European Journal of Applied Sciences – Vol. 10, No. 1
Publication Date: February 25, 2022
DOI:10.14738/aivp.101.11802.
Sultana, R., Shemu, S., Ali, A., Ahiduzzaman, Rahman, M., Rafiquzzaman, S. M., & Haque, M. A. (2022). Burmese Grape is an Underexploited Fruit Crop Potential to Use in Phytonutrient Enriched Yogurt Formulation. European Journal of Applied Sciences, 10(1). 525-543.

Burmese Grape is an Underexploited Fruit Crop Potential to Use in Phytonutrient Enriched Yogurt Formulation

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ABSTRACT

The present study aimed to investigate the phytonutrients present in Burmese grape (*Baccaurea ramiflora)* fruit (BGF) followed by an antioxidant-enriched yogurt preparation. The fruit peel was extracted in 100% ethanol, 50% ethanol and water. The total phenolic content (TPC) and total flavonoid content (TFC) were determined as a quantitative measure of phytonutrients. The antioxidant activities both in extracts and yogurt were assessed using ABTS and DPPH radical scavenging assays. The BGF juice contained the highest concentration of TPC (57.25 ± 0.68 µg GA/g) and TFC (73.36 ± 0.75 µg QE/g). The peel extracts exhibited radical scavenging activities according to the order of 100% ethanol > 50% ethanol > water. The yogurt samples containing 0%, 10% and 12% BGF juice secured the overall scores indicating Like moderately to Like very much. Therefore, addition of 12% BGF juice

is recommended to formulate a phytonutrient enriched yogurt with acceptable sensory properties.

Keywords: Burmese grape, phytonutrients, total phenolic content, total flavonoid content, antioxidant activities, yogurt.

INTRODUCTION

The biological system always induces the production or discharge of various reactive constituents or free radicals. These free radicals attack electron-rich lipid membranes, proteins, and nucleic acids, thereby damaging the body's cells and tissues when consumed or inhaled. In response, the body protection system removes or neutralizes these unstable molecules by certain compounds collectively known as antioxidants. The antioxidant defense system diminishes the level of free radicals and retains the homeostatic balance for the proper functioning of the body. However, when these reactive species are overwhelming high in the body, it surpasses the capacity of the antioxidant defense system leading to a condition known as oxidative stress (Engwa, 2018). Such persistent stress might lead to certain diseases like diabetes, atherosclerosis, cardiovascular diseases, cancer, etc. The spontaneous oxidation causes food rancidity and spoilage of medicines. One of the possible remedies for this condition is to enhance the endogenous antioxidant defense system with exogenous antioxidants. Antioxidants remove or inactivate these free-radical intermediates by oxidizing them (Soory, 2012; Pellegrino, 2016).

The food and pharmaceutical industries very often use synthetic antioxidants like Butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) to prepare antioxidant-enriched products (Shebis et al., 2013). However, the researchers are aware of commercial using of synthetic chemicals because of its carcinogenic effects on human cells (Thorat et al., 2013; Caleja et al., 2017). Therefore, it is imperative to include antioxidants in our diets from natural sources. To meet the increased demand for natural antioxidants, currently, there is a strong research trend to search for available and efficient natural sources of antioxidants to replace synthetic ones (Ahmed et al., 2015; Gowe, 2015).

The fruits and vegetables are blessed with numerous phytochemical groups which are naturally used as antioxidants to protect the plants from environmental stresses such as pollution, pathogens and so on. Phytochemicals are plant-derived secondary metabolites that play important functional roles in the human and animal body in preventing chronic diseases (Abbas et al., 2015). These compounds are also helpful in the food and pharmaceutical industries as additives and flavors (Das et al., 2012; Tarko et al., 2015). These are beneficial for health, mainly due to their oxygen reduction activity. There are numerous fruits, vegetables, seeds, and plants that have been tested for their phytochemicals and antioxidant activities (Baiano et al., 2014) like tomatoes (Alarcon-Flores et al., 2015), peppers (Bhandari et al., 2016), jackfruit (Shafiq et al., 2017), mango (Kim et al., 2010), banana (Aboul-Enein et al., 2016), apple (Lata et al., 2009).

The Burmese grape (*Baccaurea ramiflora*), which belongs to the family Phyllanthaceae, is one of the common edible fruits grown in the Southeast Asian region, including Bangladesh. Apart from its convincing nutrient contents, including carbohydrate 51.9%, water 35.6%, fiber 20.4% and protein 5.58%, different parts of BGF like fruit juice and peel extract exhibit cytotoxic,

antiviral, and antioxidant activities and are rich in varieties of phytochemicals (Hawlader et al., 2012). Hence, the researchers found BGF as a good source of phenolic and flavonoid compounds (Amin and Nabi, 2015; Gunawan et al., 2016). However, most extractions were carried out using non-safe solvents like methanol, butanol, hexane, petroleum ether etc., for food preparation (Mann et al., 2016; Uddin et al., 2018). The literature lacks complete study on the extraction of the phytochemicals from BGF and their utilization in food formulation.

Yogurt is a fermented dairy product that is a delicious, nutritious, and healthy food item and has therapeutic value (Perdigon et al., 2002). It is easily digestible due to the lower lactose content that lactose-intolerant individuals can easily intake. Although this is a healthy food item regarding major food nutrients, it contains no or inadequate phytonutrients. With the advancement of food technology, value addition in food preparations by incorporating natural and synthetic micronutrients is a common practice (Dary and Mora, 2013). The BGF phytocompunds can be used in antioxidant-enriched food preparation that could be beneficial against the aforesaid health-related problems. (Wibawanti et al., 2017).

Therefore, the present study was designed to investigate the quantitative and qualitative phytonutrients present in Burmese grape fruit juice and peel. The peel portion was extracted in food-grade solvents like ethanol and water, whereas the fruit juice was used directly for investigations. The study activities were followed by preparing yogurt with higher antioxidant activities by using the BGF juice as an ingredient in the formulation.

MATERIALS AND METHODS

Preparation of Burmese grape peel extract and fruit juice

The fresh Burmese grape fruit was purchased from the local market of Gazipur, Bangladesh and washed thoroughly with potable water to remove adhered materials from the fruit surfaces. After peeling, the fruit juice and pulp were blended and filtrated through a clean nylon cloth to make fine juice. The peel was dried at 40°C in an oven dryer for 48 h followed by grinding and sieving through 1.0 mm sieve into fine powder. The peel powder was then subjected to solvent extraction in different food-grade solvents such as pure ethanol, 50% ethanol in water, and distilled water. Each extraction was performed in a beaker by dissolving 4 g powder in 100 mL solvent (Sobuj et al., 2021). For better extraction, the beakers were kept on an incubator shaker (CS-05C, Classic Scientific) and allowed intermittent shaking at 37°C in the dark for 24 h. After incubation, the solvent mixes were filtered through Whatman filter paper No 4 (20-25 µm). The ethanol extracts (Figure 1) were then concentrated by a Rotary Vacuum Evaporator (LRE-702A, Labocon, UK) at 36°C. The concentrated extracts were further dried to a 10 mg/mL concentration by bubbling nitrogen gas into the solution through nitrogen concentrator evaporator (AT-EV-50, Athena Technology, India) at 36°C.

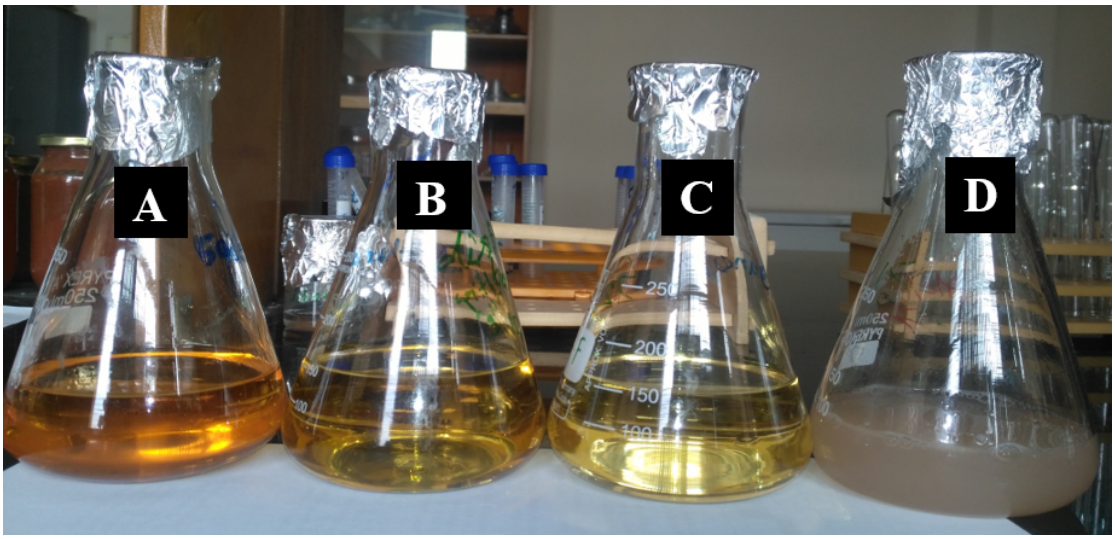


FIGURE 1 Peel extracts in different solvents and fruit juice; A) Ethanolic extract of peel, B) 50% ethanolic extract of peel, C) Water extract of peel, D) Fruit juice

The water solvent of peel was dried to 10 mg/mL by the Freeze Dryer (VaCo 2, Zirbus, UK) at -83°C. Since the Burmese grape juice is an edible product, all bioassays were performed with untreated fruit juice, i.e. without using the fruit juice in any solvent extraction. The stock solutions (10mg/ml) were preserved at 4°C. The working solutions were prepared at various concentrations from 1 to 4 mg/ml for further analyses. The analyses were performed immediately; within a week of storing the stock solutions.

Screening Tests for Phytochemicals

Terpenoids

5 mL of stock solution from each sample (Figure 1) were taken in individual test tubes and 2 mL chloroform was added to each one. 3 mL H_2SO_4 was also added to the tubes held at 45° angle to avoid any possible explosion. The formation of reddish-brown color at the interface indicated the presence of terpenoids (Rafiquzzaman et al., 2016; Ayoola et al., 2008).

Saponin

3 mL of distilled water was added to the test tubes containing 3 mL of sample extracts (Figure 1). Persistent froths were appeared after shaking the tubes for 1 min. 3 drops of olive oil were mixed into each tube and shaken vigorously for formation of emulsion which subsequently indicated the characteristic presence of saponins in the extract. A positive result was perceived by the appearance of froth of around 2 cm or higher which persisted for 10 minutes or longer (Rafiquzzaman et al., 2016).

Phlobatannins

3 mL of individual stock solution (Figure 1) was taken in separate test tubes. A few drops of 1% hydrochloric acid were added to each one. A red precipitate indicated the presence of phlobatannins in the crude extract (Suriyamoorthy et al., 2014).

Cardiac Glycoside

5 mL of each stock solution (Figure 1) was taken in the individual test tube. 2 mL of stock reagent solution (glacial acetic acid + 1 drop of ferric chloride) was added into each test tube,

followed by adding 1 mL H_2SO_4 into the tube held at 45° angle to avoid any explosion. A brown ring and a blue ring below the brown ring were appeared simultaneously at the interfaces indicating the presence of deoxysugar characteristics of cardenolides (Oloyede, 2005; Edeoga et al., 2005).

Tannins

In individual test tubes 2/3 mL of each stock solution (Figure 1) were taken. 2 mL of 5% ferric chloride was added to each stock solution in test tubes. The formation of dark blue or greenish-black indicated the presence of tannins (Suriyamoorthy et al., 2014; Krishnamoorthi, 2015).

Flavonoids

An amount of 2/3 mL of individual stock solutions (Figure 1) were taken in separate test tubes. 1 mL of 2 N NaOH was added to each solution in test tubes. The presence of yellow color indicated the presence of flavonoids.

Phenolic compounds

2 mL of each stock solution (Figure 1) was taken in individual test tubes. 2 mL distilled water was poured into each test tube, followed by adding a few drops of 10% ferric chloride to the contents of each test tubes. The formation of blue or green color indicated the presence of phenols (Suriyamoorthy et al., 2014; Krishnamoorthi, 2015).

Quinine

3 mL of each stock solution (Figure 1) was taken in individual test tubes. 2 mL of concentrated H_2SO_4 was added into each test tube slowly and the formation of red color indicated the presence of quinones.

Coumarins

In individual test tubes, 2 mL of individual stock solutions (Figure 1) were taken and 2 mL of 10% NaOH was added into each test tube. The formation of yellow color indicated the presence of coumarins.

Anthraquinones

2 mL of each stock solution (Figure 1) was taken in individual test tubes. A few drops of 10% NH3 solution were added to the contents inside each test tube. The pink color precipitate indicated the presence of anthraquinones (Suriyamoorthy et al., 2014; Krishnamoorthi, 2015).

Steroid

2.0 mL of each stock solution (Figure 1) was mixed with 2.0 mL of chloroform in individual test tubes. 1 mL of concentrated H_2SO_4 was added carefully to the mixtures in test tubes alongside the surface of the tube. The red color that appeared in the chloroform layers indicated the presence of steroids (Suriyamoorthy et al., 2014; Krishnamoorthi, 2015).

Glycosides

3 mL of chloroform and 10% NH_3 solution was added to test tubes containing 2 mL of each stock solution (Figure 1). The formation of pink color indicated the presence of glycosides (Suriyamoorthy et al., 2014; Krishnamoorthi, 2015).

Preparation of Burmese grape yogurt

The yogurt using Burmese grape juice was prepared according to the procedure stated by Parvin et al., (2019) with a slight modification. Cow's milk was heated to boil and sugar was added to a rate of 12 g sugar per 100 mL milk. The suspension was reheated to evaporate so as to reduce 1000 mL milk to 700 mL followed by cooling its temperature to 35°C. The starter culture was added to this heat-treated milk at a rate of 6 g per 1000 mL fresh milk. To prepare phytonutrient enriched yogurt Burmese grape juice was added to the suspension by six different percentages; 0%(S_1), 4%(S_2), 8%(S_3), 10%(S_4), 12%(S_5), and 14%(S_6). For fermentation and formation of end product, the suspensions were poured into food-grade cups and incubated at 40°C for at least 6 h followed by refrigeration at 9°C until serving or using for further analyses.

Sensory evaluation of Burmese grape yogurt

In order to evaluate consumer's preferences, the prepared yogurts were subjected to an evaluation of sensory attributes such as color, flavor, taste, texture and overall acceptability. A test panel, consisting of 9 panelists, was selected from the academics, staffs and students who were previously experienced in the organoleptic evaluation of food products. The yogurts were served to the panelists and asked for a rating on a 9-point hedonic scale including 'Dislike extremely = 1, Dislike very much = 2, Dislike moderately = 3, Dislike slightly = 4, Neither like nor dislike = 5, Like slightly = 6, Like moderately = 7, Like very much = 8 and Like extremely = 9'. The panelists scored the yogurts against degrees of preferences and the obtained data were analyzed statistically.

Determination of Total Phenolic Content

Total phenolic content (TPC) in the fruit juice, crude extracts of peel, and yogurt samples were determined using Folin-Ciocalteu phenol reagents and external calibration of Gallic acid (Singleton et al., 1999; Uddin et al., 2018).

0.5 mL from the working solution was taken in the individual test tube and 100 µL of Folin–Ciocalteu working reagent was added to each one and mixed thoroughly. The details of preparing the sample solutions and their absorbance measurement (λ = 760 nm) are described in the next section (*Preparation of Gallic acid equivalent curve*). The total phenolics (TPC) concentration in each working solution was determined as milligram Gallic acid equivalent per gram by putting the absorbance in Equation-1. Triplicate analyses were performed to obtain more accurate results.

Preparation of Gallic acid equivalent curve

To prepare the Gallic acid (GA) equivalent curve, GA solutions of different concentrations (10, 20, 30, 40, 50 µg/mL) were prepared from the GA stock solution (1000 mg/L). 1 ml of each concentration was taken in the individual test tube and 0.5 ml Folin–Ciocalteu reagent was added to each one and mixed thoroughly. The mixtures were allowed to stand for 15 min at room temperature (25 ± 2^0C) and then 2.5 mL of Na_2CO_3 solution (75 g/L). The test tubes were again allowed to stand for 30 min at room temperature (25 ± 2^0C) followed by measuring the absorbance (λ = 760 nm) of each mixture in test tube using Spectrophotometer (T80+ UV/VIS Spectrophotometer, UK). The GA equivalent curve (Equation-1) was prepared (with 0. 0.9986 R^2 value) by plotting the gallic acid concentrations along to X-axis and corresponding absorbance along to Y-axis on the Microsoft Excel 2016 Spreadsheet (Ali et al., 2019):

$Y = 0.0114X + 0.0160$……(1)

Determination of Total Flavonoid Content

Total flavonoid contents (TFC) in the fruit juice, crude extracts of peel, and yogurt samples were determined using aluminium chloride colorimetric method with slight modifications (Chang et al., 2002; Uddin et al., 2018).

1 mL from each sample was taken in individual test tubes, in which 3 mL methanol, 0.2 mL 10% aluminum chloride solution, 200 µL 1 M potassium acetate and 5.6 mL of distilled water were successively added and mixed vigorously. The test tubes were then allowed to stand for 30 min at room temperature (25 ± 2^0C). The absorbance of contents was measured individually at 420 nm wavelength by spectrophotometer (T80+ UV/VIS Spectrophotometer, UK). The concentrations of TFC in Burmese grape pulp juice and yogurt were individually determined as mg of quercetin equivalent by putting absorbance in Equation-2.

Preparation of Quercetin equivalent curve

A stock solution of quercetin was prepared by adding 10 mg quercetin in 10 mL water followed by mixing thoroughly so that the ultimate strength of the stock solution remained 1 mg/mL or 1000 µg/mL. From the stock solution serial dilutions such as 12.5 µg/mL, 25 µg/mL, 50 µg/mL and 100 µg/mL were made in distilled water. The dilutions' absorbance (λ = 420 nm) was measured using Spectrophotometer (T80+ UV/VIS Spectrophotometer, UK). A standard curve in terms of Quercetin equivalent curve (Equation-2) was prepared (with 0.9692 R^2 value) by plotting individual concentrations along to X-axis and corresponding absorbance along to Y-axis on Microsoft Excel Spreadsheet (Ali et al., 2019).

$Y = 0.0103X - 0.0635$ …… (2)

Evaluation of Antioxidant Activity

Antioxidant activity of BGF pulp juice, peel extracts and yogurt samples were determined by using DPPH and ABTS radical scavenging assays with slight modifications (Ghiselli et al., 2000; Pinchuk et al., 2012).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Assay

300 µL from each experimental sample (pulp juice, peel extract, yogurt) was taken in individual test tubes followed by adding 2.7 mL of 0.25 mM DPPH solution to each test tube. The mixtures were mixed thoroughly and allowed to stand in dark for 30 min at room temperature (25 ± 2^0C). The mixtures were undergone to measure absorbance at 517 nm wavelength by Spectrophotometer (T80+ UV/VIS Spectrophotometer, UK). For each experiment, 3 mL DPPH solution (0.1 mM) was considered as a control one. Different concentrations of DPPH solution were tested to obtain IC_{50} value, which is defined as the amount of antioxidant necessary to decrease the initial**.**

DPPH ion by 50%. According to Ghiselli et al., (2000) and Pinchuk et al., (2012), ascorbic acid was used as a positive control and the percent radical scavenging activity of the experimental samples were calculated using Equation-3.

%DPPH radical scavenging activity = $[(A_0 - A_1)/(A_0)] \times 100$…….. (3)

Where
A_0 = Absorbance of control
A_1 = Absorbance of samples (pulp juice, peel extract, yogurt)

ABTS Radical Scavenging Assay

50 µL stock solution of each experimental sample (pulp juice, peel extract, yogurt) was added to 950 µL ABTS reagent individually and the mixtures were covered with aluminum foil and kept in dark at room temperature (25 ± 2^0C) for 10 min. Absorbance of the samples was measured at 734 nm wavelength by Spectrophotometer (T80+ UV/VIS Spectrophotometer, UK). A blank experiment was run taking 1000 µL ABTS reagent by following the procedures mentioned above. The IC_{50} value was tested for each sample of different concentrations. Ascorbic acid was used as a positive control and the values were compared with the crude extracts (Ghiselli et al., 2000; Pinchuk et al., 2012). The percent ABTS inhibition was calculated using Equation-4.

$$\%\text{ABTS scavenged} = [(A_0 - A_1)/(A_0)] \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Where
A_0 = Absorbance of control
A_1 = Absorbance of the experimental sample (pulp juice, peel extract, yogurt)

Statistical Analysis

Data collected from the sensory evaluation were analyzed for the Analysis of Variance (ANOVA) using R statistical software. The significant differences were observed at 5% level. Ducan's Multiple Range Test (DMRT) was used to analyze the mean differences between the samples. Other experiments were carried out in triplicate, and the average values are reported in the following sections. The inbuilt program in Microsoft ExcelTM, Microsoft Office Excel 2010 was used for this purpose with the same degree of confidence level ($p<0.05$).

RESULTS AND DISCUSSIONS

Screening Tests for Presence of Phytochemicals

Plant materials are rich sources of diverse ranges of secondary metabolites namely phytochemicals which possess several biological effects and help plant metabolism (Sufian et al., 2017). Moreover, they also avert the disproportionate cholesterol absorption in the intestine and thus diminish the risks associated with cardiovascular diseases, including hypertension (Stubbs et al., 2016).

In the present study, three solvents namely ethanol, 50% ethanol and water were applied to determine the presence of different phytochemicals of Burmese grape peel. The pulp is an edible part, so in such a case, the bioassay was performed with untreated fruit juice/ pulp and not in a solvent extraction form. Pulp and ethanolic extract of the peel contained higher amount of the phytochemicals, whereas the 50% ethanolic and water extracts of peel had much lower amount. Out of 48 tests, 30 were positive and 18 were negative; among those tests, glycoside, anthraquinone and phlobatannin compounds were absent in all extracts (Table 1).

Phytochemical analyses of different extracts of Burmese grape leaves found the presence of alkaloids, carbohydrates, glycosides, tannins, phytosterols, saponins, flavonoids etc. **(**Saha et

al., 2017). Uddin et al., (2018) conducted a study on methanolic extract of Burmese grape pulp, peel and seed. They reported the existence of alkaloids, glycosides, phenols, saponins, flavonoids etc. however, glycoside and saponin were absent in their extract of Burmese grape peel. The current study results were consistent with the papaya and banana peels, which were found to contain terpenoids, tannins, alkaloids, saponins steroid, phenols, fixed oils, and fats in 80% ethanolic extract (Siddique et al., 2018).

Nevertheless, the presence or absence of phytoconstituents in any plant may depend on the solvent media used for extraction and the physiological property of the plant. Even the extraction of pigments like chlorophyll, xanthophyll, carotenoid etc may vary depending on the extraction solvents, thus would have a significant effect on the functionality of the extracts (Jeeva et al., 2012). This study suggested that among pulp and the three different crude extracts of the peel of Burmese grape, pulp and ethanolic extract of peel have superior antioxidant potential owing to the higher amount of phenols, flavonoids, saponins etc.

TABLE 1 Phytochemical analysis of crude extracts from Burmese grape

Phytochemicals	**Peel extracts**			**Fruit juice**
	Pure Ethanol	50% Ethanol	Water	
Terpenoid	+	+	+	+
Phlobatanin	-	-	-	-
Saponin	-	+	+	-
Cardiac Glycoside	+	+	+	+
Tannin	+	-	-	+
Flavonoid	+	+	+	+
Phenol	+	+	+	+
Quinine	+	+	+	+
Coumarins	+	-	-	+
Anthraquinone	-	-	-	-
Steroid	+	+	+	+
Glycoside	-	-	-	-

Where '+' indicates presence of phytochemicals and '–'indicates absence of phytochemicals

Quantitative phytonutrients in Burmese grape juice and peel extracts

Total phytochemicals in the experimental BGF pulp and peel extract were determined in terms of TPC and TFC through the development of Gallic acid and Quercetin equivalent curves. The TPC and TFC in the samples were determined in µg GAE/mL from the standard 'Gallic acid equivalent curve' and µg QE/mL from the standard 'Quercetin equivalent curve', respectively (Figure 2). The Gallic acid equivalent curve corresponding to Equation-1 best fit with the experimental values. The regression coefficient value (R2 = 0.9986) tends to a high precision level, which is reliable to determine TPC in the experiments. The Quercetin equivalent curve, described with Equation-2, best fits the TFC standard determination with 0.9692 regression coefficient (R2).

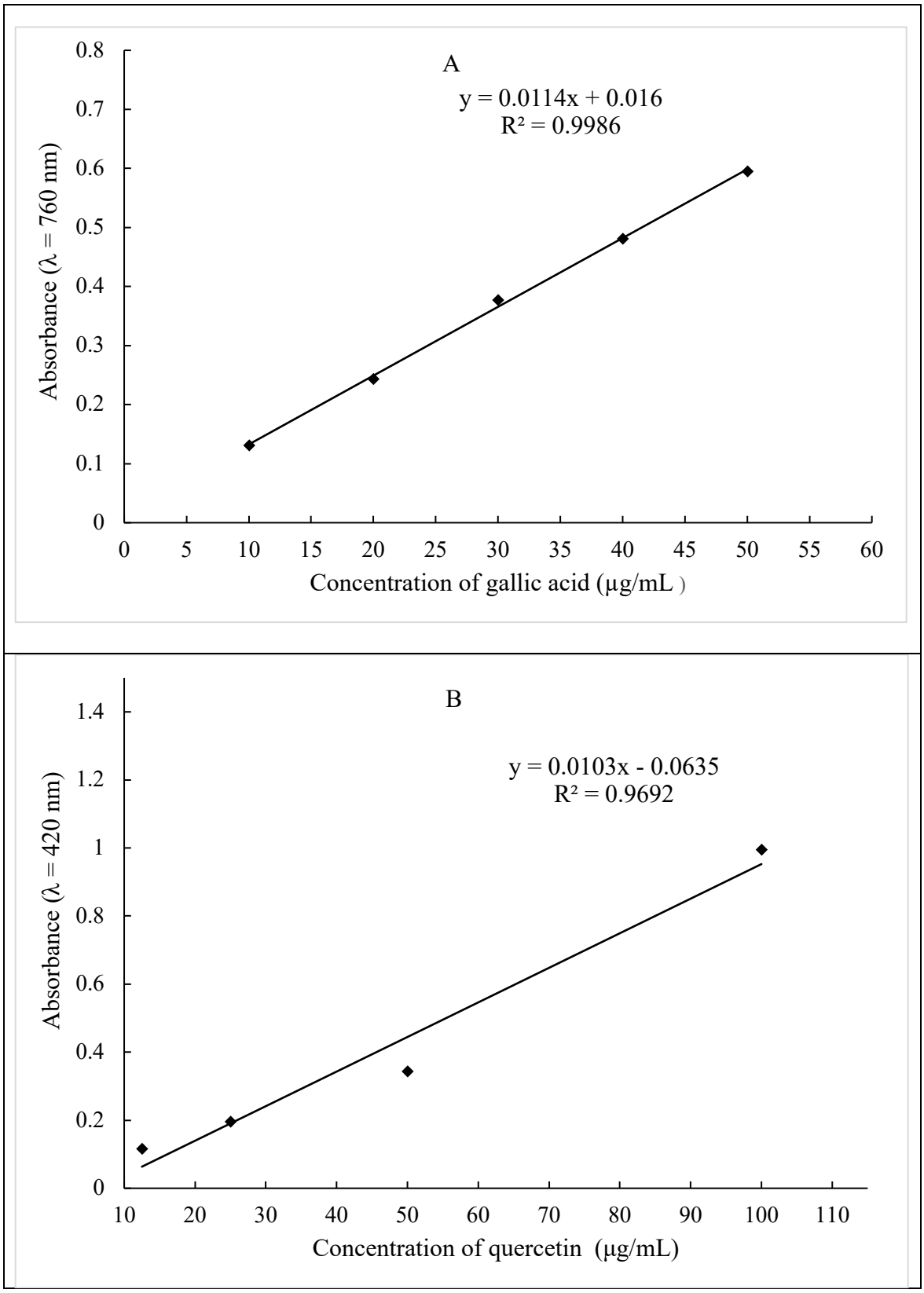


FIGURE 2 A) Gallic acid equivalent curve for total phenolic content (µg GAE/mL), B) Quercetin equivalent curve for total flavonoid content (µg QE/mL)

The TPC and TFC obtained in this study from Burmese grape fruit juice and peel extracted by different solvents are summarized in Table 2.

TABLE 2 Total phenolic and flavonoid contents in Burmese grape juice and peel

Fruit parts	Extraction solvent	Total phenols (µg GAE/g)[a]	Total flavonoids (µg QE/g)[b]
Peel	100% Ethanol	44.35 ± 0.83	36.5 ± 0.79
	50% Ethanol	37.68 ± 0.76	40.32 ± 0.53
	Water	13.32 ± 0.47	11.69 ± 0.60
Fruit juice		57.25 ± 0.68	73.36 ± 0.75

[a]GAE = Gallic acid equivalence
[b]QE = Quercetin equivalence

Total Phenolic Content (TPC)

The concentration of total phenols in BGF juice and different peel extracts were determined through external calibration with Gallic acid (Figure 2A). As in Table 2, fruit juice contained a higher amount of phenolic content (57.25 ± 0.68 µg GAE/g) followed by the peel extracted in pure ethanol (44.35 ± 0.83 µg GAE/g of peel powder). The lowest amount (13.32±.47 µg GAE/g) was obtained by the water extraction method. The results obtained in this study for TPC (Table 2) is consistent to the results as reported by Permatasari et al., (2019) who used n-hexane, ethyl acetate, water, dichloromethane and methanol for extraction of phytochemicals from Burmese grape (species *Baccurea*). They found the highest TPC (42.975±1.97 µg GAE/g) in ethyl acetate extract while the lowest one (13.538±0.47 µg GAE/g) was in the water extract. However, the variation in the quantitative extraction of TPC from BGF may be due to the complex nature of phenolic compounds and their method of extraction and extractant used (Kalt et al., 2001, Rajauria et al., 2013). The variation in this study (Table 2) might be due to the varying degree of solubility of phenolic compounds in different solvents.

Total Flavonoid Content (TFC)

Flavonoids are probably the most dynamic natural phytochemical due to their wide variety of chemical and biological activities, including antioxidant and free radical scavenging properties (Kahkonen et al., 1999). As shown in Table 2, the BGF juice showed the highest amount of TFC (73.36 ± 0.75 µg QE/g) followed by the peel extracted by 50% ethanol (40.32 ± 0.53 µg QE/g), and the lowest amount (11.69 ± 0.60 µg QE/g) was found in the water extract. The TFC obtained in this study is similar to Mann et al., (2016), who reported that TFC in BGF juice is higher than that of peel extracts where extractions are generally performed using different solvents such as methanol, acetone and water. Uddin et al., (2018) also found the highest amount of TFC (107.527 ± 1.90 µg QE/g) from BGF juice, where the extraction was in methanol.

The results obtained in this study (Table 3) suggest that among the BGF juice and three individual peel extracts, the juice and ethanolic peel extracts are superior in antioxidant potential owing to a higher amount of phenols and flavonoids.

Antioxidant activity of Burmese grape juice and peel extracts

The phytoconstituents play antioxidant roles in scavenging or stabilizing the free radicals through hydrogenation or making complexes with oxidizing species (Gengatharan et al., 2015).

The antioxidant activities of BGF juice and peel extracts were tested in terms of DPPH and ABTS inhibition assays separately. Prior to testing inhibition, IC_{50} (μg/mL) values of the assays were determined. The IC_{50} value represents the minimal concentration of a drug required for 50% inhibition *in vitro*.

IC_{50} values in DPPH and ABTS assays

The IC_{50} (μg/mL) values obtained in this study for DPPH and ABTS assays are shown in Table 3.

TABLE 3 IC_{50} values of Burmese grape peel extracts and pulp in DPPH and ABTS assays

Antioxidant assay	Ascorbic acid IC_{50} (mg/mL)	Peel extracts IC_{50} (mg/mL)			Juice IC_{50} (mg/mL)
		Pure Ethanol	50% Ethanol	Water	
DPPH assay	(2.887 ± 0.4) $\times 10^{-3}$	2.50 ± 0.09	2.84 ± 0.11	3.23 ± 0.35	**2.13 ± 0.03**
ABTS assay	0.159 ± 0.007	3.65 ± 0.16	4.12 ± 0.98	4.73 ± 0.42	**3.11 ± 0.08**

According to Table 3, the IC_{50} (mg/mL) values of ascorbic acid (control) were much lower in the DPPH assay than that in the ABTS assay. The IC_{50} (mg/mL) values of different peel extracts and BGF juice (Table 3) slightly differ from previous studies (Zheleva-Dimitrova, 2013), where the authors obtained IC_{50} (μg/mL) = 9.83 and 2.90 respectively in DPPH and ABTS assays. They have had a 30 min reaction of *Amorpha fruticosa* fruit methanol extracts using butylated hydroxytoluene (BHT) as the positive control. As shown in Table 3, the BGF peel exhibited IC_{50} values (DPPH assay) according to extraction orders with pure ethanol (2.50 mg/mL) < 50% Ethanol (2.83 mg/mL) < Water (3.23 mg/mL). The IC_{50} values of BGF juice and all crude extracts showed lower DPPH radical scavenging effects compared to ABTS using ascorbic acid [IC_{50} = (2.887 ± 0.4) $\times 10^{-3}$ mg/mL] as the positive control (Table 3). In this study (Table 3), the BGF juice and peel extracts containing high levels of TPC play an effectual DPPH radical scavenging role, which denotes that plant polyphenol is the important constituents for the inhibition properties of the juice and peel extracts.

DPPH radical scavenging activity

The DPPH radical scavenging activity (% Inhibition) obtained in this study for BGF juice and different peel extracts are graphically presented in Figure 3A. The DPPH radical-scavenging aptitude varies significantly depending on types and concentrations of solvents used in extractions. The fruit juice having 4 mg/mL showed the highest inhibition [(76.57 ± 1.87)%] followed by peel extracts in pure ethanol [(66.52 ± 2.66)%], 50% ethanol [(58.53 ± 3.27)%] and water [(54.90 ± 3.43)%]. Similar trends of variations were found in all 4 experimental concentrations (1 mg/mL, 2 mg/mL, 3 mg/mL and 4 mg/mL) of the extracts (Figure 3A). The presence of antioxidants in the BGF juice and extracts (Table 3) led to scavenging the DPPH radical chromogens detected in this study at 517 nm Spectrophotometry.

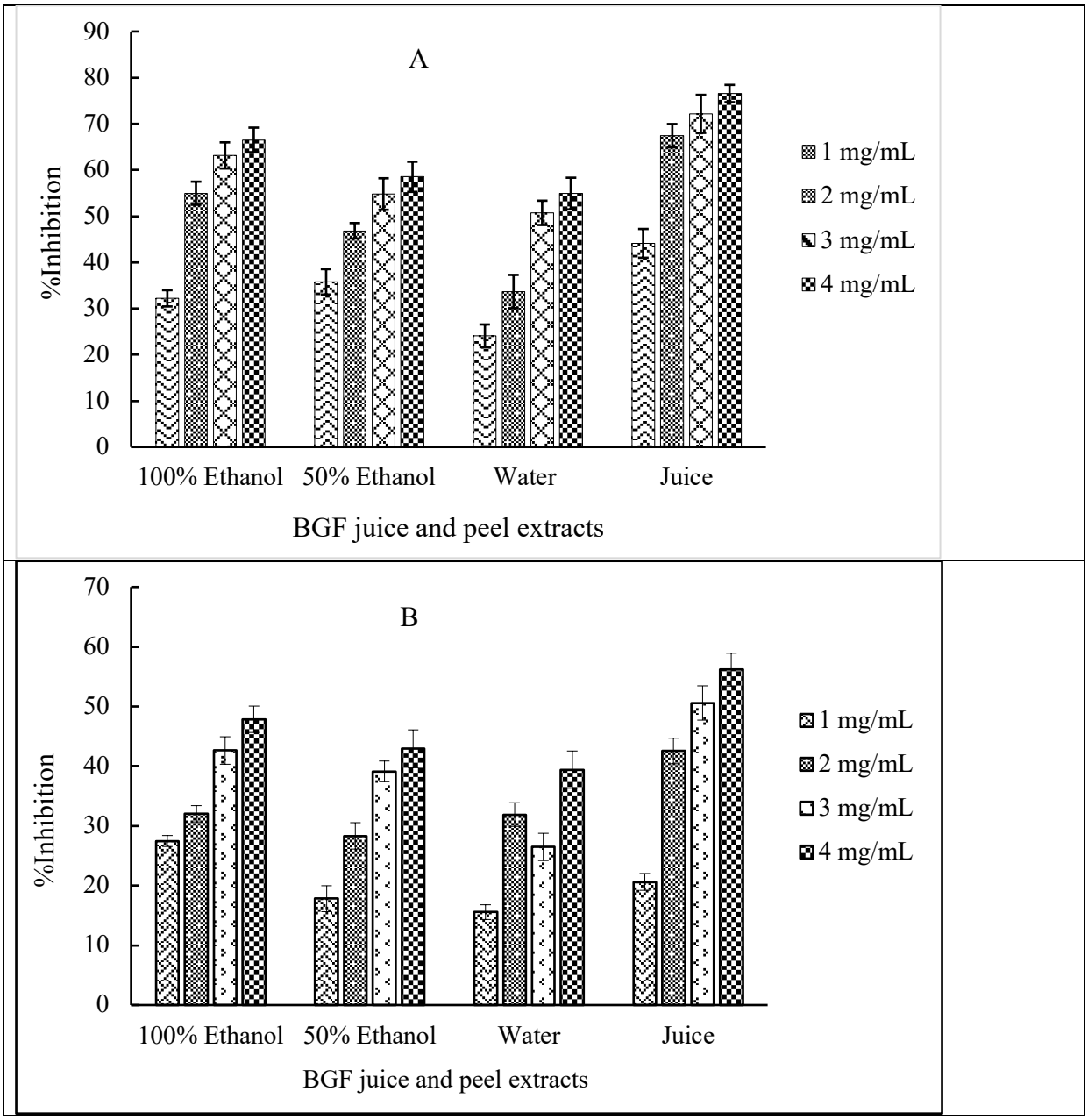


FIGURE 3 The radical scavenging performance of BGF juice and peel extracts. The extractions in different solvents (along X-axis) indicates the percent inhibition of A) DPPH B) ABTS radicals (along Y-axis) by BGF juice and peel extracts.

ABTS radical scavenging assay

As seen in Figure 3B, the BGF juice had the ABTS free radical scavenging activity in an order as (56.16 ± 2.75)% > (50.58 ± 2.87)% > (42.54 ± 2.14)% in 4 mg/mL, 3 mg/mL and 2 mg/mL, respectively. The BGF juice inhibition performance was followed by the peel extracts in pure ethanol as an order of (47.85 ± 2.21)% > (42.62 ± 2.30)% > (32.03 ± 1.36)%) > (27.47 ± 0.92)% in 4 mg/mL, 3 mg/mL, 2 mg/mL and 1 mg/mL, respectively. The 50% ethanol and water extracts showed a reduced percentage of inhibition including (42.91 ± 3.17)% and (39.42 ± 3.12)%, respectively in 4 mg/mL (Figure 3B).

The better scavenging performance of BGF juice presented in Figure 3 complies other experimental findings of this study presented in Table 2 and 3. According to IC_{50} values shown in Table 3, the ABTS radical scavenging ability of BGF juice is 3.11, which is more effective compared to the BGF peel extracts indicating the higher amount of TPC and TFC in BGF juice leads to a higher antioxidant activity. There is a positive correlation between the amount of phenolic compounds in an extract and its antioxidant capability (Alothman et al., 2009). Our findings are also consistent with the literature (Saha et al., 2016; Uddin et al., 2018; Ahmed et al., 2015) where the BGF juice exhibited a higher antioxidant activity than other parts of this fruit. As expected, we found that the radical scavenging activity of BGF juice and peel extracts is dose-dependent. The percentage of radical scavenging activities increased with the concentration of the substances in the reaction medium (Figure 3). Besides, the effect of the type of extraction solvents on the scavenging performance is evident. As in literature, the n-hexane fraction has higher ABTS free radical scavenging activity than the methanol and water extracts of *Barringtonia racemosa* pulp because the n-hexane fraction contains a lipophilic compound that can play a role to neutralize the free radical (Permatasari et al., 2019).

Sensory evaluation of phytonutrient enriched yogurt

The scores secured in this study by BGF juice enriched yogurt for individual sensory attributes were statistically analyzed and tabulated (Table 4). The analysis of variance ($p<0.05$) showed a varying degree of acceptability in terms of color, flavor, texture, taste and overall acceptability. The juice mixed sample S2 and control yogurt S1 scored the same 'overall acceptability,' 7.4. Other enriched yogurt samples S3, S4, S5 also secured satisfactory rating 'a'. The S6 with the highest mixture of BGF juice was rated 'b', which means not adequately liked by the sensory panel.

Therefore, mixing the Burmese grape juice up to 12% (S_5) in the formulation did not significantly reduce yogurt's organoleptic qualities. Further addition (S_6) harmed the sensory properties. Hence, a clear suggestion from the results is that the maximum percentage of Burmese grape juice to add in the yogurt formulation is 12%. Therefore, this study further investigated the highest two phytonutrient enriched samples S_4 (10%) and S_5 (12%), which were compared with the control sample S_1 (0%).

TABLE 4 Sensory evaluation of Burmese grape juice yogurt
Secured scores by individual sensory attributes (Mean ± SD)

Samples	Color	Flavor	Texture	Taste	Overall acceptability
S_1	7.1 ± 0.35^a	7.4 ± 0.16^a	7.6 ± 0.15^a	7.7 ± 0.21^a	7.4 ± 0.22^a
S_2	7.2 ± 0.20^a	7.2 ± 0.31^a	7.5 ± 0.18^a	7.6 ± 0.23^a	7.4 ± 0.21^a
S_3	7.0 ± 0.30^a	7.2 ± 0.22^a	7.5 ± 0.31^a	7.4 ± 0.26^a	7.3 ± 0.27^a
S_4	7.2 ± 0.29^a	7.1 ± 0.28^a	7.3 ± 0.27^a	7.2 ± 0.26^a	7.2 ± 0.22^a
S_5	7.1 ± 0.23^a	6.9 ± 0.15^b	7.2 ± 0.16^a	7.0 ± 0.27^a	7.0 ± 0.26^a
S_6	6.8 ± 0.26^b	6.4 ± 0.15^b	6.3 ± 0.17^b	6.2 ± 0.16^b	6.4 ± 0.18^b

Similar superscripts in a column denote no significance difference in means at $p < 0.05$
S_1 = Yogurt enriched with 0% BGF juice (control)
S_2 = Yogurt enriched with 4% BGF juice
S_3 = Yogurt enriched with 8% BGF juice

S_4 = Yogurt enriched with 10% BGF juice
S_5 = Yogurt enriched with 12% BGF juice
S_6 = Yogurt enriched with 14% BGF juice

Phytonutrient analysis of BGF juice yogurt

Analysis of TPC

The TPC determined in plain yogurt (S_1) was 2.68 ± 0.30 mg GAE/g (Table 5). This result is supported by Verma and Tripathi (2017), who found 4.5 mg GAE/g TPC in their plain yogurt. El-Said et al., (2014) reported 3.09 ± 0.02 mg GAE/g TPC in their control yogurt. In the case of phytochemical enriched samples, TPC in S_4 and S_5 were 11.61 ± 0.81 mg GAE/g and 13.20 ± 0.69 mg GAE/g, respectively (Table 5). These results agree with the results reported by Senadeera et al., (2018), who obtained 11.22 ± 0.83 to 15.53 ± 0.46 mg GAE/100 g TPC in Annona species pulp yogurt. According to Goyal et al., (2013), TPC in BGF juice and wine ranged from 141.27 mg GAE/L and 181.48 mg GAE/L, respectively. The current study was performed using fresh BGF juice without adapting special extraction solvents, resulting in less TPC than in literature (Goyal et al., 2013; Paul and Bhattacharyya, 2015; Senadeera et al., 2018).

TABLE 5 Total phytochemicals and antioxidant activity in BGF juice enriched yogurt

Sample	TPC (mg GAE/g) (Mean ± SD)	TFC (mg QE/g) (Mean ± SD)	DPPH (%) (Mean ± SD)	ABTS (%) (Mean ± SD)
S_1	2.68 ± 0.30	3.02 ± 0.45	11.16 ± 0.10	6.78 ± 0.22
S_4	11.61 ± 0.81	16.37 ± 0.62	25.50 ± 0.40	22.96 ± 0.35
S_5	13.20 ± 0.69	18.54 ± 0.56	30.26 ± 0.49	25.98 ± 0.17

S_1 = Yogurt enriched with 0% BGF juice (control)
S_4 = Yogurt enriched with 10% BGF juice
S_5 = Yogurt enriched with 12% BGF juice

Analysis of TFC

The control yogurt (S_1) contained 3.02 ± 0.45 mg QE/g TFC (Table 5), which is approximately similar to the results reported by Verma and Tripathi (2017), who found 3.95 mg QE/g TFC in plain yogurt. According to El-Said et al., (2014) the TFC in control yogurt is 1.11 ± 0.01 mg QE/g. Table 5 showed that TFCs in BGF juice enriched yogurts such as S_4 and S_5 were 16.37 ± 0.62 mg QE/g and 18.54 ± 0.56 mg QE/g, respectively. The results obtained in this study is consistent with literature where Paul and Bhattacharyya (2015) reported 14.52 ± 4.21 to 19.53 ± 1.41 μM QE/g TFC in cookies fortified with pomegranate juice.

Antioxidant activity of BGF juice yogurt

According to Table 5, the control yogurt (S_1) exhibited (11.16 ± 0.10)% DPPH scavenging activity. This result is consistent with Kim et al., (2010). They reported an inhibition of (10.07 ± 0.69)% DPPH assay in a control yogurt sample, whereas El-Said et al., (2014) found (17.91 ± 1.54)% DPPH scavenging activity in a similar yogurt sample. The BGF juice incorporated yogurts S_4 and S_5 show (25.50 ± 0.40)% and (30.26 ± 0.49)% inhibition of DPPH assay, respectively (Table 5). It indicates a significant increase in scavenging activity due to the presence of BGF juice in the formulated yogurts. The DPPH scavenging activity reportedly increased from (22.17 ± 1.40)% to (43.81 ± 2.54)% and from (10.07 ± 0.69)% to (24.73 ± 1.12)% while the pomegranate peel extract (El-Said et al., 2014) and *Nelumbo nucifera* leaf

powder (Kim et al., 2019) were incorporated in yogurt preparations. The inhibition of ABTS radical in control yogurt (S_1) was (6.78 ± 0.22)%, and in S_4 and S_5 were (22.96 ± 0.35)% and (25.98 ± 0.17)%, respectively (Table 6).

CONCLUSIONS

The present study was undertaken to investigate the phytochemicals present in the BGF juice and peel extract, followed by incorporating BGF juice in yogurt preparation. The chemical screen tests confirm the presence of essential phytochemicals in BGF except Phlobatanin, Anthraquinone and Glycoside. The BGF juice and pure ethanolic extract of BGF peel exhibit higher amount of phytonutrient compounds, including phenol and flavonoids. An in-vitro assessment of the antioxidant activities of different BGF extracts shows an increasing trend of activities against an increase in concentration of the extracts. BGF juice exhibits a higher antioxidant activity than that of BGF peel extracts. Both the DPPH and ABTS assays exhibit the radical scavenging roles in order of pure ethanol>50% ethanol>water. A significant positive correlation exists among the TPC, TFC contents and their scavenging performance. The BGF juice enriched yogurt formulated with 12% BGF juice secured the maximum phytochemical properties with satisfactory sensory responses.

FUNDING STATEMENT

This work was supported by the Research Management Wing, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh [Project number: Agriculture, 19 (2018-2021)].

ACKNOWLEDGEMENTS

The Authors thankfully acknowledge the research wing of the Bangabandhu Sheikh Mujibur Rahman Agricultural University for funding this study. The first and second authors express their gratitude to the Ministry of Science and Technology, Bangladesh for NST fellowships during their Master studentships.

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