IJP (2018), Vol. 5, Issue 9 **(Research Article)**

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 South Asian folk medicine. They are potent in treating dermatological disorders and constipation. The study aims 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 the disk diffusion method. *In-vitro* antibacterial activity was evaluated against *E. coli*, *P. aeruginosa*, and *S. aureus* and Azithromycin was used as the reference compound. IC_{50} was 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, cardio-vascular diseases, *etc*. The 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 molecule [3]. The key property of an antioxidant compound is its ability to trap free radicals.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.5(9).612-15

The article can be accessed online on www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(9).612-15

Phenolic acids, polyphenols as well as flavonoids are the antioxidant compounds which scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to the progression of degenerative diseases [4]. Since ancient times, herbal plants are considered a good antioxidant source for treating acute and chronic diseases.

Baccaurea ramiflora, also known as Burmese grape, is an evergreen tree that 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 durability [5]. 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* has the efficacy to treat constipation [6]. 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 the antibacterial 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 the shed and crushed manually with the 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 and dried in an oven at reduced temperature (< 70 ºC) 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 an electrical water bath.

Evaluation of Anti-oxidant Activity: The antioxidant activity of the plant seed extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by UV spectrophotometer at 517 nm. The antioxidant activity was evaluated according to the method described by the Clinical and Laboratory Standards Institute [7]. 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, the 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:

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

Where Ab is the absorption of the blank solution and Aa is the 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 were investigated by the disc diffusion method mentioned by Alzoreky and Nakahara [8]. 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}) were used, and ethanol solvent of the plant extract was used as the negative control. To obtain a result, each study were done in triplicates. Growth inhibitory activity was calculated by measuring the diameter of the clear zone around the disc using a ruler [9].

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 compared 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 (IC_{50}) values; the results are reported in **Table 1**. Lower IC_{50} 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 IC_{50} value of 27.57 μgml^{-1},

while IC_{50} 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 extract [10]. DPPH is a compound which has significant free radical scavenging ability and shows good absorbance at 517 nm [11]. Ascorbic acid (vitamin C) is usually used as a standard anti-oxidant and it has a strong DPPH scavenging property [12]. Our findings indicated that *B. ramiflora* extract showed promising antioxidant activity with 27.57 µgml^{-1} IC_{50} value.

TABLE 1: INHIBITION PERCENTAGE OF DPPH AND IC_{50} 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 *B. ramiflora*	Extract of *B. ramiflora*
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 the 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)
S. aureus	7.07 ± 0.58	10.67 ± 0.33	14.83 ± 0.53
P. aeruginosa	8.40 ± 0.75	9.34 ± 0.63	19.44 ± 0.51
E. coli	9.4 ± 0.35	10 ± 0.47	16.45 ± 0.30

From previous studies, the plant has been identified as an important source for development of new therapeutic approaches. To achieve this target, the *in-vitro* antibacterial test was the initial step [13]. 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 to 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 Jahangir-nagar 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.