO₂) extraction and cold press extraction (Stratakos and Koidis, 2016) [77].

4.1 Cold pressing

Cold pressing is also known as cold extraction and is an oldest known technique for essential oil extraction. This method is usually used for citrus oil extraction. The extraction is done in a cold press, in which the mechanical force applied by this physical method will help in bursting the oil glands and thereby expel out the oil (Stratakos and Koidis, 2016) [77]. The main attraction of this technology is the absence of heat treatment as well as chemical treatment during the oil extraction period. Also the oil collected will not be subjected to any refining process and hence the higher level of lipophilic phytochemicals like natural antioxidants (Kiralan *et al.*, 2014) [20]. But the need of volatile oil separation out of the crude oil, it is subjected to hydrodistillation in Clevenger apparatus (Edris, 2011) [20].

4.2 Hydrodistillation

Hydrodistillation is considered to be a green solvent extraction method for essential oils as the solvent involved is water and can be conducted in a Clevenger type apparatus (Gavahian and Farahnaky, 2018) [25]. It is widely used over the other methods because of its low cost and low temperature application which helps in preserving the volatile components of the oil from adverse effect of high temperature, despite being a slow process. This process may take 1.5 to 8 hour duration to complete the process and the yield of product (essential oil) extracted depends on various factors like distillation time, temperature, pressure, type of material etc. (Stratakos and Koidis, 2016) [77]. The yield usually exist in the range of 0.1-2.1% (Erdoğan, 2020; Hassanien *et al.*, 2015) [23, 31]. Some studies have proven that, though the yield of essential oils are less in hydrodistillation compared to some other solvent extraction techniques, the antioxidant activity and total phenol content of the oil is high for hydrodistilled oil (Saha *et al.*, 2016) [71] and also is a better process for food applications. Thymoquinone (Erdoğan *et al.*, 2020; Edris *et al.*, 2016) [21, 23] and p-Cymene (Bourgou *et al.*, 2012) [15] are

found as the major bioactive compounds in hydrodistilled black cumin essential oil.

4.3 Soxhlet extraction

This is the process in which the essential oil or the volatile oils of the plant materials are extracted by means of organic solvents like ethanol, methanol, hexane and petroleum ether and the apparatus used are generally known as soxhlet apparatus. The extraction proceeds for a time of about 4-12 hour, at a temperature range of 40-70°C (Hassanien *et al.*, 2015; Boudiaf *et al.*, 2016) [14, 31]. Compared to the steam distillation method the temperature applied is low, thereby the thermolabile components can be preserved to a certain extent. Even though the phytochemical quantity and yield is pretty good for this extraction technique, the food applications of solvent extracted oils are not promoted due to the presence of unwanted toxic solvent residue in the extracted essential oil (Stratakos and Koidis, 2016) [77]. The yield of essential of *Nigella sativa* by this extraction techniques ranges between 1 and 45% (Boudiaf *et al.*, 2016; Ashraf *et al.*, 2011; Bornare *et al.*, 2015) [10, 14, 18] and thymoquinone is the most active biocompound identified in essential oil extracted by this method with a concentration of 30-48% (Boudiaf *et al.*, 2016; Ashraf *et al.*, 2011) [10, 14].

4.4 Supercritical fluid extraction

This is considered as the most efficient method for the extraction of essential oil. The extraction time is between 2 to 5 hours at a temperature of 20-70°C and under a pressure range of 100-400 bar (Ashraf *et al.*, 2011; Gurganova *et al.*, 2010; Parhizkar *et al.*, 2011) [10, 28, 60]. As the solvent extraction techniques using several organic solvents results in the formation of unwanted residues in the final product, there is huge limitation in their applications. If the extracted oils are subjected to the residue removal, it may results in the deterioration of the essential oil properties. But the use of supercritical fluids for the extraction process has removed the limitations efficiently. The low temperature application of supercritical CO₂ extraction process helped in preventing the loss of thermolabile bioactive compounds as well. Along with this benefits one of the main advantage of SC – CO₂ extraction is the high yield of the product (Solati *et al.*, 2012) [75] which is usually in the range of 0.84-36.4% (Ashraf *et al.*, 2011; Parhizkar *et al.*, 2011) [10, 60] and another speciality is the high content of thymoquinone, a monoterpenoid (Bornare *et al.*, 2015; Gurganova *et al.*, 2010) [14, 28]. Non-toxicity, lack of odor and taste, lower extraction time, low critical temperature are also the other pronounced benefits of SCFE which demands their use in food industries (Stratakos and Koidis, 2016) [77].

5. Therapeutic potentials of *Nigella sativa*

Studies that have been chosen show the wider applications of black cumin in pharmacology as well as culinary. The antioxidant, antibacterial, antifungal, anti-inflammatory effects (Fig. 4) of black cumin are well known (Ramadan, 2016) [62]. These various essential oil constituents and their potential effects are given in Table 6.

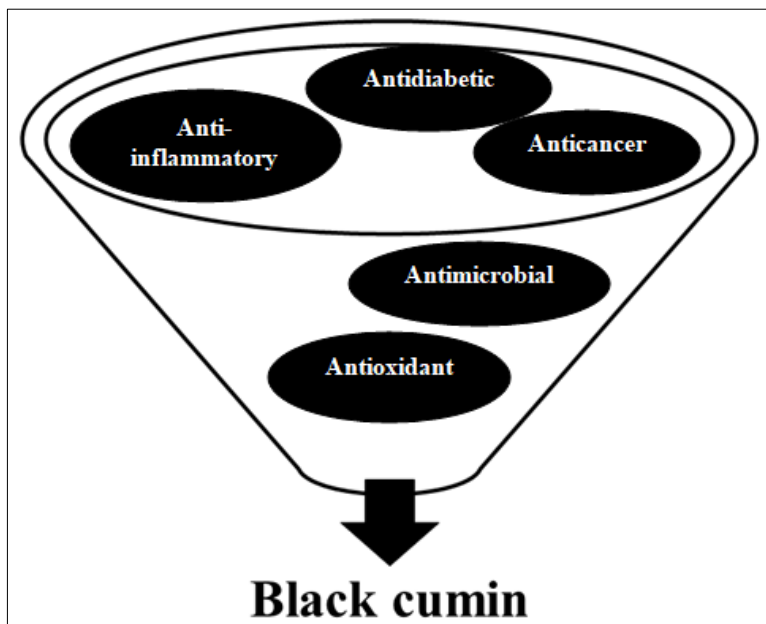


Fig 4: Uses of *Nigella sativa* (Ramadan, 2016) [62]

Table 5: Different extraction techniques and some of the characteristics of respective oil

Extraction method	Extraction condition	Solvent	Yield of essential oil (%)	Main active component in essential oil	Concentration of main active component	Major fatty acid	References
Hydrodistillation	95-96°C 1.5-8 hours	Water	0.1 - 2.1	Thymoquinone P - Cymene	45-50% 56.2-65.8%	Linoleic acid	(Amin and Hosseinzadeh 2016; Cakmakci <i>et al.</i> , 2014; Halawani, 2009) [8, 17, 21]
Soxhlet extraction	70°C, 5 hours	Hexane	1	Thymoquinone	30-48%	Linoleic acid	(Boudiaf <i>et al.</i> , 2016; Kazemi, 2014; Kiralan <i>et al.</i> , 2017) [14, 37, 40]
	30-35°C, 7 hours	Ethanol	12.4				
	100°C, 6 hours	Hexane	19.1				
	40-45°C, 8-12 hours	Hexane/ethanol	28.1/29.2				
Supercritical CO ₂ extraction	40-60°C, 8 hours	Petroleum ether	37.33	Thymoquinone	33.12 -76.7%	Linoleic acid	(Khosravi <i>et al.</i> , 2011; Lutterodt <i>et al.</i> , 2010; Kokoska <i>et al.</i> , 2008) [39, 41, 44]
	50°C, 100 bar	Carbon dioxide	0.84				
	50°C, 200 bar		9.03				
	60°C, 250 bar		12				
50°C, 300 bar	24.4						

Table 6: *Nigella sativa* essential oil constituents and their potential effects

Potential effects	Oil constituent	References
Antioxidant activity	Thymoquinone, thymol, 4-terpineol, thymohydroquinone, t-anethole, linoleic acid, oleic acid	(Yimer <i>et al.</i> , 2019; Ozdemir <i>et al.</i> , 2018) [57, 81]
Antimicrobial	Thymoquinone, thymohydroquinone, p-Cymene, thymol, longifolene, Carvacrol, melanin	(Ibrahim <i>et al.</i> , 2017; Khoddami <i>et al.</i> , 2011; Ozdemir <i>et al.</i> , 2018) [33, 38, 57]
Anticarcinogenic activity	Thymoquinone, Dihydrothymoquinone, p-Cymene, α -pinene, α -hederin	(Amin and Hosseinzadeh, 2016; Dimitrios, 2006) [8, 19]
Anti-inflammatory effects	Thymoquinone, p-Cymene, α -pinene, α -thujene, γ -terpinene	(Ozdemir <i>et al.</i> , 2018; Parhizkar, Latiff, and Rahman 2011) [57, 60]
Antidiabetic	Thymoquinone	(Pokorný, 2007; Randhawa and Alghamdi, 2011) [61, 65]
Analgesic activity	Thymoquinone	(Ashraf <i>et al.</i> , 2011; Kokoska <i>et al.</i> , 2008) [10, 41]

5.1 Antioxidant activity

Food processing industries are in search of products to delay the changes taking place in food products, during the preparation, storage and distribution, due to the lipid oxidation (Ramadan, 2016) [62]. Reactive Oxygen Species (ROS) are mainly responsible for the initiation of oxidation reaction in foods which will change the functionalities of proteins, lipids and carbohydrates. Superoxide radical, hydrogen peroxide, peroxy radicals, hydroxyl radical, as well as reactive nitrogen species such as nitric oxide (NO) and

peroxynitrite etc. are some of the compounds that are included in ROS. Due to their action, food products become less acceptable or unacceptable to consumers and also the reactions reduce the overall nutritional, chemical and physical qualities of food during storage and marketing (Bourgou *et al.*, 2012) [15]. Antioxidative compounds are those which are used to prevent the oxidation and thereby the rancidity. Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT) and tert-butyl hydroquinone (TBHQ) etc. are used for preventing the

oxidative reactions in foods, pharmaceuticals and cosmetics etc. But nowadays, there is a deviation from the habit of using synthetic antioxidants and the food sector is efficiently trying to replace them with the natural antioxidants. It is the consumer's concern over the use of synthetic antioxidants and their ill effects has given rise to the use of natural antioxidants in food as these are safe and health-friendly. As a result the demand for natural bioactive compounds with antioxidant effects are highly exploited. Some of the most common dietary natural antioxidants include ascorbates, tocopherols and carotenoids (Dimitrios, 2006) [19].

Natural antioxidants are essentials in human nutrition because of high concentrations of free lipid radicals both in food and *in vivo* after food ingestion (Pokorný, 2007) [61]. The major antioxidant components present in black cumin essential oil as revealed by Gas chromatography-mass spectrometry (GC-MS) analysis were thymoquinone (37.6%) followed by p-cymene (31.2%), α -thujene (5.6%), thymohydroquinone (3.4%), and longifolene (2.0%), while linoleic acid was the major component in the oleoresins extracted in different solvents. Black cumin oil and ethyl acetate oleoresin at 20 $\mu\text{g mL}^{-1}$ concentrations showed the scavenging activity of 95.4% and 89.75%, respectively, which was comparatively higher than BHT and BHA but lower than PG (Singh *et al.*, 2014) [74]. The antioxidant activity of black cumin seed (BCS) extract in comparison with BHT was evaluated and concluded that BCS extract at higher concentration can be used in place of BHT with comparable effects and also the BCS extract showed similar radical scavenging activity as that of BHT when applied in soybean oil containing no antioxidants. So the black cumin is a promising antioxidant for the stability of edible oils (Soleimanifar *et al.*, 2019) [76]. It was reported that *Nigella sativa* species are also rich in other compounds like tannin, flavonoids and alkaloids (Kooti *et al.*, 2016) [43]. The pronounced antiradical effect and antioxidant effect of these mentioned compounds, add onto the superoxide radical scavenging activity. The extract of shoots, roots and seeds of *Nigella sativa* showed strong antioxidant potential when analysed by oxygen radical absorbance capacity (ORAC) method and a cell based method. In this study it was also found out that the seed water fraction possess a maximum inhibition of t-BuOOH-induced DCFH (dichlorodihydrofluorescein) oxidation among all other extracts with an IC_{50} value of 0.11 mg/mL (Bourgou *et al.*, 2012) [15]. The antioxidant activities of crude methanolic extract (CME) and its fractions using ethyl acetate (EAF), hexane (HF) and water (WF) of black cumin seedcake was studied (Mariod *et al.*, 2009) [50]. The total phenolics were found to be 78.8, 27.8, 32.1 and 12.1 mg gallic acid equivalents (GAE)/g in EAF, CME, WF and HF, respectively. The CME and EAF exhibited the highest DPPH followed by WF and HF. The extract/fractions showed high effect on reducing the oxidation of β -carotene. It has been studied the ability of black cumin seeds in reducing the oxidant malondialdehyde (MDA) in plasma of the normal postmenopausal women (Mostafa *et al.*, 2013) [55]. After the consumption of *Nigella sativa* and *Allium sativum* on a specified dose for 8 weeks resulted in a reduction of plasma MDA and inferred that, the effect may be due to the increased activity of antioxidants, especially thymoquinone, which is obtained from the black seeds. In a study conducted, rats nourished with atherogenic suspension were provided with methanolic extracts and volatile oil obtained from the black seeds. It was found on examination after 30 days that those

hyperlipidemic rats which treated with the methanolic extract and volatile oil had a great potential to prevent oxidation (88%) in their plasma when compared with the control rats (Ahmad and Beg, 2016) [5].

5.2 Antimicrobial activity

Antimicrobial agents are used to prevent the attack of microorganisms like bacteria, fungi, mold etc. and thereby extend the shelf life of the food products. But the use of synthetic antimicrobial compounds are being restricted in foods due to their adverse effects on the human health as well as the microbes are getting resistant against them. Thus, in the present scenario the demand for natural antimicrobial substances are increasing. Spices and herbs, their essential oils or active compounds are found to be a best source of antimicrobial agents against a wide range of gram positive and gram negative bacteria, yeast and molds, that can be safely used in foods for preserving their quality and shelf-life (Gottardi *et al.*, 2016) [27].

In *Nigella sativa* (black cumin), thymoquinone (TQ), thymohydroquinone (THQ), dithymoquinone and thymol was found as the major compounds imparting the antibacterial and antifungal effects and their efficiency was studied. By the broth dilution method the antimicrobial activity of black cumin essential oil is examined and stated that the gram positive bacteria were highly susceptible with MIC (maximum inhibitory concentration) as low as 3 $\mu\text{g/ml}$ whereas the gram negative bacteria showed 200 to 1600 $\mu\text{g/ml}$ MIC. It was also reported that *Staphylococcus aureus* is highly susceptible to TQ as it only require 3 $\mu\text{g/ml}$ for inhibition and 6 $\mu\text{g/ml}$ for killing. But the susceptibility is less towards THQ as higher concentrations are preferred (Halawani, 2009) [29].

The antibacterial activity of different extracts (methanol, petroleum ether and distilled water) of *Nigella sativa* and *Cassia angustifolia* was analysed against some bacteria like *E. coli* (ATCC 25922), *S. aureus* (ATCC 25932), *B. subtilis* (ATCC 6633) and *K. pneumonia* using broth dilution method and found out that methanol extracts were of high effectiveness than the petroleum ether extracts and distilled water extracts against both gram positive and gram negative bacteria. It was also identified that comparing both the herbs, black cumin shown the high antibacterial activity against gram negative and gram positive bacteria. The inhibition zone offered by methanolic extract for gram positive bacteria was 14.66 mm and 19.66 mm for *Bacillus subtilis* and *Staphylococcus aureus* respectively while its inhibition zone was 10.33 mm and 18.66 mm for gram negative bacteria *Escherichia coli* and *Klebsiella pneumonia* respectively (Reddy *et al.*, 2018) [67]. It was also proved that the antimicrobial (bactericidal and fungicidal) effects of black cumin oil against microorganisms, especially *Escherichia coli* (MIC-2.5 \pm 0.3) and *Candida albicans* (MIC- 2 \pm 0.6), which is efficient than the action of antibiotics like streptomycin and fluconazole (Kazemi, 2014) [37].

It has been reported that the black cumin oil can be used effectively against the growth and production of aflatoxin B1 by *Aspergillus parasiticus* (CBS 921.7) and *Aspergillus flavus* (SQU 21) strains (Adegbeye *et al.*, 2020) [3]. Usage of 1 and 2 ml/100 ml of oil hindered the production of aflatoxin B1 by 49.7–58.3% through interruption of the biosynthesis pathway of aflatoxin. It is stated that thymoquinone content in black cumin is majorly responsible for its antimicrobial action, though other phytochemicals are also present. In another

study, synthesised platinum nanoparticles (Pt NPs) using *Nigella sativa* extract also exhibited antibacterial effect against both gram positive and gram negative bacteria, analysed by disc diffusion method (Aygün *et al.*, 2020) [11].

5.3 Anti-inflammatory activity

Inflammation is a reaction of human body whenever it suffers from any kind of injury. The injuries will stimulate the arterioles of the encompassing tissues and results in their dilation. as a result the affected area will experience a pain, redness, swelling and a burning sensation. This oedema is characterized by piled up fluids and will result in the scrunching of the nerves. Thus the individual will be affected with severe pain. Anti-inflammatory drugs are usually prescribed to overcome such situations (Apu *et al.*, 2012) [9]. But the intake of these drugs are having certain side effects on the human body and may even lead to the disfunctioning of internal organs like liver, kidney etc.

Phytochemicals are an alternative source of anti-inflammatory chemicals and many medicinal plant extracts are used for the same. The essential (volatile) oil of black cumin is proved to be fruitful as an anti-inflammatory agent. A study related to black cumin oil evinced their anti-inflammatory activity against carrageenan-induced rat hind paw oedema and cotton seed pellet granuloma (Majdalawieh and Fayyad, 2015) [46]. They demonstrated thymoquinone in black cumin oil as the potent constituent responsible for the activity and their anti-inflammatory activity was comparable to the drug indomethacin.

A study was conducted to prove the anti-inflammatory activity of *Nigella sativa*. The anti-inflammatory effect of the alcoholic extract of both seeds and callus on the glial cells were analysed. They were of the opinion that both the seed and callus extracts were a moderate anti-inflammatory agent and their effects can be compared to the drug acetaminophen in terms of their concentration. The inflamed mix glial cells, on treatment with the *Nigella sativa* oil of concentration at a range of 1.25 to 5 µl/ml was found to be cured due to its high anti-inflammatory effects (Alemi *et al.*, 2013) [6].

5.4 Anticancer activity

Cancer is a drastic condition which is lethal in most cases. To combat with this deadly sick, several naturally occurring stuffs are in search. To prevent these tumorigenesis, various herbal extracts and bioactive phytochemicals are used nowadays (Majdalawieh and Fayyad, 2016) [47]. Anticancer activity is one of the most important properties of black seed and its volatile oil which has been studied extensively. Many studies were carried out to analyse the anticancer potential of black cumin and the main component responsible for the activity.

The ability of black cumin oil and extract as an anticancerous agent was examined against human lung cancer cell line. After exposure of the cancer cells to black cumin extract and oil, there occurred a sound reduction in the viability of those cells and also their size get reduced. The percentage cell viability was found to be 75%, 50%, and 26% at 0.25, 0.5, and 1 mg/ml of *Nigella sativa* oil and it was 73%, 48%, and 23% at 0.25, 0.5, and 1 mg/ml of *Nigella sativa* extract. The effect was high at higher concentration of both extract and oil and they proved to be an active treatment aid against cancer. When talking about the morphology of the cancer cells, it also got changed under the effects of black cumin oil and extract. When the cells were exposed to a concentration of 0.25, 0.5

and 1mg/ml of extract and oil, there size got reduced and attained the normal cell size. It was also denoted that oil showed higher anticancerous effect compared extract (Al-Sheddi *et al.*, 2014) [7].

The active component possessing highest anticancer activity in black cumin was found to thymoquinone. It was also revealed that a controlled thermal processing results in increase of the anticancerous activity. Seeds which were heated to a temperature of 50°C showed improved activity (90%) in inhibiting the tumor cells compared to the non-heated seeds (40%), in this study. At the same time over heated seeds had no effect. This is attributed to the activity of thymoquinone content in the seeds. It is advocated that the thymoquinone content in controlled heated seeds were enhanced than that of non-heated samples, which may happened due to the oxidation of persisting thymohydroquinone to thymoquinone under thermal processing as denoted in Fig. 5 (Agbaria *et al.*, 2015) [4].

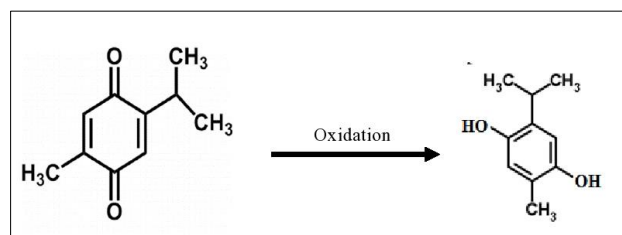


Fig 5: Conversion of thymohydroquinone to thymoquinone on heating (Agbaria *et al.*, 2015) [4]

It has been reported that the active compounds in *Nigella sativa* which possess the ability to destroy cancer cells include thymoquinone and alpha hederin. Both these compounds interpret the cell cycle of tumor cells and cause damage to them, thereby wipe out their activity. Due to these bioactive compounds, black cumin came out a potent source of anticarcinogenic substances and can be used in it treatments along with other established chemotherapeutic agents (Randhawa and Alghamdi, 2011) [65].

6. Applications of black cumin in food

Spices and herbs are precious substances in which a huge amount of benefits are concealed within it that are beneficial for mankind. They have been used both in pharmaceuticals and foods from earlier times itself (Panpatil *et al.*, 2013) [59]. In foods, they have been used for imparting color, flavour, aroma etc. from long years ago. The main properties such as antioxidant and antimicrobial effects of spices and their essential oils are being explored in food industries. This is aimed at enhancing the shelf stability, nutritional properties etc. of the foods in which they are applied. The main matrix for their application, chosen are fat rich products that are highly susceptible for microbial spoilage and lipid oxidation process (Embuscado, 2015) [22].

Black cumin (*Nigella sativa* L.) is one among the spices that have high free radical scavenging activity and microbial destruction ability due to the constituents within them. The various bioactive compounds in *Nigella sativa* (black cumin) seeds are entrusted with the highly pronounced antioxidant and antimicrobial activity, which made their effective applications in various food products of plant origin as well as animal origin.

It was reported that the nutrient quality and oxidative stability of rapeseed oil were improved by blending the oil with

different concentrations of black cumin oil and rice bran oil. The blends on evaluation for nutritional properties and shelf stability, resulted that the blend of rapeseed oil and black cumin were enriched with omega-6 and omega-3 fatty acids. Also the α - and γ -tocopherols as well as all isomers of tocotrienols were in increased amount. The blending also reduced the polyunsaturated fatty acid (PUFA) to saturated fatty acid (SFA) ratio and thereby improved the stability of oils. Usage of a concentration of 10% of black cumin oil and 20% of rice bran oil was found to be more effective against oxidation reaction of rapeseed oil. It shows the higher antioxidant activity offered by black cumin oil at low concentrations (Rudzińska *et al.*, 2016) [69]. Similarly the stability and nutritional quality examination of corn oil was studied by blending it with coriander seed oil and black cumin oil. The addition of black cumin oil to corn oil improved the contents of certain minor fatty acids and also a pronounced change was found in the tocopherols content in the range of (821–850 mg/kg). Even though the protection offered by black cumin oil in the blend was lagging behind the coriander seed oil, their effects in improving the oxidative stability and enhancing the nutritional properties are well pronounced (Ramadan and Wahdan, 2012) [63].

As dairy products are nutritious rich, they are always prone to microbial attack and the ample fat content in them make a good chance for spoilage by lipid oxidation as well. Butter, one of the mostly consumed dairy products around the world. The high nutrition leads to increased attack of spoilage microorganisms as well as reduced storage life. On application of black cumin oil in butter at various concentration (0.05, 0.1 and 0.2 weight%) found an effective way of preventing the microbial spoilage as well as rancidity in them without using synthetic preservatives (Çakmakçı *et al.*, 2014) [17]. It is analysed and found in the study that, among various bioactive compounds in black cumin, it is p-cymene and thymoquinone mainly responsible for the radical scavenging activity and antimicrobial activity. Growth of many spoilage bacteria have been hindered by the essential oil but the fungal growth was not very well prevented. The antioxidant activity performed by black cumin essential oil was comparable with that of butylated hydroxyl toluene (BHT). For both antioxidant and antimicrobial effects, it was the concentration of 0.1% and 0.2% showed high efficiency.

Cheeses are also a highly consumed dairy product around the globe. Many studies in cheeses have been carried out to investigate the bioactivity of black cumin and their oils. Tulum cheese is a kind of cheese made out of sheep's milk. Black cumin has been added in those cheese at different concentrations of 0, 1 or 2%, before ripening stage and their effects on the microbiological, physicochemical and ripening properties was examined. On analysis it was found that the addition of whole black cumin reduced the attack of spoilage coliform bacteria, yeast and moulds and also, the increase in concentration of black cumin and reduction in microbial count had a direct effect. When talked about the chemical characteristics, there was an appreciable decrease in the moisture content as well as a good increment in the dry matter, ash, salt, protein and fat content on the addition of black cumin seeds (Çakır and Çakmakçı, 2018; Mahgoub *et al.*, 2013) [16, 45]. Similarly in another study conducted, the antimicrobial effect of essential oil of Iranian black cumin was examined by applying them in Gouda cheese. It was found out that the maximum antimicrobial effect was at a concentration of 0.4% of essential oil. Yeast was highly

susceptible to essential oil effects that got a 2 log reduction and the least effect was upon Enterobacteriaceae that got reduced by 0.75 log. The addition also improved the organoleptic properties of cheese such as flavour and aroma whereas texture and color was not much affected (Taherkhani *et al.*, 2015) [78].

Yogurt is a nutritious dairy product and its acceptability can be positively used as a food base for value addition. The high content of thymoquinone in *Nigella sativa* oil has been utilized by the scientists to make functional yogurt with improved health benefits. Thymoquinone, one of the main monoterpene in black cumin seed oil has a great amount of therapeutic effects. Their addition to food product will help the consumers to acquire such benefits. But the problem with their addition was that, their potential effects were getting degraded during the processing and storage time. But as a solution to this, it was found that the microencapsulation of *Nigella sativa* using spray drying technology will improve the thymoquinone retention within the food material during the whole storage period (Mohammed *et al.*, 2017) [54]. The cold pressed *Nigella sativa* oil was microencapsulated using spray drying technology with modified starch (MS) and maltodextrin (MD) mixture as wall material and developed functional yogurt (Abedi *et al.*, 2016) [2]. The microencapsulation efficiency sounded good (89.48%) and thymoquinone retention was also acceptable (61.12%). Their analysis found that the pH and acidity of the samples were not varied much from control sample and also the thymoquinone retained in the yogurt during the storage period. The consumer acceptance evaluated by sensory analysis was also good for the functional yogurt. A study was done about the action of nanoemulsions of *Nigella sativa* oil added to icecream at different concentration to develop a fortified product. Gum arabic, sodium caseinate, and Tween-20 were used to stabilize the nanoemulsions prepared (Mohammed *et al.*, 2020) [53]. Supercritically extracted *Nigella sativa* oil is used to prepare the nanoemulsions, which is of high content of bioactive compounds and is prepared with the help of a hot plate with a magnetic stirrer. The nanoemulsions were prepared at various concentrations of 3%, 5% and 10%. The icecream added with the *Nigella sativa* oil nanoemulsions were subjected to several analyses and found that the icecream in which the nanoemulsions added at 5% was having high value of overall acceptability among the consumers. Thus a functional icecream has been developed successfully with the addition of *Nigella sativa* essential oil.

Nigella sativa seed oil was extracted with soxhlet extraction method and was incorporated into cookies to impart a nutraceuticals effect in it. With the aim of improving the value of normal cookies a concentration of black cumin oil was added with an acceptable organoleptic properties. The specific taste, aroma and flavour of the *Nigella sativa* oil prevented the use of its higher concentration in cookies, which may make the product unacceptable. The addition of the oil at a concentration of 6% made a functional cookies with increased nutritional and health benefits (Bornare *et al.*, 2015) [18].

A work has been carried out on nutraceuticals pizza base enriched with black cumin essential oil to analyse the antioxidant properties. Essential oil obtained by conventional technique (solvent extraction) and supercritical fluid extraction has been used. In the study they revealed that the antioxidant potential was high in pizza dough enriched with essential oil, among which the one incorporated with

supercritically extracted essential oil found to be more effective. The addition of essential oil improved the storage life of the pizza base to a sound level (Iqbal *et al.*, 2018) [34].

Mayonnaise is a well-known and typically used sauce which is rich in oil. The high amount of oil itself is responsible for their reduced shelf stability. Black cumin oil had used in preparation of mayonnaise at various concentrations (5%, 10% and 20%) and analysed their storage quality, nutritional properties as well as their sensory parameters (Ozdemir *et al.*, 2018) [57]. The results came out explained the inevitable role of black cumin oil in preventing the lipid oxidation of the product and thereby preventing rancidity. The results express the intimacy of thymoquinone in providing this function which act as a hydrogen donor and scavenger of free radicals. In case of sensory parameters, lowest concentration of black cumin oil of 5% was most acceptable. Though the mayonnaise having lowest amount of black cumin oil was having overall acceptability, the efficacy of black cumin oil as an antioxidant remains dominant.

In case of non-vegetarian products also the black cumin can be used as a preservative as a whole or their extracts. The antimicrobial potential of black cumin oil has been exploited in the storage of *Barbus grypus* fish fillets. The fillets were prepared and treated with different concentrations of black cumin oil such as 0.2%, 0.4% and 0.6% and examined their microbial quality at different periods of storage. Also their thiobarbituric acid (TBA) value was referred for determining the effects on lipid oxidation. On analysing the results, there were no influential difference in their TBA value compared to the control. But the storage stability improved with increased oil dosage, also the sensory analysis gave a good acceptance of black cumin oil incorporated fillets than the control. In terms of their microbial growth, at a concentration of 0.4% and 0.6% of black cumin oil, found a great reduction in spoilage microorganisms. For Enterobacteriaceae, yeast and mold the concentration was same for maximum log reduction (Ozpolat and Duman, 2017) [58].

The storage period of the flesh of the fish rainbow trout (*Oncorhynchus mykiss*) was improved when the fish was fed for 144 days with feed containing black cumin oil at different concentrations of 0.00%, 0.10%, 0.40%, 0.70%, 1.00%, and 1.30%. Though there was no noticeable change in the sensory characteristics for the fish even after feeding with black cumin oil, the microbial safety was well established during the storage period, and it was concluded that the black cumin feeding in fishes helps to enhance the keeping quality of the fish's flesh in microbial aspects (Öz *et al.*, 2017) [56].

Chicken meat, due to high moisture content and protein, considered a good substrate for microbial growth. Active packagings can be used to provide an antimicrobial effects in these spoilage prone foods. Black cumin essential oil is applied in multiple layers (5, 10 and 15) of the package in which chicken meat is confined and was analysed for their antioxidant and antimicrobial effects on the meat during 5 days of storage. The antioxidant activity found increasing with increasing number of layers coated with black cumin oil, also 10 layers found to be effective along with feasibility. In this study, it was successfully proved that the active packages made with incorporation of black cumin oil was efficient in preventing the bacterial growth, especially gram negative bacteria (*E. coli*) than gram positive bacteria (*Staphylococcus aureus*). They also helped in preventing lipid oxidation of the fat in the meat. At the same time, the control pack lacking black cumin oil was not effective in both of these functions.

So it is concluded that black cumin oil is a highly potent natural active agent against bacterial growth and rancidity in chicken breast meat (Konuk *et al.*, 2019) [42].

7. Conclusion

Black cumin is a spice of supreme qualities. They are good source of many biologically active compounds. The same has given them an inevitable role in food and pharmacology from ancient times itself. The active components within them help in curing many diseases as well as are helpful in improving the nutritional and keeping qualities of food products. In this study, various aspects related to black cumin and their potential functions and its application have been analysed. The antioxidant, antimicrobial activities of black cumin and its essential oil is widely exploited by food industries. They always give an augmentation for food products in terms of nutritional, sensory and storage qualities. The phytochemicals in black cumin, scientifically known as *Nigella sativa*, are highly efficient to be used in food matrix as they impart several functions like free radical scavenging activity, antimicrobial effect, antidiabetic potency, anti-inflammatory, anticarcinogenic, analgesic activity etc. it is the bioactive components like thymoquinone, p-cymene, terpenole, thymol etc. provide them all these potencies. The most efficient technique for oil extraction is found to be supercritical fluid extraction compared to steam distillation and solvent extraction with organic solvents. This is because of the higher quality and quantity of the volatile oil offered by this technique. Through this investigation, it is well understood that the need for natural additives are increasing as the consumers are giving high concern to their health and are even trying to avoid synthetic additives in food in view of their adverse health effects. Anyway the capabilities of black cumin as a natural food additive can be maximally used up in foods without any dilemma.

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