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Research Article

Black Cumin (*Nigella sativa* L.): Studies on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety

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Abstract

The significance of natural flora is well known by the scientific community. The medicinal plant gifted by nature have been explored by the humans to find out their respective values in the medical field. *Nigella sativa* has been used as traditional medicine for centuries. The aim of this study is that to test the phytochemical screening and antioxidant capabilities of methanolic extract of *Nigella sativa* seeds. In the present study the seeds were powdered and the best methanol extracts were screened for Preliminary phytochemical investigations. Seed powder showed the presence of alkaloids, flavonoids, saponins, carbohydrates, tannins, sterols, glycosides, phenolic compounds, protein and amino acids and absence of gums, mucilage, oil and fats. The experimental data indicated that *Nigella sativa seeds* displayed the highest DPPH scavenging effect (82%) with the help of standard graph plotted using standard antioxidant compound. Finally, the results obtained in this study that *Nigella sativa* possess significant amount of antioxidants and phytochemical compounds.

Keywords: *Nigella sativa*; Phytochemistry

Introduction

Medicinal plants have always been a major source of therapeutic agent since ancient time for curing human diseases. Plant products that have been derived from barks, flowers, roots, leaves, fruits, seeds are the key constituents of phytomedicines.[1]The seeds of the *Nigella sativa* plant have been used to promote good health and immunity for fighting against disease for centuries especially in the medicine system of Middle East and southeast. *Nigella sativa as* oriental spice have been highly used as a natural medicine for the treatment of multiple acute as well as chronic conditions. *Nigella sativa* is an herbaceous plant which is colloquially known as black seed, its habitat is found in Southeast Asia and Mediterranean countries. Indian folks used this plant as a food preservative as well as a protective and curative treatment for numerous disorders [1-3].

Nigella sativa seed, commonly known by the term black seed, has been used as a natural remedy for centuries in many different cultures. *Nigella sativa* is an important annual herb of the

Ranunculaceae family. This plant is known by numerous names, for example black cumin (English), black caraway seeds (USA), shonaiz (Persian) and kalajira (Bangali) Al-Habba Al-Barakahand(Arabic) [3]. It also has traditional medical applications and considered to be a characteristic traditional herbal medicine for diverse diseases in Unani, Arabic, Prophetic and Indian traditional medicines [4-6].

The Seeds of *N. sativa* are frequently used in folk medicine in the Middle East and some Asian countries (Unani, Ayurveda, Chinese and Arabic Medicines) for the promotion of good health and the treatment of many ailments including fever, the common cold, headache, asthma, rheumatic diseases and microbial infections and to expel worms from the intestines as well as cancer. The Islamic Prophet Mohammad had described the healing powers of the Black Seeds against a variety of diseases. He stated that the uses of black seeds, cures all diseases except dath" [3,5]. *N. sativa* seeds have been used traditionally in the middle eastern folklore medicine as a medicine for various diseases for more than 2000 years ago [6,7].

Nigella sativa contain more than 100 active chemical components including phytochemicals such as thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellidine and alpha-hederin, vitamins, minerals etc. [7]. The secondary metabolites of *N. sativa* seeds possess potent therapeutic features like immune, cardiovascular, respiratory, gastric and endocrinal health [8,9]. A large number of *in vivo* and *in vitro* studies have been experimented on laboratory animals and humans for the investigation of its pharmacological properties, like hypoglycemic, immunostimulation, anti-inflammatory, antihypertensive, antioxidant, antimicrobial, antiparasitic, as well as anticancer properties [8-10]. *Nigella sativa* has a very pungent smell and bitter taste. It is used primarily in the synthesis of confectionery and liquors. Peshwari nam is, as a rule, is topped with kalonji seeds. *Nigella sativa* is also used in the manufacturing of Armenian string cheese, a braided string cheese called Majdouli or Majdouleh in the Middle East.

N. sativa seeds have gained attention due to their potential health benefits, including antioxidant, antimicrobial, and anticancer properties. Phytochemical screening of *N. sativa* seed extracts has revealed the presence of various bioactive compounds, including flavonoids, alkaloids, and phenolic compounds.

Materials and Methods

Plant sample collection

Nigella sativa seeds were collected from the local market of Velachery, Chennai, Tamilnadu, India. The seeds were identified and authenticated by Dr. Jayaraman, Director, Plant Anatomy Research Center, Chennai.

Preparation of powder (Harborne, 1973)

The collected seeds were washed three times with distilled water to remove the dust particles and dried on a blotting paper in the laboratory at 37ºC for 24 hrs. After drying, the seeds were powdered by using a kitchen blender and stored in an airtight container. These powdered materials were used for further studies.

Preparation of plant extract with different solvents

The solvents such as chloroform, ethyl acetate, methanol and water were used for direct extraction of the samples (Eloff, 1998) for the purpose of preliminary screening. In this method, finely ground material 5 g was extracted separately with 50 ml of direct solvents in conical flask in shaking condition. The extracts were decanted into pre-weighed glass vials. The process was repeated 3 times using fresh solvent. The solvent was removed by placing the extracts in front of a steam of air in a fume hood at room temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10 mg/ml concentration for the further analysis.

Phytochemical screening procedures (Brindha., *et al*. 1981)

The following tests were performed on the extracts to identify various phyto-constituents present in them.

Test for alkaloids (Mayer's test)

A little amount of extract was taken and a few drops of Mayer's reagent were added. The formation of precipitate indicates the presence of alkaloids.

Test for flavonoids (Ferric chloride test)

1 ml of extract was taken and a few drops of dilute ferric chloride solution were added. The colour change to pale green or red brown colour indicates the presence of flavonoids.

Test for saponins (Foam test)

1 ml of extract was diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of air bubbles indicates the presence of saponins.

Test for carbohydrates (Molisch's test)

Small quantity of extract was dissolved separately in 4 ml of distilled water and filtered, 2 ml of filtrate and 2 drops of alcoholic napthol solution are added. The mixture is shaken well and 1 ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. Formation of reddish brown ring or violet ring indicates the presence of carbohydrate.

Test for tannins (Lead acetate test)

To 5 ml of extract solution, 1 ml of lead acetate solution was added. Flocculent brown precipitate indicates the presence of tannins.

Test for sterols (Leibermann burchard reaction)

A small amount of sample extract and a few crystals of sodium nitrate were taken in a dry test tube and heated gently for a minute. It was cooled and added 0.5 ml of concentrated sulphuric acid. Orange colour indicates the presence of sterols.

Test for glycosides

A portion of the extract was hydrolysed with hydro chloric acid for few hours on a water bath and the hydrolysate was subjected to legal's test to detect the presence of different glycosides.

Legal's test

1 ml of sodium nitro prusside solution was added and then it was made alkaline with sodium hydroxide solution. If the extract produced pink to red colour, which indicates the presence of glycosides.

Test for oil and fats

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soaps or particle neutralization of alkali indicates the presence of fixed oil and fats.

Test for phenolic compounds

Few drops of extracts were taken separately in water and tested for the presence of phenolic compounds with dilute ferric chloride solution (5%) which gives violet colour.

Test for protein and amino acids (Biuret test)

A few drops of extract were taken in water and 1 ml of 4% copper sulphate was added to it. Violet or pink colour confirmed the presence of proteins.

Test for gums and mucilage

About 10 ml of the extract was added to 25 ml of absolute alcohol with stirring and then filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Fluorescence spectrometer analysis

Fluorescence analysis was carried out with shade dried seed powder of *Nigella sativa.* The powder was separately treated with 1N aqueous NaOH, 1N ethanolic NaOH, 1N H_2SO_4, 1N HCl, $FeCl_2$, and KOH. The supernatants were examined under ultraviolet light and ordinary day light (Maluvendar Viji, 2010).

Antioxidant activity (Estimation of Radical Scavenging Activity (RSA) Using the DPPH Assay)

The RSA activity was determined using DPPH assay according to the modified method of Nenadis and Tsimidou (2002). The decrease of the absorption at 517 nm of the DPPH solution after the addition of antioxidant (seed extract) was measured in a cuvette containing 2960 µl of 0.1 mM ethanolic DPPH solution mixed with 40 µl of 20 - 200 µg/ml of seed extract. Blank containing 0.1 mM ethanolic DPPH solution without seed extract and vortexed thoroughly, the setup was left at dark at room temperature. The absorption was then monitored after 20 minutes. Ascorbic acid (AA) and Butylated hydroxyl toluene (BHT) were used as reference controls. The DPPH radical scavenging activity was calculated using the formula:

$$\%\ \text{of DPPH radical scavenging} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Measurements were performed in triplicate. Absorbance values were corrected for radical decay using blank solutions. The IC50 was calculated using a calibration curve vs. percentage of inhibition.

Ferric thiocyanate method (Osawa and Namiki, 1981)

The plant seed sample of 4 mg in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 ml), pH 7.0 (8 ml) 0.05 M phosphate buffer, and distilled water (3.9 ml) and kept in screw cap containers under dark conditions at 40°C. To 0.1 ml of this solution, about 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate were added. After 3 min, 0.1 ml of 2 M ferrous chloride (dissolved in 3.5% HCl) was added and the absorbance was measured at 500 nm regularly until one day after absorbance of the control reached maximum. The control and the standard were subjected to the same procedure as the sample, but the control was not added with sample and the standard was added with 4 mg of α - tocopherol instead of sample.

Thiobarbituric acid (TBA) test (Kikuzaki and Nakatani, 1993)

The samples as prepared for the above method were used in TBA test. To 1 ml of sample solution, 2 ml of 20% aqueous trichloroacetic acid was added. This mixture was then incubated in a boiling water bath for 10 min. After cooling, it was then centrifuged around at 3000 rpm for 20 min and the absorbance of supernatant was read at 532 nm.

Superoxide Anion Radical Scavenging Assay (Nishikimi., et al. 1992)

One milliliters of NBT solution (156 µM NBT in 100 mM phosphate buffer, pH 8.0) was added with 1ml of NADH solution (468 µM in 100 mM phosphate buffer, pH 8.0). Then it was mixed with 0.1 ml of sample solution (10 mg/ml). The reaction was started by adding 100 µl of PMS solution (60 µM PMS in 10 mM, Phosphate buffer, pH 8.0). The mixture was incubated for 5 min at 25ºC. For control, the reaction mixture was not added to the sample. Absorbance was measured spectrophotometrically at 560 nm.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the *Nigella sativa* extract was measured (Klein., *et al.* 1991). Different concentrations: 50, 100, 150 and 200 µg/ml of extracts were added with 1.0 ml of iron-EDTA solution, 0.5 ml of EDTA solution (0.018%), and 1.0 ml of dimethyl sulphoxide (DMSO). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated for 15 min in a water bath at 90°C. The reaction was terminated by the addition of 1.0 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent was added and incubated for 15 min at room temperature. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm against the blank.

The percentage of the hydroxyl radical scavenging activity (RSA) was calculated by the following formula:

$$\% \text{ HRSA} = \frac{A0 - A1}{A0} \times 100$$

Where, A0 = absorbance of the control, A1 = absorbance of the extract.

Phosphomolybdenum assay

The antioxidant activity of samples was then evaluated by the green phosphomolybdenum complex formation according to the method of Prieto., *et al*. (1999). An aliquot of 100 µl of sample solution was added with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial and incubated in a water bath for 90 min at 95°C. The samples were cooled and the absorbance was read at 695 nm. The results were expressed as g of ascorbic acid equivalents/100 g extract.

Results

Antioxidant activity (Estimation of Radical Scavenging Activity (RSA) by using DPPH Assay)

The experimental data indicated that ethyl acetate extract of *Nigella sativa* displayed the highest DPPH scavenging effect with the help of standard graph plotted using standard antioxidant compound (Table 1).

Superoxide anion radical scavenging activity

Ethyl acetate tuber extract of *Nigella sativa* had shown scavenging activity on the superoxide radicals in a dose dependent manner (1000 µg in the reaction mixture). The ethyl acetate extract exhibited strong superoxide radical scavenging activity standard was 21.38 ± 2.70 and extract was 9.42 ± 2.17 (Table 2).

Hydroxyl radical scavenging activity

The OH scavenging activity of *Nigella sativa* ethyl acetate extract at different dose levels (50, 100, 150 and 200 µg/mL). Theextract showed the highest OH scavenging potential of 69% at 200 µg/

ml. The ability of *Nigella sativa* extract to quench hydroxyl radicals seems to directly relate to the prevention of propagation of the process of lipid peroxidation, and seems to be good scavengers of active oxygen species thus reducing the rate of chain reaction (Table 3).

Phytchemical screening of *Nigella sativa*

The preliminary phytochemical screening of *Nigella sativa* revealed the presence of alkaloids, flavonoids and reducing sugars in high amounts, phenols, glycosides and saponins in moderate followed by terpenoids and tannins in trace (Table 5).

Antioxidant activity (%)		
Standard	**Control**	**Extract**
0.098	0.236	0.143

Table 1: Antioxidant activity assay of *Nigella sativa* ethyl acetate extract by Thiobarbituric acid (TBA) test.

Concentration (µg)	**Superoxide Anion Radical Scavenging Activity (%)**	
	Standard	**Extract**
1000	21.38 ± 2.70	9.42 ± 2.17

Table 2: Superoxide Anion Radical Scavenging Assay of *Nigella sativa* ethyl acetate extract (% activity).

Concentrations (µg)	**Hydroxyl radical scavenging activity (%)**	
	Standard	**Extract**
50	34.44 ± 1.32	26.53 ± 3.34
100	49.50 ± 1.57	37.87 ± 3.56
150	67.12 ± 1.41	52.54 ± 3.45
200	73.56 ± 2.32	69.64 ± 2.51

Table 3: Hydroxyl radical scavenging activity of *Nigella sativa* ethyl acetate extract.

Control	**Extract**
0.612 ± 0.71	2.0 ± 0.70

Table 4: Phosphomolybdenum assay of *Nigella sativa* ethyl acetate extract (1 g of ascorbic acid equivalent to 100 g extract).

Detection of phytochemicals	**Test**	**Result**
Alkaloids	Mayer's	+++
Phenols	Ferric chloride	++
	Lead acetate	++
Glycosides	Borntrager's	++
Terpenoids	Salkowski	+
Flavonoids	Salkowski	+++
Tannins	Salkowski	+
Reducing sugars	Fehling's	+++
	Benedict's	+++
Saponins	Foam	++
Proteins	Millon's	-
	Biuret	-

Table 5: Phytochemical screening of *Nigella sativa* ethyl acetate extract.

+ + +: High concentration

++: Moderate concentration

+: Trace concentration

-: Constituents not detectable using the specified assay.

Discussion

Herbal formulations have gained global level recognition for the bioremediation of humanity. Phytodrugs have been consistently proved to be potential source of remedies for many fatal ailments. Hence, the medicinal plants have drawn the attention of the pharmcologists and medical practitioners. Traditional Indian systems of medicines largely depend upon the herbals for their formulations. The native principles of medicine started to diminish due to several factors, one of the factors being lack of knowledge for those who work on the herbal medicines, the correct diagnosis, and identity of the herbals handled by them. This seems to be a serious problem in employing the herbals in clinical pharmacology [1-4]. Due to convergence in evolutionary process of biological species, especially plant species, there are considerable similarities among the taxa, at species level or generic level. Taxonomic diagnosis of taxa is largely relied upon external characters such as phyllotaxy, foliar characters and floral features. The herbal botanists have very often to deal with fragmentary market plant samples which ought to be verified for its purity and genuineness. These tasks involve

microscopic examinations of the plant samples and compare the data thus obtained with already established data for the samples. The microscopic characterization of medicinal plants is one of the essential aspects of pharmacognostical investigation [2,6,7].

Natural products once served man kind of as the source of all drugs and their derivatives still represent over 50% of all drugs in clinical use. The world health organization estimates that 80% of the people in the developing countries of the world rely on traditional medicine for their health care and about 85% of the traditional medicine involves the use of plant extracts [7,11,12].

Between 1960 and 1982 The National Cancer Institute screened around 1, 14,000 extracts from as estimated 35,000 plant samples for anticancer activity. Further they initiated a new natural products program in which 23,800 plant species collected from 20 different countries have been screened for anticancer activity. Hepatocellular carcinoma is the fifth most common cancer in the world and the third most common cause of deaths each year.[9,12] The number of deaths each year amount to about 550,000 and are most often in the developing countries. Drug discovery from medicinal plants has played an important role in the treatment of cancer and indeed most new applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer [10,11,13].

Medicinal plants are the backbone of traditional medicine and the beneficial activity of plant extract on human welfare is due to the presence of bioactive compounds in that extract [6,12,14]. These compounds may exert their activity by acting as antioxidant agent, detoxification agent, immune system enhancing agent, antibacterial agent, antiviral agent, etc. Initial screening of plants for bioactivity in particular antimicrobial activities typically begins by using crude aqueous or alcohol extraction and can be followed by various organic extraction methods. Extraction of crude extract from medicinal plant using different solvents is very important in point of choosing the solvent for the extraction [6,9,11].

The extracts of *Nigella sativa* obtained with ethyl acetate revealed that, when DPPH was added, purple color was observed for all the concentrations. After incubation for 20 min in dark, the purple color of DPPH was turned to yellow for high concentration showing the capacity of the plant extract to scavenge the free radical DPPH owing to the nature of phenolics and their hydrogen donating ability [8,10].

The presence of various compounds such as vitamins, carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, enzymes, minerals, etc. might be responsible for the antiproliferative activity of such medicinal plants. Phenolic compounds, including flavonoids are especially promising candidates for cancer prevention [9,12].

The results of the present study indicated that ethyl acetate extract of *Nigella sativa* was high in phenolic contents and the extract exhibited strong antioxidant activity. The scavenging activities observed against DPPH and hydroxyl radicals, peroxidation inhibition, lead us to propose *Nigella sativa* as a promising natural source of antioxidants suitable in the application of nutritional/ pharmaceutical fields, in the prevention of free radical- mediated diseases.

Conclusion

Nigella sativa demonstrates higher levels of antioxidant capability and phenolic content in the investigated material, while the phytochemical content. Therefore, *Nigella sativa* is confirmed as a medicinal plant abundant in phytochemicals.

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