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Antimicrobial effects of Nigella sativa L.: Review

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Abstract. In recent decades, the phenomenon of resistance of microorganisms to anti-infectious molecules used in therapy has become a health problem throughout the world. Therefore, it is necessary to adopt new approaches to overcome this phenomenon of resistance with the suggestion of finding the solution by researching bioactive molecules in medicinal plants and exploiting them in the pharmaceutical industry in order to develop new antimicrobial drugs. Nigella sativa is used in many regions in traditional medicine, has broad therapeutic effects, and has been reported to have significant antimicrobial effects. This review article attempts to describe some antimicrobial effects such as antiviral, antibacterial, antifungal and antiprotozoal effects, and which have been carried out by various researchers.

Keywords: Nigella sativa, Antiviral, Antibacterial, Antifungal, Antiprotozoal

1 Introduction

Nigella sativa, known as black seed or black cumin in English and habbat albarakah in Arabic. N. sativa is an annual herbaceous plant in family Ranunculaceae which grows well on loamy soils in tropical and subtropical regions. Also, in Asia, Europe, the Middle East and other Mediterranean countries, N. sativa has been regularly andabundantly cultivated for various purposes [1].

N. sativa is widely used natural remedy and the seeds are extensively used as spice, carminative, condiment and aromatic. Traditionally, they have been used as diuretic, diaphoretic, stomachic, liver tonic, digestive, emmenagogue and galactagogue. Also, they are used in diarrhoea, indigestion, dyspepsia, sour belching, loss of appetite, vomiting, dropsy, puerperal diseases [2].

The seeds of N. sativa are composed of 66.5% linoleic acid (18: 2) and 23.5% oleic acid (18: 1) as the main fatty acids. Their content of trace elements like Co, Ni, Fe, Zn, Cu, Mn and Cr is 0.12, 1.48, 117.32, 41.42, 30.26, 28.56 and 2.55 µg/g respectively, and their vitamin content is 10.19 µg/g for -tocopherol, 2.28 µg/g for -tocopherol, 0.18 µg/g for retinol, 1.38 µg/g for vitamin D2, 1.85 µg/g for vitamin K1 and 2.15 µg/g for vitamin K2 [3]. N. sativa is rich in

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steroids, alkaloids, flavonoids, glycosides, saponins and terpenoids [4]. The main compounds of the fixed oil and essential oil of N. sativa are thymoquinone, p-cymene and longifolene [5, 6]. Also, oleoresins extracted in different solvents (ethanol, ethyl acetate and n-hexane) contain linoleic acid as a major component [7].

Various pharmacological aspects of N. sativa extracts have been reported, including its anti-inflammatory [8, 9], analgesic and antipyretic [8], Antipsoriatic [10], Antitumor [11], immunopotentiating [12], Antioxidant [13], Antidiabetic [14], Hepatoprotective [15], Neuroprotective [16], Anti-ischemic [17], Gastroprotective [18], antihypertensive [19], Diuretic and hypotensive [20] properties.

This review investigates the antimicrobial effects of N. sativa of which the most important antiviral, antibacterial, antifungal and antiprotozoal effects are discussed below. It is not possible to discuss all of its antimicrobial effects here because the literature on this plant is very extensive.

2 Antiviral activity

In a study of patients with hepatitis C ineligible for interferon (IFN)-, administration of N. sativa was tolerable, safe, decreased HCV viral load and improved oxidative stress, clinical and glycemic control in diabetic patients [21]. In another study, administration of ethanolic extract of N. sativa to patients with HCV showed potential therapeutic benefits via the decrease in viral load and the relief of impaired liver function, with an effect more powerful offered by their mixture with the ethanolic extract of Zingiber officinale [22].

Dithymoquinone (DTQ) the major constituent of N. sativa showed in a study a strong potential for binding to the SARS-CoV-2: ACE2 interface and could therefore be predicted as a plausible inhibitor to disrupt viral-host interactions [23]. Also, it was reported in a study that an adult person diagnosed with HIV positive became HIV negative after receiving treatment with N. sativa for several months, since this case is considered surprising [24].

N. sativa is of importance in agriculture since in in-vitro experience, decoction and infusion of N. sativa seeds inhibited the symptoms of Zucchini Yellow Mosaic Virus (ZYMV) on squash plants by 85% and 80%, respectively. In post-experiment, decoction and infusion gave 70% and 65% inhibition of ZYMV, respectively. In addition, the soaking of pumpkin seeds in decoction showed higher activity against ZYMV than the infusion. The decoction showed a maximum percentage of viral inhibition (95%) after soaking for 24 hours, while the infusion gave 90% inhibition after soaking for 24 hours [25]. In another study, the volatile oil and acetone extract of N. sativa showed effective inhibitory activity against papaya ringspot virus (PRSV), using Chenopodium amaranticolor coste and Reyn, a host of the lesion [26].

Moreover, N. sativa can be exploited in the veterinary field since in an experiment, the ethanolic extract of black seed was used in the embryonated eggs inoculated with the Newcastle disease virus (NDV), it increased the level of survival, and gross and histopathological lesions were not intense in embryos treated with

this extract, which justifies its antiviral and immunotherapeutic effect against NDV infection [27]. Also, N. sativa has shown antiviral activity against infectious laryngotrachietis virus (ILTV) [28]. In another experiment, supplementation of the commercial diet with seeds of N. sativa resulted in turkeys experimentally infected with the H9N2 avian influenza virus a considerable improvement in immune reactivity and suppression of the pathogenicity of this virus [29]. Also, intraperitoneal administration of N. sativa oil to BALB/c mice experimentally infected with murine cytomegalovirus (MCMV) showed an effective antiviral effect which may be mediated by increased number and function of Mf and IFN-production [30]. In addition, treatment with alcoholic extract of N. sativa of Vero cell lines infected with PPRV resulted in an increase in the viability of the infected cells and a reduction in the cytopathic effects of PPRV [31].

3 Antibacterial activity

N. sativa essential oil and its compounds (thymoquinone and carvacrol) have shown substantial antibacterial activity against Listeria monocytogenes, and the essential oil may even have potential to control antibiotic resistance in Listeria [32], and even N. sativa oil has shown strong antibacterial activity against twenty strains of Listeria monocytogenes [33]. Also, N. sativa oil was found to be more effective against several species of Salmonella for which even ceftriaxone and ciprofloxacin were ineffective [6]. In another study, the essential oil and methanolic extract of N. sativa had antibacterial activity in-vitro against Salmonella enterica isolates of human and poultry origin resistant to antibiotics, in contrast the aqueous extract had no anti-salmonella activity [34].

In a study, aqueous and ethanolic extracts of N. sativa inhibited the growth of methicillin-resistant Staphylococcus aureus (MRSA) [4] and in another study an extract of N. sativa prepared by the method reflux extraction had an inhibitory effect on strains of MRSA [35].

N. sativa essential oil and its compound thymoquinone have demonstrated significant anticariogenic activity against 30 clinical cariogenic bacteria. The strongest activity of the essential oil was observed against Streptococcus mitis, Streptococcus mutans, Streptococcus constellatus and Gemella haemolysans. However, thymoquinone was active against all the strains studied, in particular Streptococcus mutans and Streptococcus mitis [36]. The seed methanolic extract of N. Sativa was more effective than the ether extract in inhibiting the growth of two cariogenic bacteria Streptococcus mutans and Streptococcus mitis [37].

N. sativa is of great importance in dermatology since more than half of the 19 multidrug-resistant Staphylococcus aureus isolates isolated from diabetic wounds were sensitive to different concentrations of N. sativa oil [38]. In a study designed to explore the antimicrobial effect of N. sativa seed extract against skin pustules, the antibacterial effect of N. sativa seed extract on skin pustules with staphylococcal infections was evaluated in-vivo and compared to mupirocin in newborns, this extract was as effective as mupirocin and without any side effects [39]. In another study, three crude extracts (methanolic, hexane and ethyl acetate) of N.

sativa seeds were evaluated for their anti-acne potential against certain bacterial species responsible for acne; among these three crude extracts tested, the highest antibacterial activity against Staphylococcus aureus was observed in the ethyl acetate extract. Subsequently, topical gels were formulated incorporating the ethyl acetate extract at three different concentrations. These three formulations were able to inhibit the growth of Staphylococcus aureus and Propionibacterium acnes, with the highest antibacterial activity in the formulation comprising 15% of the seed extract [40].

N. sativa seed oil has been shown to have bactericidal activity in-vitro against bacteria commonly involved in otitis media and otitis externa (clinical isolates of Streptococcus pneumoniae, Moraxella catarrhalis, and Haemophilus influenzae). But this oil has not been shown to be effective against clinical isolates of Pseudomonas aeruginosa [41].

The seed crude extract of N. sativa has demonstrated antibacterial activity against Bacillus subtilis, Klebsiella pneumoniae, Mycobacterium phlei and methicillin-resistant and methicillin-sensitive Staphylococcus aureus [42]. Also, 37 isolates of Shigella dysenteriae 1, Shigella flexneri, Shigella sonnei and Shigella boydii and 10 strains of Vibrio cholerae and Escherichia coli showed promising sensitivity to volatile oil of N. sativa [43]. In another study, the fixed and volatile oils of N. Sativa showed antibacterial activity in-vitro against all standard strains (ATCC) of Escherichia coli, Pseudomonas aeroginosa, Acinetobacer baumannii, Staphylococcus aureus and Enterococcus faecalis, and anti-mycobacterial activity against two standard strains of Mycobacterium tuberculosis H37Rv and Mycobacterium avium (ATCC). Usually, volatile oil was much more efficient than fixed oil. On the other hand, these oils were judged ineffective against the isolated bacterial strains [44].

Thymoquinone (TQ) the major active ingredient of N. sativa seed has shown significant antimicrobial activity against standard strains (ATCC) of four anaerobic bacteria which are Clostridium difficile, Clostridium perfringens, Bacteroides fragilis and Bacteroides thetaiotaomicron [45]. In another study, thymoquinone showed antibacterial activity against eleven reference bacterial strains considered to be human pathogens and which are Gram-negative bacilli: Escherichia coli ATCC 35218, Salmonella enterica serovar Typhimurium ATCC 14028, Pseudomonas aeruginosa ATCC 27853, Vibrio alginolyticus ATCC 33787, Vibrio paraheamolyticus ATCC 17802; Gram-positive bacilli: Bacillus cereus ATCC 14579, Listeria monocytogenes ATCC 19115 and Gram-positive cocci: Enterococcus faecalis ATCC 29212, Micrococcus luteus NCIMB 8166, Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis CIP 106510. In addition, it has shown that it has antibiofilm potential against Pseudomonas aeruginosa ATCC 27853, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923 and Staphylococcus epidermidis CIP 106510 [46].

In a study aimed at evaluating the antibacterial activity of total crude extracts and essential oil of N. sativa seed in male mice infected intraperitoneally with Staphylococcus aureus or Escherichia coli; the methanolic and chloroform extracts as well as the essential oil had dose-dependent antibacterial activities

on these bacteria, while the aqueous extract showed no inhibitory effects on any of these bacteria [47].

4 Antifungal activity

The essential oil and various extracts (aqueous and methanolic) of N. sativa and especially thymoquinone have demonstrated powerful antifungal effects on pathogenic dermatophyte strains which are Trichophyton mentagrophytes, Microsporum canis and Microsporum gypseum [5]. Also, the seed ether extract of

N. sativa and its active ingredient thymoquinone demonstrated antifungal activity against eight species of dermatophytes: four species of Trichophyton rubrum and one of Trichophyton interdigitale, Trichophyton mentagrophytes, Epidermophyton floccosum and Microsporum canis [48]. In another study, the essential oil of N. sativa demonstrated strong anti-dermatophytic activity against Microsporum gypseum, Trichophyton rubrum, Trichophyton simii, Chrysosporium tropicum and Chrysosporium evolceanui [49].

N. sativa can be used to treat candidiasis since N. sativa seed oil had strong antifungal activity against several isolates of Candida albicans, Candida dubliniensis, Candida glabrata and Candida krusei [50] and even ethanolic extract of N. sativa has demonstrated effective antifungal activity against Candida albicans [51]. In an in-vivo study, the seed aqueous extract of N. sativa exhibited an inhibitory effect against experimental candidiasis caused by Candida albicans in mice, in which it considerably inhibited the growth of this pathogen in the kidneys, liver and spleen [52].

Different fractions (aqueous, chloroform, n-hexane, ethyl acetate and n-butanol) of the methanolic extract of N. sativa vegetative parts inhibited the production of fungal biomass from two soil fungal species, Fusarium oxysporum and Macrophomina phaseolina [53]. Also, the oil and oleoresins (ethanolic, ethyl acetate and n-hexane) of N. sativa have demonstrated inhibitory activity against Aspergillus niger, Aspergillus flavus, Fusarium moniliforme, Fusarium graminearum and Penicillium viridicatum [7]. In addition, the essential oil of N. sativa has shown fungicidal activity against eight fungi which are Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme, Fusarium nivale, Fusarium semitectum, Drechslera hawiinesis and Alternaria alternata [54].

5 Antiprotozoal activity

In an in-vitro study aimed to evaluate the anti-leishmanial effects of N. sativa against Leishmania tropica and Leishmania infantum; the essential oil, the methanolic extract and in particular thymoquinone, had a powerful antileishmanial activity on the promastigotes of these two species since these extracts induced a significant decrease in the growth rate of the amastigote forms of the two species [55]. In another in-vitro study, the essential oil of

N. sativa and its main bioactive component thymoquinone showed cytotoxic effects on promastigotes of Leishmania tropica [56] and even the chloroform extract of N. sativa seed showed effective leishmanicidal activity against this Leishmania species in an in-vitro model [57]. In addition, the aqueous extract of the N. sativa plant has shown a leishmanicidal effect in-vitro against Leishmania major [58].

N. sativa is effective in controlling intestinal-tropic protozoa since in an experiment, supplementation of the diet with 1% whole crushed seeds of N. sativa resulted in broilers experimentally infected with the field strain Eimeria tenella improved biochemical parameters and cecal lesions and reduced oocyst excretion [59]. Also, the oil and alcoholic extract of N. sativa seed resulted in reduced oocyst excretion of Cryptosporidium spp. in Swiss Albino mice experimentally infected with these protozoa [60]. In other in-vitro studies, the ethanolic extract of N. sativa seed was shown to have potent antiprotozoal activity against the trophozoites of Entamoeba Histolyica and Giardia lamblia [61, 62]. In addition, thymoquinone the active component of N. sativa has been shown to have more potent in-vitro anti-protozoan activity against Entamoeba histolytica than against Giardia lamblia [63].

N. sativa oil showed a trypanocidal effect in rats experimentally infected with Trypanosoma brucei as there was a reduction in parasitaemia and an extension of the lifespan of these rats [64]. Also, aqueous and ethanolic extracts of N. sativa seed showed antitrypanosomal activity in an in-vitro test against Trypanosoma evansi [65].

In albino mice experimentally infected with the virulent Toxoplasma RH strain, N. sativa oil alone has significant immunostimulatory and antioxidant properties and it has no direct anti-toxoplasma effect, whereas its combination with pyrimethamine (PYR) produced a potent therapeutic effect since it considerably increased the survival rate and decreased parasite density and pathological damage in the liver and spleen [66]. In another experiment, N. sativa oil showed promising prophylactic and therapeutic effects on murine toxoplasmosis in mice experimentally infected with brain cysts of Toxoplasma gondii (strain Me49). It dramatically improved the protection of infected mice from death and reduced the burden of brain cysts. The brains of mice that received N. sativa oil showed milder lesions and higher iNOS expression [67].

In Swiss albino mice experimentally infected with Plasmodium yoelli nigeriensis (Plasmodium yoelli), the methanolic extract of N. sativa seed was found to be more effective than chloroquine (CQ) in clearing the parasite and restoring biochemical indices modified by infection [68]. The methanolic and ethyl acetate extracts of N. sativa showed good in-vitro activity against the Chloroquine Sensitive Sierra Leone 1 (D6) and Chloroquine-resistant Indochina 1 (W2) strains of Plasmodium falciparum, and in-vivo against the ANKA strain of Plasmodium berghei [69]. Also, intraperitoneal and oral administrations of ethanolic, aqueous and chloroform extracts of N. sativa seed in mice experimentally infected with Plasmodium berghei showed suppression activity since they decreased parasitaemias and increased survival times of infected mice [70]. In another ex-

periment, the methanolic and ethyl acetate extracts of N. sativa seed showed in-vivo antimalarial activity against the Plasmodium berghei ANKA strain by the parasitaemia suppression test in a mouse model [71]. Also, seeds and oil of N. sativa have shown in-vivo antimalarial activity against chloroquine-sensitive Plasmodium berghei NK65 strain by parasitaemia suppression assay in albino mice, and they have potential to improve the efficacy of chloroquine (CQ) [72]. In addition, the aqueous extract of N. sativa seed increased the survival rate and induced a reduction in the number of parasitaemias and the level of nitric oxide in mice infected with the parasite Plasmodium berghei NK65 [73].

The alcoholic extract, the aqueous extract and the seed oil of N. sativa showed in-vitro inhibitory activity on the growth and activity of Trichomonas vaginalis [74, 75, 76]. Also, N. sativa oil showed a high toxic effect in-vitro on trophozoites of Trichomonas vaginalis since it induced severe cell damage with cytoplasmic and nuclear destruction, while the effect of alcoholic extract of N. sativa was moderate [77].

Finally, the aqueous extract of N. sativa has been shown to be effective in the treatment of experimentally induced Acanthamoeba keratitis (AK) [78].

The difference in antimicrobial activity between the different extracts can be explained by several factors since the physicochemical properties of N. sativa extracts vary according to regions or geographic areas [79] and the method of extraction [80]. Also, the anti-microbial potential of N. sativa seed extracts varies according to regions or geographic areas [81, 82]. In addition, the chemical composition and antimicrobial activity of N. sativa seed essential oils and thymoquinone content vary depending on the extraction methods [83, 84].

The whole plant, seeds, extracts and bioactive molecules of Nigella sativa were concerned for the study of the antimicrobial effects of which these studies were carried out by different methods: in-vitro and in-vivo (patients, farm animals and experimental animals). Figure 1 represents a summary of the studies mentioned in this review.

6 Conclusion

The black seed is a well-documented medicinal plant and the present study showed that N. sativa extracts have a broad spectrum of antimicrobial activity. It is a pity that this miracle medicinal plant is not more widely exploited in public health, veterinary and agricultural field since it can be used as a readily available source of antimicrobial molecules in food, pharmaceutical and cosmetic products.

As many experiments illustrate its potential for many infectious diseases, more clinical and pathological studies must be carried out to investigate the untapped potential of this plant. Thus, more work is needed to determine the bioactive molecules, the mechanisms of action, the dosage and the ideal doses for the different extracts.

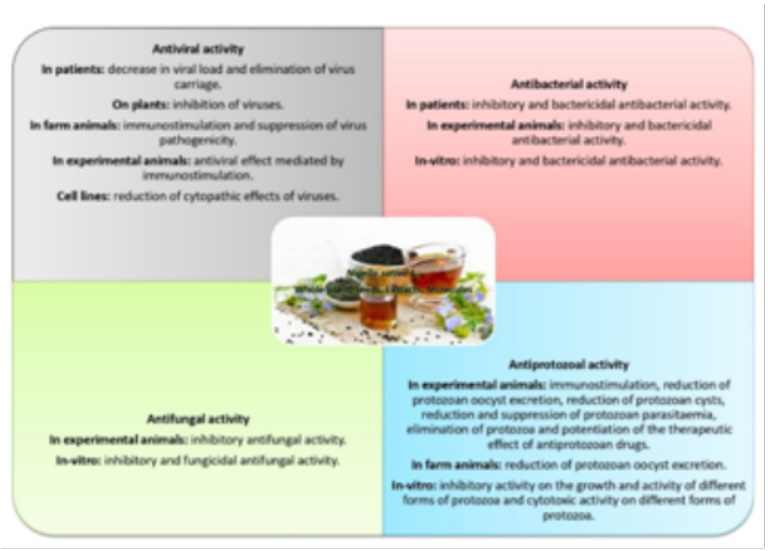

Fig. 1. Summary diagram of the antimicrobial effects of N. sativa.

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