



# Aut Ecology, Total Phenol and Total Flavonoid Content, Antioxidant Activity and Ethno-pharmacological Survey of *Nigella sativa* Linn. in Traditional Medicine of Golestan Province, North of Iran

Masoumeh Mazandarani\*

## Abstract

**Objective:** *Nigella sativa* Linn., which grows wildly in a small region of the national park of Golestan has been used in traditional medicine of this province. In the present study we evaluated the ecological requirements, antioxidant activity, phytochemistry and conducted an ethno-pharmacological survey of *Nigella sativa* in traditional medicine of Golestan province.

**Materials and Methods:** Ecological requirements and ethno-pharmacological survey were obtained in many field observation through interviews with practitioners and housewives in September 2013. The ripen seeds of *Nigella sativa* were collected from its natural habitat (560 m) in North east of national park of Golestan and ethanolic extract was obtained by maceration. Total phenolics (TPs) and total flavonoids (TFs) contents were determined spectrophotometrically and antioxidant activity was measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging method.

**Results:** *Nigella sativa* is usually grown wildly in a small region of the national park of Golestan (500-760 m), that has an average rain fall of 575.9-614.8 mm, Ec (electrical conductivity of soil) 0.73 in sandy loam soils. The TP content is  $121.3 \pm 0.3$  mg GAE g<sup>-1</sup>, TF contents was  $194.04 \pm 1.65$  mg QUE g<sup>-1</sup> and antioxidant activity (IC<sub>50</sub>) was measured in  $0.13 \pm 0.05$  mg/ml-1. Results showed that the seeds of *Nigella sativa* has high content of TP and TF compounds as well as antioxidant activity, confirming the traditional use of *Nigella sativa* by rural healers as analgesic, anti-inflammatory, antiseptic, anticonvulsant, antidiabetes and antitumour agent against breast cancer, hypertension and hyperglycaemia in north of Iran.

**Conclusion:** Our results demonstrated that the seed extract of *Nigella sativa* L. could have good antioxidant potentials to prevent or control free radicals, which can produce many current inflammatory diseases such as cancer, diabetes, coronary diseases, infection, etc. So these data confirm the traditional use of this plant with its terpenoides, flavonoid and phenolic compounds.

**Keywords:** Antioxidants, Ethnopharmacology, *Nigella sativa* Linn., Phytochemical

## Introduction

Reactive oxygen species (ROS) can induce changes in different biological tissues causing many current disorders in humans such as atherosclerosis, arthritis, ischemia, central nervous system injury, hypertension, cancer, AIDS, Alzheimer's and Parkinson's (1). Therefore, natural antioxidant compounds and secondary metabolites play main role in inhibiting and scavenging free radicals as antipathogen, anti-inflammatory and anticancer activities (2,3). *Nigella sativa* Linn. black seeds belonging to *Ranunculaceae* family, is a herbaceous plant which is better known as black seed, a habitat of Southeast Asia and Mediterranean countries. It has been used in Iranian folks medicine as a food and drug in treatment for numerous disorders (4). In many researches, *Nigella sativa* seeds have been

reported to be used as analgesic, anti-inflammatory, anti-convulsant, antidiabetic, anticancer, antioxidant agent and considered as decreasing serum levels of cholesterol and triglycerides (5).

Phytochemical analysis of *Nigella sativa* seeds of Khorasan province has demonstrated that the seeds contain active ingredients including flavonoids such as kaempferol, quercetin, and quercetin-3, alkaloids nigellone, beta-sitosterol, and thymoquinone, anthraquinones, saponins, glycosoides, melanthin, essential oil, fixed oil, tannins, protein, mucilage resins and glucose. Several studied reported that flavonoides, especially quercetin and kaempferol, have anti-inflammatory and antioxidant effects (6-9). Black seeds with honey have been mentioned to disintegrate the calculi in the kidney and bladder to small pieces thus remov-

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Department of Botany, Gorgan Branch, Islamic Azad University, Gorgan, Iran.

\*Corresponding author: Masoumeh Mazandarani, Tel: +989111719477, Email: mazandarani.m@gorganiau.ac.ir

ing them from the body (10,11).

Therefore, due to numeric medicinal uses of *Nigella sativa* extract against breast cancer, vaginal infection, hypertension and hyperglycemia in traditional medicine of Golestan province, we decided to investigate the phytochemical, antioxidant activity and conducted an ethno-pharmacology survey of *Nigella sativa* seeds in Golestan province (North of Iran).

## Materials and Methods

### Ecological Requirements and Ethno-pharmacology

Ethno-pharmacological data on *Nigella sativa* were collected through interviews with practitioners and housewives. Each practitioner was interviewed about its local name, current diseases, its effects and preparation methods of drug and finally the obtained data from questionnaires were compared with the findings in vivo and in vitro experiments in other similar reports. *Nigella sativa* seeds were collected from a local farm in Minoudasht region (570 m, in September 2013) in Southeast of Golestan province (North of Iran) and the plant was authenticated at the RCMP herbarium (Research Center of Medicinal Plants) of Islamic Azad University, Gorgan branch.

### Extract Preparation

Maceration extraction assembly was used for this purpose. Each of 60 g dried and powdered seeds were mixed with 300 ml hydro-alcohol (ethanol 80%) and continuous extraction was done for overnight in shaker. After that extracts were filtered and were dried in a rotary evaporator at reduced pressure, then stored in refrigerator at 4°C until use.

### Total Flavonoids Determination

TF content was determined by aluminium chloride method (12). Extract of different parts (0.5 ml) were separately mixed with 1.5 ml of solvent, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. They were kept at room temperature for 30 minutes; the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. Quercetin was used as a standard for calibration curve. TF values were expressed in terms of mg equal quercetin in 1 g dry plant powder.

### Total Phenol Determination

Total Phenolic (TP) content was estimated by the Folin-Ciocalteu method, based on the procedure suggested by Pourmorad et al (12). Then 0.5 ml of plant extracts or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml) and aqueous Na<sub>2</sub>CO<sub>3</sub> (4 ml, 1M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765 nm. Gallic acid was used as a standard for calibration curve. TP values were expressed in terms of mg equal gallic acid in 1 g dry plant powder.

### Antioxidant Activity tests

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

capacity assay: The ability of the extract for free radical scavenging was assessed by the method of Arabshahi and Urooj (13). The aliquots of plant extract (20 to 40 to 60 to 80 to 100 µl) were mixed with a methanolic solution of DPPH- (1 mM, 600 µl) and brought to 6 ml with solvent. After incubating in dark and room temperature that absorbance was measured at 517 nm. A DPPH-blank sample (containing 5.4 ml of methanol and 600 µl of DPPH-solution) was prepared. The percent decrease in absorbance was recorded for each concentration and percentage inhibition was calculated according to the following formula: %inhibition= [(ADPPH - AExtract) / ADPPH] ×100 ADPPH is the absorbance value of the DPPH-blank sample and extract is the absorbance value of the test solution. The plots of the 'percentage inhibitions amounts of dried plants (mg) in the extract' were used to find the concentration at which 50% radical scavenging occurred (IC50).

## Results

The finding indicated that *Nigella sativa* with locally name "Siah daneh" is an annual herb (25-550 cm), which grows in temperate climate and sandy loam soils (500 m), in an area with an average rain fall of 575.9 mm and Ec 0.73 (electrical conductivity of soil). The TP content 121.3 ± 0.3 mg GAEg<sup>-1</sup> and TF content 194.04 ± 11.65 mg QUEg<sup>-1</sup> and antioxidant activity (IC50) was measured in 0.13 ± 0.05 mg/ml<sup>-1</sup> in DPPH method.

Ethno-pharmacological data showed that the *Nigella sativa* has been considered in tradition as a food, spice and a tonic drug, pungent, stimulant, analgesic, anti-inflammatory, diuretic, antihelmentic, carminative, expectorant, antiseptic, anticonvulsant, antidiabetic, antitumour and antihypertension agent and used in treatment of cough, sinusitis, jaundice, fever, diarrhoea, dysentery, amenorrhoea and breast tumour with combination with other herbs as below:

**Amenorrhoea:** The tea mix of *Nigella sativa*, *Cuminum cyminum*, *Mentha longifolia*, *Hypericum perforatum* and distilled of *Achillea micrantha* and *Foniculum vulgare*.

**Breast tumour:** The poultice of *Nigella sativa* + *Tanacetum parthenium* + the gum of *Ferula assa foetida* + *Malva neglecta* + *Stachys lavandulifolia* and the fruit of *Melilotus officinalis*.

**Anxiety of women in menopause period:** The powder of seeds of *Nigella* in honey (Dusin) and the tea of *Lavandula angustifolia*, *Melissa officinalis* and *Echium amoenum*.

**Stomach ache and diarrhea:** The infusion of *Descurainia sophia* with *Nigella sativa* and *Mentha aquatica* powders in honey.

**Vitiligo:** Dusin (*Nigella sativa* powder in honey) with a cup of distilled *Lavand* per night and rubbing of *Nigella sativa* oil on the affected area.

**Poly cystic ovary (PCO):** Dusin (*Nigella sativa* powder in honey) with distilled *Foeniculum vulgare* with 1 tea spoon of *Peganum harmala*.

**Osteoporosis:** Orally the powders of *Elaeagnus angustifolia* and *Nigella sativa* seeds in milk or *Nigella sativa* in bread with the buds of beans.

**Nose polyp and sinusitis:** Powder of *Nigella sativa* in vinegar and 2 drops in nose.

**Paroxysm:** One tea spoon of *Nigella sativa* + 1 cup of *Foeniculum vulgare* + *Lavandula* + Wey.

**Frequent urination:** The rub of (oil mixture of *Chamomile* + *Nigella sativa* + bone oil of cow) behind the bladder.

**Rheumatoid arthritis:** The rub of *Nigella sativa* oil + *Viola* oil on affected painful joint.

**Tendon inflammation:** Rub infusion of *Nigella sativa* oil + *Chamomilla* + salt and lemon juice.

**Heavy menstrual bleeding:** Dusin + distilled *Foeniculum vulgare* + infusion of *Coriandrum sativum*.

**Diabetes:** One tea spoon of *Nigella sativa* + 2 spoon of *Urtica dioica* + pulverized of *Pistacia lentiscus* after per meal

**Ulcer:** Dusin + gum of *Ferula assa foetida*.

**Hypertension:** 1 tea spoon of Dusin + 1 cup of distilled *Foeniculum vulgare* and *Urtica dioica*.

## Discussion

Antioxidant compounds and secondary metabolites in medicinal herbs play main role in inhibiting and scavenging free radicals as antipathogenic, anti-inflammatory and anticancer activities (2), so these results can confirm that *Nigella sativa* rich in TF and TP content had strong potential in free radical scavenging. Thus the above findings confirm the usage of *Nigella sativa* in the traditional medicine as a tonic, analgesic, anti-inflammatory, antiseptic, anticonvulsant, antidiabetic, antitumor and antihypertensive agent in combination with other herbs to prevent and treat many current diseases in Golestan province.

In relation to these results, another research showed that methanol extract of *Nigella sativa* Linn. has maximum inhibitory potential against fungi and bacteria. They reported that the *Nigella sativa* seeds contain 36%-38% fixed oil, proteins, alkaloids, saponin and essential oils to possess antimicrobial activity, antioxidant, antitumor activity (14). Gharby et al (14) showed that *Nigella sativa* L. (*Ranunculaceae* family) is an annual herbaceous plant whose growth area extends from the countries of the Mediterranean basin to Iran, Pakistan and India. It has been used to cure gastro-intestinal disorders as well as skin or respiratory ailments, cardiovascular, respiratory, immune, and endocrine systems (14-17). Most of these activities have already been attributed to poly phenols, flavonoids, thymoquinone, a major component of the essential oil of the seeds (18,19).

Botnick et al (20) showed that *Nigella sativa* volatiles consist largely of oxygenated monoterpenes, mainly p-cymene, carvacrol, thymohydroquinone, thymoquinone,  $\gamma$ -terpinene and  $\alpha$ -thujene, with lower levels of sesquiterpenes, mainly longifolene and its monoterpene composition.

The seeds contain about 35% to 41% fixed oil, mainly composed of the non-saturated linoleic, oleic and palmitic acids. Thymoquinone is considered as the major active compound of *Nigella sativa* which has antioxidant activity to induce apoptosis and adversely affect cell division in cancer cells, block angiogenesis in vivo, and inhibit the

growth of prostate and colon tumors implanted in nude mice with no noticeable side effects (20).

Breast cancer is the most commonly diagnosed cancer in women in the world and is one of the leading causes of death due to cancer. *Nigella sativa* with rich antioxidant and anti-inflammatory metabolites has been shown to possess anti-carcinogenic activity, inhibiting growth of several cancer cell lines in vitro. *Nigella sativa* extracts significantly inhibit human breast cancer MDA-MB-231 cell proliferation at doses of 2.5-5  $\mu\text{g}/\text{mL}$  ( $P < .05$ ). Apoptotic induction in MDA-MB-231 cells was observed in a dose-dependent manner after exposure to *Nigella sativa* extracts for 48 hours (21). There are several published articles on the efficacy of *Nigella sativa* and its antitumor capabilities especially in breast, colon and pancreatic cancer in those who took nine capsules a day of *Nigella sativa* for brain cancer (22).

Arabian and Asian countries such as Iran and Egypt are also very rich in historical knowledge in particular about the medicinal use of black cumin seed (*Nigella sativa*) to treat headaches and toothaches and wide range of ailments including headaches, asthma, colds, paralysis, pain, gastrointestinal problems, eczema, obesity and diabetes (22,23).

Alimohammadi et al (24) showed that hydro alcoholic extract of *Nigella sativa* is rich in polyphenolic compounds and at low doses has hypoglycemic effect and ameliorative effect on regeneration of pancreatic islets and may be used as a therapeutic agent in the management of diabetes mellitus. Thus it is being used in countries bordering the Mediterranean sea, Pakistan, India and Iran, as a natural remedy for over 2000 years as bronchodilatory, anti-inflammatory, antibacterial, hypoglycaemic and immune-potentiating agent.

Thymoquinone (TQ) and polyphenols are believed to be the main active constituent of black cumin seed oil and responsible for suppressing the proliferation of tumor cells including colon, breast, bone, ovarian, prostate and pancreatic carcinoma (22).

The seeds of *Nigella sativa* Linn. (*Ranunculaceae*) commonly known as Black cumin seed, are reported to possess hepatoprotective, anti-inflammatory, hypoglycemic, antibacterial, carminative and stimulant activities (25). Clinical research showed that *Nigella sativa* extract appears to induce DNA damage of liver tissue and decrease the DNA damage as compared to carboplatin alone (22,25). Another result indicated that in diabetic rats treated with *Nigella sativa* the frequency of micronuclei in the erythrocytes of bone marrow decreased ( $P < .05$ ) and the antioxidant status was enhanced ( $P < .05$ ) in the treated diabetic rats as compared to controls. Thus the observations indicated that diabetic patients are more prone to cell mutations which are related to the level of cellular oxidative status which could be reduced by *Nigella sativa* (26).

## Conclusion

Our results demonstrated that the seed extract of *Nigella sativa* L. could have good antioxidant potentials to prevent

or control free radicals, which can produce many current inflammatory diseases such as cancer, diabetes, coronary diseases, infection, etc. So these data confirm the traditional use of this plant with its terpenoids, flavonoid and phenolic compounds.

Hence, we believe that the seed extract absolutely had antioxidant and anti-inflammation effect to treat many inflammatory diseases and for further justification more in vivo and in vitro evaluations are required. The investigations confirm that higher plants used as antioxidants may serve as a valuable source as anti-inflammation and antioxidant agents.

### Ethical issues

Ethical of this research work was approved by Gorgan Branch, Islamic Azad University.

### Conflict of interests

The authors declare that they have no conflict of interests.

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