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Original Research Article

Nutritional, Phytochemical Composition and Antimicrobial Activity of Market and NRCSS *Nigella sativa* (Seed and Oil) Samples for Healthier Being

Deepti Giri[1*], Sunanda Sharan[2], Suvarna V. Chavannaavar[3] and Ambreen Fatima[3]

[1]Department of Food Science and Nutrition, N.D.U.A & T, Kumarganj, Faizabad, India
[2]Department of Food Science and Nutrition, [3]Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bangalore 560065, Karnataka, India
**Corresponding author*

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Nigella sativa, Phytochemicals composition, Antimicrobial activity

A B S T R A C T

Nigella sativa is an herbaceous and annual spice of the rannunculaceae family. Investigation was undertaken to study the nutrient, phytochemical and antimicrobial activity of *Nigella sativa* seed spice from samples procured from NRCSS (National Research Center on Seed Spices, Ajmer) and local market. It is estimated that there may be as many as 10,000 different phytochemicals present in *Nigella sativa* having the potential to affect diseases such as cancer, stroke or metabolic syndrome etc. The seeds of *Nigella sativa* commonly known as black seed which are used in herbal medicine for the treatment and prevention of a number of diseases and conditions including asthma, diarrhea and dyslipidaemia. The *Nigella sativa* oil possesses antibacterial activity against several bacteria due to the presence of several phytochemicals. The results of the study indicated that this *Nigella sativa* spice contained substantial amounts of macro nutrients *i.e.*, 16.31-16.52 per cent protein, 34-37 per cent fat, 6-7 per cent crude fiber and 4.15-4.18 per cent ash. It was also found to be abundant in several micronutrients. The study present on phytochemical composition and antimicrobial activity of *Nigella sativa* revealed that the two samples of *Nigella sativa* contained 53-57 mg total polyphenol, 63-64 mg phytic acid, 38-40 mg tannic acid, 77-78 mg/100 g oxalic acid and total alkaloids were less than one per cent. *Nigella sativa* provided 75-78 per cent antioxidant activity. The seeds of *Nigella sativa* had antimicrobial activity against Gram positive bacteria *i.e Staphylococcus aureus* as well as Gram negative bacteria *i.e. Escherichia coli* to the extent of 120.86 sq.mm and 82.79 sq.mm area of zone inhibition respectively. The results studied indicated the presence of various phytochemicals which play an important critical function of seed spice *Nigella sativa* in the management of ageing, cardiovascular disorders, diabetes, cancer and contribution in the prevention of infectious diseases caused by these pathogenic microorganisms.

Introduction

Spices and herbs have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (Beuchat, 1994; Nakatani, 1994; Cutler, 1995). In addition to imparting characteristic flavors, certain spices and herbs prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bactericidal activity (Beuchat and Golden,

1989). Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (Smid and Gorris, 1999). Being natural foodstuffs, spices and herbs appeal to many consumers who question the safety of synthetic food additives. Some spices and herbs used today are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities. The extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications. The antimicrobial activities of plant extracts form the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies (Lis-Balchin and Deans, 1997).

Pharmacologically active seeds of *Nigella sativa* L. (*Ranunculaceae* family), is found in southern Europe, northern Africa and Asia Minor. The seeds are small, black and possess aromatic odor and taste. The seeds commonly known as black seed or black cumin or kalunji have been extensively investigated in recent years and used in folk medicine as a natural remedy for a number of diseases such as asthma, hypertension, diabetes, inflammation, cough, eczema, fever and gastrointestinal disturbances. Seed oil also has antipyretic, analgesic and antineoplastic activity (Ali and Blunden, 2003). Thymoquinone, an active constituent of *Nigella sativa* seeds, is a pharmacologically active quinone, which possesses several pharmacological properties including analgesic and anti-inflammatory actions (Abdel- Fattah *et al*., 2000; Randhawa and Al- Ghamdi, 2002). Thymoquinone (TQ) as an antioxidant, TQ attenuates hypercholestreolemic atherosclerosis and this effect was attributed to decrease in lipids and oxidative stress *i.e*., Molondialdehyde (MDA) and protein carboxyl as biomarkers (Ahmad *et al.*, 2008).

Phytochemical screening of *Nigella sativa* indicated that these spices are also rich in phytonutrients including alkaloid, tannin, carotenoids, saponin and flavonoids. The spices had low concentrations of steroids and cardenolides. The findings indicated that the spices are good sources of nutrients, mineral and phytochemical could be exploited as great potentials for preparations of drugs and/or nutritional supplements (Otunola *et al.*, 2010).

Antioxidant effect of black seed was attributed to be due to its oil- thymoqinone (TQ) and flavonoids. It has been shown that the *Nigella sativa* L. oil and TQ inhibit non-enzymatic lipid peroxidation in liposomes and both of them especially TQ, work as a scavenger of various reactive oxygen species, including superoxide and hydroxyl radicals (Padhye *et al.*, 2008).

Extracts of *N. sativa* have shown promising effects against bacteria, fungi, viruses, parasites and worms. In 1975, the purified compound THQ from Nigella sativa oil was found to have high antimicrobial effect against Gram positive microorganisms (El-Fatatry, 1975). In later studies, seed extracts of N. sativa were found to inhibit the growth of Escherichia coli, Bacillus subtilis and Streptococcus feacalis (Saxena and Vyas, 1986). The antimicrobial activity of N. sativa was further established against several species of pathogenic bacteria and yeast (Topozada *et al.*, 1965; Hanafy and Hatem, 1991). In the latter study, filter paper discs impregnated with the diethyl ether extract of N. sativa seeds caused concentration-

dependent inhibition of Gram-positive Staphylococcus aureus and Gram-negative Pseudomonas aeruginosa and E. coli and a pathogenic yeast Candida albicans. *Nigella sativa* L. is an herbaceous plant, used for centuries for the treatment of various ailments, including infectious diseases.

Materials and Methods

Samples and oil

One seed sample was collected from NRCSS (National Research Center ON Seed Spices) (Ajmer) and another seed sample from local market of Bangalore. The seeds were dried in oven at 40^0C for three hrs, cooled and ground to a fine powder and stored in a glass bottle in ambient condition. For studying antimicrobial activity *N. sativa* oil was procured from local market of Banagalore. As well as oil extracted from *Nigella sativa* was used.

Extraction of oil from *Nigella sativa* seed

Nigella seed powder was extracted with a sufficient volume of 96% ethanol using Soxhlet extraction apparatus. Ethanol was evaporated at 40-50^0C under reduced pressure and the yield of extract was used for antimicrobial activity.

Analysis of macro and micro nutrient of *Nigella sativa* seeds

Moisture, protein, fat, crude fiber, ash, carbohydrate, calcium, phosphorus and sulphur composition was determined by AOAC (1980) methods and energy was computed by differential method. The samples for mineral analysis were subjected to acid digestion and following the procedure described by AOAC (1980). Iron, copper, zinc and manganese were analysed by using AAS (Atomic Absorption Spectrophotometry). Sodium and potassium were analysed by flame photometry

Analysis of phytonutrients from *Nigella sativa* seeds

Total polyphenol was estimated by Bray and Thorpe method (1954), antioxidant activity by DPPH (Diphenyl picrylhdrazyl) method, phytic and tannic acid were determined by AOAC, 1982 and oxalate was determined by Raguramulu *et al.* (2003) method.

Antimicrobial activity

Agar diffusion assay method was followed for testing the antimicrobial activity of *Nigella sativa* oil (Prabha, 1984) of both NRCSS and market sample against *E. coli.* and *S. aureus.*

Results and Discussion

Nutritional composition

Analysis of proximate composition of the two samples of *Nigella sativa* is presented in Table I. Results obtained showed that the moisture content ranged from 3.70 to 4.25 percent, protein 16.31 to 16.52 percent, fat 34.12 to 37.04 percent, crude fiber 6.25 to 7.11 percent, ash 4.15 to 4.18 percent, carbohydrate 30.93 to 35.44 percent and energy 510.7 to 530.5 Kcal. The results obtained from this study showed that *Nigella sativa* seeds contained considerable amount of fat and proteins. Slight difference in proximate nutrients contents were found among the two samples of *Nigella sativa*. It was reported that, the composition and nutritional values vary with the country of origin, stage of maturity, growth, storage conditions and due to the analytical techniques used (Maccane and Widdowson's, 1992).

Minerals composition

Mineral composition of the two samples of *Nigella sativa* seeds studied is shown in Table II. The average results indicated that 100 g of seeds contained 357.9 to 366.5 mg of calcium. From the result, it becomes evident that *N.sativa* seeds provide an abundance of many minerals and could be considered as a good source of calcium as reported by Nergiz and Otles (1993). Results also showed that *Nigella sativa* were found to be rich in iron, phosphorus, sodium and potassium at 10.87 to 11.27, 84.20 to 87.13, 309.0 to 319.3 and 493.0 to 507.3mg respectively. While zinc content was found to be 4.65 to 4.79mg and copper content was 2.61 to 2.83mg, manganese at 1.11 to 1.12 and at 1.11 to 1.16mg sulphur. Data of minerals analysed revealed that seeds are an abundant source of all the major and minor minerals. The high iron content in seed makes it a good source of iron particularly for menstruating and lactating women. Phosphorus, sodium and manganese were lower than the value of Sultan *et al.,* 2009. Whereas potassium content was higher than the reported value of Sultan *et al.,* 2009. Variations in mineral contents may be related to the variations of cultivated regions, storage conditions, maturity stage, geographical and climatic differences where the sample seeds had been grown (Atta, 2003).

Phytochemical composition

Phytochemical composition of the two samples of *Nigella sativa* is presented in Table III. Results obtained showed that the total polyhenol content ranged from 53.01 to 57.29 mg which is similar to reported value of 52.8 to 87.6 mg GAE per 100 g of dry material (Rababa *et al.,* 2011). Antioxidant activity was found to be 74.93 to 77.80 µg of Vit C Eq/g which is close to reported value by Khattak *et al.,* 2008 *i.e.* 79.4, 79.1 and 92.0 per cent for water, acetone and methanol extracts, respectively for *Nigella sativa*. Phytic acid ranged from 63.18 to 63.34 which are lower than the value reported by Yewelsew *et al.,* (2007). Tannic acid was found to be 38.24 to 40.37mg which is lower than the value reported by Rebey *et al.,* 2011 *i.e.* 83.23 and 80.23 mg CE/g DW in a cumin seed. Oxalic acid was found to be in the range of 77.61 to 78.67 mg/100g. Total alkaloids were found to be less than one percent in both the samples studied.

Table.1 Macro-nutrient composition of *Nigella sativa* (per 100 g)

Varieties	Moisture (g)	Protein (g)	Fat (g)	Crude fibre (g)	CHO (g)	Energy (kcal)	Ash (g)
Market sample	4.25	16.52	37.04	7.11	31.93	530.53	4.15
NRCSS(Ajmer) sample	3.70*	16.31	34.12*	6.25*	35.44*	510.77*	4.18
SEm±	0.03	0.14	0.87	0.02	0.37	2.23	0.12
CD at 5% level	0.13		1.44	0.11	1.45	8.78	

*Significant at 5% level NS-Non significant

Table.2 Mineral composition of *Nigella sativa* (per 100 g)

Varieties	Market sample	NRCSS(Ajmer) sample	SEm±	CD at 5% level
Calcium (mg)	357.93	366.56*	3.04	11.96
Iron (mg)	10.87	11.27*	0.15	0.60
Zinc (mg)	4.79	4.65*	0.02	0.11
Copper (mg)	2.83	2.61*	0.01	0.05
Manganese (mg)	1.11	1.12	0.008	-
Phosphorus (mg)	84.20	87.13*	0.67	2.63
Sodium (mg)	319.33	309.00*	1.74	6.68
Potassium (mg)	507.33	493.00*	2.86	11.25
Sulphur (mg)	1.16	1.11*	0.01	0.05

*Significant at 5% level

Table.3 Phytochemical composition of *Nigella sativa* seed (per100g)

Varieties	NRCSS(Ajmer) sample	Market sample	SEm±	CD at 5% level
Total polyphenol(mg)	57.29	53.01*	1.56	3.13
Phytic acid (mg)	63.34	63.18*	0.01	0.06
Tannic acid (mg)	38.24	40.37*	0.12	0.49
Oxalic acid (mg)	78.67	77.61*	0.11	0.46
Antioxidant Activity (µg of Vit C Eq/g)	77.80	74.93*	0.31	1.22
Total alkaloids (%)	<1	<1	-	-

*Significant at 5% level

Table.4 Antimicrobial activity of Nigella sativa oil

Microorganism	Area of zone inhibition (sq.mm)	
	E.coli	Staphylococcous aureus
Market sample	35.55	35.70
NRCSS (Ajmer) sample	82.79	120.86

Antimicrobial activity of *Nigella sativa* oil

Antimicrobial activity of *Nigella* oil was tested against *Escherichia coli* and *Stapylococcus aureus*. Nigella sativa oil extracted from NRCSS (Ajmer) seed sample contained highest antimicrobial activity against both the microorganisms. NRCSS seed oil sample had antimicrobial activity against *Staphylococcus aureus* with an area of zone inhibition at 120.86 sq.mm and 82.79 sq.mm. of area of zone inhibition for *E.coli*. The zone inhibition area was found to be more in NRCSS (Ajmer) seed extracted oil compared to market oil. Which showed desirable effect on *Staphylococcous aureus* compared to *Escherichia coli* (Table IV). It may be that the market oil possibly adultrated and the extraction procedure were different could be the reason. It could have been affected by the variety of seed, soil under which grown, storage length and condition. Tagos *et al.*, 2002 reported that *Nigella sativa* oil was found to be more effective on gram +ve than gram –ve bacteria. A number of compounds derived from plants often showed considerable activity against Gram +ve bacteria but not Gram –ve species.

It can be concluded that there was no noticeable differences in macro and micro nutrients composition in the two samples studied. Results showed that *Nigella sativa* seeds are rich sources of protein, fat, calcium, iron, copper, manganese, sodium and potassium. Evidence indicates that *N. sativa* seeds have a potential medicinal value and are relatively safe to consume. The present study demonstrated that many of the extracts (especially medicinal herbs) contained high levels of antioxidant activity and possessed strong antibacterial activity. They could be a potential source for inhibitory substances against some foodborne pathogens as well as antioxidant agents. Future research should focus on the mechanisms by which *N. sativa* seeds exert their medicinal effects. With the increased understanding of its mechanism of bioactivity, the incorporation of this medicinal herb as complementary medicine into mainstream medical science can be achieved in the future.

The antimicrobial activity of *Nigella sativa* being an important property which plays a critical role in prevention and management of infectious diseases.

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