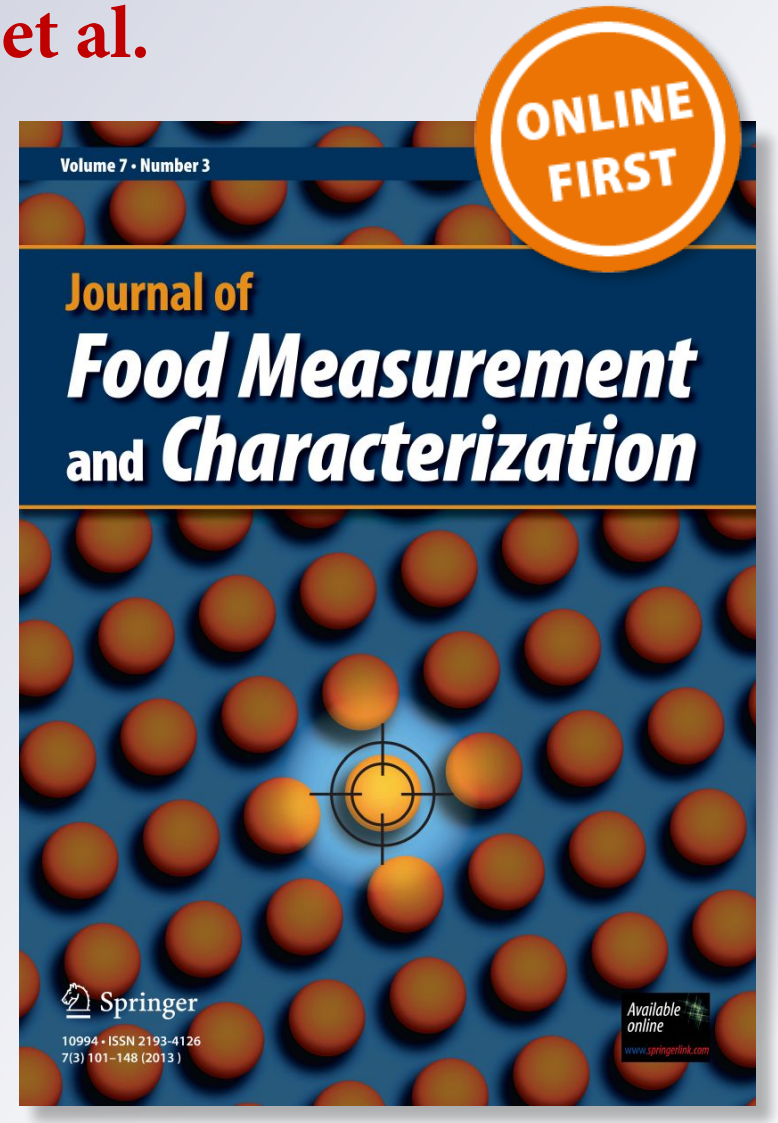
Characterization of quality and pharmacological assessment of Pimpinella anisum *L. (Anise) seeds cultivars*

Apu Ghosh, Md. Moshfekus Saleh#e# In, Mirza Md. Abukawsar, Md. Aminul Ahsan, Md. Matiur Rahim, Md. Nurul Huda Bhuiyan, et al.

Journal of Food Measurement and Characterization

ISSN 2193-4126

Food Measure
DOI 10.1007/s11694-019-00188-3



Springer

Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-019-00188-3

ORIGINAL PAPER

Characterization of quality and pharmacological assessment of *Pimpinella anisum* L. (Anise) seeds cultivars

Apu Ghosh[1] · Md. Moshfekus Saleh-e-In[2] · Mirza Md. Abukawsar[1] · Md. Aminul Ahsan[2] · Md. Matiur Rahim[3] · Md. Nurul Huda Bhuiyan[3] · Sudhangshu Kumar Roy[4] · Shamsun Naher[1]

Received: 2 March 2019 / Accepted: 6 June 2019

Abstract

Pimpinella anisum L. seeds of the two cultivars were assessed for their nutritional quality and safety assessment through proximate, chemical and pharmacological studies. The proximate composition along with rich mineral elements as well as minor level of toxic elements indicated the good quality of seeds. The GC–MS analyses of hydro distilled volatile oils of two cultivars contained 16 constituents. The main constituents in BSRC (Bangladesh Spice Research Centre) seeds were *trans*-anethole (83.67%), fenchone (5.29%) and 1,2-diisopropenylcyclobutane (6.31%) whereas, *trans*-anethole (69.94%), fenchone (11.184%) and D-limonene (13.007%) were the main constituents in local market seeds. The IC_{50} values of DPPH antioxidant activity showed at 48.71 and 52.48 mg/mL, in BSRC and local market seeds cultivars essential oils respectively. The antimicrobial activity of the essential oils showed moderate activity in both cultivars against Gram-positive, Gram-negative bacteria and fungi. Both oils had some similarity in physiochemical properties and antimicrobial activity. The brine shrimp cytotoxic activity of the essential oils exhibited weaker activity than the standard drug at 3.06 and 2.86 µg/mL (IC_{50}) in BSRC and local market seed cultivars respectively. The present investigation demonstrated good nutritional quality with rich bioactive phytoconstituents as well as low level of toxic elements of anise seeds. The research findings can open up a new possibility for exportation of indigenously cultivated spices as well as recommendations for food and pharmaceutical industries.

Keywords *Pimpinella anisum* L. · Anise seed · Essential oil · *p*-anethole · Elemental composition · Antimicrobial · Antioxidant · Cytotoxicity

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

✉ Md. Moshfekus Saleh-e-In
saleheen.sosthe@gmail.com

1 Department of Chemistry, Jagannath University, Dhaka 1100, Bangladesh

2 Institute of National Analytical Research and Services (INARS), BCSIR Laboratories, Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Dhaka 1205, Bangladesh

3 Food Toxicology Research Section, IFST, Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Dhaka 1205, Bangladesh

4 IFRD, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka 1205, Bangladesh

Introduction

Aromatic spices are the storehouse of several bioactive compounds and elements over a wide range of health impact in the human body [1–4]. Pungency and flavor of the spices are an important economic factor in the world trade and economy [5, 6]. Nonetheless, spices have played diverse role in medicine and pharmaceutical formulation [7, 8]. *Pimpinellaanisum* L. is a member of the genus *Pimpinella*

Published online: 10 July 2019

and belongs to the family Apiaceae is an economically important vegetable and condiment plant. It is well known as anise (Bangla name Mouri) and comprises 150 species of mostly distributed in the northern hemisphere. It is largely cultivated in Europe, South East Asian countries, Africa and South America [9–11]. The major anise producing countries are Spain, Mexico, Egypt, Italy, India, Bulgaria, Tunisia, Syria, and France [12]. In Bangladesh, it is mostly cultivated in Northern districts. Anise seeds are used as a food flavouring, preservative, medicinal, biochemical agents and vast spectrum applications in pharmaceutical industries. Its leaves are used in salads and in pickling. Traditionally, the seeds are used as carminative, diuretic, sedative, appetizers, and disinfectant, analgesic in migraine, increase milk production, seizure and in the treatment of epilepsy [13–16]. The pharmacological studies of the essential oil were also reported to have antibacterial, antifungal, antioxidant, insecticidal, antiviral, muscle relaxant of tracheal chain, anticonvulsant, analgesic and anti-diabetic activities [17–29]. Chemical studies of *P. anisum* essential oils have demonstrated that the anise seed comprises the predominant amount of *trans*-anethole, *cis*-isoeugenol, linalool, estragol, methyl chavicol and anisaldehyde [30–32]. Other minor components are β-caryophyllene, anisic acid (oxidation products of anethole), limonene, α-pinene, acetaldehyde, *p*-cresol, creosol, hydroquinine, β-farnasene, γ-himachalene and *ar*-curcumene [33, 34]. Carcinogenic effects are an important safety issue in the food and pharmaceutical industries. Therefore, this issue encouraged scientist to investigate biological activity of components for manufacturing safe product. The toxicity and cancerogenic effect of anethole are well investigated. The *cis*-isomer of anethole being 15–38 times more toxic than the *trans* (E) isomer to animals [35]. *cis*-Anethole (c.a. 0.3–0.4%) contained with *trans*-anethole in anise seeds which may not cause of toxicity but possible in daylight to form *cis*-anethole during storage period [33]. Moreover, the other issue is heavy metals toxicity, which is well known toxic roles in various biochemical reactions in human body [36]. Toxic heavy metals cause serious health risk like as kidney injury, renal failure and liver damage [37]. More than twenty elements are responsible for humane physiological activities. Among them, minor and trace elements are conjugated with proteins to form metalloproteins or small molecules like polyphenols, phosphates, phytates as well as chelating compounds. Also, these elements have important enzymatic function by transporting the bound proteins to their target sites in the human body [36, 38]. Several techniques are available to evaluate the elemental composition of the plants and foods. Nonetheless, the ICP-MS is a sensitive multi-element analysis technique which is widely using in plant, food and pharmaceutical industries. In this regards, the elemental analysis of the anise seeds varieties has been employed by Varian ICP-MS technique to assess the minor and trace elemental contents in the present study.

Numerous spices are commonly consumed by the Bangladeshi people for their daily culinary purposes. Generally, in Bangladesh, anise seeds are obtained from local market; most of them are imported and old spices of different varieties without any quality specification and source of origin. Moreover, many works have been done on volatile compounds as well as their pharmacological effects of anise seeds and very few reports have been published on their toxic and trace elemental contents as well as toxicity of essential oils and compounds. The data relating to nutritional quality composition and safety assessments are really scarce. So, it is very important to determine their comprehensive quality assessment and toxicological studies. In this view, the objective of the present research was to investigate in vitro antioxidant, antimicrobial and cytotoxic activities of the essential oils of *P. anisum* seeds in addition to their nutrition, elemental and essential oil compositions in the quality perspective for the possibilities of exportation. The permissible and critical levels of proximate parameters, necessary dietary intakes of minor nutritional elements and toxic elements have been also compared by WHO and USDA recommended limits.

Material and methods

Plant material

Pimpinella anisum L. (anise) seeds were collected from the local market (Chakbazar) Dhaka and Bangladesh Spice Research Centre (BSRC), Bogra. The samples were cleaned in water, freed from others specimen and air-dried in shade at ambient temperature. The dried samples were powdered by the ball mill and preserved in airtight high-density double lined polyethylene bag until analysis. The seeds samples are shown in Fig. 1.

Physico-chemical and proximate studies

The physicochemical and proximate parameters were carried out by the standard methods with three replications [39–43]. The total content of carbohydrate and food energy (FE) was estimated by the following equation:

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

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

Fig. 1 **a** *P. anisum* L. seeds from BSRC (Bogra) cultivar and **b** *P. anisum* L. seeds from local market (Dhaka)

Elemental analyses

Reagents, water and standard

HNO_3 and HCl (37%) acids were trace metal analysis grade of BDH (Analar Grade, England), high purity de-ionized water (Barnstead purification system) and Multi-element stock solutions containing 10 mg/L of each element (Accu Trace TM Reference Standard, USA) were used. Aliquots of ICP multi-element standard solution (10 mg/L) containing the metals such as Be, Bi, Cd, Cs, Cr, Co, Cu, Ga, In, Li, Ni, Pb, As, Se and Ag. The tuning solution (10 ppb) contains Ba, Be, Ce, Co, In, Pb, Mg, Tl and Th for instrument tuning and verification of performance. Working standard solutions (1, 5, 10, 20 and 50 µg/L) were prepared by dilution of the stock standard solutions in 1% HNO_3 from 100 µg/L as the intermediate standard for elemental analyses.

Sample preparation for ICP-MS and AAS

A certain amount of moisture less ash samples were taken from Local market (1.0082 g) and BSRC (1.0098 g) for elemental analyses. On the other hand, moisture less powder samples were taken from local market (2.5060 g) and BSRC (2.5005 g) for Hg estimation by AAS. Ashing and subsequent sample preparation were performed as per AOAC method [41, 42].

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.

Atomic absorption spectrometry (AAS) analyses

Mg, Fe, Mn and Ca were analysed by Flame Atomic Absorption Spectrometer (Varian AA 240 FS). Al was analysed by Zeeman Atomic Absorption Spectrometer (Varian AA 240 Z) in graphite furnace and the total Hg was analysed by cold vapour hydrate generation Atomic Absorption Spectrometer (Varian AA 220 FS) followed by the Varian operating manual. The metals stock standard was 1000 mg/L (Reference/Traceable) for Mg, Fe, Ca, Al and Mn and the quantification limit was at ppm levels for individual element. The detection limit of 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, equivalent to 100 mg/L and quantification limit was 0.01 µg/L (ppb). All calibrated standard, quality control standard and check standard are traceable according to National Institute of Standard and Technology (NIST). Recovery of the quality spiked sample, duplicate samples and quality control sample were observed and the recovery ranges for each parameter was $100 \pm 10\%$.

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 the total quantities in solution of samples were calculated.

Essential oil analysis

Anise seeds were subjected to hydro-distillation using Clevenger's apparatus for 4 h. The extracted oils were dried over anhydrous sodium sulphate to remove traces of moisture and stored in a refrigerator at 4 °C until analyses.

GC–MS analysis

The essential oils of anise 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.

Identification of the compounds

The constituents of the essential oils 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.

In vitro antioxidant activity

The in vitro antioxidant activity of the anise seed essential oils were determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) method with some modifications [44]. The oils were dissolved in methanol and the applied 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. The reaction mixture was vortexed and kept in the dark box for 25 min 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 was calculated from the following equation.

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

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 10 Software for windows. The experiment was performed triplicate and the results were expressed as mean ± SD with 95% confidence interval in every case.

Antimicrobial screening

The disc diffusion method [45] was used to test antimicrobial and antifungal activities 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 millimetre.

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 anise seed essential oils [46]. The Brine Shrimp eggs were collected from local pet shops. In this experiment, eggs were hatched within 48 h providing a 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 (Dimethyl Sufoxide) (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 h, 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 (LC_{50}) of dose-response data were calculated by MS-Excel (version 7) software.

Data analysis

The data analyses were carried out by MS Excel (version 10) software. The results were evaluated by statistically significant with 95% confidence interval.

Results and discussion

Physico-chemical and proximate composition

The physico-chemical and proximate composition of *P. anisum* seeds (Table 1) show fresh weight and dry weight basis of the two cultivars. The highest content of total ash, acid soluble ash, water in-soluble ash, nitrogen, protein, essential oil and food energy were observed in the BSRC cultivated seeds than the local market seeds and same was true in local market seeds for crude fibre, fatty

Table 1 Physico-chemical and proximate composition of anise seed cultivars (g/100 g)

Parameters	Market seeds	BSRC	USDA limit[c]
Moisture	9.41 ± 0.02[b]	9.27 ± 0.01 [b]	9.54[c]
Dry mater	90.58 ± 0.02	90.72 ± 0.01	
Organic matter	91.74 ± 0.01	91.61 ± 0.00	
Total ash	8.25 ± 0.01[a]	8.38 ± 0.01[a]	
	7.47 ± 0.01[b]	7.61 ± 0.01 [b]	6.95[c]
Acid in-soluble ash	0.30 ± 0.01	0.21 ± 0.01	< 1[d]
Acid soluble ash	7.95 ± 0.01	8.17 ± 0.01	
Water in-soluble ash	5.58 ± 0.2	6.14 ± 0.02	
Water soluble ash	2.67 ± 0.02	2.24 ± 0.02	
Nitrogen	3.14 ± 0.11[a]	3.27 ± 0.04[a]	
	2.84 ± 0.1[b]	2.97 ± 0.03[b]	
Protein	19.63 ± 0.74[a]	20.49 ± 0.26[a]	
	17.79 ± 0.67[b]	18.59 ± 0.24[b]	17.60[c]
Crude fiber	13.14 ± 0.05[a]	12.64 ± 0.01[a]	14.6[c]
	11.90 ± 0.04[b]	11.46 ± 0.01[b]	
Fatty oil	9.72 ± 0.09 [a]	9.44 ± 0.02[a]	15.90[c]
	8.80 ± 0.07[b]	8.56 ± 0.01[b]	
Essential oil	2.22 ± 0.01[a]	2.32 ± 0.01[a]	
	2.01 ± 0.01[b]	2.10 ± 0.01[b]	
Carbohydrate	49.24 ± 0.71[a]	49.03 ± 0.20[a]	50.02[c]
	44.60 ± 0.65[b]	44.48 ± 0.18[b]	
Food energy (kcal/100 g)	362.99 ± 0.19[a]	363.07 ± 0.04[a]	337[c]
	328.81 ± 0.12[b]	329.39 ± 0.08[b]	

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

BSRC Bangladesh Spice Research Centre, Bogra

[a]On dry weight basis

[b]On the fresh weight basis

[c]USDA (2018) https://ndb.nal.usda.gov

[d]Suggested limit (Tainter and Grenis 1993)

oil and carbohydrate contents. There are no apparent variations were observed among the cultivars of the proximate parameters. Most of the results are close to those already reported data [47, 48] whereas the variation was observed in the essential oil content which was reported to have higher value (2.49 and 3.98%) than the current studies [48, 49]. The differences observed may be due to variety, climatic variation, cultivar and agronomic practice differences. The results have also complied with USDA [50] limit for moisture, ash, protein, carbohydrate and food energy contents which have indicated good quality seeds as well as good nutritional source. The ash content of both cultivars indicated high amount of minerals which have found higher than the USDA limit. On the other hand, low acid insoluble ash indicated poor silicate impurity, as well as high water soluble ash, indicated soluble minerals contents [51]. Lower acid insoluble ash and higher water soluble ash are present in BSRC seeds cultivar which marks its rich sources of minerals. The high protein content in BSRC seeds indicated good source of quality protein. Although, there are numerous reports were disseminated around the web but the proximate properties of the present cultivars have been reported in the first time. Overall, the BSRC seeds cultivars meet the best quality standards of USDA nutritional limit. Therefore, it can be considered as good quality spice for human consumption as well as it would be considered for possible exportation.

Elemental composition

The elemental compositions of anise seeds of two cultivars were analyzed by ICP-MS, AAS and FP methods and the results are shown in Tables 2 and 3 respectively. A total of 21 elements were detected including 4 toxic elements. The concentration of the detected elements have been calculated as dry (DWB) and fresh weight basis (FWB) within the significance level ($p \leq 0.05$) and the relative standard deviations (RSD%) were ≤ 5%. K and Mg were detected as the highest concentration in local market seeds cultivar whereas Ca and Na were the highest level in BSRC seeds cultivar followed by Fe and Al on the dried basis. The trace elements of Ba, Se, Ga, V, Co, Ag and Cs were detected higher level in BSRC seeds cultivar than the local market seeds cultivar. On the contrary, Li, Ni and Bi were detected predominant amount in local cultivar seeds in comparison to the BSRC seeds cultivar. By comparing with the reported data, the content of Ca (6.0 0.60, 10.135, 2.63), K (15.47, 15.61, 0.887, 0.252), Mg (3.33, 0.270, 0.147) and Na (0.087, 0.365) (g/kg), Fe (104.2, 5.40, 44.4), Co (0.061) and Ni (0.114) (mg/kg) were found lower than the current studies [48, 52–55]. On the contrary, Na (5.83) (g/kg), Al (2962, 492, 269), Fe (1799.5, 211, 156), Li (5.76, 0.4), Ni (18.42), Ba (191) and Se (2.86) (mg/kg) were reported higher values than the present studies [52–54, 56]. Some data of elemental contents of anise seeds showed minor differences with literature. The observed differences may responsible for the soil composition, climate condition, maturity, harvesting period and biochemical synthesis of the plant body which affect the mineral composition of the present results [57]. Mineral elements are an important quality parameter of food products due to its pharmacological effect in human metabolism because many organometallic compounds have reported biological effect [58]. For example, Co is a part of vitamin B_{12} compound which has many physiological actions in human and animal body. It requires a small amount (7–50 µg/day) in human body though it is regarded sometimes as toxic element at the elevated level [59, 60]. Li is regarded as another beneficial element with many pharmacological effects such as it was reported for the treatment of manic depressive

Table 2 Essential elemental composition of anise seed cultivars

Elements	Conc. in solution	Market anise seeds		RSD (%)	Conc. in solution	BSRC anise seeds		RSD (%)	Recommended limit
		DWB	FWB			DWB	FWB		
Li (µg/kg)	93.6928	383.56	347.45	0.91	87.1731	362.09	328.5	2.54	20.0 mg/kg[b]
Be (µg/kg)	0.2386	0.97	0.88	30.97	0.287	1.19	1.08	25.75	
V (µg/kg)	32.3656	132.5	120.02	0.76	40.1214	166.65	151.19	1.36	
Co (µg/kg)	16.3808	67.06	60.75	1.05	16.4576	68.36	62.02	0.85	0.2–0.3 mg/kg[b]
Ni (µg/kg)	130.7678	535.34	484.94	0.85	126.7391	526.44	477.6	0.72	
Ga (µg/kg)	7.372	30.18	27.34	1.13	8.0071	33.26	30.17	1.76	
Se (µg/kg)	73.4551	300.71	272.4	2.37	166.845	693.03	628.74	2.84	5 µg
Ag (µg/kg)	0.7067	2.89	2.62	3.08	1.3759	5.72	5.18	0.46	
Cs (µg/kg)	0.9911	4.06	3.67	1.49	1.0268	4.26	3.87	0.48	
Ba (µg/kg)	216.468	886.18	802.75	1.21	283.4041	1177.19	1067.98	0.43	
Bi (µg/kg)	6.989	28.61	25.92	1.04	4.346	18.05	16.38	2.03	
Al (mg/kg)	25.4	103.98	94.19	0.1	28.23	117.26	106.38	0.1	
Fe (mg/kg)	29.9	122.4	110.88	0.1	33.5	139.15	126.24	0.1	36.96 mg[a]
Ca (g/kg)	2580	10.56	9.56	0.1	3080	12.79	11.6	0.1	646 mg[a]
Mg (g/kg)	920	3.766	3.41	0.1	773	3.21	2.91	0.1	170 mg[a]
Na (g/kg)	289	1.18	1.07	0.1	353	1.47	1.33	0.1	16 mg[a]
K (g/kg)	8100	33.16	30.03	0.1	6200	25.75	23.36	0.1	1441 mg[a]

ND not detected, *RSD* relative standard deviation, $p \leq 0.05$, *Conc. in solution* concentration of the elements in solution, *DWD* dry weight basis, *FWB* fresh weight basis, *BSRC* Bangladesh Spice Research Centre, Bogra Dilution factor 0.05. Fe, Ca, Mg were done by AAS, Na, K by FP, other elements by ICP-MS

[a]Recommended limit for anise seeds (per 100 g) by USDA, 2018

[b]Toxicity level of recommended intake Li (Aral and Vecchio-Sadus 2008), Co (WHO 2005)

Table 3 Toxic elemental composition of anise seed cultivars

Elements	Conc. in solution	Market anise		RSD (%)	Conc. in solution	BSRC anise		RSD (%)	Permissible limit (mg/kg)[a]
		DWB	FWB			DWB	FWB		
As (µg/kg)	0	ND	ND	ND	0	ND	ND	ND	10
Cr (µg/kg)	27.8712	115	103.35	1.66	27.6856	115	104.33	0.89	2
Pb (µg/kg)	27.3075	111.79	101.26	0.07	19.9683	82.94	75.25	0.33	10
Cd (µg/kg)	1.4078	5.76	5.22	4.89	1.2048	5.004	4.54	4.02	0.3
Hg (µg/kg)	23.71	473.06	428.52	0.4	51.65	1032.8	936.97	0.9	1

ND not detected, *RSD* relative standard deviation, $p \leq 0.05$., *Conc. in solution* concentration of the elements in solution *DWD* dry weight basis, *FWB* fresh weight basis, dilution factor 0.05. As, Cr, Pb, Cd by ICP-MS and Hg by AAS, *BSRC* Bangladesh Spice Research Centre, Bogra

[a]WHO (2005)

disorders though it has a toxicological effect at 20 mg/kg [61, 62]. Vegetables, grains, meat and dairy products are the main sources of Li and can fulfil the Li deficiency. The recommended daily dietary intake of Li is 0.65–3.1 mg/day for adult [63, 64]. On the other hand, Se is a biologically important element in the aspect of antioxidant nutrient as well as a modulator in inflammatory and immune responses [65]. It binds with different enzymes, protein and fatty acids to form selenoprotein P, type 1 iodothyronine deiodinase metalloproteins and glutathione peroxidase. The Se has been found higher amount in our present study than the reported data (4, 5 µg/kg) [50, 66]. Moreover, Ca, Fe, K and Mg contribute many important biological processes and met the daily dietary requirement in human metabolism [67]. The elements can influence the taste of spice as well as quality of anise seeds. The human body cannot able to synthesize essential elements which supplemented by external sources but over intake cause of toxicity in the body. The current results have complied with the USDA [50] recommended limit of quality parameters of anise seeds (Table 3) of both cultivars. Moreover, the mineral composition of the current

Table 4 Essential oil composition (% w/w) of *Anise* seeds cultivars

Compounds	RI	Retention time		Composition (%)	
		Local market seeds	BSRC seeds	Local market seeds	BSRC seeds
β-Thujene[a]	873	–	8.352	ND	0.261
1,2-Diisopropenyl-cyclobutane[a]	934	–	9.69	ND	6.315
α-Pinene[a]	948	7.373	7.374	1.851	0.85
β-Myrcene[a]	958	8.77	8.771	0.378	0.204
Trans-β-Ocimene[a]	976	–	9.902	ND	0.403
β-Terpinen[a]	993	8.35	–	0.353	ND
γ-Terpinen[a]	998	10.417	10.418	0.394	0.286
D-Limonene[a]	1018	9.69	–	13.007	ND
p-Menthatrienea	1029	-	9.588	ND	0.449
β-Cymene[a]	1042	9.587	–	1.418	ND
Cineole[b]	1059	9.768	9.772	0.438	0.292
Fenchone[b]	1121	11.137	11.14	11.184	5.291
trans-Anethole[b]	1190	13.667	13.669	69.949	83.671
R-(-)-Carvone[b]	1190	14.664	–	0.313	ND
Fenchyl acetate[b]	1277	14.437	14.436	0.329	0.134
α-Carcumene[c]	1524	19.41	–	0.239	ND
Total (%)				99.853	98.156
Compounds Identified (%)					
Monoterpene hydrocarbons[a]				17.401	8.768
Oxygenated monoterpenoid hydrocarbons[b]				82.213	89.388
Sesquiterpene hydrocarbon[c]				0.239	ND

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. BSRC: Bangladesh Spice Research Centre, Bogra

[a]Monoterpene hydrocarbons

[b]Oxygenated monoterpenoid hydrocarbons

[c]Sesquiterpene hydrocarbon

experiment might have to provide a sufficient amount of minerals to meet the recommended dietary allowances [68].

In the view of toxic heavy metals (Table 3), As was not detected any of the samples under investigation. Hg was detected as high amount in BSRC seeds cultivars. On the other hand, Pb and Cd were estimated higher amount in local market seed cultivar than the BSRC seeds cultivar. Whereas, Cr was found the same amount in both cultivars in the current experiment. The present results of toxic elements were found difference with the reported data. Cd (6, 54, 90, 660), Cr (64, 156, 9100, 24680) and Pb (215, 221, 1210) µg/kg were reported higher than the current study [52–55]. However, the results of toxic elements also found below the recommended level of WHO [69] established data for herbal medicine. The heavy metal in spice mainly contaminated by polluted soil and contaminated water. Hence, it could be a great concern to the consumer and also in pharmaceutical manufacturer without assessment of their safety profile. The present levels of toxic elements may not create any toxicity alarm over safety of this spices consumption, especially in BSRC seeds cultivar. In the nutritional point of view, present findings would also be valuable information to the consumer and spice traders as a good source of essential nutrients.

Essential oils analysis

The essential oils of both cultivars were obtained by hydro-distillation method and the analyses were carried out by GC and GC–MS. The oils were homogeneous, transparent and pale yellow liquid with pungent and characteristics spicy odour. The oils were found lighter than water and freely miscible with polar organic solvents. As a result of essential oil analyses (Table 4, Fig. 2), a total of 12 (representing 99.853%) and 11 compounds (representing 98.156%) were identified and quantified in local market and BSRC seeds cultivars respectively. The oils are characterized by high contents of oxygenated monoterpenoid hydrocarbons (89.388%) in BSRC seeds which denoted higher value than the local market seed cultivar. On the other hand, monoterpene hydrocarbons (17.401%) were identified higher in local

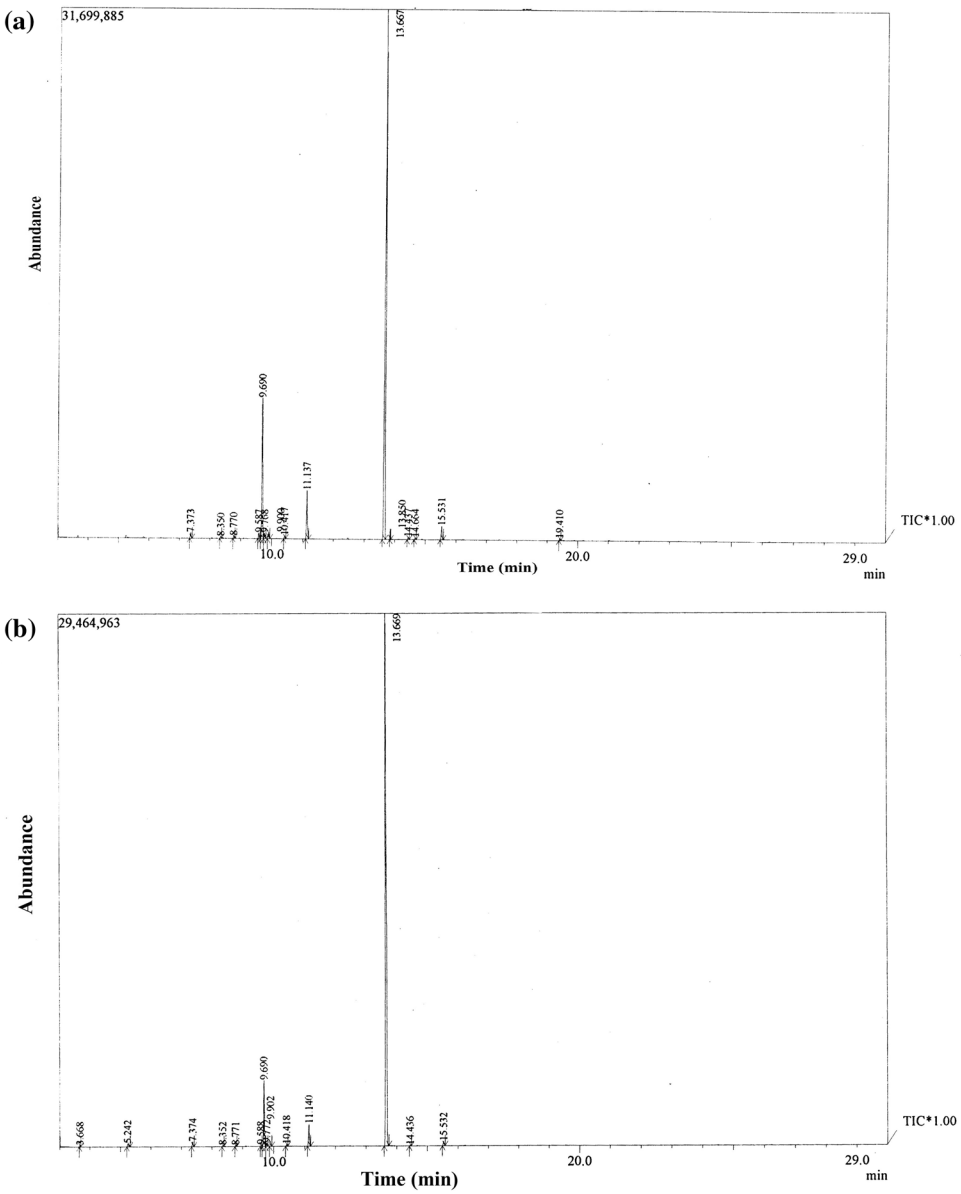


Fig. 2 GC–MS chromatogram of *P. anisum* L. seed essential oil. **a** Essential oil from local market and **b** Essential oil from BSRC, Bogra cultivar

market seed cultivar than the BSRC seeds. *trans*-Anethole (83.671%) was the most oxygenated monoterpene rich compound in BSRC seed cultivar along with a monoterpene hydrocarbon 1, 2-diisopropenylcyclobutane (6.315%). In addition, other major compounds D-limonene (13.007%), fenchone (11.184%) and α-pinene (1.851%) were detected in local market seed cultivar. The major components are displayed in Fig. 3. In the case of 1,2-diisopropenylcyclobutane, β-thujene, *trans*-β-ocimene and p-menthatriene, these compounds were absent in local market seed cultivar. Moreover, few reports have been published on the chemical compositions of the essential oils of *P. anisum* seeds and found difference in the major compounds. In this context, *trans*-Anethole (74.58–96.3), limonene (1.1), methyl chavicol (14.5) and estragole (1.89–2.4) were reported as prominent

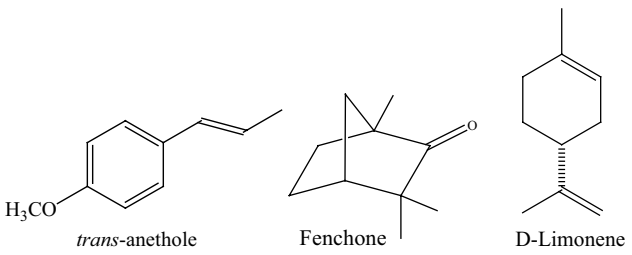


Fig. 3 Major components of *P. anisum* in the seeds of essential oils

terpenoids in the oils [30, 49, 70–74]. D-limonene was higher and *trans*-anethole was lower contents than the reported data in local market seed cultivar. Whereas, *trans*-anethole has been detected within the range of the reported data in BSRC

seed cultivar. Furthermore, fenchone and 1,2-diisopropenylcyclobutane have been found the predominant amount for the first time but methyl chavicol and estragole are totally absent in the present study. The variations of the oil compositions are due to the same geochemical and environmental factor stated above along with different isolation techniques [75]. *trans*-Anethole is a derivative of alkoxypropenylbenzene as well as key fragrance and bioactive compound of more than 20 plant species essential oils [76]. The oil quality of the anise seed oil mostly depends on the crystallization point of *trans*-anithole content. The crystallization temperature of the oil is 15–20 °C which increases with *trans*-anethole content in the essential oil [77]. The congealing point of the oils observed at 15–17 °C at time of storage in the freeze. However, the present results are in good agreement with reported data compared with various cultivars.

Antioxidant activity

Antioxidant activity of the two essential oils was evaluated through DPPH-free radical scavenging assays using ascorbic acid and BHT as positive controls. The assays were carried out at concentrations of 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50 and 100 mg/mL. The inhibition per cent and concentrations that inhibited 50% in each test (IC_{50} values) are shown in Table 5. The antioxidant activity of the oils exhibited significant dose dependent activity with concentrations. The IC_{50} value of local market seed cultivar was calculated as 52.48 mg/mL with the highest inhibition of 61.27%. On the other hand, the BSRC seed cultivar observed 48.71 mg/mL (IC_{50}) with the highest inhibition of 63.78%. The activity of the oils were weak than that of either ascorbic acid or BHT (IC_{50} 3.73 and 11.84 µg/mL) respectively. Present experiment was coincide with reported value of anise seeds essential oil at 48.5 mg/mL (48%) [78] and found higher at the concentration of 86.88 mg/mL and 250 µL (c.a. 25%) [79, 80]. On the other hand, the highest activity was reported at 5.62 µg/mL and 65.22% at 25 µL concentration than the current study [70, 81]. Such variations in the antioxidant activity might be attributed to the varied chemical composition, structure of the predominant compounds and agro-climatic factors. Fennel essential oil reported as good DPPH radical scavenging activity due to its major components *trans*-anethol, fenchone, estragole and limonene [82]. The double bond of the propenyl side chain is conjugated with the aromatic ring in trans-anethole. It can form a conjugated radical cation, delocalized with benzene ring and later stabilized by the $–OCH_3$ group through 1, 4 interaction [83]. Besides, fenchone is a colorless, with a pungent camphoraceous odor and bitter taste [77]. It was reported [84] as the major compound (11.32%) in *F. vulgare* oil and responsible for antioxidant activity which is similar of our current studies. This methoxy ($-OCH_3$) group act as strong electron donating group as well as CH_3 group has an inductive effect into the benzene ring that can increase the stability of the benzene ring and enhance the radical scavenging activity. Moreover, essential oils are a complex mixture of multiple components and therefore, the antioxidant action is responsible for their predominant components as well as broad spectrum synergistic action of the multiple components [85–87]. The exact reason of the antioxidant action of the oils is quite troublesome to explain without isolation of the individual components as well as synergistic assay. Further study would be a footstep to unfold the reason of the antioxidant action of the individual components as well as synergistic study of the oils. In this connection, the antioxidant action of the oils may be due to the predominant amount of individual components such as *trans*-anethol, fenchone and limonene as well as minor components or synergistic action.

Antimicrobial activity

The results of antimicrobial activity of the two essential oils showed (Table 6) moderate activity against all tested organism compared to the standard antibiotic ciprofloxacin. Gram-negative bacteria of *E. coli*, *S. paratyphi*, *S. dysenteriae* and *V. mimicus* and all tested fungi showed the same moderate inhibition with the zone of 18 mm of both cultivars than the Gram-positive bacteria except *S. lutea*. Overall, all the tested oils were less effective against microorganism than ciprofloxacin. The present results were comparatively higher than the reported data of various seed cultivars [70, 78, 81, 87] however, some studies were revealed good activity than the present study [28, 73]. The oils contain high amount of *trans*-anethole, fenchone and D-limonene. The *trans*-anethole was reported as high anti-bacterial activity against 8 microorganism that was very similar with sample contained high anethole [88]. Moreover, *trans*-anethole reported to have anti-inflammatory, antidiabetic, and chemopreventive, neuro protective, anti-carcinogenic, immunomodulatory and many pharmacological effects [76]. Besides, fenchone and D-limonene were also reported as potent anti-microbial activity [89, 90]. The higher resistance of Gram-negative bacteria may be due to the differences in the cell membrane of the tested organism. It possesses outer membrane and unique periplasmic space which is absent in the Gram-positive bacteria. Strong substances can easily damage the bacterial cell wall and cytoplasmic membrane and cause cell leakage in bacterial cellular and mitochondria membranes. The Gram-positive bacteria do not have such type of cell wall structure to protect [91, 92]. Therefore, Gram-negative bacterial group has displayed good activity than that of Gram-positive bacteria. Nonetheless, the antimicrobial activity is generally considered as dose dependent concentration of the oil. This variation is due to the major component as well as the synergistic action of the minor constituents. On the

Table 5 DPPH antioxidant activity of anise seed essential oils

Assay concentrations (mg/mL)	100	50	25	12.5	6.25	3.125	1.5625	0.78125	IC_{50}
DPPH Inhibition (%)									
Local market seed oil	61.27 ± 0.44	50.31 ± 0.65	44.54 ± 0.86	24.14 ± 0.82	17.94 ± 0.740	14.57 ± 0.38	7.69 ± 0.08	1.63 ± 0.50	52.48 ± 0.40 mg/mL
BSRC seed oil	63.78 ± 1.46	51.37 ± 0.66	43.29 ± 1.0	23.47 ± 0.22	17.17 ± 0.66	12.79 ± 0.65	7.21 ± 5.43	0.76 ± 0.58	48.71 ± 570.57 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

Mean ± SD, $P < 0.05$ of independent sample T-test

ASA ascorbic acid, *BHT* tert-butyl-1-hydroxytoluene

Table 6 Antimicrobial activity of the anise seed essential oils and standard

Samples	Gram positive bacteria					Gram negative bacteria								Fungi		
	B.meg	*B.sub*	*S.aur*	*S.lut*	*B.cer*	*E.col*	*P.aur*	*S.par*	*S.typ*	*S.dys*	*S.boy*	*V.mim*	*V.par*	*C.alb*	*A.nig*	*S.cer*
Zone of inhibition (mm)																
Local market seeds oil	15	15	17	18	15	18	19	18	17	18	15	18	17	18	18	18
BSRC, Bogra cultivar oil	16	16	17	18	16	18	18	18	17	18	16	18	17	18	18	18
Ciprofloxacin	45	46	46	45	45	46	46	46	45	46	45	46	46	45	46	45

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 cereus*, *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*

Table 7 Cytotoxic effect on Brine Shrimp nauplii of *P. anisum* seed essential oils

Dose concentrations (µg/mL)	0.039	0.078	0.156	0.3125	0.625	1.25	2.5	5	10	IC_{50} (µg/mL)
Mortality (%)										
Market seeds Oil	0	8.67 ± 1.19	9.14 ± 0.83	14.01 ± 1.18	15.01 ± 0.63	27.93 ± 1.09	34.12 ± 1.37	48.71 ± 2.22	100 ± 00	2.86 ± 0.08
BSRI seeds Oil	0	10.31 ± 3.43	10.31 ± 3.43	19.39 ± 1.04	20.0 ± 0.0	22.61 ± 2.06	27.77 ± 4.81	48.88 ± 1.92	100 ± 00	3.06 ± 0.09
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

whole, both oils exhibited good antibacterial activity at the dose of 400 µg/mL. Further studies would be appreciated to investigate synergistic action as well as the mechanism of action of these essential oils and isolated components that were responsible for antimicrobial action by means of in vivo studies on animal model.

Cytotoxic activity

The cytotoxic activity of the two essential oils on brine shrimp nauplii shown significant activity compared with positive control vincristine sulphate (Table 7). Local market and BSRC seed cultivars exhibited potent activity with the highest mortality rate of 100% and LC_{50} values were 2.86 and 3.06 µg/mL respectively whereas, vincristine sulphate displayed strong activity at 0.50 µg/mL. DMSO (the negative control) had not any mortality at the dose of 50 µL/mL. The Brine Shrimp cytotoxic activity of anise seed essential oil reported higher than 1000 µL/mL in the microplate assay at low concentration [93]. The present study showed strong activity than the reported data. The difference observed of the two oils may be due to the prominent component of the oils together with synergistic action of the minor components. *trans*-Anethole, fenchone and D-limonene were the major component in the essential oils by GC–MS study. *trans*-Anethole and D-limonene were reported as anticancer activity [94–98]. Moreover, fenchone was also reported for its fumigant toxic activity [99]. The observed cytotoxic activity on Brine Shrimp naupli might be responsible for those components present in the oils. On the other hand, the brine shrimp cytotoxicity is a very simple bioassay technique for anti-tumor and anticancer predictor as well as important parameter of the safety profile assessment of foods and medicinal plants. It marks also antiplasmodial, antimalarial, antifilarial and antiviral activities [91, 100]. On the other hand, the relationship between cytotoxic assay and antimicrobial assay is very clear. Though cytotoxic assay is a predictor of vast array of bioactivities, nonetheless it cannot predict the antimicrobial assay because of the cell structure of the shrimp nauplii and microorganism. The bacterial cells are prokaryotic type and brine shrimp cells are eukaryotic type structure [93]. Therefore, both are potential tools to understand the activity of the essential oils and extract or compound for its safety and potential use in food and pharmaceutical industries. The degree of mortality depends on the applied concentration of the oils and related pure compounds. At the elevated doses, it can be toxic since the degree of mortality is proportional to the concentration. The reported recommended toxicity level for plant extracts is less than 1.0 mg/mL (LC_{50}) [46]. The current investigation of the oils have exhibited toxic on brine shrimp larvae cells at the highest dose of 10 µL/mL. The results revealed that the oils

can be safe at certain concentration level. Therefore, further in vivo toxicity studies on cell line and animal model of the essential oils and its isolated pure compound also suggested for safety profile as well as possible anticancer effect.

Conclusion

The quality parameters of the spices and condiments are the important health factor in human and important criteria for spice importers as well as food industries due to the adulteration and toxicological effects. Therefore, it should be taken necessary steps to ensure nutritional quality and safety by the extensive physicochemical, chemical and pharmacological investigation. Therefore, the present study incited interest in evaluating imported (local market) and indigenous anise seeds for its physiochemical, chemical and proximate analysis along with pharmacological evaluation. The physiochemical and proximate parameters such as protein, fibre, and carbohydrate and food energy have been complied and agreed with the quality recommendation of USDA and WHO. The ICP-MS is a quite efficient technique to determine minor and trace elements of medicinal plants and food products. The anise seeds of two cultivars were found to be the rich source of nutritionally essential trace elements and very low amount of toxic elements that were within the recommended limits specified by WHO and USDA and could not pose any risk to the consumer though the daily consumption of this spice is very low. The present results suggest that many of these essential elements have vital importance in metabolism as well as mineral supplement in the body. In addition, the essential oils were comprised with high amount of *trans*-anethole, fenchone and D-limonene of both cultivars. The pharmacological studies of these compounds are well recognized importance of various activities against different diseases. The antioxidant activity of the essential oils promises substantial nutraceutical importance associated with age related disorder. Besides, both antimicrobial and brine shrimp cytotoxic activities of the essential oils were displayed valuable information concerning the safety issue either in food and pharmaceutical formulation. The results can be considered as the first information on the chemical and pharmacological properties of anise seeds of the two cultivars. The present study delineates comparative information of the two cultivars and also contributes knowledge to the consumer and scientist pertaining to nutritional quality and safety profile. This might open up a new prospect of possible exportation of indigenously cultivated spices. Further, in vivo studies are recommended on animal model with active chemical components for discovering new bioactive drug lead molecules.

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

Compliance with ethical standards

Conflict of interest The authors indicate no potential conflicts of interest.

Human and animal participants This article does not contain any studies with human or animal subjects.

References

1. M.R. Panuccio, A. Fazio, C.M. Musarella, A.J. Mendoza-fernández, J.F. Mota, G. Spampinato, Plant Biosyst. **152**(3), 398–406 (2018)
2. A.B. Cutillas, A. Carrasco, R. Martinez-Gutierrez, V. Tomas, J. Tudela, Plant Biosyst. **152**(6), 1282–1292 (2018)
3. T.L. Miron, I. Gazi, M.P.D. Moral, Innov. Rom Food Biotechnol. **6**, 18–24 (2010)
4. C. Proestos, T. Varzakas, Foods **6**(4), 28 (2017)
5. S. Zuhdi, J. Marit. Stud. Nat. Integr. **2**(1), 31–44 (2018)
6. Z. Lakner, E. Szabó, V. Szűcs, A. Székács, Food Control **83**, 141–146 (2018)
7. C. Musarella, I. Paglianiti, A. Cano-Ortiz, G. Spampinato, Atti Soc. Toscana Sci. Nat. **126**, 5 (2019). https://doi.org/10.2424/ASTSN.M.2018.17
8. G. Maruca, G. Spampinato, D. Turiano, G. Laghetti, C.M. Musarella, Genet. Resour. Crop. Evol. **5**, 4 (2019). https://doi.org/10.1007/s10722-019-00768-8
9. N.K. Leela, T.M. Vipin, Chemistry of Spices, in ed. by A.V. Parthasarathy, B. Chempakam, T.J. Zachariah (CAB International, Cambridge, 2008), p. 331
10. Z.X. Wang, S.R. Downie, J.B. Tan, C.Y. Liao, Y. Yu, X.J. He, Nordic J. Bot. **32**, 642–657 (2014)
11. Ko¨nemann, In Botanica; The illustrated A–Z of over 10,000 garden plants and how to cultivate them. (Gordon Cheers Publication, Hong Kong, 1999), pp. 51–53.
12. G. Reineccius, *Source Book of Flavours*, 2nd edn. (Chapman and Hall, New York, 1994)
13. G.R. Amin, *Popular Medicinal Plants of Iran, Vice-Chancellorship of Research* (Tehran University of Medical Science Press, Tehran, 2005)
14. H. Mirheydar, *Herbal Information: Usage of Plants in Prevention and Treatment of Diseases* (Islamic Culture Press Center, Tehran, 2001)
15. A. Shojaii, M.A. Fard, ISRN Pharm. (2012). https://doi.org/10.5402/2012/510795
16. C.P. Khare, *Indian Medicinal Plants. An Illustrated Dictionary* (Janak Puri, New Delhi, 2007), p. 487
17. I.B. Rebey, W.A. Wannes, S.B. Kaab, S. Bourgou, M.S. Tounsi, R. Ksouri, M.L. Fauconnier, Sci. Hortic. **246**, 453–461 (2019)
18. N. Martins, L. Barros, C. Santos-Buelga, I.C.F.R. Ferreira, Ind. Crops Prod. **79**, 188–194 (2016)
19. G. Singh, I.P.S. Kapoor, P. Singh, C.S. de Heluani, C.A.N. Catalan, Int. J. Essent. Oil Ther. **2**(3), 122–130 (2008)
20. A. Tas, Ind. Vet. J. **86**(2), 145–147 (2009)
21. M.H. Pourgholami, S. Majzoob, M. Javadi, M. Kamalinejad, G.H.R. Fanaee, M. Sayyah, J. Ethnopharmacol. **66**(2), 211–215 (1999)

22. J.B. Lee, C. Yamagishi, K. Hayashi, T. Hayashi, Biosci. Biotechnol. Biochem. **75**(3), 459–465 (2011)
23. H.S. Lee, Planta Med. **70**(3), 279–281 (2004)
24. I. Tunc, B.M. Berger, F. Erler, F. Dagli, J. Stored Prod. Res. **36**(2), 161–168 (2000)
25. F. Erler, I. Ulug, B. Yalcinkaya, Fitoterapia **77**(7–8), 491–494 (2006)
26. V. Prajapati, A.K. Tripathi, K.K. Aggarwal, S.P.S. Khanuja, Biores. Technol. **96**(16), 1749–1757 (2005)
27. I.K. Park, K.S. Choi, D.H. Kim, I.H. Choi, L.S. Kim, W.C. Bak, J.W. Choi, S.C. Shin, Pest Manage. Sci. **62**(8), 723–728 (2006)
28. F.A. Al-Bayati, J. Ethnopharmacol. **116**(3), 403–406 (2008)
29. U. Rajeshwari, I. Shobha, B. Andallu, Spatula DD **1**(1), 9–16 (2011)
30. M.M. Ozcan, J.C. Chalchat, Ann. Microbiol. **56**(4), 353–358 (2006)
31. A. Orav, A. Raal, E. Arak, Nat. Prod. Res. **22**(3), 227–232 (2008)
32. M.B. Embong, D. Hadziyev, S. Molnar, Can. J. Plant Sci. **57**, 681–688 (1997)
33. M.O. Zgüven, in *Handbook of Herbs and Spices*, ed. by K.V. Peter (Woodhead Publishing Limited, Abington, Cambridge, England, 2001), p. 41
34. I. Gulcin, M. Oktay, E. Kirecci, O.I. Kufrevioglu, Food Chem. **83**(3), 371–382 (2003)
35. A.Y. Leung, S. Foster, *Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics* (Wiley, New York, 1996)
36. C. Karadas, D. Kara, Food Chem. **130**(1), 196–202 (2012)
37. A.A.K. Abou-Arab, M.S. Kawther, M.E. El-Tantawy, R.I. Badeaa, N. Khayria, Food Chem. **67**, 357–363 (1999)
38. N. Khan, J.Y. Choi, E.Y. Nho, N. Jamila, G. Habte, J.H. Hong, I.M. Hwang, K.S. Kim, Food Chem. **158**, 200–206 (2014)
39. A.B. Khattak, G.S.S. Khattak, Z. Mahmood, N. Bibi, I. Ihsanullah, Int. J. Food Sci. Technol. **41**(Suppl. 2), 1–5 (2006)
40. M. Zia-ul-Huq, S. Iqbal, S. Ahmad, M. Imran, A. Niaz, M.I. Bhanger, Food Chem. **105**, 357–363 (2007)
41. AOAC. Official methods of analysis. 14th edn. (Association of official analytical chemist Sydney: William, 2005), pp. 503–532.
42. British Pharmacopoeia, *British Pharmacopoeia, Appendix: Vol. IV*. (British Pharmacopoeia Commission, London, 2004), pp. 248–250
43. USP. United States of Pharmacopoeia: United States of Pharmacopoeia National Formulary USP30-NF25. General Test and Assay. (12601 Twin brook Parkway, Rockville, Maryland, 2007).
44. W. Brand-Williams, M.E. Cuvelier, C. Berset, LWT **28**, 25–30 (1995)
45. A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Turck, Am. J. Clin. Pathol. **44**, 493–496 (1966)
46. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, J.B. Jacobsen, D.E. Nicholsand, J.L. Mclaughlin, Planta Med. **45**, 31–34 (1982)
46. R.D. Tainter, T.A. Grenis, *Spice and Seasonings: A Food Technology Handbook* (Wiley, Hoboken, 1993)
48. F. Khanum, S. Krishna, A.D. Semwal, K.R. Vishwanathan, Ind. J. Nutr. Dietet. **38**, 93 (2001)
49. N. Tabanca, B. Demirci, T. Ozek, Kirimer, N. Baser, K.H. Bedir, I.A. Khan, D.E. Wedge, J. Chromatogr. A. **1117**(2), 194–205 (2006).
50. USDA, USDA Food Composition Databases (https://ndb.nal.usda.gov, 2018).
51. M.M. Abukawsar, M.M. Saleh-e-In, M.A. Ahsan, M.M. Rahim, M.N.H. Bhuiyan, S.K. Roy, A. Ghosh, S. Naher, J. Food Biochem. **42**(6), e12590 (2018). https://doi.org/10.1111/jfbc.12590
52. S. Kumaravel, K. Alagusundaram, Orient. J. Chem. **30**(2), 631–636 (2014)
53. M.M. Ozcan, M. Akbulut, Food Chem. **106**, 852–858 (2007)
54. M. Ozcan, Food Chem. **84**, 437–440 (2004)
55. A.A.K. Abou-Arab, M.A. Abou-Donia, J. Agric. Food Chem. **48**, 2300–2304 (2000)
56. B.A. Al-Bataina, A.O. Maslat, M.M. Al-Kofahil, J. Trace Elem. Med. Biol. **17**(2), 85–90 (2003)
57. M.M. Saleh-e-In, N. Sultana, M.M. Rahim, M.M. Ahsan, M.N.H. Bhuiyan, M.N. Hossain, M.R. Islam, BMC Comp. Alt. Med. **17**, 127 (2017)
58. I.D.L. Calle, M. Costas, N. Cabaleiro, I. Lavilla, C. Bendicho, Food Chem. **138**(1), 234–241 (2013)
59. A. Lesniewicz, K. Jaworska, W. Zyrnicki, Food Chem. **99**, 670–679 (2006)
60. D.G. Weir, J.M. Scott, Encycl. Hum. Nutr. **1**, 394–401 (1999)
61. H. Aral, A. Vecchio-Sadus, Ecotoxicol. Environ. Saf. **70**, 349–356 (2008)
62. R. Macrae, R.K. Robinson, M.J. Sadler, Vol. 7. (Academic Press, San Diego, 1993).
63. M.L. Weiner, Overview of lithium toxicology, in *Lithium in Biology and Medicine. Hydrogen Substitution in Lithium-Aluminosilicates*, ed. by G.N. Schrauzer, K.F. Klippel (VCH Verlag, Hoboken, 1991), pp. 83–99
64. G.N. Schrauzer, J. Am. Coll. Nutr. **21**(1), 14–21 (2002)
65. J. Neve, Experimentia **47**, 187–193 (1991)
66. A. Kumar, K. Krishnaswamy, J. Agric. Food Chem. **45**, 2565–2568 (1997)
67. T. Brody, *Nutritional Biochemistry*, 2nd edn. (Academic Press, San Diego, 1994)
68. NRC/NAS, B. Recommended dietary allowances, 10th edn. (National Academy Press, Washington DC, 1989). p. 302.
69. WHO. Quality control methods for medicinal plant materials. (World Health Organization, Geneva, 2007).
70. B. Tepe, H.A. Akpulat, M. Sokmen, D. Daferera, O. Yumrutas, E. Aydin, M. Polissiou, A. Sokmen, Food Chem. **97**, 719–724 (2006)
71. P.M. Santos, A.C. Figueiredo, M.M. Oliveira, J.G. Barroso, L.G. Pedro, S.G. Deans et al., Phytochem **48**, 455–460 (1998)
72. S.I. Kreydiyyeh, J. Usta, K. Knio, S. Markossian, S. Dagher, Life Sci. **74**, 663–673 (2003)
73. L.B. Gende, M.D. Maggi, R. Fritz, M.J. Eguaras, P.N. Bailac, M.I. Ponzi, J. Essent. Oil Res. **21**(1), 91–93 (2009)
74. K.S. Skalicka-Wozniak, M. Walasek, A. Ludwiczuk, K. Głowniak, J. Sep. Sci. **36**, 2611–2614 (2013).
75. I.P.S. Kapoor, B. Singh, G. Singh, C.S.D. Heluani, M.P.D. Lampasona, C.A.N. Catalan, J. Agric. Food Chem. **57**, 5358–5364 (2009)
76. A.C. Aprotosoaie, I. Costache, A. Miron, Anethole and its role in chronic diseases. Drug Discovery from Mother Nature. ed. by S.C. Gupta, S. Prasad, B.B. Aggarwal (Springer, New York, 2016), pp. 247–268.
77. L.A. Shelef, Herbs. Herbs of the Umbelliferae Encyclopedia of Food Sciences and Nutrition, 2nd edn. (Elsevier Science Ltd., Amsterdam, 2003), pp. 3090–98.
78. E. Fitsiou, G. Mitropoulou, K. Spyridopoulou, A. Tiptiri-Kourpeti, M. Vamvakias, H., Bardouki, M.I. Panayiotidis, A. Galanis, Y. Kourkoutas, K. Chlichlia, A. Pappa, Molecules **21**(8), E1069 (2016)
79. S. Nanasombat, P. Wimuttigosol, Food Sci. Biotechnol. **20**(1), 45–53 (2011)
80. P. Marimuthu, N.A. Shakil, D.B. Saxena, Food **1**(1), 65–67 (2007)
81. G. Singh, S. Maurya, P. Marimuthu, H.S. Murali, A.S. Bawa, Nat. Prod. Rad. **6**(2), 114–121 (2007)
82. S. Škrovánková, L. Mišurcová, L. Machů, Adv. Food Nutr. Res. **67**, 75–139 (2012)
83. S. Chang, A.M. Nafchi, A.A. Karim, J. Essent. Oil Res. **28**(4), 357–363 (2016)

84. N. Mimica-Dukic, B. Bozin, M. Sokovic, B. Mihajlovic, M. Matavulj, Planta Med. **69**, 413–419 (2003)
85. A. Kamal-Eldin, L.A. Appelqvist, Lipids **31**, 671–701 (1996)
86. F. Lu, L.Y. Foo, Phenolic antioxidant component of evening primrose. In A.S.H. Ong, E. Niki, & L. Packer (Eds.), Nutrition, lipids, health and disease (American Oil Chemists Society Press, Champaign, 1995).
87. I. Kosalec, S. Pepeljnjak, D. Kustrak, Acta Pharm. **55**, 377–385 (2005)
88. F. Senatore, F. Oliviero, E. Scandolera, O. Taglialatela-Scafati, G. Roscigno, M. Zaccardelli, E.D. Falco, Fitoterapia **90**, 214–219 (2013)
89. R.L. Zyl, S.T. Seatlholo, S.F. Vuuren, J. Essen, Oil Res. **18**, S129–S133 (2006)
90. W. Bei, Y. Zhou, X. Xing, M.R. Zahi, Y. Li, Q. Yuan, H. Liang, Front. Microbiol. **6**, 1010 (2015)
91. M.M. Saleh-e-In, N. Sultana, M.N. Hossain, S. Hasan, M.R. Islam, B.M.C. Complement, Alt. Med. **16**, 464 (2016)
92. C.F. Duffy, R.F. Power, Int. J. Antimicrob. Agents **17**, 527–529 (2001)
93. I. Khafagi, A. Dewedar, S. Farouk, Egyptian. J. Biol. **2**, 20–27 (2000)
94. A.I. Elkady, Med. Chem. **18**(2) (2018). https://doi.org/10.2174/1871520617666170725165717
95. J.A. Elegbede, C.E. Elson, A. Qureshi, M.A. Tanner, M.N. Gould, Carcinogenesis **5**, 661–664 (1984)
96. C.E. Elson, T.H. Maltzman, J.L. Boston, M.A. Tanner, M.N. Gould, Carcinogenesis **9**, 331–332 (1988)
97. T.H. Maltzman, L.M. Hurt, C.E. Elson, M.A. Tanner, M.N. Gould, Carcinogenesis **10**, 781–783 (1989)
98. L.W. Wattenberg, Cancer Res. **43**, 2448S–2453S (1983)
99. E.H. Eshra, Y. Abobakr, G.M. Abddelgalil, E. Ebrahim, H.I. Hussein, A.S. Al-Sarar, Egypt. Sci. J. Pest. **2**(2), 91–95 (2016)
100. P.N. Solis, C.W. Wright, M.M. Anderson, M.P. Gupta, J.D.A. Phillipson, Planta Med. **59**(3), 250–252 (1993)