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PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL POTENTIAL OF FERMENTED *BAMBUSA BALCOOA* SHOOTS

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ABSTRACT
Preliminary phytochemical screening of solvent extracts viz. methanol and ethyl acetate of fermented *Bambusa balcooa* shoots were carried out. Study reveals the presence of tannins, steroids, phenols, glycosides, flavanoids, carbohydrates and proteins. FT-IR spectrum in the mid infrared region (4000-400cm^{-1}) shows the presence of N-H, C=O, C-H, C=C and C-O, C-C, C-O bonds responsible for alkyl groups, methyl groups, alcohols, ethers, esters, ketones, carboxylic acid, anhydrides and deoxyribose confirming the qualitative results. Further, the therapeutic potential was confirmed by the antimicrobial property of the methanolic extracts against 4 bacterial strains viz., *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* and 3 fungal strains viz., *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum*. Maximum activity was observed against *Staphylococcus aureus* (18mm), *Bacillus subtilis* (15mm) followed by *Pseudomonas aeruginosa* (13mm) and *Escherichia coli* (11mm). While no activity was observed against *Aspergillus niger* and *Candida albicans*, significant activity was seen against *Fusarium oxysporum* (9mm).

INTRODUCTION

Bamboo is intricately associated with humans from times immemorial. It belongs to the family *Poacea*, is widely distributed and grows wild in the fields and mountains from the temperate zone of Japan to the tropical zone of India. Bamboo shoots are young, new canes that are generally 8-12inches long, taper to one end and grow extraordinarily. Bamboo shoots have a long history of being used as a source of both food and medicine in China and Southeast Asia (Bao, 2006). Bamboo shoots are low in calories, rich in nutrient components, mainly proteins, carbohydrates, minerals, and dietary fiber and are low in fat and sugars. The nutritional value of edible shoots of different bamboo species has been worked out by several workers (Tripathi, 1998; Chen *et al*., 1999; Sharma *et al*., 2004; Xu *et al*., 2005; Nirmala *et al*., 2008; Singh *et al*., 2011). Modern research has revealed that bamboo shoots have a number of health benefits: improving appetite and digestion, weight loss, curing cardiovascular diseases, antioxidant activities and anti-inflammatory effects (Hu *et al*., 2000; Lu *et al*., 2005) and anti-cancer property (Shi and Yang, 1992). Pyrolysates-derived from 3 bamboo species viz., *Phyllostachys bambusoides, P. nigra* and *P. pubescens* have anti-apoptotic effects and can be useful as a supplement for ischemic injury treatment (Hong *et al*., 2010). Bamboo shoots, both fresh and fermented, are a good source of phytosterols that are the precursors of many pharmaceutically active steroids found in plants (Srivastava, 1990; Sarangthem and Singh, 2003a) and act as nutraceuticals (Miettinen, 2003).

The young and tender bamboo plant is utilized as one of the food items in many countries. It is consumed in dried, canned, boiled, fermented or medicinal forms. In Manipur, a state located in the north eastern part of India, bamboo shoot is consumed as fresh or fermented form, locally called *Soibum* (Jeyaram *et al*., 2009). With recent wave of economic depression and its attendant effect on the purchasing power of the population of less developed nations, it has become obvious that the local food stuffs will play increasing role in the food, nutrition and health security of the rural people and the increasing urban poor. As popular as this vegetable is in Manipur, there is still paucity of information on the phytochemical constituents and antimicrobial activity of *Soibum*. Hence the present study was carried out to evaluate the phytochemical constituents of *Soibum* (fermented *Bambusa balcooa* shoots). FT-IR analysis was done to support the qualitative results. Further, the antimicrobial potential of the methanolic extract was evaluated against pathogenic microbes.

MATERIALS AND METHODS

Fermented *Bambusa balcooa* shoots were purchased from its production centre at Andro; Manipur, India located about 25km from the laboratory. The collected samples were packed in 500mL coded PET bottles and transported and stored in the laboratory refrigerator for further analysis. The samples were washed with distilled water and dried then powdered with mechanical grinder and stored in air-tight containers.

The dried powered materials were extracted using methanol and ethyl acetate. 10g of dried powder was taken in 100mL of each solvent in a conical flask, plugged with cotton wool and then kept on a rotary flask at 190-220rpm for 24h. After 24h the supernatant was collected and the solvent was evaporated through a rotary evaporator to ¼ its original volume and preserved in airtight container at 5ºC which was further used for analysis. The extract was evaluated for the presence of carbohydrate, tannins, flavanoids, cardiac glycosides, saponins, alkaloids, steroids and phenols using simple qualitative methods (Trease and Evans, 1989).

FT-IR analysis was carried out in the range 4000-400cm^{-1} using a Shimadzu FT-IR spectrometer- 8400S model. 2mg of the finely powdered sample free from moisture was mixed with 200mg KBr (FT-IR grade) and pressed onto pellet. The pellet was immediately put into the sample holder and a FT-IR spectrum was recorded.

Antimicrobial activity of the methanolic extract was determined using agar well diffusion method. Sterile Nutrient Agar and Potato Dextrose Agar was poured into a petri dish in uniform thickness and kept aside for solidification. Using sterilized swabs, even distribution of lawn culture was prepared using test bacteria such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* and fungi such as *Aspergillus niger, Candida albicans* and *Fusarium oxysporum*. After diffusion with 100μL/well of the methanolic extract, the plates were incubated at room temperature for 24-48h. After incubation, the inhibition of growth was analysed and the results were recorded.

RESULTS AND DISCUSSION

In the present study, phytochemical analysis of fermented *Bambusa balcooa* shoots were carried out using two different solvent extracts namely methanol and ethyl acetate. FT-IR spectrum was measured to support the qualitative results. Further, the antimicrobial activity of the methanol extract was investigated in selected pathogenic strains of bacteria and fungi to prove its therapeutic potential.

Phytochemicals are physiologically active compounds produced via secondary metabolism in relatively small amounts (Rodriguez *et al.,* 2006). They are essential and non-essential compounds that occur in nature, are part of the food chain and have effect on human health (Biesalski *et al.,* 2009). Some groups of phytochemicals that have significant health potentials are carotenoids, phenolic compounds (flavonoids, phytoestrogens, phenolic acids), phytosterols and phytostanols, saponins, tocotrienols, organosulfur compounds (allium compounds and glucosinolates), and nondigestible carbohydrates (dietary fiber and prebiotics). Preliminary phytochemical analysis of the aqueous leaf extract of *Bambusa vulgaris* revealed the presence of alkaloids, tannins, phenolics, glycosides, saponins, flavonoids and anthraquinones (Yakubu *et al.,* 2009). Bamboo shoots are a rich source of protein. The protein content ranges from 2.31 to 3.72g/ 100g fresh weight (Sundriyal and Sundriyal, 2001; Bhatt *et al.,* 2005). Qiu, 1992 reported bamboo shoots contain 17 amino acids, 8 of which, serine, methionine, isoleucine, leucine, phenylalanine, lysine and histidine are essential for the human body. Nirmala *et al.,* 2009 reported high amount of dietary fibre (non-digestible carbohydrate), ranging from 2.23 to 4.20g/ 100g fresh weight of shoot in some bamboo species. Bamboo shoots have also been reported to be rich in both phenols and phytosterols. Eight phenolic compounds, viz., protocatechuic acid, p-hydroxybenzoic acid, catechin, caffeic acid, chlorogenic acid, syringic acid, p-coumaric acid and ferulic acid were identified from *P. pubescens* and *P. nigira* (Park and Jhon, 2010). Quitain *et al.,* 2004 has identified ethoxyquin, a sesquiterpene, and a cyclohexanone derivative from *P. heterocycla*. Kim *et al.,* 2007 reported α- tocopherol and γ-tocopherol in bamboo shoots. Bamboo shoots, both fresh and fermented are good source of phytosterols. The level of total phytosterols in bamboo shoots ranges from 0.12% to 0.19 % on a dry weight basis in different species of bamboos (Sarangthem and Singh, 2003a). Lu *et al.,* 2009 reported major sterols, β-sitosterol, campesterol, stimastanol, ergosterol, cholesterol and stigmastanol present in 4 bamboo species viz., *P. amarus, P. pubescens, D. latiflorus* and *P. praecox*. Sarangthem and Singh 2003b reported that sitosterols, the most widely distributed phytosterols, are abundant in fermented bamboo shoots. Here we report the preliminary phytochemical screening of fermented *Bambusa balcooa* shoots (summarized in Table 1), which revealed that the solvents have the extraction of phytochemical constituents. Among the two solvents methanolic extract showed maximum number of phytochemical constituents when compared to ethyl acetate extract.

Table 1: Screening of phytochemical constituents in methanolic and ethyl acetate extracts of fermented *Bambusa balcooa* shoots

Phytochemical	Methanol extract	Ethyl acetate extract
1. Tannins	+	-
2. Steroids	+	+
3. Alkaloids	+	-
4. Saponins	+	-
5. Glycoside	+	+
6. Flavonoids	-	+
7. Phenols	+	+
8. Amino Acids	+	+
9. Carbohydrates	+	-

FT-IR revealed the existence of various characteristics functional group in fermented *Bambusa balcooa* shoots (Fig. 1). The absorption bands, the wave number (cm^{-1}) of dominant peak obtained from absorption spectra are defined in Table 2. The FT-IR spectrum exhibits the characteristic finger print band features. The very strong absorption bands at 3770, 3310 and 2927 cm^{-1} are representative for C-H, O-H and N-H

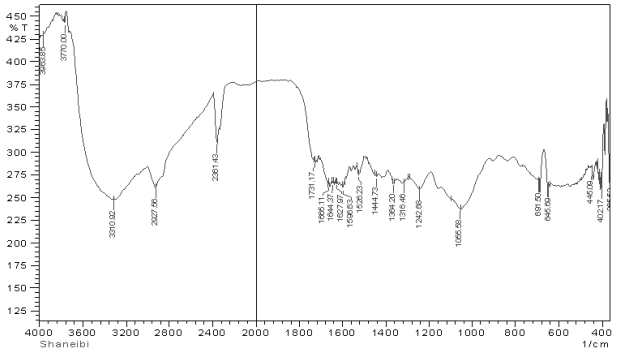

Figure 1: FT-IR spectra of fermented *Bambusa balcooa* shoots

Table 2: General band assignments of the FT-IR spectra of biological tissue

S. No.	Peak	Assignment
1.	3770	NH_2 Stretching- Characteristic of amino acid
2.	3308	Bonded O-H Stretching
3.	2926	CH stretching of CH_2 group indicating the presence of various amino acids
4.	1728	Ketones, Ester carboxyl group
5.	1664	C=O, Aldehyde
6.	1643	C=O Stretching phenyl ring amino acid-1
7.	1625	C=O, Chelate
8.	1549	N-H deformation
9.	1444	C-N Stretching- in- plane OH bending
10	1371	CH_3 (asymmetric/deformation)
11.	1242	Esters carboxyl group, Phenol
12.	1050	C-H deformation or C-O or C-C stretching pertaining to carbohydrates, Glycogen
13.	600-900	C-O-O, P-O-C bonding (aromatics) phosphate

Table 3: Antimicrobial activity of methanolic extract of fermented *Bambusa balcooa* shoots

Name of the Organism	Zone of Inhibition (mm)	
Bacteria	Control	Methanolic Extract
Escherichia coli	No zone	11
Staphylococcus aureus	No zone	18
Bacillus subtilis	No zone	15
Pseudomonas aeruginosa	No zone	13
Fungi		
Aspergillus niger	No zone	No zone
Candida albicans	No zone	No zone
Fusarium oxysporum	No zone	9

stretching vibrations, characteristics of the presence of various amino acids (Rao *et al.,* 1963). The 1664, 1643 and 1625cm^{-1} bands are due to stretching vibration of carbonyl group characteristic of the secondary amides and other compounds containing C=O groups (Mueen *et al.,* 2005). The strong band at 1728 and 1242cm^{-1} predicts the presence of ester carboxyl group characteristics of ketones and phenols (Suresh *et al.,* 2002). The absorption band at 1050cm^{-1} in the fingerprint region indicates several modes such as C-H deformation or C-O or C-C stretching, pertaining to carbohydrates (Li *et al.,* 2004). The strong bands at 600-900cm^{-1} represent C-O-O and P-O-C bending of aromatic compounds (phosphates).

Most research studies have investigated the functional activities of bamboo leaves and stems. Kim *et al*. (2001) reported that extracts of bamboo leaves and stems of *Phyllostachys* spp. showed strong antibacterial activities. Wang and Ng (2003) reported the isolation of an antifungal protein, dendrocin, isolated from *D. latiflora*. Antimicrobial compounds have also been isolated from *P. heterocycla* by superficial CO_2 extaction and subsequent hydrothermal treatment of the residues (Quitain *et al.,* 2004). Vijay *et al*. (2010) reported that the aqueous extracts of bamboo leaves of *B. arundinaceae* showed activity against *E. coli* while the ethanolic extracts showed activity against *S. aureus, E. coli, P. aureginosa* and *B. subtilis*. The antibacterial and antioxidant activities of the essential oils isolated from the leaves of *Phyllostachys heterocycla cv. pubescens* and three more species of bamboo have been demonstrated. The oils showed marked antimicrobial and antioxidant activities. The result equally showed that there were no significant differences among varieties and related with respect to their antioxidant and antimicrobial activities (Jin *et al.,* 2011). Akinobu *et al*. (2011) has reported that *Phyllostachys pubescens* shoot skin itself and its dichloromethane extract has antibacterial activity against *S. aureus*. Antimicrobial activity of chitin binding peptides, Pp-AMP1 and Pp-AMP2 of *Phyllostachys pubescens* shoots has also been reported (Fuzimura *et al.,* 2005). There are many effective components in bamboo leaves, shavings and shoots including flavonoids, phenolic acids, polysaccharides, anthraquinones, coumarins, special amino acids and peptides, etc which have multiple biological activities such as antioxidant, scavenging oxygen radicals, protecting human being from cardiovascular disease and cancer, as well as anti-bacteria and antivirus (Lu *et al.,* 2005, 2006; Zhang *et al.,* 2004, 2007). Our work focuses on the antimicrobial assay of the methanolic extract of fermented *Bambusa balcooa* shoots (Table 3) which has not been reported so far. The level of inhibition was observed for seven organisms (4 bacteria and 3 fungi). Methanolic extract of fermented *Bambusa balcooa* shoots showed activity against most of the selected strain of organisms. Significant activity was observed against *Escherichia coli* (11mm) while maximum activity can be seen against *Staphylococcus aureus* (18 mm), *Bacillus subtilis* (15 mm) and *Pseudomonas aeruginosa* (13 mm). While no activity was observed against the two fungal strains *Aspergillus niger* and *Candida albicans,* significant activity was seen against *Fusarium oxysporum* (9 mm). The above results indicated that fermented *Bambusa balcooa* shoots has a spectrum of phytochemicals which acts as a potential antimicrobial agents, when extracted with methanol.

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