ABSTRACT

Background: Bambusa arundinacea (Bamboo) belonging to family (Graminae) is used in number of disease including liver ailments in various parts of India. The present study was done to evaluate antioxidant activity of different fractions of methanolic extract of young shoots of Bambusa arundinacea. Material and method: Antioxidant activity of different fractions (n-hexane, chloroform, ethylacetate and n-butanol) of methanolic extract was evaluated by DPPH, Lipid peroxidation and Ferric reducing power assay. The Total Phenolic (TPC) and Total Flavonoid contents (TFC) of all the fractions were also determined. Results: The results of the present analysis demonstrated that different fractions of methanolic extract of young shoots of Bambusa arundinacea contained appreciable levels of total phenolic and flavonoid contents and also possess good antioxidant activity. Of all the fractions tested, ethylacetate fraction was found to be more effective with total phenolic content (30.8±1.5 mg gallic acid equivalent (Gallic acid)/g DW) and total flavonoid content (47±2.6 mg Quercetin equivalent/g DW). Conclusion: The results of the present study shows that different fractions of methanolic extract of young shoots of Bambusa arundinacea are a viable source of natural antioxidants and might be exploited for functional foods and nutraceutical applications.

KEYWORDS: Bambusa arundinacea, DPPH, Antilipid peroxidation activity, Reducing power assay.
1. INTRODUCTION

Free radicals, reactive oxygen species and reactive nitrogen species are highly reactive and are linked with the pathophysiology of majority of diseases like aging, atherosclerosis, carcinogenesis, diabetes, liver cirrhosis and cardiovascular disorders. It damages the macromolecules of the body and produce cellular damage.\textsuperscript{[1,2]} Antioxidants are radical scavengers which give protection to human body against free radicals by inhibiting the oxidizing chain reactions.\textsuperscript{[3]} An imbalance between Reactive oxygen species and the inherent antioxidant capacity of the body, directed the use of antioxidants from dietary and or medicinal supplements particularly during the disease attack.\textsuperscript{[4]}

Many medicinal plants have been extensively studied for their antioxidant activity in recent years. It is believed that an increased intake of food rich in natural antioxidants lowers the risks of degenerative diseases.\textsuperscript{[5]} Phytochemicals which mainly includes secondary metabolites like carotenoids, flavonoids, glycosides, alkaloids, volatile oils have crucial role in the maintenance of human health.\textsuperscript{[6,7]}

\textit{Bambusa arundinacea} belonging to family Gramineae is a native Indian plant used in folk medicine as tonic for heart, liver and brain.\textsuperscript{[8,9]} It fortifies the heart and calms down heart palpitations. It is recommended in cases of diarrhea and chronic liver ailments. It is also used as rejuvenator, hemostatic and antispasmodic.\textsuperscript{[10]} It is helpful in bronchitis, colds, convulsions, cough, enuresis, fever, gallbladder problems, inflammation, vomiting and urinary tract infection as well as in urinary problems.\textsuperscript{[11,12]}

\textit{Bambusa arundinacea} contains phytochemicals like resins, lignin, waxes, silica, uronic acid, reducing sugars such as galactose, glucose, arabinose, mannose and xylose.\textsuperscript{[13]}

Choline, betain, urease, nuclease, proteolytic enzymes, diastatic and emulsifying enzyme, alkaloids and glucoside.\textsuperscript{[14]} Flavonoids like orientin, homoorientin, vitexin and isovitexin. Phytosterols like stigmasterol and β-sitosterol, Stigmast-5, 22-dien-3β-ol, Stigmast-5-en- 3β-ol-β-D glucopyranoside, triterpenes and steroidal glycosides, 17, 20, 20-tri demethyl-20α-isopropyloleanane, eicosanyldicarboxylic acid, α-amyrin acetate, urs-12-en-3β-ol-β-D-glucopyranoside are also present.\textsuperscript{[15-17]}

\textit{Bambusa arundinacea} is reported to possess in vivo antifertility, anti-inflammatory, antiulcer, antihyperglycemic, antiarthritic, anthelmintic and antihyperlipidemic activities.\textsuperscript{[18-25]} It also possess antioxidant and antimicrobial potential in in vitro studies.\textsuperscript{[26]}
Despite a long tradition of use for the treatment of various ailments, no systematic phytochemical and pharmacological work has ever been carried out on this potentially useful plant. Thus, the present investigations were planned with an objective to estimate total phenols, flavonoids and antioxidant activity of different fractions of methanolic extract of young shoots of *Bambusa arundinacea*.

2. MATERIALS AND METHOD

**Chemicals**

2,2-diphenyl-1-picrylhydrazyl (DPPH), were purchased from Hi-media. TCA (trichloroaceticacid), TBA (thiobarbituric acid), Thioacetmide, Ascorbic acid was purchased from Sigma and all other chemicals were of analytical grade.

**Plant Collection and preparation of extract**

Young shoots of *Bambusa arundinacea* were collected in the month of August from Assam. They were dried under shed at room temperature (25-30°C). After drying the shoots (10 Kg) were powdered with dry grinder and sieved through sieve 40mesh. The powder was packed in soxhlet apparatus and defatted with petroleum ether. The powder was extracted with methanol to obtain methanolic extract. The percentage yield was found to be 15.5% (w/w).

**Fractionation of methanolic extract of young shoots of Bambusa arundinacea in increasing polarity**

Methanolic Extract (12 gm) was column chromatographed using silica gel (100-200 mesh) as the stationary phase and eluted with solvents of increasing polarity.

**Toluene:** Ethyl acetate (5:5) was used as mobile phase.

![Scheme](image)

**Figure 1:** Scheme for fractionation of methanolic extract of young shoots of *Bambusa arundinacea* in increasing polarity.
Quantitative Determination of Phytochemicals\cite{27}

**Total phenolic content**

An aliquot of 0.1 ml of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) and standard was combined with 2.8 ml 10% sodium carbonate and 0.1 ml of 0.2 mol/l Folin-Ciocalteu reagent. After 40 min absorbance at 725 nm was checked by UV-visible spectrophotometer. Total phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample using the standard calibration curve constructed for different concentrations of gallic acid. Results were expressed in GAE mg/g.

**Total flavonoid content**

50 μl of all the four fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) and standard were made up to 1 ml with methanol, mixed with 4 ml of distilled water and then 0.3 ml of 5% NaNO₂ solution. 0.3 ml of 10% AlCl₃ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 ml of 1 mol/ NaOH solution were added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg Quercetin equivalent per g dry weight.

**Animals**

All experiments were carried out after the approval of Institutional Animal Ethics Committee (IAEC) with protocol number KBRCP 2013/06/07 in accordance with the guidelines laid by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Male wistar rats (200-250g) were housed in standard cages (48 cm×35 cm×22 cm) at room temperature (20±2 °C) relative humidity (55±5%) on a 12-h light–dark cycle. Animals had access to standard pellet diet (certified Amrut brand rodent feed, Pranav Agro Industries, Pune, India) and filtered tap water *ad libitum*.

**Antioxidant activity**

**Free radical scavenging activity by DPPH method\cite{28,29}**

Different concentrations (25, 50, 100, 200 and 250μg/ml) of all the fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) and standards were prepared. To this 3 ml of methanolic solution of DPPH was added, this mixture was incubated at 37°C for 30 min. A blank was prepared in the similar way and absorbance was
measured at 517nm. Free radical scavenging activity was expressed as the percentage inhibition calculated by using the following formula:

\[
\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

**Anti-lipid peroxidation effect**\[^{30}\]

The 0.5ml of liver homogenate was taken and to it 1ml of 0.15M KCL and 0.5ml of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) and standard (Ascorbic acid) at different concentrations (25, 50,100, 200 and 250µg/ml) were added. Lipid peroxidation was initiated by adding 100µl of 1mM ferric chloride. The reaction was stopped by adding 2ml of ice cold 0.25N HCL containing 15% TCA, 0.38% TBA, and 0.2 ml of 0.05% butylated hydroxyl toluene. These reaction mixtures were heated for 60min at 80°C then cooled and centrifuged at 6900 rpm for 15min. The absorbance of supernatant was measured at 532nm against blank, which contained all reagents except liver homogenate and drug. Same experiments were performed to determine the normal (without drug and FeCl\(_3\)) and induced (without drug) lipid peroxidation level in the tissue. The percentage of anti-lipid per oxidation effect (%ALP) was calculated by the following formula:

\[
\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

**Reducing power assay**\[^{31}\]

Reducing power of all the four fractions was determined on the ability of antioxidants to form colored complex with potassium ferricyanide. Different concentrations(25, 50,100, 200 and 250µg/ml)of fractions(n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) and standard (Ascorbic acid) were mixed with 2.5ml phosphate buffer (pH 6.6) and 2.5ml potassium ferricyanide (1%). The mixture was incubated at 50°C for 20min. 2.5ml TCA (10%) was added to it and centrifuged at 3000rpm for 10min. 2.5ml of supernatant was mixed with 2.5ml of water and 0.5ml of FeCl\(_3\) (0.1%) were added to it and absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power.

**Statistical Analysis**

The IC\(_{50}\) values were calculated by regression analysis. Results were expressed as mean ± S.D. Total variation, present in a set of data were estimated by one way analysis of variance (ANOVA). A p-value lower than 0.05 was considered to be significant.
3. RESULTS

Total phenolic and Flavonoid content
Table 1 shows the total phenolic and flavonoid content of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* calculated by using calibration curve.

<table>
<thead>
<tr>
<th></th>
<th>Phenolic content mg Gallic acid equivalent/g DW</th>
<th>Flavonoid content mg Quercetin equivalent/g DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane fraction</td>
<td>3.5±1.4</td>
<td>9.5±1.1</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>10.2±1.8</td>
<td>8.5±0.7</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>30.8±1.5</td>
<td>47±2.6</td>
</tr>
<tr>
<td>n-butanol fraction</td>
<td>56.6±2.4</td>
<td>20±1.6</td>
</tr>
</tbody>
</table>

Antioxidant activity

Free radical scavenging activity by DPPH method

Figure 2 and Table 2 depicts DPPH free radical scavenging ability of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* and ascorbic acid as standard.

Figure 2: DPPH free radical scavenging ability of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* and Ascorbic acid. Data are represented as mean ± SD (n = 3).
Table 2: DPPH free radical scavenging ability of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* and ascorbic acid. Each value is represented as mean ± SD (n = 3).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Standard and fractions</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>30.69±2.1</td>
</tr>
<tr>
<td>2</td>
<td>n-hexane fraction</td>
<td>149.6±0.7</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform fraction</td>
<td>224±0.12</td>
</tr>
<tr>
<td>4</td>
<td>Ethylacetate fraction</td>
<td>40.33±0.63</td>
</tr>
<tr>
<td>5</td>
<td>n-butanol fraction</td>
<td>93.94±3.07</td>
</tr>
</tbody>
</table>

Anti-lipid peroxidation effect

Fig. 3 and Table 3 shows Anti-lipid peroxidation effect of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* and Ascorbic acid.

![Anti-lipid peroxidation Activity](image)

**Figure 3**: Anti-Lipid peroxidation activity of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* and Ascorbic acid. Each value is represented as mean ± SD (n = 3).
Table 3: Anti-Lipid peroxidation activity of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* and ascorbic acid. Each value is represented as mean ± SD (n = 3).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Standard and fractions</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>37.37±3.1</td>
</tr>
<tr>
<td>2</td>
<td>n-hexane fraction</td>
<td>152.17±2.6</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform fraction</td>
<td>216.59±1.3</td>
</tr>
<tr>
<td>4</td>
<td>Ethylacetate fraction</td>
<td>61.89±0.44</td>
</tr>
<tr>
<td>5</td>
<td>n-butanol fraction</td>
<td>95.89±2.97</td>
</tr>
</tbody>
</table>

Reducing power assay

Reducing power has been used as one of important antioxidant capability for medicinal herbs. Increased absorbance of the reaction mixture indicated increased reducing power. Figure 4 shows reducing power of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* and Ascorbic acid.

![Reducing Power Assay](image)

Figure 4: Reducing power of different fractions (n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction) of methanolic extract of young shoots of *Bambusa arundinacea* and Ascorbic acid. Each value is represented as mean ± SD (n = 3).

4. DISCUSSION

Phenolic compounds are secondary metabolic products containing the phenolic hydroxyl group, which has an anti-oxidative effect via interactions with the phenol ring and its
resonance stabilization effect.\textsuperscript{32} Flavonoids are also potent antioxidants and they inhibit lipid peroxidation. This activity is believed to be mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.\textsuperscript{33} As shown in Table. 1 n-hexane fraction, Chloroform fraction, Ethyl acetate fraction and n-butanol fraction contain variable amount of total phenolic and flavnoid content. Among these n-butanol fraction have highest phenolic (56.6±2.4 mg gallic acid equivalent (GAE)/g DW) and ethylacetate fraction contain highest flavonoid (47±2.6 mg Quercetin equivalent/g DW) content. Since the phenolic and flavonoid compounds are considered to be major contributors to the antioxidant capacities of plants, we further evaluated antioxidant activity of these fractions by DPPH, Lipid Peroxidation and Reducing power assay.

DPPH is a stable nitrogen-centered free radical, and its color changes from violet to yellow when it is reduced by either the process of hydrogen- or electron donation. Substances to perform this above reaction can be considered as antioxidants and therefore radical scavengers.\textsuperscript{34} Figure 2 shows the comparison of DPPH free radical activity of different fractions of methanolic extract of young shoots of \textit{Bambusa arundinacea}. Among all the fractions Ethylacetate fraction (IC\textsubscript{50} = 40.33±0.63) exhibit highest free radical scavenging activity comparable with ascorbic acid (IC\textsubscript{50} = 30.69±2.1).

Lipid peroxidation can inactivate cellular components and plays an important role in oxidative stress in biological systems.\textsuperscript{35} Figure 3 shows the comparison of anti-lipid peroxidation effect of different fractions of methanolic extract of young shoots of \textit{Bambusa arundinacea}. Anti lipid peroxidation effect of Ethylacetate fraction (IC\textsubscript{50} = 61.89±0.44) was superior to other fractions.

Reducing power has been used as one of important antioxidant capability for medicinal herbs. Increased absorbance of the reaction mixture indicated increased reducing power.\textsuperscript{36} As shown in Figure 4 Ethylacetate fraction shows better reducing power which is comparable to Ascorbic acid.

\textbf{5. CONCLUSION}

This study reveals that different fractions of methanolic extract of young shoots of \textit{Bambusa arundinacea} possess good antioxidant activity. Among all the fractions ethylacetate fraction shows superior antioxidant activity. Ethylacetate fraction contains highest amount of
flavonoids as compared with other fractions which may be responsible for its better antioxidant activity.

6. REFERENCE

19. Musa TY, Bimbo BB. Abortifacient potentials of the aqueous extract of Bambusa vulgaris leaves in pregnant Dutch rabbits, Contraception, 2009; 80: 308–313.


