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Nutritional profile and physicochemical analysis of two indigenous cultivars of black cumin (*Nigella sativa*) seeds and oil in Bangladesh

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Abstract

The black cumin (*Nigella sativa*) seed oil has a long history of use for food ingredients. The goal of this study is to figure out the nutritional and physicochemical properties of two indigenous black cumin cultivars (locally known as "Kali Jira" and "Kalonji") seed and oil. Additionally, the effects of extraction time and particle size on oil yield generation was investigated. The values of moisture content, crude protein content, crude fat, crude fiber, total ash, and carbohydrate of Kali Jira seed were 5.29%, 19.83%, 41.3%, 18.96%, 5.10%, and 9.52% whereas Kalonji had 5.43%, 19.95%, 40.3%, 14.28%, 4.35%, and 14.69%, respectively. In the Kali Jira and Kalonji types, the mineral analysis revealed significant potassium levels (829.11 mg/100 g, 746.27 mg/100 g), followed by phosphorus, calcium, sodium, iron and zinc. The minimum oil yield was 46.77% in the Kalonji variety compared to Kali Jira, which had 49.57% after the extraction time of 2 hrs for 1.4-2.5 mm particle size. On the other hand, a maximum oil yield of 91.08% and 94.77% was found in Kalonji seed and Kali Jira seed, respectively, following a 6-hour extraction time for 0.25-0.5 mm particle size. Characterization of extracted seed oil (iodine value, saponification value, and acid value) indicated the abundance of unsaturation in oil. FTIR analysis was used to investigate functional groups. According to the findings of this study, black cumin and extracted oil are rich in nutrients and can be used to treat lifestyle problems.

1. Introduction

Nigella sativa is a spicy annual herbaceous plant belonging to the *Ranunculaceae* family that grows in Mediterranean nations. *Nigella arvensis*, *Nigella ciliaris*, *Nigella damascene*, *Nigella hispanica*, *Nigella integrifolia*, *Nigella nigellastrum*, *Nigella orientalis*, and *Nigella sativa* are among the 14 species that make up the *Ranunculaceae* family of flowering plants. *Nigella sativa* is the species that has been studied the most extensively for therapeutic purposes. Other species, however, have been linked to medical applications.

Black cumin seeds, also known as *Nigella sativa* L., have amazing healing powers. Because of its pungent, peppery flavor, black cumin seeds have long been used in cooking (Ramadan, 2007). Seed oil or extract has been shown to have therapeutic properties and is one of the more recent edible oil sources (Cheikh-Rouhou *et al.*, 2007). Seeds and oils are frequently utilized as dietary supplements because of their many health benefits, including antioxidant activity (Burits and Bucar, 2000) and immunostimulation (Salem, 2005).

In black cumin extracts, studies have found remarkable proximate results for moisture, oil, protein, ash, and total carbohydrate (Takruri and Dameh, 1998; Atta, 2003). Potassium, calcium, phosphorus, and magnesium are the most prevalent minerals in *Nigella sativa* seeds (Sultan *et al.*, 2009). Unsaturated fatty acids like oleic, linoleic, and linolenic acids are abundant in both the seeds and the oils of *Nigella sativa* (Atta, 2003). The phenolic chemicals in *Nigella sativa*, which have significant antioxidant activity, are thought to be responsible for the plant's wide range of therapeutic qualities. Except for olive oil, the phenolic content of *Nigella sativa* seeds has been observed to be higher than

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that of most edible oils (Salvador *et al.*, 2001).

Unsaturated fatty acids, particularly polyunsaturated fatty acids, make up a significant component of *Nigella sativa* seed fixed oil, ranging from 48 to 70%, whereas monounsaturated (18-29%) and saturated fatty acids (12-25%) present in smaller proportions (Nickavar *et al.,* 2003). Aside from a superior fatty acid composition, it also contains significant amounts of tocopherols and other bioactive chemicals that help the body's overall antioxidant capacities. Furthermore, pharmacological studies have explored the efficacy of essential oils and their active constituent, thymoquinone, against a variety of ailments such as oxidative stress, cancer, immunological dysfunction, and diabetic complications (Gali-Muhtasib *et al.,* 2004). The composition of *Nigella sativa* varies according to geographic distribution, harvest time, and agronomic approaches, as it does with most herbs. We aimed to characterize the native variety of *Nigella sativa* seeds and the nutritional profile of extracted oils to investigate if they could be used to treat lifestyle diseases in Bangladesh.

2. Materials and methods

2.1 Sample collection and preparation

Indigenous black cumin seeds were obtained from several local markets of Chattogram. Kali Jira (Sirajgonj, Bangladesh) and Kalonji (Naogaon, Bangladesh) were collected from the Khatungonj spices market in Chattogram, Bangladesh. Seeds were characterized in the Quality Assurance Laboratory of the Department of Applied Chemistry and Chemical Technology, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. The dust, stone, and other foreign components were manually removed to obtain the pure seed. The seeds were dried for one day by the sun and in an oven at 50°C for 18 hrs. The dried black cumin seed was milled in a Cross Beater Miller (Cross Beater Mill SK300, Retsch, Germany) with a size of 3 mm after being dried in an oven at 50°C for 18 hrs The sample was then shaken for 8 min with a 10 mm amplitude on a vibrating shaker. To acquire the specific sizes of 2.5-1.4 mm, 1.4-0.5 mm, and 0.5-0.25 mm, the sieve size was set in descending sequence of mesh sizes 3 mm, 2.5 mm, 2 mm, 1.8 mm, 1.4 mm, 1 mm, 0.85 mm, 0.5 mm, and 0.25 mm. This size range was chosen since literature suggested that oil particle sizes should be less than 5 mm and greater than 0.2 mm for a higher yield (Henry, 1983).

2.2 Extraction of seed oil

In a Soxhlet extractor, 40 g of black cumin seeds from each variety were extracted with n-hexane at 70°C. The experiment was repeated using the same amount of sample, but with different particle sizes (2.5-1.4 mm, 1.4-0.5 mm, and 0.5-0.25 mm) and time intervals (2 hrs, 4 hrs and 6 hrs). A rotary evaporator was used to separate the solvent (n-hexane) from the extracted oil mixture, which was weighed (Lawson *et al*., 2010). The oil yield percentage was calculated using the formula (Warra *et al.*, 2011).

2.3 Proximate composition of seeds

Proximate composition for moisture, carbohydrates, crude protein, crude fat, crude fiber, and ash was carried out by following the methods of the Association of Official Analytical Chemists (AOAC) (2000).

2.4 Minerals analysis of seeds

The seed samples were digested by using Microwave Digestion System (Topwave PM60, Analytik Jena, Germany). Digested samples were then diluted with 50 mL of demineralized water and filtered by using Whatman No. 5B filter paper. The filtrates were collected for mineral analysis. The minerals (Potassium, Phosphorus, Sodium, Calcium, Iron, and Zinc) were analyzed by using Shimadzu AA-6800 Atomic Absorption Spectrophotometer (Japan).

2.5 Physicochemical properties of extracted oil

2.5.1 Specific gravity

The density of oil was determined using the density bottle method. A clean and dry density bottle of 25 mL capacity at 30°C was weighed in grams (W_0). Then the bottle was filled with water and reweighed at 30°C (W_1). Melted oil was brought to 300°C, and the water was substituted with this oil after drying the density bottle and weighed again (W_2) and the specific gravity was determined (AOAC, 2000).

2.5.2 Saponification value

In a 250 mL flask, 2.5 g of the extracted oil sample was combined with 25 mL of alcoholic potassium hydroxide solution. The system was hooked up to a reflux condenser and gently boiled for 1 hr. The condenser was cleansed with 10 mL of hot ethyl alcohol after cooling. The surplus potassium hydroxide was then titrated with 0.5 N hydrochloric acid to the endpoint, followed by a few drops of phenolphthalein indicator. For the blank, the same technique was used. The AOAC formula was used to compute the saponification value (SV) (AOAC, 2000).

2.5.3 Iodine value

To dissolve the oil, 0.4 g of the sample was placed in a conical flask with 20 mL of carbon tetrachloride. After that, 25 mL of iodine monochloride solution in glacial

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acetic (Wij's solution) was added to the flask using a safety pipette in the fume chamber. The cork was then inserted, and the contents of the flask were swirled vigorously. The flask was then left for 2 hrs and 30 mins in the dark. At the end of the session, 20 mL of 10% aqueous potassium iodide and 125 mL of water were put into a measuring cylinder. The content was titrated with 0.1 M sodium-thiosulfate solutions until the yellow tint was almost completely gone. The titration was continued by adding thiosulphate dropwise until the blue coloring vanished after vigorous shaking after adding a few drops of 1% starch indicator. Blank testing followed the same procedure. The standard formula was used to calculate the iodine value (Warra *et al.*, 2011).

2.5.4 Acid value

In a 250 mL conical flask, 10 g of cooled oil sample was mixed with 50 mL of freshly neutralized hot ethyl alcohol and 1 mL of phenolphthalein indicator solution. The mixture was warmed for 5 min and titrated against standard alkali solution (0.1 N KOH) while hot, shaking vigorously throughout the titration. The titration process ended with lasting pink color and the acid value was calculated (Ranganna, 1986).

2.6 Functional group analysis by Fourier Transform Infrared

In the Bangladesh Council of Scientific and Industrial Research (BCSIR) laboratory in Chattogram, Bangladesh, the essential oil's Fourier Transform Infrared (FTIR) spectrum was acquired using a Perkins Elmer Spectrum 65 FTIR spectrometer (Perkin Elmer 1600 Spectrometer, USA). The functional groups were then established using IR correlation charts. The percent transmittance of the infrared spectra was recorded. The wavenumber range studied was 4000 - 400 cm^{-1} (in the mid-infrared range). FTIR spectroscopy was performed on a Perkin Elmer FTIR with Spectrum software version 10.3.2.

2.7 Statistical analysis

All the experiments were done in triplicate, and the results were expressed as means with standard deviations. Microsoft Excel 2010 was used to examine data. The significance threshold was set at $p<0.05$.

3. Results and discussion

3.1 Proximate compositions

The proximate composition of two black cumin seed varieties is summarized in Table 1. The moisture content (%) of black cumin seed (Kali Jira) was 5.29±0.82 while black cumin seed (Kalonji) had 5.43±0.11. In this study, Kali Jira contained 9.52±2.31%, 19.83±0.10%, 41.3±0.11%, 18.96±0.21%, and 5.10±0.11% of carbohydrates, crude protein, crude fat, crude fiber and ash contents respectively while Kalonji had 14.69±1.45%, 19.95±0.14%, 40.3±0.08%, 14.28±0.22% and 4.35±0.12% respectively. The result obtained from both varieties was similar to the study reported in the literature (Dandik and Aksoy, 1992; Abdel-All 1993; Salem, 2005). But slight differences in ash content and carbohydrate were found that might be due to environmental factors like climate and location. In comparison with other previous study results, there was a major difference found in crude fat, crude fiber and carbohydrate content. The crude fat content of Kali Jira and Kalonji seeds was much higher than the fat content found in the study reported elsewhere (Sultan *et al.,* 2009; Iqbal *et al.,* 2011; Mamun and Absar, 2018). But the crude fat of the study varieties was lower than the value reported by Kabir *et al.* (2019). The crude fiber of Kali Jira and Kalonji seeds was remarkably higher than the value studied by Mamun and Absar (2018). Among these two study varieties, Kalonji had a higher carbohydrate content but was remarkably lower than the value conducted by other researchers (Sultan *et al.,* 2009; Iqbal *et al.,* 2011; Mamun and Absar, 2018). Generally, Kali Jira varieties had higher crude fat, crude fiber and ash content than Kalonji varieties. However, moisture, crude protein and carbohydrate contents were higher in Kalonji compared to Kali Jira.

Table 1. Proximate composition of black cumin seeds.

Proximate Composition (%)	Experimental Data (Mean±SD)	
	Kali Jira	Kalonji
Moisture	5.29±0.82	5.43±0.11
Crude Protein	19.83±0.10	19.95±0.14
Crude Fat	41.3±0.11	40.3±0.08
Crude Fiber	18.96±0.21	14.28±0.22
Ash	5.10±0.11	4.35±0.12
Carbohydrate	9.52±2.31	14.69±1.45

3.2 Minerals analysis

The mineral composition of test seed varieties showed in Table 2. In this study, potassium was found as a dominant mineral with a concentration of 829.11±7.6 mg/100 g and 746.27±5.8 mg/100 g in Kali Jira and Kalonji seeds, respectively. Among these two seed varieties, Kali Jira had a higher amount of sodium content, calcium content, and iron content than Kalonji seed (Table 2). Furthermore, Kali Jira and Kalonji seeds had significant amounts of phosphorus and zinc. Some research findings were similar to the present results regarding mineral contents such as potassium (510.3 – 1498.3 mg/100 g), phosphorus (91.5 – 543 mg/100 g), calcium (366.7 – 579.33 mg/100 g), sodium (17.6 – 100 mg/100 g), iron (9.70 – 42.6 mg/100 g) and zinc (6.23 –

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6.7 mg/100 g) (Sultan *et al.,* 2009; Mamun and Absar, 2018; Kabir *et al*., 2019). Cheikh-Rouhou *et al.* (2007) found minor changes in mineral composition when comparing black cumin seeds, but they also discovered that potassium is the major mineral in black cumin seeds. Kali Jira varieties had a higher value than Kalonji varieties in all minerals content except phosphorous. Possible causes of this difference could be climatic variations and genotype.

Table 2. Mineral analysis of black cumin seeds.

Mineral Analysis (mg/100 g)	Experimental Data (Mean±SD)	
	Kali Jira	Kalonji
Potassium (K)	829.11±7.6	746.27±5.8
Phosphorus (P)	698.89±5.4	710.67±8.6
Sodium (Na)	110.55±3.5	37.31±2.8
Calcium (Ca)	498.45±16.3	481.57±14.9
Zinc (Zn)	5.39±0.12	5.40±0.14
Iron (Fe)	76.48±4.3	56.45±3.2

3.3 Effect of particle size and time on oil yield

The percentage oil yield is shown in Table 3. In this study, the highest percentage oil yield was attained as 91.08±1.31 and 94.77±0.81 for Kalonji and Kali Jira respectively, with a particle size range of 0.25-0.5 mm and an extraction time of 6 hrs. With a particle size range of 1.4-2.5 mm and an extraction length of 2 hrs, the minimum percentage of oil production for Kalonji and Kali Jira was 46.77±1.60 and 49.57±1.61 respectively. In this present study, particle size had a vital role in the black cumin seed essential oil yield. Smaller particle sizes yielded more oil, but higher particle sizes yielded less. Thus, the percentage of essential oil yield was inversely related to the particle size. The reason was that larger particles had a smaller surface area of contact and a larger distance to the solvent entrance and oil diffusion than smaller particles using n-hexane solvent. The results of n-hexane extracts were similar to the previous studies (Nickavar *et al.,* 2003; Atta *et al*., 2003; Ebewele *et al.,* 2010). The oil yield falls when the particle size is too small or very fine, which might be attributed to particle agglomeration, which reduces the contact surface area. The current study result was also comparable to the outcome of oil from seed extraction for three distinct particle size ranges (0.5 mm, 0.5-0.75 mm, and 0.75 mm) (Nickavar *et al.,* 2003; Atta, 2003; Ahsan, 2014). The intermediate particle size (0.5-0.75 mm) produced the highest percentage of oil yield, indicating that decreasing the particle size below a specific size does not increase the percentage of oil yield and may even reduce it. The percentage of oil yield was directly proportional to extraction time, while the extraction time increased, the yield increased as well (Meziane *et al*., 2008; Nickavar *et al.,* 2003; Atta, 2003). The yield of oil increased substantially with time up to 4 hrs for smaller particle sizes in the 0.25-0.5 mm range, then remained steady. The highest oil yield (94.77% and 91.08%) for Kali Jira and Kalonji was found at the time of 6 hrs. The oil in the seed was nearly depleted at 4 hrs, resulting in low oil outputs. On the other hand, at the time ranges from 4 hrs to 6 hrs the oil yield was 2.91% and 2.82% for Kali Jira and Kalonji, respectively. The yield was lower at the start of the extraction for larger particle sizes, such as 1.4-2.5 mm, but gradually increased as the extraction duration increased. In comparison to large particle size, the results of this study showed that smaller particle size required a shorter extraction time to achieve maximal yield. For optimal yield production for the smaller particle, we discovered that a 6-hour extraction period was ideal. As a result, generating a 97% yield would take 4-6 hrs. Any extraction time of more than 6 hrs is, therefore, a waste of time and resources.

3.4 Physicochemical properties of oil

At 25°C, the specific gravities of Kali Jira and Kalonji seed oils were 0.91768 and 0.90790, respectively, as reported in Table 4. This result was extremely comparable to the study result (0.9071 at 25°C for *Nigella sativa*) reported by Ali *et al*. (2012). The calculated iodine values for Kali Jira and Kalonji seed

Table 3. Oil extraction from black cumin seeds.

No. of run	Factors		Oil yield (%) (Mean±SD)		Extraction yield (%) (Mean±SD)	
	Particle size (mm)	Time (hrs)	Kali Jira	Kalonji	Kali Jira	Kalonji
1	2.5-1.4	2	49.57±1.61	46.77±1.60	2.22±0.08	1.89±0.05
2	2.5-1.4	4	66.67±1.52	52.56±0.68	2.35±0.05	2.13±0.03
3	2.5-1.4	6	76.00±1.00	73.67±1.53	2.6±0.05	2.51±0.02
4	1.4-0.5	2	55.23±1.62	51.60±1.26	2.28±0.06	2.04±0.11
5	1.4-0.5	4	75.13±3.82	72.60±3.67	2.44±0.04	2.29±0.04
6	1.4-0.5	6	84.29±2.67	81.37±2.90	2.7±0.10	2.65±0.05
7	0.5-0.25	2	62.33±0.69	59.77±1.36	2.54±0.03	2.15±0.03
8	0.5-0.25	4	82.00±1.00	79.00±1.00	2.73±0.02	2.5±0.10
9	0.5-0.25	6	94.77±0.81	91.08±1.31	2.91±0.01	2.82±0.01

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oils were 118±0.922 and 116±0.132 respectively, but the lower iodine value of *Nigella sativa* oil was investigated in previous studies (Sultan *et al.,* 2009; Ali *et al.,* 2012). In contrast to the findings of the current investigation, Farhan *et al*. (2021) found that the iodine value of extracted oil was 122.7 mg/100 g. The present findings of iodine value were within the investigated level of earlier studies (Atta, 2003; Cheikh-Rouhou *et al*., 2007). The high iodine values found in Kalonji seed oils imply significant unsaturated bonds, according to the results of this investigation. As a result, the samples in this study have a higher risk of becoming rancid due to oxidation. Saponification values of seed oil (Kali Jira and Kalonji) were estimated to be 192.6±1.81 mg KOH/g and 189.8±1.80 mg KOH/g respectively which were lower than the study findings of Ali *et al.* (2012) but higher than the study of Sultan *et al.* (2009). Furthermore, the results are more similar to the Saudi *Nigella sativa* seed oil results reported by Farhan *et al.* (2021). Kali Jira and Kalonji seed oils had acid levels of 0.61±0.4 mg KOH/g and 0.53±0.3 mg KOH/g respectively, as reported in Table 4. The findings are greater than Sultan *et al.* (2009) findings and substantially lower than Farhan *et al.* (2021). The high acid value indicates that the oil was mostly hydrolyzed by enzymes. The high acidity of the oil may be due to the nature of the *N. sativa* seed, which, like many other oil-bearing seeds like olive, palm, and rice bran, contains high acidity oils (Patterson, 1989).

Table 4. Physicochemical parameters of black cumin seed varieties.

Physicochemical parameter	Kali Jira	Kalonji
Specific Gravity (g/cm^3)	0.91768	0.9079
Iodine value (g/100 g)	118±0.922	116±0.132
Saponification Value	192.6±1.81	189.8±1.80
Acid Value (mg KOH/g)	0.61±0.4	0.53±0.3

3.5 Functional groups in oil

A Perkin-Elmer FTIR spectrometer was used to capture the infrared (IR) spectrum. The IR spectra were recorded using about 1 mg of the isolated chemical as KBr pellets (frequencies between 4000 and 400 cm^{-1}). By comparing the vibration frequencies in wavenumbers of the sample spectrograph generated from an FTIR spectrophotometer with those of an IR correlation chart, the functional groups present in the essential oil were determined. Both oil's FTIR spectra appear to be quite similar. The range of wave number 1740 – 1760 cm^{-1} esterified carboxyl group C=O, from 1600 – 1650 cm^{-1} asymmetric carboxyl, stretching, and the peak is 1630 - 1650 cm^{-1} free carboxyl group compound. Alkenes C=C stretch were found in a medium-weak band between 1680 - 1600 cm^{-1}. The carbohydrate radical from the triglyceride structure of oils has a wavenumber of 1230 – 700 cm^{-1}. The bands at 1130, 1180, and 915 cm^{-1}, which correspond to the stretching vibration of the C-O ester group, are visible in the spectra of selected oils. But from the 1500 – 400 cm^{-1} fingerprint region, a significant absorption band between 900 cm^{-1} and 675 cm^{-1} suggested aromatic C=C. Both oils appear to have identical FTIR spectra. However, a close examination of the FTIR spectra of both oils reveals some significant differences in the number of peaks in the region 3100 – 2800 cm^{-1} (assigned with) or peak intensities at frequencies of especially at 2982 and 3000 cm^{-1}, as shown in Figures 1 and 2, except for the above peak Kali Jira and Kalonji black cumin essentials, which were almost identical functional group peaks. The characteristic wavelength of the commercial black cumin lies in the wavelengths between 400 and 4000 cm^{-1} (Perkin Elmer 1600 Spectrometer, USA). The free and esterified carboxyl groups are indicated by the carbonyl bands at 1630 - 1650 and 1740 - 1760 cm^{-1}, respectively (Gnanasambandam and Proctor, 2000; Kittiphoom, 2001). The intensities and band size of the esterified carboxyl groups will increase as DE levels rise. This could be used to compare the various black cumin seed oils. The ether (R-O-R) and cyclic C-C bonds in the ring structure of black cumin molecules were responsible for the absorption bands between 1100 and 1200 cm^{-1}. The FTIR spectra of black cumin (Kali Jira and Kalonji)

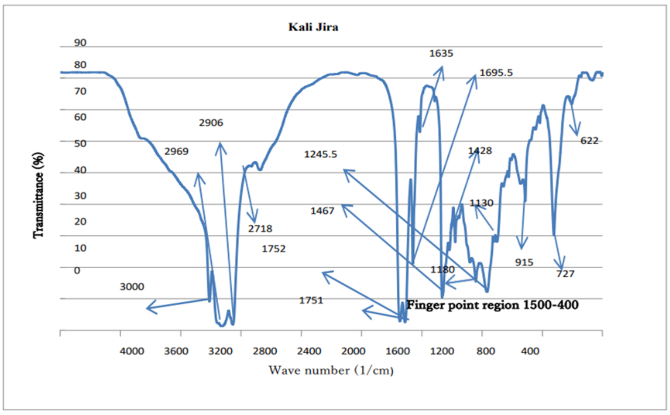

Figure 1. FTIR analysis of extracted oil from Kali Jira variety.

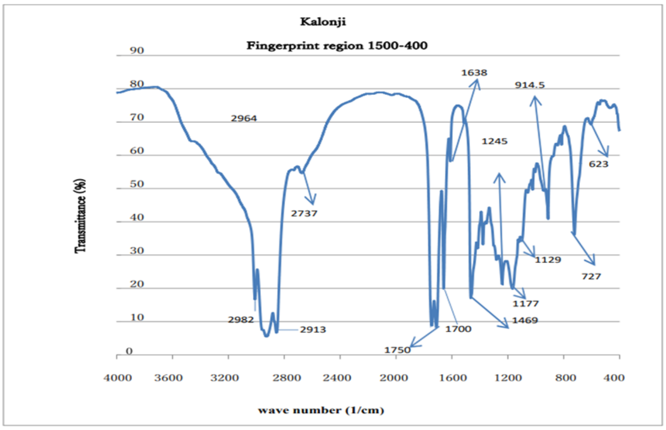

Figure 2. FTIR analysis of extracted oil from Kalonji variety.

samples obtained under varied extraction procedures showed no major structural differences. The structures of the indigenous black cumin seed oil (Kali Jira and Kalonji) were similar to the commercial black cumin sample. Because of absorbed moisture in the black cumin samples, the bandwidth ranged from 2400 to 3000 cm^{-1}.

4. Conclusion

Both types of *Nigella sativa* seeds (Kali Jira and Kalonji) have identical nutritional values, according to the research. Additionally, the extracted oil's physicochemical properties were better. As a result, the extracted oil falls into the essential unsaturated category. Furthermore, Kali Jira and Kalonji seeds can be used as food supplements that can help to avoid a variety of ailments.

Conflicts of interest

All authors declare that there are no conflicts of interest regarding the research.

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