

Effect of Boiling on the Nutritional Composition and Antioxidant Properties of Beniseed (*Sesamum indicum L.*)

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Abstract

Beniseed has been considered to be a valuable oilseed, not only because of its high oil content, but also because of its medical effects. The present study sought to investigate the effect of boiling on the proximate and mineral composition of beniseed. In addition, the effect of boiling on the antioxidant properties of beniseed extract was evaluated. Proximate and mineral composition of raw beniseed and beniseed boiled for 5, 10, 20 and 30 min were assessed. The amount of phenols, flavonoids and vitamin C were determined in the aqueous extracts of raw and boiled beniseed. In addition, the antioxidant mechanisms of the extracts of raw and boiled beniseed were assessed by measuring their reducing property, iron (II) chelating ability and their ability to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Results showed that boiling significantly ($p < 0.05$) improved the crude fat (49.23 to 56.78%) and calcium content (757.13 to 975.54 mg/100g). However, boiling caused a significant ($p < 0.05$) reduction in levels of protein (18.87 to 14.12%), fiber (6.17 to 4.45%) and potassium (831.47 to 727.42 mg/100g) while iron levels were unchanged. The total phenolics levels of the raw beniseed (0.15 mg/g) showed a remarkable increase as the boiling time was increased to 30 min with a level of 0.35 mg/g. In addition, boiling caused a significant ($p < 0.05$) increase in the total flavonoid levels from 0.22 mg/g to 0.55 mg/g while a decrease in the vitamin C content of raw beniseed was observed within the period of boiling. Furthermore, the aqueous extracts of boiled beniseed exhibited greater antioxidant properties than that of the raw seeds. It was concluded that boiling for 30 min caused a significant loss of some nutrients but potentiated the antioxidant activities of the aqueous extract of the seed.

Keywords: Antioxidant, beniseed, boiling, radicals, nutritional, oilseed

1. Introduction

There is considerable interest in the potential health benefits of oilseeds, such as beniseed, soybean seed and flaxseed especially regarding cardiovascular disease and cancer. This interest in oilseeds relates to their high content of polyunsaturated fatty acids, vegetable protein, soluble fibre, flavonoids and related compounds which may possess cholesterol lowering and antioxidant activities (Jenkins *et al.*, 1999).

Beniseed also known as sesame (*Sesamum indicum L.*) is a rich source of protein and minerals such as calcium and phosphorus (Salunkhe *et al.*, 1991). Beniseed is found in tropical, subtropical and southern temperate areas of the world, particularly in India, China, South America and Africa. It has utmost economic importance and is primarily grown by small farmers in developing countries. It has been cultivated for centuries particularly in Africa and Asia, due to its high content in edible oil and protein.

Beniseed is considered not only to have nutritional value but also medicinal properties. The seed has been long regarded in the orient as a health food for increasing energy and prevention of aging (Hajimahmoodi *et al.*, 2008). The seeds are used as a demulcent in respiratory infections, infantile cholera, diarrhoea, dysentery, bowel infections and bladder diseases. The seed powder is useful in amenorrhoea, dysmenorrhoea, ulcers and bleeding piles (Visavadiya and Narasimhacharya, 2008). The seed and oil are being utilized as important food ingredients since about 6, 000 years ago. The Thebes Medicinal Papyrus (1552 B.C.), an old text found in Egypt, describes the medicinal effect of beniseed as a source of energy. In traditional Indian medicine, beniseed oil has been used as the basal oil for human body massage since 1100 B.C. (Weiss, 1983; Joshi, 1961). The Japanese traditionally believe that beniseed is very good for health (Namiki, 1990; Namiki, 2007).

The use of natural antioxidants in foods such as flavonoids, tannins, coumarins, phenolics and terpenoids is arousing special attention because of the world wide trend to avoid or minimize the use of synthetic food additives (Mohamed and Awatif, 1998). Some components from fruits, vegetables and seeds extracts have shown strong antioxidant effect in model systems (Salunkhe *et al.*, 1992; Hochstein and Atallah, 1998; Sadeghi *et al.*, 2009). Phenolic compounds are widely distributed in the plant. These compounds are known as important antioxidants because of their ability to donate hydrogen atom or an electron in order to form stable radical intermediate. They

prevent the oxidation of various biological molecules. In fact, several oilseeds and their byproducts have been investigated for phenolic compounds for safe sources of natural antioxidants (Namiki, 1995; Jeong *et al.*, 2004).

Beniseed contains a lot of nutrients and one of the pressing problems in developing countries such as Nigeria is how to prevent great losses of nutrients. Preservation of nutrients is important in the processing of beniseed. Little research has been carried out on the effects of boiling on the nutritional composition and antioxidant potential of beniseed.

Several studies have been carried out to determine the antioxidant potential of beniseed. These studies have clearly shown that the seeds are rich sources of antioxidants which have the capacity to scavenge free radicals when consumed in diet (Namiki, 2007). Generally, boiling is commonly used in folkloric medicine for the extraction of plants phytochemicals without any knowledge of their effect on the bioactive constituents of these plants. Hence, it is important to investigate the effect of boiling on the antioxidant properties of beniseed. In Nigeria and other parts of the world where beniseed is consumed, different processing methods are used for beniseed which include roasting, toasting and boiling. Beniseed is a cherished soup condiment in some northern states in Nigeria and some parts of Cross-river state (Agiang *et al.*, 2010). The soup is eaten with carbohydrate foods such as pounded yam, garri and other flours made into foofoo. The preparation of beniseed soup involves boiling. Boiling is one of the domestic food processing methods that involve using heat to prepare food for consumption. Soup making involves the application of moist heat method which employs relatively low temperatures (Fox and Cameron, 1984). Boiling, apart from making food palatable and safe practically inactivates all the antinutritional factors that are heat labile and improves digestibility and may result in the reduction of available nutrients (Umoh and Bassir, 1981).

Previous work carried out on beniseed showed that fermentation attenuates the free radical scavenging and antioxidant activities of beniseed (Ibukun, 2012). Some studies have also been undertaken to evaluate the effects of seed roasting conditions on the antioxidant activity which revealed that roasting improved the antioxidant activity of beniseed meal extract at 200°C for 60 minutes (Jeong *et al.*, 2004). This study therefore focuses on discovering the effect of boiling on the nutrients and antioxidant activities of beniseed and also seeks to unravel the chemical changes that take place during boiling of the seeds. Studying the influence of boiling on the nutrients and antioxidant activities of the seeds can contribute to the information on how to derive maximum health benefits from consumption of beniseed.

2. Materials and Methods

2.1 Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH), 1-10 phenanthroline, trichloroacetic acid (TCA), potassium ferrocyanide, ferric chloride (FeCl₃), ferrous sulfate (FeSO₄), thiobabitoric acid (TBA), Deoxyribose, Tris-HCl, sodium dodecyl sulfate (SDS) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and were obtained from standard chemical suppliers.

2.2 Seed Collection and Identification

White beniseeds (*Sesamum indicum L.*) were purchased from a local market in Akure, Ondo State, Nigeria and were identified at the Department of Crop Soil and Pest Management of the Federal University of Technology, Akure, Nigeria. The seeds were separated from undesirable materials such as stones, sand and plant parts. The seeds were thereafter washed, sun-dried and grinded into coarse powder by an electric blender and kept in clean plastic cans until use.

2.3 Preparation of Boiled Seed Samples

Powdered seeds were divided into five parts weighing 20 g each. The first part represents the raw sample for proximate and mineral analysis determination. The other four parts were placed into beakers containing 400 ml of distilled water, sealed with an aluminum foil (closed system) and boiled for 5, 10, 20 and 30 min respectively at boiling temperature (100°C). The boiled samples were dried in a hot air circulating oven at 70°C for 12 h and were stored for analysis. The four parts represent the boiled sample for proximate and mineral analysis determination.

2.4 Preparation Seed Extracts

Powdered seeds were divided into five parts each weighing 20g. The first part was placed into a beaker containing 400 ml of distilled water and left for 24 h to allow for extraction. Thereafter, the sample was decanted and filtered.

The filtrate was then kept in the refrigerator and used as stock of raw extract of sample for all antioxidant determinations. The other four parts were poured into four beakers containing 400 ml of distilled water, sealed with an aluminum foil (closed system) and heated for 5, 10, 20 and 30 min respectively at boiling temperature (100°C). These were allowed to cool, decanted and extract was filtered using a Whatman filter paper. The filtrates were stored in the refrigerator and used as stock of boiled samples for all antioxidant determinations.

2.6 Determination of Nutritional Composition

Moisture content, crude protein, crude fat, ash content and crude fibre of raw beniseed and boiled beniseed samples were determined according to AOAC (1995). Carbohydrate content was calculated by difference. Mineral analysis was also done according to AOAC (1995).

2.7 Quantitative Antioxidant assay

2.7.1 Total Phenolic Content Determination

The total phenol contents of the extracts were determined as described by Singleton *et al.* (1999). 0.2 ml of the extracts was mixed with 2.5 ml of 10% Folin-Ciocalteu's reagent and 2 ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45°C for 40 min, and the absorbance was measured at 765 nm in the spectrophotometer. The amount of phenols in the seed extract was expressed as gallic acid equivalent (GAE).

2.7.2 Determination of Total Flavonoid

The total flavonoid content of the extracts was determined using a colorimeter assay as described by Bao *et al.* (2005). 0.2 ml of the extracts was added to 0.3 ml of 5% NaNO₃ at zero time. After 5 min, 0.6 ml of 10% AlCl₃ was added and after 6 min, 2 ml of NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the blank reagent. The amount of flavonoids in seed extract was expressed as rutin equivalent (RE).

2.7.3 Determination of Vitamin C

The vitamin C content was determined colorimetrically as described by Benderitter *et al.* (1998). 200 µl of the extracts were pipette and mixed with 300 µl of 13.3% of TCA and 75 µl of DNPH. The mixture was incubated at 37°C for 3 h and 500 µl of H₂SO₄ was added and the absorbance was read at 520 nm. The content of ascorbic acid is related per gram of dried sample.

2.8 Qualitative Antioxidant assay

2.8.1 DPPH free radical scavenging activity

The free radical scavenging abilities of the extracts against DPPH (1, 1-diphenyl-2-picrylhydrazyl) were evaluated according to the method of Gyamfi *et al.* (1999). 1 ml of the extracts was mixed with 1 ml of the 0.4mM methanolic solution of the DPPH radicals. The mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

$$\text{DPPH radical scavenging activity} = \left(A_0 - \frac{A_1 - A_s}{A_0} \right) * 100$$

Where A₀ = Absorbance of the control solution containing only DPPH

A₁ = absorbance in the presence of extract in DPPH solution and

A_s = the absorbance of the sample extract solution without DPPH

2.8.2 Ferric Reducing Property

The reducing property of the extracts was determined as described by Pulido *et al.* (2000). 0.25 ml of the extracts was mixed with 0.25 ml of 200 mM Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% Potassium ferrocyanide. The mixture was incubated at 50°C for 20 min, thereafter 0.25 ml of 10% trichloroacetic acid was added and centrifuge at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power.

2.8.3 Fe^{2+} Chelating assay

The ability of the extracts to chelate Fe^{2+} was determined using a modified method described by Puntel *et al.* (2005). Briefly, 150 mM $FeSO_4$ was added to a reaction mixture containing 168 μ l of 0.1M Tris-HCl pH 7.4, 218 ml saline and extract and the volume was made up to 1 ml with distilled water. The reaction mixture was incubated for 5 min, before the addition of 13 μ l of 1, 10-phenantroline. The absorbance was read at 510nm. The Fe (II) chelating ability was subsequently calculated with respect to the reference which contains all the reagents without seed extract.

2.9 Statistical Analysis

The results were expressed as mean \pm SEM for three to four independent experiments performed in triplicate and were analyzed by appropriate analysis of variance, followed by Duncan's multiple-range test. Differences between groups were considered significant when $p < 0.05$.

3. Results and Discussion

3.1 Proximate Composition

The proximate analysis of raw and boiled beniseed as presented in Table 1 revealed an increase in moisture content, fat content, carbohydrate content and energy values while there were decreases in the protein, fibre and ash contents with increase in boiling time. The slight differences in proximate analysis of raw beniseed in comparison with proximate compositions reported by Joshi (1961), Smith (1971) and Weiss (1983) could be due to the differences in growing conditions and soil types. The comparison of raw and boiled beniseed revealed that boiling significantly increased the crude fat (Table 1). The result shows that the mean crude fat content of the raw beniseed (49.23%) is significantly different at ($p < 0.05$) from that of boiled beniseed for 30 min (56.78%). The higher level of fat content in boiled beniseed could be attributed to the disruption of the cell structures and membrane partitions of the seeds by heat during boiling causing the fat to melt and be easily released from the seeds. Fat is important in diets because it promotes fat soluble vitamin absorption. It is a high energy nutrient and does not add to the bulk of the diet (Bogert *et al.*, 1994). These values are in agreement with the values of 41.3-56.8% reported by Oresanya and Koleoso (1990), Achinewhu (1998), Biwas *et al.* (2001) and Kanu (2011) for raw beniseed. The result also showed that the high fat contents of raw and boiled beniseed are comparable with that of other commercial oil seeds (Ojiako *et al.*, 2010). Therefore, commercial extraction of oil from beniseed can be said to be economically viable.

The moisture content of the raw and boiled beniseed ranged from 9.67-10.34% (Table 1). Boiling did not have a significant effect on the moisture content of the beniseed when compared to that of the raw sample. This is however different from the 4.1% moisture of dry beniseed reported by Oresanya and Koleoso (1990).

The crude fibre is the amount of indigestible sugars present in a food sample. The amount of crude fibre varied with increase in boiling time among the samples. There was a reduction in fibre content corresponding to an increase in boiling time. It was lowest in beniseed boiled for 30 min (4.45%) and highest in the raw beniseed (6.17%). Fibre is important for the physiological role of maintenance of internal distension for a normal peristaltic movement of the intestinal tract. It has been reported that a diet low in fibre is undesirable as it could cause constipation and such diets have been associated with diseases of the colon such as piles, appendicitis and cancer (Okon, 1983).

The crude protein ranged from 14.12% in boiled beniseed to 18.87% in raw beniseed (Table 1). The level of crude protein found in raw beniseed can qualify it as a good source of protein, if bio-available and easily digestible by the body. The results show that there was a significant difference in crude protein of raw beniseed and boiled beniseed at 30 min ($p < 0.05$). The reduction in protein content in boiled beniseed with increase in boiling time could be attributed to the denaturation of protein by heat (Fox and Cameron, 1984; Wardlaw, 1999; Potter and Hotchkiss, 2006). The results showed that moderate boiling for about 5 to 10 min would preserve the proteins.

The carbohydrate content did not undergo any significant change. The carbohydrate ranged from 11.12% in raw beniseed to 12.05% in boiled beniseed. The gradual increase in the carbohydrate in boiled beniseed agreed with the report of Onyeike and Oguike (2003) on the effect of cooking on the carbohydrate content of groundnut seed, an oil seed. Boiling has been reported to cause the granules to break down, soften the cellulose and make the starch more available (Agiang *et al.*, 2010).

The energy values of the samples were increased correspondingly to an increase in boiling time. Boiled beniseed contained a higher energy value than the raw beniseed. The energy values of the raw and boiled beniseed were similar to 563 kcals reported by Weiss (1983) for whole beniseed and comparing with that of groundnut (605 kcals), soybean (452.4 kcals), water melon (576.1 kcals) among others (Oyenuga, 1968).

3.2 Mineral Composition

The results of the mineral analysis of boiled and raw beniseed as presented in Table 2 shows that boiling resulted in some increases in calcium and phosphorus. There were however, some decreases in sodium and potassium. There were no significant changes in zinc, magnesium and iron. The decrease in some minerals observed may be attributed to losses caused by discarding the water used in boiling the beniseed. Calcium was the most abundant element varying from 757.13 mg/100g in raw beniseed to 975.54 mg/100g in beniseed boiled for 30 min. Zinc was the least among the elements studied and it varied from 3.21 mg/100g in raw beniseed to 3.25 mg/100g in beniseed boiled for 30 min. The results imply boiling improved the concentration of some of the minerals (Umoren *et al.*, 2007; Obiajunwa *et al.*, 2005). However, raw beniseed had higher or same values in potassium, magnesium, iron and zinc. In all cases however, the values obtained for the minerals in both raw and boiled beniseed meet the recommended daily requirements for both children and adults (Food and Nutrition Board, 2004).

3.3 Antioxidant contents

3.3.1 Phenolic, Flavonoid and Vitamin C content

The antioxidant constituents of beniseed were determined in this study as shown in Figure 1 include total phenols, flavonoids and vitamin C respectively. The total phenol and flavonoids contents of the seed extracts appear to increase with a corresponding increase in boiling time. There was however a reduction in the vitamin C content of aqueous extracts of boiled beniseed obtained at different boiling times. This reduction in vitamin C content could be due to the instability of vitamin C at boiling temperature; hence it probably gets oxidized at boiling temperature with consequent depletion of its content. Aqueous extracts of boiled beniseed contained a higher phenol and flavonoid content than aqueous raw extracts of beniseed. This increase in total phenol and flavonoid could be attributed to the fact some phytochemicals which are insoluble at room temperature get solubilised and extracted at increased temperature (Kolodziej and Hemingway, 1991). Hence, some medicinal plants are better exploited when extracted at increased temperature (Hemingway *et al.*, 1992). Jannat *et al.* (2010) reported that the total phenol contents of beniseed increased significantly with roasting temperature while Lee *et al.* (2005) reported that far-infrared irradiation of beniseed for 30 min increased the total phenol content of defatted beniseed meal extracts. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts of beniseed may be due to these compounds (Okudu *et al.*, 1994; Tepe *et al.*, 2006). This activity is believed to be mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Zheng and Wang, 2001). Phenols and flavonoids possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson *et al.*, 2001; Djeridane *et al.*, 2006). The results of the determination of total phenol and flavonoids suggest that they are important components of the seed and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

3.4 Antioxidant activities

3.4.1 Free radical scavenging activities

One important routine *in-vitro* antioxidant parameter used for testing the potency of agents is their ability to scavenge 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radicals. The reaction involves protonation of the unstable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radicals turning it to stable diamagnetic molecule which is visually noticeable as a discoloration from purple to golden yellow. The effect of antioxidants on 1, 1-diphenyl-2-picryl hydrazyl (DPPH) is thought to be due their hydrogen donating ability (Baumann *et al.*, 1979).

The free radical scavenging ability of both raw and boiled extracts of beniseed as presented in Figure 2 clearly shows that all extracts possess significant scavenging ability. However, boiled extracts of beniseed possess higher free radical scavenging abilities than the raw extract. The reason for this increase in radical scavenging ability could be due to the higher phenolic and flavonoid content in the boiled extracts. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (Oktay *et al.*, 2003). Phenolics and flavonoids are commonly known for their antioxidant effects. They

react and capture free radicals thereby inhibiting oxidative stress. They are also commonly known to exhibit anti-allergic, anti-inflammatory, anti-microbial and anticancer activity (Balch and Balch, 2000). It is therefore rational to believe that extracts which contain a higher content of these important phytochemicals would exhibit a higher free radical scavenging ability. Since boiling leads to an increase in the phenolic and flavonoid content of beniseed, it would be logical to believe that higher free radical scavenging ability is attributed to the increased phenolic and flavonoid content of extracts of boiled beniseed.

3.4.2 Ferric Reducing Property

The ferric reducing/antioxidant power (FRAP assay) is widely used in the evaluation of the antioxidant component in dietary polyphenols (Luximon-Ramma *et al.*, 2005). Antioxidants can also act by reducing and deactivating transition metals specifically by the reduction of Fe^{3+} to Fe^{2+} . Figure 3 shows that the boiled extracts of beniseed demonstrated potent ferric reducing power than the raw extract. This observation could be attributed to the difference in the levels of the phytochemicals in the extracts which are responsible for metal reduction probably due to their highly nucleophilic nature that enables them to readily donate proton to electron deficient centers to cause reduction (Ogunmoyole *et al.*, 2012).

3.4.3 Fe^{2+} Chelating Assay

Antioxidants can act by chelating and deactivating transition metals especially iron. Ions of transition metals such as copper and iron are involved in many free radical reactions, and often these lead to the generation of very reactive species (Halliwell and Gutterbridge, 1989). Figure 4 shows that boiled extracts of beniseed had greater Fe^{2+} chelating ability than the raw extracts. The study revealed that there was an increase in the iron chelating ability of the extracts of beniseed with increase in boiling time. This observation may be explained by the differences in the total phenol and flavonoid contents of the extracts since earlier reports have indicated that polyphenols exhibit potent iron chelating ability (Omololu *et al.*, 2011). Duthie *et al.* (1997) reported that several flavonoids are known to be very effective metal chelators. Hence, the increased amount of these polyphenols in the boiled extracts of beniseed must have conferred it with more potent iron chelating property than the raw extract. The results obtained from these study clearly shows that despite the reduction of vitamin C content in boiled extracts of beniseed, the extracts still exhibited potent antioxidant and free radical scavenging activities. This may be explained by the fact that boiling caused an increase in the total phenol and flavonoid contents of extracts. Amie *et al.* (2003) reported that phenolics such as flavonoids have antioxidant capacities that are much stronger than those of vitamin C and E and have been found to possess antioxidant and free radical scavenging activity.

4. Conclusion

This study revealed that boiling had significant effects on the nutritional composition of beniseed and also on the antioxidant properties of beniseed extracts. There were considerable losses of some nutrients due to a longer time of boiling especially protein and crude fibre while other nutrients such as fats, carbohydrates, calcium and iron were sufficiently retained. However, the levels of nutrients are adequate and compare well with those of other oil seeds. Boiling of beniseed for long periods of time should be discouraged to avoid significant loss of proteins and crude fibre when prepared as a soup to be consumed with carbohydrates foods such as garri, pounded yam and other flours. However, this study has been able to show that boiling which incidentally is a common practice of traditional medical practitioners potentiates the antioxidant properties of beniseed aqueous extract. Therefore, boiled extracts of the seed can be useful for therapeutic purposes.

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Table 1: Proximate composition of raw beniseed and boiled beniseed at different boiling times (% dry matter)

PARAMETERS	BOILING TIME OF SAMPLE				
	Raw	5 min	10 min	20 min	30 min
Moisture (%)	9.67±0.05a	9.93±0.07a	10.19±0.04a	10.23±0.06a	10.34±0.03a
Fat (%)	49.23±0.11a	50.97±0.06a	52.73±0.13ab	54.33±0.16b	56.78±0.14b
Protein (%)	18.87±0.22a	18.32±0.12a	16.69±0.14ab	15.56±0.13ab	14.12±0.08b
Crude Fibre (%)	6.17±0.03a	6.00±0.04a	5.6±0.05ab	5.32±0.04ab	4.45±0.05b
Ash (%)	4.94±0.23a	3.50±0.13b	3.23±0.06b	2.69±0.03c	2.26±0.08c
Carbohydrate (%)	11.12±0.06a	11.28±0.05a	11.56±0.07a	11.87±0.08a	12.05±0.04a
Energy Value (kcal/100g)	539.68±1.23a	553.22±2.21ab	562.66±1.45ab	572.48±1.34ab	575.71±1.28b

Values are Mean ± SEM of triplicate determinations. a, b, c: Mean values in each row with different alphabets are significantly different.

Table 2: Mineral elements in raw beniseed and boiled beniseed at different boiling times (mg/100g)

MINERALS	BOILING TIME OF SAMPLE				
	Raw	5 min	10 min	20 min	30 min
Calcium (mg/100g)	757.13±31.96a	798±2.11a	854±10.12a	921.23±5.43b	975.54±2.57b
Sodium (mg/100g)	296.79±20.24a	256.45±6.79a	196.53±3.73b	185±2.16b	174.34±4.23b
Potassium (mg/100g)	831.47±17.22a	802.22±3.12a	786.34±2.23ab	754.46±0.13b	727.42±3.28b
Phosphorus (mg/100g)	678.32±1.23a	692.24±2.14a	735.78±1.15ab	789.67±4.24b	843.46±5.13b
Magnesium (mg/100g)	385.26±12.53a	386.17±5.13a	388.21±4.56a	387.41±0.03a	386.56±2.13a
Zinc (mg/100g)	3.21±0.15a	3.14±0.12a	3.07±0.07a	3.20±0.11a	3.25±0.24a
Iron (mg/100g)	36.23±0.23a	36.45±0.43a	35.76±1.01a	36.13±0.27a	36.67±1.11a

Values are Mean ± SEM of triplicate determinations. a, b, c: Mean values in each row with different alphabets are significantly different.

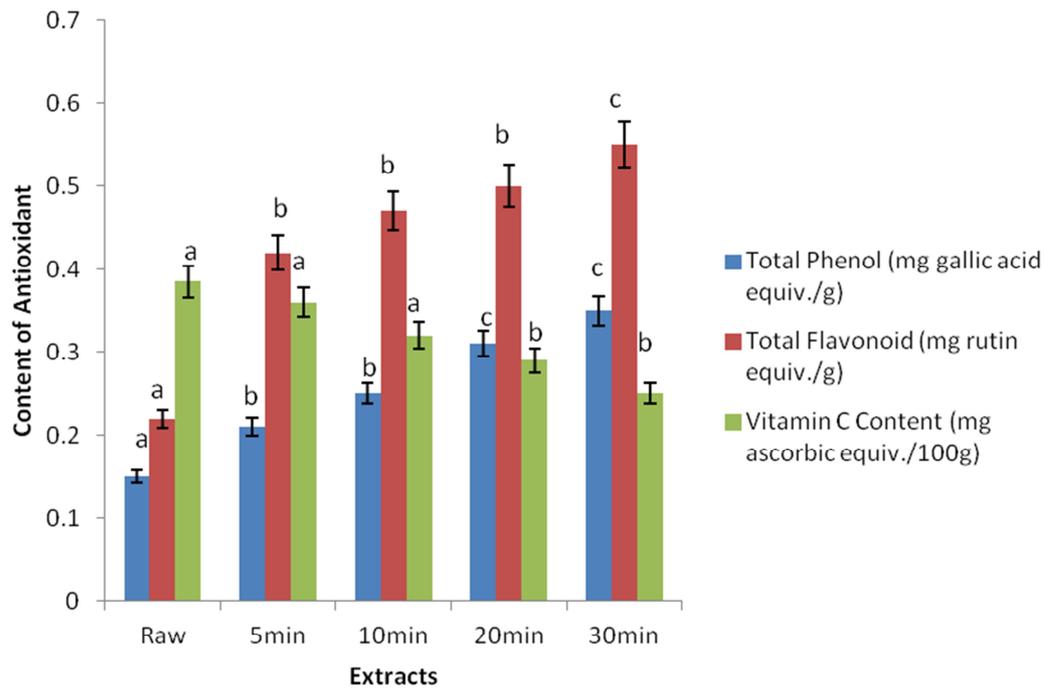


Figure 1: Antioxidant contents in aqueous extracts of raw and boiled beniseed. Values are mean \pm SEM of triplicate determinations. “b” and “c” indicate a significant difference from control “a” at $p < 0.05$.

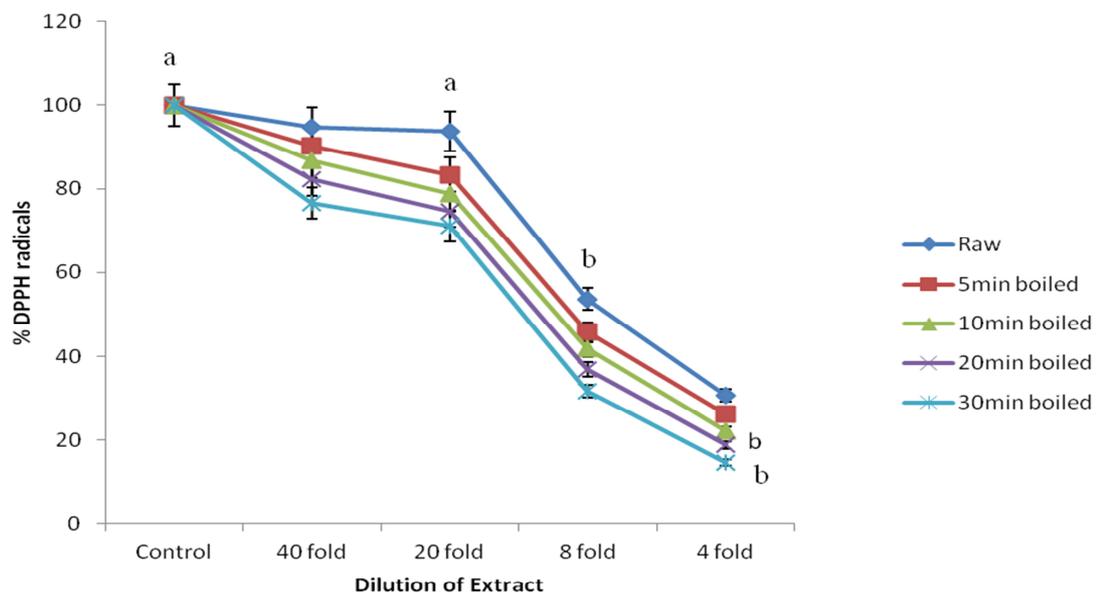


Figure 2: Free radical scavenging ability of raw and boiled beniseed extracts. Values are mean \pm SEM of triplicate determinations. “a” indicates a significant difference from “b” at $p < 0.05$.

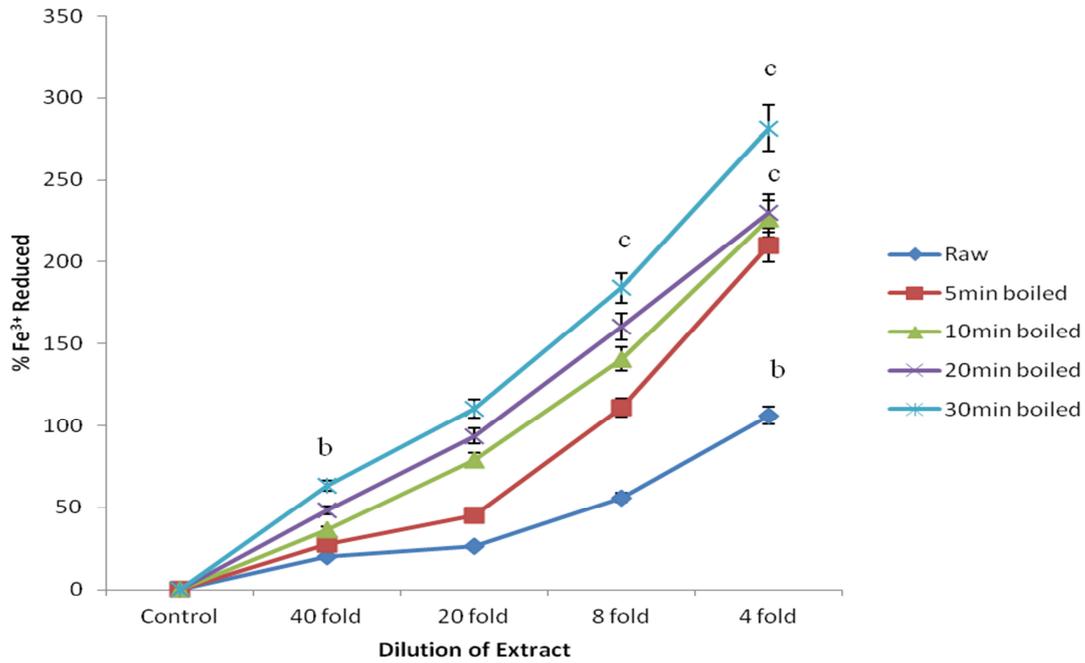


Figure 3: Ferric reducing antioxidant properties of raw and boiled beniseed extracts. Values are mean \pm SEM of triplicate determinations. “a” indicates a significant difference from “b” at $p < 0.05$.

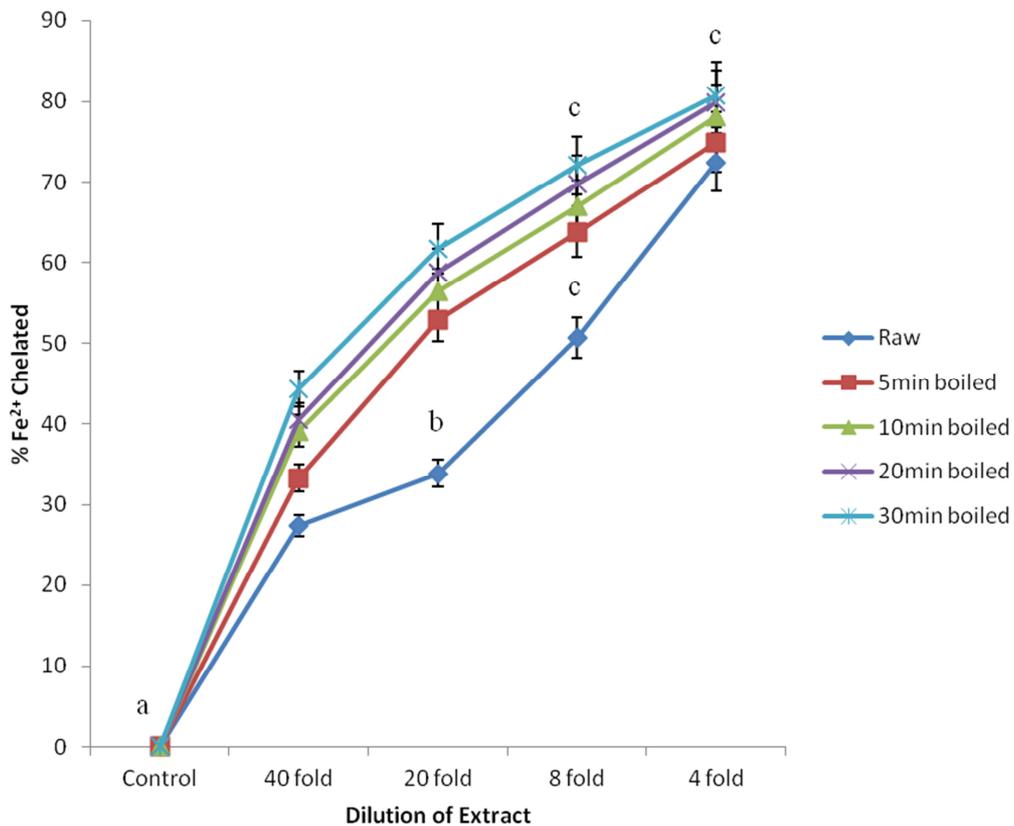


Figure 4: Fe²⁺ Chelating ability of raw and boiled beniseed extracts. Values are mean \pm SEM of triplicate determinations. “a” indicates a significant difference from “b” and “c” at $p < 0.05$.

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