Received: 22 February 2018 | Revised: 8 May 2018 | Accepted: 15 May 2018

DOI: 10.1111/jfbc.12590

FULL ARTICLE

WILEY Journal of Food Biochemistry

Chemical, pharmacological and nutritional quality assessment of black pepper (*Piper nigrum* L.) seed cultivars

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Abstract

The present research has been performed on black pepper of two cultivars to evaluate their nutritional quality and safety issue on the basis of proximate, chemical and pharmacological properties. The proximate results were compiled with the standard USDA limits. K, Ca, Mg, Na, Fe, Al and Se were detected as major elements, whereas the toxic elements were found within the recommended limit. The GC-MS analysis of the essential oils showed the presence of δ-3-carene (32.61%) as the major component in Kerala cultivar whereas β-caryophyllene (18.39%) was the major components in Indigenous cultivar. The IC_{50} values of DPPH antioxidant activity of the essential oils were found to be 44.16 and 22.88 mg/mL in Indigenous and Kerala cultivars, respectively. The antimicrobial activity of the essential oils showed good activity in both cultivars. The LC_{50} values of the brine shrimp cytotoxic activity were 1.03 and 1.21 μg/mL in Indigenous and Kerala cultivars, respectively.

Practical applications

Spice quality is an important issue in the food industries as well as export markets. Due to the toxicological effect of imported spices, much attention has been paid to the safety issue especially on cytotoxicity and toxic metal contaminations. The present study reveals that both cultivars possess rich amount of nutritional components, essential minerals, phyto and flavouring components in their essential oils and recommended level of toxic elements as well as decent antioxidant and antimicrobial properties. This study promises to scaffold a new window for exportation of quality spice and confer potential for use in food, nutraceutical and pharmaceutical industries.

KEYWORDS

antimicrobial, antioxidant, cytotoxicity, essential oil, mineral element, *Piper nigrum* L.

Abbreviations: AAS, Atomic Absorption Spectrometry; AOAC, Association of Official Analytical Chemist; ASA, Ascorbic acid; BHT, tert-butyl-1-hydroxytoluene; DMSO, Dimethyl Sufoxide; DWD, Dry Weight Basis; GC-MS, Gas Chromatography and Mass Spectroscopy; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; NA, Nutrient Agar; PDA, Potato Dextrose Agar; RI, Retention Index (Kovalts index); Rt, Retention time.

1 | INTRODUCTION

Spices and culinary herbs play an important role in the civilization and the economy of nations. The flavour and pungency of spices make the dishes palatable and delightful in food preparation. In addition, spices are reported to have indispensable medicinal and pharmacological properties (Parthasarathy et al., 2008). *Piper nigrum* L. (Piperaceae) commonly known as black pepper (Bengali name Gol morich) belongs to genus Piper. The genus contains more than 1000 species. Various species of the Piper genus are important for the medicinal and aromatic constituents and bioactive properties. Commonly, three different forms of *P. nigrum* seeds are available in the market such as black, green and white peppers depending on the processing method and maturity level. Black pepper widely grows in the tropical and subtropical regions of the world. It is grown especially in the tropical evergreen forest of Southern India, Malaysia, Brazil, Indonesia and Sri Lanka. It is considered as the king of spices for its highest volume of international trade (Jelen et al., 2015). Black Pepper is used as food flavouring, preservative, medicinal and biochemical agents since ancient times. Traditionally, it has been used as a nerve tonic, relief of pain, atrophic arthritis, apathy, influenza and febricity. Nowadays, it is also used in perfumery and insecticide as a flavouring agent (Parthasarathy et al., 2008; Zachariah et al., 2005). It has also reported in treating respiratory disorders including asthma, cold fever, digestive disorder like chronic indigestion, intestine toxins and diarrhea as well as reported to have antimicrobial and antioxidant activities (Ao et al., 1998; Dorman & Deans, 2000; Kapoor et al., 2009; Ravindran & Johny, 2001). Moreover, it also displays favourable results by the Ames test of the mutagenic and carcinogenic properties (Deans, 2001). The quality of pepper depends on its characteristic flavour and pungency, chemical constituents and toxic level. These constituents make it commercially important in food, cosmetics, medical and technological application for its aroma flavour, antibacterial, antifungal and antioxidant action. More than 273 volatile constituents were reported by comprehensive GC-MS analysis (Cardeal et al., 2006). The essential or volatile oils of black pepper comprise of terpenoids, phenols, aliphatic and aromatic ester constituents. The most important reported essential oil components are α- and β-pinene, sabinene, myrcene, *p*-cymene, β-sesquiphellandrene, β-phellandrene, piperonal, dihydrocarvon, β-caryophyllene, caryophellene oxide and limonene. The alkaloid compound piperine has been isolated from crude extract which is the principle odorant component of the black pepper seed. Moreover, terpenoids and phenolic compounds in the essential oils have been reported for the antioxidant properties (Singh et al., 2004; Menon et al., 2000; Gopalakrishnan et al., 1993; Jagella & Grosch, 1999; Zachariah et al., 2005; Jirovetz 2002). Synthetic antioxidants have been used since a long time in food industries but some carcinogenic effects are reported as a safety issue in food industry. Therefore, this issue encouraged scientist to extract biologically active components from plant origins for alternative natural food antioxidants (Bakkali et al., 2008). Although, there is a great number of publications on the chemical constituents, antimicrobial, antioxidant and larvicidal properties of volatile oil, data pertaining to nutritional quality composition and safety assessments are scarce. Moreover, most of the published reports are on commercial spices and collected from the local spice shop without verification of source and origins are also very old imported seeds. Therefore, there is a need for comprehensive experimental and theoretical evaluation for its food value, medicinal as well as commercial importance in the present perspective. Moreover, standardization and quality control of spice product must comply some primary and major quality indices, such as identification of perfect species, foreign organic matter, moisture content, food value parameters (crude fibre, protein carbohydrate and food energy), ash parameters (total ash, sulphated ash, water soluble ash and acid insoluble ash), qualitative and quantitative chemical evaluation and toxicological studies. Therefore, the objective of this work was to determine the nutritional quality and safety profile of black pepper by comparative chemical studies of the oils and elements of two growing variety as well as to pharmacological assessments of the essential oils.

FIGURE 1 (a) *P. nigrum* L. seeds from Indigenous cultivar and (b) *P. nigrum* L. seeds from Kerala cultivar (India)

2 | MATERIAL AND METHODS

2.1 | Plant material

The black pepper seeds of indigenous cultivar were collected from Bangladesh Tea Research Institute (BTRI) (Sreemangal 3210, Moulvi Bazar District), Bangladesh and Indian spice market (Spice market, Jew Town, Kappalandimukku, Mattancherry Kochi, Kerala 682002, India). The samples were cleaned, freed from dirt and others specimen, air-dried in shade at room temperature, powdered by the ball mill and preserved in airtight high-density double lined polyethylene bag until analysis. The collected materials are depicted in Figure 1.

2.2 | Physico-chemical and proximate studies

The physical characteristics of the seeds (seed volumes, seed density, hydration capacity, hydration index and swelling capacity) were measured according to Khattak et al., (2006) & Zia-Ul-Haq et al., (2007) with slight modification. The physicochemical and proximate characteristics of the two cultivars were carried out with three replications by the standard methods (AOAC, 2005; British Pharmacopoeia 2004; USP, 2007). The gross carbohydrate and food energy (FE) contents were estimated by the following equation:

$$\text{Carbohydrate}(\%) = 100 - (\text{moisture} + \text{total ash} + \text{crude Protein} + \text{crude fiber} + \text{fats and oil})$$

$$\text{FE}(\text{kcal}/100\text{g}) = (\%\,\text{crude protein} \times 4) + (\%\,\text{fats and oils} \times 9) + (\%\,\text{charbohydrate} \times 4).$$

2.3 | Elemental analyses

2.3.1 | Reagents, water and standard

Nitric acid (HNO_3) of trace metal analysis grade, hydrochloric acid (HCl) 37% of BDH, analar grade and high purity de-ionized water from the Barnstead purification system were used throughout the study. Multi-element stock solutions containing 10 mg/L of each element were obtained from USA (Accu Trace TM Reference Standard).

ICP-MS tuning solution: Contains 10 ppb Ba, Be, Ce, Co, In, Pb, Mg, Tl and Th for instrument tuning and verification of performance.

*Metals stock standard for ICP-MS:*10 mg/L (Reference/Traceable) of metals Be, Bi, Cd, Cs, Cr, Co, Cu, Ga, In, Li, Ni, Pb, As, Se and Ag.

Preparation of working standard for calibration: Working standard were prepared of 1, 5, 10, 20 and 50 μg/L from 100 μg/L intermediate standard by 1% HNO_3 diluents for carrying out analysis.

2.3.2 | Ashing procedure and sample preparation for ICP-MS and AAS

A certain amount of moisture less sample powder was taken. Ash 1.0005 g. was taken for Indigenous cultivar and 1.0017 g. for Kerala cultivar. For Hg, oven dried 2.5031 g powder was taken for Indigenous cultivar and 2.5043 g for Kerala cultivar. Ashing and subsequent sample preparation were performed as per AOAC method (AOAC, 2005).

2.3.3 | ICP-MS instrument and operating condition

The elemental analyses were done by Varian UltraMass™ ICP-MS system (Varian Optical Spectroscopy Instruments, Melbourne, Australia). The plasma source was 99.998% argon (Carbagas 3097, Liebefeld, Bern, Switzerland). The instrument and operating conditions were as follows: Instrumental: Sampler cone: Nickel (0.5 mm orifice diameter), Skimmer cone: Nickel (1.0 mm orifice diameter); Plasma conditions: RF power: 1.40 kW, Plasma Ar flow rate: 18.0 L min^{-1}, Auxiliary Ar flow rate: 2.25 L min^{-1}, Sheath gas flow: 0.20 L min^{-1}, Nebulizer gas flow: 1.0 L min^{-1}, Sampling depth: 6.50 mm, Pump Rate: 5 rpm.

2.3.4 | Atomic absorption spectrometry (AAS) analyses

Mg, Fe, Mn and Ca were analysed by Flame Atomic Absorption Spectrometer (Varian AA 240 FS). Al was analyzed by Zeeman Atomic Absorption Spectrometer (Varian AA 240 Z) in graphite furnace and the total Hg was analyzed by cold vapor hydrate generation Atomic Absorption Spectrometer (Varian AA 220 FS) followed by the Varian operating manual. The metal stock standard for Mg, Fe, Ca, Al and Mn were 1000 mg/L (Reference/Traceable) and quantification limit for the elements were at ppm levels. The detection limit for Fe, Mn and Al were 0.027, 0.005 and 0.00196 mg/mL respectively. On the other hand, Hg stock standard was AR grade of equivalent 100 mg/L. The quantification limit for the total Hg was 0.01 g/L (ppb). All calibrated standard, quality control standard and check standard are traceable to National Institute of Standard and Technology (NIST). Recovery of quality spiked sample, duplicate samples and quality control sample were observed. The recovery ranges for each parameter were 100% ± 10%.

2.3.5 | Flame photometry analyses

The concentration of Na and K (Certified Reference Material) were analysed by Flame Photometer (Jenway PFP-7, England, UK). Standard Na^+ and K^+ solutions (1–5 ppm) were used in the serial dilution method for standard curve within linear calibration range and total quantities in solution of samples were calculated.

2.4 | Essential oil analysis

2.4.1 | Extraction of essential oils

Black pepper seeds were subjected to hydro-distillation using Clevenger's apparatus for 4 h. The extracted oils were dried over

anhydrous sodium sulfate to remove traces of moisture and stored in a refrigerator in the dark at 4°C until analyses.

2.4.2 | GC-MS analysis

The essential oils of black pepper seeds were analysed by Electron Impact Ionization (EI) method on GC-2010 Shimadzu Gas Chromatograph, coupled to a GC-MS QP 2010 plus Shimadzu Mass Spectrometer fitted with RTX–5 MS fused silica capillary column (Supelco Inc.) (30 m × 2.5 mm; 0.25 μm film thickness). The column temperature was 40°C (hold 2 min) to 220°C (hold 5 min) at the rate of 10°C/min, maintained with carrier gas helium at a constant pressure of 90 kPa (Acquisition parameters full scan; scan range 40–550 amu). The split ratio was 10. Mass spectra were taken at 70 eV.

2.4.3 | Identification of the compounds

The constituents of the essential oil were identified by retention indices under temperature-programmed conditions based on co-injection of homologous n-alkanes (C_6-C_{24}) on the RTX-5 MS capillary column. Compounds were identified by comparison of their mass spectra with those of the internal reference mass spectral NIST-107 library.

2.5 | FTIR spectrometric finger print profiling

The functional groups of *P. nigrum* seeds powder were profiled by FTIR Spectrometer (Shimadzu FTIR 8900) for the finger print identification. Pellet (KBr pressed disk) technique was used for this purpose. The spectral range was 4,600–400 cm^{-1}.

2.6 | In vitro antioxidant activity

The antioxidant activity of the *P. nigrum* essential oils were determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) by the Brand-Williams method (Brand-Williams 1995) with some modifications. The oils were dissolved in methanol and the applied dose of concentrations were 100 to 0.781 mg/mL by serial dilution technique. Ascorbic acid (ASA) and tert-butyl-1-hydroxytoluene (BHT) (200-1.562 μg/mL) were used as positive control. Briefly, 200 μL of a sample solution (extracts or control) at different concentration and 800 μL methanol (1.0 mL) were mixed with 1.0 mL of a DPPH solution. Vortexed the reaction mixture thoroughly and kept in the dark box for 25 minutes at the room temperature. The absorbance of the mixture was measured at 517 nm using an UV-Visible spectrophotometer (UV-VIS 1650, Shimadzu Corporation, Japan). The per cent of inhibitions were calculated from the equation:

$$\text{DPPH Scavenging activity}\,(\%) = \left(1 - \text{ABS}_{\text{sample}}/\text{ABS}_{\text{control}}\right) \times 100.$$

2.6.1 | IC_{50} value of the oils and standards

The actual decrease in absorbance was measured against controls. The data of IC_{50} values were transformed into a straight line by means of a trend line fit linear regression analysis by MS Excel version 7 Software for windows. The experiment was performed triplicate and the results were expressed as mean ± SD with 95% confidence interval in every case.

2.7 | Antimicrobial screening

The disc diffusion method (Bauer 1966) was used to test antimicrobial and antifungal activity against five Gram-positive, eight Gram-negative organisms and three fungi species. The bacterial and fungal strains were collected as pure culture from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The test samples were made by dissolving in calculated volumes of solvents separately and applied to sterile discs (6 mm diameter) at a concentration of 400 μg/disc and carefully dried to evaporate the residual solvents. The test material (disc containing) were placed on nutrient agar medium and seeded uniformly with test microorganisms. Standard antibiotic ciprofloxacin (5 μg/disc) discs and blank discs (impregnated with solvents) were used as positive and negative control respectively. The antimicrobial activity of the test samples were determined by measuring the diameter of the inhibited zone on the disc and expressed in millimeter.

2.8 | In vitro cytotoxic activity

In vitro cytotoxic activity was carried out on the Brine Shrimp nauplii by the lethality bioassay to detect the toxicity level of the black pepper essential oils (Meyer et al., 1982). The Brine Shrimp eggs were collected from local pet shops. In this experiment, eggs were hatched within 48 hours providing large number of larvae (nauplii in a tank with 3.8% w/v sea salt in distilled water) at 30°C in front of a lamp. The test samples (essential oil) were prepared by dissolving in DMSO (not more than 50 μL in 5 mL solution) and it was applied in 5 mL brine solution (3.8% NaCl in water) to attain concentrations of 0.039 to 10.0 μg/mL. A vial containing 50 μL DMSO diluted to 5 mL of brine solution was used as a control. Standard vincristine sulfate was used as positive control at the same concentration. Then matured shrimps (10-20 of each vial) were applied to all experimental and control vials. After 24 hours, the vials were inspected using a magnifying glass in front of lamp and the number of surviving nauplii in each vial were counted. The lethal concentrations of essential oil resulting in 50% mortality of the brine shrimp (LC_{50}) from the 24 hours counts and the dose-response data were calculated by MS Excel (version 7) software.

2.9 | Data analysis

The data analyses were carried out by MS Excel (version 7) software with the statistical significant (95% confidence interval).

3 | RESULTS AND DISCUSSION

3.1 | Physical characteristics

The physical characteristics of black pepper seeds (Table 1) show a considerable variation between two cultivars. Seed volume was found the higher in Kerala cultivar than Indigenous cultivar. On the other hand, seed density, hydration capacity, hydration index, swelling capacity and swelling index were found higher in Indigenous cultivar than the Kerala cultivar. The difference observed of the two cultivars may be due to geographical conditions, harvesting, drying and different temperature condition during storage. So far, we know, these experiments have been done for the first time of these seed cultivars. These are important parameters of the seed quality and also for shelf life. High hydration as well as swelling capacity causes the growth of mold in the storage environment. This result may be helpful to identify the physical status of seed and quality before storage for long time along with commercial aspect.

TABLE 1 Physical characteristics of black pepper cultivars

Parameters	Indigenous cultivar	Kerala cultivar
Seed Volume (mL/g)	1.1978 ± 0.0006	1.1989 ± 0.0001
Seed Density (g/mL)	0.8363 ± 0.0008	0.8348 ± 0.000
Hydration Capacity (g)	0.5272 ± 0.0009	0.2664 ± 0.0004
Hydration Index	0.5262 ± 0.0006	0.2663 ± 0.0004
Swelling Capacity (mL/g)	0.5822 ± 0.0002	0.1998 ± 0.003
Swelling Index	0.4860 ± 0.0003	0.1666 ± 0.002

Notes. Each value represents the average value from three experiments (n = 3). Mean ± SD, p < 0.05 (95% of confidence interval) on fresh weight basis.

3.2 | Proximate properties

Proximate composition of *P. nigrum* seeds of two cultivars were calculated in fresh weight (FWB) and dry weigh basis (DWB) and shown in Table 2. The moisture, total ash, acid in-soluble & water-soluble ash and carbohydrate contents of the analysed seeds were higher in the Kerala cultivar than the Indigenous cultivar. On the other hand, protein, crude fiber, fatty acid, essential oil and food energy were found to be higher in Indigenous cultivar than Kerala cultivar. No significant differences were observed between the cultivars in the carbohydrate and essential oil contents. Our results are almost similar and somewhat varied

TABLE 2 Proximate composition of *P. nigrum* seed cultivars (g/100 g)

Parameters	Indigenous cultivar	Kerala cultivar	[c]USDA limit
Moisture[b]	11.48 ± 0.01	12.09 ± 0.02	10.510
Dry mater	88.52 ± 0.01	87.91 ± 0.03	
Organic matter	95.92 ± 0.01	94.45 ± 0.01	
Total ash	4.08 ± 0.00[a]	5.55 ± 0.01[a]	
	3.61 ± 0.00[b]	4.88 ± 0.00 [b]	4.330
Acid in-soluble ash	0.0952 ± 0.00	0.1282 ± 0.00	
Acid soluble ash	3.9797 ± 0.00	5.4217 ± 0.00	
Water in-soluble ash	2.0108 ± 0.00	2.7369 ± 0.00	
Water soluble ash	2.064 ± 0.00	2.813 ± 0.00	
Nitrogen	2.69 ± 0.01 [a]	2.50 ± 0.04 [a]	
	2.38 ± 0.01 [b]	2.19 ± 0.03 [b]	
Protein	16.86 ± 0.07 [a]	15.64 ± 0.25 [a]	
	14.92 ± 0.07 [b]	13.74 ± 0.22 [b]	10.950
Crude fiber	14.33 ± 0.005 [a]	12.94 ± 0.02 [a]	
	12.68 ± 0.003 [b]	11.38 ± 0.02 [b]	
Fatty acid	8.20 ± 0.008 [a]	7.89 ± 0.07 [a]	
	7.27 ± 0.008 [b]	6.94 ± 0.06 [b]	3.26
Essential oil	2.42 ± 0.01 [a]	2.31 ± 0.04 [a]	
	2.15 ± 0.01 [b]	2.03 ± 0.04 [b]	
Carbohydrate	46.06 ± 0.08 [a]	46.93 ± 0.15 [a]	
	40.77 ± 0.05 [b]	41.25 ± 0.13 [b]	64.810
Food energy (kcal/100 g)	316.80 ± 0.07 [a]	312.31 ± 0.38 [a]	
	280.43 ± 0.09 [b]	274.55 ± 0.33 [b]	255.0

Notes. Each value represents Mean ± SD (n = 3), p < 0.05 (95% of confidence interval).
[a]On dry weight basis, [b]on the fresh weight basis. [c]USDA (1977) Agricultural Handbook 8–2.

from the reported data. The results of dry mater (88.52), protein (14.92), fat (7.27) and acid soluble ash (3.9797) (g/100 g) were found to be higher in Indigenous cultivar and ash (4.88) content in Kerala cultivar than the reported data but was observed to be lower in carbohydrate (65.75) and food energy (500) content (Pradeep, Geervani & Eggum, 1993; Pruthi, 1993). Similarly, the essential oil contents were reported to possess lower (1.10%–1.5%) proportion than the current studies (François et al., 2009; Jirovetz et al., 2002; Perakis, Louli & Magoulas, 2005). On the contrary, Kapoor et al., (2009) reported 2.6% oil of their studied sample which is slightly higher than the both cultivars. The variation of the proximate parameters of the two cultivars might depend on the variety, climatic variation and geographical difference and cultivation process. The level of carbohydrate was lower than that of reported data due to the higher levels of crude protein, crude fat and fiber in the seed samples of the both cultivars. By comparing to the USDA (United States Department of Agriculture) (2017) limit for moisture, fat, protein and food energy, it has indicated good quality of seeds as well as good nutritional source (Ravindran & Johny, 2001). The ash values indicate the rich source of essential minerals which was higher than the reported data. Moreover, the acid insoluble ash was found to be significantly lower which indicated poor silicate impurity as well as water soluble ash indicated soluble minerals contents present in the ash samples (Saleh-e-In et al., 2017). Dietary fibre is other important parameter in the digestion and absorption processes in the body (Cherbut et al., 1995). The protein content of Indigenous cultivar have higher value which indicates good quality protein and would be a suitable source for food formulations as well as considerable as export quality. Data of current studies have been reported for the first time for the proximate composition on the two cultivars of black pepper seeds, though some reports were published on the different cultivars and varieties. Overall, both the cultivars comply with the USDA quality parameters of proximate composition and can be recommended as quality seeds for the human diets in the different form of food preparation as well as make it commercial importance.

3.3 | Elemental composition

The elemental composition of black pepper seed of two cultivars were carried out for the essential and toxic elements and the results are shown in Tables 3 and 4 respectively. A total of 20 elements were detected which noted 15 elements are essential and 5 elements are toxic. The concentration of the detected elements were done by most sensitive ICP-MS, AAS and FP instruments and have been presented as dry (DWB) and fresh weight basis (FWB) within the significance level ($p \leq 0.05$). The relative standard deviation (RSD%) values for each elements were found to be ≤ 5%. In the present study, essential element of Ca and Mg were detected as highest level in Indigenous cultivar among the elements. On the other hand, K was determined and contained the highest level in the Kerala cultivar followed by Fe, Al, Na and Se. Other trace elements like V, Li, Cs, Ga, Co and Bi were found higher in Kerala cultivar in comparison to

TABLE 3 Essential elemental composition of black pepper cultivars

	Indigenous cultivar				Kerala cultivar				
Elements	Solution conc.	DWB	FWB	RSD (%)	Solution conc.	DWB	FWB	RSD (%)	Recommended limit
Li (μg/kg)	87.7275	178.87	158.33	0.79	79.8001	221.07	194.38	1.20	20.0 mg/L**
Be (μg/kg)	3.0933	6.30	5.58	5.44	0.5719	1.58	1.39	35.48	–
V (μg/kg)	38.4544	78.40	69.40	1.03	108.0987	299.46	263.31	0.47	-
Co (μg/kg)	20.9695	42.75	37.85	0.62	18.8198	52.13	45.84	1.12	0.2–0.3 mg/kg**
Ga (μg/kg)	10.0879	20.57	18.20	0.36	16.4735	45.63	40.12	1.22	–
Se (μg/kg)	335.2435	683.56	605.08	4.11	424.297	1175.42	1033.52	1.08	4.90*
Ag (μg/kg)	8.7289	17.78	15.75	0.65	2.3619	6.54	5.75	0.75	–
Cs (μg/kg)	22.9664	46.83	41.45	1.02	29.5733	81.92	72.03	0.58	–
Bi (μg/kg)	3.6709	7.48	6.62	2.07	3.6491	10.10	8.88	1.42	–
Al (mg/kg)	44.34	90.40	80.02	0.1	91.11	252.40	221.93	0.1	–
Fe (mg/kg)	67.86	138.36	122.48	0.1	124.2	344.07	302.53	0.1	9.71*
Ca (g/kg)	45300	9.23	8.17	0.1	2270	6.28	5.52	0.1	443.00*
Mg (g/kg)	1151	2.34	2.07	0.1	844	2.33	2.05	0.1	171.00*
Na (mg/kg)	76	154.96	137.18	0.1	58.5	162.06	142.50	0.1	20.00*
K (g/kg)	6850	13.96	12.36	0.1	7500	20.77	18.26	0.1	1.32*

Notes. ND: Not Detected, RSD: Relative Standard Deviation, $p \leq 0.05$., DWD: dry weight basis, FWB: Fresh weight basis, (μg/kg): ppb, (mg/kg): ppm,Dilution factor 0.05. Fe, Ca, Mg were done by AAS, Na, K by FP, other elements by ICP-MS.

*Recommended limit for black pepper (mg/100 g) by USDA, 2017

**Toxicity level of recommended intake Li (Aral & Vecchio-Sadus, 2008), Co (WHO, 2005)

TABLE 4 Toxic elemental composition of black pepper cultivars

Elements	Indigenous cultivar				Kerala cultivar				Permissible limit (mg/kg)*
	Solution conc.	DWB	FWB	RSD (%)	Solution conc.	DWB	FWB	RSD (%)	
As (μg/kg)	0.6515	1.328	1.17	78.17	1.7525	4.854	4.27	21.33	10.0
Cr (μg/kg)	130.396	265.875	235.35	1.15	144.9117	401.44	352.98	0.09	2.0
Pb (μg/kg)	0	ND	ND	1.69	57.7536	159.99	140.67	1.23	10.0
Cd (μg/kg)	5.5603	11.337	10.03	6.41	1.7236	4.77	4.19	2.36	0.3
Hg (μg/kg)	24.06	480.604	425.43	0.5	39.02	779.06	684.87	0.6	1.0

Notes. ND: Not Detected, RSD: Relative Standard Deviation, $p \leq 0.05$., DWD: dry weight basis, FWB: Fresh weight basis, (μg/kg): ppb, (mg/kg): ppm,Dilution factor 0.05. As, Cr, Pb, Cd by ICP-MS and Hg by AAS.
*WHO (2005)

the Indigenous cultivar. Moreover, Ag and Be were found in higher amount in Indigenous cultivar in comparison to Kerala cultivar. The present results are also comparable to the reported values. Ca (6.59, 4.96, 2.38, 2.63), Mg (1.80, 1.97, 1.48) and K (17.79, 18.44,10)(g/kg on dry basis) were reported to have lower than our present findings (Calle et al., 2013; Fathivand et al., 2017; Gupta et al., 2003; Lavilla et al., 1999; Ozcan & Akbulut, 2007). Na was reported higher values (208, 1892.12 mg/kg) (Fathivand et al., 2017; Ozcan & Akbulut, 2007) and Fe had the lower values 0.64, 60 and 89.24 mg/kg (Gupta et al., 2003; Lavilla et al., 1999; Ozcan & Akbulut, 2007) than the current findings. On the contrary, Fe (1264 mg/kg) was found to be higher (Calle, et al., 2013) than the present study. Moreover, Al was reported to be 275 mg/kg (Fathivand et al., 2017) which was quite high compared to values (70, 167.67 mg/kg) reported by Ozcan and Akbulut (2007) & Gupta et al., (2003). Li (1.44 mg/kg), V (2.80, 6.28 mg/kg) and Co (0.19, 0.3 mg/Kg) were also reported to have higher (Fathivand et al., 2017; Gupta et al., 2003; Ozcan & Akbulut, 2007; Sherif et al., 1980) than the present experiments. Se is an important element in considering to the biological point of view. Selenium (Se) is regarded as an antioxidant nutrient in the present world. It forms selenoprotein P, fatty acid binding protein, type 1 iodothyronine deiodinase metalloproteins as well as enzymes glutathione peroxidase which was found in higher content in the present study than the reported (4 μg/kg) data (Kumar & Krishnaswamy, 1997). Other essential elements play vital role in daily dietary intake as well as human metabolism. Elemental composition is a part of quality issue of the plant product because it affects the pharmacotherapeutic properties (Calle et al., 2013). Current results have complied with the USDA (2017) recommended limit of quality parameters of black pepper (Table 3) of both cultivars. Moreover, Li and Co are regarded as toxic elements in some cases at the elevated level though Co is a form of vitamin B_{12} which is active in physiological form and body required small amount (7-50 μg/day) (Lesniewicz, et al., 2006, Weir et al., 1999). Dairy products (0.50 mg Li/kg food), meat (0.012 mg Li/kg food), grains and vegetables (0.5–3.4 mg Li/kg food) are the major dietary sources of Li (Weiner, 1991). The daily Li intake of a 70 kg adult averagely between 0.65 and 3.1 mg/day (Schrauzer, 2002). The current results did not exceed the recommended permissible limit of those elements (WHO, 2005). These essential elements present in the black pepper may influence taste in the food preparation as well as provide essential nutrients that body unable to synthesize them but over intake may cause of toxic effect in the body. The variation of elemental composition of the plant and its different parts depends on the several factors that affect the biochemical synthesis in the plant body including type of plant, maturity, soil composition, climate, agricultural practices and harvesting (Saleh-e-In et al., 2017). Therefore, the observed variation is due to said factors in the elemental composition. The main parameters of quality spice are identity, avoiding the presence of foreign particle, agrochemicals, heavy metals and microbial contamination as well as aroma flavour (WHO, 1998).

In the case of toxic elements (Table 4), Hg was the highest toxic elements followed by Cr and As in the Kerala cultivar than Indigenous cultivar whereas, Cd was found higher in Indigenous cultivar than the Kerala cultivar. Pb was not detected in Indigenous cultivar but found in Kerala cultivar in the present study. The investigation of the toxic elements was carried out by different researchers for different cultivar samples. As (0.36, 0.33–0.51 mg/kg), Cr (5.27, 11.19 mg/kg), Pb (0.30–0.34, 0.88 0.73–0.82, 0.17–0.82, 1.1) and Cd (0.79, 0.15–0.32,0.01–0.07, 1.16 mg/kg) were reported to have higher values than our current study (Sherif et al., 1980; Blagojević et al., 2015; Ozcan & Akbulut, 2007; Abou-Arab & Abou-Donia, 2000; Ziyaina et al., 2014; Krejpcio, Krol & Sionkowski 2007). Moreover, Cr (0.03, 0.3 mg/kg) content reported to have low amount (Seddigi et al., 2016; Sherif et al., 1980). The content of Hg (0.96 mg/kg) was reported by Ndelekwute et al., (2014) which was higher than the present study. Nevertheless, the present result of toxic elements also complied with WHO (2007) established data for herbal medicine and found the below level of the analysed toxic elements. Spices can be contaminated by air, water, soil, fertilizer and pesticide residues. Therefore, consumption of spices may be a potential threat to human health through food chain by metal contamination especially heavy metal or toxic metals without knowing the quality parameters. On the other hand, safety issue of herbal product is a major factor of every stage in herbal drug preparation. Therefore, this study will provide important information regarding food safety as well as assess its quality issue in the commercial aspect.

TABLE 5 Essential oil composition (w/w) of *Piper nigrum* seed cultivars

Compounds	R.I	Retention time		Composition (%)	
		Indigenous cultivar	Kerala cultivar	Indigenous cultivar	Kerala cultivar
δ-Carene[a]	919	11.132	11.135	0.434	0.756
β- Pinene[a]	943	8.437	8.436	13.618	13.204
α- Pinene[a]	948	7.386	7.382	16.685	7.350
δ-3-Carene[a]	948	9.253	9.261	9.228	32.611
Myrcene[a]	958	8.777	8.781	2.896	3.108
β-Phellandrene[a]	964	8.359	–	3.167	ND
α-Phellandrene[a]	969	9.102	9.107	2.877	8.889
D-Limonene[a]	1018	9.713	9.705	16.168	15.222
m-Cymene[a]	1042	–	9.599	ND	3.446
β-Linalool[b]	1082	11.370	–	0.348	ND
α-Copaene[c]	1221	17.441	–	3.139	ND
δ-Elemene[c]	1377	16.627	16.627	2.871	0.903
β-Elemene[c]	1398	–	17.733	ND	0.488
α-Muurolene[c]	1440	19.819	–	0.925	ND
δ-Cadinene[c]	1469	20.235	–	1.361	ND
β-Selinene[c]	1469	–	19.616	ND	0.720
β-Caryophyllene[c]	1494	18.388	18.352	18.393	10.661
β-Humulene[c]	1574	–	19.771	ND	0.500
cis, cis, cis-1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene[c]	1579	18.995	18.993	3.439	2. 143
δ-Cadinol[d]	1580	18.505	–	4.451	ND
Total (%)				100	100
Compounds Identified (%)					
Monoterpene hydrocarbons[a]				65.073	84.586
Oxygenated monoterpenoid hydrocarbon[b]				0.348	ND
Sesquiterpene hydrocarbons[c]				30.128	15.414
Oxygenated sesquiterpene hydrocarbons[d]				4.451	ND

Notes. RI = Retention Index, determined with reference to a homologous series of normal alkanes on RTX-5 MS column. Minimum detection limit is 0.001%,ND stands for not detected.

3.4 | Essential oils analysis

The isolation of essential oils was carried out by hydro-distillation method. The oils were found transparent, colorless and lighter than water with characteristics pungent spicy odour, bitter taste and freely miscible most of the organic solvents. The compositional analysis of black pepper samples of two cultivars were done by GC-MS. The results of oil constituents are summarized in Table 5 and the GC-MS spectra of the corresponding peaks are shown in Figure 2. The characteristic odour and known biological activities of the individual components are displayed in Table 6. A total of 21 compounds were identified and quantified in the oils. Among the identified compounds, 17 and 13 components were detected from Indigenous and Kelara cultivar respectively that contributed 100% of the total weight. In the current experiment, monoterpene hydrocarbons contained 65.073% and 84.586%, oxygenated monoterpenoid hydrocarbons 0.348% and 0%, sesquiterpene hydrocarbons 30.128% and 15.414% and oxygenated sesquiterpene hydrocarbons 4.451% and 0% in Indigenous and Kelara cultivars respectively. The major components in Indigenous cultivar were β-caryophyllene (18.393%), α-pinene (16.685%), D-limonene (16.168%), β-pinene (13.618%), *cis*, *cis*, *cis*-1,1,4,8-tetramethyl-4,7,10-cycloundecatriene (3.439%) and δ-elemene (2.871%), those are higher than Kerala cultivar. δ-3-carene (32.61%) was the highest component in Kerala cultivar followed by α-phellandrene (8.889%) and myrcene (3.108%) than the indigenous cultivar. In case of β-phellandrene, β-linalool, α-muurolene, δ-cadinene, α-copaene, torreyol and δ-cadinol, these compounds were absent in Kerala cultivar. Several studies have been published regarding the oil composition of the black pepper oils and found the variation in the major components. α-pinene, β-pinene, D-limonene, α-phellandrene and β-caryophyllene have complied with different cultivars those were reported as predominant components (Gopalakrishnan et al., 1993; Jagella & Grosch, 1999; Jirovetz et al.,

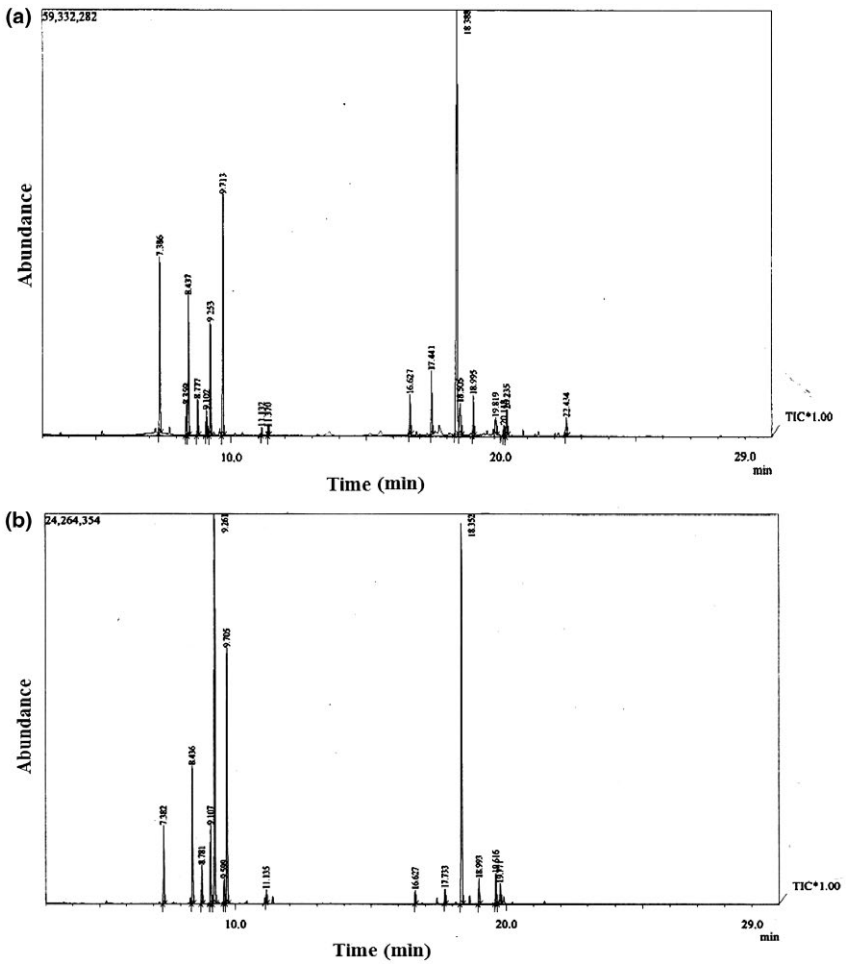

FIGURE 2 GC-MS chromatogram of *P. nigrum* L seed essential oil. (a) Essential oil from Indiginous cultivar and (b) Essential oil from Kerala cultivar.

2002; Kerscher & Grosch, 1997). Moreover, β-caryophyllene (57.6%, 12.8%, 15.96, 29.9, 8.91, 2.36, 16.6), α-pinene (3.3%, 5.6%, 9.12%, 4.5, 2.15, 2.15, 21.11), D-limonene (8.8%, 14.7, 24.07, 13.2, 6.32, 6.30, 11.11), δ-3-carene (18.5%, 27.85, 4.7, 4.34, 4.30), β-pinene (6.7%, 2.11, 7.9, 4.21, 4.20, 11.1) and δ-cadinene (1.3, 2.14, 2.15) were reported as prominent terpenoids (François et al., 2009; Kapoor et al., 2009; Kumoro, Hasan & Singh, 2010; Lewis et al., 1969; Mageed et al., 2011; Perakis, Louli & Magoulas, 2005; Politeo, Jukic, & Milos, 2006). Besides these, β-caryophyllene (25.8, 18.64%), δ-3-carene (15.6, 8.56%), D-limonene (12.2, 14.95%) and β-pinene (8.4, 9.71%), were also reported as the highest constituents in hydro-distilled oil (Bagheri et al., 2014; Jelen & Gracka, 2015). In composition to the 17 cultivars of Indian variety, it was reported that monoterpene hydrocarbons ranged from 69.4% to 85%, sesquiterpene hydrocarbons from 15% to 27.6% and the rest were oxygenated constituents. The major components were α-pinene (12.8%), β-pinene (35.5%), D-limonene (31.1%) and β-caryophyllene (22.4%) (Lewis et al., 1969). The current experimental data regarding the Kerala cultivar was found to be lower than the reported data. Furthermore, δ-3-carene (32.611%) in Kerala cultivar and *cis*, *cis*, *cis*-1,1,4,8-tetramethyl-4,7,10-cycloundecatriene in Indigenous cultivar have been found to be in higher amount in the oils and not reported previously. Zachariah (1995) reported α-pinene (3.8%–16.6%), D-limonene (3.6%–21.2%) and β-caryophyllene (11.8%–41.8%) as prominent components by evaluating 42 accessions of black pepper (*P. nigrum*) germplasm for essential oil and chemical constituents. Some variations were observed in the chemical composition of the black pepper oils by different researchers due to the difference of cultivation areas, geographical factors, maturity and nutritional status of the plant body, different chemotype, different methods of oil isolation as well as various co factor (Kapoor et al., 2009). Moreover, the indigenous sample was collected from the eastern part of Bangladesh which is a very suitable and popular area for tea plantation. On the other hand, oil of Kerala cultivar was collected from spice market Kerala (India). Therefore, the variations can be explained by the aforesaid factors. Nevertheless, the present studies are in good agreement with previously reported data.

3.5 | IR assignments

The IR vibrational band assignments of black pepper powder of two cultivars are summarized in Table 7 and their corresponding spectra are shown in Figure 3. The sharp broad band at 3367 and 3328 cm^{-1} are assigned for the O–H stretching vibration due to the phenolic and alcoholic OH groups (Colthup et al., 1964;

TABLE 6 Bioactivities and sensory properties of compounds detected from *P. nigrum* seeds

Compounds	Sensory properties	References	Bioactivities	References
δ-Carene	Floral	Miyazawa et al. (2012)	Anticancer	Bordoloi et al. (2017)
β- Pinene	Dry-woody, pine-like, resinous-terpene-like, spicy	Jirovetz et al. (2002)	Antibacterial	Leite et al. (2007)
			Antifungal	Hammer et al. (2003)
			Cytotoxic (human tumor cells)	Ramos et al. (2014)
			Analgesic	Liapi et al. (2007)
			Antidepressants	Guzmán-Gutiérrez et al. (2012), Guzmán-Gutiérrez et al. (2015)
			Antinoxious ffect	Liapi et al. (2007)
			Antioxidant	Kelen and Tepe (2008)
			Insecticidal	Yang et al. (2004)
			Anti-inflammatory	Ocete et al. (1989)
α-Pinene	Pine-like, sharp, woody, turpentine-like	Jirovetz et al. (2002)	AChE inhibition	Lomarat et al. (2015), Perry et al. (2000)
			Antibacterial	Leite et al. (2007)
			Antinociceptive (analgesic)	Him et al. (2008), Orhan et al. (2006), Santos et al. (1998)
			Cytotoxic (human tumor cells)	Ramos et al. (2014)
			Anti-inflammatory	Gil et al. (1989)
			Bronchodilatory in humans	Falk et al. (1990)
			Antidepressants	Guzmán-Gutiérrez et al. (2012)
			Anti-inflammatory	Ocete et al. (1989)
δ-3-Carene	Sweet, refined-limonene-like, spicy	Jirovetz et al. (2002)	Antibacterial	Montanari et al. (2012)
			Cytotoxic (human tumor cells)	Carvalho-Freitas and Costa (2002)
			Anti-inflammatory	Ocete et al. (1989)
Myrcene	Mild, sweet, balsamic, plastic-note	Jirovetz et al. (2002)	Protective cardiac effect (oxidative and histological damage)	Burcul et al. (2016)
			Analgesic	Lorenzetti et al. (1991), Rao et al. (1990)
			Sedatiive	do Vale et al. (2002)
β-Phellandrene	Peppery, minty, refreshing, citrus-like	Jirovetz et al. (2002)	Genotoxic	Cheng et al. (2017)
			Acetylcholinesterase	Bonesi et al. (2010)
α-Phellandrene	Herbaceous, minty, peppery-woody, fresh, citrus	Jirovetz et al. (2002)	Larvicidal	Cheng et al. (2009)
			Antioxidant	Dorman et al. (2000)
			Antibacterial	Iscan et al. (2012)
			Antifungal	Iscan et al. (2012)
			Antidepressants	Piccinelli et al. (2015)

TABLE 6 (Continued)

Compounds	Sensory properties	References	Bioactivities	References
D-Limonene	Fresh, citrus-like, mild lemon-and orange-notes	Jirovetz et al. (2002)	Antimicrobial	Bei et al. (2015)
			Anti-oxidant and antidiabetic	Murali et al. (2013)
			Anti-inflammatory and anti-oxidant	Yu et al. (2017)
			Anticancer	Elegbede et al. (1984), Elson et al. (1988), Maltzman et al. (1989), Wattenberg (1983)
			Immunostimulant	Komori et al. (1995)
			Anxiolytic	Carvalho-Freitas and Costa (2002)
			Analgesic	De Sousa et al. (2007
			Antidepressants	Piccinelli et al. (2015
m-Cymene or β-Cymene	Not available		Not available	
β-Linalool	Fresh, floral, clean, sweet, lemon-notes	Jirovetz et al. (2002)	Anti-oxidant and antimicrobial	Zengin et al. (2014)
			Anticancer	Sun et al. (2015), Chang et al. (2015)
			Anti-oxidant	Celik and Ozkaya (2002), Seol et al. (2016
			Anticonvulsant	De Sousaa et al. (2010), Elisabetsky et al. (1995)
			Anti-infllammatory	Peana et al. (2002)
			Anti-oxidant and cytotoxicity	Liu et al. (2012)
			Antimicrobial	Herman et al. (2016)
			Antidepressants	Guzmán-Gutiérrez et al. (2012)
			Anti-anxiety	Russo (2001)
			Sedative	Buchbauer et al. (1993)
			Anesthetic	Re et al. (2000)
			Analgesic	Peana et al. (2006)
			Antidermatophytic	Houel et al. (2014)
α-Copaene	Wood, spice, earthy	Souza et al. (2016)	Antidermatophytic	Houel et al. (2014)
			Antibacterial	Solıs et al. (2004)
			Antioxidant and cytotoxic activity	Turkez et al. (2014)
δ-Elemene	Wood	Souza et al. (2016)	Anticancer	Wang et al. (2005), Guan et al. (2014)
			Anti-oxidant	Guerrini et al. (2016)
β-Elemene	Herb, wax, fresh	Souza et al. (2016)	Anticancer	Zhang et al. (2015)
			Reduce atherosclerosis progression	Zhong et al. (2015)

TABLE 6 (Continued)

Compounds	Sensory properties	References	Bioactivities	References
α-Muurolene	Woody, green	Miyazawa et al. (2012)	Anti-oxidant	Guerrini et al. (2016)
δ-Cadinene	Thyme, wood	Souza et al. (2016	Anticancer	Hui et al. (2015)
			Acaricidal	Guo et al. (2017)
			Anti-oxidant	Guerrini et al. (2016)
β-Selinene	Orange, Herb	Rega et al. (2004), Souza et al. (2016)	Antioxidant	Chandra et al. (2017)
β-Caryophyllene	Woody, spicy, terpene-notes	Jirovetz et al. (2002)	Anticancer	Dahham et al. (2015)
			Antimicrobial	Dahham et al. (2015)
			Local anaesthetic anticancer	Ghelardini et al. (2001)
			Synergistic	Legault and Pichette (2007)
			Anti-oxidant	Lourens et al. (2004), Singh et al. (2006)
			AChE inhibition	Lomarat et al. (2015)
			Anti-malarial	Campbell et al. (1997)
			Antidepressants	Bahi et al. (2014)
β-Humulene	Not available		Non-steroidal anti-inflammatory agent	Rayne, 2011)
cis, cis, cis-1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene	Not available		Not available	
δ-Cadinol	Herb	Acree and Arn (2004)	Not available	

TABLE 7 IR bands and their assignment of black pepper seeds powder

	Indigenous Cultivar		Kerala Cultivar	
Peak No.	IR bands at cm^{-1}	Assignment	IR bands at cm^{-1}	Assignment
1	3367.5	$\upsilon_{O-H,}$ Hydrogen bonded alcohols, phenols	3328.9	$\upsilon_{O-H,}$ Hydrogen bonded alcohols, phenols
2	2923.9	υ_{C-H} (aliphatic), CH_2 of alkanes	2923.9	υ_{C-H} (aliphatic) alkanes
3	2852.5	υ_{C-H} of $-OCH_3$ (ether) or O-CH_2–O group of alkanes	2852.5	υ_{C-H} of $-OCH_3$ (ether) or O–CH_2–O group of alkanes
4	2378.1	υ_{N-H} tertiary amino salt	2376.1	υ_{N-H} tertiary amino salt
5	2154.3	$\upsilon_{C=C}$ alkynes (weak)	2343.4	υ_{N-H} tertiary amino salt
6	1635.5	$\upsilon_{C=C}$ of benzene (aromatic) or O–C=N stretching	2169.8	$\upsilon_{C\equiv C}$ alkynes (weak)
7	1488.9	$\upsilon_{C=C}$ (aromatic and alkane system), υ_{C-C} band in alkenes	1635.5	$\upsilon_{C=C}$ of benzene (aromatic) or O–C=N stretching
8	1444.6	δ (CH_2) scissors deformation	1542.9	$\upsilon_{C=C}$ in aromatic or conjugated lactone.
9	1321.1	υ_{C-N} in amines	1488.9	$\upsilon_{C=C}$ (alkenes)
10	1251.7	$\upsilon_{=C-O-C}$ (ether, OCH_3), N–H in amine, -COOH	1446.5	δ (CH_2) scissors deformation
11	1020.3	υ_{C-C} band in carbohydrate or cycloalkane, υ_{C-N} for amine	1321.1	υ_{C-N} in amines
12	927.7	δ_{C-H} in plane bending, long chain alkenes or fatty oils	1251.7	$\upsilon_{=C-O-C}$ (ether, OCH_3), N–H in amine, –COOH
13	854.4	δ_{C-H} out of plain	1020.3	υ_{C-C} band in carbohydrate or cycloalkane, υ_{C-N} for amine
14	522.7	δ_{C-H} in plane	927.7	δ_{C-H} in plane bending, long chain alkenes or fatty oils
15		–	854.4	δ_{C-H} out of plain
16		–	524.6	δ_{C-H} in plane

Notes. δ: bending vibration, υ: stretching vibration.

Silverstein et al., 2005). The peaks at 2923 and 2852 cm^{-1} of both cultivars are due to C–H asymmetric and symmetric stretching of methylenedioxy group like piperine since piperine is one of the major constituent of black pepper (Deepthi et al., 2012). The strong band at 1635 cm^{-1} attributed to the C=C stretching vibration in the aromatic ring and conjugated diene or –CO–N stretching like piperine. The band at 1542 cm^{-1} assigned for C=C stretching due to the aromatic system or conjugated lactone. The sharp strong band at 1444 and 1446 cm^{-1} may be due to CH_2 scissors deformation (Barua et al., 2008). A medium band at 1321 cm^{-1} indicated for the amines groups in the samples (Silverstein et al., 2005). A clear and sharp band at 1251 cm^{-1} assigned for methylene ether (=C–O–C) asymmetric stretching may be due to ether group in the fatty acids chain. The strong IR band at 1020 cm^{-1} corresponding to –C–O stretching of carbohydrates (Silverstein et al., 2005) because black pepper contains high amount of carbohydrates in the proximate analysis. The band at near 854 cm^{-1} is due to C–H bending out of the plane for lipid and protein. Band at 522 and 524 cm^{-1} for C–H in plane bending (Silverstein et al., 2005). Most of the bands are resemble for the characteristics piperine alkaloid due to the high concentration in pepper seed.

3.6 | Antioxidant activity

The DPPH antioxidant activity of black pepper essential oils of the two cultivars exhibited significant dose dependent action that rapidly increase with the concentrations (0.781–100 mg/mL) oil and has been shown in Table 8. The inhibition of concentration (IC_{50}) of Indigenous cultivar was found to be 44.16 mg/mL with the highest inhibition of 88.38%, while Kerala cultivar showed 22.88 mg/mL with the highest inhibition of 95.71%. Both cultivars were shown to have remarkable weak activity than the positive controls ASA and BHT (IC_{50} 3.73 and 11.84 μg/mL) respectively. Politeo, Jukic, and Milos (2006) reported that the highest inhibition (61%) was observed at 50 mg/mL followed by 37% at 20 mg/mL, 22% at 10 mg/mL and 14% at 5 mg/mL which were higher than the Indigenous cultivar (46.09%) and lower than the Kerala cultivar (70.33%) at the concentration of 50 mg/mL. The variation of the antioxidant activity of the black pepper oils in both cultivars might have accounted for their chemical constituents. The monoterpene compounds limonene of lemon essential oil and β-caryophyllene of *Marrubium pergrinum* essential oil are reported by their strong antioxidant activity (Kaurinovic et al., 2010; Yang et al., 2010). β-caryophyllene was also reported to have strong

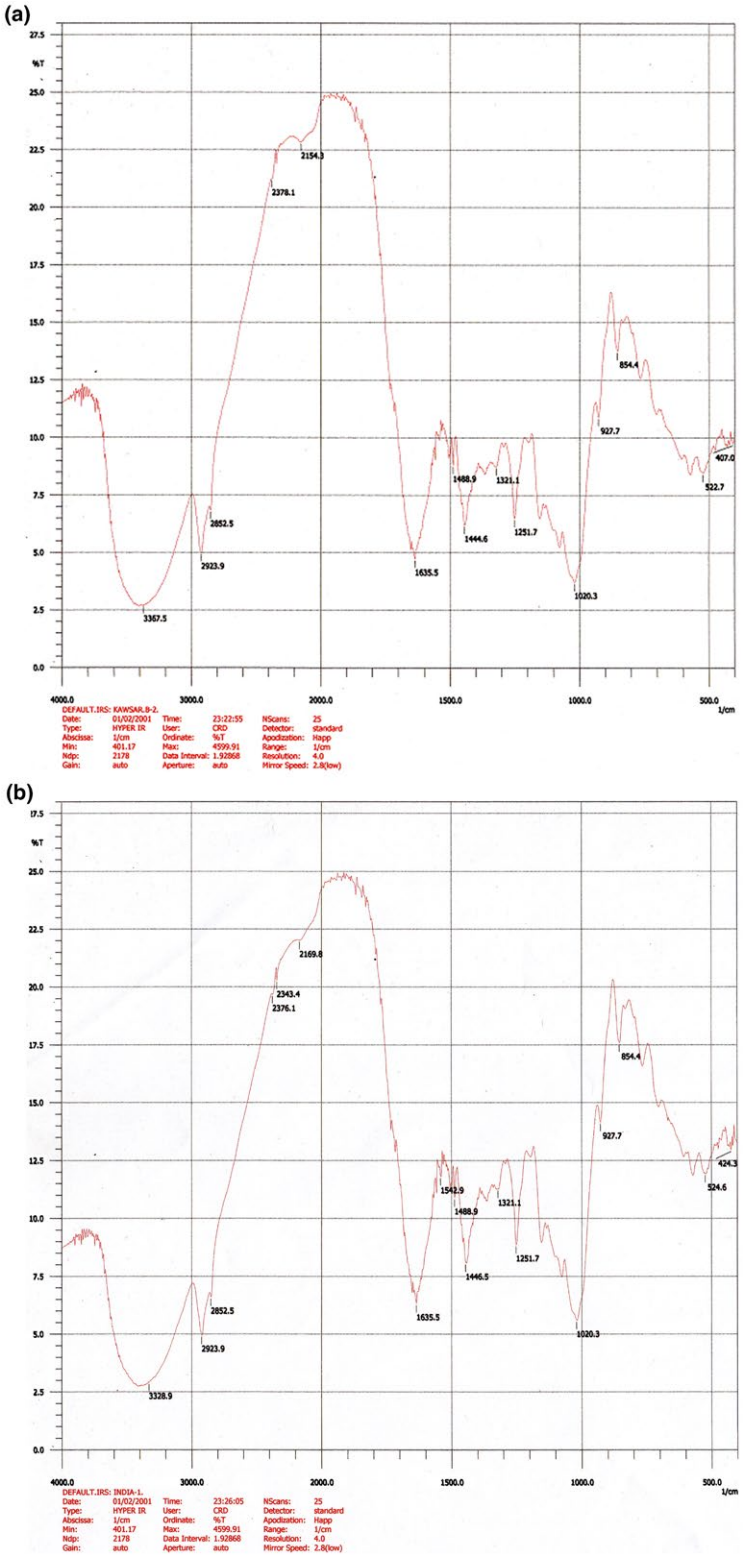

FIGURE 3 IR Spectrum of *P. nigrum* L. seed: (a) Indigenous cultivar), (b) Kerala cultivar

scavenging activities against hydroxyl radical and superoxide anion (Calleja et al., 2013). Generally, essential oils are a complex mixture of various chemical compounds. Therefore, the antioxidant action is responsible for their higher components present in the oil. On the other hand, natural compounds show antioxidant action synergistically in broad spectrum that produces active defense system against

TABLE 8 DPPH (2, 2-diphenyl-1-picrylhydrazyl) antioxidant activity of black pepper seeds essential oils

Assay concentrations (mg/mL)		100	50	25	12.5	6.25	3.125	1.5625	0.78125	IC_{50}
DPPH Inhibition (%)	Indigenous cultivar	88.38 ± 0.69	46.09 ± 0.11	31.39 ± 0.48	14.43 ± 0.41	8.08 ± 0.69	5.15 ± 0.18	1.35 ± 0.16	0.77 ± 0.67	44.16 ± 0.58 mg/mL
	Kerala cultivar	95.71 ± 0.18	70.33 ± 0.16	40.80 ± 0.53	21.51 ± 0.51	12.71 ± 0.43	8.01 ± 0.35	7.07 ± 0.20	4.87 ± 0.42	22.88 ± 0.008 mg/mL
Assay concentrations (µg/mL)		200	100	50	25	12.5	6.25	3.125	1.5625	IC_{50}
DPPH Inhibition (%)	ASA	99.05 ± 0.21	98.35 ± 0.17	90.71 ± 0.60	85.31 ± 0.60	79.79 ± 0.18	69.67 ± 0.68	37.26 ± 1.12	27.02 ± 1.19	3.73 ± 0.05 µg/mL
	BHT	93.02 ± 0.50	91.98 ± 0.19	85.51 ± 0.76	69.54 ± 0.60	55.87 ± 0.82	29.69 ± 0.97	25.63 ± 0.51	9.05 ± 0.51	11.84 ± 0.29 µg/mL

Notes. ASA = Ascorbic acid, BHT = tert-butyl-1-hydroxytoluene, Mean ± SD, $p < 0.05$ of independent sample T-test.

TABLE 9 Antimicrobial activity of the black pepper seed essential oils and standard

	Zone of Inhibition (mm)															
	Gram-positive bacteria					Gram-negative bacteria								Fungi		
Samples	*B.meg:*	*B.sub*	*S.aur*	*S.lut*	*B.ser*	*E.col*	*P.aur*	*S.par*	*S.typ*	*S.dys*	*S.boy*	*V.mim*	*V.par*	*C.alb*	*A.nig*	*S.cer*
Indigenous cultivar	7	8	9	9	8	8	9	8	8	9	8	8	8	7	8	8
Kerala cultivar	8	8	9	9	8	9	10	8	8	10	9	8	8	8	9	9
Ciprofloxacin	45	46	46	45	45	46	46	46	45	46	45	46	46	45	46	45

Notes. Essential oil (400 µg/disc), ciprofloxacin (5 µg/disc), Microorganisms: *B.meg: Bacillus megaterium, B.sub: Bacillus subtilis, S.aur:Staphylococcus aureus, S.lut: Sarcina lutea, B.ser: Bacillus sereus, E.col; Escherichia coli, P.aur: Pseudomonas aureus, S.par: Salmonella paratyphi, S.typ:Salmonella typhi, S.dys: Shigella dysenteriae, S.boy: Shigella boydii, V.mim: Vibrio mimicus, V.par:Vibrio parahemolyticus, C.alb:Candida albicans, A.nig: Aspergillus niger, S.cer: Sacharomyces cerevaceae.*

free radical attack (Kamal-Eldin & Appelqvist, 1996; Lu & Foo, 1995). In the present study, the essential oil contains predominant amount of limonene, pinene, phellandrene, β-caryophyllene, δ-3-carene and δ-cadinol. These constituents may able to show antioxidant action as synergistically or individually in the system. Moreover, the antioxidant power of the oils might have been increased by terpenoid and phenolic compounds though they identified as small amount in the studied oils. The better antioxidant action of the Kerala cultivar oil may be due to the synergistic effect of the constituents. Actually, it is difficult to give an exact explanation of the antioxidant action of the studied oils without assessment of individual components or combination due to lack of limitation or suitable scope of the present investigation. Nonetheless, the antioxidant action of the both oils might be responsible for both major constituents and also minor constituents for possible synergistic action.

3.7 | Antimicrobial activity

The antimicrobial activity of the essential oils showed weak activity against all tested strains in comparison to the standard antibiotic ciprofloxacin (Table 9). In the current study, the Gram-positive strains *S. aureus* and *S. lutea* showed moderate activity with the zone of 9 mm in both cultivars. In the case of Gram-negative strains, *P. aureus* and *S. dysenteriae* showed strong activity in both cultivars with the zone of 9–10 mm. On the other hand, fungi strains of *A. niger and S. cerevaceae* were found to display moderate activity with the zone of 9 mm. The present results are higher than reported data (Bag & Chattopadhyay, 2015; Nikolic et al., 2017) whereas, some oils were reported to have higher activity (Morsy & El-Salam, 2017; Singh et al., 2005). This variation is due to the major component present in the oils. Overall, both oils found to have best activity against Gram-negative bacteria than Gram-positive and fungi at the dose of 400 μg/mL of the tested oil. The oil contains predominant amount of monoterpenoid constituents. Therefore, the antimicrobial activity may be due to the major monoterpenes constituents, chemical structure of the compounds as well as the synergistic action. Generally, antimicrobial action is a dose dependent manner. In that case, it could be found more active by increasing oil concentrations. Moreover, the antibacterial activity depends on the cell wall composition of the bacterial species. Strong bioactive compounds can disrupt the lipid organization and cause cell leakage in bacterial cellular and mitochondria membranes. The Gram-positive bacteria create resistance to essential oil due to its thicker membrane also theicoic acid (Perigo et al., 2016). In the present study, the antimicrobial activity of the oils may be responsible for the α and β-pinene, δ-3-carene, D-limonene and β-caryophyllene due to their reported strong activity (Bei et al., 2015; Dahham et al., 2015; Leite et al., 2007; Montanari et al., 2012). Further studies would be helpful to identify the components which are responsible for antimicrobial action through in vivo studies.

TABLE 10 Cytotoxic effect on Brine Shrimp nauplii of black pepper essential oils

Assay concentrations (μg/mL)		0.039	0.078	0.156	0.3125	0.625	1.25	2.5	5	10	IC_{50}
Mortality (%)	Indigenous cultivar	9.14 ± 0.83	11.94 ± 1.73	22.24 ± 0.82	30.30 ± 1.55	32.63 ± 1.20	45.68 ± 0.40	48.71 ± 2.22	80.12 ± 1.62	100 ± 00	1.03 ± 0.07 μg/mL
	Kerala cultivar	7.56 ± 0.84	10.31 ± 3.17	20.22 ± 1.77	29.78 ± 1.11	31.31 ± 3.49	44.59 ± 2.60	49.76 ± 4.20	68.78 ± 4.66	100 ± 00	1.21 ± 0.21 μg/mL
	Vincristine sulphate	10.0 ± 00	20.0 ± 00	30.0 ± 00	40.55 ± 0.95	50.00 ± 00	60.51 ± 0.88	80.12 ± 1.62	90.85 ± 0.83	100 ± 00	0.50 ± 00 μg/mL

Notes. Each value represents Mean ± SD ($n = 3$), $p < 0.05$ (95% of confidence interval).

3.8 | Cytotoxic activity

Essential oils of two cultivars have shown significant cytotoxic activity on brine shrimp nauplii (Table 10). Indigenous and Kerala cultivar have exhibited potent activity with LC_{50} 1.03 and 1.21 μg/mL respectively with the highest mortality rate of 100% in comparison with vincristine sulphate (LC_{50} value 0.50 μg/mL). The negative control (DMSO) did not exhibit any mortality at the dose of 50 μL/mL. Bajracharya and Tuladhar (2011) reported the cytotoxic activity of black pepper oil and observed 6%, 36% and 100% mortality at the dosages of 10, 100 and 1000 μg/mL respectively with the LC_{50} 113.58 μg/mL. Moreover, Krishnaraju et al. (2006) reported the LC_{50} 30 μg/mL at the dose of 1–5000 μg/mL. In another report, the LC_{50} values were found to have 25.57–56.74 μg/mL on the cell line assay (Nikolic et al., 2017). The observed difference may be due to the chemical composition of the oils as well as synergistic action. GC-MS study of the oils showed the presence of δ-cadinene, β-caryophyllen, δ-elemen, δ-carene, α and β-pinene and δ-3-carene predominantly. These constituents have been reported to possess anticancer activities (Bordoloi et al., 2017; Dahham et al., 2015; Guan et al., 2014; Hui et al., 2015; Montanari et al., 2012; Ramos et al., 2014; Wang et al., 2005). The cytotoxic activity of the present study may be due to those components present in the oils. The brine shrimp cytotoxicity is a very simple bioassay technique for toxicity evaluation. It is not only pre idea for anti-tumor, anticancer, enzyme inhibition, pesticidal, trypanocidal and ion regulation activities but also it is very important regarding for the quality issue of the plant extract (Saleh-e-In et al., 2016). At the high doses, bioactive compounds and extracts are always toxic since the degree of mortality is directly proportional to the concentration of the applied extracts or pure compounds. The recommended toxicity level for plant extracts (LC_{50}) are: strong at 0–100 μg/mL, moderate at 100–500 μg/mL and weak at 500–1000 μg/mL (Oketch-rabah et al., 1999). On the other hand, Mayer et al., (1982) reported the toxicity level of the plant extracts which should be $LC_{50} \leq 1.0$ mg/mL. In the current investigation, the oils have exhibited toxic on brine shrimp larvae cells, on the basis of lethal concentration (LC_{50}) at certain dose level. The results suggested that, the oils can be used for flavouring of food and pharmaceutical agent at certain concentration level. Moreover, at early developmental stages, *A. salina* embryos are highly vulnerable to the toxins. Therefore, further in vivo acute toxicity studies need to undertake on mice to assess the toxicity level for the safety and quality requirements.

4 | CONCLUSIONS

The quality issue is a great concern of black pepper due to its extensive use as culinary spice and potential for the pharmaceutical preparation. The results of nutritional profile by the physiochemical and proximate analysis indicated potential source of protein, fibre, carbohydrate and food energy which are in good agreement with USDA quality requirements. Moreover, the essential oils of both cultivars have composed of mainly monoterpene and sesquiterpenes along with their oxygenated terpenes. The pharmacological importance of these compounds is well documented for their numerous activities. The antioxidant, antimicrobial and brine shrimp cytotoxic activities of the essential oils exhibited sigtnificant activity as compared to the standard drug. The cytotoxic activity of black pepper oils could be helpful for further investigating cytotoxic molecule as a source for new drugs in the field of cancer drug discovery. Moreover, the elemental composition showed a rich source of essential mineral elements as well as the low concentration of toxic elements which has complied with WHO recommendation levels. Generally, the daily consumption of spice by adults is very low. Therefore, the consumption of spices is not harmful as per recommended daily intake. The present investigations provide vital data of the two cultivars on the nutritional availability, necessity of the quality and safety profile pertaining to their consumption. Although in vitro studies are inadequate to evaluate safety and efficacy, however, further safety studies are required through in vivo studies before launching a final product. This investigation promises to be of great value pertaining to food quality, safety pharmaceutical formulation and might open up new possibilities in the domain of spice exportation.

ACKNOWLEDGEMENTS

Authors are thankful to the Director IFST and the Director BCSIR Laboratories, Dhaka, Bangladesh for giving the permission to conduct the research work.

CONFLICT OF INTEREST

The authors indicate no potential conflicts of interest.

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How to cite this article: Abukawsar MM, Saleh-e-In MM, Ahsan MA, et al. Chemical, pharmacological and nutritional quality assessment of black pepper (*Piper nigrum* L.) seed cultivars. *J Food Biochem*. 2018;42:e12590. https://doi.org/10.1111/jfbc.12590