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## SHORT COMMUNICATION

# *Bambusa vulgaris* Schrad. ex J. C. Wendl. var. *vittata* Riviere & C. Riviere leaves attenuate oxidative stress- An *in vitro* biochemical assay

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*Bambusa vulgaris* Schrad. ex J. C. Wendl. var. *vittata* Riviere & C. Riviere (BVV) leaves are consumed as food and have antimicrobial activity. Positive health effect of its consumption is attributed to the bioactive substances. In the present study, the influence of BVV antioxidants on oxidative stress that can be used as an alternative option has been studied. The total phenol and flavonoids content in the aqueous and acetone extract were determined by calorimetric assay, as well as their antioxidant activity through various chemical assays like, DPPH radical, Hydrogen peroxide scavenging and reducing power assay. Both the aqueous and acetone extracts displayed their antioxidantactivity in a dose dependent manner by scavenging hydrogen peroxide and DPPH radical. The total phenolic and total flavonoids compounds in both aqueous and acetone extract were 345.910.071 and 325.630.051 mg GAE/g of dry extract and 289.20.05 and 179.20.002 mg quercetin/g of dry extract, respectively. This may be the first report to provide evidence that the crude aqueous and acetone extract of *Bambusa vulgaris* var. *vittata* leaf is a potential source of natural antioxidants.

**Keywords:** *Bambusa vulgaris* 'Vittata', DPPH, Flavonoids, FRP, $H_2O_2$ scavenging, Phenolics.

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## Introduction

Rendering the side effects of synthetic antioxidants, plant-derived phytochemicals are safer and regular consumption of them may drift the balance towards a sufficient antioxidant status[1-2]. Thus in recent years interest on natural antioxidant, especially of plant origin has increased many folds[3]. Bamboo species find their importance as a novel foods additive due to high natural antioxidant contents[4]. It is a source of complementary and alternative medicines, used world wide as rich antioxidant resource and specific functional factors[5-6].

*Bambusa vulgaris* Schrad. ex J. C. Wendl. var. *vittata* Riviere & C. Riviere (BVV)belonging to the subfamily Bambusoideae (Poaceae) is commonly known as yellow bamboo, tiger bamboo or painted bamboo[7], because of its golden yellow clumps interspersed with green streaks. The culms attain a height of about 15 m, diameter of about 15.3 cm, thick walled and densely tufted[8]. It is also cultivated as an ornamental plant, though; it flowers sporadically but fails to produce seed[9]. The newly formed shoots are consumed as food in North eastern states of India, China and Taiwan[10]. The leaves of BVV have been reported to have antimicrobial activity[11].The culms of this species have been used in China for the treatment of oedema[12].

Our previous studies on BVV methanolic extract had shown a promising antioxidant activity[13]. However, till date there are no comparative studies reported. Therefore, the aim of this study was to evaluate *in vitro* antioxidant activity of aqueous (BAQE) and acetone (BAE) extract of BVV leaves. For this purpose total free radical scavenging activity, preliminary phytochemical assay, hydrogen peroxide and hydroxyl inhibitory activity has been assayed. Attempts have also been made to quantitatively estimate the phenolics and flavonoids content.

## Materials and Methods

### Plant material and extraction

BVV leaves were collected from Siliguri in June 2009 and identified by Dr. P.P. Paudyal (Consultant, Bamboo Mission Sikkim, India). A voucher specimen (SUK/KRR/BVVOOI) has been submitted in Bambusetum, Kurseong Reasearch Range, Sukna, Darjeeling[14]. Dried, ground leaves of BVV (10 g) were extracted in a Soxhlet apparatus using water and acetone, respectively[15]. The extracts obtained were evaporated under pressure (12 Torr) at 50°C to constant weight and was stored at 4°C until further use. The yield was calculated as per author's previous studies[13]. For experimental purpose the extracts were dissolved in double-distilled water (DDW) in desired concentrations.

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**Determination of total phenolic content**

Total phenolic content was determined using Folin-Ciocalteu CFC) reagent method at 730 nm[16]. The total phenolic content was expressed as gram of Sallie acid equivalents (GAE) per 100 g.

**Determination of total flavonoid content**

The total flavonoid content was determined with aluminium chloride ($AlCl_3$) method at 510 nm[17] and calculated from a quercetin standard curve.

**DPPH radical scavenging activity**

The free radical scavenging capacity of the extracts was determined using DPPH[18]. Discoloration was measured at 517 nm after incubation of 30 min in dark (Themo UVI spectrophotometer). Ascorbic acid was used as a reference standard. Control sample was prepared containing the same volume without any extract, 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1)/ A_0 \times 100$$

where, $A_0$ and $A_1$ are the absorbance of the control and the sample, respectively

**Reducing power assay**

The reducing power of BVV leaf extracts was determined according to the method previously described by Ruch method[19]. The absorbance was measured at 700 nm. Ascorbic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture was taken and expressed as mean standard deviation.

**Hydrogen peroxide scavenging activity**

This activity was determined according to a previously described method with minor changes[20]. After incubation, absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity of hydrogen peroxide by BVV leaves extracts was calculated as follows:

$$\text{Percentage scavenging activity } [H_2O_2] = [\text{Abs (control) - Abs (standard) / Abs (control)}] \times 100$$

Where, Abs (control): Absorbance of the $H_2O_2$ (2 mM) as control and Abs (standard): Absorbance of the extract/standard. All the experiments were performed in triplicate.

## Statistical Analysis

Results are expressed as mean ±S.E. of triplets. The groups were compared by two-way ANOVA using Graph Pad Prism, Version 5.0 (Graph Pad Software, San Diego, CA, USA). $P< 0.001$ was considered significant.

## Results

The yield of BAQE and BAE extract was 3.37 % and 2.94 %, respectively. The total phenolic content of BAQE was 345.910±.071 and BAE was 325.63 ± 0.051 mg GAE/g of dry extract. The total flavonoid content of the BAQE and BAE was 289.2±0.05 mg quercetin/g and 179.2±0.002 mg quercetin/g of dry extract, respectively. Comparison of the antioxidant activity of the extracts and ascorbic acid is shown in Fig. 1. BAQE exhibited above 55% and BAE shows 28% scavenging activity at 200µg/mL concentration.

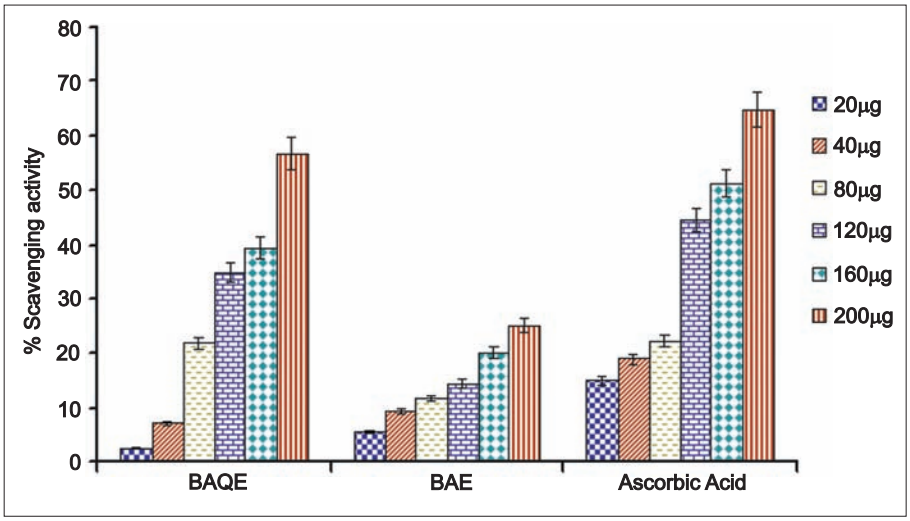


Fig. 1—DPPH scavenging activity of different leaf extract of *B. vulgaris* var. vittata compared with standard (ascorbic acid).

Fig. 2 depicts the $H_2O_2$ scavenging activity of BAQE and BAE. A high amount of $H_2O_2$ scavenging was found. Fig. 3 depicts the reductive capability of the plant extracts compared to ascorbic acid. The reducing power of extract of BVV leaves was found to be notable, which increased gradually with a rise in concentration. As illustrated in Fig. 3, $Fe^{3+}$ was transformed to $Fe^{2+}$ in the presence of extract and ascorbic acid which is a measure of reductive capability. From the figure it is evident that even a low dose of the extract had maximum reducing power, when compared with standard.

## Discussion

It is known that the biomolecules are damaged by the free radicals generated during oxidative stress and

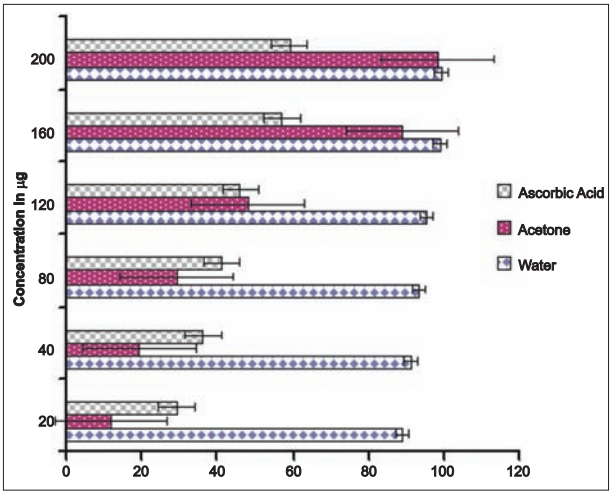


Fig. 2—Hydrogen peroxide scavenging of different extract of *B. vulgaris* var. *vittata* in comparison with a standard (ascorbic acid).

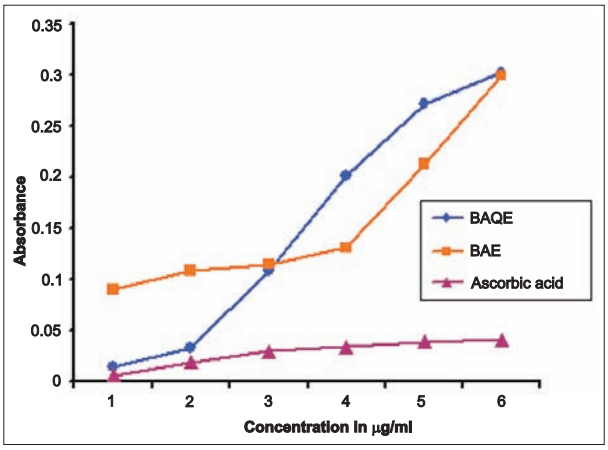


Fig. 3—Reducing power activities of different extract of *B. vulgaris* var. *vittata* in comparison with a standard (ascorbic acid) at $\lambda$ = 700 nm.

exposure to radiation[21-22]. The amount of antioxidants in BAQE and BAE are in accordance with previous reports [12,13,23]. The phenolic and flavonoid content was found to be higher in BAQE than BAE. This might be due to the difference in the polarity of the solvent used, depending upon which selective phenolic compounds percolate in the extract. Similar type of results has been observed in parallel studies reported elsewhere[12,13,24].

The DPPH antioxidant assay is based on the ability of DPPH to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 540 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Considerable difference in DPPH scavenging activity of BAQE and BAE was observed. Since BAQE had the higher antioxidant activity, it can be inferred that water could be a suitable solvent to prepare the extract in comparison to acetone.

Findings from our result revealed that the BAQE had high effects on scavenging OH- as reported earlier[25-26] compared to BAE. The presence of phenolic components, such as phenolic acids, and flavone C-glucosides may be responsible for the antioxidative effect of BVV[27]. Phenolic compounds maycontribute directly to antioxidative action. The antioxidant activity of phenolics are primarily due to their redox properties, which may play a key role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[28]. In this respect, polyphenolic compounds, like flavonoids and phenolic acids, naturally occurring in plants have been reported to have numerous biological effects ,including antioxidant activity[29-30].

In the reducing power assay, it has been found that the $Fe^{3+}$ -$Fe^{2+}$ transformation occurred in the presence of the extract[19]. Similar trends have been observed with both BAQE and BAE extracts. The absorbance of BAQE and ascorbic acid was found to be almost similar at 20 and 40 µg/mL concentrations. The absorbance of BAQE and BAE showed parallelism at 80 and 200 µg/mL concentrations and then differ considerably. A direct correlation between antioxidant activity and reducing power of certain plant extracts have been observed by many scientists[31].

## Conclusion

Both the aqueous and the acetone extracts of BVV showed strong antioxidant activity by reducing free

radicals, inhibiting DPPH and hydrogen peroxide. The high amount of total phenols and flavonoids in both the extracts of BVV could have played a crucial role in controlling oxidation. The outcome of this study illustrates that the BVV leaves can be used as easily accessible source of natural antioxidant. However, the phyto-constituents responsible for the antioxidant activity of BVV are yet to be elucidated. Hence, a detailed study is `required to trace out the mechanism behind antioxidant activity of this plant.

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