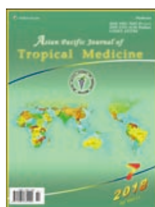

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# Phytochemical analysis and antioxidant profile of methanolic extract of seed, pulp and peel of *Baccaurea ramiflora* Lour.

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ABSTRACT

**Objective:** To analyze the phytochemical constituents responsible for the plausible antioxidant effect of methanolic extract of the seed, pulp and peel of *Baccaurea ramiflora* Lour. **Methods**: Fresh seed, pulp, and peel of *Baccaurea ramiflora* fruits were extracted with methanol (MEBRse, MEBRpu, MEBRpe) and evaluated by phytochemical analysis for their content of innumerable metabolites (primary and secondary) *viz.* carbohydrates, alkaloids, glycosides, tannins, phenols, terpenoids, flavonoids, proteins, and fixed oils. The antioxidant efficacy was assessed through different assay methods *viz.* 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, total antioxidant capacity (TAC) and reducing power capacity (RPC). Estimation of total phenolic content (TPC), and total flavonoid content (TFC) was also done to confirm the presence of these phytochemicals. **Results**: It was revealed from the phytochemical analysis of MEBRse that alkaloids, glycosides, carbohydrates, phenols, and flavonoids were present, while that of MEBRpu showed the existence of carbohydrates, proteins, alkaloids, glycosides, phenols, saponins, flavonoids, and fixed oils. Presence of carbohydrates, alkaloids, phenols, tannins, flavonoids, and terpenoids were found in the MEBRpe. A significant antioxidant activity was revealed by the MEBRpu [$EC_{50}$: (27.612 ± 1.375) μg/mL], compared to MEBRpe, and MEBRse in DPPH assay. The ranking order for RPC was MEBRpu > MEBRpe > MEBRse respectively. The $EC_{50}$ value of TAC of the MEBRpu, MEBRpe, and MEBRse were (25.107 ± 0.744) μg/mL, (241.127 ± 7.463) μg/mL and (372.364 ± 11.030) μg/mL, respectively. Quantity of TPC and TFC were the highest in the MEBRpu (124.360 ± 2.078 mg GAE/g and 107.527 ±1.900 mg QRE/g extract) rather than MEBRpe and MEBRse extracts. **Conclusions**: This study suggests that MEBRpu has a significantly higher antioxidant property than MEBRpe and MEBRse. These extracts might be advantageous in prevention or decelerating the progress of different diseases related to oxidative-stress/damage. Moreover, detailed analysis of these extracts is required to identify the presence of promising compound(s) responsible for their antioxidant activity.

## 1. Introduction

Use of plants as medicinal substances is as old as human civilization and mankind continues to rely on them for healthcare[1]. At present, around 80% population residing in the developing or underdeveloped countries still use plant-based medicines to combat their ailments[2]. Naturally-derived compounds have significantly contributed in the discovery of new chemical entities. The process

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of drug discovery from nature involves multi-disciplinary approach and is interconnected with many disciplines like ethnobotany, phytochemistry, biology, and various chemical separation processes along with combinatorial synthetic techniques. It is currently estimated that around 87% of drugs are derived directly or indirectly from nature. Approximately, 420 000 plant species occur in nature[3].

Oxidative stress is considered as the principal cause of human ailments. Oxidation of lipids, proteins, and DNA is related to several life-threatening diseases like cancer[4], atherosclerosis[5], heart disease[6], diabetes[7], preeclampsia[8], and neurodegenerative diseases like Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease, celiac disease[9-12] and Parkinson's disease[13]. Several free radicals are produced throughout metabolic process, however, the body balances oxidation and antioxidation using its multiple defense mechanisms[14-16]. Aging process is directly linked to systemic oxidative stress. Declined nutritional antioxidants availability, and accumulation of oxidation products have been recognized as main contributors in human aging[17]. According to the Denham Hartman's free radical theory of aging, it is believed that consequences of building-up of bio-molecules, spoiled through free radicals leads to aging[18,19]. Antioxidants are substances that are accountable for the prevention of reactive oxygen species formation or scavenge them[20]. Most of the dietary antioxidants are derived from plants. Moreover, antioxidants, obtained from medicinal plants, have attracted the researchers' attention due to the risks, associated with several available synthetic antioxidants including butylated hydroxyanisole and/or butylated hydroxytoluene[21].

The plant *Baccaurea ramiflora* (*B. ramiflora*) Lour. of Euphorbiaceae family is known as "Latkan" in Bengali. The plant is a semi-evergreen tree that grows in few districts of Bangladesh[22]. This fruit tree is instinctive to Southeast Asia region and is growing under cultivation in Bangladesh, India, Nepal, Myanmar, Thailand, Indo-China, South China, and Peninsular Malaysia[22,23]. The detailed description about the plant is available elsewhere[24,25]. The leaves and flowers of this plant can be consumed[26], whereas fruit juice is utilized against constipation[27]. The fruit extract of *B. ramiflora* exhibits cytotoxic, antiviral and antioxidant activities[28].

Previous research has revealed that *B. ramiflora* fruit-extracts have a significant DPPH radical scavenging activity[25]. Although, *B. ramiflora* fruit is considered as a new food additive due to high vitamin C, proteins and minerals content, no studies have yet examined the comparative phytochemical composition and the antioxidant effect of different parts (seed, pulp, and peel) of the fruits of this plant[29]. Therefore, here we explored the responsible phytochemical constituents, and antioxidant potential of methanolic extract of *B. ramiflora* fruit seed (MEBRse), pulp (MEBRpu), and peel (MEBRpe).

## 2. Materials and methods

### 2.1. Chemicals

Ascorbic acid (AA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, ammonium molybdate, trichloroacetic acid, Folin-Ciocalteu's reagent, quercetin (QT) and gallic acid (GA) were acquired from Sigma-Aldrich, USA and other remaining chemicals were used of analytical grade unless otherwise specified.

### 2.2. Plant materials

Fresh fruits (about 8 kg) of *B. ramiflora* were collected from Hotapara, Gazipur (Latitude: 23.911522, Longitude: 90.388962) of Bangladesh during December, 2014 to January, 2015. The fruits were washed to remove foreign matters, and then sun-dried for 1 h. Later, the identification was done as *B. ramiflora* (Accession number: DACB-42084) by an expert taxonomist from Bangladesh National Herbarium, Dhaka, Bangladesh.

### 2.3. Processing of plant materials

The seed, pulp, and peel of *B. ramiflora* fruit (about 2 kg each) were separated manually, followed by sun-dried and finally hot air drying in an oven (temperature $\leqslant$ 40 ℃). Dried seed, pulp, and peel were grounded with the help of a grinding machine into coarse powder. The generated powders (about 320 g each) were preserved in hermetic containers and placed in a cool, dry and dark place till extraction.

### 2.4. Preparation of plant extract

The extraction power of methanol is the highest owing to polarity and based on the literature review in this study as a solvent methanol was used[25]. About 250 g of individual powdered sample was placed in reagent bottle (amber-colored) and water-logged in 1 L of 95% methanol at 25 ℃. The reagent bottle along with the contents was sealed, set aside for a week with occasional stirring. Thereafter, mixture was filtered with the help of cotton followed by Whatman (No.1) filter paper. Filtrate thus obtained was concentrated under reduced pressure by means of a rotary evaporator at 50 ℃ to yield crude extracts (*i.e.* 6.57 g for MEBRse, 4.95 g for MEBRpu, 5.28 g for MEBRpe). The crude methanolic extracts thus obtained were kept at 4 ℃ for further evaluations.

### 2.5. Qualitative phytochemical screening

Crude extracts were screened to identify the occurrence of primary and secondary metabolites, *viz.* carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, saponins, terpenoids, proteins and fixed oils, using standard screening test and phytochemical procedures[30-39].

### 2.6. Antioxidant activity

The samples were dissolved in methanol (95% v/v) to get 1 mg/mL concentration and utilized for antioxidant assays.

#### 2.6.1. DPPH radical scavenging activity (FRSA)

The antioxidant effect of seed, pulp, and peel of *B. ramiflora*

fruit was evaluated by DPPH FRSA according to the method described by Molyneux[40] with minor modifications. The free radical's reduction is trailed by a decline in the absorbance at 517 nm. The DPPH solution was prepared in methanol (95%) toward getting a concentration of 240 μg/mL. The stock solution of 1 mg/mL concentration was prepared by mixing of *B. ramiflora* crude extracts with 95% methanol. The stock solution was used for the preparation of test solution through dilution with methanol to get the appropriate concentrations (25, 50, 100, 200 and 300 μg/mL). A standard solution of AA was prepared in the same way as described above. A recently prepared DPPH solution (3 mL) was mixed in each of the test tubes already having 100 μL extracts. The mixture was vigorously shaken and placed aside for the 30 min reaction period at room temperature in a dark room. After incubation, the absorbance of the mixture was recorded by UV spectrophotometer at 517 nm against methanol as a blank and experimental procedure was repeated for three times. The control used for the study was DPPH solution without sample solution. DPPH free radical scavenging % was measured by following equation:

$$\text{DPPH radical scavenging activity (\%)} = ([1-(As/Ac)] \times 100)$$

where, Ac = Absorbance of control and As = Absorbance of sample/standard solution.

### 2.6.2. Reducing power capacity (RPC)

The RPC of all extracts was determined depending upon the transformation of Fe (Ⅲ) to Fe (Ⅱ) as described by Oyaizu[41,42]. The higher reducing power is followed by an intensification in the absorbance at 700 nm. For the estimation of RPC, stock solution (1 mg/mL) was prepared according to the method described earlier in DPPH assay. AA was used as a standard and the solution was prepared similarly. For this test, different concentrations (*i.e.* 25, 50, 100, 200 and 300 μg/mL) of 2 mL extracts were mixed along with equal volume of 0.2 M phosphate buffer (pH 6.6) and 2 mL of 10 mg/mL concentration's potassium ferricyanide. This blend was then incubated for 20 min at 50 ℃, followed by adding of 2 mL trichloroacetic acid with a concentration of 100 mg/L. After that, the obtained solution mixture was centrifuged (3 000 rpm) for 10 min and the supernatant thus obtained was gathered. Two mL from each of the previously mentioned mixtures was allowed to mix with equal amount of distilled water and 0.4 mL of ferric chloride (0.1% (w/v)). After a reaction time of 10 min, the absorbance was recorded using UV spectrophotometer at 700 nm. All the analysis were performed in triplicate and results were averaged.

### 2.6.3. Total antioxidant capacity (TAC)

The TAC of the extracts was estimated by phosphomolybdenum assay, the basic principle of which is the reduction of Mo (Ⅵ) to Mo (Ⅴ)[43]. The stock solution (1 mg/mL) was prepared by addition of the extract to 95% methanol. Similarly, standard solution of AA was prepared as described above. For this test, various concentrations (25, 50, 100, 200 and 300 μg/mL) of 0.1 mL of the crude extract was mixed with a reagent solution (1 mL), 28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate. After capping, incubation was done for a period of 90 min in a water bath at 95 ℃. The samples were then allowed to cool at room temperature and the absorbance was measured at 765 nm against a reagent blank with the help of a UV spectrophotometer. Estimation of total antioxidant capacity was done by using following formula:

$$\text{Total antioxidant capacity (\%)} = ([(Ac - As/Ac)] \times 100)$$

where, Ac = Absorbance of control and As = Absorbance of sample/standard solution.

### 2.6.4. Total phenolic content (TPC)

The TPC was estimated according to Cheung *et al.*[44,45]. *B. ramiflora* crude extracts were mixed with methanol (95%) for preparation of the stock solution (1 mg/mL). A standard, GA was also mixed with 95% methanol to prepare the 1 mg/mL concentration standard solution. For this test, 1 mL of crude extract with 1 000 μg/mL concentration was mixed along with 1 mL Folin-Ciocalteu's reagent, 5 min later 10 mL volume of sodium carbonate (7%) solution was added to the mixture, and then deionized distilled water (13 mL) was added and thoroughly mixed. This blend was kept for 90 min in the dark at 23 ℃, and then the absorbance was recorded at 750 nm by UV spectrophotometer. Standard curve for estimation of TPC was prepared using GA standard solution (*i.e.* 6.25 μg/mL to 300 μg/mL) using the similar procedure as described earlier. The TPCs were expressed as mg of gallic acid equivalents (GAE) per g of the dried sample.

### 2.6.5. Total flavonoid content (TFC)

The TFC was estimated by method described by Park *et al.*[46] The stock solution was prepared as mentioned in TPC. Similarly, the standard solution of QT was prepared through mixing it with 95% methanol (*i.e.* 1 mg/L). To estimate the TFC, 0.3 mL of the crude extract (1 000 μg/mL), 3.4 mL of methanol (30%), 0.15 mL of 0.5 moL/L sodium nitrate, and 0.15 mL of 0.3 moL/L aluminum chloride were mixed. Then after 5 min, 1 mL of 1 moL/L sodium hydroxide was supplemented. The obtained solution was thoroughly mixed and absorbance was recorded at 506 nm against the reagent blank. TFCs were expressed as mg of quercetin equivalents (QRE) per g of the dried sample.

### 2.7. Statistical analysis

Obtained results were recorded from triplicate observations and articulated as mean ± SD. The Student's $t$ test applied to determine the significance of the standard and sample for $EC_{50}$ values. SPSS 14.0 (Chicago, IL, USA) and Microsoft Excel 2010 (Roselle, IL, USA) were used for the statistical and graphical analyses and $P<0.05$ was considered statistically significant.

## 3. Results

### 3.1. Estimation of phytochemical constituents

The phytochemical analysis of *B. ramiflora* fruits exhibited the

existence of many important bioactive secondary metabolites in different extracts, such as alkaloids, glycosides, phenols, flavonoids, saponins, tannins, terpenoids, carbohydrates, proteins, and fixed oils that were confirmed by color reaction tests as shown in Table 1. Based on the intensity of the color reaction, the MEBRpu contained the highest amount of phenols, flavonoids, and saponins, compared to MEBRse and MEBRpe.

**Table 1**

Phytochemical analysis of *B. ramiflora* fruit extracts

| Phytochemical constituents | MEBRse | MEBRpu | MEBRpe |
|---|---|---|---|
| Alkaloids | | | |
| Wagner's test | ++ | + | + |
| Mayers test | ++ | | + |
| Test for glycosides | + | + | - |
| Carbohydrates | | | |
| Fehling's test | ++ | ++ | + |
| Benedict's test | ++ | ++ | + |
| Phenols | | | |
| Ferric chloride test | + | +++ | ++ |
| Flavonoids | | | |
| Alkaline reagent test | + | +++ | + |
| Lead acetate test | + | +++ | + |
| Saponins | | | |
| Froth test | - | ++ | - |
| Foam Test | - | ++ | - |
| Tannins | | | |
| Ferric chloride test | - | - | ++ |
| Gelatin test | - | - | ++ |
| Terpenoids | | | |
| Horizon test | - | - | + |
| Libermann test | - | - | + |
| Proteins | | | |
| Xanthoproteic test | - | + | - |
| Ninhydrin test | - | + | - |
| Fixed oils | | | |
| Spot test | - | + | - |

where, +: present (mild amount), ++: present (moderate amount), +++: present (large amount), : absent, based on the power of generated color reaction.

### 3.2. Estimation of DPPH FRSA

Table 2 depicts the FRSA of the methanolic extracts of seed, pulp and peel of *B. ramiflora* fruits on DPPH free radicals which were in the following order: AA > MEBRpu > MEBRpe > MEBRse. It was found that the extracts exhibited a dose-dependent activity which indicates that DPPH scavenging activity was increased proportionately to the increase in the extracts' concentration. Additionally, the $EC_{50}$ values of scavenging DPPH radicals for the AA, MEBRpu, MEBRpe, and MEBRse were shown in Table 2. Comparing with AA, the $EC_{50}$ value for DPPH radical activity of MEBRpu was significant higher ($P<0.05$). Thus, the present results demonstrated that among the three extracts, MEBRpu exerted a 75.9% scavenging activity at 300 μg/mL concentration.

### 3.3. Estimation of RPC

Table 3 shows the reducing power activities on absorbance by all extracts from *B. ramiflora* fruits. It was found that a significant dose-dependent reducing activity at concentrations, ranging between 25 μg/mL and 300 μg/mL was exhibited by the extracts. The ranking order for the reducing powers was AA > MEBRpu > MEBRpe > MEBRse. It was also found that higher [(2.48 ± 0.10) nm at 300 μg/mL) reducing power was recorded in MEBRpu than MEBRpe, and MEBRse.

### 3.4. Estimation of TAC

All extracts of *B. ramiflora* fruits was found to decrease the antioxidant capacity in the following order: AA > MEBRpu >

**Table 2**

DPPH radical scavenging activity, $EC_{50}$ values for both DPPH radical and TAC of standard and *B. ramiflora* fruit extracts

| Standard /Extracts | DPPH Scavenging (%) | | | | | $EC_{50}$ values for DPPH radical (μg/mL) | $EC_{50}$ values for TAC (μg/mL) |
|---|---|---|---|---|---|---|---|
| | 25 μg/mL | 50 μg/mL | 100 μg/mL | 200 μg/mL | 300 μg/mL | | |
| AA | 56.52 ± 2.83 | 62.98 ± 1.66 | 65.01 ± 1.76 | 76.45 ± 1.83 | 79.11 ± 3.45 | 22.152 ± 1.110 | 18.439 ± 0.780 |
| MEBRpu | 45.34 ± 2.26 | 54.01 ± 1.76 | 60.50 ± 2.52 | 73.27 ± 2.15 | 75.90 ± 2.46 | 27.612 ± 1.375$^{*}$ | 25.107 ± 0.744$^{**}$ |
| MEBRpe | 15.23 ± 0.77 | 31.29 ± 1.56 | 38.93 ± 1.93 | 43.29 ± 1.94 | 46.85 ± 1.04 | 320.276 ± 7.158 | 241.127 ± 7.463 |
| MEBRse | 13.75 ± 0.69 | 25.42 ± 1.37 | 31.10 ± 1.33 | 37.60 ± 1.69 | 40.21 ± 1.01 | 373.130 ± 9.421 | 372.364 ± 11.030 |

Values were expressed as mean ± SD ($n$ = 3). where, AA = Ascorbic acid, MEBRse = Methanolic extract of seed of *B. ramiflora*, MEBRpu = Methanolic extract of pulp of *B. ramiflora*, MEBRpe = Methanolic extract of peel of *B. ramiflora*. $^{*}P < 0.05$, $^{**}P < 0.01$ significant difference from the standard.

**Table 3**

Reducing power activities of standard and crude methanolic extracts of *B. ramiflora* fruits (nm).

| Standard/Extracts | 25 μg/mL | 50 μg/mL | 100 μg/mL | 200 μg/mL | 300 μg/mL |
|---|---|---|---|---|---|
| AA | 1.32 ± 0.03 | 1.46 ± 0.03 | 1.65 ± 0.04 | 1.98 ± 0.03 | 2.49 ± 0.07 |
| MEBRpu | 1.30 ± 0.03 | 1.41 ± 0.03 | 1.54 ± 0.06 | 1.93 ± 0.05 | 2.48 ± 0.10 |
| MEBRpe | 0.69 ± 0.02 | 0.76 ± 0.03 | 0.82 ± 0.04 | 1.02 ± 0.05 | 1.35 ± 0.02 |
| MEBRse | 0.39 ± 0.02 | 0.43 ± 0.02 | 0.66 ± 0.03 | 0.65 ± 0.03 | 0.73 ± 0.03 |

Values were expressed as mean ± SD ($n$ = 3). where, AA = Ascorbic acid, MEBRse = Methanolic extract of seed of *B. ramiflora*, MEBRpu = Methanolic extract of pulp of *B. ramiflora*, MEBRpe = Methanolic extract of peel of *B. ramiflora*.

**Table 4**

Total antioxidant capacities of standard and crude methanolic extracts of *B. ramiflora* fruits(%).

| Standard /Extracts | 25 μg/mL | 50 μg/mL | 100 μg/mL | 200 μg/mL | 300 μg/mL |
|---|---|---|---|---|---|
| AA | 67.87 ± 2.89 | 78.31 ± 3.99 | 77.34 ± 2.87 | 87.68 ± 2.42 | 96.54 ± 1.83 |
| MEBRpu | 49.81 ± 1.45 | 75.47 ± 2.08 | 78.44 ± 1.91 | 83.81 ± 3.16 | 96.1 ± 3.14 |
| MEBRpe | 31.23 ± 1.11 | 38.64 ± 0.76 | 40.62 ± 2.53 | 48.18 ± 0.56 | 62.24 ± 1.91 |
| MEBRse | 10.19 ± 0.43 | 13.05 ± 0.47 | 17.28 ± 1.48 | 32.67 ± 0.99 | 40.30 ± 1.19 |

Values were expressed as mean ± SD ($n$ = 3). where, AA = Ascorbic acid, MEBRse = Methanolic extract of seed of *B. ramiflora*, MEBRpu = Methanolic extract of pulp of *B. ramiflora*, MEBRpe = Methanolic extract of peel of *B. ramiflora*.

MEBRpe > MEBRse as shown in Table 4. The antioxidant capacity increased with concentration of each sample. The $EC_{50}$ value of TAC for the AA, MEBRpu, MEBRpe, and MEBRse were shown in Table 2 which revealed that the value of MEBRpu ($P$<0.01) was comparable to AA and significantly lower than MEBRpe and MEBRse.

### *3.5. Estimation of TPC and TFC*

TPC was estimated from QA standard curve (y = 0.014 6x + 0.093 7) and the results were represented in milligrams of GAE. Table 5 shows that the TPC in the MEBRse, MEBRpe, and MEBRpu varied largely and MEBRpu exhibited the highest TPC. The content of flavonoid was estimated from the QT standard curve (y = 0.012 8x + 0.099 6) and the results were expressed as mg of QRE (Table 5). The MEBRpu showed the maximum amount of flavonoid contents followed by MEBRse, and MEBRpe.

**Table 5**

Total phenolic and flavonoid contents of *B. ramiflora* fruit extracts

| Extracts | Total phenolic (mg GAE/g) | Total flavonoid (mg QRE/g) |
|---|---|---|
| MEBRpu | 124.360 ± 2.078 | 107.527 ± 1.900 |
| MEBRpe | 47.230 ± 3.703 | 20.720 ± 1.226 |
| MEBRse | 12.960 ± 0.767 | 6.942 ± 0.649 |

Values were expressed as mean ± SD ($n$ = 3).

## 4. Discussion

Natural products, specifically plants are believed as the pillar of all traditional medicine systems[48]. These are tremendously rich sources of diverse range of phytochemicals, which possess several biological effects, like antioxidant, antimutagenic, anti-diabetic, anti-inflammatory, and antimicrobial activities[47]. Moreover, phytomedicine serves as a natural blueprint for development of new drugs[49]. Drugs are obtained from almost all forms of natural products in general and plants in particular. These plants range from unicellular yeasts to highly-differentiated plants[50]. In present study, the phytoconstituents, and the antioxidant activities of the methanolic extracts of seed, pulp, and peel of *B. ramiflora* fruit were evaluated.

Phytochemical evaluation showed the existence of many bioactive compounds like alkaloids, glycosides, carbohydrates, phenolssaponins, tannins, flavonoids, terpenoids, proteins, and fixed oils. Several polyphenolic compounds like flavonoids, phenolic acids, and tannins are deemed as the chief constituents of plants[51,52]. Flavonoids are water-soluble polyphenolic molecules that have antioxidant, free radical scavenging, antimutagenic, antibacterial, antifungal, and antiviral activities[53]. Diverse pharmacological activities are exerted by plant saponins, these include expectorant, immunomodulatory, vasoprotective, anti-inflammatory, hypocholesterolemic, hypoglycaemic, antifungal, and antiparasitic activities[54,55]. Moreover, they also avert the disproportionate cholesterol absorption in intestine, and therefore diminish the risk associated with cardiovascular diseases, including the risk of hypertension[56]. Tannins, the complex organic, non-nitrogenous compounds and polyhydroxy benzoic acids (polyphenols) derivatives having anticancer, antimutagenic, antimicrobial, astringent and anti-diarrheal properties. They have also been reported as healing agents in different inflammatory conditions, gonorrhoea, burns and to promote blood clotting, reduce blood pressure and modulate immunoresponses[57]. This study suggested that among the methanolic crude extracts of seed, pulp, and peel of *B. ramiflora* fruits, MEBRpu have superior antioxidant potential owing to the presence of higher amount of phenols, flavonoids, and saponins. Plant phenols, flavonoids and saponins significantly associated to the antioxidant potential of these extracts. The literature suggests that these phytoconstituents exert their antioxidant activity via scavenging or stabilizing free radicals through hydrogenation or complexion with oxidizing species[58].

Scavenging of DPPH is one of the imperative parameter to assess the antioxidant effect of crude extracts. The degree of change in color of the test solution from purple color to colorless is straightly proportionate to the scavenging potency and concentration of the extracts. Decline in the absorbance at 517 nm is a clear indicative of FRSA of the extract[59]. In this study MEBRpu exhibited higher percentage of DPPH scavenging activity than MEBRse, and MEBRpe. The study suggested that the plant extract that contains flavonoids, and related polyphenols are able of donating hydrogen atom to a free radical to neutralize it. A previous study suggested that the chloroform soluble fraction from methanol extract of *B. ramiflora* fruits exhibited the uppermost DPPH radical scavenging potential[25].

The RPC of extracts was estimated by reducing power assay in which,

yellow coloration of the test solution converts to greenish color. This is due to the presence of one or many reducing agents in test compound which is responsible for the reduction of iron, Fe (Ⅲ) to Fe (Ⅱ) form. An increase in the absorbance at 700 nm is an indicative of reducing capacity of the extract[60]. Current study demonstrated that MEBRpu had a higher reducing power than the other *B. ramiflora* fruit extracts. This may be related to the content of biologically active compounds of the extracts, particularly total polyphenols, which exert electron donating activities. Earlier findings highlighted that the plant extracts with iron reducing power are able to prevent oxidative stress by inhibiting lipid peroxidation[61].

The TAC of the extracts can be quantitatively analyzed by estimation of antioxidant capacity, through phosphomolybdenum complex formation. This assay depends on reduction of molybdenum, Mo (Ⅵ) to Mo (Ⅴ) by the test compounds and succeeding complex formation of green phosphate Mo (Ⅴ) at acidic pH. Amplification in the absorbance at 765 nm against reagent blank indicates the antioxidant potential of the plant extract[62,63]. The present study showed that MEBRpu possess higher antioxidant capacity than MEBRse, and MEBRpe. Moreover, it confirmed that antioxidant effect of plant extracts is correlated to their bio-active constituents content mostly polyphenols and ascorbic acid. In a former study by Usha *et al.*, the methanolic extract of *B. ramiflora* leaves had a potent antioxidant activity[23].

Plants are the greatest source of natural antioxidants due to the presence of various biophenolic compounds like phenolic acids, saponins, flavonoids, and tocopherols[64,65]. Plant materials which are rich in phenol contents, are widely used as medicinal remedies due to their various pharmacological properties[65]. Flavonoids are naturally-occurring compound of plants and account for more than half of the 8 000 different phenolic compounds[66]. They have been shown to effectively scavenge most oxidizing molecules, which include singlet oxygen and other free radicals[67]. Additionally, plant flavonoids have anti-inflammatory, anti-hypertensive, and cardioprotective activities[68, 69]. This study also demonstrated that the TPC, and TFC of MEBRpu were higher than MEBRse, and MEBRpe. The consequences of this study recommended that polyphenolic constituents may be the chief agents for the antioxidant action. The variation in the polyphenolic contents may be due to the existence of volatile/essential oils occurring in the plant. Numerous studies have also suggested that the medicinal plants showed antioxidant activity due to the presence of ploy-phenolic and flavonoid compounds[70, 71].

In recent past, rising interest in search for phytochemicals, possessing antioxidant properties has been observed due to adverse effects of synthetic antioxidants on human health. The present study suggests that methanolic pulp extracts of *B. ramiflora* fruit have a potent antioxidant activity.

In summary, this study clearly revealed that the pulp extracts of *B. ramiflora* fruit have a significant antioxidant activity as compared to seed, and peel extracts and may be beneficial for preventing free radicals mediated oxidative stress. As a result, the pulp extracts of *B. ramiflora* fruit may serve as a possible source of natural antioxidant. However, there is a need of further studies to characterize the active compound(s).

## Conflict of interest statement

The authors declare that they have no competing interests.

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