



Research article

Relative evaluation of in-vitro antioxidant potential and phenolic constituents by HPLC-DAD of *Brassica* vegetables extracted in different solvents

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ABSTRACT

Cabbage, cauliflower and broccoli are well-known vegetables from the *Brassica* family having functional effects on human health. This study was carried out to identify different antioxidant properties and to quantify phenolic compounds by HPLC-DAD in different extracts (methanol, ethanol and water: acetic acid: acetone) of these vegetables. The results showed that, the methanolic dry extract of cabbage possessed the highest antioxidant activity ($549 \pm 7.30 \mu\text{g/g}$) and IC_{50} was $90 \pm 2.52 \mu\text{g/mL}$ than others. Whereas the ethanolic dry extract of cauliflower had $348 \pm 5.20 \mu\text{g/g}$ of flavonoid, which was the highest among all. The maximum levels of total tannin ($414 \pm 5.20 \mu\text{g/g}$) and total phenolic content ($465 \pm 3.25 \mu\text{g/g}$) was found in broccoli dry extract. Several polyphenolic compounds were identified in different extracts of the vegetables and they were Cauliflower (8) > Cabbage (10) > Broccoli (9) in total. Therefore, the use of total vegetables rather than extracts in the food industry would be more appropriate to get greater health benefit.

1. Introduction

Cauliflower, cabbage and broccoli are three popular winter vegetables of the *Brassicaceae* genus in the *Cruciferae* family. Cauliflower (*Brassica oleracea* var. *botrytis*) is served as a curry, soup, or fried dish. It includes cancer-fighting sulforaphane, as well as glucosinolates, carotenoids, indole-3-carbinol, isothiocyanates, dithiolethiones, and phenols, which improves DNA repair, functions as an estrogen antagonist and reduces cancer cell proliferation [1]. Additional bioactive breakdown products, including nitriles, thiocyanates, epithionitriles, and oxazolidines, are produced when cauliflower myrosinase hydrolyzes glucosinolates during cellular disruption [2]. The most prevalent hydrophilic compound in cauliflower is ascorbic acid, which is known to detoxify reactive oxygen species directly [3].

Cabbage (*Brassica oleracea* var. *capitata*) is another common vegetable consumed either raw as salads or processed in different ways, e.g., boiled or, fermented. Cancer patients who follow such diets also benefit from an increase in the bioavailable content of non-heme iron and from the use of complementary and alternative medicine [4]. Clinical research also shows that eating cabbage can help with peptic ulcer healing and lowering LDL levels in the blood [5]. Moreover, they have been used for

centuries in traditional medicine to treat a variety of conditions, including minor cuts and wounds, mastitis, and gastrointestinal disorders such as gastritis, peptic and duodenal ulcers, and irritable bowel syndrome.

Broccoli (*Brassica oleracea* L. var. *italica*) sprouts, florets, flour, fiber, flakes, powder, crisps and so on are gaining popularity for their preventive role in noncommunicable diseases like hypertension, atherosclerosis and cancer [6]. It is also a good source of isothiocyanates as well as sulforaphane (SF), known for its chemopreventive properties [7]. It is also rich in vitamins (C and K), beta-carotenes, dietary fiber, polyphenols and fatty acids, with considerable beneficial health effects [6, 8]. Consumption of this vegetable may decrease the risk of gastric cardiac and esophageal adenocarcinomas [9], but also colon and colorectal cancers in human [10].

Various structural components like lignin, cellulose is present in these vegetables. Among them, cellulose is important, which contains a long chain of covalently linked glucose units and gives tensile strength in plant cells [11]. This cellulose is important as a coating material in food processing techniques, especially in the microencapsulation process. Moreover, vegetables are a good source of bioactive polyphenols which show different functional properties. There is not enough research on the

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polyphenolic content and antioxidant properties of these three vegetables of the brassica genus extracted with both polar and non-polar solvents. Hence, this study was designed to determine the polyphenolic compounds and antioxidant profiles of these three vegetables extracted in three different solvents based on polarity. In the end, this will give a thorough summary of bioactive polyphenols with functional qualities as well as the antioxidant activities of extracts of broccoli, cabbage, and cauliflower.

2. Materials and methods

2.1. Sample collection

The cabbage (n = 25), broccoli (n = 28), cauliflower (n = 21) were collected from different wholesale markets around Dhaka city, where vegetables were gathered from different agricultural regions of Bangladesh and the cultivars were identified by a botanist. Then, those samples were cut into about 2cm pieces and dried at 60 °C in an oven (Mettler UNB 100). After drying, samples were ground in a blender and the powder was kept in an air-tight container. A single composite sample of a homogeneous mix was prepared from of same type of item.

2.2. Sample extraction

The maceration process was employed to extract bioactive chemicals from dry materials based on the polarity of the solvents [12]. It has long been recognized that ethanol, which is safe for consumption, makes a good solvent for extracting polyphenols. Lower molecular weight polyphenols can generally be extracted more effectively with methanol, whilst higher molecular weight can be extracted more effectively with aqueous acetone [13].

2.2.1. Methanol extract (ME) and ethanol extract (EE)

About 25 g of dry powder of each sample was put into 250 mL of methanol and ethanol separately in a conical flask. Then, flasks were put on the shaker (GFL orbital shaker 3005) and were shaken for 72 h. Solutions were filtered through filter paper (Whatman, no. 1) and filtrates were evaporated in a rotary evaporator (IKA RV 10, USA) at 60 °C. Dried extracts of broccoli (BCME, BCEE), cabbage (CAME, CAEE) and cauliflower (CUME, CUEE) were obtained after evaporation and were stored at -20 °C [14].

2.2.2. Water, acetic acid and acetone extract (WAA)

Solvent polarity is a key factor in enhancing phenolic solubility. Hexane, dichloromethane (ratio = 50:50), and acetone: water: acetic acid (70: 29.5: 0.5) were utilized subsequently to boost the extraction of water-soluble polyphenols. The disruption of the cell matrix of the sample for maximum extraction was enhanced by acetone: water: acetic acid solvent [14, 15]. About 25–30 g of dried powder was put into 250 mL of solvent (hexane: dichloromethane = 50:50) in a conical flask and was shaken on a shaker for 72 h. The solution was filtered through filter paper (Whatman, no. 1) and the residue was dried in an oven at 60 °C. Then this dried powder was mixed with 200 mL of solvent (water:acetic acid:acetone = 0.5:29.5:70) and was put on shaker for 72 h [16]. After filtration, the filtrate was dried using a freeze dryer at -42 °C (ThermoFisher Modulyod-230). Properly dried extracts of broccoli (BCWAA), cabbage (CAWAA) and cauliflower (CUWAA) were powdered and were stored at -20 °C.

2.3. Chemicals

The following ingredients were acquired from Sigma (St. Louis, Mo, USA): 1,1-Diphenyl-2-picryl Hydrazyl (DPPH), Folin-Ciocalteu reagent, Aluminum Chloride (AlCl₃), Sodium Acetate, Ascorbic acid, Tri-Sodium Hydrogen Phosphate (Na₃PO₄), Sodium Carbonate (Na₂CO₃), Ammonium Molybdate, H₂SO₄, Potassium Di-Hydrogen Phosphate (KH₂PO₄),

HPLC grade solvent (acetonitrile, methanol, acetic acid, IPA), and standards. Gallic acid, chlorogenic acid, catechol, catechin hydrate, vanillic acid, caffeic acid, (-) epicatechin, vanillin, trans-ferulic acid, p-coumaric acid, rutin hydrate, rosmarinic acid, myricetin, quercetin, trans-cinnamic acid, naringenin, and kaempferol were used as standards.

2.4. Yield determination

The yield percentage was determined to observe the effect of the solvent system on the extraction. The yield was calculated using the equation, Yield (%) = 100*(A-B)/W, where A = weight of flask containing extract after evaporation, B = weight of dry empty flask, W = weight of dry sample.

2.5. Determination of antioxidant properties

2.5.1. Sample preparation

The dried extract was dissolved in a corresponding solvent (methanol, ethanol or water: acetic acid: acetone) to prepare a 10 mg/mL concentration, and the solution was vigorously shaken and sonicated. This solution was used as a stock solution and kept at 4 °C.

2.5.2. Determination of total flavonoid content (TFC)

Total flavonoid content was determined colorimetrically [17]. First, 0.3325 g of AlCl₃ and 1 g of sodium acetate were mixed in 100 mL of DI water. The thawed sample (0.2 mL) was mixed with 4.8 mL of water and the mixture was kept for 5–6 min after adding the 2.5 mL of reagent mix. A Thermo Scientific double beam UV-VIS spectrophotometer (Model: Evolution 300) was used to measure absorbance at 430 nm. Quercetin (0.01 g) was dissolved in 100 mL of methanol for the preparation of the calibration curve. Total flavonoids were expressed as µg of Quercetin equivalent per gram of dry extract.

2.5.3. Determination of total tannin content (TTC)

The method used by Haile & Kang (2019) with a few modifications was used to determine the tannins [18]. A sample extract (0.5 mL) was combined with 8.5 mL of distilled water and 0.5 mL of the Folin-Ciocalteu Phenol reagent and maintained at room temperature for 5 min. A 35% sodium carbonate solution (1 mL) was then added, and it was allowed to sit at room temperature for 20 min after thoroughly stirring the mixture. The absorbance at 725 nm was measured and the total tannin concentration was stated as µg of tannic acid equivalent per gram of dry extract.

2.5.4. Determination of total phenolic content (TPC)

Samples were made according to section 2.5.3's, instructions. After 20 min of incubation at room temperature, the absorbance was measured at 765 nm. A blank was read against a collection of Gallic acid standard solutions and the phenolic values were represented in terms of Gallic acid in µg/g of dry extract [19].

2.5.5. Determination of total antioxidant activity (TAA)

The phosphomolybdenum assay method, which is based on the reduction of Mo (VI) to Mo (V) and the subsequent formation of a green phosphate-Mo (V) complex in acidic conditions, was used to assess the extract's overall antioxidant activity [20]. The sample extract (0.5 mL) was mixed with 3.0 mL of reagent solution (0.6 M H₂SO₄, 28 mM Na₃PO₄, 4 mM ammonium molybdate) and incubated at 95 °C for 90 min. The absorbance of the solution was measured at 695 nm against a reagent blank, and the ascorbic acid equivalent per gram of dry extract was used to evaluate antioxidant activity.

2.5.6. Determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The experiment that investigated the DPPH radical-scavenging activity was carried out using the modified approach described by Sanna et

al. (2012) [21]. During this procedure, 2 mL of 0.2 mM methanol DPPH solution was added to 2 mL of extract solution at varied concentrations. The solutions were left in a dark, room-temperature setting for 30 min to react and the absorbance at 517 nm was measured after 10 min.

DPPH radical-scavenging activity (%) = $(Ac-As)/Ac \times 100$, where Ac and As is the absorbance of control solution's and DPPH solution with plant extracts respectively. The IC₅₀ was estimated by plotting DPPH radical-scavenging activity (%) versus sample extract concentration [22]. Positive control in this investigation was ascorbic acid.

2.6. Identification of bioactive polyphenols

2.6.1. Standard preparation

A stock standard solution (100 µg/mL) of each phenolic compound was prepared in methanol. The mixed standard solution was prepared by diluting the stock standard solutions in methanol to give a concentration of 5 µg/mL for each polyphenol. All standard solutions were stored in the dark at 4 °C. The calibration curves of the standards were made by a dilution of the stock standards (five sets of standard dilutions). The calibration curves were constructed from chromatograms as peak area vs. concentration of standard [14].

2.6.2. HPLC system

Thermo Scientific Dionex UltiMate 3000 Rapid Separation Liquid Chromatography (RSLC) system (Thermo Fisher Scientific Inc., MA, USA) was used for the chromatographic analyses. It is equipped with a quaternary rapid separation pump (LPG-3400RS), Ultimate 3000RS auto-sampler (WPS-3000), and rapid separation diode array detector (DAD-3000RS). An Acclaim® C18 (4.6 × 250 mm; 5 µm; 120 Å) column (Dionex, USA) was used to separate phenolic compounds. The temperature of the temperature-controlled column compartment (TCC-3000) was maintained at 30 degrees Celsius. For data acquisition, peak integration, and calibrations, the Dionex Chromeleon software (Version 6.80 RS 10) was used.

The phenolic content was determined using Sarunya and Sukon's (2006) approach [23]. Acetonitrile (solvent A), acetic acid solution (solvent B), methanol (solvent C), and IPA (solvent D) were used in the mobile phase. The system was run with the gradient elution program described in Table 1. A 5-minute post-run at initial conditions was performed to equilibrate the column. The flow rate varied throughout the analysis from 1 to 0.75 mL/min, and the injection volume was 20 µL. The wavelength program was adjusted to monitor phenolic chemicals at 280 nm for PDA detection. Calibration curves were generated using standards, and the R² for these curves was more than 0.995%.

2.7. Statistical analysis

All experimental results were expressed as mean ± standard deviations (SD). Data was analyzed by ANOVA, followed by the Tukey HSD

Table 1. Chromatographic conditions.

Sl no.	Retention [min]	Flow [mL/min]	A%	%B	%C
1	0.000	1.000	0.00	100	0.0
2	4.000	1.000	3.00	95.0	2.0
3	10.00	1.000	3.00	92.0	2.0
4	14.00	0.800	6.00	90.0	4.0
5	20.00	0.800	10.00	85.0	5.0
6	24.00	0.750	14.00	80.0	6.0
7	30.000	0.750	15.00	75.0	10.0
8	39.00	0.750	20.00	65.0	15.0
9	45.00	0.750	25.00	55.0	20.0
10	55.00	1.00	0.00	100	0.0

Where, A = Acetonitrile, B = Acetic acid solution of pH 3.0, C = Methanol.

test to analyze multiple comparisons using IBM SPSS Statistics (version 25). $P < 0.05$ was regarded as statistically significant. A Pearson correlation analysis was used to determine the association of test parameters. The figures were plotted with RStudio 2022.02.3 + 492 for Windows.

3. Results and discussion

3.1. Yield of the extract

The solvent system affects the yield percentage of the extract. The yield percentage based on the solvent follows the order: methanol > ethanol > acetone: water: acetic acid (Table 2). The highest yield percentage was observed for broccoli: methanol (18.54%), ethanol (16.02%), and acetone: water: acetic acid (15.29%). The results of the study were in good accord with Do et al. (2014)'s investigations on the role of different polarity solvents in bioactive component extraction [13].

3.2. Total flavonoid content (TFC)

The total amount of flavonoid content varied according to plant extract and solvent (Figure 1). Ethanolic extract of cauliflower had significantly higher amounts of TFC than other vegetables ($p = 0.03$). CUEE contains the highest amount of TFC among all the extracts (348 ± 5.20 µg/g dry extract). Whereas, flavonoid content in CUME and CUWAA were 118 ± 3.20 µg/g and 113 ± 2.20 µg/g, respectively. Drabińska et al. (2021) showed 290 µg/g of TFC in methanolic extract of cauliflower [24]. Broccoli had moderate amount of TFC and the amount in BCEE, BCME, and BCWAA was 186 ± 7.10 µg/g, 115 ± 3.2 µg/g and 109 ± 5.6 µg/g respectively. Bhandari et al. (2018) showed the level of TFC value ranged from 20 to 80 µg/g in methanolic extract of broccoli, which supported the findings of the current study [25]. Cabbage showed a significantly lower amount of TFC than the other two brassica vegetables.

Table 2. Yield and DPPH radical-scavenging activities of different extracts.

Samples	Yield (%)	DPPH scavenging activity (IC ₅₀ , µg/mL)	Regression equation, Inhibition (R ²)
CUME	15.14 ± 1.52 ^{ab}	380 ± 4.50 ^b	$y = 21.834\ln(x) - 80.54$ R ² = 0.9927
CUEE	14.69 ± 1.2 ^{bc}	160 ± 5 ^{fg}	$y = 14.385\ln(x) - 22.24$ R ² = 0.9904
CUWAA	12.69 ± 0.69 ^{cd}	400 ± 10 ^a	$y = 20.122\ln(x) - 72.512$ R ² = 0.9812
CAME	14.73 ± 0.84 ^{bcd}	90 ± 2.52 ^h	$y = 28.317\ln(x) - 77.61$ R ² = 0.995
CAEE	13.62 ± 0.76 ^{cd}	180 ± 4.05 ^d	$y = 23.073\ln(x) - 70.73$ R ² = 0.9657
CAWAA	12.15 ± 0.42 ^d	200 ± 5 ^c	$y = 14.385\ln(x) - 22.24$ R ² = 0.9904
BCME	18.54 ± 1.29 ^a	170 ± 3.60 ^e	$y = 15.033\ln(x) - 26.62$ R ² = 0.9596
BCEE	16.02 ± 1.29 ^{ab}	165 ± 2.51 ^f	$y = 13.193\ln(x) - 18.77$ R ² = 0.9717
BCWAA	15.29 ± 1.36 ^{bc}	155 ± 1.73 ^g	$y = 18.302\ln(x) - 43.21$ R ² = 0.9523
Ascorbic Acid	-	19.5 ± 0.15 ⁱ	$y = 16.082\ln(x) + 3.33$ R ² = 0.8538

Values are mean ± SD, n = 3 (three independent extractions).

Means containing same letter (s) in the column did not differ significantly at 5% level of significance.

Where, CAME = Methanol extract of cabbage, CAEE = Ethanol extract of cabbage, CAWAA = water, acetone and acetic acid extract of cabbage, CUME = Methanol extract of cauliflower, CUEE = Ethanol extract of cauliflower, CUWAA = water, acetone and acetic acid extract of cauliflower, BCME = Methanol extract of broccoli, BCEE = Ethanol extract of broccoli, BCWAA = water, acetone and acetic acid extract of broccoli.

nd = not detected.

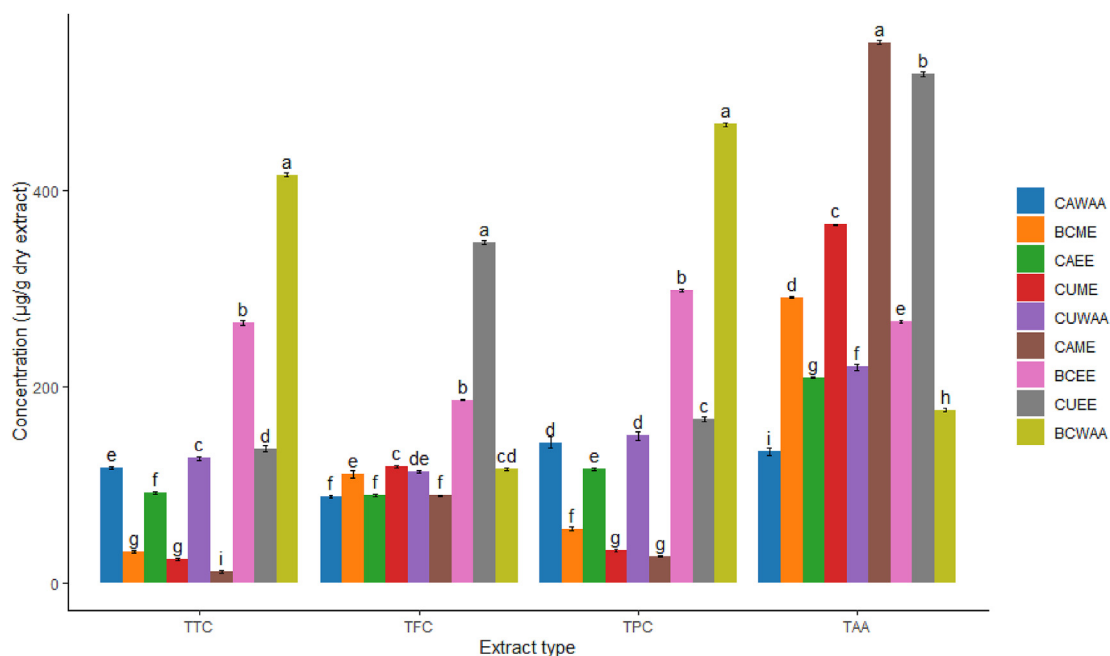


Figure 1. Antioxidant properties of cabbage, cauliflower and broccoli extracts. The same letter (s) on the top of the bar of the same experiment did not differ significantly at the 5% level of significance. Where, CAME = Methanol extract of cabbage, CAEE = Ethanol extract of cabbage, CAWAA = water, acetone and acetic acid extract of cabbage, CUME = Methanol extract of cauliflower, CUEE = Ethanol extract of cauliflower, CUWAA = water, acetone and acetic acid extract of cauliflower, BCME = Methanol extract of broccoli, BCEE = Ethanol extract of broccoli, BCWAA = water, acetone and acetic acid extract of broccoli.

CAWAA illustrated the lowest amount of TFC ($87 \pm 5.20 \mu\text{g/g}$) than CAME ($88 \pm 3.20 \mu\text{g/g}$) and CAEE ($89 \pm 2.20 \mu\text{g/g}$). A study by Singh *et al.* (2006) reported similar levels of total phenolic content in ethanolic extract of cabbage [26].

3.3. Total tannin content (TTC)

Broccoli demonstrated a significantly higher amount of TTC than cabbage and cauliflower ($p = 0.04$) in all types of extracts (Figure 1). BCWAA had the highest concentration of TTC ($414 \pm 5.20 \mu\text{g/g}$ dry extract). Whereas, the tannin content of BCME and BCEE were $31 \pm 1.36 \mu\text{g/g}$ and $262 \pm 5.70 \mu\text{g/g}$ respectively. A previous study conducted by Manchali *et al.* (2012) showed $410 \mu\text{g/g}$ of TTC in ethanolic extract, which concurred with the findings of the current study [27]. Cauliflower had moderate amounts of TTC in CUEE, CUME and CUWAA, which were $139 \pm 8.10 \mu\text{g/g}$, $25 \pm 1.2 \mu\text{g/g}$ and $127 \pm 5.6 \mu\text{g/g}$ respectively. However, in a study in Algeria showed higher levels of tannin ($526 \mu\text{g/g}$) in methanolic extract of cauliflower [28]. Cabbage had a significantly ($p = 0.02$) lower amount of TTC than the other two extracts, and the amount in CAME was $11 \pm 1.25 \mu\text{g/g}$. The TTC in CAEE and CAWAA were $90 \pm 3.70 \mu\text{g/g}$ and $116 \pm 5.30 \mu\text{g/g}$ respectively. Although Deepa *et al.* (2020) found almost double TTC ($285 \mu\text{g/g}$) in ethanolic extract of cabbage [29].

3.4. Total phenolic content (TPC)

BCWAA contains the highest amount of TPC among all the extracts and it was $465 \pm 3.25 \mu\text{g/g}$ dry extract (Figure 1). TPC in BCME and BCEE were $54 \pm 1.75 \mu\text{g/g}$ and $297 \pm 4.55 \mu\text{g/g}$ respectively. Jaiswal *et al.* (2012) reported that methanolic and ethanolic extracts of broccoli showed TPC value of $236 \mu\text{g/g}$ and $195 \mu\text{g/g}$, respectively [30].

Cauliflower showed moderate TPC values in CUEE, CUME and CUWAA, which were $165 \pm 3.25 \mu\text{g/g}$, $33 \pm 2.25 \mu\text{g/g}$ and $146 \pm 2.35 \mu\text{g/g}$ respectively. Ahmed *et al.* (2013) and Drabińska *et al.* (2021) found $267 \mu\text{g/g}$ and $276 \mu\text{g/g}$ of TPC in methanolic extracts of cauliflower, respectively [24, 31]. Cabbage showed significantly ($p = 0.02$) lower

amount of TPC in CAME ($27 \pm 1.15 \mu\text{g/g}$) than CAEE ($115 \pm 2.45 \mu\text{g/g}$) and CAWAA ($140 \pm 3.25 \mu\text{g/g}$). Whereas, Jaiswal *et al.* (2012) reported that the TPC of methanolic extract of cabbage was $187 \mu\text{g/g}$ [30]. In addition, TTC and TPC showed strong positive ($r = 0.953$) correlation in all extracts.

3.5. Total antioxidant activity (TAA)

TAA varied according to vegetable and solvent (Figure 1). The ethanolic extract of cabbage showed significantly higher amounts of TAA than the other two vegetables ($p = 0.04$). The highest amount of TAA was found in CAME, which was $549 \pm 7.30 \mu\text{g/g}$. Whereas, the TAA of CAEE and CAWAA were $209 \pm 5.70 \mu\text{g/g}$ and $131 \pm 3.30 \mu\text{g/g}$, respectively. On the other hand, the TAA of CUEE, CUME and CUWAA were $515 \pm 5.25 \mu\text{g/g}$, $364 \pm 6.15 \mu\text{g/g}$ and $217 \pm 3.65 \mu\text{g/g}$ respectively. Moreover, the antioxidant activity of BCEE, BCME and BCWAA were $265 \pm 3.20 \mu\text{g/g}$, $290 \pm 4.20 \mu\text{g/g}$, $175 \pm 5.25 \mu\text{g/g}$ respectively. Bahorun *et al.* (2004) presented $188 \mu\text{g/g}$, $748 \mu\text{g/g}$, $499 \mu\text{g/g}$ of TAA in methanolic extracts of cabbage, broccoli, and cauliflower [32]. Podse *et al.* (2006) also showed $250 \mu\text{g/g}$ of TAA in methanolic extract of cabbage, which supports the findings of our study [33].

3.6. DPPH radical scavenging activity

At the tested levels, all of the vegetable extracts and the reference compound (ascorbic acid) were capable of directly interacting with and quenching the DPPH radical (Table 2). In the case of cabbage, methanolic extract was found to have the highest DPPH radical-scavenging capacity with an IC_{50} value of $90 \pm 2.52 \mu\text{g/mL}$ (Figure 2). On the other hand, Cauliflower was found to have the lowest DPPH radical-scavenging capacity with IC_{50} $400 \pm 10 \mu\text{g/mL}$ in CUWAA followed by $380 \pm 4.50 \mu\text{g/mL}$ in CUME. There was no significant difference in IC_{50} value between BCME, BCEE and BCWAA. According to Turkmen *et al.* (2006), solvent polarity may be possible reason of efficiency of different extracts on scavenging of free radicals [34]. This antiradical activity was similar to the extracts of broccoli, cabbage and cauliflower [35, 36, 37, 38].

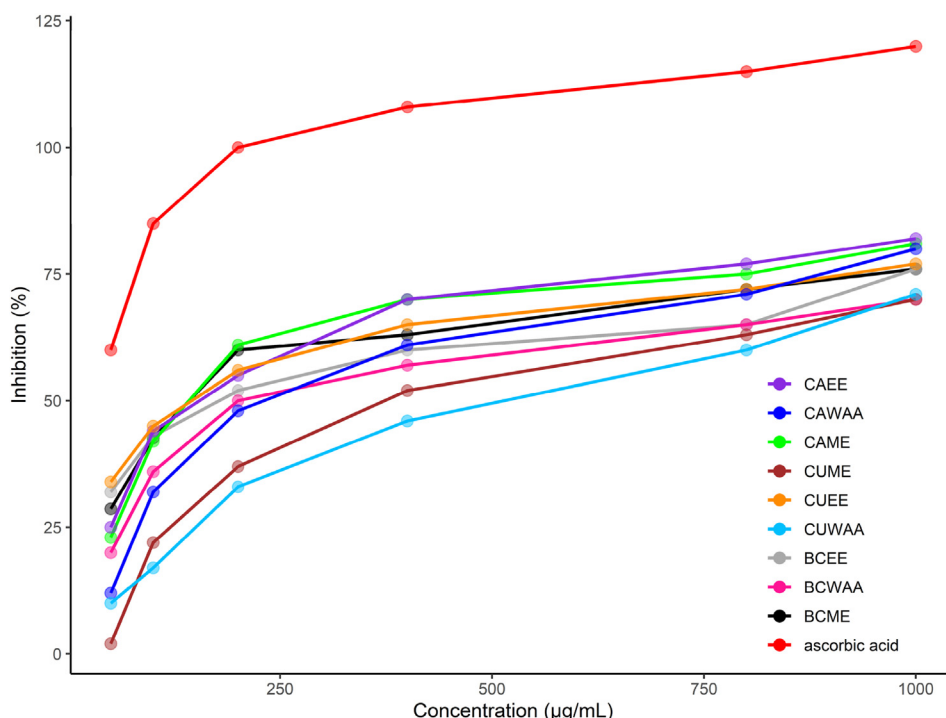


Figure 2. Logarithmic trendline for DPPH radical-scavenging activity of cabbage, cauliflower and broccoli extracts and Ascorbic acid. Where, CAME = Methanol extract of cabbage, CAEE = Ethanol extract of cabbage, CAWAA = water, acetone and acetic acid extract of cabbage, CUME = Methanol extract of cauliflower, CUEE = Ethanol extract of cauliflower, CUWAA = water, acetone and acetic acid extract of cauliflower, BCME = Methanol extract of broccoli, BCEE = Ethanol extract of broccoli, BCWAA = water, acetone and acetic acid extract of broccoli.

3.7. Bioactive polyphenols

The various phenolic compounds that were found in the extracts of these three brassica vegetables are presented in Table 3, and the corresponding chromatograms are shown in Figure 3. The panels labeled A–J of Figure 3 represent the standards, CUME, CUEE, CUWAA, CAME, CAEE, CAWAA, BCME, BCEE, and BCWAA, respectively. Gallic acid was the most abundant phenolic acid in all types of extracts. CUWAA had

significantly ($p = 0.03$) the highest amount of Gallic acid ($881 \pm 4.24 \mu\text{g/g}$) than others. CAWAA had the lowest amount of Gallic acid ($112 \pm 2.31 \mu\text{g/g}$). A study by Bahorun et al. (2004) found $270 \mu\text{g/g}$, $150 \mu\text{g/g}$ of Gallic acid in methanolic extracts of cauliflower and cabbage respectively [32].

Chlorogenic acid was found in cabbage and cauliflower. CUEE contained $540 \pm 2.31 \mu\text{g/g}$ of Chlorogenic acid which was the highest among all. The level of Chlorogenic acid in cabbage was $115 \pm 1.25 \mu\text{g/g}$. In a

Table 3. Bioactive polyphenolic compounds ($\mu\text{g/g}$ dry extract) of different extract of three vegetables.

Compounds ($\mu\text{g/g}$ dry extract)	Retention time (minute)	CUME	CUEE	CUWAA	CAME	CAEE	CAWAA	BCME	BCEE	BCWAA
Gallic acid	8.33	451 ± 3.26^c	518 ± 6.2^b	881 ± 4.24^a	141 ± 3.5^g	122 ± 1.23^b	112 ± 2.31^c	245 ± 2.5^f	290 ± 2.30^e	320 ± 4.15^d
Chlorogenic Acid	13.70	nd	502 ± 1.7^a	282 ± 1.24^c	nd	115 ± 1.25^d	nd	nd	nd	331 ± 3.60^b
Catechol	19.20	nd	nd	nd	nd	nd	nd	nd	nd	nd
Catechin hydrate	22.6	48 ± 2.51^e	89 ± 1.5^d	113 ± 2.94^c	115 ± 1.5^{bc}	125 ± 2.5^b	90 ± 2.84^d	440 ± 1.45^a	92 ± 1.20^d	35 ± 1.20^f
Vanillic acid	20.10	80 ± 3.54^b	540 ± 2.31^a	40 ± 5.1^c	nd	35 ± 3.26^d	20 ± 2.59^f	nd	nd	22 ± 0.50^e
Caffeic acid	24.06	nd	nd	nd	31 ± 1.45^a	nd	10 ± 1.51^b	nd	nd	nd
(-) Epicatechin	29.08	158 ± 2.1^b	135 ± 1.36^c	90 ± 1.24^e	210 ± 1.05^a	nd	26 ± 3.20^g	36 ± 1.30^f	29 ± 1.10^g	94 ± 1.05^d
Vanillin	31.87	nd	nd	nd	nd	nd	nd	nd	nd	nd
Trans-Ferulic acid	32.81	Nd	nd	nd	nd	nd	nd	nd	nd	nd
p-Coumaric acid	31.34	58 ± 0.76^e	340 ± 1.59^b	286 ± 1.7^c	nd	380 ± 3.25^a	nd	nd	nd	65 ± 1.1^d
Rutin Hydrate	37.28	63 ± 1.23^c	350 ± 2.31^b	nd	nd	551 ± 2.12^a	nd	nd	nd	21 ± 0.75^d
Rosmarinic acid	29.76	122 ± 1.23^d	202 ± 1.24^a	53 ± 0.81^f	150 ± 2.10^b	142 ± 1.50^c	75 ± 3.5^e	nd	nd	nd
Myricetin	43.59	nd	nd	nd	nd	nd	nd	nd	34 ± 1.20^b	52 ± 0.65^a
Quercetin	49.47	nd	nd	nd	nd	33 ± 0.90^b	nd	nd	186 ± 1.5^a	22 ± 0.75^c
trans-Cinnamic acid	46.05	nd	nd	nd	nd	nd	nd	nd	nd	nd
Naringenin	52.03	nd	nd	nd	nd	nd	nd	nd	nd	nd
Kaempferol	53.80	nd	nd	nd	nd	nd	nd	nd	nd	nd

Values are mean \pm SD, $n = 3$ (three independent extractions).

Means containing same letter (s) in row did not differ significantly at 5% level of significance.

Where, CAME = Methanol extract of cabbage, CAEE = Ethanol extract of cabbage, CAWAA = water, acetone and acetic acid extract of cabbage, CUME = Methanol extract of cauliflower, CUEE = Ethanol extract of cauliflower, CUWAA = water, acetone and acetic acid extract of cauliflower, BCME = Methanol extract of broccoli, BCEE = Ethanol extract of broccoli, BCWAA = water, acetone and acetic acid extract of broccoli.

nd = not detected.

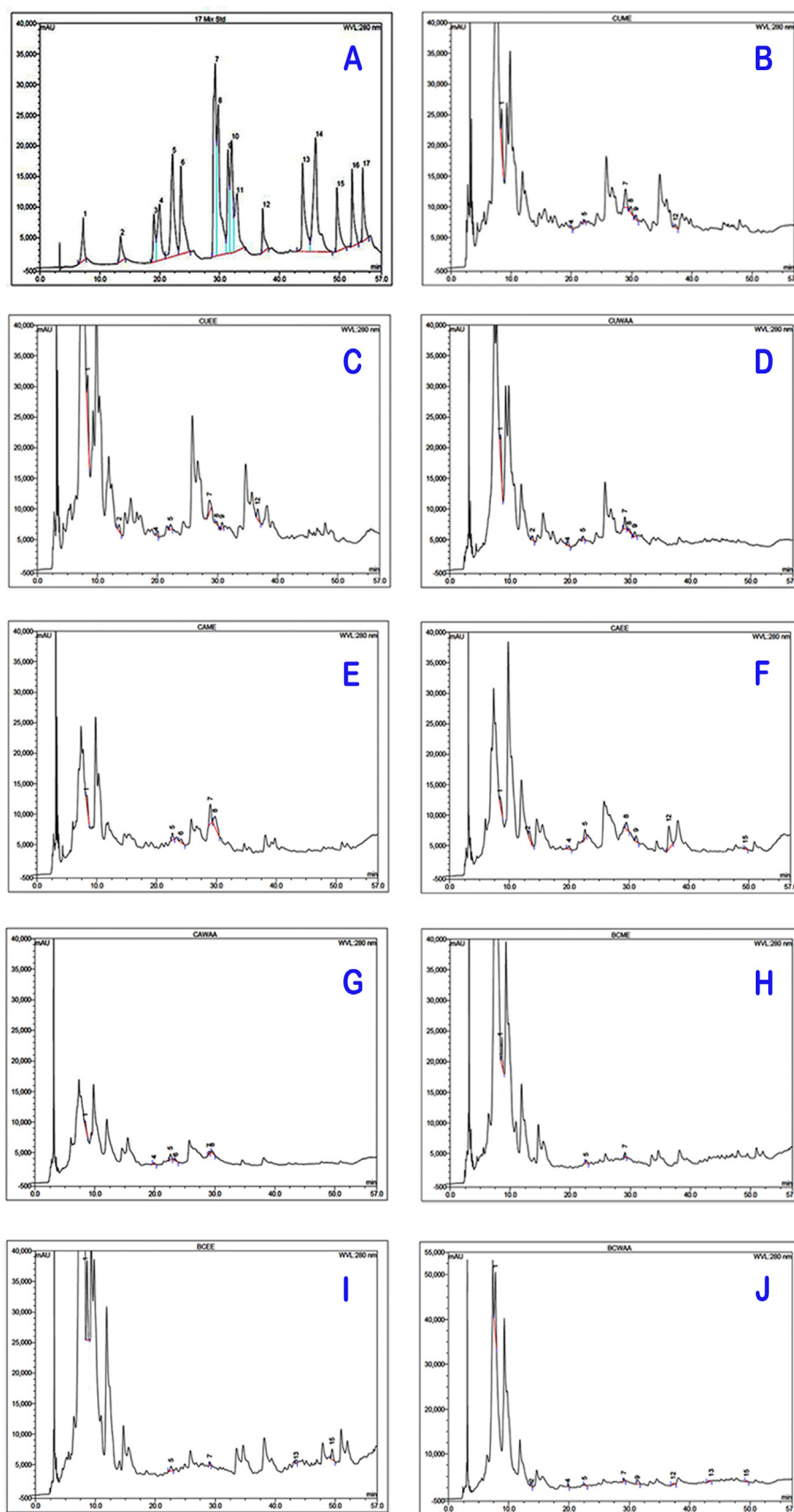


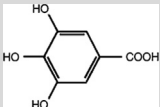
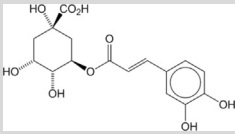
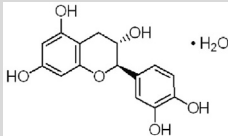
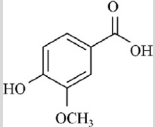
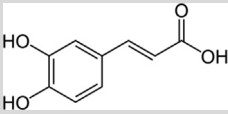
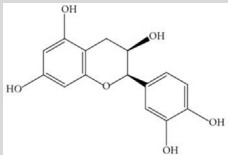
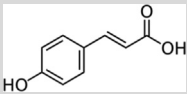
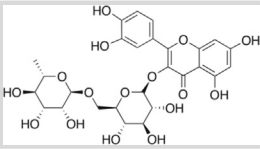
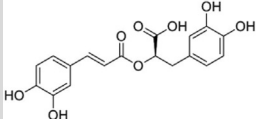
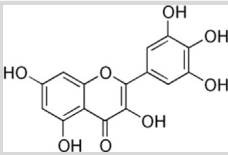
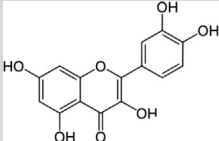
Figure 3. Chromatogram of polyphenols: A) Standards, B) CUME, C) CUEE, D) CUWAA, E) CAME, F) CAEE, G) CAWAA, H) BCME, I) BCEE, J) BCWAA. Where, 1 = Gallic acid, 2 = Chlorogenic Acid, 3 = Catechol, 4 = Vanillic acid, 5 = Catechin hydrate, 6 = Caffeic acid, 7 = (-) Epicatechin, 8 = Rosmarinic acid, 9 = p-Coumaric acid, 10 = Vanillin, 11 = Trans-Ferulic acid, 12 = Rutin Hydrate, 13 = Myricetin, 14 = trans-Cinnamic acid, 15 = Quercetin, 16 = Naringenin, 17 = Kaempferol.

similar report, Park *et al.* (2019) showed that Chlorogenic acid in cabbage was 37 $\mu\text{g/g}$ [39].

Catechin hydrate is the second most available phenolic compound in all types of extracts. The highest amount of Catechin hydrate was found

in BCME ($440 \pm 1.45 \mu\text{g/g}$). Cabbage contained Catechin hydrate in the range of 113–125 $\mu\text{g/g}$ dry extract. Park *et al.* (2019) reported that the methanolic extract of cabbage had 11 $\mu\text{g/g}$ of Catechin hydrate [39]. The level of Vanillic acid in CUEE ($540 \pm 2.31 \mu\text{g/g}$) was higher than other

Table 4. Review on the functional properties of bioactive polyphenols available in the extract of three brassica vegetables (cabbage, cauliflower and broccoli).

Polyphenol compounds	Structure	Source	Functional properties
Gallic acid		Cabbage (CAME, CAEE and CAWAA) Cauliflower (CUME, CUEE and CUWAA) Broccoli (BCME, BCEE and BCWAA)	<ul style="list-style-type: none"> antioxidant anti-inflammatory antineoplastic properties antihyperlipidemic cardioprotective [44]
Chlorogenic Acid		Cabbage (CAME, CAEE and CAWAA) Cauliflower (CUEE) Broccoli (BCWAA)	<ul style="list-style-type: none"> anti-diabetic effect anti-obesity effect anti-inflammatory hepatic steatosis anti-carcinogenic [45]
Catechin hydrate		Cabbage (CAME, CAEE and CAWAA) Cauliflower (CUME, CUEE and CUWAA) Broccoli (BCME, BCEE and BCWAA)	<ul style="list-style-type: none"> liver damage prevention cholesterol lowering effect anti-obesity activity inhibiting the ovarian cancer potential chemo preventive agent [46, 47]
Vanillic acid		Cabbage (CAEE and CAWAA) Cauliflower (CUME, CUEE and CUWAA) Broccoli (BCWAA)	<ul style="list-style-type: none"> anti-inflammatory Anti-analgesic [48]
Caffeic acid		Cabbage (CAME and CAWAA)	<ul style="list-style-type: none"> anti-diabetic effect anti-carcinogenic prevent premature aging prevent neurodegenerative diseases, like Parkinson's disease reduce inflammation [49]
(-) Epicatechin		Cabbage (CAME and CAWAA) Cauliflower (CUME, CUEE and CUWAA) Broccoli (BCME, BCEE and BCWAA)	<ul style="list-style-type: none"> anti-diabetic effect anti-carcinogenic prevention and treatment of Parkinson's disease [50]
p-Coumaric acid		Cabbage (CAEE) Cauliflower (CUME, CUEE and CUWAA) Broccoli (BCWAA)	<ul style="list-style-type: none"> anti-inflammatory antiplatelet aggregation antipyretic analgesic anti-arthritis activities anxiolytic [51]
Rutin Hydrate		Cabbage (CAEE) Cauliflower (CUME and CUEE) Broccoli (BCWAA)	<ul style="list-style-type: none"> Anticonvulsant activity Antidepressant effects Prevention of neuroinflammation Improve blood circulation [52]
Rosmarinic acid		Cabbage (CAME, CAEE and CAWAA) Cauliflower (CUME, CUEE and CUWAA)	<ul style="list-style-type: none"> anti-diabetic anti-allergic anti-inflammatory hepato- and renal-protectant agent Anti-Inflammatory [53]
Myricetin		Broccoli (BCEE and BCWAA)	<ul style="list-style-type: none"> anticancer, antidiabetic anti-inflammatory activities anti-amyloidogenic [47, 54]
Quercetin		Cabbage (CAEE) Broccoli (BCEE and BCWAA)	<ul style="list-style-type: none"> anti-carcinogenic anti-inflammatory antiviral activities Reducing the risk of heart disease Preventing neurological diseases [55]

extracts. Ahmed and Ali (2013) reported 119 µg/g of Vanillic acid in methanolic extract of cauliflower [31]. Caffeic acid was identified only in cabbage and the amount in CAME and CAWAA was 31 ± 1.45 µg/g, 10 ± 1.51 µg/g respectively. Park *et al.* (2019) also found similar levels of Caffeic acid (5 µg/g) in the methanolic extract of cabbage [39]. CAME had the highest amount of Epicatechin (210 ± 1.05 µg/g) followed by CUME (158 ± 2.1 µg/g). Park *et al.* (2019) found 35 µg/g of Epicatechin in methanolic extract of cabbage [39]. P-Coumaric acid was found in all vegetables of the *Brassicaceae* but it was mostly abundant in ethanolic extracts of cabbage and cauliflower. CAEE had 380 ± 3.25 µg/g of P-Coumaric acid, which was the highest amount of all and was followed by CUEE (340 ± 1.59 µg/g). Whereas, CUWAA (286 ± 1.7 µg/g) contained the highest P-Coumaric acid, followed by BCWAA (65 ± 1.1 µg/g). Paško *et al.* (2018) found 270 µg/g of p-Coumaric acid in methanolic extract of broccoli [40]. The level of p-Coumaric acid in CUEE was greater than the findings of Ahmed and Ali (2013), which was 70 µg/g [31]. The highest amount of Rutin Hydrate was found in CAEE (551 ± 2.125 µg/g) followed by CUEE (350 ± 2.31 µg/g). Conversely, Park *et al.* (2019) showed that cabbage contained 50 µg/g of Rutin Hydrate [39]. Broccoli had small amount of Rutin Hydrate in BCWAA (21 ± 0.75 µg/g). Rosmarinic acid was not detected in broccoli, but nevertheless found in both cabbage and cauliflower. The highest level of Rosmarinic acid was determined in CAME (150 ± 2.10 µg/g) and the lowest in CUWAA (53 ± 0.81 µg/g). Ahmed and Ali (2013) showed that 17.3 µg/g of Rosmarinic acid was present in methanolic extract of cauliflower [31]. Myricetin was found only in Broccoli, ranging from 34 ± 1.20 µg/g (BCEE) to 52 ± 0.65 µg/g (BCWAA). Miesan *et al.* (2001) expressed that 62.5 µg/g of myricetin was estimated in a methanolic extract of broccoli [41]. Quercetin was not detected in Cauliflower but was found in both cabbage and broccoli. The highest amount of Quercetin was recorded in BCEE (186 ± 1.5 µg/g). USDA (2022) claimed 28 µg/g and 33 µg/g of quercetin in methanolic extracts of cabbage and broccoli [42]. Catechol, Vanillin, trans-Cinnamic acid, trans-Ferulic acid, Naringenin and Kaempferol were not detected in any of the extracts of three vegetables.

In addition, Caffeic acid had a strong positive association ($r = 0.932$) with Gallic acid. At the same time, Gallic acid also had a well-built positive association ($r = 0.932$) with Myricetin. Whereas, Caffeic acid showed a strong affirmative association ($r = 0.932$) with Epicatechin and Catechin hydrate. Moreover, Epicatechin showed a moderate positive association ($r = 0.602$) with Chlorogenic Acid and Quercetin. Catechin hydrate showed a strong positive association ($r = 0.952$) with p-Coumaric acid and a moderate negative association ($r = -0.522$) with Quercetin and Myricetin.

In the case of cabbage and broccoli, aqueous extract shows more efficiency in extracting antioxidant compounds, whereas ethanol has good properties for cauliflower. The combination of organic solvent and water may help extract all constituents that are soluble in both water and organic solvents, which may explain why overall extraction efficiency improved. Several variables, including the variation in plant matrix, the composition of antioxidant compounds, the amount of hydroxyl groups in components, the temperature and duration of extraction, as well as solvent polarity, affect the antioxidant capabilities and bioactive compound extraction [13].

Different epidemiological studies over recent years and their linked meta-analyses strongly recommended that intake of diets loaded with plant polyphenols for the long term provides defense against the development of non-communicable chronic health problems like cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [43]. A short review of the functional activity of the analyzed phenolic compounds was presented in Table 4 and as well depicting the scenario of available polyphenols in different extract to understand the extraction behavior of extracts.

4. Conclusion

Cauliflower, cabbage and broccoli contained bioactive polyphenols along with remarkable in vitro antioxidant properties. The uniqueness of

this work was that it presents a polarity-based comparison of the polyphenolic components and antioxidant profiles of these three brassica vegetables that were extracted in three different solvents. The discussion revealed that the relative polarity of the solvent had an impact on the extraction ability of particular bioactive polyphenols. The funding of this study supported the functional properties of brassica vegetables which might play an important role in human health. It could be said in support of the literature that these vegetables as a whole are perhaps more appropriate to the value-added food products in the food industry. In order to promote the health advantages of such veggies, the extraction solvent may choose to produce functional food by focusing on a particular bioactive polyphenol.

Declarations

Author contribution statement

Mohammad Mahfuzur Rahman: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Abu Tareq Mohammad Abdullah: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Miskat Sharif: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sharmin Jahan; Md. Alamgir Kabir; Md. Motalab: Performed the experiments; Analyzed and interpreted the data.

Tanzir Ahmed Khan: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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