A Comparative Assessment of Biochemical Potential of Fresh and Fermented *Bamboosa balcooa* from Narengi (Guwahati), Assam

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Abstract

Traditional health practices and cultural heritage are filled with numerous underutilized plants having high healthcare potential that can prevent ailments. Bamboo is one such plant, distributed globally and is used in various cultural as well as food habits. Bamboo shoots have been consumed as fresh as well as fermented. The work was planned to assess the potential of both fresh and fermented bamboo shoots with their phytochemical profile. The fresh and fermented bamboo shoots were collected from Narengi (Guwahati), Assam and crushed for extract preparation. The extract was evaluated for respective antioxidant and antimicrobial potential along with the comparative study of the phytochemical profile.

The extracts have shown commendable antioxidant properties with IC_{50} 31.62±1.67 $mg.mL^{-1}$ for fresh and 18.55±2.78 $mg.mL^{-1}$ for fermented bamboo shoot extract. The extracts were also effective in inhibiting the growth of bacterial pathogens, especially S. aureus. Phytochemical analysis by GCMS identified the dominance of alcohols in fermented samples along with salicylic acid derivatives which may be crucial for bioactivities. Bamboo shoots have shown positive results in terms of healthcare applications. However, still there is a lot of space to fill to make a commercial formulation to cure disease or malnutrition.

Keywords: Fermented shoot extract, phytochemical analysis, antioxidants, antimicrobial.

Introduction

Bamboos, grass of the Poaceae family with more than 148 species under 29 genera are distributed all over the world and are spread over an area of 9.57 million hectares all over India[26]. Bamboo is mostly found in tropical, subtropical, semi-evergreen, moist deciduous areas of forest. It is also referred to as poor man's timber and rich man's delicacy due to its widespread availability and multifaceted applications[2]. It plays a crucial role in the prevention of soil erosion and conservation of soil moisture. Bamboo shoots are rich in proteins, carbohydrates, vitamins, fibers and minerals and are very low in fat[10], hence shoots or sprouts are edible and are used as a traditional delicacy mostly in Southeast Asian and East Asian countries[2] including China, Japan, the US, North East India, Thailand, Nepal, Bhutan, Korea, Australia, New Zealand, Malaysia and Indonesia[24].

Fresh bamboo shoots also contain taxiphyllin, cyanogenic glycoside that is toxic and bitter. Bamboo shoots of all the species are not edible[22]. The most commonly edible bamboo shoots in India are *Bambusa pallid, Bambusa tulda, Bambusa polymorpha, Bambusa balcooa, Dendrocalamus giganteus, Dendrocalamus hamiltoni* and *Melocanna bambusoides*[11].

Bamboo shoots exhibit great potential as a nutritious food resource, thus most commonly consumed as well as used as stored reserve food in North Eastern India[7,23]. Mostly, bamboo shoots are consumed in raw, dried, canned, boiled and fermented forms, depending upon the traditional and local culture and food habits[10,28]. It is considered nutritious and Rich in antioxidant compounds and vitamins including thiamine, niacin, vitamin A, vitamin B6, vitamin E, minerals and electrolytes including iron, calcium and phosphorus that can potentially be helpful in preventing metabolic disorders[15]. Besides raw and dried form consumption, bamboo shoots are also fermented before consumption to extend shelf life and enhance the flavour[28]. In addition, bamboo shoots also contain unsaturated fats that help in fighting against bad cholesterol[4].

Available literature has proven the health benefits including antioxidant, anti-aging and anti-cancer properties, cardiovascular disease prevention, weight loss, improved digestion, decreased blood pressure and antimicrobial activity due to flavones and glycosides[1,14,23]. However, their consumption is currently limited to tribal people in India and there is a need to create awareness among the general public about their nutritional benefits. The proposed work was planned to evaluate the potential of fresh and fermented bamboo shoots.

Material and Methods

Plant sample collection: The fresh sample, *Bambusa balcooa* shoots and fermented bamboo shoots were collected from Narengi, Guwahati, Assam in September 2021 (Fig. 1). The fresh bamboo shoots were cleaned using tap water and were weighed in the electronic balance. After weighing, both fresh bamboo shoots and fermented bamboo shoots were crushed using mortar and pestle and were also weighed.

A.

B.

C.

Fig. 1: A. *Bamboosa balcooa*, B. collected sample of *Bamboosa balcooa* Shoot and C. fresh *Bambusa balcooa* Shoot

Preparation of plant extract: The shoot extract was prepared by solvent-assisted extraction as defined earlier[11]. 40 g of both fresh and fermented crushed plant was added in six beakers and was dipped in 60 ml of methanol, ethanol and acetone respectively. The soaked samples were kept for 72 hours to ensure complete extraction. The extracts were then filtered using filter paper for 24 hours. After the filtration process, the filtrates were transferred into six test tubes respectively and were covered in parafilm and aluminium foil and stored in the refrigerator.

Antioxidant potential: Antioxidant substances and mechanisms are required to protect cells against free radicals and oxidative stress and subsequently play a vital role in lowering the risk of cardiac ailments, cancer and other associated diseases. Measuring the antioxidant activity of bioactive formulations is therefore essential to ensure the quality of functional foods. The antioxidants potential of fresh and fermented bamboo shoot extract was assessed by DPPH, ABTS, FRAP and CUPRAC methods against different types of free radicle species.

For the DPPH assay, fresh and fermented shoot extracts were screened against 1,1-diphenyl-2-picryl hydrazyl (DPPH). The stock of 0.1µg/10µl was used for assessment in different concentrations. 10-1000µl stock solution was mixed separately with 3 mL DPPH workable solutions, mixed and kept at room temperature for 30 min in the dark. The absorbance of solutions was recorded at 517 nm[5]. A similar strategy was also adopted for ABTS assay[19], FRAP assay[13] and CUPRAC assay[3]. The antioxidant potential was calculated in comparison to 100µl of 1mg.mL^{-1} TROLOX, as the positive control and distilled water as a negative control. The antioxidant potential was calculated using eq. 1:

$$Antioxidant\ potential\ (\%) = \frac{Abs_{control} - Abs_{Sample}}{Abs_{Control}} 100$$

Antimicrobial potential: The antimicrobial potential of extract of fresh and fermented bamboo shoots was determined with different concentrations of extract against clinical microbial pathogens by CFU count[6,9]. Test bacterial pathogens were revived on nutrient broth and incubated at 37°C to attain a microbial load of 1.5x10^{8} CFU.ml^{-1}. Shoot extracts of 1mg.10ml^{-1} stock, prepared in dimethyl sulfoxide were used for evaluation. Different concentrations of sheet extract were added to each well and incubated at 37^{o}C. 1mg.ml^{-1} of antimicrobial was used as positive control while DMSO was used as negative control. The plates were inoculated for 24-36 hours at 37°C (bacteria) and 27°C (fungi). Inhibition (%) was assessed by CFU and compared with negative control (100%).

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Comparative analysis of phytochemicals in fresh and fermented bamboo shoot: The plant extract was characterized by different phytochemicals including tannins, phenolics, proteins, lipids and carbohydrates. Total phenolic content in plant extract was determined by Folin-Ciocalteu (FC) reagent as defined by Singleton and Rossi[29]. 100µL of plant extract was mixed with 500µL diluted FC reagent solution and kept at 22°C for 5 min. Post incubation, 2 mL Na_2CO_3 (20% aqueous solution) was added to the solution and further incubated at 22°C for 90 min. The absorbance was recorded at 650 nm and phenolics was quantified with gallic acid standard ($1 mg.mL^{-1}$). Similarly, total flavonoid content was quantified by using aluminum chloride ($AlCl_3$) method[27]. For quantification, quercetin was used as a standard and absorbance was recorded at 510 nm. The total tannin content of plant extract was determined by titrimetric method as defined in Ayurvedic Pharmacopoeia of India (https://www.ayurveda.hu/api/API-Vol-1.pdf).

The total saponin content in plant extract was determined with the vanillin-sulfuric acid colorimetric method. The absorbance of the sample was determined at 544 nm and quantified with diosgenin equivalent (DE).g^{-1} [16]. Total and reducing sugar content and protein content were quantified by phenol sulfuric[20], dinitro-salicylic acid[21] and Bradford method[8].

Statistical analysis: The statistical analysis of antimicrobial and antioxidant analysis was done by using MS-Excel with a data analysis pack using ANOVA. The p-value was considered for model suitability which must be below 0.05 for model fitness.

Results and Discussion

Bamboo shoot dried powder was extracted with hydro-alcohol (1:1) for 72 hours at room temperature and filtered to remove solid fraction. The extract was characterized for phytochemical constituents like phenolics, carbohydrates and other metabolites. The bioactivities of both fresh and fermented bamboo shoots were assessed for antioxidants and antimicrobial activity. The results pertaining to various analyses have been summarised.

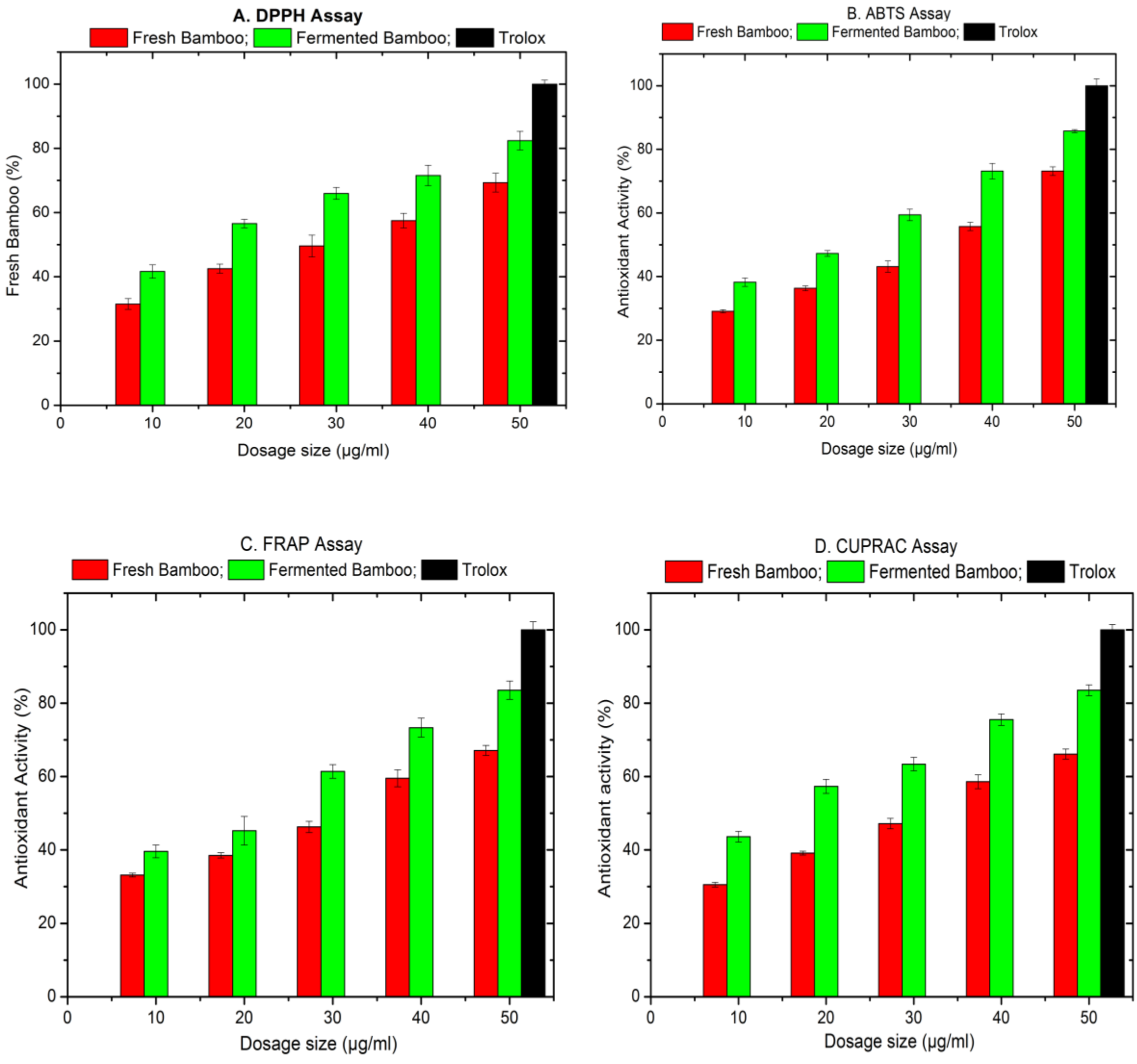

Fig. 2: Antioxidant potential of fresh and fermented bamboo shoot extract by A. DPPH assay, B. ABTS assay, C. FRAP assay and D. CUPRAC assay.

Antioxidants: The antioxidant potential of plant extract was determined against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium radical cation (ABTS), Fe^{3+}-TPTZ (FRAP) and $Cu(Nc)_2^{2+}$ (CUPRAC). The test involved different types of radicals including organic and metal-based structures to determine the total antioxidant potential. For comparison, trolox was used as a positive control (commercial antioxidant compound) and distilled water as a negative control.

As depicted in fig. 2, both fresh and fermented bamboo shoot extract have shown antioxidant potential in a dose-dependent manner in all four assays i.e. DPPH (Fig.2A), ABTS (Fig.2B), FRAP (Fig. 2C) and CUPRAC (Fig. 2D). However, the antioxidant potential of fermented bamboo shoot was higher than fresh bamboo shoot extract. The maximum antioxidant potential of shoot extract was exhibited at a higher concentration range of 50µg.mL^{-1}. The antioxidant potential was still high in comparison to plant extract. Bamboo shoots have been identified as a rich source of antioxidant compounds including phenols, flavonoids, vitamins and minerals which contribute significantly to its antioxidant activity[22].

Iwansyah et al[18] reported that the ethanolic extract of bamboo shoots contained a high amount of 27.83 mg GAE.g^{-1} phenolics and 2.49 mg QE.g^{-1} flavonoids. The IC_{50} of ethanolic extract was 347.48 µg.mL^{-1} for the Kuning bamboo shoot. Santosh et al[25] reported IC_{50} of fortified bamboo shoot 2100.95 µg.L^{-1}. IC_{50} values of bamboo shoot extract in current work were 18.55±2.78 µg.mL^{-1} (fermented shoot) and 31.62±1.67 µg.mL^{-1} (fresh shoot). The values were quite lower in comparison to previous work and clearly visible that fermented shoot extract has higher antioxidant potential.

Antimicrobial analysis: Fresh as well as fermented bamboo shoot extracts have shown a wide range of phytochemicals that are mainly responsible for antimicrobial activity. The antimicrobial activity of extracts has shown a similar trend as fermented bamboo shoot extract has higher inhibitory potential than fresh bamboo shoots (Fig. 3). Both fresh and fermented bamboo shoot extracts were effective against microbial pathogens and inhibited the growth of bacterial as well as fungal pathogens. The Antimicrobial activity was maximum against *S. aureus* with 75.39±1.92 % (fermented bamboo shoot) and 67.51±1.11 % (fresh bamboo shoot) while minimum against *Aspergillus niger* with 17.83±0.35 % (Fresh bamboo shoot) and 19.89±0.76 % (fermented bamboo shoot).

The results from the current work coincide with the previous researches. Ethanolic extract of bamboo shoot has antimicrobial activity against salivary *Streptococcus mutans* and *Lactobacillus acidophilus* with inhibition zones of 23.00 ± 0.816 mm and 22.00 ± 0.816. The maximum inhibition was reported at a bamboo shoot extract concentration of 60 µg.mL^{-1} [12]. In another work, fermented shoot extract has shown antimicrobial activity against *S. aureus*, *P. aeruginosa and E. coli*[30]. The antimicrobial activity of bamboo shoots was possibly due to chitin-binding proteins (Pp-AMP1 and Pp-AMP2), flavones and glucoside[12].

Statistical analysis: The antioxidant and antimicrobial analysis were evaluated for fitness by two-way ANOVA. The 'p' value must be below 0.05 to make a model fit. Regarding antioxidant analysis, the p-values for DPPH, ABTS, FRAP and CUPRAC analysis were well below 0.05. For DPPH the p values were 0.00005 and 0.03, for ABTS, 0.00004 and 0.01, for FRAP, 0.00005 and 0.01 and CUPRAC, 0.00005 and 0.03. The ANOVA analysis supported the model's fitness. Similar kind of observations was reported with antimicrobial analysis. The p-value for antimicrobial analysis was 0.039 which was also below 0.05 (Table 1).

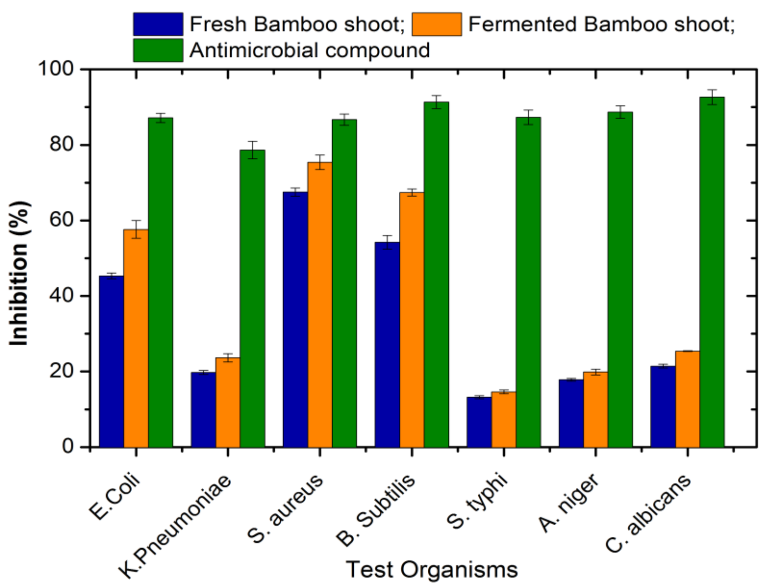

Fig. 3: Antimicrobial activity of fresh and fermented bamboo shoot

Table 1
ANOVA analysis of antioxidant and antimicrobial activity of bamboo plant extract

Source of Variation	***SS***	***Df***	***MS***	***F***	***P-value***	***F crit***
DPPH Assay						
Rows	0.55	2	0.27	43.12	5.19E-05	4.46
Columns	0.13	4	0.03	5.04	0.03	3.84
ABTS Assay						
Rows	0.62	2	0.31	44.65	4.57E-05	4.46
Columns	0.19	4	0.05	6.89	0.01	3.84
FRAP Assay						
Rows	0.53	2	0.27	43.74	4.93E-05	4.46
Columns	0.17	4	0.04	7.15	0.01	3.84
CUPRAC Assay						
Rows	0.58	2	0.29	44.64	4.58E-05	4.46
Columns	0.13	4	0.03	5.02	0.03	3.84
Antimicrobial assessment						
Rows	1740.93	6	290.16	2.47	0.039	2.32
Columns	81481.78	7	11640.25	98.95	5.13E-24	2.24

Table 2
Phytochemical analysis of fresh and fermented bamboo shoot extract

Phytochemicals	**Bamboo shoot extract ($g.100g^{-1}$)**		**Change**
	Fresh	Fermented	(Rise/reduction)
Carbohydrate	9.63±0.23	1.11±0.01	-88.47
Proteins	2.89±0.11	5.83±0.2	101.73
Phenolics	3.1±0.19	2.9±0.01	-6.45
Flavonoid	0.3 ±0.02 (ruetin equivalent)	0.34±0 (ruetin equivalent)	13.33
Tannins	0.03±0	0.03±0	0.0
Saponins	0.15±0.0	0.14±0.03	-6.67
Total sugars	4.51±0.19	2.91±0.17	-35.48

- Reduction and + Increase

Comparative analysis of phytochemical profiling: The phytochemicals are the root factor for bioactivities. Both fresh as well as fermented bamboo shoot extracts were characterized for the presence of various phytochemicals including carbohydrates, proteins, tannin, flavonoids, phenolics etc. (Table 2). Fresh shoot extract contains a significant amount of saponins, phenolics and flavonoids. However, the concentration of different phytochemicals has changed significantly in fermented shoot extract due to microbial action. Carbohydrate and sugar content have been reduced by more than 88% and 35% respectively as it is the main substrate for microbial fermentation while protein content was increased by more than 101% due to the additional production of proteins by microorganisms in fermentation.

Literature has also reported the presence of diverse phytochemicals including phenols, derivatives, acids, alcohols and flavonoids. Iwansyah et al[18] have compared different types of bamboo shoots like Kuning, Gombong, Betung and Buluh among which maximum phenolics and flavonoids were reported from Buluh and Betung respectively. The maximum antioxidant potential was reported from Betung shoots ethanolic extract. Comparative GCMS profiles of both fresh and fermented shoot extract also revealed the dominance of alcohols[31]. The bamboo shoot extracts have also shown the hepato-protective effects of fresh and fermented bamboo shoot extract in diabetic mice models.

In comparison to fresh, fermented shoot extracts have shown a better reduction in liver enzyme levels including serum glutamic pyruvic transaminase, serum glutamic-oxaloacetic transaminase, alkaline phosphatase and bilirubin[17]. Some of the recent studies have also reported the valuable contribution of plant-based products and phytochemicals in health care and in improving food nutrition[32-34]. The difference in activity is mainly attributed to diverse phytochemicals.

In addition, bamboo shoots also contain a good amount of vitamins and minerals that become a conducive factor for bioactivities. The results seem promising but still, cell line-based and animal model-based analysis are required to conclude its commercial feasibility as health care products.

Conclusion

Phytochemicals including primary and secondary metabolites have shown a diverse range of bioactivity and have shown a brighter side of healthcare and herbal formulations. Fresh and fermented bamboo shoot extracts have shown the presence of phenolics, alcohols, acids, flavonoids, tannins and proteins which are mainly responsible for bioactivities including antioxidants and antimicrobial, anti-aging and anti-fatigue. The fermented bamboo shoot extract has a significantly higher potential than fresh.

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