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Phytochemical and comparative biological studies of *Baccaurea ramiflora* (Lour) extract

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Abstract

The aim of this study was to evaluate the phytochemical and *in vitro* antioxidant activity along with central nervous system (CNS) depressant, analgesic and anti-inflammatory activities of ethanol extract of *Baccaurea ramiflora* fruits. Qualitative phytochemical screening confirmed the presence of alkaloid, steroid, saponin, phenolic and flavonoid compounds. Total phenolic and flavonoid content measured by Folin-Ciocalteu and Aluminium chloride method was observed maximum for peel (93.05 ± 0.33 mg GAE /gm and 34.33 ± 0.24 mg CA /gm of dried extract respectively. In DPPH assay method, peel showed significant ($P < 0.05$) antioxidant activity based on IC_{50} value. Total antioxidant capacity and reducing power assay result also demonstrated potential antioxidant capacity of *B. ramiflora* peel. The seed with flesh extract significantly ($P < 0.01$) inhibited writhing 46.51% induced by acetic acid in mice at 200 mg/ kg doses. The anti-inflammatory activity measured by carrageenan induced mice paw edema was observed significant ($P < 0.01$, $P < 0.05$) at 2nd, 3rd and 4th hour. The percent of inhibition of peel and seed at 200 mg/ kg dose was 36.19% and 36.73% which was very close to standard Ibuprofen (37.80%). Our investigation also revealed significant reduction of locomotor activity of mice in open field and whole cross test at 25 and 75 mg/kg doses compared to standard diazepam at 5 mg/kg. Based on present study, it could be accomplished that *B. ramiflora* may be the promising source of natural antioxidant as well as for analgesic, anti-inflammatory and CNS depressant agent.

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Introduction

About 250 species of medicinal plants were used for preparing traditional medicines which is the half of total species of plants grown in Bangladesh (Ghani, 2003). About 87% of drugs originated from natural sources comprise 25% of prescribed drugs (Khatun *et al.,* 2014). Bangladesh is also blessed with 70 various kinds of fruits (Hossian *et al.,* 2011). Minor fruit occupies 3.01% of area and 8.38% of production compared to the total fruit production of Bangladesh (BBS, 2011).

Many bioactive compounds such as alkaloid, tannin, flavonoid, glycoside, saponin, terpenoid, steroid, carbohydrate and phenolic compounds are obtained from medicinal plants which lead to modern drug design and development (Begum *et al.,* 2015; Singh *et al.,* 2015; Hakemi Singh *et al.,* 2012) and play an important role for curing many diseases (Yadav *et al.,* 2011; Hegde *et al.,* 2016). These phytochemical screening gives us evidences for discovery of new valuable drugs (Khan *et al.,* 2011). Antioxidants are substances that prevent and stabilize the damage caused by free radicals by supplying electrons from antioxidants to these damage cells. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body. Consumption of antioxidant enriched fruits have countless benefits in improving quality of life by lowering the risk of several diseases caused by free radicals (Hamid *et al.,* 2010) like cancer, stroke, cardiovascular disease, and various factors associated with ageing (Nile *et al.,* 2015; Megala *et al.,* 2015; Sharma *et al.,* 2015). Compared to other fruits, minor fruits possess high antioxidant activity and are naturally rich in a variety of phytochemicals particularly phenolic compounds (Francesca *et al.,* 2012).

Baccaurea ramiflora Lour. (syn. *Baccaurea sapida*) commonly known as Burmese grape belongs to the Phyllanthaceae family, is a valuable nutritional minor fruits. The fruit tree is native to Southeast Asian countries (Goyal *et al.,* 2013) and found growing in China, Nepal, Bangladesh, Thailand, Myanmar, Indonesia, India and Malaysia (Abdullah *et al.,* 2005). This fruit has been put into the category of underutilized fruit among 675 wild edible fruits and harvested during the month of May to July. This fruits are yellowish pink to purple in color with leathery pericarp, glabrous, 2-4 cm in diameter having 3-4 seeds, embedded in pale- rose colored delicious pulp (Saha *et al.,* 2016).

Baccaurea ramiflora fruits are largely exploited as a good source of vitamin C and several nutrients like protein, iron (Hossain *et al.,* 2017). The fruit juice is mainly used for the treatment of constipation, whereas different parts of the plant are used to treat arthritis, abscesses and injuries (Lin *et al.,* 2003).The hydro methanol extract of the fruit pericarp of *B. ramiflora* showed significant DPPH scavenging activity with IC_{50} of 31.38 µg/ml (Hasan *et al.,* 2009) which indicates presence of phenolic compounds such as flavonoids, polyphenols, tannins and phenolic terpenes (Rahman *et al.,* 2007). Recent study has reported the presence of rosmarinic acid in *B. ramiflora* leaf extract that is responsible for anti-inflammatory action by inhibiting the biosynthesis of prostaglandin (Usha *et al.,* 2014). The fractions of ethanol extracts of *Baccaurea ramiflora (*Lour.) leaves and stems showed potential cytotoxic activity (Rahman *et al.,* 2007).The whole plant is used as an anti-inflammatory and anodyne against rheumatoid arthritis, cellulitis, and abscesses to treat injuries in Chinese Dai medicine (Lin *et al.,* 2003). Phytochemical research identified essential oil from *B. ramiflora* root, leaf & fruit. 10 essential oils were isolated from leaves and roots of *B. ramiflora* whose relative contents were 76.66%. 7 essential oils from fruits and roots of *B. ramiflora* were the same whose relative contents were 69.82% (Jing *et al.,* 2007). A new sesquiterpene lactone called Epidihydrotutin was isolated from root (Jing *et al.,* 2007).Two new phenolic compound 6′- O-vanilloylisotachioside and 6′- O-vanilloyltachioside, together with nine known compounds, were isolated from the leaves of *B. ramiflora* (Yang *et al.,* 2007). A study (Ullah *et al.,* 2012) reported that the methanol leaf extract of *B. ramiflora* have hypoglycemic, hypolipidemic and antioxidant activity. The DNA protective activity,

lipid per-oxidation and superoxide radical scavenging activity of dried fruit extract of *B. ramiflora* was also reported (Prakash *et al.,* 2012). Another study also showed significant hypoglycemic activity of bark extract (Howlader *et al.,* 2009).The antiviral properties of the fruit and diuretic activity of stem bark has also been reported (Goyal *et al.,* 2013). Various study reported the use of fruits in skin diseases (Hasan *et al.,* 2009).

In addition to medicinal value, young leaves are used as vegetable, flavoring agent with curries and minced meat in Bangladesh (Hasan *et al.,* 2009). The peel of mature but unripe fruits yields 14.1 percent pectin and this pectin is useful in preparation of jellies and jams (Hossain *et al.,* 2017). In animal models, phytochemical studies showed the presence of appreciable amount of saponins and alkaloids in pulps (8.27 and 7.48%). Therefore, there is increasing relationship between antioxidant activity and diseases. So, this study was aimed to analyze the crude ethanol extract of *B.ramiflora* peel and seed with flesh (BRP, BRS) for phytochemical and antioxidant activity and also for finding out new sources of CNS depressor, analgesics and anti-inflammatory agents.

Materials and methods

Plant collection

The fresh fruits of *B. ramiflora* were collected from Shaheb Bazar, Rajshahi, Bangladesh on May 2017. The plant was identified by an expert taxonomist at the Department of Botany, University of Rajshahi, where a voucher specimen (Voucher No.78) was deposited. Then peel and seed were separated and shade-dried for several days with occasional sun drying. Then the dried plant material is pulverized into coarse powder with a grinding machine. The powder material was poured in an airtight container and placed in a cool, dark and dry place for extraction.

Preparation of the extract

About 500 g peel and seed powdered materials were placed in fresh, amber colored extraction bottles separately and soaked with 1.5 L of ethanol. The sealed bottles were preserved for 15 days with frequent shaking and stirring. The whole mixture was filtered separately through a fresh cotton plug and finally with a Whatman No. 1 filters paper. Then the filtrate were concentrated using a rotary evaporator (Bibby Sterilin Ltd., UK) under reduced pressure.

The mixture was looked like a gummy black color concentrate which referred to as crude ethanol extract of peel and seed. The extract was stored in air tight glass container for further use.

Drugs and Chemicals

All the chemicals used in this study were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Diazepam (Square Pharmaceuticals Ltd.), Ibuprofen (ACI Pharmaceuticals Ltd.), Diclofenac Sodium (Beximco Pharmaceuticals Ltd.) was used for conducting the tests.

Experimental Animals

Healthy Swiss albino mice, six weeks of age and weighing about 25-30 g of both sexes were obtained from the central animal house of the department of Pharmacy, Jahangirnagar University, Savar, Dhaka - 1342, Bangladesh. Under ambient temperature all animals were kept with 12 hour light followed by a 12 hour dark cycle. Prior to research work, the animals were acclimatized for 7 days and were fed formulated rodent food and water.

The study was conducted following all the rules governing the use of laboratory animals. The experimental protocol was approved by the Animal Ethics Committee of the Department of Pharmacy of Varendra University, Rajshahi, Bangladesh.

Qualitative phytochemical screening

Phytochemical screening tests were carried out for the detection of various bioactive compounds like alkaloid, flavonoid, tannin, phenolic, saponin, glycoside, steroid, fixed oil and fat. Procedure of tests was given below:

Alkaloids detection test

Mayer's test (Potassium mercuric iodide solution): Mayer's reagent was added drop wise in 25 ml of each ethanol extract solution. A creamy white precipitate indicated the presence of alkaloids (Deore *et al.,* 2015).

Phenol detection test

Ferric chloride test: The extract was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds (Mir *et al.,* 2013).

Flavonoid detection test

Lead Acetate test: Few drops of lead acetate solution were mixed with plant extract solution. Yellow color precipitate appearance in solution indicated the presence of flavonoid (Onocha *et al.,* 2011).

Fixed oil and fat detection test

A drop of concentrated extract of peel and seed of *B. ramiflora* was kept in filter paper and pressed. Oil stain on the paper indicated the presence of oils and fats (Onocha *et al.,* 2011).

Tannin detection test

Ferric chloride test: To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution were added. Formation of blue color will show the presence of tannin (Somkuwar *et al.,* 2013).

Saponin detection test

Hydrochloric acid test: In this test few drops of HCl were added in extract solution, appearance of pink colour indicates the presence of saponin. If addition of ammonia solution with HCl in extract solution change color of extract solution into deep violet, will show presence of saponin (Tiwari *et al.,* 2011).

Steroid detection test

20mg of the extract was treated with 2.5 ml of acetic anhydride and 2.5 ml of chloroform. Then concentrated solution of sulfuric acid was added slowly and red violet color was observed for terpenoids and green bluish color for steroids (Talukder *et al.,* 2010).

Glycoside detection test

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added and observed for raddish brown coloration at the junction of two layers and the bluish green color in the upper layer (Chhetri *et al.,* 2008).

Quantitative phytochemical screening

Determination of total phenolic

The total phenol content in the extract was determined with Folin- Ciocalteu's assay using gallic acid as standard (Wolfe *et al.,* 2003). In this procedure, 0.5 ml of plant extract was mixed with 2 ml Folin- Ciocalteu's reagent (FCR) (Previously diluted with water 1:10 v/v) and 2 ml of 7.5% sodium carbonate solution was added. The tubes were vortexes for 1 min and allowed to stand for 20 min at 25° C for color development. Then, absorbance of sample was measured against blank at 760 nm using UV- Spectrophotometer (Shimadzu, USA).

The experiment was repeated three times at each concentration for precision. Total phenol content was expressed in terms of gallic acid equivalent GAE per gm of dry extract.

Determination of total flavonoid

Determination of total flavonoid content in all experimental parts of *B. ramiflora* was done by aluminiun chloride colorimetric method (Olajire *et al.,* 2011) using catechin as standard. 0.5 ml of samples/ standard was taken in a test tube and added 1.5 ml of methanol and 2.8 ml of distilled water. After 5 min, 100 µl of 10% $AlCl_3$ and 1M potassium acetate solution were added thoroughly. The test tubes were then incubated at room temperature for 30 minutes to complete the reaction. Absorbance of the solution was measured at 420 nm against blank using a spectrophotometer. The experiment was repeated three times at each concentration for precision. Total flavonoid content of the extractives was expressed as mg of CAE/gm of dried extract.

Antioxidant activity

The antioxidant activity of *B. ramiflora* peel and seed extract was determined by the following procedure.

Determination of total antioxidant capacity

Total antioxidant capacity of *B. ramiflora* peel and seed extract was determined (Prieto *et al.,* 1999) using catechin as standard. The assay was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex at acidic p^H. 0.5 ml of sample/standard at different concentrations (20-100 µg/ml) was mixed with 3 ml reaction mixture containing 0.6 M sulphuric acid, 28Mm sodium phosphate and 1% ammonium molybdate into test tube. All the test tubes were incubated at 95°C for 10 min to complete the reaction. Sample absorbance was measured spectrophotometrically at 695nm using reaction mixture as blank solution. The experiment was repeated three times at each concentration.

Reducing power capacity assay

The reducing power of the test samples was determined according to the method of Oyaizu using ascorbic acid as standard (Oyaizu, 1986). The reductive ability was measured by the reduction of $FeCl_3$ in presence of antioxidant. Ascorbic acid dissolved in distilled water having concentration 5 – 40 µg/ml used as positive control. 0.25 ml sample/standard solution was taken in test tube at different concentration. 0.625 ml of 0.2M phosphate buffer & 0.625 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] was added and incubated at 50°C for 20 min. Then 0.625 ml solution of 10 % of Trichloroacetic acid (TCA) was added into test tubes. Total mixture was then centrifuged at 3000 rpm for 5 min, after which 1.8 ml of supernatant was collected & mixed with 1.8 ml of distilled water and 0.36 ml of 0.1% ferric chloride solution. The absorbance of the solution was measured at 700 nm against blank using spectrophotometer.

DPPH radical scavenging assay

The DPPH (1,1-diphenyl-2-picryl hydrazyl) was used to detect the free radical scavenging potency of experimental parts (Blois, 1958; Desmarchelier *et al.,* 1997) .The hydrogen atom donating ability of plant extract was determined by decolorization of methanol solution of DPPH. In the presence of antioxidant, DPPH changes violet / purple color in methanol solution to shades of yellow color. A solution of 0.1 mM DPPH in methanol was prepared. 2.4 ml of this solution was mixed with 1 ml methanol solution of extract at various concentrations (12.5 – 150 µg/ml). The reaction mixture was vortex thoroughly and left in dark place at room temperature for 30 min to complete the reaction. The absorbance was measured against blank at 517 nm using a spectrophotometer. For this test, butylated hydroxyl toluene (BHT) was used as standard. Percentage of DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = \{(A_o - A_1) / A_o\} \times 100$$

Where, A_o is the absorbance of the control and $A_1$is the absorbance of the extract/standard. Then % of inhibition was plotted against concentration and IC_{50} was calculated from the graph. The experiment was repeated three times at each concentration.

Determination of analgesic activity

Acetic acid induced writhing method

Evaluation of analgesic activity of the extract was conducted according the procedure of acetic acid induced writhing model in mice (Whittle, 1964). The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice.

The test samples (ethanol extract of *B. ramiflora* peel and seed at 100 and 200 mg/kg body weight), standard (Diclofenac Sodium 10 mg/kg body weight) and control (1% Tween 80 in water at the dose of 10 ml/kg body weight) were administered orally. After 30 min, 0.7% acetic acid was injected intra-peritoneally. Approximately 5 minutes after the injection of acetic acid, a wave of contraction and elongation of abdominal musculature referred to as writhing was started and the number of writhing for

the next 10 minutes were counted for each mouse. The percent inhibition (% analgesic activity) was calculated by:

% Inhibition = [(A-B)/A] X 100

Where, A=Average number of writhing of the control group, B=Average number of writhing of the test or standard groups.

Determination of anti-inflammatory activity

Carrageenan-induced paw edema method

Evaluation of anti-inflammatory activity of the extract was conducted according the procedure of carrageenan-induced paw edema method in mice (Winter *et al.,* 1962). The six groups of mice (each containing 3 mice) were taken for the test. 0.1 ml of 1% carrageenan was injected intraperitoneally into the plantar surface of mice left hind paw of each animal to create edema. After 30 minutes of carageenan injection, the test samples, control (1% tween 80 in water) and standard (Ibuprofen) were administered orally with the help of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. The volume of paw edema was measured at 1h, 2h, 3h and 4h using a vernier caliper to determine the diameter of edema.

Determination of CNS depressant activity

Open field test

This experiment was carried out in accordance with a modified method of open field test (Gupta *et al.*, 1971). The mice in the control group received the vehicle 1% Tween 80 in water (at the dose of 10 ml/kg body weight) and standard group received diazepam at the dose of 5 mg/kg body weight orally. The test group received the crude ethanol extract of *B. ramiflora* seed and peel at the doses of 25 and 75 mg/kg body weight respectively. The animals were then placed on the floor of an open field (100 cm x 100 cm x 4 cm) divided into a series of squares with alternative color black and white. After administering test drugs, the number of squares visited by each animal was counted for 3 min duration started at 0, 30, 60, 90 and 120 min.

Whole cross test

The method described by hole cross (Takagi *et al.,* 1971) was followed to conduct this test using a cage (30 cm x 20cm x 14cm) with a steel partition fixed in the middle. A hole of 3 cm diameter was made at a height of 7.5 cm in the middle of the cage. The animals were divided into control, standard and test group. The test groups received crude ethanol extract at the dose of 25 mg/kg and 75 mg/kg body weight orally whereas control group receive vehicle (1% Tween 80 in water) at 10ml/kg body weight orally and standard group received diazepam at the dose of 5mg/kg body weight orally with the help of a feeding needle. The number of passage of a mouse from one chamber to another through the hole was recorded for a period of 3 min at the 0 min, 30 min, 60 min, 90 min and 120 min after oral administration of test drugs.

Statistical analysis

Results of all chemical analysis were expressed as mean ± standard deviation and for biological analysis were mean ± S.E.M. One way analysis of variance (ANOVA) followed by Dunnett's post Hoc test and Independent sample t- test was carried out with SPSS 20.0 for windows software and the results obtained were compared with the control group. *P*-value (***P* < 0.01, **P* < 0.05) was considered to be statistically significant.

Results

The qualitative phytochemical screening shown in table 1 confirmed the presence of alkaloid, glycoside, phenol, flavonoid, steroid, saponine and fixed oil of ethanol extract of *B. ramiflora* peel and seed with flesh.

The phenolic and flavonoid compounds in the ethanol extract of BRP and BRS were calculated using standard curve for gallic acid and catechin (Table 2). The screening of plant part revealed that the amount of total phenol content were higher in peel extract 93.05 ± 0.33 mg of GAE /gm of dried extract in

compared to seed extract 75.13 ± 1.77 mg of GAE /gm of dried extract with reference to standard curve(Y = 0.0657x + 0.0481, R^2 = 0.9984). Flavonoid were also reported in plant part of *B. ramiflora*. The flavonoid content were higher in peel extract 34.33 ± 0.24 mg of CA /gm of dried extract and lower in seed extract 14.84 ± 0.21 mg of CA /gm of dried extract with reference to standard curve (Y = 0.0033x -1E-04, R^2 = 0.9829). These phytochemical compounds were known to support bioactive chemicals in medicinal plants and thus responsible for the antioxidant activities of this plant extract.

Table 1. Phytochemical analysis of ethanol extract of *B. ramiflora* fruit.

Sample	Phytochemical group							
	Alkaloid	glycoside	Tannin	Steroid	Flavonoid	Phenol	Saponin	Fixed oil and fat
BRP	+	-	-	+	+	+	+	+
BRS	-	+	-	+	+	+	-	+

(+) shows the presence of compound; (-) shows absence of compound.

Table 2. Phenolic and flavonoid content analysis of ethanol extract of *B. ramiflora* peel (BRP) and seed (BRS).

Polyphenols	Peel extract	Seed extract
TPC (mg of GAE /gm of dried extract)	93.05 ± 0.33	75.13 ± 1.77
TFC (mg of CA /gm of dried extract)	34.33 ± 0.24	14.84 ± 0.21

TPC = Total phenolic content; TFC = Total flavonoid content.

n=3, Each value is the mean of three analysis ± standard deviation.

Total antioxidant capacity (TAC) and Ferrous reducing power capacity (FRPC) of ethanol extract of different parts of *B. ramiflora* were shown in table 3. Ethanol extract of different parts of *B. ramiflora* showed considerable antioxidant activity compared to standard catechin (CA). At the concentration of 80 µg/ml, the absorbance of CA, BRP and BRS extract was in the range of 0.493 ± 0.003, 0.277± 0.002 and 0.147 ± 0.002 while at 100 µg/ml, the absorbance was 0.619 ± 0.002, 0.422 ± 0.003 and 0.306 ± 0.003 respectively. Increase the extractive concentration increased the total antioxidant activity.

The ethanolic extract of *B. ramiflora* showed moderate to high FRPC with increased concentration of extract. At 30 µg/ml concentration, the absorbance of AA, BRP and BRS was in the range of 2.440± 0.001, 1.365 ± 0.005 and 0.734 ± 0.004 whereas at 40 µg/ml, the absorbance was 3.434 ± 0.003, 1.891 ± 0.002 and 1.114 ± 0.003 respectively. Higher the absorbance value indicated higher reducing power. These results demonstrated that the ethanol extract of BRP possess the higher TAC and FRPC than BRS which was almost resemble to the standard CA and AA (Table 3).

Table 3. Absorbance of total antioxidant capacity (TAC) and Ferrous reducing power capacity (FRPC) of *B. ramiflora* peel (BRP) and seed (BRS) extract.

Extractives	TAC		FRPC	
	At 80 µg/ml	At 100 µg/ml	At 30 µg/ml	At 40 µg/ml
BRP	0.277± 0.002	0.422 ± 0.003	1.365 ± 0.005	1.891 ± 0.002
BRS	0.147 ± 0.002	0.306 ± 0.003	0.734 ± 0.004	1.114 ± 0.003
CA	0.493 ± 0.003	0.619 ± 0.002	-	-
AA	-	-	2.440± 0.001	3.434 ± 0.003

BRP and BRS are representing *B. ramiflora* peel and seed, CA and AA representing standard Catechin and Ascorbic acid respectively.

Each value is the average of three analysis (n= 3, X ± SD).

Free radical scavenging activity of peel and seed of *B. ramiflora* was shown in figure 1 in the form of percent of scavenging using BHT as a standard. Among the extracts BRP possessed the higher activity. At a concentration of 100µg/ml, the scavenging activity BHT, BRP and BRS was 82.04 ± 0.23 %, 60.59 ± 0.46% and 51.77 ± 0.72 %. On the other hand, at 150 µg/ml concentrations, the scavenging activity of BRP and BRS was 69.22 ± 0.40% and 57.68 ±1.20%, whereas at the same concentration, the standard BHT was 88.51 ± 0.57%. The IC_{50} value of ethanol extract of BHT, BRP and BRS was 11.5, 52 and 92 µg/ml respectively. Therefore, the free radical scavenging activity of BHT and different extracts were in the order of BHT >BRP> BRS.

Table 4. Analgesic activity *B. ramiflora* peel and seed by acetic acid induced writhing in mice.

Animal group	Dose (mg/kg body weight)	No of writhing ± SEM	Percent inhibition (%)
Control	1ml/10gm	81 ± 0.58	
Diclofenac Na	10 mg/kg	42.33** ± 0.67	47.74
BRP	100 mg/kg	55.33** ± 0.88	31.69
BRP	200 mg/kg	44.66** ± 0.67	44.86
BRS	100mg/kg	49** ± 0.58	39.50
BRS	200 mg/kg	43. 33** ± 0.88	46.51

All values are expressed as mean ± standard error mean (SEM). Differences with respect to the control group were calculated using the independent sample T –test (**P < 0.01).

The effect of ethanolic extract of different parts of *B. ramiflora* (BRP and BRS) on acetic acid induced writhing in mice was shown in table 4. All doses of extract showed significant (**P < 0.01) reduction of writhing provoked by the intraperitoneal administration of acetic acid in a dose dependent manner. At 200 mg/kg doses, BRP and BRS showed almost same percent of inhibition (44.86% and 46.51% respectively) compared to standard Diclofenac Na (47.74%) while at 100 mg/kg doses, it exhibited 31.69% and 39.50% of inhibition respectively.

Table 5. Effect of ethanol extract of *B.ramiflora* peel and seed on carrageenan induced mice paw edema.

Animal group	Dose	Paw edema diameter in mm					% of inhibition of paw edema			
	(mg/kg)	Before treatment	1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr
Control	1ml/10gm	8.17 ±0.60	15.83±0.20	15.37±0.26	14.00±0.53	13.07± 0.15	-	-	-	-
Ibuprofen	10mg/kg	10.27±0.50	12.20±0.17**	10.23±0.24**	9.10±0.17*	8.13± 0.23**	22.93	33.44	35	37.80
BRP	100 mg/kg	9.77±0.39	12.83±0.17**	11.00± 0**	9.83±0.60*	9.10± 0.15**	18.95	28.43	29.79	31.14
BRP	200 mg/kg	9.83±0.17	12.73±0.26**	10.37±0.07**	9.27±0.09*	8.43± 0.12**	19.77	32.53	33.79	36.19
BRS	100 mg/kg	10.23±0.09	13.77±0.43*	11.17±1.01*	10.27±0.39*	9.57± 0.47*	13.07	27.33	26.64	30.15
BRS	200 mg/kg	9.83±0.17	13.50±0.87	10.77±0.15**	9.77±0.15*	8.27± 0.38**	24.72	29.93	30.21	36.73

All values are expressed as mean ± standard error mean (SEM). Differences with respect to the control group were calculated using the independent sample T –test (**P < 0.01, *P < 0.05).

Carrageenan induced paw edema test were performed to evaluate the anti-inflammatory activity of ethanolic extract of *B. ramiflora* (BRP and BRS) fruits. There was a dose dependent significant reduction (**P < 0.01, *P < 0.05) on carrageenan induced mice paw edema at 100 and 200 mg/kg of extract compared to Ibuprofen (10mg/kg) over a period of 4 hours as shown in table 5. The percent inhibition activity of BRP and BRS at 200 mg/ kg was 36.19% and 36.73% and Ibuprofen at 10mg/kg was 37.80% after 4 hours. From experimental data, it was observed that in open field test, the number of squares travelled by the mice was suppressed significantly in the test group throughout the study period (Table 6).

Table 6. CNS depressant activity of *B. ramiflora* peel and seed on open field test in mice.

Animal group	Dose (mg/kg)	Number of movement				
		0 min	30 min	60 min	90 min	120 min
Control	10ml/kg	49.67±1.20	78±1.52	40.67±1.20	26.66±0.88	4.33±0.67
Diazepam	5 mg/kg	98.33±1.45**	68.67±0.88*	50.33±1.20*	18.67±0.89*	8.33±0.33*
BRP	25 mg/kg	55±1.15*	48.33±1.45**	35±1.00*	8.33±0.88**	5±0.58
BRP	75 mg/kg	35.67±0.88*	11.33±0.33**	6.67±0.67**	3±0.58**	1.66±0.67*
BRS	25 mg/kg	73.33±0.33**	48.69±1.20**	42.67±1.20	12±0.57**	9.66±0.88*
BRS	75 mg/kg	46.33±0.88	36.67±0.88**	17.33±1.20**	6±0.58**	2±1.00

All values are expressed as mean ± standard error mean (SEM). Differences with respect to the control group were calculated using the independent sample T –test (**P < 0.01, *P < 0.05).

The CNS depressant activity observed for the extract was dose dependent and a noticeable decline in locomotion of test animals at 30 min was observed which continued upto 120 min. Test animals showed significant (**P < 0.01, *P < 0.05) decrease in number of movement at the doses of 75 mg/kg for BRP and BRS (1.66 ± 0.67 and 2±1.00) as compared to 4.33 ± 0.67 for the control group and 8.33±0.33 for the standard group after 120 min. Results of the hole cross test of BRP and BRS were shown in table 7. The locomotors activity reducing effects was manifested at the 2nd observation (30 min) period and was sustained upto the 5th observation period (120 min) for the plant extract. The extract diminished the movement of the tested animals in a dose dependant manner. After 120 min of administration, BRP and BRS showed significant (*P < 0.05) depressant activity at 75 mg/kg body weight. Same activity was observed for standard drug diazepam at the dose of 5 mg/kg.

Table 7. CNS depressant activity of *B. ramiflora* peel and seed on hole cross test in mice.

Animal group	Dose (mg/kg)	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Control	10ml/kg	4.3±0.67	2.33±1.20	2.33±0.88	3.67±0.67	3.33± 0.88
Diazepam	5 mg/kg	12.67±1.20*	3.67±0.88	1.33±0.67	0*	0*
BRP	25 mg/kg	11±1.00*	4±0.58	3.33±0.88	3±1.15	2±0.58
BRP	75 mg/kg	6.67±0.88	2.67±1.20	2.33±1.33	0.33±0.33*	0*
BRS	25 mg/kg	6.67± 0.67*	5±0.58	3.66± 0.88	2.33±0.67	1.33±0.33
BRS	75 mg/kg	5.33±1.20	2.67±0.67	1.33±0.33	0.33±0.33*	0*

All values are expressed as mean ± standard error mean (SEM). Differences with respect to the control group were calculated using the independent sample T –test (*P < 0.05).

Discussion

In our research work, we tried to explicate diverse pharmacological potency of ethanolic extract of *B. ramiflora* fruits in mice along with phytochemical and antioxidant analysis. The secondary metabolites (phytochemical) present in plant extract was responsible for the pharmacological action and so estimation of these bioactive compounds may be useful for treating chronic as well as infectious disease (Shukla *et al.,* 2015). Our study revealed the presence of alkaloid, flavonoid, phenol, saponin, steroid and carbohydrate. Phenols and flavonoids have been shown to exhibit their actions through effects on membrane permeability and the inhibition of membrane bound enzymes such as ATPase phospholipase A2 (LiH *et al.,* 2003).This property is regarded as the ant oxidative action of phenolic and flavonoid compound. The plant extract also revealed the presence of saponins which was known to produce inhibitory effect on inflammation (Just *et al.,*

1998). Carbohydrate and reducing sugar are essential nutrients for the body as they produce energy required and supplies energy to brain, muscle and blood (Ejelonu *et al.,* 2011). Various studies reported that alkaloid and their synthetic compound possessed analgesic, antispasmodic and antibacterial activity (Achi *et al.,* 2017). Further, quantitative analysis of phytochemicals reported the amount of phenolic and flavonoid compound. The significant antioxidant activity shown by the extract can be attributed due to the presence of phenolic in the extract.

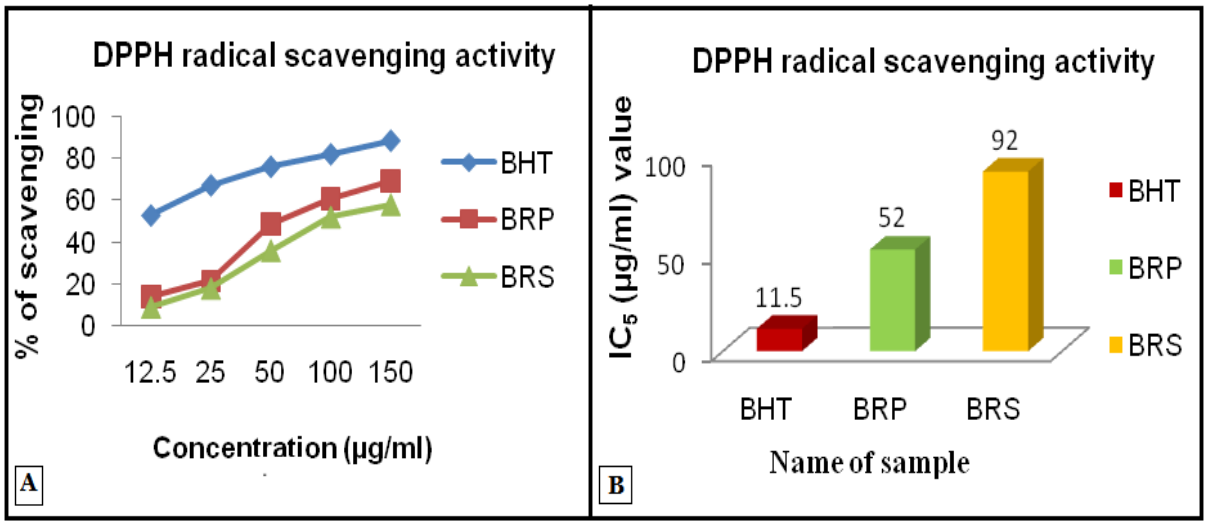

Fig. 1. Determination of A) DPPH radical scavenging activity and B) IC_{50} value of ethanol extract of *B. ramiflora* peel and seed.

Experiments were performed as triplicate (n=3, $p< 0.05$) for all tested doses.

The result of DPPH scavenging activity assay in this study indicated that the plant was potentially active. This result suggested that the plant extract contained compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. DPPH can also inhibit the formation of ABTS+.

The scavenging activity of ABTS+ radical by the plant extract may be useful for treating radical related pathological damage (Wang *et al.,* 1998). In our study, the IC_{50} value of ethanol extract of BHT, BRP and BRS was 11.5, 52 and 92µg/ml. In another study, the chloroform soluble fraction of methanol extract of *B. ramiflora* fruits showed IC_{50} value of 49.78 µg/ml and petroleum ether soluble fraction 75.31µg/ml (Amin *et al.,* 2015).

According to the result of total antioxidant capacity and reducing power assay, we can suggest that *B. ramiflora* fruits had potential antioxidant capacity which is comparable to that of the standard. The electron donation capacity of the extract stabilizes the free radical and break free radical chain reaction, thus producing reducing power of extract (Gangadharan *et al.,* 2013). Phenolic and flavonoid compounds exhibited antioxidant activity through their reductive capacity in a Fe^{3+} - Fe^{2+} system indicating that these compounds might produce the reducing capacity of the extract (Aliyu *et al.,* 2013).

The acetic acid induced writhing method was found efficient to assess peripherally active analgesics effect. The agent reducing the number of writhing will render analgesic effect by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Ferdous *et al.,* 2008). *B. ramiflora* peel and seed exhibited 44.86% and 46.51% analgesic activity at the doses of 200 mg/kg which is almost similar with that of standard Diclofenac Na. Therefore, it is likely that our extract might exert peripheral antinociceptive action by interfering with the irritant or by inhibiting the synthesis, release or antagonizing the action of pain mediators at the target sites.

Carrageenan induced paw edema method is a well-known experimental animal model for acute inflammation and is believed to be biphasic. The early phase is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotriene's, polymorph nuclear cells and prostaglandin produced by tissue macrophages (Gupta *et al.,* 2006). Our extract significantly reduced paw edema in second phase and the inhibition rate for BRP and BRS was 36.19% and 36.73% at 200 mg/kg after 4 hours while the standard ibuprofen reported 37.80% inhibition. This finding suggested that the possible mechanism of the observed anti-inflammatory activity might be its ability to reduce the release of histamine, serotonin or kinin-like substances or biosynthesis of prostaglandins. Two different neuropharmacological models, named open field and whole cross test were used to study the CNS depressant activity of *B. ramiflora* fruit extract. The Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect. Locomotor activity lowering effect was evident in the 2nd observation (30 min) and continued upto 5th observation period (120 min). Maximum depression activity was observed from 3rd to 5th observation period. Gamma-aminobutyric acid (GABA) is evidenced to be the major inhibitory neurotransmitter of CNS and several anxiolytic, muscle relaxant and sedative- hypnotic drugs show their effect via GABA. Therefore, it can be assumed that *B. ramiflora* fruit extract may act by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of cortical neurons in the brain or may be direct activation of GABA receptor by the extracts (Kolawole *et al.,* 2007).The result of our study illustrated that the plant extract significantly (**P < 0.01, *P < 0.05) induced sedative-hypnotic activity in test animals confirming their CNS depressant properties. Previous investigation on phytoconstituents and plant suggested that flavonoids and neuroactive steroid were found to be ligands for the GABA receptors in the CNS which assume that they act as benzodiazepine like molecules (Yadav *et al.,* 2013).

Conclusion

On the basis of the findings, it can be stated that the ethanolic extract of *B. ramiflora* fruit contains alkaloid, steroid, saponin, phenolic and flavonoid compounds. The amount of phenolic and flavonoid was also confirmed by quantitative phytochemical analysis. This study also affirmed the presence of promising antioxidant activity which was comparable to that of the standard compound.

The results of our research work also suggested that the fruits have potent analgesic, anti-inflammatory and CNS depressant activity. Therefore, *B. ramiflora* fruit is the natural source of antioxidant substance of high significance and consumption of the fruit can offer health benefits in prevention of disease caused by oxidative stress, diabetic and cancer. However, further research is needed in order to find out the responsible bioactive components for the above mentioned pharmacological activities.

Conflict of interest

The authors hereby declare no conflict of interest for publication of this manuscript.

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