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Phytochemical, Antioxidant, And Antimicrobial Properties Of Fermented Shoot Extracts Of Bambusa Tulda Found In Northeastern India

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ABSTRACT

Medicinal plants provide a significant supply of natural compounds that can be used to develop new therapeutic medicines for treating severe illnesses including microbial infections. The genus Bambusa, which belongs to the Poaceae family, encompasses a vast and widely distributed collection of plants that possess a wide range of traditional uses in the treatment of various diseases. This study examined the ethanolic extracts of Bambusa tulda, along with its fractions such as ethyl acetate, petroleum ether, n-butanol, and water, to investigate their qualitative and quantitative phytochemical, antioxidant, and antibacterial properties. FTIR analysis of ethanolic powdered shoot extracts shows, presence of different functional groups including C-H, C-O, O-H and aromatic groups by showing stretching's in different wavelengths. The extracts underwent testing to evaluate their antimicrobial activity against various strains of bacteria by using agar disc diffusion method and micro-dilution techniques to determine the minimum inhibitory and bactericidal concentrations. Additionally, the antioxidant activity of the extracts was assessed using the scavenging activity of DPPH radical methods. The extracts exhibited a robust positive connection between their antioxidant activity and the combined amounts of phenolics and flavonoids. The IC₅₀ values of n-butanol, ethanol, water, petroleum ether, and ethyl acetate fraction show in ascending order 10.77+0.24 µg/ml, 11.35+0.13 µg/ml, 13.36+0.11 µg/ml,13.93+0.71 µg/ml and14.25+ 0.27 µg/ml respectively. The extracts have been shown effective efficacy against Escherichia coli and Staphylococcus aureus bacteria. However, it did not show any responses against Klebsiella pneumoniae and Pseudomonas aeruginosa. Escherichia coli exhibited greater susceptibility to the extracts compared to S. aureus, with the n-butanol fraction being the most potent extract. **CC License** CC-BY-NC-SA 4.0 Keywords: Anti-microbial properties, Antioxidant properties, Bambusa Tulda, *Phytoconstituents*, *Sustainability*

INTRODUCTION

Rich bioactive natural resources are highly demandable by people nowadays for their high efficacy in pharmaceutical potentials and therapeutic values with nutritional components. Bioactive chemicals and antioxidants are highly valued by the food and pharmaceutical sectors due to their pivotal role in avoiding oxidation and enhancing health. These substances are used in nutritional supplements, nutraceuticals, and functional food additives. Bamboo shoots, the emerging young culms of a bamboo plant, are widely enjoyed as a vegetable in various Asian countries. Many literatures stated the different nutritional components about fermented and non-fermented bamboo shoots. The Chinese and Southeast Asians have traditionally eaten and utilized bamboo shoots as medicine. The calorie count, carbohydrate content, mineral content, dietary fibre content, and sugar and fat content of bamboo shoots are all rather low [1]. The nutritional value of different species of bamboo shoots has been assessed by several researchers. Bamboo shoots improve hunger and digestion, weight reduction, improve cardiovascular disease, antioxidant activity, anti-inflammatory activity , and anti-cancer properties, according to modern studies. Pyrolysates from Phyllostachys bambusoides, P. nigra, and P. pubescens bamboo species show anti-apoptotic properties and can cure ischemia damage [2]. Phytosterols are found in both fresh and pickled bamboo leaves. Many countries eat young, delicate bamboo. Dry, canned, boiled, fermented, or medicinal versions are eaten. Bamboo stalk, called Khorisa in Assam India, is eaten fresh or fermented [3]. Laxmikant S Badwaik et al the total phenolic content and the antioxidant activity is higher in fermented bamboo shoots as compared to fresh bamboo shoots.

Several scientific studies have been reported on the pharmacological potential of Bambusa tulda. The phytochemical, phenolic composition antioxidant and antimicrobial study has been carried out in the leaves. The methanolic extract of fresh leaves established the highest yield (22%), TPCs (221), TFCs (135), and total antioxidant capacity (EC50 values: DPPH. scavenging, 194 µg/ml; reducing power, 1343 µg/ml; hydroxyl radical scavenging, 466 µg/ml; metal chelating, 2 mg/ml) after fractionation with petroleum ether, ethyl acetate and n-butanol The aforesaid experiments indicated greater TPCs (679, 637), TFCs (156, 119), and shows highest antioxidant capacity in n-butanol and ethyl acetate fractions. Anti-hyperlipidaemic activity of Bambusa tulda leaves are established. For the acute toxicity study median lethal dose was found 6088.13 mg/kg body weight in mice. In between 100 mg/kg to 200 mg/kg of leaf extract of Bambusa tulda shows significant increase of superoxide dismutase (24.81%) and glutathione peroxidase (31.60%) enzyme and decrease malondialdehyde levels (21.90%), as compared with alloxan induce diabetic rats. Fermented bamboo shoots have been highly consumed by the people of north eastern region of India and as very popular vegetable is in Assam, there are literature review shows that, there are various pharmacological activities has been found in different parts of Bambusa tulda species, the quantification of enzymatic antioxidant study has been done by researchers which may be have a good future prospect in pharmaceuticals and nutraceutical field of study [4,5].

One of the biggest problems in modern medicine is treating infectious diseases like e coli infections, tuberculosis, urinary tract infections caused by resistant bacterial strains; this is particularly true when it comes to long-term pharmacological therapy, which is often ineffective. These diseases have a disproportionately big and significant impact in underdeveloped nations due to the relative lack of treatment availability and the proliferation of multi-resistant bacterial strains. Researchers have been on the lookout for new molecules generated from natural sources that can either replace or work in conjunction with existing antibacterials as a means to treat microbial diseases [6]. Thus, this present study investigated the phytochemical composition of ethanolic extracts and their fractions using ethyl acetate, petroleum ether, nbutanol, and water. Additionally, the study evaluated the antioxidant and antibacterial activities of these extracts against selected pathogenic microorganisms [7].

MATERIALS AND METHODS

Collection and Fermentation of Plant Materials

The shoots of Bambussa tulda were collected from the Kamrup district of Assam in the month of July 2023 and subsequently submitted for authentication to the Department of Botany at Guwahati University Assam India, accompanied by reference number GUBH20043 The newly sprouted shoots were rinsed with clean water, sliced into small pieces, and subsequently placed in a glass container for a duration of one month to undergo the fermentation process [8]. The chemicals, solvents, and reagents used in this investigation were of analytical grade and are procured from recognized sources such as Hi Media, SRL, and Finar Chemicals. Process of Extraction: The fermented shoots underwent the drying process in a tray dryer at a temperature of 40°C until they achieved the appropriate degree of dryness. The dehydrated slices are crushed using a Available online at: <u>https://jazindia.com</u> 250 blender. The dried and ground shoots were subjected to maceration process by a 48-hour treatment with ethanol. Further, the ethanolic extract was fractionated with different solvents like ethyl acetate, petroleum ether, n-butanol and water according to the polarity level [9].

Phytochemical Analysis

Qualitative Method

The qualitative phytochemical analysis tests for various phytochemicals were carried out with existing protocols which is mentioned in table no 1.

SN	Phytochemical	Test employed	Reference
	Test		
1	Test for Alkaloids	Dragendorff's Test, Mayer's Test, Ammonia Test	[10]
2	Test for Tannins	Ferric Chloride Test	[11]
3	Test for Saponins	Foam test	[12]
4	Test for	Lead Acetate Test, Fehling's Test	[13]
	Glycosides		
5	Test for Steroids	Salkowski Test, Libermann-Burchard Test, Acetic Anhydride Test,	[14]
		Liebermann–Burchard test	
6	Test for	Salkowski Test, Vanillin Test, Borntrager's Test, Ferric Chloride Test	[15]
	Terpenoids		
7	Test for Phenols	Ferric chloride test	[16]

 Table 1 List of phytochemical analysis for qualitative study

Quantitative Analysis

Determination of Alkaloid Content

A 5 gram of dried powder was dispersed in a solution of 10% acetic acid and ethanol and prepare a suspension. A subsequent temperature of 28° C for the period of 4 hours was maintained for the mixture. Afterward, suspension was filtered with using Whatman filter paper no 42. The volume of the filtrate suspension was reduced to 25% of its original volume and then added concentrated aqueous NH₄OH dropwise to accumulate the alkaloids. All the precipitate was rinsed with a 1% ammonia solution and then dried at a temperature of 80 °C in a hot air oven. The alkaloid content was measured and reported as milligrams per gram of the sample [17].

Determination of Flavonoids Content

For the quantification of flavonoid content, in a 2 M hydrochloric acid solution, a total of 5 grams of dried fermented bamboo shoots was added which were boiled for 30 minutes using reflux equipment. After cooling, the mixture was then filtered. Afterward, the ethyl acetate with similar quantity was added slowly in very minimal amount to the liquid filtrate. The mass of the precipitated flavonoid was measured and later expressed in milligrams per gram. (mg/gm) [18].

Determination of Tannins Content

Total 5 grams of finely ground Bambusa tulda shoots were added to a beaker having 20 mL of 50% concentrated ethanol with constant stirring and heated up to 80°C. The extract was quantitatively filtered using a double-layered Whatman Number 1 filter paper and then washed with a 50% ethanol solution. 20 ml distilled water was added to the extract along with 2.5 mL of Folin-Denis reagent, and 10 mL of a 17% sodium bicarbonate solution. The purpose of this treatment was to stimulate the formation of a bluish-green hue in the sample, which was thereafter left undisturbed for a period of 20 minutes. The absorbance was measured at a wavelength of 760 nm. The tannin concentration was quantified by comparing the absorbance value with a standard curve established within the 0-10 ppm range [19].

Determination of Saponins Content

Total 5 gram of dried powdered sample was mixed with 100 mL of isobutyl alcohol. The concoction was subsequently agitated for a period of 5 hours. A 20 mL of 40% saturated MgCO₃ solution was added to the mixture and then filtered. A solution was created by mixing 2 mL of a 5% FeCl₃ solution with 50 mL of distilled water. To induce the formation of a crimson hue, 1 mL of a transparent solution was introduced into the mixture and left undisturbed for 30 minutes. The samples, together with the standard, were tested for

absorbance values at a wavelength of 380 nm and then converted to mg/g units. A solution of saponin was made within the specified range of 0-10 ppm range [20].

Determination of Total Phenols Content:

A quantity of 5 grams of powdered bamboo shoot were subjected to boiled with 50 mL of ether for a duration of 15 minutes. The resultant combination mixture was subsequently divided in a proportion of 1:2, with one portion consisting of the extract and the other portion consisting of distilled water. A mixture consisting of 2 mL of NH₄OH and 5 mL of pentanol was produced and then kept at room temperature for 30 minutes. The absorbance was measured at a wavelength of 505 nm [21].

FTIR Analysis:

The Dried powder of ethanolic extract of Bambusa tulda was used for FTIR analysis by flowing the method. 1 mg of the dried powder of bamboo shoots was taken e. The powdered sample was analysed in FTIR spectroscopy (Bruker, Jermany) with ATR technology, with a Scan range from 400 to 4000 cm-1 with a resolution of 4 cm-1[22].

Antioxidant Activity study

DPPH free Radical Scavenging Activity [23,24].

The modified version of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, as detailed by Joyeux, Mortier, and Fleurentin (1995) and Robards, Sanchez-Moreno, Larrauri, and Sauracalixto (2002), was performed to determine radical scavenging activity. Four hundred millilitres of an ethanolic DPPH solution containing 0.4 mol/L were mixed with eight hundred millilitres of each extract. After 30 seconds of aggressively stirring at maximum speed, the mixes were left undisturbed in a dark place to settle for 30 minutes. After that, with MeOH as the standard solution, the absorbance was measured at 517 nm. The formula was used to determine the DPPH radical's scavenging activity:

Scavenging activity (%) =
$$\frac{100 (A1 - A2)}{A1}$$

where A1 denotes the absorbance of the ethanol control, whereas A2 signifies the absorbance of bamboo shoots extracts. The IC_{50} , also known as the inhibition concentration, represents the amount of extract required to achieve a 50% reduction in free scavenging activity. The IC_{50} values were calculated from the inhibition curves that were produced. The results were compared to the activity of ascorbic acid, obtained from Sigma in St. Louis, MO, USA, which was utilized as the control antioxidant.

Antimicrobial Activity

The antimicrobial potential of bamboo shoot extracts was evaluated in this study using the agar diffusion method. The Gram-positive bacteria Staphylococcus aureus (ATCC25923) and the Gram-negative bacteria Escherichia coli (ATCC25922), Klebsiella pneumoniae (NCTC13440), and Pseudomonas aeruginosa (ATCC27853) were cultured for an overnight period at a temperature of 37°C in 20 mL of Mueller-Hinton broth (MHB). The cultures were diluted with sterile saline solution until they reached a turbidity equivalent to that of 0.5 McFarland standards [25]. The microbial suspensions were inoculated onto sterilized Mueller-Hinton agar in 90 mm petri plates, with each dish holding 12 mL of agar. Individually, sterile Whatman No.1 discs with a diameter of 6 mm were put on the surface of agar plates that had been seeded. The filter paper discs were then treated with ethanolic extracts in dimethyl sulfoxide (DMSO) at concentrations of 50, 100, and 200 mg/mL. The plates that were treated with a substance to prevent disease were placed in a controlled environment with a temperature of 37°C and left undisturbed for a period of 24 hours. For this investigation, ampicillin and gentamicin (10 mg/disk) employed as positive control to test bacteria. In addition, dimethyl sulfoxide (DMSO) served as the negative control. The tests were conducted in triplicates. Antimicrobial activity was measured by zone of inhibition. Growth in each well was compared to the control well. Compared to the control well, the lowest inhibitory concentration of the substances inhibited growth by > 95% [26].

STATISTICAL ANALYSIS

All the experiments were done in triplicate trials. The variance of analysis and the means of the data were illustrious using ANOVA technique. The mean average values were recorded for all analysis and experiments and distinguished with t- test. Significant disparities in results were detected at a significance level of P<0.05 [27].

RESULT AND DISCUSSION

Phytochemical Profiling

The quantitative phytochemical estimation specifies that Bambusa tulda contains medicinally important phytochemicals in the ethanolic shoots extracts. The numerous phytochemicals such as tannins, steroids, saponins, flavonoids, alkaloids, amino acids, carbohydrate, and glycoside are reported in the present sample as mentioned in table 2. The positive sign indicates the presence of the phytochemicals and negative sign indicates the absence of phytochemicals. The occurrence of different phytochemicals and the antimicrobial activity of ethanolic, petroleum ether, chloroform, and methanolic extract of a single anonymous variety of Bambusa tulda fermented shoots have been formerly not stated yet however, the study is first ever reported to the best of literature review on qualitative and quantitative relative analysis that available in India. [28].

SN	Phytochemical	Ethanol extract	Ethyl acetate fraction	Petroleum ether fraction	n-butanol fraction	Water fraction
1	Tannins	+	+	-	+	+
2	Steroids	+	+	-	+	+
3	Alkaloids	+	+	-	+	+
4	Saponins	+	+	-	+	+
5	Glycosides	+	+	-	+	+
6	Flavonoids	+	-	+	-	-
7	Phenol	+	+	-	+	+
8	Amino acids	+	-	+	-	-
9	Carbohydrate	+	+	-	+	+

Table 2 Qualitative analysis of phytochemicals in ethanolic extract of Bambusa tulda with different fractions

Quantitative method

The quantitative analysis of phytochemicals in Bambusa tulda shoots reveals a substantial presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds mentioned in table 3. The ethanolic fraction contains highest number of alkaloids saponins and tannins accordingly 04.01 ± 0.02 mg/gm, 7.26 ± 0.14 mg/gm and 5.47 ± 0.75 mg/gm. N-butanol fraction contains highest number of flavonoids and phenols accordingly 5.21 ± 0.21 mg/gm and 8.06 ± 0.77 mg/gm of flavonoids which is the highest concentration as compared to the other fractions [29]. All results are calculated on triplicates of each fraction.

Name of	the	Ethanolic	Ethyl acetate	Petroleum ether	N-butanol	Water			
Phytochemicals		extract	fraction	fraction	fraction	fraction			
		(mg/gm)	(mg/gm)	(mg/gm)	(mg/gm)	(mg/gm)			
Alkaloids		4.01 <u>+</u> 0.02	2.06 <u>+</u> 0.41		03.01 <u>+</u> 0.02	2.06 <u>+</u> 0.86			
Flavonoids		3.24 <u>+</u> 0.24			5.21 <u>+</u> 0.21	3.27 <u>+</u> 0.55			
Tannins		5.47 <u>+</u> 0.75	2.14 <u>+</u> 0.12			2.08 <u>+</u> 0.74			
Saponins		7.26 <u>+</u> 0.14	4.89 <u>+</u> 0.06	1.02 <u>+</u> 0.56	3.99 <u>+</u> 0.29	1.55 <u>+</u> 0.32			
Phenols		6.46 <u>+</u> 0.31	4.05 <u>+</u> 0.44		8.06 <u>+</u> 0.77	6.04 <u>+</u> 0.67			

Table 3 Quantitative Analysis of different phytochemicals in different ethanolic fractions of Bambusa tulda

FTIR Analysis:

From the FTIR spectrum of extract of Bambusa tulda, the functional groups of the active compounds have been identified depending on the peak values in the infrared regions. The peak values of FTIR spectra have been shown in table 4 and figure 1. The absorption spectra mainly indicate the presence of different functional groups in the extract. The absorption at 2958.29cm-1, 2924.23 cm-1 and , 2852.30 cm-1 shows C-H stretch, 1019.54 cm-1 shows C-O stretch and aromatic C-H groups, 1367.68 cm-1 shows presence of Trimethyl, Nitro groups, 1277.67 cm-1 indicates the presence of CN Strech and Aromatic primary ammine, 1125.66 cm-1 reflects the presence of Aromatic C-H groups and C-F, C-O groups, 743.09 cm-1 indicates presence of Methylene $-(CH_2)_n$ groups[30].

 Table 4: Structural features of the Bambusa tulda extract by FTIR analysis

Plant Extract	Wave numbers (cm-1)	Functional Groups
Ethanolic extract of Bambusa tulda	2958.29cm-1	C-H stretch
	2924.23 cm-1	C-H - stretch
	2852.30 cm-1	C-H stretch
	1019.54 cm-1	C-O*, Aromatic C-H

1367.68 cm-1	Nitrates
1277.67 cm-1	O-H stretch
1125.66 cm-1	Aromatic C-H, C-F, C-O
743.09 cm-1	Methylene –(CH ₂) _n



Figure 1: FTIR spectra of Bambusa tulda ethanolic extract

Antioxidant Activity

Determination of DPPH free Radical Scavenging Activity

The results concerning the DPPH radical scavenging activity of shoots with the standard reference ascorbic acid are shown in Figure: 2 and Figure: 3. From the below data, it has been established that the n-butanol fraction of extract of fermented shoots has a higher DPPH radical scavenging activity. with the IC₅₀ value was calculated within the different concentration of extracts from 50 to 350 µg/ml. The mean IC₅₀ value of n butanol fraction shows 10.77 ± 0.24 µg/ml which shows the lowest IC₅₀ value with a standard deviation as compared to the others. The ethanol extract shows 11.35 ± 0.13 µg/ml, ethyl acetate shows 14.25 ± 0.27 µg/ml, petroleum ether shows 13.93 ± 0.71 µg/ml, and water shows 13.36 ± 0.11 µg/ml as shown in the table 5.The results are calculated on triplicates with standard deviations [31].

Table 5	5: IC ₅₀	value	of ethar	olic ex	stract of	Bambusa	tulda	and its	different	fractions.
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Solvent Extracts	$IC_{50}(\mu g/ml)$
Ethanol	11.35 <u>+</u> 0.13
N butanol	10.77 <u>+</u> 0.24
Ethyl acetate	14.25 <u>+</u> 0.27
Petroleum Ether	13.93 <u>+</u> 0.71
Water	13.36 <u>+</u> 0.11



Figure 2: Concentration-response curve against % scavenging of different fractions of ethanolic extract and their fractions of Bambusa tulda



Figure 3 Concentration-response curve against IC_{50} value of different fractions of ethanolic extract of Bambusa tulda

Antimicrobial Activity

The phenolic compounds like 4 -hydroxy benzoic acid, gallic acid, vanillic acid, protocatechuic acid shows good antimicrobial resistance against different gram positive and gram-negative bacteria like S. arues and E.colli. [35].Other phytoconstituents like alkaloids and tannins also shows very good responses against both gram positive and gram negative bacteria[32,33].As per the literatures the extracts having higher antioxidant activity can claim as a potent anti-microbial activity against microbes. Here, the antimicrobial activity of ethanolic plant extracts and n-butanol fraction of fermented shoots has been investigated as compared to their antioxidant potency [34]. The zones of inhibition of Bambusa tulda by ethanolic plant extracts and n-butanol fraction at 24 and 48 hours were measured with different concentrations and mentioned in table 6. The n-butanol fraction shows the strongest growth inhibition of Bambusa tulda with concentrations of 50,100 and 200 µg/ml respectively $14\pm0.52,18\pm0.63$ and 17 ± 0.03 mm against Escherichia coli, and also shows the highest zone of inhibition against Staphylococcus aureus with $11\pm0.13,13\pm0.22$ and 15 ± 0.20 mm at 48 h, respectively shown in Figure 4. Both plant extracts not able to demonstrate any antimicrobial activity against Klebsiell a pneumoniae and Pseudomonas aeruginosa in any concentrations in both 24 hours and 48 hours of time [35]. Overall, all of the plant extracts exhibited antibacterial properties for a duration of 48 hours, with a significant difference of 5% (P < 0.05).

Type of Bacteria	f Bacteria Name of Concentration Zone of Inhibition				Zone of Inhibition o	
	Bacteria	(mL)	Ethanol Pla	ant Extract	butanol Pla	nt extract
			(mm)		fraction (mm)	
			24 h	48 h	24 h	48 h
Gram Positive	Staphylococcus	50	8 <u>+</u> 0.24	10 <u>+</u> 0.04	9 <u>+</u> 0.42	11 <u>+</u> 0.13
	aureus (ATCC25923)	100	9 <u>+</u> 0.08	11 <u>+</u> 0.74	11 <u>+</u> 0.21	13 <u>+</u> 0.22
		200	12 <u>+</u> 0.21	14 <u>+</u> 0.07	12 <u>+</u> 0.24	15 <u>+</u> 0.20
Gram Negative	Escherichia coli (ATCC25922)	50	11 <u>+</u> 0.32	11 <u>+</u> 0.04	14 <u>+</u> 0.17	14 <u>+</u> 0.52
		100	13 <u>+</u> 0.07	13 <u>+</u> 0.11	16 <u>+</u> 0.21	17 <u>+</u> 0.03
		200	14 <u>+</u> 0.55	15 <u>+</u> 0.24	17 <u>+</u> 0.04	18 <u>+</u> 0.63
	Klebsiella pneumoniae (NCTC13440)	50	-	-	-	-
		100	-	-	-	-
		200	-	-	-	-
	Pseudomonas	50	-	-	-	-
	aeruginosa	100	-	-	-	-
	(ATCC27853)	200	-	-	-	-

Table 6 Antimicrobial activity of Bambusa tulda with different gram-positive and gram-negative bacteria



Figure 4: Zone inhibitions of N-butanol fractions of Babusa tudla extract against: (A). Staphylococcus aureus (ATCC25923); (B). Escherichia coli (ATCC25922)

CONCLUSION

In the present study, all the fractions of extracts Bambusa tulda showed the presence of tannins phenols, alkaloids, flavonoids and saponins. This study also leads to further research in the way of isolation and identification of the bioactive compounds from the selected fractions using chromatographic and spectroscopic techniques. The plant extracts also show good antioxidant activity and also potentially effective against Staphylococcus aureus and Escherichia coli which can be used as a potential antimicrobial source.

Conflict of Interest None

Financial Disclosure NA

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