



Black cumin (*Nigella sativa*) and its constituent (thymoquinone): a review on antimicrobial effects

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ABSTRACT

Nigella sativa seeds have wide therapeutic effects and have been reported to have significant effects against many ailments such as skin diseases, jaundice, gastrointestinal problems, anorexia, conjunctivitis, dyspepsia, rheumatism, diabetes, hypertension, intrinsic hemorrhage, paralysis, amenorrhea, anorexia, asthma, cough, bronchitis, headache, fever, influenza and eczema. Thymoquinone (TQ) is one of the most active constituent and has different beneficial properties. Focus on antimicrobial effects, different extracts of *N. sativa* as well as TQ, have a broad antimicrobial spectrum including Gram-negative, Gram-positive bacteria, viruses, parasites, schistosoma and fungi. The effectiveness of *N. sativa* seeds and TQ is variable and depends on species of target microorganisms. The present review paper tries to describe all antimicrobial activities that have been carried out by various researchers.

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Introduction

Nigella sativa is an annual flowering plant. It grows to 20–30 cm (7.9–11.8 inch) tall and has linear lanceolate leaves. The delicate flowers have 5-10 petals and the colors are usually yellow, white, pink, pale blue or pale purple. The fruit of plant is large and inflated capsule composed of 3-7 united follicles, that each of them has numerous seeds. The black colored seeds are flattened, oblong and angular, funnel shaped, with the length of 0.2 cm and 0.1 cm wide (1).

This plant is known by numerous names, for example black cumin (English), black caraway seeds (USA), shonaiz (Persian) and kalajira (Bangali) (2).

Chemical constituents

Extensive studies were done to identify the composition of the black cumin seed, the ingredients of *N. sativa* seed includes: fixed oil, proteins, alkaloid, saponin and essential oil.

The fixed oil (32-40 %) contains: unsaturated fatty acids which includes: arachidonic, eicosadienoic, linoleic, linolenic, oleic, almitoleic, palmitic,

stearic and myristic acid as well as beta-sitosterol, cycloecalenol, cycloartenol, sterol esters and sterol glucosides (3-5).

The volatile oil (0.4-0.45 %) contains saturated fatty acids which includes: nigellone that is the only component of the carbonyl fraction of the oil, Thymoquinone (TQ), thymohydroquinone (THQ), dithymoquinone, thymol, carvacrol, α and β -pinene, d-limonene, d-citronellol, *p*-cymene volatile oil of the seed also contains: *p*-cymene, carvacrol, t-anethole, 4-terpineol and longifoline (3, 4, 6).

Black cumin seed have two different forms of alkaloids: isoquinoline alkaloid that includes: nigellicimine, nigellicimine n-oxide and pyrazol alkaloid that includes: nigellidine and nigellicine (3, 4).

The nutritional compositions of *N. sativa* are vitamins, carbohydrates, mineral elements, fats and proteins that include eight or nine essential amino acids.

By sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) the fractionation of whole *N. sativa* seeds was done which shows the bands ranged from 94 to 100 kDa molecular mass (7).

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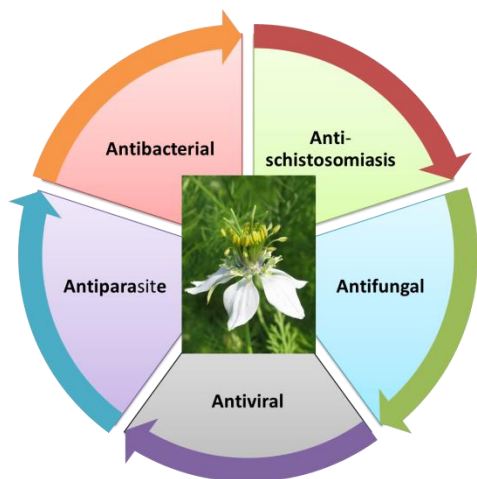


Figure 1. Different effects of *Nigella sativa* against microorganisms

Black cumin seeds also have saponin and alpha hederine and in trace amount has carvone, limonene and citronellol, as well as provide relatively good amounts of different vitamins and minerals such as Fe, Ca, K, Zn, P, Cu (3, 4).

Most of the pharmacological effects are due to quinine constituent, of which TQ is the mainly abundant. TQ possess anticonvulsant activity (8-10), antioxidant (11), anti-inflammatory (12), anti-cancer (13), antibacterial (14) and antifungal activity (15).

Traditional uses of folk remedies

N. sativa seeds have been used traditionally in middle eastern folk medicine as a treatment for various diseases for more than 2000 years ago (16).

The seeds were used as pungent appetizer, aromatic, thermogenic, diuretic, expectorant, purgative, stimulant, sudoriferous, sedative and carminative (17-23).

Black cumin seeds have a history of use in traditional Arabic herbal medicine to treat many diseases such as skin diseases, jaundice, gastro-intestinal problems, anorexia, conjunctivitis, dyspepsia, rheumatism, diabetes, hypertension, intrinsic hemorrhage, paralysis, amenorrhea, anorexia, asthma, cough, bronchitis, headache, fever, influenza and eczema (17-23).

Pharmacological properties

In recent years huge number of studies have been carried out, acclaimed medicinal properties emphasized on different pharmacological effects of *N. sativa* seeds such as antioxidant (24), anti-tussive (25), gastroprotective (26), anti-anxiety (27), anti-ulcer (28), antiasthmatic (29), anti-cancer, anti-inflammatory, immunomodulatory and anti-tumor properties (30-32), hepatoprotective effect (33), also gastric ulcer healing (34), tumor growth suppression (35), men infertility improvement (36), cardiovascular disorders (37), memory improvement (38), stimulate milk production (39), protective effects on lipid

peroxidation (40), antibacterial activity (41), anti dermatophyte (42), antiviral activity against cytomegalovirus (43), have been reported for this medicinal plant.

In this paper, we describe the antimicrobial effects of *N. sativa* and its constituents. Selected studies showing the different extracts and microorganisms tested in experimental models *in vivo* and *in vitro* (Figure 1) for antibacterial, antifungal, anti-schistosomiasis and antiviral also demonstrated in Tables 1, 2, 3 and 4, respectively.

Antibacterial activity

The antimicrobial properties of herbal plants and their extracts have been recognized since ancient times, while attempts to illustrate these qualities in the laboratory date back to the early 1900s (44). Now, the development of resistance via a pathogen to several of the usually used antibiotics provides a drive for additional attempts to find new antimicrobial agents to eradicate the infection and defeat the problems of resistance and side effects of the antimicrobial drugs that are currently used (45,46).

The mechanism of the antimicrobial effect of *N. sativa* seeds has not been reported, its antimicrobial property could be attributed to the active constituents particularly TQ and melanin (47). Their broad spectrum of activity may be the reason of that the key processes of the organisms are affected (48).

In vitro studies for antibacterial activity

Concentration dependent inhibition of Gram-positive; *Staphylococcus aureus* and Gram-negative; *Pseudomonas aeruginosa*, *Escherichia coli* and pathogenic yeast *Candida albicans* in filter paper discs impregnated with ethyl ether extract of *N. sativa* (25-400 micrograms/disc) was observed, the extract showed antibacterial synergism with streptomycin and gentamicin and exhibited additive antibacterial action with doxycycline, spectinomycin, erythromycin, tobramycin, ampicillin, chloramphenicol, nalidixic acid, lincomycin and sulfamethoxazole trimethoprim combination (49).

To investigate the antibacterial effect of crude extracts of *N. sativa*, various bacterial isolates which included of 16 Gram-negative and 6 Gram-positive, these isolates, particularly Gram-negative bacteria, showed multiple resistance against antibiotics. Crude alkaloid and water extracts were the most effective extracts, and especially against Gram-negative isolates they were effective (45).

A population of 7.0 log CFU of each strain of *Listeria monocytogenes* was inoculated on duplicate plates having antibiotic medium one agar. Each discs (6 mm diameter), impregnated with 10 μ l of black seed oil, or gentamicin (positive control).

N. sativa seed oil had a strong antibacterial activity against all the strains of *L. monocytogenes*, yielding a significantly greater inhibition zone

Table 1. Antibacterial effects of *Nigella sativa* and its constituents

Treatment	Method	Microorganism	Main results	References
Diethyl ether extract (25-400 µg/disc)	Filter paper discs impregnated	Gram-positive bacteria, Gram-negative bacteria and <i>Candida albicans</i>	Effective against Gram-positive (<i>Staphylococcus aureus</i>), Gram-negative bacteria (<i>Pseudomonas aeruginosa</i> and <i>Escherichia coli</i>), <i>C. albicans</i> , (not effective on <i>Salmonella typhimurium</i>), also effective against staphylococcal infection in mice	(49)
Methanol extract, aqueous extract, chloroform extract, essential oil	Specimens aspiration of infected mice with <i>Staph. aureus</i> or <i>Esch coli</i> (0.1 ml from 10 ⁶ colony forming units/ml suspension), cultured on a soybean casein digest agar plate surface	<i>Staph. aureus</i> (ATCC 29737) and <i>Esch. coli</i> (ATCC 8739)	Methanol, chloroform extract and essential oil showed significant antibacterial activity against both microorganisms	(41)
Ethanol extract (4 mg/disc)	Disc diffusion and agar dilution	Methicillin resistant <i>Staph. aureus</i> (MRSA)	All tested strains of MRSA were sensitive to extract and the extract had an MIC range of 0.2-0.5 mg/ml	(46)
TQ and THQ	Disc diffusion	<i>Esch. coli</i> , <i>Pseudo. aeruginosa</i> , <i>Shigella flexneri</i> , <i>Sal. typhimurium</i> , <i>Salmonella enteritidis</i> and <i>Staph. aureus</i>	In the case of <i>Staph. aureus</i> the MIC and MBC for TQ were 3 and 6 µg/ml respectively, the MIC and MBC for THQ were 400 and 800 µg/ml respectively Gram-negative bacteria were less susceptible to both TQ and THQ and their MIC and MBC variety between 200 and 1600 µg/ml	(14)
TQ (0 to 512 µg/ml)	Broth microdilution	Gram-negative bacilli: <i>Esch. coli</i> ATCC 35218, <i>Salmonella enteric</i> serovar <i>typhimurium</i> ATCC 14028, <i>Pseudo. aeruginosa</i> ATCC 27853, <i>Vibrio liginolyticus</i> ATCC 33787, <i>Vibrio paraheamolyticus</i> ATCC 17802; Gram-positive bacilli: <i>Bacillus cereus</i> ATCC 14579, <i>Listeria monocytogene</i> ATCC 19115 and Gram-positive cocci: <i>Enterococcus faecalis</i> ATCC 29212, <i>Micrococcus luteus</i> NCIMB 8166, <i>Staph. aureus</i> ATCC 25923, <i>Staphylococcus epidermidis</i> CIP 106510	TQ showed a significant bactericidal activity against the majority of the tested bacteria (MICs values ranged between 8 to 32 µg/ml), the best effect was seen especially in Gram-positive cocci (<i>Staph. aureus</i> ATCC 25923 and <i>Staph. epidermidis</i> CIP 106510)	(51)
Aqueous extract and methanol extract	Disc diffusion	Gram-positive bacteria: <i>Pseudo. aeruginosa</i> , <i>Klebsella pneumoniae</i> , <i>Proteus vulgaris</i> and Gram-positive bacteria: <i>Streptococcus pyogene</i>	Aqueous extract and methanol extract were effective but the aqueous extract was less effective; 20 mg/ml the methanol extract of <i>N. sativa</i> is effective against <i>Strep. pyogenes</i> (10 mm zone of inhibition), and it induced 15 mm zone of inhibition at 100 mg/ml which in this concentration is effective against <i>Strep. pyogene</i> , <i>Pseudo. aeruginosa</i> and <i>Pro. vulgaris</i> . Aqueous extract of <i>N. sativa</i> at 100 mg/ml is effective against <i>Pseudo. aeruginosa</i> (20 mm zone of inhibition), <i>Strep. pyogenes</i> (15 mm zone of inhibition) and at concentration of 50 mg/ml is effective against <i>Strept. pyogenes</i> (10 mm zone of inhibition), <i>K. pneumoniae</i> (11 mm zone of inhibition), <i>Pro. vulgaris</i> (12 mm zone of inhibition)	(53)

than that of gentamicin ($P < 0.01$). The mean zones of inhibition produced by *N. sativa* seed oil and gentamicin were 31.50 ± 1.0 and 14.80 ± 0.50 mm, respectively (50).

Methicillin resistant *Staph. aureus* (MRSA) is one of the commonest pathogens encountered in

laboratory and in clinic. All tested strains of MRSA were sensitive to ethanolic extract of *N. sativa* seeds at a concentration of 4 mg/discs, the extract had minimum inhibitory concentration (MIC) range of 0.2-0.5 mg/ml (46).

Table 2. Antifungal effects of *Nigella sativa* and its constituents

Treatment	Method	Microorganism	Main results	References
Aqueous extract (6.6 ml/kg once daily for 3 days)	Candidiasis in mice	<i>Candida albicans</i>	Aqueous extract showed inhibitory effect against candidiasis.	(60)
Ether extract (2.5 – 40 mg/ml) TQ (0.062-.5 mg/ml)	Agar diffusion method	Four species of <i>Trichophyton rubrum</i> and one each of <i>Trichophyton interdigitale</i> , <i>Trichophyton mentagrophytes</i> , <i>Epidermophyton floccosum</i> and <i>Microsporium canis</i>	The MICs of the ether extract of <i>Nigella sativa</i> and TQ were between 10 and 40 and 0.125 and 0.25 mg/ml, respectively	(42)
Oil (1, 2 and 3 ml/ 100 ml)	Afla Test-P affinity column	On the growth and aflatoxin B1 production by <i>Aspergillus parasiticus</i> (CBS 921.7) and <i>Aspergillus flavus</i> (SQU 21) strains	The inhibition of aflatoxin B1 production by <i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i> strains with different concentrations of <i>N. sativa</i> oil was in the range of 49.7- 58.3% and 32-48% respectively, but different concentrations of <i>N. sativa</i> oil displayed no significant effect on the growth of <i>Aspergillus</i> species	(57)
Oil (0.35% (v/w) yield)	Disc diffusion method and microdilution method	<i>Chrysosporium evolceanui</i> , <i>Chrysosporium tropicum</i> , <i>Trichophyton simii</i> , <i>Trichophyton rubrum</i> and <i>Microsporium gypseum</i>	The maximum zone of inhibition was seen in <i>Micro. gypseum</i> with diameter of inhibition zone and activity index 38 mm (AI: 1.90), and the inhibition against <i>Tricho.rubrum</i> , <i>Tricho. simii</i> were (IZ: 20 mm ,AI: 1.33 and IZ:35 mm, AI: 1.09), respectively. For <i>Chrysos. tropicum</i> was (IZ: 26 mm, AI: 0.86) and against <i>Chrysos. evolceanui</i> was (IZ: 25 mm, AI: 0.71)	(58)
Cream containing TQ with different concentrations (1%, 2%, 4%, 6%, 8% and 10%)	Vaginal candidiasis in mice	<i>Can. albicans</i>	The most effective concentration of TQ was 10%	(15)

TQ has antibacterial activity that could be potentiated by antibiotics especially in case of *Staph. aureus*. In a study the antibacterial effect of TQ and HQ against *Esch coli*, *Pseudo. aeruginosa*, *Shigella flexneri*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Staph. aureus* was investigated. *Staph. aureus*, was very susceptible to TQ, as, 3 and 6 µg/ml were sufficient to inhibit and kill the bacteria respectively. On the other hand, the concentration of THQ required to inhibit and kill *Staph. aureus* was 400 and 800 µg/ml respectively which is 100 times more than that of TQ. Gram-negative bacteria were less susceptible to TQ and THQ, their MIC and minimum bactericidal concentration (MBC) were in the range between 200 and 1600 µg/ml. When TQ and THQ combined with antibiotics (ampicillin, cephalixin, chloramphenicol, tetracycline, gentamicin, and ciprofloxacin) showed synergistic properties especially in case of *Staph. aureus* (14).

Chaieb and coworkers reported that, TQ had a

significant bactericidal activity (MICs values in the range of 8 to 32 µg/ml) that is especially effective against Gram-positive cocci (*Staph. aureus* ATCC 25923 and *Staphylococcus epidermidis* CIP 106510) (51).

In MIC determination, TQ was active against all the strains that studied. The main antibacterial activity was seen against *Streptococcus constellatus* (MIC 4 µg/ml). The essential oil showed the strongest activity against *Streptococcus mitis*, *Streptococcus mutans*, *Strep. constellatus* and *Gemella haemolysans* (MIC 2.13 mg/ml), but it was not effective against *Enterococcus faecalis* and *Enterococcus faecium* (MIC > 8.5 mg/ml) (52).

By the disc diffusion method, TQ (150 µg/disk) was effective especially against *Strep. mutans* and *Strep. mitis* (zone of inhibition were: 24.5 ± 0.71 and 22 ± 1.41 mm, respectively). TQ showed also weak antibacterial activity against *Entero. faecalis*, *Entero. faecium* and *Streptococcus salivarius* (9 ± 0.00, 9.5 ± 0.71 and 9.5 ± 0.71 mm, respectively) (52).

Table 3. Anti-schistosomiasis effects of *Nigella sativa*

Treatment	Experimental model	Microorganism	Main results	References
Oil (2.5 and 5 ml/kg, orally for two weeks)	<i>Schistosoma mansoni</i> infected mice	<i>Sch. mansoni</i>	The oil was effective against the alterations caused by <i>Sch. mansoni</i> infection	(62)
Crushed seeds	Laboratory tissue culture plates	Different stages of <i>Sch. mansoni</i> (miracidia, cercariae, and adult worms)	<i>N. sativa</i> seeds could turn render the parasite vulnerable to damage by the host and may play a role in the anti-schistosomal potency	(64)
Oil (0.2 mg/ kg)	<i>Sch. mansoni</i> infected mice	<i>Sch. mansoni</i> cercariae (Egyptian strain)	The oil prevented most of the hematological and biochemical changes and markedly improved the antioxidant capacity of schistosomiasis mice compared to the infected untreated ones	(65)

Table 4. Antiviral and antiparasitic effects of *Nigella sativa*

Treatment	Experimental model	Microorganism	Main results	References
Oil (100 µg/100 µl/mouse for 7 consecutive days)	Murine Cytomegalovirus (MCMV) as a model, viral plaque forming assay, cell preparation and flow cytometry, cytolytic activity of NK cells, ELISA for cytokines assay, suppressor function assay, cytolytic T lymphocyte (CTL) activity assay	Smith strain of MCMV was used in all experiments	The oil treatment increased IFN- gamma production and augmented numbers of CD4+ helper T cells, suppressor function and numbers of macrophages.	(43)
Oil administered continuously for 3 months a dose of (450 mg three times daily)	Patient with hepatitis C virus (HCV) infection who were not eligible for IFN-α therapy	HCV	The oil significantly improved HCV viral load.	(68)
Methanolic extract of <i>N. sativa</i> seeds (1.25 g/kg)	Malaria infection in vivo using the Swiss albino mice	<i>Plasmodium yoelii</i>	Improving the oxidative status in red blood cells and hepatocytes of infected mice.	(70)
Aqueous suspensions and oil emulsions (400 mg/kg)	<i>Eimeria stiedae</i> infection in rabbit	<i>Eimeria stiedae</i>	The anti coccidial effects were seen with both treatments, but the more rapid antiparasite effect was seen with the <i>N. sativa</i> oil emulsion.	(71)

The essential oil (2.43 mg/disc) have high activity against *Strept. mitis*, *Streptococcus. oralis*, *Strep. mutans*, *Strep. constellatus* and *G. haemolysans* with a zone of inhibition ranged from 13.5 to 15.5 mm, besides, it was not effective against *Entero. faecalis*, *Entero. faecium* and *Strep. salivarius* (52).

An obvious inhibition of the growth of *Staph. aureus* was seen at concentration of 300 mg/ml of *N. sativa* seeds compared with distilled water as control. The inhibitory effect was confirmed with azithromycin as positive control. The inhibitory effect may be due to TQ (47).

The aqueous extract of *N. sativa* seed showed less antibacterial effect compared to the methanol extract. At concentration of 20 mg/ml the methanol extract of seed was effective against *Strep. pyogenes* (10 mm zone of inhibition), and it induced 15 mm zone of inhibition at 100 mg/ml which in this concentration was effective against *Streptococcus. pneumoniae*, *Pseudo. aeruginosa* and *Proteus vulgaris* (53).

The aqueous extract of *N. sativa* seed at 100 mg/ml was effective against *Pseudo. aeruginosa* (20 mm zone of inhibition), *Strep. pyogenes* (15 mm zone of inhibition) and at concentration of 50 mg/ml exhibited modest effective against *Strep. pyogenes* (10 mm zone of inhibition), *Klebsiella pneumoniae* (11 mm zone of inhibition), *Pro. vulgaris* (12 mm zone of inhibition) (53).

Thermosensitive N-Isopropyl acryl amide-N-Vinyl 2-pyrrolidone (NIPAAm-VP) co-polymeric micelle synthesized by radical copolymerization, and the extract of *N. sativa* entrapped in this polymeric system for checking the release of the bioactive compound and estimated its antibacterial effect (54).

N. sativa seed extract has been loaded into the polymeric micelle and its effectiveness has been evaluated against Gram-positive strain of *Staph. aureus*, *Bacillus subtilis* and a Gram-negative *Esch. coli*.

N. sativa loaded polymeric micelles were hundred times more effective than the naked one (54).

These findings suggest that this thermosensitive polymeric system would more effectively release the drug in the body when there is an infection such as higher temperature conditions (54).

In vivo studies for antibacterial activity

In an animal study, it was shown that the methanol and chloroform extracts of *N. sativa* seed total extract (TE) as well as the essential oil (EO) caused a dose dependent antibacterial activity on Gram-positive and Gram-negative organisms. In this research, *Staph. aureus* and *Esch. coli* (0.1 ml from 10⁶ colony forming units/ml suspension) were injected intraperitoneally to male mice. After 24 hours infected mice were exposed to different doses of TE or EO. The specimens aspiration of intraperitoneal fluid were cultured on a soybean casein digest agar plate surface, finally it was observed that the EO and TE are effective against both Gram-positive and Gram-negative bacteria (41).

To assess whether treatment with TQ prior to or during inoculation of *Esch. coli* can prevent oxidative damage in an acute pyelonephritis (PYN) ascending obstructive rat model. TQ was injected intraperitoneally (10 mg / kg), 24 hr prior to bacteria inoculation and repeated at 24 hr intervals through the indicated time. The results showed that TQ diminished the oxidative damage that occurred in PYN. The protective effect of TQ in kidney tissue also was confirmed with histological examination, because of the releasing free radicals through PYN, these properties can attribute to antioxidant effect of TQ (55).

The methanol extract of *N. sativa* seed used to examine *in vitro* and *in vivo* antibacterial effects against pathogenic bacteria that cause mastitis in cows through the year 2010-11. The cows that have clinically confirmed mastitis were treated with local

injection of the extract to the breast. For *in vitro* antimicrobial experiment, the extract was used against the pathogen that collected from infected breast. Agar dilution and disk diffusion methods were the technique of investigation. The results showed that the extract have significant *in vitro* and *in vivo* inhibitory effect (48).

***In vitro* antifungal activity**

A study was done to investigate the antidermatophyte effects of ether extract of *N. sativa* seeds and TQ. In this research, the test was performed by using the agar diffusion method with serial dilutions of ether extract of *N. sativa* seed, TQ and griseofulvin. The species were used consists of eight species of dermatophytes, four species of *Trichophyton rubrum* and one each of *Epidermophyton floccosum*, *Trichophyton interdigitale*, *Microsporum canis*, and *Trichophyton mentagrophytes*. The results showed that the MICs of the ether extract of *N. sativa* seed and TQ were in range of 10-40 and 0.125-0.250 mg/ml, respectively, whereas for griseofulvin was between 0.00095 to 0.01550 mg/ml (42).

Two novel defensins named Ns-D1 and Ns-D2 from seeds of *N. sativa*, were isolated, purified and sequenced. The Ns-D1 and Ns-D2 peptides exhibited strong divergent antifungal activity against a number of phytopathogenic fungi. The mechanism is due to expected interaction of defensins with specific sphingolipids on the fungal membranes (56).

The growth of *Aspergillus parasiticus* (CBS 921.7) and *Aspergillus flavus* (SQU 21) strains, and also the production of aflatoxin B1 by this fungus, was evaluated. The inhibition of aflatoxin B1 production by *A. flavus* and *A. parasiticus* with different concentrations of *N. sativa* oil (1, 2 and 3 ml/ 100 ml) were in the range of 49.7- 58.3% and 32-48% respectively, but different concentrations of *N. sativa* oil displayed no significant effect on the growth of other *Aspergillus* species. *N. sativa* oil might have metabolic effects on biosynthesis pathways of aflatoxin (57).

In a study to evaluate the antidermatophytic effects of *N. sativa* essential oil, microdilution and disc diffusion methods were used. *N. sativa* essential oil represented a significant antidermatophytic activity, that the maximum zone of inhibition was seen in *Microsporum gypseum* with inhibition zone of (IZ) 38 mm and activity index (AI): 1.90. Also NSO showed antidermatophytic activity against *T. rubrum*, *Trichophyton simii* (IZ:20 mm, AI: 1.33 and IZ:35 mm, AI: 1.09, respectively) when compared to standard. Whereas, it was seen that the inhibition effect against *Chrysosporium tropicum* (IZ: 26 mm, AI: 0.86) and *Chrysosporium evolceanui* (IZ: 25 mm, AI: 0.71) were slightly comparable to ketoconazole, the standard drug (AI = IZ of sample / IZ of standard) (58).

***In vivo* antifungal activity**

When *Candida albicans* inoculated into mice will produce colonies in the liver, spleen and kidneys. In a study, 24 hours after inoculation, the infected mice were administered the aqueous extract of *N. sativa* seeds (6.6 ml/kg daily for the 3 days), the results showed markedly inhibition of the growth of these pathogens in all studied organs (59, 60).

The candidacidal effect is related to nitric oxide (NO) dependent pathway in rat neutrophils. NO is responsible for protection against pathogens that are living and proliferating in the intracellular environment of several different kinds of somatic cells (59, 60).

N. sativa seed extract has active ingredient (s), that may directly stimulate the granulocytes and monocytes to produce NO, that kills *Can. albicans* (59, 60).

The *Can. albicans* intravascular inoculation, produces colonies of the *Can. albicans* in the liver, spleen and kidneys (61).

Treatment of mice with the extract 24 hr after the inoculation produced a significant inhibitory effect on the growth of the organism in these organs. Histopathological examination also confirmed this finding, methanolic extract of *N. sativa* seeds was the most effective extract against different strains of *Can. albicans*, followed by the chloroform extract. The antifungal was not found with the aqueous extract (61).

A research was done to evaluate the effect of TQ against vaginal candidiasis in prednisolone induced immune suppressed mice. The mice were immunosuppressed via the subcutaneous injection of methyl prednisolone (150 mg/kg) on days 1 and 3 before the induction of infection. Then for inducing the infection via the vaginal route, a swab saturated with cell suspension of *Can. albicans* was used. To estimate the effect of TQ, 5 days later cream containing TQ administered once daily for 6 days. Treatment with TQ showed lower number of *Can. albicans* colonies as compared to control group. The reduction of yeast colonies was proportional to increase the TQ concentration. At the concentration of 10% TQ, the cream was able to successfully kill most of *Can. albicans* cells as well as all of the mold cells. Complete eradication of *Rhizopus* sp also was seen, at concentration of 2%, 4%, 6% and 8% TQ. The average numbers of colonies were 55, 21, 21 and 8 CFU/mouse. Histological analysis also confirmed the antifungal activity of TQ. The mechanism is attribute to that TQ lead to inactivate the plasma membrane protein of microorganism and loss of the function occurs followed by yeast cells death, whereas TQ had no effect on the vaginal cells (15).

Few studies have been conducted on the effects of antischistosomiasis, antiviral and antiparasite activity

of *N. sativa* seed. In the following section we discuss these effects.

Antischistosomiasis activity

Two weeks treatment with *N. sativa* oil (NSO) in mice that infected with *Schistosoma mansoni* leads to diminish the number of *Sch. mansoni* worms in the liver and also reduced the whole number of ova deposited in both the liver and the intestine. Moreover, it augmented the number of dead ova in the intestinal wall and obviously diminished the granuloma diameters. The infected mice with *Sch. mansoni* produced a marked rising in the serum activity of L-alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), with a low increase in alkaline phosphatase (AP) level, while reduced serum albumin level. Administration of NSO partially be successful to correct the prior changes in ALT, GGT, AP activity, in addition to correct the albumin content in serum, when praziquantel administered with NSO, further reduces the dead ova number in comparison with praziquantel alone seen. These changes were related mostly with the ability of NSO to improve liver function and the immunological system of infected mice and partly to its antioxidant effects (62).

The protection is also due to the ability of NSO and TQ to reduce the cytogenetic damage caused by schistosomiasis infection (63).

The karyotype of the bone marrow and spleen cells of infected mice shown that the main chromosomal abnormalities were gaps, fragments and deletions on chromosome 2, 6, and some in chromosomes 13 and 14, compared to the control level. Decline in the percentage of chromosomal aberrations and the incidence of deletions and tetraploidy was observed in treated group with NSO or TQ. So that, *N. sativa* has therapeutic effects against *Sch. mansoni* (63).

The results of *in vitro* tests of *N. sativa* seeds against *Sch. mansoni*, miracidia, cercariae, and adult worms show its strong effects against all stages of the parasite and an inhibitory effect on egg laying of adult female worms. Besides, *N. sativa* seeds induced an oxidative stress against adult worms which shown with diminish in the activities of glutathione reductase, antioxidant enzymes, glutathione peroxidase, superoxide dismutase and enzymes of glucose metabolism, glucose-6-phosphate dehydrogenase and hexokinase. Disturbing of these enzymes of adult worms using *N. sativa* seeds could turn render the parasite vulnerable to damage by the host and may play a role in the anti-schistosomal potency (64).

The antioxidant and anti-schistosomal effects of the garlic extract (AGE) and NSO on normal and *Sch. mansoni* infected mice was studied. The results presented that, treatment with AGE and NSO prevented most of the hematological and biochemical changes and markedly improved the

antioxidant capacity of schistosomiasis mice compared to the infected untreated ones (65).

Antiviral activity

Apoptosis is caused by viral infections leading to lymphocyte depletion in the host cell, and antioxidants can inhibit apoptosis which induced by viruses in addition to inhibit the viral replication in target cells, so antiviral and antioxidant effects can be linked together (66).

To investigate the antiviral effect of NSO, murine cytomegalovirus (MCMV) as a model were used. Intraperitoneal administration of NSO to mice completely inhibited the virus titers in spleen and liver on day 3 of infection. Viral load in the liver and spleen of the control had a high difference with NSO treated mice, 45×10^4 vs. 7×10^4 and 23×10^3 vs. 3×10^3 , respectively. This antiviral effect accorded with raising the serum level of interferon-gamma and increased numbers of CD4+ helper T cells, suppressor function and numbers of macrophages. On the tenth day of infection, the virus titer was undetectable in spleen and liver of NSO treated mice, whereas it was detectable in control mice, so the *in vivo* treatment with *N. sativa* oil induced a remarkable antiviral effect against MCMV infection (43).

By nonspecific cells including natural killer cell (NK cells), and specific cells including CD4 and CD8 T cells, immunity produced to viral infection is controlled (67). The antiviral effect of the NSO is related with increasing response of CD4 cells (43).

In a research, the patient with hepatitis C virus (HCV) infection who was not eligible for IFN- α therapy, had received the capsule of NSO (450 mg) for three successive months, for three times in a day and the findings showed that the significantly decreased of the viral load and also improvement of the oxidative stress due to augmented total antioxidant activity, total protein and albumin, improved RBC and platelet counts in HCV patients. The augmented RBC count can attributed to the lowering of the membrane lipid peroxide level, leads to reduce the incidence of hemolysis (68).

Diminish the blood glucose levels, implying that it may provide a potential modulatory influence on HCV induced glucose intolerance, improve in the lower limb edema also was seen (68).

Antiparasite activity

The effects of *N. sativa* seeds in children who naturally infected with cestode worms investigated. A single oral administration of 40 mg/kg of ethanolic extract of *N. sativa* without side effect in the doses tested diminished the fecal eggs percentage (69).

1.25 g/kg of methanolic extract of *N. sativa* seeds (MENS) lead to suppression of *Plasmodium yoelii* infection (94%, $P < 0.05$) whereas chloroquine, the choice drug, lead to 86%. So MENS is more effective than chloroquine for the treatment of *Plas. yoelii*

infection. The antimalarial effect is due to MENS have antioxidant effect in *Plasmodium* infected mice, improving the oxidative status in red blood cells, and hepatocytes of infected mice was seen (70).

A 400 mg/kg of aqueous suspensions and oil emulsions of *N. sativa* seeds used for the treatment of coccidiosis in rabbits. The anticoccidial effects were seen with both treatments, but the more rapid antiparasite effect was seen with the *N. sativa* oil emulsion. Both treatment increased weight gain and decreased fecal oocyst shedding and histopathology the liver tissue improved remarkably. The improvements was included a significant reduction in infiltration of the inflammatory cells in the portal area, also the different stages of parasites in the bile ducts were diminished and bleeding between hepatic lobules disappeared, the hepatocytes returns to the natural radial arrangement and all severe symptoms disappeared. The *N. sativa* oil emulsion has higher concentrations of alkaloid nigellicine that has a deadly influence on parasites (71).

Conclusion

All findings discussed above indicate that *N. sativa* seeds have antimicrobial effects against different pathogens, including bacteria, viruses, schistosom and fungus.

Black cumin seed in traditional medicine and in recent years for the treatment of microbial diseases has been used without any reported side effects. Therefore, this plant can provide a valuable agent for microbial diseases. However, additional studies are required to evaluate and explore the specific cellular and molecular mechanisms of the antimicrobial effects of *N. sativa*, alone or in combination with other drugs.

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