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RESEARCH ARTICLES

Phytochemical screening, antioxidant and antibacterial activity of bamboo leaf collected from agroecosystem of the Central Siwalik region, Nepal

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Abstract
Bamboo are the fastest growing, versatile, perennial woody grasses. Naturally provided with the essential phytochemicals, various species of bamboo have even been used as medicine by the tribal people since ancient times. To elucidate the medicinal value of bamboo (*Bambusa tulda* and *Dendrocalamus strictus*), the total phenolic and flavonoid contents of their leaves were quantified by spectrophotometer, and an in-vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was measured. The antibacterial activity was analyzed by the agar well diffusion method. The phytochemical screening in the methanol solvent extracts of *D. strictus* and *B. tulda* showed the presence of alkaloids, glycosides, saponins, phenols, terpenoids, flavonoids, and steroids, while *D. strictus* exhibited steroids in the hexane extract. Both the flavonoids and phenolic content of *D. strictus* (65.11 ± 1.33 µg QE/ml and 16.05 µg GAE/ml) were found to be higher than that of *B. tulda* (57.97 ± 0.22 µg QE/ml and 11.53 µg GAE/ml). The highest DPPH radical scavenging activity was exhibited by *D. strictus* (IC_{50} = 26.05 ± 0.09), and remarkable antibacterial activity was shown against four pathogenic bacteria (*Bacillus subtilis, Enterococcus faecalis, Pseudomonas aeruginosa* and *Staphylococcus aureus*) with a zone of inhibition ranging from 10.66 ± 0.57 to 9.0 ± 0.18 mm at a maximum concentration of 200 mg/ml. This study concludes that *D. strictus* and *B. tulda* have a wide spectrum of phytochemicals and could serve as complementary medicines along with mainstream drugs.

Keywords Antioxidant · Bamboo · Fodder · Food · Siwalik

Introduction

Bamboo leaves have long been used to feed cattle, particularly during times of drought when other crops are in short supply. Almost all parts of the bamboo plants and their rhizospheric soil are useful. Bamboo roots bear beneficial plant growth promoting rhizobacteria (KC et al. 2020b, 2021, 2022) while their young leaves contain good sources of plant growth regulators including indole-3-acetic acid (IAA) (KC et al. 2020a). The fresh and processed bamboo shoots are rich sources of essential minerals Ca, Mg, K, P and Na (Sadananda et al. 2021). Bamboo has superior value in traditional indigenous Chinese, Ayurveda, Siddha and Unani medicines (Kimura et al. 2022). Their leaves are a sustainable source of fodder for dairy cattle and other ruminants (Bhardwaj et al. 2019) while the shoots are good sources of micro minerals. They have a wide array of nutrients like proteins, amino acids, carbohydrates, minerals, vitamins, dietary fibre, polyphenols, phytosterols, cyanogenic glycosides, and a comparatively small amount of cholesterol (Cheng et al. 2023), and are endowed with antiviral, antibiotic, antiulcer, and antioxidant and antimicrobial properties (Ma et al. 2020). Plethora of bioactive compounds purified from bamboo plants are potentially antioxidants that are pharmacologically palliative in certain oxidative stress-associated illnesses like inflammatory disorders, cardiovascular diseases, neurodegenerative diseases and even cancer (Cheng et al. 2023). According to pharmacological research, bamboo leaf extracts have been found to have significant influence on antioxidation, antiosteoporosis, antiviral action, improving blood circulation, regulating blood lipids, protecting liver, antifatigue and anti-inflammatory based on modern

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pharmacological research (Kimura et al. 2022). Based on the field visit and questionnaire survey, the available bamboo species in the study area are *Bambusa balcooa*, *B. nutans* subsp. *cupulata*, *B. tulda* and *Dendrocalamus strictus*, of which *B. tulda* and *D. strictus* are highly prioritized by the local residents for traditional medicines, food and fodder. All of these bamboo species are commonly distributed in tropical and subtropical regions of Nepal (Poudyal 2006). The major ethnic communities of the study area are *Dome*, *Mushahar*, *Majhii*, *Kumal*, *Tamang*, *Magar*, *Chhetri*, and rarely *Yadav* and *Sah*. Although constant use of bamboo is seen in their lifestyles, awareness and knowledge about the pharmaceutical application of bamboo resources is lacking among the residents of this region. *B. tulda* and *D. strictus* were selected for the phytochemical study. These two species have been taxonomically described. *B. tulda* is characterized by an erect culm that is green, glabrous, 7.2–24 m length and 5.6–11 cm in diameter, an internode length of 30–68 cm, prominent nodal rings, aerial roots developed from the basal node, a brown and hairy sheath, a tall and round auricle, and branching from the 3rd node above 22 cm from the base. The leaf sheath is acute, prominent, straight, glabrous with whitish bristles and measures 14–38 cm length and 1.1–7 cm width. Similarly, *D. strictus* culm is erect but curved half of its height, green and glabrous and measures 7.2–24 m length, and in diameter 1.9–7 cm. The basal culm is a solid, dull green color with an internodal length of 12–26 cm, and in diameter 1.9–7 cm. The aerial roots are present up to the 4th node, the auricle is present in the sheath, ligule is absent, number of branches is 10, leaf sheath measures 30 cm in length and 2–4 cm in width, leaf is lanceolate, linear, entire, and has a deep green color. The leaves of *B. tulda* and *D. strictus* serve as forage for ruminants, and contain flavonoids that can potentially prevent cancer, diabetes and heart disease (Laley et al. 2015). Despite the multiple benefits obtained from bamboo, scanty works have been done on the phytochemical screening and antibacterial activities of bamboo in Nepal. The determination of the fodder value is necessary for assessing the nutritional levels of bamboo leaves, as the fodder source is an important aspect of ruminant feeding. The objective of this research was to identify the phytochemicals, antioxidants and antibacterial properties of bamboo leaves growing in the agroecosystem of the Central Siwalik region of Nepal.

Material and methods

Plant collection, identification and preservation

Leaf samples and voucher specimens of *B. tulda* and *D. strictus* were collected from the Sasapur area of Sarlahi district, Central Siwalik Region, Nepal (Fig. 1). These two bamboo species are frequently used as food, fodder and medicines by the local people. Collected bamboo specimens were identified according to taxonomic keys (Stapleton 1994; Tewari 1994; Poudyal 2006). The herbarium specimens were prepared (Jain and Rao 1977) and categorized based on the altitude, latitude, longitude and locality from where they were sampled. Vaucher specimens of identified bamboo have been deposited in the Organic Farming and Natural Product Research Center (ONRC), Kathmandu University, Dhulikhel, Kavre. Leaf samples were dried at room temperature under shade, crushed into powder using a mechanical grinder, and stored in air-tight containers in a dry place until laboratory analysis.

Preparation of bamboo leaf extracts

Ten grams of finely powdered leaf samples of each bamboo species were soaked in 500 ml of methanol, ethyl acetate, and hexane (HiMedia) separately. The mixtures were incubated at 4 °C for 72 h followed by sonication for 10 min to obtain a homogenized solution, and filtered through Whatman No. 1 filter paper. Then, they were evaporated through a rotary evaporator at 37–39 °C. The dried extracts were carefully suspended separately in methanol, ethyl acetate and hexane, and kept at 4 °C for further experiments.

Qualitative screening of phytochemicals

The phytochemical screening of the bamboo leaf extract was carried out as described by Hu et al. (2016).

Estimation of total phenolic content (TPC)

TPC of the methanol extract of *B. tulda* and *D. strictus* leaves was estimated (Neupane and Lamichhane 2020) with slight modifications. In brief, 300 µl of methanol extracted solution was taken in a test tube and added with 1 ml methanol, 3.16 ml distilled water and 200 µl Folin-Ciocalteu reagent, and incubated for 8 min at room temperature. Then, 465 µl of 10% sodium carbonate solution was added, and the test tube was covered with aluminium foil and incubated in a water bath at 36 °C for 60 min. A blank was prepared by replacing the plant extract with an equal volume of methanol. The absorbance of the sample was determined using a UV visible spectrophotometer (Shimadzu UV-1800) at 765 nm. The standard curve of Gallic acid (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg/ ml) was obtained using the same procedure. The experiment was carried out in triplicates. TPC was expressed in micrograms of Gallic acid

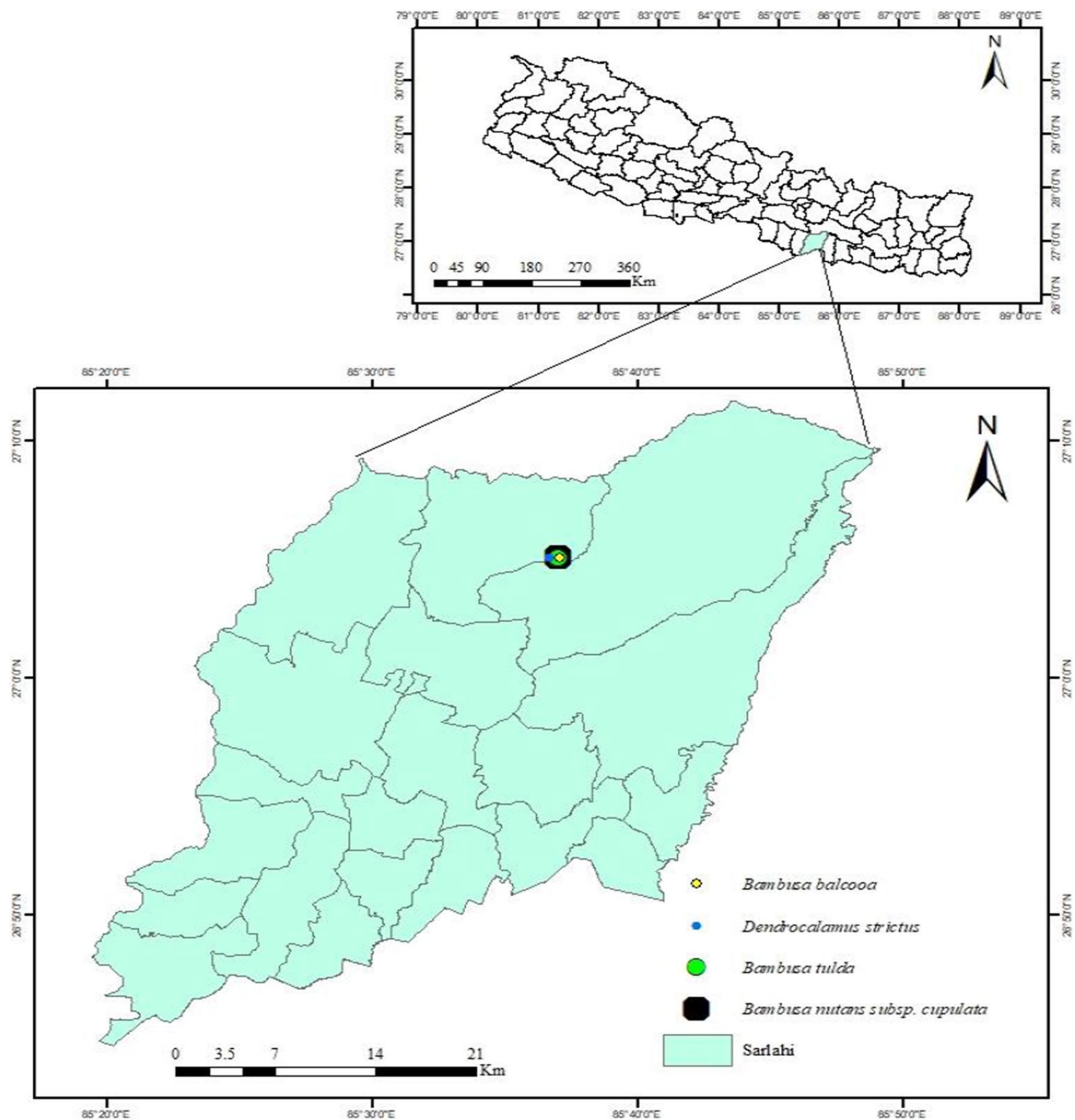

Fig. 1 Location map of sampling sites

equivalents (GAE) per millilitre, which was calculated using the equation: Y = 0.0027 X + 0.2256 (R2 = 0.8897).

Estimation of total flavonoid content (TFC)

TFC of methanol extracts from bamboo leaf samples was measured according to Zhishen and Jianming (1999) and Neupane and Lamichhane (2020). The bamboo leaf extract was dissolved in methanol to prepare its stock solution at a concentration of 1 mg/ml. Serial dilutions at various concentrations (20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 µg/ml) were made from the stock solution of methanol extracts. 0.3 ml of a 5% sodium nitrite solution and 4 ml of distilled water were added to each test tube. After five minutes, 0.3 ml of 10% aluminium chloride was added and allowed to stand for 6 min. Then, 2 ml of 1 M sodium hydroxide was added to the mixture and the final volume was made up to 10 ml with the addition of distilled water. After mixing well, yellowish colour was observed, and the mixture was incubated for 30 min at room temperature. The absorbance was taken at 765 nm on a UV spectrophotometer (Shimadzu UV-1800) against a blank. The absorbance curves of the blank and quercetin were obtained by using the above mentioned procedure, however, the plant extract was replaced with an equal volume of methanol. The experiments were carried out in triplicates. The total flavonoid content in bamboo leaves was expressed

as mg quercetin equivalents (QE) per gram dry weight (dw). The equation used for calculation was $Y = 0.0007X + 0.0882$ ($R2 = 0.9235$), where Y is the absorbance at 765 nm and X is the amount of quercetin equivalent μg QE/ml.

Determination of antioxidant activity

The antioxidant activities of crude methanol extracts of *D. strictus* and *B. tulda* leaves were assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich) free radical activity (Clarke et al. 2013) with some modification. The DPPH reagent was freshly prepared in Methanol (0.04 mg/ml). Ascorbic acid was used as a positive control and methanol as a blank. Methanol fractions of *D. strictus* and *B. tulda* leaves (20-200 μg/ml) and ascorbic acid (10–100 μg/ml) were prepared in methanol. 0.5 ml of each concentration of ascorbic acid and plant extract were taken in clean test tubes separately. To this sample, 0.5 ml of the 0.2 mM DPPH solution was added, properly mixed and incubated in the dark at room temperature for 30 min. The absorbance was measured on a spectrophotometer (Shimadzu, UV-1800) at 517 nm. The regression curve was plotted by taking the concentration of ascorbic acid at the X-axis and per cent of radical scavenging activities at the Y-axis (Fig. 2). Additionally, a measure of half-maximal inhibitory concentration (IC_{50}) for 50% DPPH scavenging activity was carried out for standards and a methanol extract of *D. strictus* and *B. tulda* leaves by fitting a linear regression to DPPH scavenging curves. The degree of radical scavenging activity was calculated by the following equation:

$$\text{Radical scavenging activity}(\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

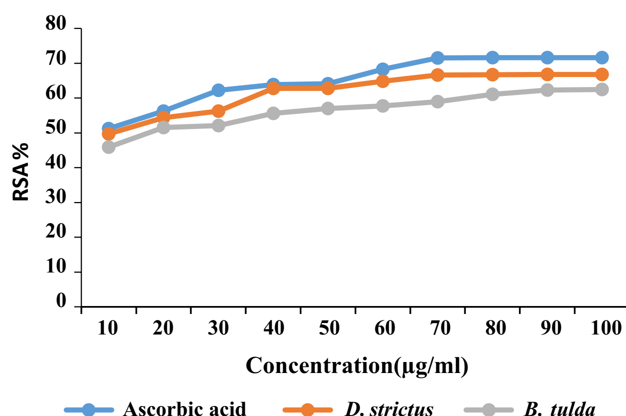

Fig. 2 DPPH radical scavenging against the concentration of bamboo leaf extracts. The % radical scavenging ability of methanol fraction from bamboo leaves compared to ascorbic acid

Determination of antibacterial activities

From each bamboo, 10 g of bamboo leaf powder was extracted with 50 ml of methanol for 48 h in a dark place at room temperature, followed by evaporation in a rotary evaporator at 40 °C. Each plant extract was dissolved in dimethyl sulfoxide (DMSO), and 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml sterilized concentrations were prepared by filtration using a sintered glass filter, centrifuged at 10,000 rpm for 20 min at 4 °C and used for in-vitro screening of antibacterial activity. The extracts were individually tested against *Bacillus subtilis* ATCC6633, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC12600, and *Enterococcus faecalis* ATCC19433. The antibacterial activity of each extract was tested using the agar-well diffusion method. Each culture strain was streaked onto nutrient agar and incubated for 24 h at 37 °C to obtain pure colonies. The isolated colonies were transferred to Mueller Hinton (MH) broth (HiMedia), and incubated for 24 h at 110 rpm in a shaking incubator (RIS-24BL) at 37 °C. MH broth containing 10^6 colony-forming units (CFU/ml) of bacteria was spread on the surface of MH agar plates. Wells with a 6 mm diameter were cut off and filled with 25 μl of different concentrations (25–200 mg/ml) of each extract. Gentamicin 10 μg per disc (GEN^{10}) (HiMedia) was used as a positive control (antibiotics), and DMSO as a negative control. Each antibacterial assay was performed in triplicates. The plates were placed for incubation at 37 °C for 24 h. The assessment of antibacterial activity was based on the measurement of inhibition zones (in millimeter) on the agar surface around the well.

Statistical analysis

For the quantitative analysis of TPC and TFC of bamboo leaves in methanol extracts, the data were recorded as the mean ± standard error (SE) of absorbance for each concentration, from which the linear correlation coefficient (R2) value was calculated using MS Office Excel 2013. The linear regression for a straight line is: $y = mx + c$. The concentration of the extract was calculated using the above given regression equation. Analysis of variance (ANOVA) was performed to compare the phytochemical, antioxidant, and antibacterial characteristics of bamboo leaves using the IBM Statistical Package for Social Sciences (SPSS, 2015). Mean values were compared by Tukey's Honestly

Significant Difference (HSD) ($P < 0.05$). The values were then presented as the mean along with the standard error.

Results and discussion

Qualitative screening of phytochemicals

The qualitative phytochemical analysis of the methanol extract of bamboo leaf (*B. tulda* and *D. strictus*) revealed the presence of terpenoids, flavonoids, saponins, phenols, alkaloids, glycosides, and steroids, which are known to exhibit medicinal value as well as physiological activities. The methanol extract showed better results for the presence of phytochemicals compared to other solvents concluding the fact that only polar phytochemicals are present. Extract of *B. tulda* showed only a positive result for steroid in the hexane extract. No phytochemical compounds were detected in ethyl acetate extracts (Table 1). Our findings are in support with Singh et al. (2012) who reported tannins, steroids, phenols, glycosides, flavonoids, carbohydrates, and protein during phytochemical screening of fermented *B. balcooa* shoot.

Estimation of total flavonoid content (TFC) and total phenolic content (TPC)

Flavonoids are water soluble polyphenolic compounds that are extremely common and widespread in the plant kingdom, whereas phenolic compounds are considered to be the most important antioxidants. Our experiment has shown that the TFC and TPC of *B. tulda* and *D. strictus* are comparable to those of quercetin and Gallic acid. The methanol extracts of *D. strictus* and *B. tulda* leaf were found to contain considerable quantities of total flavonoids, 65.11 ± 1.33 μg QE/ml and 5 7.97 ± 0.22 μg QE/ml, compared to the total phenolic content of 16.05 μg GAE/ml and 11.53 μg GAE/ml respectively. *D. strictus* showed a significantly higher value for both TFC and TPC than that of *D. strictus* (Table 2). These plants are in continuous use against infectious diseases by local ethnic communities in traditional ways. Eight phenolic compounds such as protocatechuic acid, p-hydroxybenzoic acid, catechin, caffeic acid, chlorogenic acid, syringic acid, p-coumaric acid,and ferulic acid were recognized by Park and Jhon (2010) from *Phyllostachys pubescens* and *P. nigira.* Flavonoid antioxidant activities can prevent oxidative cell damage and carcinogenesis (Abd EIslam et al. 2013) which justifies the use of bamboo plants against skin diseases. Therefore, the leaf extracts of these two bamboo species can be added to food to achieve the desired antioxidant activity. During the quantification of flavonoids and phenolic compounds, we found that flavonoids were comparatively more abundant than phenolic compounds in methanol extracts of bamboo leaf.

Antioxidant activity

The percentage scavenging effect on the DPPH radical was concomitantly increased with the increase in concentration of *B. tulda* and *D. strictus* leaf extracts from 10 to 100 μg/ml (Fig. 2). Our result is supported by Rai et al. (2020). The IC_{50} values of methanol extracts of leaves for DPPH free radical scavenging activity of *B. tulda* and *D. strictus* were

Table 1 Qualitative test of phytochemicals of bamboo leaves

Solvent	Bamboo species	Extracted phytochemicals						
		Terpenoids	Flavonoids	Saponins	Phenols	Alkaloids	Glycosides	Steroids
Methanol	*B. tulda*	+	+	+	+	+	+	+
	D. strictus	+	+	+	+	+	+	+
Ethyl acetate	*B.tulda*	–	–	–	–	–	–	–
	D. strictus	–	–	–	–	–	–	–
Hexane	*B. tulda*	–	–	–	–	–	–	+
	D. strictus	–	–	–	–	–	–	–

(+) for positive tests and (–) for negative in tests

Table 2 Total phenolic and flavonoid content of methanol extracts of bamboo leaves (n = 3)

Solvent	Bamboo species	Flavonoid content (μg QE/ml ± SE)	Phenolic content (μg GAE/ml ± SE)	Radical scavenging activity (IC_{50})
Methanol	*D. strictus*	65.11 ± 1.33^{a}	16.05 ± 0.72^{c}	26.05 ± 0.09^{e}
	B. tulda	57.97 ± 0.22^{b}	11.53 ± 1.22^{d}	65.87 ± 1.03^{f}

Data represent mean ± SE of three replicates. Means sharing the different letter in each column differ significantly at $P < 0.05$ (Tukey HSD test)

Table 3 Antibacterial activity of methanolic extract of bamboo leaves

Sample	Microbes	Extract concentration (mg/ml) and ZOI (mm)				
		25 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml
D. strictus leaf extract	*Bacillus subtilis*	2 ± 0.17^{c}	6.33 ± 0.87^{b}	8.33 ± 0.88^{a}	9.66 ± 0.66^{a}	10.66 ± 0.33^{a}
	Enterococcus faecalis	1.66 ± 0.33^{c}	3 ± 0.43^{c}	5 ± 0.57^{b}	8.33 ± 1.20^{a}	9 ± 0.57^{a}
	Pseudomonas aeruginosa	2 ± 0.57^{c}	5 ± 0.36^{b}	7 ± 0.57^{b}	9.33 ± 0.33^{a}	10 ± 0.57^{a}
	Staphylococcus aureus	1.33 ± 0.33^{c}	2 ± 0.43^{c}	5 ± 0.57^{b}	7.33 ± 0.66^{b}	9 ± 0.18^{a}
B. tulda leaf extract	*Bacillus subtilis*	1.33 ± 0.33^{c}	3 ± 0.57^{c}	3.33 ± 0.33^{c}	9 ± 0.57^{a}	9.66 ± 1.2^{a}
	Enterococcus faecalis	1.33 ± 0.33^{c}	2.0 ± 0.57^{c}	2.66 ± 0.33^{c}	6.33 ± 1.45^{b}	7.33 ± 1.20^{b}
	Pseudomonas aeruginosa	2 ± 0.58^{c}	5 ± 0.47^{b}	7 ± 0.57^{b}	9.33 ± 0.33^{a}	10 ± 0.57^{a}
	Staphylococcus aureus	1.3 ± 0.32^{c}	2 ± 0.52^{c}	5 ± 0.57^{b}	7 ± 0.66^{b}	9 ± 0.47^{a}
Gentamicin (antibiotic)	*Bacillus subtilis*	6.0 ± 0.49^{f}	8.66 ± 0.34^{e}	10.33 ± 0.33^{e}	13.0 ± 0.54^{e}	16.0 ± 0.57^{d}
	Enterococcus faecalis	5.66 ± 0.33^{c}	8 ± 0.57^{a}	10.6 ± 0.33^{a}	11.0 ± 0.57^{h}	13.66 ± 0.33^{g}
	Pseudomonas aeruginosa	4.33 ± 0.39^{c}	7.33 ± 0.32^{b}	10.66 ± 0.33^{a}	11 ± 0.52^{a}	14 ± 0.57^{g}
	Staphylococcus aureus	4 ± 0.57^{c}	5.66 ± 0.34^{c}	7 ± 0.52^{b}	9.66 ± 0.31^{a}	12 ± 0.57^{g}

Data represent mean ± SE of three replicates. Means sharing the different letter in each column differ significantly at $P < 0.05$ (Tukey HSD test)

recorded to be 65.87 ± 1.03 and 26.05 ± 0.09 respectively (Table 2), which were in accordance with the levels of phenolic content in the extracts. The lower IC_{50} value indicates higher antioxidant capacity, thus *D. strictus* leaf extract showed significantly higher radical scavenging activity as its IC_{50} value was close to standard ascorbic acid (19.61 ± 0.05) compared to *B. tulda*. In a previous study, the crude aqueous methanolic extract obtained from *B. tulda* leaf was found to be a potential source of natural antioxidants (Goyal and Brahma 2014).

Antibacterial activities

We focused on the antibacterial assay of the methanol extracts of *B. tulda* and *D. strictus* leaves (Table 3) as these bamboo leaves have been frequently used by the local people to cure fresh cuts and wounds. The antibacterial activities of the bamboo leaf extract along with the positive control (gentamicin) measured in the form of ZOI were found to be in an increasing trend with correspondingly increased concentrations of the extract. However, the ZOI in both species of bamboo extract could not meet the strength of the positive control in various concentrations (Table 3). ZOI exhibited by the extract of *B. tulda* at its highest concentration against the bacterial species was statistically nonsignificant, but in the case of *D. strictus*, the ZOI observed was lowest against *E. faecalis* (7.33 ± 1.20), which was statistically significant as compared to the rest of the bacterial species in our experiment. In the case of the positive control, the antibacterial activities were found to be the highest against *B. subtilis* (16.0 ± 0.57), followed by *P. aeruginosa* (14 ± 0.57), *E. faecalis* (13.66 ± 0.33) and *S. aureus* (12 ± 0.57). The antibacterial activity of bamboo leaves justified the presence of potential bioactive compounds in the methanol extract of the leaves. We observed that methanol leaf extracts of *B. tulda* and *D. strictus* demonstrated antibacterial properties against highly pathogenic bacteria (*B. subtilis, P. aeruginosa. E. faecalis* and *S. aureus*). Singh et al. (2012) observed significant activity of fermented shoots of *B. balcooa* against *S. aureus, B. subtilis, P. aeruginosa* and *E. coli.* Similarly, the *Phyllostachys heterocycla* cv. *pubescens* leaf extract exhibited antibacterial activities against pathogenic bacteria (*E. coli* and *B. subtilis*) and fungus (*Saccharomyces cerevisiae*) (Tao et al. 2018).

Conclusions

Phytochemical screening of leaf extracts of *B. tulda* and *D. strictus* demonstrated the presence of flavonoids and phenolic compounds. These constituents are known to exhibit medicinal value as they successfully exhibit antioxidant and antibacterial activities. Our study also confirms the potential antibacterial properties of leaf extracts of *B. tulda* and *D. strictus* since it unveiled the antibacterial response against highly pathogenic bacteria such as *B. subtilis, P. aeruginosa*, *E. faecalis* and *S. aureus*. The leaves of these bamboos can therefore represent a potential source of fodder during times of drought. These bamboo species could be studied further to screen other bioactive compounds with the prospective of pharmacological application. Therefore, a detailed phytochemical study of traditionally used bamboo is required.

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Data availability All data mentioned in the article has been provided in the supplementary files.

Declarations

Conflict of interest Authors declare no conflict of interest.

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