



Received on 09 June, 2018; received in revised form, 04 July, 2018; accepted, 09 July, 2018; published 01 September, 2018

IN-VITRO ANTIOXIDANT AND ANTIBACTERIAL STUDY OF *BACCAUREA RAMIFLORA* SEEDS

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Keywords:

Baccaurea ramiflora,
Anti-oxidant, Anti-bacterial,
DPPH scavenging activity

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ABSTRACT: *Baccaurea ramiflora* which is commonly known as Burmese grape is a commonly used medicinal plant in the South Asian folk medicine. They are potent in treating dermatological disorders and constipation. The aim of the study is to investigate the possible biological activities of *B. ramiflora* by determination of antioxidant activities of total ethanolic extracts through the free radical scavenging activity over vitamin C and the antibacterial activity using disk diffusion method. *In-vitro* antibacterial activity was evaluated against *E. coli*, *P. aeruginosa*, and *S. aureus* and Azithromycin was used as reference compound. IC₅₀ were found to be 27.57 and 13.66 µg/ml for *B. ramiflora* and vitamin C respectively and the ethanolic plant seed extract exerted significant antibacterial activity at 500 µg, 1000 µg/disc concentration over the standard antibiotic drug which demonstrates the efficacy of *B. ramiflora* plant seeds extract.

INTRODUCTION: Oxidative stress is considered as an important risk factor in the pathogenesis of several chronic diseases like diabetes, cardiovascular diseases *etc.* Increased amount of free radicals and other reactive oxygen species are found to be involved in the pathogenesis of conditions such as atherosclerosis, diabetes and neurodegenerative diseases like Parkinson's and Alzheimer's diseases even cancers. Evidence also shows that, reactive oxygen species (ROS) are responsible for the human aging^{1,2}. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule³. The key property of an antioxidant compound is its ability to trap free radicals.

Phenolic acids, polyphenols as well as flavonoids are the antioxidant compounds which scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to the progression of degenerative diseases⁴. Since ancient times, herbal plants are considered as good antioxidant source for treating acute and chronic diseases.

Baccaurea ramiflora also known as burmese grape is an evergreen tree belongs to the Euphorbiaceae family which is found throughout Asia, most commonly cultivated in south Asian countries like Bangladesh, India and Malaysia. For south Asian people, the fruits of *B. ramiflora* is one of the valuable nutritional sources due to its enriched vitamins and minerals and the woods of the tree are used in furniture production because of its durability⁵. In Chinese Dai medicine, the plant has been reported to possess anti-inflammatory and anodyne against rheumatoid arthritis, cellulitis and abscesses to treat injuries.



The fruit juice of *B. ramiflora* have the efficacy to treat constipation⁶. Hence, the aim of the current study was to evaluate the antioxidant activity of alcoholic extracts of seed of *B. ramiflora* by using DPPH scavenging assay and evaluation of anti bacterial activity.



FIG. 1: *BACCAUREA RAMIFLORA* TREE WITH FRUITS

MATERIALS AND METHODS:

Collection of Plant Material: The *B. ramiflora* seeds were collected from Botanical garden of Jahangirnagar University campus, Bangladesh. The seeds were dried in shed and crushed manually with wooden arrangement and reduce in fine powder form. A voucher specimen has been archived in the herbarium Prof. Khairul Kabir (CBOT/JU) with code JU 15.318 ID.

Extraction: The plant seeds were sun-dried first and then dried in an oven at reduced temperature (< 70 °C) to make the item suitable for grinding. The powdered plant seeds were submerged in sufficient volume of ethanol and methanol in an air-tight flat bottomed container for a week, with occasional shaking and stirring. The seed extracts were then filtered and dried on electrical water bath.

Evaluation of Anti-oxidant Activity: The anti-oxidant activity of the plant seed extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by UV spectrophotometer at 517 nm. The anti oxidant activity was evaluated according to the method described by Clinical and Laboratory Standards Institute⁷. A number of concentrations of the plant extracts were prepared using analytical ethanol solution (5, 10, 20, 40, 60, 80 and 100 µgml⁻¹). Ascorbic acid (vitamin C) was used as an anti-oxidant standard. 1 ml from each extract and 3ml of ethanol were mixed by 0.5 ml of 1.0 mM DPPH in methanol and allowed the mixture to react

at room temperature for 30 min. To prepare the blank solution, same amount of ethanol and DPPH was mixed. For each analysis, all determinants were prepared in triplicate and the mean value of absorbance was obtained. The DPPH scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = 100 \times (\text{Ab} - \text{Aa}) / \text{Ab}$$

Where, Ab is absorption of the blank solution and Aa is absorption of the plant extract.

Evaluation of Anti-microbial Activity: The *in-vitro* antimicrobial tests of ethanolic extract of *B. ramiflora* against Azithromycin were done at Bangladesh Livestock Research Institute (BLRI), Bangladesh. Antibacterial activities of the ethanolic seed extracts was investigated by the disc diffusion method mentioned by Alzoreky and Nakahara⁸. The ethanolic plant extracts were dissolved in 0.1 dimethyl sulfoxide (Gaylord Chemical. Inc., USA) in addition to MeOH. Four well-isolated colonies of the similar morphological type were chosen and inoculated into tubes containing 5 ml Muller-Hinton agar plate (Sigma-Aldrich. Inc., Germany) and incubated at 37 °C followed by shaking at 150 rotation per min for 24 h. The bacterial cells were counted using hemocytometer. For positive control *S. aureus*, *P. aeruginosa* and *E. coli*, Azithromycin discs (60 µgml⁻¹) was used and ethanol solvent of the plan extract was used as negative control. To obtain result each studies were done in triplicates. Growth inhibitory activity was calculated by measuring the diameter of clear zone around the disc using a ruler⁹.

RESULTS AND DISCUSSION:

Antioxidant Activity: The antioxidant activity of ethanolic extract of *B. ramiflora* seed was measured by the ability to scavenge DPPH free radicals comparing with ascorbic acid (vitamin C). The free radical scavenging effects of *B. ramiflora* plant extract and the standard substance on the DPPH radical were expressed as half maximal inhibitory concentration (IC₅₀) values; the results are reported in **Table 1**. Lower IC₅₀ value reflects higher DPPH radical scavenging activity. According to the results obtained, the ethanolic extract of *B. ramiflora* seeds showed significant DPPH activity with the IC₅₀ value of 27.57 µgml⁻¹,

while IC₅₀ of ascorbic acid (vitamin C) as standard was 13.66 µgml⁻¹.

Different experiments have been performed to identify the free radical scavenging activities of the plant extract¹⁰. DPPH is a compound which has significant free radical scavenging ability and shows good absorbance at 517 nm¹¹. Ascorbic acid (vitamin C) is usually used as a standard antioxidant and it has a strong DPPH scavenging property¹². Our findings indicated that *B. ramiflora* extract showed promising antioxidant activity with 27.57 µgml⁻¹ IC₅₀ value.

TABLE 1: INHIBITION PERCENTAGE OF DPPH AND IC₅₀ FOR ETHANOLIC EXTRACT OF *B. RAMIFLORA* AT DIFFERENT CONCENTRATIONS (g/ml) COMPARED WITH VITAMIN C

Concentration (µg/ml)	% Inhibition of ethanol extract and standard at different concentration	
	Extract of <i>B. ramiflora</i>	Extract of <i>B. ramiflora</i>
5	31 ± 0.45	42 ± 0.38
10	38 ± 0.34	49 ± 0.44
20	45 ± 0.64	58 ± 1.05
40	65 ± 0.47	65 ± 1.25
60	76 ± 0.28	73 ± 0.12
80	89 ± 0.43	88 ± 0.23
100	95 ± 0.18	101 ± 0.12
IC ₅₀ value	27.57	13.66

Antimicrobial Activity: The antibacterial activity of seed extract of *B. ramiflora* against the tested bacteria strains was evaluated using disk diffusion method. The inhibition zone produced by the plant extract on selected bacterial strains was between 7mm and 18 mm. Our antimicrobial study revealed that the ethanolic extract of *B. ramiflora* showed inhibitory effects on *S. aureus*, *P. aeruginosa* and *E. coli* as shown in **Table 2**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF *B. RAMIFLORA* PLANT EXTRACT AT DIFFERENT CONCENTRATIONS ON THE BACTERIAL GROWTH

Bacteria	Zone of inhibition (mm)		
	500 µg/disc	1000 µg/disc	Azithromycin (60 µg/disc)
<i>S. aureus</i>	7.07 ± 0.58	10.67 ± 0.33	14.83 ± 0.53
<i>P. aeruginosa</i>	8.40 ± 0.75	9.34 ± 0.63	19.44 ± 0.51
<i>E. coli</i>	9.4 ± 0.35	10 ± 0.47	16.45 ± 0.30

From previous studies, the plant has been identified as a important source for development of new therapeutic approaches. To achieve this target, the *in-vitro* antibacterial test was the initial step¹³. The result of our *in-vitro* study clarified that the crude

extract of *B. ramiflora* proved its efficiency to be used as a potential source for antibacterial compounds due to its inhibitory effects on *S. aureus*, *P. aeruginosa* and *E. coli*.

CONCLUSION: Ethanolic extracts of *B. ramiflora* seeds have different levels of both antioxidant and antibacterial activity. Several studies suggest that, there is a strong relationship exists between total phenolic content of plants extracts and antioxidant and antibacterial activity.

So the plant could be subjected for further studies to purify the active components which are responsible for the antioxidant and antimicrobial activities.

ACKNOWLEDGEMENT: The key researcher, Pritam Saha Podder, is sincerely grateful to Pharmaceutical chemistry laboratory of Jahangirnagar University for providing research facilities too.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:

Podder PS, Das R and Kundu SK: *In-vitro* antioxidant and antibacterial study of *Baccaurea ramiflora* seeds. Int J Pharmacognosy 2018; 5(9): 612-15. doi link: [http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5\(9\).612-15](http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(9).612-15).

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