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In vitro antioxidant profiling of *Bambusa tulda* Roxb. aqueous methanolic leaf extract growing in the forests of Kokrajhar district, BTAD, Assam, India

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Abstract

Background: From an age-old period bamboo has been an important ingredient of traditional Asian medicines in general and Chinese medicine in particular. Today people are searching over the use of herbal medicine instead of the synthetic drugs. **Objective: The a**im of this study was to evaluate phytochemical, biochemical constituent and *in vitro* antioxidant activity of aqueous methanolic leaf extract of *Bambusa tulda. Bambusa tulda* (Poaceae) is widely distributed in North Eastern parts of the Country. **Methodology:** Phytochemical constituents of leaves were extracted by hot extraction method. Aqueous methanolic extract showed that the presence of phenolic compound, alkaloids, saponnins, flavonoids, steroids, anthraquinones, glycosides. The total phenolic, flavonoid and flavonol content in the aqueous methanolic extract were determined by calorimetric assay as well as their antioxidant activity through various chemical assays like DPPH radicals, hydrogen peroxide scavenging and reducing power assay. **Results:** The total phenolic, flavonoids and flavonol content in aqueous methanolic extract were 17.494±0.01 mg GAE/g, 176.35±0.03 mg QE /g and 96.2±0.01 mg QE /g respectively. **Conclusion:** This may be first report to provide evidence that the crude aqueous metanolic extract of *Bambusa tulda* leaf is a potential source of natural antioxidant.

Keywords: *Bambusa tulda*; antioxidants; DPPH, Kokrajhar; *Jati banh*; correlation; FRP, phenolics, *Owa gubwai*

1. Introduction

In spite of diverse Innovations and improvements in remedial sciences, modern world is yet to overcome various diseases like HIV, neurodegenerative, diabetes and many more. Many of these ailments are because of overproduction of ROS and RNS due to various metabolic reactions which in turn damages our tissues as a result of oxidative stress. Therefore, the secondary metabolites of plants as phytomedicine that could produce a definite physiological action on human body have been an imperative element of the diet since the days of yore[1]. Thus researchers are attempting to find cure using these natural antioxidants occurring in food and medicinal resources to substitute synthetic ones.

Thus in recent years interest on natural antioxidant especially of plant origin has increased many folds[2]. Bamboo species is a source of traditional medicines, used world-wide as rich antioxidant resources and specific functional factor[3]. Previous authors insisted different species of bamboo such as *Phyllostachys edulis*[4], *Dendrocalamus hamiltonii*[1], *Sasa borealis*[5], *Dendrocalamus strictus*[3], *Bambusa vulgaris* 'Vittata'[6,7] are rich source of antioxidants[8].

Bambusa tulda Roxb., (Family: Poaceae; Bengal bamboo)[9] locally addressed as *Owa gubwai* (Bodo) *Jati banh* (Assamese)[10], is considered to be one of the most useful bamboo species and is extensively in paper pulp industry in India. It can grow up to 15m height, 8cm thickness[11] and commonly found in southeastern Asia. In India, it is endemic North Eastern parts of the country and West Bengal[12]. In North-eastern part of India, this species of bamboo is also valued as medicine. The fresh juice of shoots is applied to the injury of nails while decoction of fermented shoots is prescribed for tumors[12].

However, as per our knowledge there are no studies on leaf extract of *Bambusa tulda* from North Eastern province, India on its antioxidant potential. So, the aim of this study was to evaluate *in vitro* antioxidant activity of aqueous methanolic extract of *Bambusa tulda* leaves along with preliminary phytochemical analysis. Attempts

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have also been made to quantitatively estimate the phenolics and flavonoids content.

2. Materials and Methods

2.1 Plant material collection and extraction

Bambusa tulda leaves were collected from the forests of Kokrajhar District, BTAD, Assam, India during winter session, 2013. Identified and authenticated by a taxonomist in university and a voucher specimen (Voucher No. DBT/BU/ Bamboo/007) was deposited to Bamboo technology, Bodoland University, Kokrajhar, BTAD, Assam, India. 10 grams of mechanically grinded dry leaf samples were extracted in a Soxhlet apparatus using 150ml of 70% aqueous methanol (*w/v*) separately at boiling temperature for 6 h. The extract was then filtered using whatman filter paper (no 42). The obtained extract was reduced at rotary evaporator at 60-80°C under reduced pressure, followed by lyophilization using a freeze drier until constant weight was obtained then stored at 4°C until required[13]. Before use, the methanolic extract (BME) was dissolved in double-distilled water (DDW) in desired concentrations.

2.2 Preliminary Phytochemical Screening

The presence or absence of the preliminary phytochemical constituents such as reducing sugars, carbohydrates, saponnins, tannins, alkaloids, anthraquinones, steroids, flavonoids, glycosides of the sample were analyzed using previously explained standard methods[14,15].

2.3 Determination of Biochemical Constituents

The total soluble phenolics (TPC) were determined by Singleton and Rossi[16] method with slight modifications using gallic acid equivalent (mg GAE/g) as a standard[6]. The total flavonoid content (TFC) was determined according to Zhishen *et al.*[17] with minor modifications using quercetin equivalent (mg QE/g) as a standard[18]. The modified method of Goyal *et al.*[19] was used to estimate the total flavonols using quercetin equivalent (mg QE/g) as a standard.

2.4 In vitro Antioxidant properties of the extracts

Three different *in vitro* test systems viz. DPPH scavenging activity, ferric reducing power assay and hydrogen peroxide scavenging activity were used to access the antioxidant potential of the BME.

2.4.1 Free Radical Scavenging Activity (DPPH Method)
The antioxidant activity of *B. tulda* leaf extracts and standard were assessed on the basis of the radical scavenging effect of the stable DPPH free radical as per the modified protocol by Goyal *et al.*[6].

2.4.2 Ferric reducing power assay (FRP)

The reducing power of the extracts was determined according to the method of Oyaizu[20]. Different concentrations of BME (0.2–2.0 mg/mL) in 1 mL of DDW was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of TCA (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with DDW (2.5 mL) and FeCl3 (0.5 mL, 0.1%) and 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.

2.4.3 Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al.[21]. An aliquot of H_2O_2 (2mM) and sample at various concentrations (0.2–2.0 mg/mL) were mixed (1:0.6 v/v) and incubated for 10 min at room temperature. After incubation, absorbance was read at 230 nm was determined against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity of hydrogen peroxide was calculated using the following equation.

H_2O_2 activity (%)= Abs (control)- Abs (sample)/ Abs (control) *100

Where, Abs (control): Absorbance of the control and

Abs (sample) : Absorbance of the extracts/standard.

2.5 Statistical analysis

Results were calculated as the mean_s.d. for each sample. Statistical analysis was done with one-way ANOVA with Graph Pad Prism, ver. 4.0 (Graph Pad Software, San Diego, CA, USA). A significant difference was judged to exist at a level of $p < 0.05$.

3. Results

3.1 Preliminary phytochemical screening

The preliminary phytochemical screening of aqueous methanolic exact of *B. tulda* leaf was depicted in table I.

Table I: Preliminary phytochemical screening of *Bambusa tulda* leaf extract

Chemical compounds	Results
Saponins	+
Steroids	+
Alkaloids	+
Tannins	+
Carbohydrates	+
Flavonoid	+
Anthraquinone	+
Glycosides	+
Reducing sugars	+

- = Compound not detected; + = Compound detected

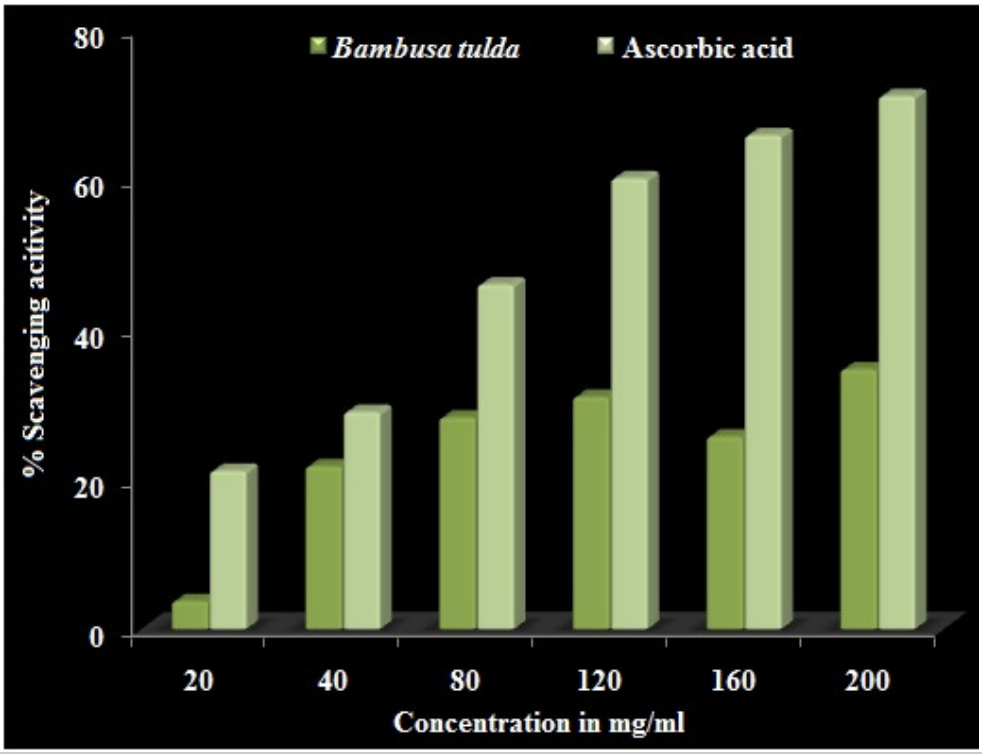

Figure I: DPPH scavenging activity of aqueous methanolic extract of *Bambusa tulda* leaf

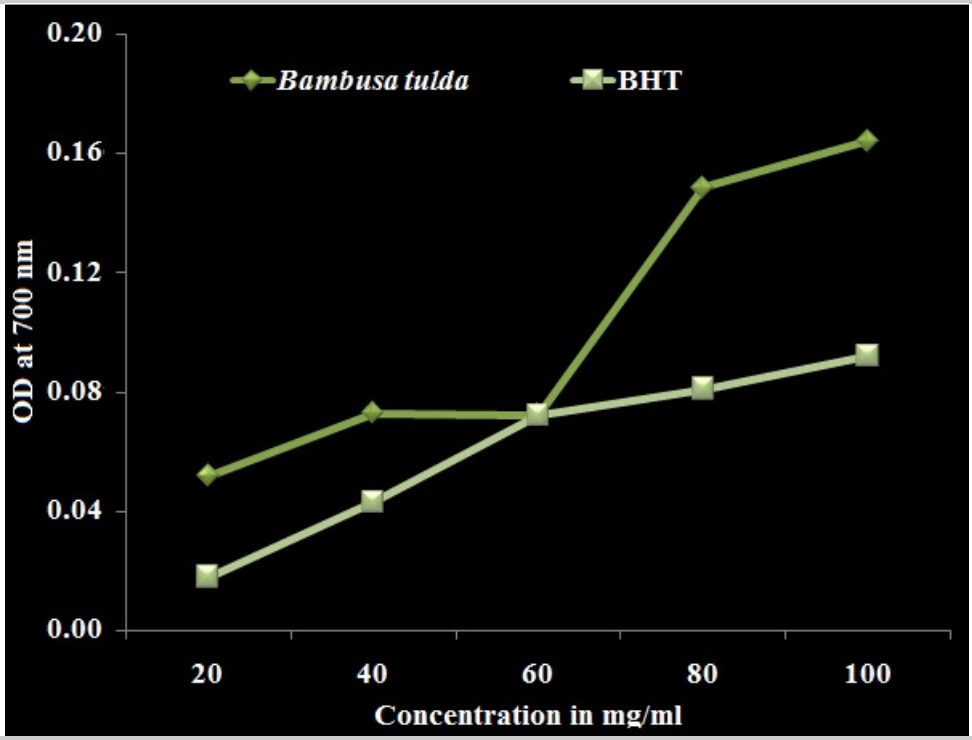

Figure II: Reducing power assay of aqueous methanolic extract of *Bambusa tulda* leaf

3.2 Determination of Biochemical constituents

The biochemical constituent such as total phenolic, flavonoid, flavonol content in aqueous methanolic extract of *Bambusa tulda* were found to be 17.494±0.01 mg GAE /g, 176.35±0.03 mg QE /g and 96.2±0.01 mg QE /g respectively.

3.3 DPPH Scavenging activity The DPPH scavenging activity of different concentration of leaf extracts compared to the standard ascorbic acid is shown in figure I.

3.4 Reducing power assay

The ferric reducing power capability of plant extracts compare to BHT as depicted in figure II.

3.5 H_2O_2 Scavenging activity

The H_2O_2 scavenging activity of different concentration of leaf extracts compound to standard ascorbic acid is

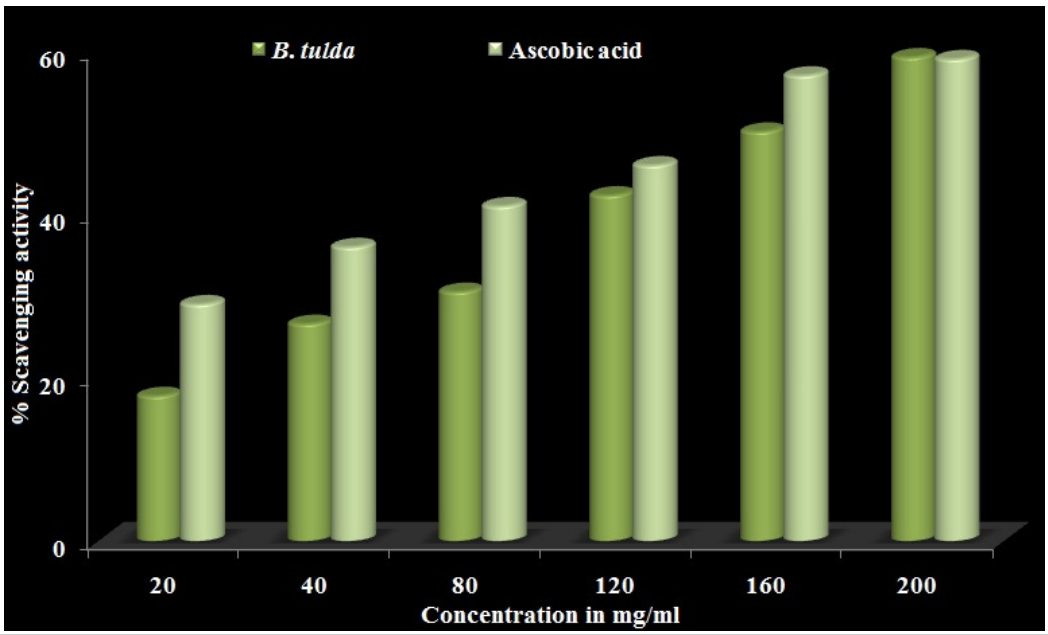

Figure III: H_2O_2 scavenging activity of aqueous methanolic extract of *Bambusa tulda* leaf

shown in figure III.

3.6 Correlation study

To determine the relationship between antioxidant activity and biochemical constituents of *Bambusa tulda* extract, a positive linear correlation was established between them at $P < 0.05$ (Table II).

4. Discussion

In the present study, preliminary phytochemical screening of 70% aqueous methanolic fraction of *Bambusa tulda* leaf revealed the presence of various bioactive components like saponins, steroids, alkaloids, tannins, carbohydrates, flavonoids, anthraquinone, glycosides.

Saponins are a glycoside of triterpenes and most of the anticancer agent comes from this group of compound[22].

The alkaloids have been long recognized as an important group to metabolite because of various biological activities like analgesic properties[23].

Table II: Linear correlation between phenolics, flavonoids and antioxidant activities (DPPH, H_2O_2, FRP) of aqueous methanolic extract of *Bambusa tulda* leaf

	TP	TF	DPPH	H_2O_2	FRP
TP	1				
TF	0.521	1			
DPPH	0.791	0.272	1		
H_2O_2	0.692	0.793	0.369	1	
FRP	0.882	0.249	0.371	0.489	1

Tannins have stringent properties that hasten the healing of around and inflammed mucous membranes[24].

Antraquinones have associated with anticancer, laxative and anti-arthritic properties[25].

In the present study it was found that the biochemical constituents such as phenolic, flavonoid and flavonol were abundant in 70% aqueous methanolic of *Bambusa tulda* leaf extract. The flavonoid content is found to be most abundant (176.05±0.03 mg QE/g) among them where as phenolic and flavonol contents were 17.494±0.01 mg GAE/g and 96.2±0.01 mg QE/g, respectively present in the extract.

Flavonoids have been reported to passes many useful properties, included anti inflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumor activity[26].

The presence of phenolic compounds in the leaf may have the ability as an antimicrobial agent[27].

The radical scavenging activity using a DPPH generated radical was tested with 70% aqueous methanolic leaf extract. It was observed that the aqueous methanolic leaf extract of *Bambusa tulda* exhibited the radical scavenging activities. It was already reported that high DPPH scavenging activity attribute to a higher antioxidant activity[28].

The reducing power of the extract was also found to be substantial, which increases gradually with a rise in concentration. From the figure III, it is evident that 60mg/ml of the extract had shown the equal reducing power, when compare to standard BHT. This might be

due to the release of more phenolic compounds which act as potent antioxidants along with some of their pharmacological effect[29]. Moreover, the higher the inhibitory action, more powerful is the antioxidant activity[30,31].

H_2O_2 is transformed to hydroxyl radical which might be the key to its toxic effect[32]. Thus the amount of hydrogen peroxide accrue in the cells should be monitored. H_2O_2 scavenging activity of *Bambusa tulda* leaf extract is depicted in figure III and found to be higher. H_2O_2 although being a weak oxidizing agent has the potential to few enzymes directly in the presence of redox active transition metals Fe2+ and Cu2+. Our study indicated a positive linear correlation ($P < 0.05$) among the various biochemical constituents and the antioxidant test systems. This might be because the antioxidant activities of phenolic compounds are primarily due to their redox properties, which allow them to function as a reducing agent, hydrogen donor and singlet-oxygen quencher[33].

5. Conclusion

To conclude, this study revealed that the 70% aqueous methanolic extract of Bambusa tulda leaves contain various phytoconstituents that might be responsible for its proven antioxidant activity. Moreover aqueous methanolic extract of the leaves postulated strong antioxidant activity as revealed by the different test systems. Since, these phenolics and flavonoids contain an extensive scope of medicine and pharmacological properties, so in future they can be make the most use for further studies.

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Conflict of interest

The author's declares none.

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