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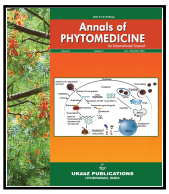

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Nutritional composition and antioxidant profiles of *Nigella sativa* L. seeds

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Abstract

This study was aimed to investigate the nutritional, antinutritional composition of *Nigella sativa* L. (NS) seeds and to analyse the antioxidant profiles of aqueous, methanolic and ethanolic extract of the sample. The nutritional composition of NS seeds was analysed using the AOAC method. Antioxidant content was measured in terms of total phenolic contents (TPC) and total flavonoid contents (TFC). Antioxidant activity was displayed by the DPPH (2,2-diphenyl-1-picrylhydrazy) radical scavenging activity, metal chelating activity, reducing capacity and FRAP (ferric reducing antioxidant power) assay. NS seeds contained 6.48-7.18% moisture, 35.91-38.41% fat, 5.31-5.61% crude fiber, 4.11-5.41% ash, 18.82-20.84% protein, and 29.14-33.66% carbohydrates. Micronutrients were also found to be in substantial amounts; however, antinutritional factors were found within the reported range. 60% methanolic extracts showed highest TPC (29.99 ± 1.16 mg GAE/g) and TFC (0.39 ± 0.02 mg QE/g). Antioxidant activity was also found highest with 60% methanolic extracts. NS being a good source of antioxidants might be recommended against the treatment of various human ailments.

1. Introduction

Plants have always found a major importance in Indian rituals. In India, around 45,000 species of plants are documented. Among them, about 20,000 have some therapeutic potential; however, traditional researchers use only 7,000-7,500 plants for treating human ailments (Sharma and Chakraborty, 2019). Plants are the major sources of natural products and these products are different in their structures, biological properties and mode of action (Rajeshwari and Andallu, 2015; Ansari, 2016).

Nigella sativa (NS) (Family Ranunculaceae) seeds are used all over the world since ancient times for edible purposes such as for seasoning in various food items like bread, pickles, yogurt, salads, sauces and as traditional remedies for treatment of some ailments (Anwar *et al.,* 2020; Sobhi *et al.,* 2016). In the Indian system of medicine, namely: Unani and Ayurveda the seeds are reported to possess immunomodulative, antibacterial, antitumor, diuretic, hypotensive, hepatoprotective, antidiabetic, bronchodilator and estrogenic potential (Ardiana *et al.*, 2020; Hamdan *et al.*, 2019; Ahmad *et al.*, 2014). The oil of NS seeds has been shown to be effective as functional food and used in treating skin conditions, earaches and chronic colds (Yimer *et al.,* 2019). In consideration of possible use, detailed knowledge on the composition of NS seed is of great importance. Like most herbs, the nutritional value of NS seeds vary with the cultivated regions, maturity stages, storage conditions and analytical techniques used for estimation (Giri *et al.*, 2018).

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Scientific investigations have depicted that NS seeds are high in macronutrients as well as micronutrients (Kabir *et al.*, 2019) and are the source of major active compounds like thymoquinone, thymohydroquinone, dithymoquinone, thymol, nigellone, tocopherols, trans-retinol and selenium (Shokri, 2016; Khan *et al.*, 2016). Thymoquinone is the most abundant phytoconstituent, predominantly present in fixed and essential oils of NS seeds and play a significant role as antioxidants (Varghese and Mehrotra, 2020; Salmani *et al.*, 2014).

Anti-nutrients such as phytic acid, oxalic acid, and alkaloids present in spices interfere with the bioavailability of minerals and vitamins (Borquaye *et al.*, 2017). It is therefore, very essential that the antinutritional component of every ingredient in a diet is properly evaluated. Literature data on the nutritional composition of NS seeds cultivated in India is very limited (Giri *et al.*, 2018; Thilakarathne *et al.*, 2018). The present study was conducted to evaluate the nutritional, antinutritional, and antioxidant profiles of selected NS seeds cultivar grown in India namely: Azad Kalaunji-1.

2. Materials and Methods

2.1 Materials

A cultivar of NS seeds namely: Azad Kalaunji-1 was procured from C. S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India. The species of NS (Azad Kalaunji-1) was identified at Department of Botany, University of Allahabad, Prayagraj, and a herbarium specimen was deposited in the department (Voucher No.: 01). The seeds were cleaned by hand to remove dirt, grit and then packed in airtight plastic containers. All the chemicals and reagents used were obtained from HiMedia and Merck, India.

2.2 Proximate analysis

Finely grounded seeds of NS were analysed for their proximate composition, *i.e.*, moisture, fat, protein, total ash and crude fiber using standard AOAC (2005) methods. The total carbohydrate was calculated by the differential method.

2.2.1 Estimation of moisture content

Moisture was estimated by oven drying method. In oven drying method, the sample is heated under specific condition and the loss of weight is used to calculate the moisture content of the sample. Empty dishes and lids (made of porcelain) were dried using hot air oven for one hour at 100 °C and then transferred to the desiccator (with granular silica gel), cooled for 30 min and weighed (W_1). 2 g of samples were transferred to the dried and weighed dishes and reweighted (W_2). The dishes with their contents were placed in the hot air oven and dried for 3 h at 105 °C and then the dishes and their contents were cooled in a desiccator to room temperature and reweighed (W_3). Moisture content was calculated by subtracting the dried weight from the sample weight and was expressed as percentage of moisture present in the sample.

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \text{ X } 100$$

where,

W_1 = Mass of the empty dish

W_2 = Mass of the dish along with the sample before drying

W_3 = Mass of the dish along with the sample after drying

2.2.2 Estimation of protein content

The protein content of sample was determined by Kjeldahl method. Sample (0.5 g) was digested with 5 g of digestion mixture (10 parts potassium sulphate and 1 part copper sulphate) and 20 ml of concentrated sulphuric acid in kjeldahl flask until the contents were carbon free. The digested sample was made upto 100 ml with distilled water. An aliquot of 10 ml was distilled with 10 ml of 30% sodium hydroxide and liberated ammonia was collected in 20 ml of boric acid containing 2-3 drops of mixed indicator (0.1% methyl red and 0.1% bromocresol green of 95% ethyl alcohol separately and mixed in the ratio of 1:5, respectively). The entrapped ammonia was titrated against 0.1N hydrochloric acid. The nitrogen content in the sample was calculated by the following expression:

$$\text{Nitrogen (\%)} = \frac{\text{(Sample titre – Blank titre) X Normality of HCl X 14 X 100}}{\text{Weight of sample X Aliquot taken for distillation X 1000}} \text{ X } 100$$

$$\text{Protein (\%)} = \text{Nitrogen (\%) X } 6.25$$

A conversion factor of 6.25 was used to convert nitrogen into protein content.

2.2.3 Estimation of fat content

Fat content in the sample was estimated by Soxhlet extraction method. Moisture free sample was transferred to thimble which was then fixed into a stand and transferred to a pre-weighed soxhlet beaker. The beaker was filled with petroleum ether. The beaker was then attached to soxhlet apparatus and the sample was extracted for two hours at 80 °C. At 120 °C, the ether was evaporated for 2 hours after extraction. At the end of 4 h, the ether left was dried in hot air oven at 105 °C for 30 min. The beaker was then cooled in desiccator and weighed. Percent crude fat was calculated by following formula:

$$\text{Fat (\%)} = \frac{W_2 - W_1}{W_3} \text{ X } 100$$

where,

W_1 = Weight of empty round bottom flask

W_2 = Weight of flask + extracted crude fat

W_3 = Weight of the sample

2.2.4 Estimation of total ash content

Ash determination was followed by the charring method. Sample (2 g) taken in a silica crucible was ignited on a Bunsen burner till the fumes stop coming and then shifted to muffle furnace until clean ash was obtained. The temperature of furnace was raised to 550 °C. The weight of residue was noted and percent ash was calculated as under following formula:

$$\text{Ash (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \text{ X } 100$$

2.2.5 Estimation of crude fiber content

Moisture and fat free sample (2 g) was transferred to pre-weighed capsules tray and placed in the extraction vessel. The sample was first washed with boiling dilute H_2SO_4 (1.25%, v/v) to remove carbohydrate from the sample. Washing is completed when the colour of acid turns transparent. It was then washed with hot water. The sample was then washed with NaOH (1.25%, w/v) till the washed solution exhibit no colour. It was followed by hot water washing. Finally washing of capsule was done with petroleum ether to remove all organic compounds. It was followed by hot water washing. The capsules were then dried for 1-2 h in hot air oven at 100 °C, cooled to room temperature and weighed. The capsules were then kept in pre-weighed crucibles for ashing in muffle furnace at 550 °C for 2 h, till ashing is complete. Crucibles were cooled slowly in desiccator to attain room temperature. The percent loss in weight was expressed as crude fiber.

$$\text{Crude fiber (\%)} = \frac{W_3 - (W_1 \text{ X } C) - (W_5 - W_4 - D)}{W_2} \text{ X } 100$$

where,

W_1 = Weight of pre-dried empty capsule with lid

W_2 = Weight of the sample

W_3 = Weight of capsule with residue after extraction

W_4 = Weight of pre-dried empty ashing crucible

W_5 = Total weight of crucible including ash

C = Weight of blank capsule after extraction / Weight of blank capsule at start

D = Ash weight obtained from the blank capsule itself

2.2.6 Computation of carbohydrate content

Carbohydrate content was calculated by subtracting the total of moisture, fat, protein, and ash from 100.

2.3 Mineral analysis

For preparing ash solution, 1g of sample was digested in the acid mixture (10 ml of nitric acid and 3 ml of 60% perchloric acid) at 250 °C for 2-3 h in kjeldahl digestion unit. After that digested sample was cooled to room temperature and then the solution was transferred to 100 ml volumetric flask and 20 ml of 1:1 HCl was added and volume made up with deionized water. Finally, it was filtered through Whatman filter paper (grade 42, 2.5 µm and 125 mm diameter) and filtrates were used for mineral analysis. Ash solutions were prepared in triplicates. Magnesium, iron, zinc, chromium, copper, manganese were determined by using atomic absorption spectrophotometry (AAS) (AOAC, 2010). Calcium and phosphorus were analysed by using titration and colorimetric methods, respectively (Ranganna, 2005). All the estimations were carried out in triplicates.

2.4 Antinutritional analysis

2.4.1 Estimation of phytic acid

The phytic acid content was estimated by method of Hassan *et al.* (2011) with slight modification. Accordingly, 4 g sample was soaked in 100 ml of 2% HCl for 3 h. Then, the sample solution was filtered. 25 ml of filtrate was taken in a conical flask, 5 ml of 0.3% aqueous NH_4SCN and 50 ml of distilled water was added and mixed. The solution was titrated against standard aqueous $FeCl_3$ solution containing 0.00195 g Fe/ml until a brownish yellow colour appears and persisted for 5 min. Similarly, the blank was titrated. phytin-phosphorus (1 ml Fe = 1.19 mg phytin-phosphorus) was determined and the phytate content was calculated by multiplying the value of phytin-phosphorus by 3.55.

2.4.2 Estimation of oxalic acid

Oxalic acid was estimated by titration method described by Adeniyi *et al.* (2009). 2 g sample was digested at 80 °C with 10 ml of 6 M HCl for 1 h. Then centrifuged and filtered. Filtrate was collected in a 250 ml volumetric flask and made up the volume up to the mark. The pH of the filtrate was adjusted with concentrated NH_4OH until the colour of the solution changed from salmon pink to faint yellow colour. Treated the filtrate with 10 ml of 5% $CaCl_2$. Centrifuged the above mixture and supernatant was decanted. The precipitate was completely dissolved in 10 ml of 20% H_2SO_4 and made up the volume to 250 ml. An aliquot of 80 ml of the filtrate was heated near to boiling point. Then titrated against 0.05 M of standard $KMnO_4$ solution to a faint pink colour which persisted for about 30 sec. Oxalic acid was obtained from the following formula:

$$\text{Oxalic acid (mg/100 g)} = \frac{\text{Titre value X M of } KMnO_4 \text{ X Volume made up X Molecular weight of oxalate}}{\text{Aliquot taken for titration X Weight of sample X 1000}} \text{ X } 100$$

2.4.3 Estimation of total alkaloids

5 g sample was dispersed in 10 % acetic acid solution in ethanol to form 1:10 w/v dispersion. The sample was stirred for every 30 min for 4 h. The mixture was filtered using Whatman filter paper and filtrate was collected in conical flask. The filtrate was concentrated by evaporation on a water bath until get reduced to one fourth of the original volume. Concentrated ammonia solution was added slowly in drops to make it alkaline and the alkaloid in the filtrate get precipitated. A pre-weighed Whatman filter paper was used to filter. The filter paper along with the precipitate was dried in the oven at 60 °C, cooled in a desiccator and weighed. The difference between the mass of filter paper plus the precipitate and the mass of filter paper along gave the mass of alkaloid (AOAC, 2005).

$$\text{Total alkaloids (\%)} = \frac{\text{Mass of alkaloid}}{\text{Mass of sample}} \text{ X } 100$$

2.5 Sample extraction for antioxidant analysis

NS seeds were washed with tap water to remove soil and dirt and milled into coarse particles about 2 mm in diameter. Then 10 g of coarse powder was extracted with 100 ml of solvent. The solution was stirred using an orbital shaker at 120 rpm for 24 h at room temperature. The extracts were then filtered, transferred into a flask and dried using a rotatory evaporator at 40 °C. Extracts were stored at 4 °C to avoid compound degradation before chemical analysis and use in experiments. The extraction process was carried out in triplicate. The solvents were methanol and ethanol at different concentrations (20%, 60%, 80%, and 100%) in distilled water and aqueous (100% millipore water).

2.6 Estimation of total phenolic content (TPC)

TPC was estimated by Folin-Ciocalteu method as previously described by Li *et al.* (2007). 200 µl of diluted sample was added to 1 ml of diluted Folin–Ciocalteu reagent (1:10 with distilled water). After 4 min, 800 µl of saturated sodium carbonate (7.5 %, w/v) was added and incubated for 2 h at room temperature and measured at 765 nm. Final results were expressed as mg gallic acid equivalents (GAE) per g of sample.

2.7 Estimation of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined by aluminium chloride colorimetric method as previously described by Bahorun *et al.* (1996) with slight modification. As per the method, 2 ml of 2% $AlCl_3$ in methanol was mixed with the 2 ml of extract and allowed to stand for 5 min at room temperature. After that absorption was measured at 430 nm against a blank sample. Quercetin was used for the standard calibration curve and the data were expressed in mg quercetin equivalent (QE) per g of sample.

2.8 Estimation of free radical scavenging activity

Free radical scavenging activity was measured in terms of % DPPH (2,2-diphenyl-1-picrylhydrazy) using the method as given by Mansouri *et al.* (2005). As per method, 50 µl of extract was added to 1950 µl of DPPH solution and then mixture was shaken and incubated for 30 min at room temperature, absorbance was read at 517 nm. The results were calculated by following equation:

$$\text{\% Scavenging activity} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \text{ X } 100$$

2.9 Estimation of metal chelating activity

Metal chelating activity was measured following the method of Le *et al.* (2007) with slight modification. Briefly, 1 ml of extract was mixed with 200 µl of 0.6 mM ferrous chloride and 1800 µl of methanol and incubated for 5 min at room temperature. 200 µl of 5 mM ferrozine was added to the mixture and allowed to stand for 10 min at room temperature. The absorbance was read at 562 nm. The percentage chelating activity was calculated by the following equation:

$$\% \text{ Chelating activity} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

2.10 Estimation of ferric reducing antioxidant power (FRAP) assay

Benzie and Strain (1996) method was used to estimate antioxidant capacity. Briefly, 3 ml of FRAP reagent was mixed with 100 µl of sample and absorbance was read at 593 nm. FRAP values were expressed as mM Fe^{2+} equivalents per g of sample.

2.11 Estimation of reducing power activity

Reducing power activity was determined by following the method of Barros *et al.* (2007). Briefly, reaction mixture contained 2.5 ml of extract, 2.5 ml of phosphate buffer (pH 6.6), 2.5 ml of 1% potassium ferricyanide and mixture was allowed to stand for 20 min at 50 °C then 2.5 ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 1000 rpm for 8 min. 5 ml of supernatant was transferred to 10 ml of volumetric flask and volume made up with deionized water and then 1 ml of 0.1% ferric chloride was added and read at 700 nm. Ascorbic acid was used as standard.

2.12 Statistical analysis

All the determinations were carried out in triplicates and results were presented as mean along with their standard deviation (Mean ± SD). Statistical package of Social Sciences (SPSS), a software programme for windows (version 16.0), was used for conducting the one-way analysis of variance (ANOVA) and Duncan's procedure was used to compare means and for determining significant difference at 5% significance level ($p<0.05$).

3. Results

3.1 Proximate composition

The results of the proximate composition of the NS seeds sample are presented in Figure 1. The NS seeds contained 6.48-7.18% moisture, 35.91-38.41% fat, 5.31-5.61% crude fiber, 4.11-5.41% ash, 18.82-20.84% protein, and 29.14-33.66% carbohydrates.

3.2 Mineral composition

Mineral analysis of the NS seeds sample is presented in Table 1. In this study, the sample contained 91.25 ± 0.3, 64.25 ± 0.22, 55.37 ± 0.1, 9.5 ± 0.02, 6.6 ± 0.03 and 2.68 ± 0.06, 610.67 ± 11.06 and 425 ± 10.19 mg per 100 g of magnesium, iron, chromium, copper, zinc, manganese, calcium and phosphorus, respectively.

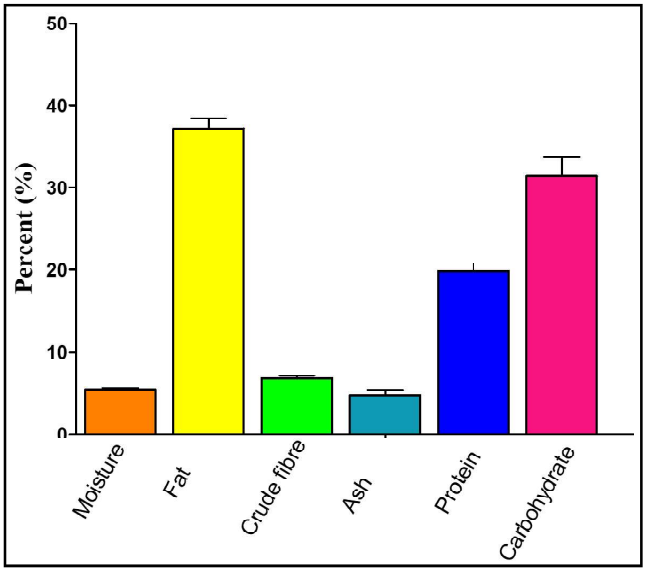

Figure 1: Proximate composition of NS seeds.

Table 1: Mineral content of NS seeds

Mineral contents	mg/100 g
Magnesium	91.25 ± 0.3
Iron	64.25 ± 0.22
Zinc	6.6 ± 0.03
Chromium	55.37 ± 0.1
Copper	9.5 ± 0.02
Manganese	2.68 ± 0.06
Calcium	610.67 ± 11.06
Phosphorus	425 ± 10.19

The values depicted are Mean ± SD where n=3.

3.3 Anti-nutritional content in NS seeds

Table 2 shows the antinutritional contents present in NS seeds. In this study, phytic acid and oxalic acid content of NS seeds ranged from 65.21 to 65.45 and 76.51 to 76.81 mg/100 g, respectively. The alkaloid content in NS seeds was found to be less than 1% (Table 2).

Table 2: Antinutritional composition of NS seeds

Antinutritional contents	Per 100 g
Phytic acid (mg)	65.33 ± 0.12
Oxalic acid (mg)	76.66 ± 0.15
Total alkaloids (%)	< 1

The values depicted are Mean ± SD where n=3.

3.4 Antioxidant content in NS seed extracts

3.4.1 Total phenolic content (TPC)

Table 3 shows the total phenolic content of NS seeds in different solvents (aqueous, ethanolic and methanolic) at different concentrations (20%, 60%, 80%, and 100%). The TPC of NS seeds extract is ranging from 7.31 ± 0.80 mg GAE/g to 29.99 ± 1.16 mg GAE/g. The highest total phenolic content (TPC) was shown in 60% methanolic extract followed by 80% methanolic, 60% ethanolic, 100% methanolic, aqueous, and 80% ethanolic extract. 20% ethanolic extract showed the lowest TPC value.

3.4.2 Total flavonoid content (TFC)

To estimate the possible effect of flavonoids on the antioxidant activity of NS seeds, the TFC of the extract was analysed, the results are depicted in Table 3. The range of TFC in extracts is from 0.05 ± 0.00 mg QE/g to 0.39 ± 0.05 mg QE/g. 60% methanolic extract showed the highest total flavonoid content (TFC) followed by 80% methanolic, 60% ethanolic, 100% methanolic extract. 100% ethanolic extract showed the lowest TFC value.

Table 3: Total polyphenol and total flavonoids content of NS seed extracts in different solvents

Solvents	TPC (mg GAE/g)	TFC (mg QE/g)
100% Aqueous	15.95 ± 1.09^{c}	0.17 ± 0.01^{b}
20% ethanol (20:80 v/v ethanol-water)	7.31 ± 1.08^{a}	0.17 ± 0.01^{b}
60% ethanol (60:40 v/v ethanol-water)	19.36 ± 1.46^{d}	0.23 ± 0.03^{cd}
80% ethanol (80:20 v/v ethanol-water)	15.26 ± 1.35^{c}	0.19 ± 0.01^{b}
100% ethanol (100:00 v/v ethanol-water)	11.52 ± 1.27^{b}	0.05 ± 0.02^{a}
20% methanol (20:80 v/v methanol-water)	13.18 ± 1.08^{b}	0.18 ± 0.02^{b}
60% methanol (60:40 v/v methanol-water)	29.99 ± 1.16^{e}	0.39 ± 0.02^{e}
80% methanol (80:20 v/v methanol-water)	20.12 ± 1.13^{d}	0.25 ± 0.01^{d}
100% methanol (100:00 v/v methanol-water)	16.92 ± 1.09^{c}	0.2 ± 0.02^{bc}

TPC, total polyphenol content; TFC, total flavonoids content; GAE, gallic acid equivalent; QE, quercetin equivalent. The results are given as Means ± SD of three measurements. Means within a column with the same superscript alphabet (a, b, c, d, e) are not significantly different (p<0.05).

3.5 Antioxidant activity of NS seed extracts

3.5.1 DPPH free radical scavenging activity

The radical scavenging activity, using a DPPH generated radical, is presented in Table 4. It was observed that 60% methanolic extract exhibited the highest radical scavenging activity (86.16 ± 1.76%), followed by 60% ethanolic (82.04 ± 1.55%), 80% methanolic (81.96 ± 1.78%), 100% methanolic (77.30 ± 1.95%), 80% ethanolic (77.22 ± 1.46%), aqueous (73.49 ± 1.38%), and 20% methanolic (70.57 ± 1.11) extract. 20% ethanolic extract showed the weakest activity (65.35 ± 1.75%).

3.5.2 Metal chelating activity

Metal chelating activity of the different extracts showed a wide variation ranging from 27.74 ± 1.49% to 88.42 ± 1.17% (Table 4). 60% methanolic extract and 60% ethanolic extract appear to have the most important chelating activity with 88.42 ± 1.17% and 85.34 ± 1.32%, respectively, followed by 80% methanolic (80.69 ± 1.42%), 20% methanolic (74.25 ± 1.26), 20% ethanolic (66.66 ± 2.24), and aqueous (51.06 ± 1.81) extract. Chelating effect of 100% methanolic extract (27.74 ± 1.49%) and 100% ethanolic extract (31.54 ± 1.70%) were found to be very low.

3.5.3 Ferric reducing antioxidant power (FRAP) assay

In the present study, large differences in FRAP values were observed among different solvents ranging from 7.56 ± 0.83 mM Fe^{2+} E/g to 22.88 ± 0.97 mM Fe^{2+} E/g (Table 4). 60% methanolic extract (22.88 ± 0.97 mM Fe^{2+} E/g) demonstrated the greatest ferric reducing power followed by 60% ethanolic extract (17.0 ± 0.49 mM Fe^{2+} E/g) (Table 4). Aqueous (8.73 ± 0.76 mM Fe^{2+} E/g), 20% ethanolic (8.7 ± 0.57 mM Fe^{2+} E/g), and 20% methanolic (7.56 ± 0.83 mM Fe^{2+} E/g) extract showed weak ferric reducing antioxidant power.

3.5.4 Reducing power activity

The reducing power of NS seeds in different solvents is shown in Table 4. In this assay, we tested the capacity of NS seed extracts to reduce Fe^{3+} to Fe^{2+}, which ranges from 17.75 ± 0.63 µM AAE/g to 43.46 ± 1.17 µM AAE/g. Among all the extracts, 60% methanolic extract showed the highest reducing power (43.46 ± 1.17 µM AAE/g) followed by 60% ethanolic (37.07 ± 0.89 µM AAE/g), 80% methanolic (36.47 ± 1.22 µM AAE/g), 100% methanolic (27.74 ± 0.49 µM AAE/g), 20% ethanolic (25.76 ± 0.82 µM AAE/g), 80% ethanolic (25.75 ± 1.11 µM AAE/g), and aqueous extract (22.08 ± 1.49 µM AAE/g). 100% ethanolic extract exhibited the least (17.75 ± 0.63 µM AAE/g) reducing power.

Table 4: Antioxidant activity of NS seed extracts

Solvents	DPPH free radical scavenging activity (%)	Metal chelating activity (%)	FRAP (mM Fe^{2+} E/g)	Reducing capacity (µM AAE/g)
100% Aqueous	73.49 ± 1.38^{c}	51.06 ± 1.81^{d}	8.73 ± 0.76^{ab}	22.08 ± 1.49^{b}
20% ethanol (20:80 v/v ethanol-water)	65.35 ± 1.75^{a}	66.66 ± 2.24^{e}	8.7 ± 0.57^{ab}	25.76 ± 0.82^{c}
60% ethanol (60:40 v/v ethanol-water)	82.04 ± 1.55^{e}	85.34 ± 1.32^{h}	17.1 ± 0.49^{e}	37.07 ± 0.89^{e}
80% ethanol (80:20 v/v ethanol-water)	77.22 ± 1.46^{d}	41.2 ± 2.87^{c}	13.46 ± 0.82^{d}	25.75 ± 1.11^{c}
100% ethanol (100:00 v/v ethanol-water)	69.38 ± 1.96^{b}	31.54 ± 1.7^{b}	11.15 ± 0.88^{c}	17.75 ± 0.63^{a}
20% methanol (20:80 v/v methanol-water)	70.57 ± 1.11^{b}	74.25 ± 1.26^{f}	7.56 ± 0.83^{a}	21.4 ± 0.85^{b}
60% methanol(60:40 v/v methanol-water)	86.16 ± 1.76^{f}	88.42 ± 1.17^{i}	22.88 ± 0.97^{f}	43.46 ± 1.17^{f}
80% methanol (80:20 v/v methanol-water)	81.96 ± 1.78^{e}	80.69 ± 1.42^{g}	14.52 ± 0.94^{d}	36.47 ± 1.22^{e}
100% methanol (100:00 v/v methanol-water)	77.3 ± 1.95^{d}	27.74 ± 1.49^{a}	9.49 ± 0.25^{b}	27.74 ± 0.49^{d}

FRAP, ferric reducing antioxidant power; AAE, ascorbic acid equivalent. The results are given as Means ± SD of three measurements. Means within a column with the same superscript alphabet (a, b, c, d, e, f, g, h, i) are not significantly different (p<0.05).

3.6 Correlation between total polyphenol content, total flavonoid content and antioxidant activities of NS seeds

Table 5 shows the correlation between TPC, TFC, and antioxidant activities. In the present study, we found a highly significant correlation (r=0.936, $p<0.001$) between TPC and DPPH radical scavenging activity. However, the lowest correlation (r=0.483) was found between TPC and metal chelating activity. Our results showed TFC was significantly correlated with DPPH radical scavenging activity (r=0.798, $p<0.001$), metal chelating activity (r=0.679, $p<0.05$), ferric reducing antioxidant power (r=0.765, $p<0.05$) and reducing capacity (r=0.907, $p<0.001$).

Table 5: Correlation between total phenolic content, total flavonoid content and antioxidant activities

	Total Phenolic Content	Total Flavonoid Content
DPPH Radical Scavenging Activity	0.936**	0.798**
Metal Chelating Activity	0.483	0.679*
Ferric Reducing Antioxidant Power	0.860**	0.765*
Reducing Capacity	0.835**	0.907**

** Significant at $p<0.001$.
* Significant at $p<0.05$.

4. Discussion

In the present study, the sample was found to contain the highest fat content which is supported by the study of Al-Naqeep *et al.* (2009). Oil bearing seeds are reported to be high in calcium and phosphorus (Ozcan, 2006). Micronutrients were also found to be in substantial amounts. Minerals play an important role in the human body. Magnesium is a cofactor of several enzymes in carbohydrate metabolism and iron plays an important role in the metabolism of all living cells (Swaminathan *et al.*, 2007).

In this study, antinutritional factors were found within the reported range. Phytic acid is a common storage form of phosphorus in seeds and is also considered as an antinutritional factor. The phytic acid content of NS seeds ranged from 65.21 to 65.45 mg/100 g which are lower than the value reported by Abebe *et al.* (2007). Many of the salts of phytic acid have low solubility and phytate has been implicated as a deterrent to the absorption of metals, principally of calcium and iron (El Anany, 2015). Oxalic acid as an antinutritional factor interferes with minerals availability particularly calcium. It reacts with calcium and forms insoluble calcium oxalate which leads to the formation of kidney stones (Hassan *et al.*, 2011). Hence, the estimation of oxalic acid becomes essential in food samples. Oxalic acid content in the sample ranged from 76.51 to 76.81 mg/100 g. Alkaloids are not stringently regarded as antinutrient but are rather known for their pharmacological potential instead of toxicity. However, when alkaloids present in high concentration in foods, it causes gastrointestinal disorder and neurotoxicity (Hussain *et al.*, 2018). The alkaloid content in NS seeds was found to be less than 1%.

The antioxidant contents of NS seeds in different solvents (aqueous, ethanolic, and methanolic) at different concentrations (20%, 60%, 80%, and 100%) were analysed. Phenolic compounds are one of the most effective natural antioxidants. In addition to antioxidant activity, phenolic compounds scavenge free radicals, induce apoptosis, reduce leukocyte immobilization, and inhibit cell proliferation (Arts and Hollaman, 2005). Flavonoid also plays a protective role against cancer, cardiovascular diseases and kidney diseases (Shoba *et al.*, 2010). *In vitro*, flavonoid has shown free radical scavenging activity and protection against reactive oxygen species (Gu and Weng, 2001).

Elevated DPPH radical scavenging ability of the NS seeds might be due to the presence of high amount of TPC and TFC. It has been well recognized that transition metal ions such as iron and copper are important catalysts for the initiation of radical chain reaction or radical mediated lipid peroxidation (Zhao *et al.*, 2008). Chelating agents stabilize transition metals by inhibiting free radical generation. To better estimate the potential antioxidant activities of the sample, chelating activity was evaluated against Fe^{2+}. Metal chelating activity of the different extracts showed a wide variation ranging from 27.74 ± 1.49% to 88.42 ± 1.17%. Our results are in accordance with the study of Meziti *et al.* (2012). They also found NS seed extracts showing appreciable chelating activity which may be due to the occurrence of phenolic components present in the NS seeds. The FRAP assay developed by Benzie and Strain (1996) was used to estimate the antioxidant potential of NS seeds extracts which has been used by Hajimahmoodi *et al.* (2008). NS seed extracts exerted remarkable FRAP value and reducing power activity. Many studies reported that reducing power was mainly associated with the antioxidant potential of the phenolic compounds (Yen *et al.*, 2000). A significant correlation was found between TPC, TFC, and antioxidant activities. This means phenolic and flavonoid contents present in NS seeds have antioxidant activity.

5. Conclusion

The results obtained from the present study reveal that NS seeds grown in India are valuable sources of nutrients due to high carbohydrate, fat, and protein content with substantial quantity of macro and micro minerals. From the findings of *in vitro* antioxidant study, it was concluded that 60% methanolic extract showed the best solvent for the extraction of TPC, TFC, and antioxidant activity of NS seeds. The correlation results suggested that the antioxidant activities of NS seeds are may be due to the presence of polyphenol and flavonoid contents. However, future studies are needed to identify the polyphenolic and flavonoid contents that are correlated with the antioxidant activity of NS seeds. The findings of the present study would provide a new approach for further research to evaluate the mechanisms which attribute the bioactive potential of NS seeds.

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Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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