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Major nutritional compositions of black cumin seeds – cultivated in Bangladesh and the physicochemical characteristics of its oil

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Abstract

Non – Conventional Seeds are being considered in the study due to their sole chemical compositions and functional properties. Black cumin seeds, frequently known as Black Seeds or Kalojeera in Bengali, are conventionally used for nutritional and medicinal purposes in Middle, Eastern and Western countries.The core objective of this analysis was to assess the foremost nutrients of Bangladeshi black cumin seeds and depiction of its oil. The proximate analysis indicated the seeds as a fibrous food containing pH 5.63 and also a good source of carbohydrate, protein, and lipid that were found subsequently in the amount of 29.18, 18.09 and 32.74 gm%. Its mineral content is also calculated to be rich where Calcium (Ca), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P) and Iron (Fe) were determined at the amount of 579.33, 510.30, 218.33, 100, 91.5 and 41.80 mg/100gm, respectively. Screening for secondary metabolites showed the presence of alkaloids, flavonoids, saponins and tannins at the amount of 10.11, 3.78, 7.58 and 2.21 mg/100gm consequently. Primary characterization of seeds extracts oil indicated it as a good source of polyunsaturated fatty acids.

Introduction

Black cumin seeds, due to their aromatic flavor and strong hot peppery taste, are extensively used as a flavoring agent in curry, a constituent in vinegar and substitute of pepper in cooking and bakery foods (Babayan *et al.*,1978). The seeds are small and black in color and possess a scented odor which is pungent and bitter in feel with a crunchy texture. Its plant is an annual herbaceous which belongs to the family of Ranunculaceae. It is indigenous to Mediterranean regions but also cultivates in Saudi Arabia, Africa, and Southwest Asia. The seeds are extensively sold in markets to be used as a condiment and native medicine.The seeds oil is also considered as one among newer sources of edible oils (Cheikh-Rouhou *et al*., 2007).

Both seeds and its oil have been a great focus of research and extensively premeditated due to its wide variety of chemical constituents and biological activities which take part in imperative roles in promoting human health and nutrition, and found versatile medicinal significances against diseases. Seeds are reported as digestive stimulants as well as carminative, diuretic, anthelmintic, asthmatic, emmenagogue and galactagogue (Hashim and Elkiey, 1962; Salama, 1973; Agarwal *et al*., 1979). In recent times, its oil has also been reported to possess antitumor (Worthen *et al.*, 1998), anti-inflammatory (Houghton *et al*., 1995), antibacterial (Morisi, 2000), antioxidant (Bruits and Bucar, 2000) activities and stimulatory effect on the immune system (Salem and Hossain, 2000). It is also used to treat the respiratory condition like asthma, emphysema, and bronchitis (Atta, 2003). Therefore, this research was initiated to explore the major constituents of Bangladeshi black cumin seeds which included the proximate nutrient analysis, mineral contents, secondary metabolites and characterization of its oil.

Materials and Methods

Sample collection and preservation

In Bangladesh, black cumin is widely known as Rabi crop. Its cultivation starts when the southeast monsoon ceasing in November and extends up to the end of March. For this analysis, black cumin plants and seeds were collected from the cultivated field of Lohagara upozila in Narail district (Geo URI 23.13^0N 89.50^0E) of the southwest region of Bangladesh at the end of February 2015. A Voucher specimen was deposited to the herbarium of Ethno

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– Botany laboratory of the Department of Botany of Chittagong University (CU), Chittagong, Bangladesh for taxonomic research and future analysis.

Sample identification

Most of the plants were found to be 22 to 25 cm in length with finely divided leaves. Leaves were 2.5 to 5.1 cm long, 2-3 pinnatisect and segmented in linear–lanceolate. The flowers were pale blue and white in color with 2.1 – 2.3 cm across on solitary long peduncles. The fruit was large and inflated capsule composed of 3 – 6 united follicles. Each contained numerous seeds which are commonly known as Black Cumin. The seeds were tiny and hairy, not more than 3 mm in length. In 1753, Black Cumin was first described by *Carl Linnaeus* (L.), a Swedish botanist and zoologist, and designated it as *Nigella sativa* L. – The Scientific name of Black Cumin (BSBI, 2007; USDA GRIN, 2000).

Sample preparation

The seeds were used for the experimental purposes. About 250 gm seeds were cleaned with fresh water to remove dust and then sundried. The seeds were then ground through the grinder (Brand: Miyako, Model: BL – 152 PF – AP, Speed: 25000 RPM, Country: China) for 3 times and then again it was grounded in mortar and pestle to make it as fine powder form. The powder was then packed and stored in a refrigerator at 4^0C prior to analysis. Solvents were procured as an analytical grade from local suppliers and standards from the local supplier of Sigma – Aldrich (Sigma – Aldrich, Tokyo, Japan).

Determination of pH

2 gm dry powder was homogenized well with 30 ml distilled water and then filtered through Whatman's No.1 filter paper. The filtrate was then centrifuged for 10 min at 5000 rpm and the clear supernatant was collected. The pH of the extracted solution was determined by a Corning 215 – pH meter using standard buffer solution.

Determination of moisture content

Moisture was determined from dry powder. Five gram of dry powder was taken in a porcelain crucible and heated at 100^0C in an oven for six hours. Then the moisture was determined by following the conventional procedure, described in ICMR (1971).

Proximate nutrient analysis

Ash and Crude fiber content were determined through the conventional procedure of AOAC (2000). Phenol Sulphuric Acid Method of Dubois *et al*. (1951) was followed for the determination of the total carbohydrate, whereas the total sugar and starch were determined by the Anthrone method of Jayaraman (1981) and the total reducing sugar by Dinitrosalicylic acid (DNS) Method of Miller (1959). Water-soluble protein content was determined through Folin – Lowry method (Lowry *et al*., 1951), the total protein contents by Micro –Kejeldahl Method of AOAC (2000) and total lipid content by Bligh and Dyer Method (1959).

Estimation of minerals

The dry powder was kept in an incubator at 105^0C for the overnight due to the presence of moisture and then digested by HNO_3 and Perchloric Acid ($HClO_4$). Then the important mineral contents of seeds were determined by Analytical Method of Petersen (2002) where Calcium (Ca), Magnesium (Mg) and Iron (Fe) were analyzed by Atomic Absorption Spectrophotometry, Phosphorus (P) by Spectrophotometry and Sodium (Na) as well as Potassium (K) by Flame photometry.

Screening for secondary metabolites

The 100 gm dry powder was soaked in 400 ml of 96% ethanol in a glass container for sixteen days with additional regular shaking and stirring. The extract was then separated from the debris by filtration using a piece of clean white cotton material and it was done for two times. The filtrate was then taken in a beaker and was wrapped by aluminum foil and perforation was done for evaporation of ethanol. The residue was designated as a crude extract of ethanol. Then different chemical group tests were performed for the screening of secondary metabolites like Alkaloids, Glycosides, Cardiac Glycosides, Anthraquinone Glycosides, Flavonoids, Tannins, Steroids, and Saponins by following the method described by Myers(1982) and Harborne (1973).

Quantitative analysis of some secondary metabolites

After screening, quantitative analyses were performed for Alkaloids, Flavonoids, Tannins, and Saponins by following the extraction method (dry weight basis) that was described by Harbone (1973); Boham and Kocipai (1974); Pearson (1976) and Obdoni and Ochuko (2001) consequently.

Extraction of seed oil and its characterization

The 25 gm dry powder was digested through 125 ml of light Petroleum Ether at 40^0 – 60^0C in a dark flask for 24 hours. It was then filtering through Whatman no. 1 filter paper after mixing for 4 hours in a shaker at the rate of 180 u/min. The total extraction

procedure repeated twice and the collected solvent was removed by rotary evaporator. The percentage of oil was then calculated and stored in an airtight dark container in a freezer at -20^{0}C for subsequent analyses. Specific Gravity, refractive index and the iodine value of extracted oil were determined by the IUPAC (1987) methods. Unsaponifiable matter, saponification value, the percentage of free fatty acids, acid value, peroxide value and acetyl value were determined by the AOCS (1990) methods.

Data analysis

All experiments were executed in triplicate except the minerals that were analyzed in duplicate. The optical density of each sample was measured with the help of spectrophotometer and was plotted on a graph of the respective standard used particularly for each biochemical's. From the graph, the concentration of biomolecules in 1ml was calculated and then converted into 100 gm and the results were the means of triplicate or duplicate ± Standard Deviation (SD).

Results and Discussion

Table 1. Proximate nutritional compositions of Bangladeshi black cumin seeds

Nutritional compositions	Result
pH	5.63±0.042
Moisture (gm %)	5.5±0.72
Ash (gm %)	4.69±0.51
Crude Fiber (gm %)	6.39±0.56
Total Carbohydrate (gm %)	29.18±0.89
Total Sugar (gm %)	0.92±0.03
Total reducing Sugar (gm %)	0.74±0.08
Total non reducing Sugar (gm %)	0.18±0.05
Starch (gm %)	2.55±0.37
Total water Soluble Protein (gm %)	6.53±0.64
Total Protein (gm %)	18.09±0.82
Total lipid (gm %)	32.74±0.76

Table 2.Mineral compositions

Minerals	Result
Iron (Fe) (mg %)	41.80±0.78
Calcium (Ca) (mg %)	579.33±4.36
Magnesium (Mg) (mg %)	218.33±3.06
Phosphorus (P) (mg %)	91.5±5
Sodium (Na) (mg %)	100±5.16
Potassium (k) (mg %)	510.30±7.45

Table 1 represents the proximate nutritional analysis where total lipid contents were found as the most dominant nutrient in Bangladeshi black cumin seeds that were resolute at the amount of 32.74 gm% which is similar to the lipid value of Turkey seeds, reported by Nergiz and Ötles(1993) but have 14.29% less than the Saudi seeds, reported by Al-Jassir (1992). Subsequently, total carbohydrate was originated as 29.18 gm% which was the second foremost nutrient in this analysis but found to be 8.52%, 21.97%, 27.05% and 10.76% less than the reported values of Saudi (Al-Jassir, 1992), Turkey (Nergiz and Ötles, 1993), Tunisian and Iranian (Cheikh-Rouhou *et al*., 2007) seeds, respectively. Following then, the total protein was estimated as the amount of 18.09 gm% whereas the Saudi, Turkey, Iranian and Tunisian seeds were determined as in the range of 20.90 to 26.7 gm% as per reported values (Al-Jassir, 1992; Nergiz and Ötles, 1993; Cheikh-Rouhou *et al*., 2007). A few numerical differences have also been observed in this study regarding moisture, ash and crude fiber than those reported values. However, the moisture, ash and crude fiber contents were found to be very similar to the other available data sources and reported as in the range of 4.6 – 6.4 gm%, 4.0 – 4.86 gm% and 6.39 – 7.9 gm%, respectively and that's clearly indicated the Bangladeshi black cumin seeds as a good source of dietary fibers which have many significant roles in human nutrition. Besides these, total sugar, reducing sugar, non-reducing sugar, starch and total water-soluble protein were also been determined at the amount of 0.92, 0.74, 0.18, 2.55 and 6.53 gm%, respectively.

Table 2 represents the major mineral contents of Bangladeshi black cumin seeds where Ca was determined as the prime element and found at the amount of 579.33 mg% that is higher than the Saudi and Turkey seeds but is very similar with the values of Iranian and Tunisian seeds as reported (Cheikh-Rouhou *et al.*, 2007). K was measured at the amount of 510.30 mg% as next in order but found to be 56.75%, 34.82% and 27.92% less than Turkey, Tunisian and Iranian seeds, respectively except Saudi seeds. Thereafter Mg, Na, P as well as Fe were determined as noteworthy amounts of 218.33, 100, 91.5 and 41.80mg% consequently and they were found to be higher than the reported values of Al-Jassir, 1992; Nergiz and Ötles, 1993; Cheikh-Rouhou *et al*., 2007. It might be also mentioned that the different mineral contents in Saudi seeds are shown to be significantly in fewer amounts than the present study as well as the other reported values.

Screening for secondary metabolites (Table 3) revealed that Alkaloids, Flavonoids, Saponins, and Tannins were presented higher than the other secondary metabolites and were determined at the amounts of 10.11±0.77, 3.78±0.31, 7.58±0.95 and 2.21±0.03 mg/100 gm, respectively.

The above experimental findings evidently demonstrated that the black cumin seeds might be

Table 3. Different chemical group tests for Qualitative (Screening) and Quantitative analysis of some secondary metabolites

Secondary Metabolites	Result
Qualitative Analysis (Screening)	
Test for Alkaloids	(++)
Test for Glycosides	(+)
Test for cardiac Glycosides	(+)
Test for Anthraquinone Glycosides	(-)
Test for Flavonoids	(++)
Test for Terpenoids	(-)
Test for Steroids	(+)
Test for Tannins	(++)
Test for Saponins	(++)
Quantitative Analysis	
Alkaloids (mg %)	10.11±0.77
Flavonoids (mg %)	3.78±0.31
Saponins (mg %)	7.58±0.95
Tannins (mg %)	2.21±0.03

Here, (++) indicates high, (+) indicates medium and (-) indicates Nil.

Table 4. Physicochemical characteristics of black cumin seeds extract oil

Parameter	Result
Seeds extract oil (%)	25
Specific gravity at 25 °C	0.61±0.01
Refractive index at 28 °C	1.42± 0.01
Iodine value (gm of I_2/100 gm of oil)	111±0.6
Saponification value (mg KOH/g)	208.6±2.3
Free fatty acids (%) as oleic	11.7±0.42
Acid Value	23.29±0.84
Unsaponifiable matter (gm/100 gm)	1.51± 0.4
Peroxide value (mEq/kg of oil)	8.4±0.7
Acetyl value(gm/100 gm)	3.4±0.45

considered as a rich source of lipid, carbohydrate, and protein including minerals and dietary fibers. Biologically important secondary metabolites are also present in a sufficient amount. According to the recommended daily intake by Cuthburtson (1989) and based on the upshot, assuming high *in-vitro* bioavailability, black cumin seeds would be better in contributing partially, to the overall daily dietary intake of the elements. The variations in nutrient concentrations that have found among the seeds produced in different regions as per reported values in literature that have shown in table 5 may be due to the geographical and climatic differences, cultivated regions, storage conditions and maturity stage.

Table 4 depicts the physicochemical characteristics of seeds extract oil where near about 25% oil was obtained after solvent extraction. Its specific gravity at 25^{0}C and refractive index at 28^{0}C was determined and found to be 0.61±0.01 and 1.42±0.01, respectively. Its saponification value was measured at the amount of 208.6±2.3

Table 5. Comparison of some major macro and micro nutrients of Bangladeshi black cumin seeds with Saudi, Turkey, Tunisian and Iranian black cumin seeds

	Experimented Results	Al-Jassir *et al.*, 1992	Nergiz and Ötles, 1993	Cheikh-Rouhou *et al.*, 2007	
	Bangladesh	Saudi Arabia	Turkey	Tunisia	Iran
Moisture (gm %)	5.5±0.72	4.6±0.45	6.4±0.15	NF	NF
Ash (gm %)	4.69±0.51	4.4±0.32	4.0±0.29	4.86±0.06	4.41±0.01
Crude Fiber (gm %)	6.39±0.56	7.9±0.93	6.6±0.69	NF	NF
Total Carbohydrate (gm %)	29.18±0.89	31.9±2.56	37.4±0.87	40.0±0.46	32.7±0.41
Total protein (gm %)	18.09±0.82	20.9±1.35	20.2±0.82	26.7±0.35	22.6±0.24
Total lipid (gm %)	32.74±0.76	38.2±2.20	32.0±0.54	NF	NF
Iron (Fe) mg %	41.80±0.78	0.15±0.04	57.5±0.5	8.65±0.65	9.42±0.88
Calcium (Ca) mg%	579.33±4.36	0.04±0.008	188±1.5	572±21.5	564±33.4
Magnesium (Mg) mg%	218.33±3.06	0.03±0.005	NF	235±4.87	260±48.70
Phosphorus (P) mg%	91.5±5	1.8±0.23	NF	48.9±0.04	51.9±0.01
Sodium (Na) mg%	100±5.16	0.75±0.10	85.3±16.07	20.8±2.21	18.5±3.17
Potassium (K) mg%	510.30±7.46	7.6±0.42	1180±10.0	783±6.61	708±7.98

NF = Not Found

mg KOH per gm whereas the unsaponifiable matters and acetyl value were found as 1.51±0.4 and 3.4±0.45gm/100gm consequently. The iodine value was indexed at 111±0.6 gm of I_2 per 100 gm of oil, which clearly indicates it as a vegetative oil as it contains high amount of unsaturated fatty acids and also found in the permissible range described by Tan *et al.*(2002), Kruatian and Jitmanee (2012) and vegetable oil products (Regulation) order (1998), where most of the commonly used vegetative oil's iodine value was studied for Soyabean oil – 120 - 141, Sunflower oil – 100 – 145 and olive oil – 79 - 90. But the presently studied oil may not be considered as edible due to its high content of acid value that is found as 23.29±0.84. The high acidity of this oil may be related to the nature of the seeds (Patterson, 1989). Although the peroxide value and FFA (Free Fatty Acid) were found as 8.4±0.7 (mEq per kg of oil) and 11.7± 0.42 (% as Oleic) that were also as acceptable for vegetative as per FAO/WHO's Codex standards to ensure the authenticity for edible oils and fats (CAC, 1983). So, this oil may be taken as crude for preparation of different food products and could also be a good alternative source of essential fatty acids as well as a potential source for commercial production. Thereafter, a standard refining process should be formulated to lower the acid value for making it as edible. Further study is also required to confirm whether the black cumin oil contains lipase enzyme, as it is one of the major enzymes for producing free fatty acids by hydrolyzing lipid and oil.

Conclusion

More *in vitro* and *in vivo* approaches are further

needed to classify its biological activities and a database of different varieties in worldwide according to their nutritional status and pharmacological activities as parameters are also needed to select the mass growing variety in different climates and to categorize the appropriate type of variety for cultivation.

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