Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2021; 9(3): 118-122

Received: 12-03-2021
Accepted: 15-04-2021

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A review on pharmacological activities and anti-microbial properties of *Nigella sativa* and isolated Thymoquinone

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DOI: https://doi.org/10.22271/plants.2021.v9.i3b.1277

Abstract
Nigella sativa has been marked as precious medicinal herb consisting various bioactive compounds including Thymoquinone and very strengthen evidences can be found in literature in this regard. *Nigella sativa* has popularized as a miracle medicinal herb against pathogenic microorganisms including human infected deleterious viral species such as Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV). Recent animal studies have proved that, *Nigella sativa* seed extract can apply against virus species including Murine Cytomegalovirus (MCMV), Papaya Ring Spot Virus, Avian influenza (H9N2), Newcastle disease virus (NDV), Peste des Petits Ruminants (PPR) Virus. It is important to focus on Thymoquinone as main bioactive compound of the *Nigella sativa*. Among them, anti-cancer effect, cardiovascular effects, gastro-protective effect, anti-diabetic activity and free radicle scavenging activities are the most beneficial effects revealed in recent research studies. Hence, more discreet investigations required for the pharmacological actions of bioactive compounds of the *Nigella sativa* specially for the drug development.

Keywords: *Nigella sativa*, Thymoquinone, Anti-viral, Bioactive compounds

1. Introduction
Some medicinal herbs exhibit miracle multi-functional activities against disease forming microorganisms as well as cure for some non-communicable diseases. *Nigella sativa* showed valuable medicinal properties proving above mentioned statement and very strengthen evidences can be found in literature in this regard and it has more populated with a rich historical and religious background. Many researches have exposed the broad range of pharmacological activities of *N. Sativa*. It is generally referred to as black seed and it is native to Southern Europe, North Africa and Southwest Asia. And it is cultivated in many countries around the world, including the Middle eastern -Mediterranean region, Southern Europe, India, Pakistan, Syria, Turkey and Saudi Arabia [1, 2]. *N. Sativa* has been extensively researched for its biological function, therapeutic activity. A wide variety of activities such diuretic, anti-hypertensive, antidiabetic, anti-cancer, immunomodulatory, analgesic, antimicrobials, anthelmintics, and anti-inflammatory, gastroprotective, hepatoprotective, renal safety and antioxidant properties [3-5].
The seed of the *N. Sativa* is commonly used in the treatment of various conditions such as bronchitis, asthma, diarrhea, rheumatism and skin conditions, too. It is also used as a tonic of the liver, digestive, antidiarrheal, stimulant of the appetite, increase milk production in nursing mothers and combat parasitic infections to support the immune system [6, 7].
N. Sativa is an annual flowering plant that grows in height 20-90 cm wide, with finely divided leaves, parts of the leaf strictly linear and threadlike. The flowers are very delicate, and white, yellow, pink, light blue or pale purple with five to ten petals. The fruit is a huge, inflated capsule composed of 3-7 unified follicles, each containing multiple numerous seeds [8].
Much of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ), a major active chemical component of the essential oil. Black seeds are also used in foods such as flavoring additive in breads and pickles because it is very low in toxicity [9].
In this review article, attention has focused on bioactive compounds of the *N. sativa* and their ailities to mitigate disease forming microorganisms and specific pharmacological potential of the thymoquinone (TQ) as main bioactive compound of the oils of the *N. sativa*.

2. Methodology

PubMed and PMC Academic Quest Completed were used to perform literature searches using the keywords "*Nigella sativa* " and "Black seed". These terms have also been entered into common search engines like Google and Google Scholar to look up secondary information about this herb

All content was reviewed, regardless of source, and a review structure was produced to represent the information available. The "MedChem designer" program was used to draw all of the phytochemicals' chemical structures.

3. Results and Discussion

3.1 Phyto-Chemistry

Seeds of *N. Sativa* analysis showed 20-85% of protein, 38.20% of fat, 4.64% of moisture, 4.37% ash, 7-9% crude fiber and 31.94% total carbohydrate. Potassium, phosphorus, sodium and iron were the predominant elements and Zinc, calcium, magnesium, manganese and copper have been found at lower levels [10].

Many bio active compounds have been isolated, and identified so far reported in different varieties of black seeds. Thymoquinone is an important active compound among them. A significant number of studies have shown that thymoquinone is the main active ingredient in *N. Sativa* oil. This constituent is responsible for most of the pharmacological properties. The beneficial organ protective role of thymoquinone has revealed in experimental animal models of various human diseases is due to the strong antioxidant and anti-inflammatory properties. Thymoquinone has also been shown to change many molecular and signaling pathways in many inflammatory and degenerative diseases such as cancers [11].

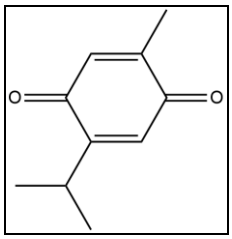

Fig 1: Chemical structure of thymoquinone, major bio active compound in of *N. Sativa* seed oil

In recent years, the natural product has gained publicity for its option to prevent cancer by Cyclooxygenase 2 (COX2) inhibitory mechanisms. Cyclooxygenase (COX) is also known as prostaglandin (PG) endoperoxide synthase catalyzes the phases of blend of proteinoids [12]. Overexpression of COX2 has been found in a wide variety of cancers, such as lung, stomach, breast, and pancreatic cancers [13]. In addition to that COX2 has been reported to inhibit some of the clinical behavior of tumors [14]. Meanwhile, thymoquinone has revealed substantial data on reticence of COX2 expression and production of PG in allergic airway inflammation in the mice [15], attenuated FLMP mediated inflammation by inhibited phosphorylation on Ser-304 and Ser-328 of p47PHOX phosphor peptides and also Defense in FMLP induced polymorphonuclear cell by decreasing gp91PHOX and CD11b expression and inhibiting myeloperoxidase [16].

Apart from that, thymoquinone has exhibits free radicle scavenging activities against free radicles which cause to many *in vitro* and *in vivo* animal models. According to that, thymoquinone has been shown to inhibit or suppress the adverse effect of various environmental toxins or xenobiotics causing oxidative damage and organ dysfunctions [17].

2.2. Anti-fungal and Anti- bacterial effects of *N. Sativa*

Table 1: Anti-fungal and anti-bacterial properties of different extracts of *N. Sativa*, seeds of *N. Sativa* and bioactive compounds of *N. Sativa*

Medicinal Property	Solvent extract or bioactive compound of *N. Sativa*	Affected Microorganism	Action	References
Anti-fungal effect	Methanolic extract	*Candida albicans*	Inhibitory effect of inoculum of *Candida albicans* produced colonies of the organism in the liver, spleen and kidneys in mice	[1]
	Aqueous extract	*Candida albicans*	Inhibitory effect against candidiasis in mice. There was a 5-fold decrease in Candida in the kidneys, 8-fold decrease in the liver and 11-fold decrease in spleen in groups of animals after treatment with plant extract.	[18]
	Ether extract and thymoquinone	*Trichophyton rubrum* and *Microsporum canis* (Dermatophytes)	Inhibition actions resulted in agar well-diffusion method. (as a source for antidermatophyte drugs)	[19]
	Quinines, Thymoquinone, Dithymoquinone, Thymohydroquinone	Yeast	Anti-yeast activities discovered in vitro using a broth microdilution system against six dairy spoilage yeast species.	[20]
Anti-bacterial effect	Grounded black seeds	*Staphylococcus aureus*	Clear inhibition of growth observed in modified paper disc diffusion method	[21]
	Ethanolic extract	methicillin resistant *Staphylococcus aureus*	Inhibition was investigated at the concentration of 4 mg/disc with an MIC range of 0.2-0.5 mg/mL of extract	[22]
	N. sativa seeds	*Helicobacter pylori*	Clinically useful for anti- *H. pylori* activity	[23]
	Thymoquinone	Gram positive cocci (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* CIP 106510)	Significant anti-bacterial activity exhibited	[24]

2.3. Immunomodulatory effect

Using BLAB/c and C57/BL6 primary cells, possible immunomodulation effects of *N. Sativa* were investigated in terms of splenocyte proliferation, macrophage and NK (Natural Killer cell) anti-tumor activity. The aqueous extract of *N. sativa* substantially increases splenocyte proliferation in a dose-dependent manner, according to the findings, the aqueous extract of *N. sativa* promotes the secretion of Th2

cytokines by splenocytes over Th1 cytokines. Apart from that, the aqueous extract of *N. sativa* substantially increases NK cytotoxic activity against YAC-1 tumor cells, implying that the observed anti-tumor effects of *N. sativa* can be due to its ability to function as a stimulant of NK anti-tumor activity [25]. The immunomodulatory effect of a group of medicinal plants, including black seed, was investigated in BALB/c mice. Treatment with five doses of methanolic extract for black seed was found to increase the total white blood cell count [up to 1.2104 cells/mm^3]. After receiving the black seed extract, bone marrow cellularity also increased significantly [26].

In a Long Evans rat model designed to analyze the impact of *N. sativa* seeds on selected immune components, the immunomodulating and cytotoxic properties of volatile oil from *N. sativa* seeds were investigated. Long-Evans rats were treated with *N. sativa* seeds after being exposed to a particular antigen (typhoid TH). As compared to control rats, treatment with *N. sativa* oil resulted in a 2-fold decrease in antibody development in response to typhoid vaccination, however there was a substantial decrease in splenocytes and neutrophils, but a significant increase in peripheral lymphocytes and monocytes in these animals [27].

2.4. Anti-viral effect

N. sativa has been shown to be very effective in treating patients infected with viruses such as the Human Immunodeficiency Virus (HIV) and the Hepatitis C Virus (HCV) in various clinical trials [28].

Table 2: Anti-Viral properties of *N. Sativa* and *N. Sativa* contained herbal

Viral Species	Herbal treatment	Action	References
Human Immunodeficiency Virus (HIV)	α-Zam (herbal concoction containing *N. sativa* and honey)	Within four weeks of starting -Zam treatment, all of the patients were free of all signs and symptoms of HIV infection. In 41 patients, the viral load was undetectable at the end of the herbal treatment, and in ten patients, the viral load was less than 1000 copies/ml. Additionally, CD4 count of all participants has been increased.	[29]
Human Immunodeficiency Virus (HIV)	*N.sativa* concoction (60% of *N.sativa* seeds and 40% of honey)	Since 24 months with *N.sativa* therapy, the patient's HIV tests have shown persistent seroreversion (undetectable viral (HIV-RNA) load) and a normal CD4 count.	[30]
Hepatitis C Virus (HCV)	Soft gelatin capsules of *N. sativa* seed oil (450 mg)	Resulted in a substantial reduction in viral load, increased Total Antioxidant Capacity (TCA), and improved laboratory biomarkers such as total protein, red blood cell, and platelet count, as well as lower fasting blood glucose and postprandial glucose.	[31]
Hepatitis C Virus (HCV)	(*N.sativa*, honey, Blue green® tablet, vitamin D_3, and linolenic acid) along with chloroquine	HCV patients who were not eligible for Interferon-α therapy, lead to negative HCV-RNA in 6 months	[32]
Murine Cytomegalovirus (MCMV)	Black seed (*N. sativa)* oil	On day 10 of infection, the virus titer was undetectable in spleen and liver of BSO-treated mice	[33]
Papaya Ring Spot Virus	The volatile oil *N. sativa*	The volatile oils of all the tested spices showed significant inhibitory activity (25-100%) at different dilutions	[34]
Hepatitis C Virus (HCV)	Alpha-zam (Herbal formulation from *N. sativa* seeds	HCV replication is selectively inhibited by this drug.	[35]
Avian influenza (H9N2	*N. sativa* seeds	Higher antibody titer against H9N2 AIV in turkeys fed 6 percent *N. sativa* seeds. Similarly, increased cytokine gene expression indicates that *N. sativa* has antiviral properties, especially in a dose-dependent manner, resulting in H9N2 virus pathogenesis suppression and decreased virus shedding and improved immune responses were more pronounced.	[36]
Newcastle disease virus (NDV)	Ethanolic extract of black seed	The embryonated eggs were examined grossly and histopathological, showing that Nigella sativa extract has a good immunotherapeutic effect against NDV infection.	[37]
Peste des Petits Ruminants (PPR) Virus	(*N. sativa*) alcoholic extract	In the plaque reduction assay, alcoholic extracts of *N. sativa* (50 g/ml) significantly decreased plaque count as compared to the negative control (P=0.05) in all modes of action studied.	[38]

2.5 Pharmacological potentials of the Thymoquinone (TQ) extracted from the *N. sativa*

Anti-cancer effect

Thymoquinone (TQ)'s antitumor and anti-angiogenic effects on osteosarcoma were studied in vitro and *in vivo*. When compared to control, TQ caused a higher percentage of growth inhibition and apoptosis in the human osteosarcoma cell line SaOS-2. In a dose-dependent manner, TQ prevented the development of human umbilical vein endothelial cell tubes. In SaOS-2 cells, TQ significantly reduced NF- κB DNA-binding activity, XIAP, survivin, and VEGF expression. Furthermore, TQ treatment increased the expression of cleaved caspase-3 and Smac in SaOS-2 cells. TQ was also discovered to inhibit tumor angiogenesis and tumor development [39].

TQ's cytotoxicity was studied in human cervical squamous carcinoma cells (SiHa). After 72 hours of incubation, TQ was cytotoxic to SiHa cells, with IC50 values of 10.670.12 and 9.330.19 g/mL determined by MTT assay and trypan blue dye exclusion test, respectively. When compared to cisplatin, TQ was found to be more cytotoxic to SiHa cells. Surprisingly [41].

Apart from that, TQ's anticancer effects on breast cancer cells, as well as its possible effect on the PPAR-activation pathway, were investigated. It was discovered that TQ had a significant anti-proliferative effect in breast cancer cells, and that cytotoxicity was increased when TQ was combined with doxorubicin and 5-fluorouracil [42].

Cardiovascular effects

The acute effects of diesel exhaust particles (DEP) on cardiopulmonary parameters in mice (at 4 and 18 hours) were studied, as well as the protective effect of TQ also investigated. TQ pretreatment of mice prevented DEP-induced systolic blood pressure drops and leukocytosis, as well as increased IL-6 levels and reduced plasma SOD activity. TQ also stopped platelet counts from dropping and prothrombotic events from occurring [43].

Gastro-protective effect

TQ inhibits the proton pump, acid secretion, and neutrophil infiltration while increasing mucin secretion and nitric oxide production, resulting in novel gastroprotective mechanisms. Ischemia/reperfusion (I/R)-induced gastric lesion has been related to the development of free radicals (FR). The antioxidant effects of Nigella sativa oil (N.O) and thymoquinone (TQ) on gastric mucosal redox state and gastric lesions, 1 and 24 hours after reperfusion, have been assessed by a research group based on above mentioned model. According to the findings, male Wistar rats were given I/R and either N.O (2.5 and 5 ml/kg, p.o) or TQ (5, 20, 50, and 100 mg/kg) injections. I/R increased lipid peroxide (LPX) and lactate dehydrogenase (LDH) levels thus decreasing reduced glutathione (GSH) and superoxide dismutase levels (SOD). These biochemical changes were followed by an increase in gastric lesion formation, which was decreased by either treatment. The levels of LDH, GSH, and SOD were all normalized by N.O. [44].

Antitussive property

The antitussive property of thymoquinone from *N. sativa* was also tested in guinea pigs who had coughs caused by a 20 % citric acid aerosol, and it was compared to codeine. The number of coughs was substantially decreased after intraperitoneal injections of thymoquinone and codeine [45].

Anti-diabetic Activity

One of the research group has investigated the anti-oxidant properties of *N. sativa* aqueous extract and oil, as well as TQ on serum insulin and glucose concentrations in streptozotocin diabetic rats. The levels of serum insulin and glucose, as well as SOD and malondialdehyde (MDA) in pancreatic tissue, were measured. Some subcellular changes were detected using electron microscopy. Diabetes raised tissue MDA and serum glucose levels while lowering insulin and superoxide dismutase (SOD) levels. Diabetes-induced rises in tissue MDA and serum glucose were significantly reduced and serum insulin and tissue SOD were significantly increased in rats treated with *N. sativa* extract and oil, as well as TQ [45].

Conclusions

Nigella sativa is a miracle plant which has variety of multi-functional activities. Specifically, it has significant bio active compounds targeting disease forming bacteria, fungi and some dangerous viral species such as Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV). Thymoquinone is the most concerned and focused bioactive compound in recent researches. As main phyto- compound of the *Nigella sativa* seed oils, Thymoquinone can be identified. Thymoquinone is responsible for enormous health effects for human. A wide range of pharmacological potentials of Thymoquinone (TQ) extracted from the *N. sativa* has been revealed in recent researches. More attention need to be focused on bioactive compounds of *N. sativa* including Thymoquinone targeting novel drug development processors.

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