

***In Vitro* Assessment of the Free Radical Scavenging Activity, Proximate and GC-MS analyses of Essential and Fixed oil of *Nigella sativa* from Pakistan**



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Abstract

The current work was carried out to study the *Nigella sativa* which is a prominent native plant and is well known for its medicinal prominence for the treatment of many diseases. By using the standard methods the nutritional values of the seed and oil of *Nigella sativa* were determined. The contiguous investigation was done to find out moisture, ash, fat, fiber, protein, carbohydrates and energy. The moisture contents of seed (7.70%) had lesser moisture content than the cake (8.48%). Ash content of the seed was 3.52% and ash content of the cake was 3.57%. The value of seed ash content was nearly same as the ash content of cake. The fiber content of seed 5.86% and fiber content of cake is 6.70%. *Nigella sativa* is considered as best seeds reducing diabetes and cholesterol level in blood. The fat contents of seed and cake was 31.02% and 11.54%. The protein contents of the seed and cake were 19.78% and 17.35%. Total energy of seed and cake was 486.78 and 382.70; there was a significant difference between energy value of seed and cake. The Antioxidant activity of the fixed & essential oil was determined by using DPPH assay and reducing power activity which showed that the antioxidant activity of fixed oil (% Inhibition DPPH 72.27+2.5) was higher than the essential oil (% Inhibition DPPH 63.39+2.3) at 20µl concentration. In the light of the above consideration, the results of the study revealed that *Nigella sativa* showed promising antioxidant activity in a dose dependent manner in both assay. GC-MS analysis of both oil were also showed number of antioxidant compounds.

Keywords: *Nigella sativa* seed/cake/oil; Nutritional facts; DPPH assay; Reducing power assay; GC-MS

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Introduction

Nutrients present in our food have unswerving influence on our health and their deficiencies mainly cause diseases which disturb the metabolism of the body. There are seven factors which are responsible for making diet balance i.e. water, carbohydrates, minerals, proteins, vitamins, fats and fiber [1]. Plant biodiversity in the world is the main source of herbal medicines. It is studied that about 60-80% of the world's population is depending on herbal medicines for the treatment of many diseases [2]. Pakistan is one of the blessed countries with exceptional plant biodiversity. About 15,000 plant species have been found to have medicinal aspects. Over the years, people have not been inclined to use plants for medicinal purpose for the treatment of technical disorders [3].

Nigella sativa belongs to kingdom Plantae. The sub kingdom is *Tracheobionta* which means it is a vascular plant. As a super division it is *Spermatophyta* which shows that it is seeds bearing plant. Its division is *Magnoliophyta* means it is well known flowering plant. It belongs to class *Magnoliopsida* means it's a dicot and its sub class is *magnoliidae*. The order is *Ranunculaceae* which means it belongs to buttercup family. It is an aboriginal herbaceous plant normally known as the fennel flower plant. It belongs to the genus *Nigella* L.-*nigella*. The species is known as *Nigella sativa* L.-black seeds, *Nigella sativa*, black cumin, black cumin, Roman coriander, onion seeds, dill seeds, Kalonji, Habbat Alabarakah (seed of blessing).

Nigella sativa belongs to *Ranunculaceae* family and is an annual plant which grows abundantly in Pakistan and India. This widespread plant is also native to some parts of the Mediterranean region and the Arab countries [4]. It has been reported our Holy Prophet Hazrat Muhammad Salallah-u-alai-hi Wasalam said, that the black seeds (*Nigella sativa*) is a cure for almost every disease except death" [5-7]. Recently, many activity values have been isolated from *Nigella sativa*. This includes thymoquinone, thyme hydroquinone, dithymol, thymol, carvacrol, phenylalanine, phenethylpropylamine-N-oxide, phenethylpropidine, and α -phenetamine [8-10].

In the past few decades, through the use of modern science and technology, the pharmacological activities of different *Nigella sativa* extracts and some of its active ingredients have been explored [11]. In fact, the chemical research on *Nigella* seeds began this year. In 1880, Greenish published the first report on the presence of 37% oil and 4.1% ash (calcium salt) in *Nigella sativa* seeds. The seeds contain 37.5% non-volatile oil, 1.5% volatile oil, 33-34% carbohydrate, 16-19.9% protein, 4.5-6.5% fiber, 0.013% saponins, 5-7% moisture [12-15]. The main component of *Nigella sativa* is fixed oil and volatile oil accounts for 0.4-0.7% of total seed weight [16,17]. The chemical composition of *Nigella sativa* fixed oil is linoleic acid (44.7-56%), linolenic acid (0.6-1.8), oleic acid (20.7-24.6%), palmitoleic acid (3%), arachidic acid (2-3%), palmitic acid (12-14.3%), stearic acid (2.7-3%), myristic acid (0.16%), eicosadienoic acid (2 -2.5%) and sterol (0.5%) [18-20]. In *Nigella* seed, the ash content showed the presence of calcium (0.5-1%), Phosphorus (0.6%), Sodium (0.1%) and Potassium (0.6%) of the total seed weight [21-23]. Three types of alkaloids are present in defatted seeds of *Nigella sativa* i.e. indazoline, isoquinolinylline and their N-oxides and the indazole alkaloid nicilidine [24].

Traditionally, black seeds have been used to treat headaches, toothaches, nasal congestion and intestinal worms. Since 1960, numerous researchers around the world have reported the different pharmacological effects of the whole seeds of *Nigella sativa*. Nigellone and thymoquinone are two of the most volatile oils found in black seed. Nigellone suppresses muscle spasms and having properties to widen the air passages in bronchi and bronchioles. It is also used as antiallergic mainly to control the seasonal allergies [25].

The active ingredients of *Nigella sativa* i.e. thyme hydroquinone, thymol, thymoquinone, dithymol, nigelmine-N-oxide, alpha-hederin, nigellicine, carvacrol and nigelidine have so many medical uses. The pharmacological properties of *Nigella sativa* were studied on animals and found that it has anti-inflammatory, anti-hypertensive, anti-parasitic, anti-oxidant, immune stimulation, anti-cancer and anti-bacterial properties [26-28]. It is also helpful in headache, cough, sore throat, chest congestion, inflammation and is helpful in regulating blood pressure [29,30]. In biological systems, this oxidizing strain affects the main cellular components. These components include lipids, proteins, and DNA. These are related to pathologies such as atherosclerosis (cardiovascular disease), inflammatory diseases (rheumatoid arthritis, asthma and

allergies) aging and Parkinson disease [31]. The literature reported the antimicrobial activity of *Nigella oleifera* extract against a variety of bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*), including multi-drug resistant Gram(+) and Gram(-) Bacteria, and a yeast (*Candida albicans*) [27,32,33].

Recently, thymoquinone has also been reported to be active against *Aspergillus niger*, a common unscrupulous saprophyte, which can infect AIDs patients and other immune deficient Personal research on *Nigella sativa* shows that the plant has traditionally been used as a medicine to treat many diseases including cough, headache, asthma, dysentery, bronchitis, infection, eczema, flu, fever and rheumatism [34]. The analysis of *Nigella sativa* showed that it is also rich in antioxidants which increases immunity against cell decomposition [35]. The purpose of the present research is to examine the basic composition of *Nigella sativa* seeds and cakes, to check Free Radical Scavenging (DPPH) and reducing power, and Gas Chromatography-Mass Spectrometry analysis of essential oil and fixed oil.

Materials and Methods

Gas Chromatography- Mass Spectrometry (GC-MS) analysis

The gas chromatograph 6890A network (Agilent technology) was used in conjunction with selective mass detector 5973 (Palo Alto, California, USA). GC-MS analysis of the essential oil and fixed oil of *Nigella sativa* was done. The ion source of a mass spectrometer is connected to column outlet directly at 230 °C and 70eV (ionization energy) was applied. Collect spectral data in the 40-540 amu mass range. Prepare samples for GC-MS analysis according to literature procedures [36].

Proximate composition

The *Nigella sativa* seed & cake were analyzed according to the protocol of Association of Official Analytical Chemists [37]. Different parameters like ash, carbohydrates, fat, protein and energy were calculated. Ash content was estimated by burning at 650±15 °C. The crude fat was determined by using Soxhlet device and extraction of powder was done with petroleum ether. The crude protein was determined by using HongKe's nitrogen determination method. The total carbohydrates were determined by the difference. Calculate energy based on energy (kcal)=4× (g protein + g carbohydrate) + 9× (g fat).

Free Radical Scavenging Activity (DPPH)

The DPPH radical scavenging activity of *Nigella sativa* oil was determined [38] with minor revision. 1mL of various concentrations of 20-100µL extracts was mixed with 3mL of methanolic DPPH solution (0.004 %). The decrease in absorbance of both the oil was measure after 30 minutes at 517nm.

Reducing power activity

The reducing power (Fe³⁺) of *Nigella sativa* extract was detected [39]. The extracts of both oils at various concentrations (20-100µl)

were mixed with phosphate buffer of 0.2M, pH 6.6 (0.75mL) and of 1%, w/v of potassium hexacyanoferrate (0.75mL). The mixture was incubated at 50 °C in a water bath for 20 minutes. 0.75mL of trichloroacetic acid solution (10%) was added to stop the reaction and centrifugation was done at 3000r/min for 10 minutes. 0.1mL of ferric chloride solution (0.1%, w/v), 1.5mL of the supernatant and 1.5mL of distilled water were mixed for 10 minutes. The absorbance was measured at 700nm by using UV-Vis spectrophotometer (1700, Shiadzu, Japan) as the reducing power. The higher the absorbance of the reaction mixture, the greater was the reducing power.

Statistical analysis

The data were presented as mean \pm standard deviation. The t-test was applied for continuous variables to differentiate mean difference.

Results and Discussion

It is very important to study different characteristics of plants and the response to process conditions. The plants characteristics are important for their handling as medicinal aspects and purpose [40]. The main function of food nutrients is to regulate different body functions, create different body structures, and provide energy for the body. The current research focuses on the nutritional value and antioxidants of *Nigella sativa*.

GC-MS of fixed oil

GC-MS of *Nigella sativa*'s fixed oil was done and following compounds were present. hexane, 4-methyle-1(1-methylethyl), benzene, 1-methyl-4-(1-metylethyl), ether p-menth-6-en-2-yl methyl, 2,5-cyclohexadiene-1,4-dione,2-methyl-5-(1-

methylethyl), 1,4-methanoazulene,decahydro-4,8,8-trimethyl-9-methylene,phenol,4-methoxy-2,3,6-imethyl, hexadecanoic acid, ledol,9,12-octadecanoic acid,9,12-octadecanoic acid, ethyl ester, hexadecanoic acid, propyl ester, 9-octadecnoic acid, methylethyl ester,9,12-octadecadienoic acid, 2,3-dihydroxypropyl ester.

GC-MS of essential oil

These are following compounds which are present in essential oil. hexane,4-methyle-1(1-methylethyl), benzene, 1-methyl-4-(1-metylethyl), ether, p-menth-6-en-2-yl methyl, 2,5-cyclohexadiene-1,4-dione,2-methyl-5-(1-methylethyl), 1,4-methanoazulene,decahydro-4,8,8-trimethyl-9-methylene,phenol, 4-methoxy-2,3,6-trimethyl,hexadecanoic acid, ledol,9,12-octadecanoic acid, 9,12-octadecanoic acid, ethyl ester, hexadecanoic acid, propyl ester, 9-octadecnoic acid, methylethyl ester,9,12-octadecadienoic acid, 2,3-dihydroxypropyl ester.

Nutritional facts

Different nutritional aspects of both *Nigella sativa* seed and cake were done including moisture, ash, fiber, fat, protein and energy was calculated as a proximate content (Table 1).

Moisture contents: It was found that the moisture of the cake is higher than the moisture of the seed. However, the moisture content of the black seed mentioned by the Atta et al. [9] is less than the present work [41]. Moisture of black seed and cake mention by Tauseef et al. was 6.64% and 7.84% respectively. These percentages are relatively less than the curent study. The moisture contents of the black seed determined by the Takruri HRH et al. [42] were 3.8% to 7% which matched our results of moisture contents. Finally, the moisture of cake is greater than seed (Table 1).

Table 1: The nutritional value of *Nigella sativa* seed and cake.

Parameters (%)	Seed	Cake
Moisture	7.70 \pm 0.5	8.48 \pm 0.6
Ash	3.52 \pm 0.3	3.57 \pm 0.5
Crude Fat	31.02 \pm 0.5	11.54 \pm 0.1
Crude Fiber	5.86 \pm 0.4	6.70 \pm 0.4
Crude Proteins	19.78 \pm 1.5	17.35 \pm 1.4
Carbohydrates	32.10 \pm 2.5	52.53 \pm 4.5
Energy (kcal/100g)	486.78 \pm 7.0	382.70 \pm 6.2

Ash contents: The amount of ash contents of cake were found relatively higher than the ash content of the seed. Total ash content determined by the Atta et al. [9] was 4.20%. Total ash content also measured by Atta MB [41] these were 3.7%.

Fat contents: The fat content of seed was much higher than the cake. Atta et al. [9] determine the fat content it was 31.16%. This value is much closer to my work after extract the oil from the seed the cake also have 11.54% fat in current study which matched with study of Babayan VK et al. [12].

Fiber contents: The fiber content of seed is less than cake. Cake has high value of fiber. Atta et al. [9] examined the fiber content in seed 6.03%. This value is higher than current study result. The less fiber contents are beneficial to human health as the dietary fiber is helpful to reduce blood sugars cholesterol levels.

Protein contents, carbohydrates and energy: The Kjeldahl method was used to determine crude protein. The protein content of the seed is higher than the cake after extract the oil from the seed its known has cake. Cake also has good percentage in it. Protein

contents are very helpful from medicinal point-of-view. The protein contents (22.80%) detected by the Tauseef et al. were relatively higher than the current findings as the protein contents vary from variety to variety. Climatic conditions also affect protein contents amount. The Protein contents (20.85%) determined by the Takruri HRH et al. [42] were nearer to present research. Carbohydrates and energy were calculated as described by AOAC Methods.

Antioxidant activity

There has been increasing interest in natural antioxidants found in medicinal plants because of the carcinogenic effects of synthetic antioxidants [43]. Medicinal plants are good foundations with antioxidant property and are used as a substitute medicine to alleviate the diseases associated with oxidative stress [44,45]. The various parts of medicinal plants those are generally rich in various phytoconstituents such as tannins, Phenolic compounds, and

flavonoids, have numerous biological effects as well as antioxidant activity. In this study antioxidant activity of were performed of two types of oil extract from the black seed one type is called fixed oil and other one is called is essential oil. In our experiment we can see the antioxidant activity of fixed oil is higher than the essential oil. These results are also agreed with Laguerre M et al. [29] and Pan Y et al. [34].

In this study, the potential *in-vitro* antioxidant activity of both oil extracts were estimated by DPPH radical scavenging and reducing power activity method. The essential and fixed showed dose dependent increased in scavenging activity on free radicals in this study. The results indicate that both essential & fixed oil showed potential free radical scavenging activity in DPPH assay and fixed oil has more DPPH radical scavenging activity than essential oil of *Nigella sativa*.

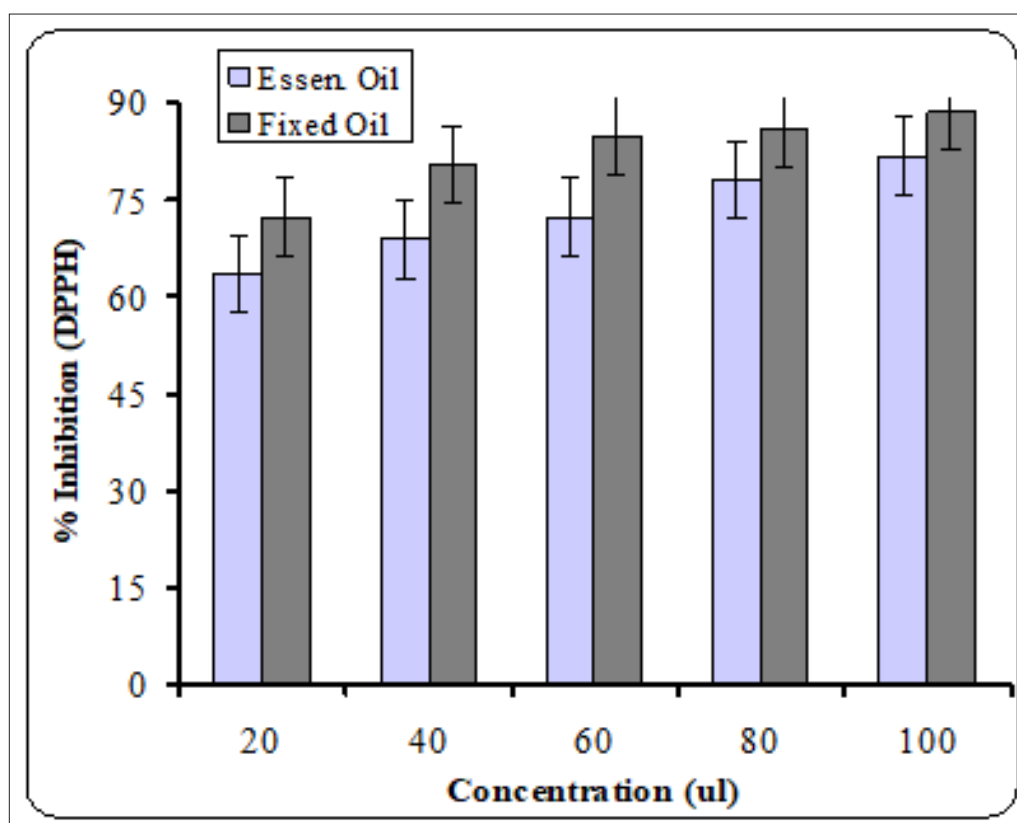


Figure 1: % Inhibition (DPPH) of essential and fixed oil of *Nigella sativa*.

Reducing power activity

The fixed and essential oil were analyzed for reducing ability. The methanol extracts of *Nigella sativa* were used for this purpose. The ferric reducing activity was determined (Figure 2). It was

found that that the fixed oil extracts exhibited stronger reducing power antioxidant activity than those of essential oil extracts and standard antioxidant BHA. In addition the essential and fixed both oils showed dose dependent antioxidant activity in reducing power assay.

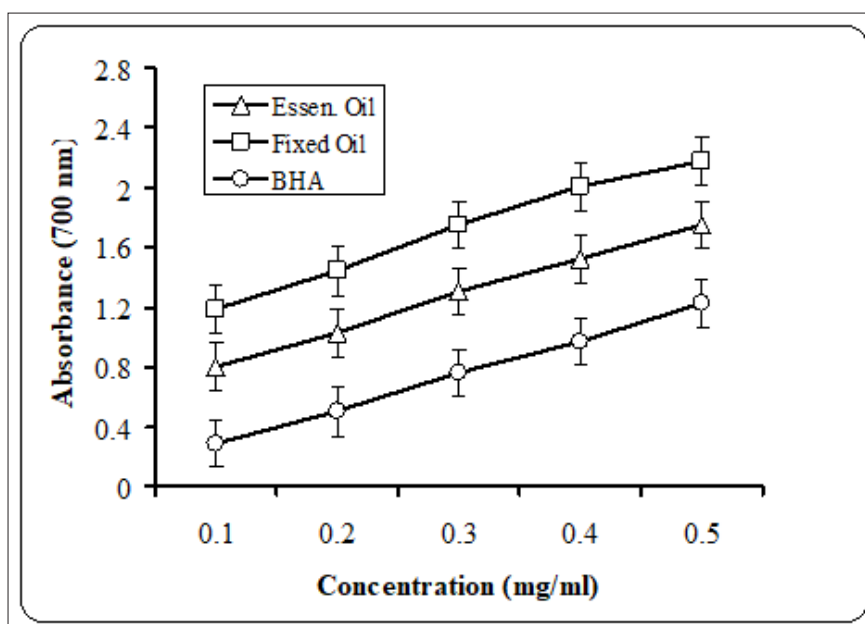


Figure 2: Reducing power activity of essential and fixed oil of *Nigella sativa*.

Conclusion

The above studies were carried out to investigate the nutrients composition of *Nigella sativa*. During the investigations and experimentations this is proved that the nutritional values of Black Seeds are very high. The remainder of black seed after extracting the oil, which is commonly known as cake has also shown the nutritional value of cake is more than adequate. The percentage of protein and fat erratically present in the cake. These things proved that the cake of the black seed is not waste or a useless product but it's a good source of minerals and nutrients which are helpful to boost body immunity against various diseases. The results have shown that it is a good source of micro as well as macro nutrients. It contains significantly high amount of ash which enriched with mineral elements. Both seed and cake of *Nigella sativa* contain only good fat and contains least amount of fiber which makes them best for reducing blood sugar and cholesterol levels. The protein content of seed was higher than cake so they could be used as good source of energy.

Oil was extract from seed of two forms; first one is termed as fixed oil and second one is known as essential oil. The anti-oxidant value of these oils is very high which showed that these oils can be used against those diseases which are caused by oxidative reactions. During experiments it was observed that the anti-oxidant activity of fixed oil is greater than the essential oil. That's why fixed oil is easily available in the markets. The extraction process of fixed oil is cheaper than the essential oil and gives high yield of antioxidant. This characteristic of oil is cured for lowering the cholesterol level and better for the heart patients.

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