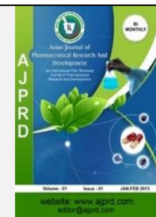

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Research Article

Evaluation of Phytochemical (Quantitative) determination & Anti-oxidant (In-Vitro) activity of ethanolic extract of *Bambusa Arundinacea* young shoots

Jitendra Chobdar[1]*, Jai Singh Vaghela[2]

[1]Research Scholar, Department of Pharmacology, B.N. College of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan- 313001

[2]Associate Professor, Department of Pharmacology, B.N. College of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan- 313001

A B S T R A C T

Bambusa Arundinaceae (Bamboo), a member of the Graminae family, is utilized in Indian folk medicine to treat a variety of inflammatory disorders. It is used to treat heart, brain, and liver problems throughout India. The current study was conducted to assess the antioxidant activity of an ethanolic extract of young shoots from Bambusa arundinaceae. Material and method: The antioxidant activity of the ethanolic extract was assessed using antioxidant assays such as DPPH, lipid peroxidation, and ferric reducing power assays. The Total Phenolic (TPC) and Total Flavonoid contents (TFC) of all fractions were also evaluated.

Result: This study found that ethanolic extracts of young shoots from *Bambusa Arundinaceae* contained significant amounts of phenolic and flavonoid content. The ethanolic extract has significant antioxidant action. The high flavonoid and phenolic content may account for its antioxidant action.

Conclusion: The current study proves that ethanolic extract of young shoots of *Bambusa Arundinaceae* are an encouraging source of natural antioxidants that could be used in functional foods and nutraceutical products.

Keywords: DPPH, antilipid peroxidation action, reducing power assay (RSA) and *Bambusa Arundinaceae.*

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*Address for Correspondence:

Jitendra Chobdar, Research Scholar, Department of Pharmacology, B.N. College of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan- 313001

INTRODUCTION

Many diseases, including aging, atherosclerosis, carcinogenesis, diabetes, liver cirrhosis, and cardiovascular problems, are associated with high levels of reactivity in the form of free radicals, reactive oxygen species, and reactive nitrogen species. It produces cellular destruction as well as harm to the body's macromolecules[1, 2]. Antioxidants are radical scavengers that prevent oxidative chain reactions, protecting the organism from free radical damage[3]. Utilizing antioxidants from dietary and/or medicinal supplements was influenced by an imbalance between reactive oxygen species and the body's natural antioxidant capacity, especially during the disease onslaught[4]. In recent years, a great deal of research has been done on the antioxidant potential of several medicinal plants. Increased consumption of foods high in naturally occurring antioxidants is thought to reduce the incidence of degenerative illnesses[5]. In order to maintain human health, phytochemicals which mostly consist of secondary metabolites like carotenoids, flavonoids, glycosides, alkaloids, and volatile oils are essential[6, 7].

Bambusa Arundinaceae, a member of the Graminae family, is a native Indian plant that is used in folk medicine as a tonic for the heart, liver and brain[8, 9]. It strengthens the heart and relieves heart palpitations. It is suggested for diarrhoea and chronic liver conditions. It is also utilized as a revitalizer, hemostatic, and antispasmodic(10). It is beneficial for bronchitis, colds, convulsions, cough, enuresis, fever, gallbladder difficulties, inflammation, vomiting, and urinary tract infections, as well as urinary issues[11, 12]. Bambusa arundinaceae contains phytochemicals such as resins, lignin, waxes, silica, uronic acid, and reducing sugars galactose, glucose, arabinose, mannose, and xylose[13].

The plant contains choline, betain, urease, nuclease, proteolytic enzymes, diastatic and emulsifying enzymes, alkaloids, and glucosides[14]. It also contains flavonoids such as orientin, homoorientin, vitexin, and isovitexin, phytosterols such as stigmasterol and β-sitosterol, Stigmast-5, 22-dien-3β-ol, Stigmast-5-en-3β-ol-β-D glucopyranoside, triterpenes and steroidal glycosides, and 17, 20, 20-tri demethyl[15-17].

In vivo antifertility, anti-inflammatory, antiulcer, antihyperglycemic, antiarthritic, anthelmintic, and antihyperlipidemic properties have all been linked to *Bambusa Arundinaceae*[18-25]. In vitro investigations have shown that it has antioxidant and antibacterial activity[26].

Although this plant has been used for a long time to treat a variety of illnesses, systematic phytochemical and pharmacological research on it has never been done. Therefore, the goal of the current studies was to determine the total phenols, flavonoids, and antioxidant activity of ethanolic extracts of *Bambusa Arundinaceae* young shoots.

MATERIALS & METHODS

Chemicals

Micro Technologies provided the 2-diphenyl-1-picrylhydrazyl (DPPH), Reagent Folin-Cio-Calteu (FC), Standard gallic acid, 20% sodium carbonate solution (Na_2CO_3), ethanol, Reagent grade quercetin, 10% aluminum chloride (AlCl3), potassium acetate (1M) and an UV spectrophotometer that were acquired. Wish Technology supplied TCA (trichloroaceticacid), TBA (thiobarbituric acid), trichloroacetmide, and ascorbic acid; all other chemicals were analytical grade.

Plant Collection & preparation of extract

The collection of *Bambusa Arundinacea* took place in September 2021. It was verified and taxonomically recognized by the University of Rajasthan's Botany Department (Identification no.: RUBL 211797). They were allowed to dry at ambient temperature (25–30°C) under the shed. The 10 kg of shoots were dried, then ground into a powder using a dry grinder and sieved through a 40 mesh screen. To help avoid from choking, the powder medication was stored in a thimble, which is a packet composed of cellulose or filter paper. In the round bottom flask (RBF), bring the solvent to a boil using the heating mantle. The solvent's vapor is transported to the condenser via a side tube. The condenser condensed the solvent vapor, which then seeps through the medication package. Through percolation, the active component was separated from the crude drug. After adding a menstruum to the extractor until it reached its maximum capacity, the liquid was siphoned into the flask with a round bottom. Again, this menstruum was cooked in RBF, maintaining the active component. The drug in the extractor is penetrated by the vaporized solvent as it goes through the side tube and condenser once more. The process won't end until the medicine runs out, which should take at least 72 hours. After filtering, the solvent was removed using a rotary evaporator set at 45°C and low pressure. the yield, or quantity, of extractable materials removed from leftover water.

Animals

The animals used for the study were 120–250 g Albino wistar rats (either sex). The B.N. College of Pharmacy in Udaipur, Rajasthan (Reg. No.870/PO/Re/S/05/CPCSEA) is the source of these animals. The IAEC of the B.N. College of Pharmacy has accepted the experimental protocol.

Phytochemical (Quantitative) determinations[27]

1.Total Phenolic Content

5ml of the FC-reagent solution was added to the test tube. The test tube was filled with a 5 ml solution of sodium carbonate (20%). For twenty minutes, the test tube was incubated at 250°C to finish the reaction. Next, using a UV-spectrophotometer against a blank, the absorbance of the solution was measured at 760 nm. The standard calibration curve developed for different amounts of gallic acid was used to quantify total phenolics in milligrams of gallic acid equivalents (GAE) per grams of sample. Three copies of the samples were inspected. GAE mg/g was used to express the results. An equation constructed from a standard gallic acid curve was used to determine the gallic acid concentration in the samples. Here is the equation:

$y = mx + b$

$x = y – b/m$

Where's:

y = absorbance

x = gallic acid concentration (μg/ml)

m = slope

b = Intercept

$x = y- 0.036/0.003$

Using the following formula, the content of all phenolic components in the sample was now determined in milligrams of gallic acid equivalent (GAE):

$A = (c \times v)/ m$

Where's:

A = Total phenol content (mg/gm gallic acid equivalent)

c = X/1000 = concentration of gallic acid in mg/ml

v = volume of extract

m = Mass of the extract (gm)

2. Total Flavonoids Content

1 milliliter of young shoot extract or standard at different concentrations was put in a test tube. Three milliliters of ethanol were added. 200 ?l of a 10% aluminum chloride solution was added. 200 microliters of potassium acetate solution (1M). A total of 5.6 milliliters of purified water were introduced. It was incubated for thirty minutes at room temperature in order to finish the reaction. The absorbance of the solution was then measured at 420 nm using a UV-spectrophotometer in comparison to a blank. A typical blank solution contained all the regents except for plant extract or standard solution. Three duplicates of the samples were inspected.

The total flavonoid component concentration in quercetin equivalents of plant extracts was determined using the following 'equation'. Here's the equation:

$y = mx - b$

$x = y + b/m$

Where's:

y = absorbance

x = quercetin concentration (μg/ml)

m = slope =0.009

b = Intercept = 0.041

$x = y + 0.041/0.009$

Using the following formula, the content of all phenolic components in the sample was now determined in milligrams of gallic acid equivalent (GAE):

$A = (c \times v)/ m$

Where:

A = total content of flavonoid compounds, mg/g plant extract, in quercetin equivalent (QE);

c = the concentration of querecetin established from the calibration curve, mg/ml;

v = the volume of extract, ml;

m = the weight of bamboo young shoots extract, gm.

Antioxidant activity

1. DPPH Free radical scavenging activity[28, 29]

Take the extracted solution and divide it into different concentrations (e.g., 25, 50, 100, 200, and 250 μg/ml) and ascorbic acid in separate volumetric flasks. Each measured sample received 3 milliliters of the DPPH solution, and each solution received 10 milliliters of ethanol (99%). Samples were kept in a dark, incubation room for thirty minutes. After preparing a blank in the same way, the absorbance was measured at 517 nm. The color changed from dark violet to yellow, and the following formulas were used to calculate the IC_{50} value (the concentration needed to prevent 50% of radical formation) and the percentage inhibition of radical scavenging activity (RSA).

Inhibition (%):

$$= \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

IC_{50}: $y = mx+b$

$x = y-b/m$

where's:

y = Concentration

b = Intercept

m = Slope

2. Anti lipid peroxidation effect[30]

The standard (ascorbic acid) was added to 0.5ml of liver homogenate [the liver samples, weighing 100 mg, were diluted in 0.5 milliliters of ice-cold phosphate-buffered saline (PBS; 8.4)] together with 1ml of 0.15M KCL and 0.5ml of ethanolic extract at varying doses (100, 200, 400, 800, and 1600μg/ml). By adding 100L of 1mM ferric chloride, the peroxidation of lipids was initiated. 0.2 ml of 0.05% butylated hydroxyltoluene, 0.38% TBA (thio barbituric acid), and 2 ml of ice-cold 0.25N HCL with 15% TCA (trichloro acetic acid) were added to stop the process. These reaction mixtures were heated for 60 minutes at 80 degrees Celsius, cooled, and centrifuged for 15 minutes at 6900 revolutions per minute. Supernatant absorbance was measured at 532 nm against a blank that contained all of the reagents, with the exception of liver homogenate and drugs.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

IC_{50}: $y = mx+b$

$x = y-b/m$

Where's:

y = Concentration

b = Intercept

m = Slope

3. Reducing power Assay(31)

Different extract and standard (ascorbic acid) quantities (ranging from 25 to 250 µg/ml) were combined with 2.5 mL of pH 6.6 phosphate buffer and 2.5 mL of 1% potassium ferricyanide. For twenty minutes, the mixture was incubated at 50ºC. After adding 2.5 ml of 10% TCA, the centrifuge was run for 10 minutes at 3000 rpm. Mix 2.5 milliliters of water with 2.5 milliliters of supernatant, which is the upper layer solution from the entire mixer material. When the absorbance at 700 nm is measured, add 0.5 ml of $FeCl_3$ (0.1%). Greater reducing power in the reaction mixture is shown by increased absorbance.

Statistical Analysis

Regression analysis was used to determine the IC_{50} values. The mean ± standard deviation (S.D.) was used to express the results. One-way analysis of variance was used to assess the total variation that existed in a collection of data (ANOVA). Significant data was defined as a p-value of less than 0.05.

RESULTS

Phytochemical (Quantitative) determinations

1. Total Phenolic Content

Figure.1 shows the calibration curve indicates that the total phenolic content of the ethanolic extract of *Bambusa Arundinaceae* young shoots was 46.33±2.4 mg gallic acid equivalents/gDW.

Table 1: Absorbance of Gallic acid

Concentration (µg/ml)	Absorbance
15.625	0.085
31.25	0.157
62.5	0.267
125	0.521
250	1.08
500	1.97

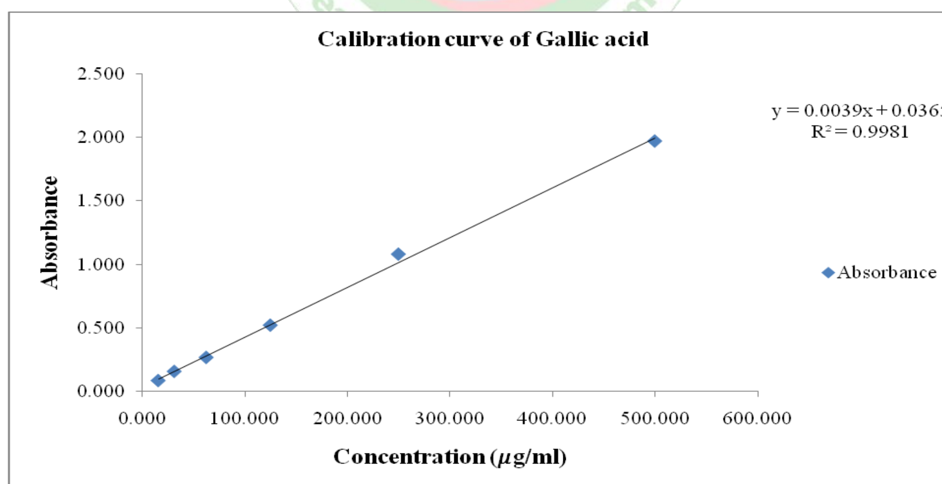

Figure 1: Calibration curve of Gallic acid for total phenol content estimination

The equation is as follows:

$y = mx + b$

$x = y - b/m$

Where's:

y = absorbance

x = gallic acid concentration (µg/ml)

m = slope

b = Intercept

x = y- 0.036/0.003

Now, the concentration of total phenolic compounds in the sample was calculated as mg of gallic acid equivalent (GAE) using the following equation:

A = (c x v)/ m

Where's:

A = Total phenol content (mg/gm gallic acid equivalent)

c = X/1000 = concentration of gallic acid in mg/ml

v = volume of extract

m = Mass of the extract (gm)

Table 2: Data for the estimination phenol content of ethanolic extract of *Bambusa Arundinaceae* young shoots. Data are represented as mean ± SD (n = 3).

Sample solution (µg/ml)	Weight of dry extract per ml m (gm)	Absorbance (y)	GAE conc. C (µg/ml)	GAE conc. C (mg/ml)	TPC as GAE, A = c x v / m (mg/ml)	Mean ± SEM
1000	0.001	0.178	47.33	0.0473	47.33	46.33 ± 2.4
1000	0.001	0.172	45.33	0.0453	45.33	
1000	0.001	0.175	46.33	0.0463	46.33	

2. Total Flavonoid Content

Figure.2 shows the calibration curve indicated that the total flavonoid content of the ethanolic extract of young shoots of *Bambusa Arundinaceae* was 20.47±3.7 mg gallic acid equivalents/gDW.

Table 3: Absorbance of Quercetin

Concentration (µg/ml)	Absorbance
6.25	0.051
12.5	0.117
25	0.197
50	0.345
100	0.996

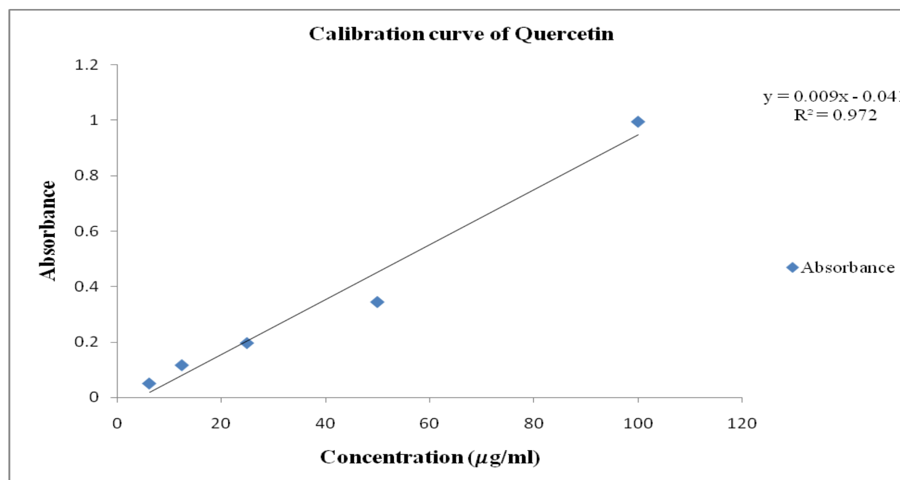

Figure 2: Calibration curve of quercetin for total flavonoid content estimination.

The equation is as follows:

y = mx - b

x = y + b/m

Where's:

y = absorbance

x = quercetin concentration (μg/ml)

m = slope =0.009

b = Intercept = 0.041

x = y + 0.041/0.009

Using the following formula, the content of all phenolic components in the sample was now determined in milligrams of gallic acid equivalent (GAE):

A = (c x v)/ m

Where's:

A = total content of flavonoid compounds, mg/g plant extract, in quercetin equivalent (QE);

c = the concentration of querecetin established from the calibration curve, mg/ml;

v = the volume of extract, ml;

m = the weight of *Bambusa Arundinaceae* young shoots extract, gm.

Table 4: Data for the estimination flavonoid content of ethanolic extract of *Bambusa Arundinaceae* young shoots. Data are represented as mean ± SD (n = 3).

Sample solution (μg/ml)	Weight of dry extract per ml m (gm)	Absorbance (y)	QE conc. C (μg/ml)	QE conc. C (mg/ml)	TFC as QE, A = c x v / m (mg/ml)	Mean ± SEM
1000	0.001	0.145	20.66	0.020	20.66	20.47 ± 3.7
1000	0.001	0.142	20.33	0.020	20.33	
1000	0.001	0.143	20.44	0.020	20.44	

Antioxidant activity

1. DPPH Free radical scavenging activity

Figure 1 showed an impressive DPPH radical scavenging activity of *Bambusa Arundinaceae* young shoots' ethanolic extract (sample) was found to be higher, with IC_{50} value (IC_{50}=94.13+3.07 µg/ml) comparable to the Ascorbic acid (control) IC_{50} value (IC_{50}=30.40+2.1 µg/ml).

Table 5: Capacity of ascorbic acid and an ethanolic extract of *Bambusa Arundinaceae* young shoots to eliminate (Scavenging) free radicals (DPPH). Data are represented as mean ± SD (n = 3).

Calculation of % Radical Scavenging and IC_{50} from DPPH Assay			
Absorbance Measurement Data			
Concentration (µg/ml)	Ascorbic Acid	BAEE	% RSA
25	41.6	31.7	23.79808
50	55.9	42.9	23.25581
100	71.9	59.4	17.38526
200	81.7	67.5	17.38066
250	89.9	76.1	15.35039
IC_{50}	**30.40+2.1**	**94.13+3.07**	

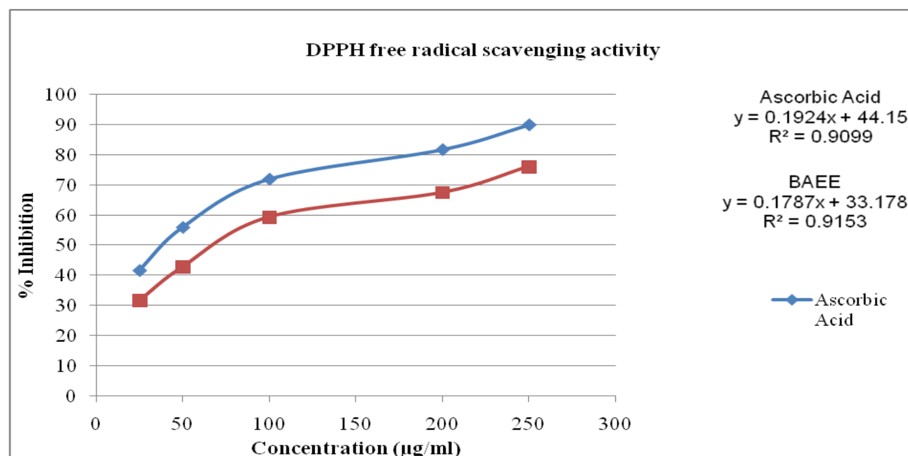

Figure 3: Free radical scavenging activity of ethanolic extract of *Bambusa Arundinaceae* young shoots. BAEE= *Bambusa Arundinaceae* ethanolic extract. Data are represented as mean ± SD (n = 3).

IC_{50}: y = mx+b

x = y-b/m

where (for Ascorbic acid)

y = 0.1924x + 44.15

R^2 = 0.9099

y = Concentration = 50

b = Intercept = 44.15

m = Slope = 0.1924

IC_{50}: x = y-b/m

IC_{50}: x = 50-44.15/0.1924

IC_{50} = **30.40+2.1**

where (for BAEE)

y = 0.1787x + 33.178

R^2 = 0.9153

y = Concentration = 50

b = Intercept = 33.17

m = Slope = 0.1787

x = 50-33.17/0.1787

IC_{50} = **94.13+3.0**

2. Anti-lipid peroxidation effect

Figure 2 showed an impressive anti-lipid peroxidation activity (IC_{50}=93.81+0.6 µg/ml) of *Bambusa Arundinaceae* young shoots in an ethanolic extract that was comparable to the Ascorbic Acid (IC_{50}=20.76+3.7 µg/ml).

Table 6: Capacity of ascorbic acid and an ethanolic extract of *Bambusa Arundinaceae* young shoots to Anti-lipid peroxidation effect. Data are represented as mean ± SD (n = 3).

Calculation of % Inhibition and IC_{50} from Anti-lipid peroxidation effect			
Absorbance Measurement Data			
Concentration (µg/ml)	Ascorbic Acid	BAEE	% Inhibition
25	47.8	32.5	23.79808
50	58.6	43.7	23.25581
100	62.7	58.8	17.38526
200	72.8	68.2	17.38066
250	88.6	72.5	15.35039
IC_{50}	**20.76+3.7**	**93.81+0.6**	

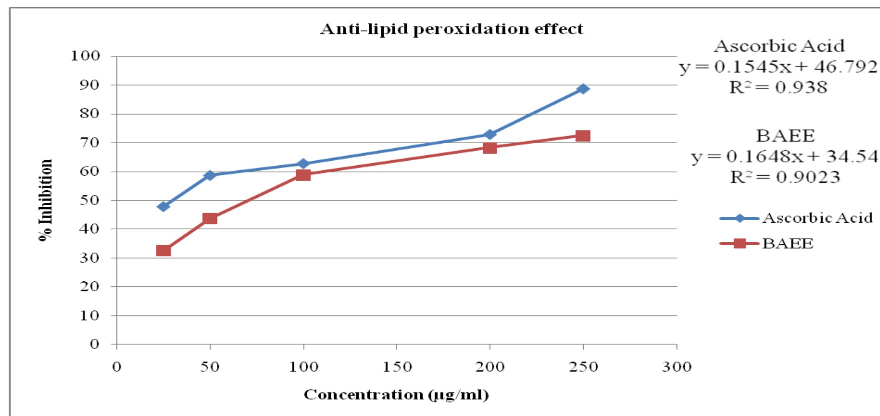

Figure 4: Anti-lipid peroxidation effect of ethanolic extract of *Bambusa Arundinaceae* young shoots. BAEE= *Bambusa Arundinaceae* ethanolic extract. Data are represented as mean ± SD (n = 3).

IC_{50}: $y = mx+b$

$x = y-b/m$

where (for Ascorbic acid)

$y = 0.1545x + 46.792$

$R^2 = 0.938$

y = Concentration = 50

b = Intercept = 46.792

m = Slope = 0.1545

IC_{50}: x = y-b/m

IC_{50}: x = 50-46.792/0.1545

IC_{50} = **20.76+3.1**

where (for BAEE)

$y = 0.1648x + 34.54$

$R^2 = 0.9023$

y = Concentration = 50

b = Intercept = 34.54

m = Slope = 0.1648

IC_{50}: x = 50-34.54/0.1648

IC_{50} = **93.81+0.6**

3. Reducing power assay

Figure 3 shows as medicinal plants have been shown to have significant antioxidant properties, including reducing power. Increased absorbance in the reaction mixture indicates more reducing power. The ethanolic extract of young shoots of *Bambusa Arundinaceae* shown comparable good reduction power to the standard.

Table 7: Reducing ability of ethanolic extract of *Bambusa Arundinaceae*

Reducing ability of ethanolic extract of *Bambusa Arundinaceae*		
Concentration (µg/ml)	Absorbance Measurement Data	
	Ascorbic Acid	BAEE
25	0.511	0.284
50	0.727	0.491
100	0.921	0.624
200	1.242	0.819
250	1.429	1.119

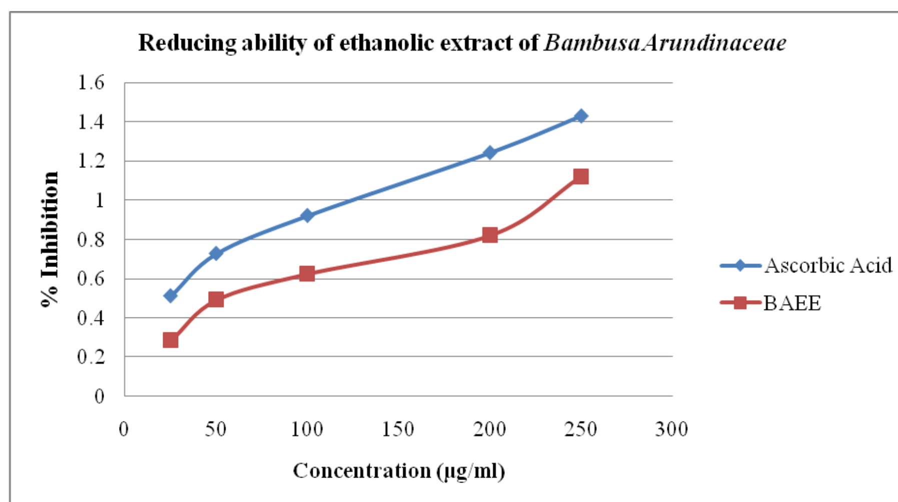

Figure 5: Reducing ability of methanolic extract of *Bambusa Arundinaceae*. BAEE= *Bambusa Arundinaceae* ethanolic extract. Data are represented as mean ± SD (n = 3).

DISCUSSION

The Phenolic compounds are residues of secondary metabolism that contain the phenolic hydroxyl group. This group interacts with the phenol ring and provides resonance stabilization, which has an anti-oxidative effect[32]. Flavonoids prevent lipid peroxidation and are strong antioxidants as well. Their redox qualities, which are thought to be primarily responsible for this action, can be crucial in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or breaking down peroxides[33]. The total phenolic and flavonoid content is displayed in Tables 2 and 4. The ethanolic extract of young shoots from *Bambusa Arundinaceae* had a total phenolic content of 46.33±2.4 gallic acid equivalents/gDW and a total flavonoid content of 20.47±3.7 quercetin equivalents/g DW. Using the DPPH, Lipid Peroxidation, and Reducing Power assays, we assessed the antioxidant activity of the phenolic and flavonoid chemicals, which are thought to be key contributions to the antioxidant capabilities of plants.

When reduced through the process of hydrogen or electron donation, DPPH, a stable nitrogen-centered free radical, turns from violet to yellow. A class of substances known as antioxidants, or radical scavengers, can be used to carry out the reaction described above[34].

The ethanolic extract of Bambusa arundinacea young shoots' DPPH free radical activity is shown in Figure 3. The free radical scavenging activity of the ethanolic extract of young shoots from *Bambusa Arundinaceae* (IC_{50} = 94.13+3.0) was found to be which is comparable to that of ascorbic acid (IC_{50} = 30.40+2.1).

Lipid peroxidation is a significant cause of oxidative stress in biological systems and has the ability to render cellular components inactive[35]. The ethanolic extract of *Bambusa Arundinacea* young shoots' anti-lipid peroxidation efficacy is displayed in Figure 4. The ethanolic extract of *Bambusa Arundinacea's* young shoots has an anti-lipid peroxidation action with an IC_{50} of 93.81+0.6.

Reducing power has been used as a key antioxidant property for therapeutic plants. Increased absorbance of the reaction mixture indicated more reducing power[36]. Figure 5 reveals that an ethanolic extract of *Bambusa Arundinacea* young shoots has comparable reducing power to Ascorbic acid.

CONCLUSION

This study concluded that the ethanolic extract of young shoots of *Bambusa Arundinaceae* had high antioxidant activity due to its phenolic and flavonoid content.

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