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In Vitro Antimicrobial and Antiproliferative Activity of *Bambusa vulgaris*

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ABSTRACT

Bambusa vulgaris common bamboo is an open clump type bamboo species. It is native to Indochina and to the province of Yunnan in southern China, but it has been widely cultivated in many other places and has become naturalized in several. Among bamboo species, it is one of the largest and most easily recognized. In this work, we evaluated the *in vitro* antimicrobial and antiproliferative activities of the acetone extract of B. vulgaris shoot against the bacteria such as *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis* and *Enterobacter aerogenes*. The fungi such as *Aspergillus fumigatus*, *Aspergillus Niger*, *Candida albicans*, *Candida glabrata* and *Candida tropicalis* using agar diffusion methods and the antiproliferative activity evaluating total growth inhibition (TGI) by MTT assay using human cancer cell line, HeLa (Human Cervical Cancer). In Acetone shoot extract the highest zone of inhibition was exhibited by *Enterococcus faecalis* 21.67±2.08(mm) and *Klebsiella pneumoniae* 21.33±1.53(mm). In fungi the highest zone of inhibition was exhibited by *Aspergillus fumigates* 23.67±2.31 (mm) while the lowest inhibitory activity was showed against *Candida tropicalis* 15.67±3.06 (mm). The extract showed activity against all the tested bacterial and fungal pathogens. MTT assay confirmed the dose-dependent antiproliferative effect i.e. as dose of extract increases, number of viable cells decreases and the percentage inhibition increases. The shoot extracts showed significant antiproliferative activity, which supports the folk claims of use of the plant as an anticancer repertoire.

INTRODUCTION

Plants have a great potential for producing new drugs for human benefit. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to a report of World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health care needs (Duraipandiyan *et al* 2006). The demand for more and more drugs from plant sources is continuously increasing. It is, therefore, essential for systematic evaluation of plants used in traditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity. In our study, we choose the shoot portion of *Bambusa vulgaris* to evaluate its biological activity. Bamboo is a large perennial grass distributed widely from tropical to subarctic zones. In Asian countries, different parts of bamboo have been used for medicinal purposes to treat hypertension, arteriosclerosis, cardiovascular disease and certain forms of cancer, antioxidant activities and are non-toxic (Xu *et al*., 2001; Lu *et al*., 2005; Lu *et al*., 2006)

Bamboo shoots have been regarded as traditional Chinese medicinal material for more than 2000 years, and according to archaic Chinese medicinal books, such as "Ben Chao Qui Zheng," "Ben Jing Feng Yuan," "Yao Pin Hua Yi," and "Jing Yue," were proclaimed to be beneficial to human health, by promoting motion and peristalsis of the intestine, helping digestion, and preventing and curing cardiovascular diseases (CVDs) and cancers. Modern research has revealed that shoots have a number of health benefits, from cancer prevention and weight loss to lowering cholesterol level, improving appetite and digestion. It is also low in sugar and therefore can be used by persons on sugar-restricted diets. The shoots also contain anticarcinogenic agents and making them a regular part of a diet effectively reduces the free radicals that can produce harmful carcinogens. It is believed that bamboo extract may have antioxidant activities and provide anti-inflammatory effects (Hu *et al*., 2000; Lu *et al*., 2005). Furthermore, bamboo-derived pyrolysates have been proposed to have antimicrobial and antifungal activities (Fujimura *et al*., 2005) and to protect neurons from oxidative stress. (Hong *et al*., 2010) studied the effects of pyrolysates-derived from 3 bamboo species, *Phyllostachys bambusoides, P. nigra and P. pubescens* indicated that pyrolysates may have antiapoptotic effects and can be useful as a supplement for ischemic injury treatment. Several antimicrobials and antioxidants have been isolated by supercritical CO_2 and subsequent

hydrothermal treatment of the residues from moso bamboo, including an ethoxyquin, a sesquiterpene, and a cyclohexanone derivative (Quitain *et al*., 2004).

In traditional Chinese medicine, bamboo shoots are used to ease labor and the expulsion of the placenta by inducing uterine contractions. A poultice of the shoots is often used for cleaning wounds and healing infections. Bamboo shoot decoction taken along with honey is used to treat respiratory disorders. However, to most people, bamboo shoots are best known as food. Fresh, dried, or fermented bamboo shoots are used in numerous Asian recipes. Nutrient analysis on freshly emerging juvenile bamboo shoots has shown high contents of amino acids, proteins, carbohydrates, vitamins, and minerals, and a low content of fat. As bamboo shoots age, the dietary fiber and moisture start to increase while vitamin and mineral contents decrease (Nirmala *et al*., 2007).

Bamboo shoots are also reported to have anticancer, antibacterial, and antiviral activity due to the presence of an important component of fiber (Fujimura *et al*., 2005). Because of its high content of potassium, bamboo helps to maintain normal blood pressure and is labeled as a heart-protective vegetable. Its relatively high content of up to 4% cellulose increases the peristaltic movement of the intestines and helps digestion. It also prevents constipation and decreases body fat.

With this approach, we studied the antimicrobial activity of acetone extract of *Bambusa vulgaris* shoot against pathogenic microorganisms, and the possible antiproliferative effect against a well-defined panel of human cancer cells lines. This is, undoubtedly, a significant contribution in the field of alternative medicine towards the elaboration of novel antimicrobial agents to prevent infections and to reduce the potential risk of systemic chronic diseases and cancer.

MATERIALS AND METHODS

Plant material

Bambusa vulgaris shoot was collected from Perunchani in Kanyakumari District, Tamilnadu, India for the proposed study. The collected plants were identified by morphological characters.

Preparation of Plant Extract

The collected fresh shoots were rinsed with distilled water and air dried in shade. The dried plant material was homogenized by electric mixer grinder to obtain coarse powder and stored in air-tight bottles for further analysis. The shade dried, powdered were extracted separately (Mukherjee, 2002) with Acetone solvents by hot extraction method using soxhlet apparatus. The extracts were evaporated in a rotary vacuum evaporator at 40°C to dryness and stored at 4°C in an airtight bottle for further analysis (Ragavendra *et al*., 2011).

Antimicrobial Activity of Acetone Extract of *Bambusa vulgaris*

Antimicrobial activity of acetone extracts of shoot of *Bambusa vulgaris* was carried out by disc diffusion method (Bauer *et al*., 1966; Murrey *et al*., 1995).

Culture Media

The media used for antibacterial test was Nutrient agar and for antifungal test was Potato dextrose agar.

Inoculum

The bacteria were inoculated into nutrient broth and incubated at 37°C for 4 hours. Similar procedure was done for fungal strains by inoculating in Potato dextrose broth for 6 hours.

Microorganisms used

The microorganisms used for antimicrobial studies were obtained from Microbial Type Culture Collection (MTCC), Chandigarh. The different bacterial strains (Gram positive and Gram negative) used are *Bacillus Subtilis* (MTCC 121), *Enterobacter aerogens* (MTCC 111), *Klebsiella pneumoniae* (MTCC 4030), *Staphylococcus epidermidis* (MTCC 6810) and *Enterococcus faecalis* (MTCC 439). The different fungal strains used for study were *Candida glabrata* (MTCC – 3984), *Candida albicans* (MTCC – 183*), Candida tropicalis* (MTCC – 184), *Aspergillus fumigatus* (MTCC – 4354) and *Aspergillus niger* (MTCC – 961).

Antimicrobial activity

Antimicrobial activity of acetone extracts of shoot was determined by disc diffusion method. Briefly, Petri plates containing 20 ml of nutrient agar (for bacteria), potato dextrose agar

(PDA) medium (for fungi) were allowed to dry in sterile chamber. The sterile filter paper discs (Whatman No.1 paper, 5 mm diameter) were impregnated with 10 µg of acetone extracts was placed on the inoculated agar surface. Acetone solvent was placed as negative controls. The plates were incubated at 37°C for 24 hours for antibacterial activity. The inoculated plates were incubated for 48–72 hours at 28°C for antifungal activity. The antimicrobial activity against each test organism was quantified by determining the zone of inhibition around the paper discs in millimeters. Each assay was replicated three times and results expressed as the mean of three replicates. All results were expressed as Mean ±Standard Deviation (SD) of three replicates.

IN VITRO ANTI-PROLIFERATIVE EFFECT OF BAMBOO SHOOT EXTRACTS ON CULTURED HeLa CELLS

HeLa (Human Cervical Cancer) cell lines were purchased from NCCS, Pune. It was maintained in Dulbecco's modified eagle's medium (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, EPPENDORF, GERMANY). The cells were trypsinized (500 µl of 0.025% Trypsin in PBS/ 0.5 mM EDTA solution (Himedia)) for 2 minutes and passaged to T-flasks in complete aseptic conditions. Extracts were added to grown cells at a final concentration of 6.25, 12.5, 25, 50 and 100 µg/ml from a stock of 1 mg/ml and incubated for 24 hours. The % difference in viability was determined by standard MTT assay after 24 hours of incubation.

MTT ASSAY (Arung *et al*., 2009).

MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilized formazan product was measured at 540 nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The cells were washed with 1x PBS and then added with 30µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at 37^0C for 3 hours. MTT was removed by washing with 1x PBS and 200µl of DMSO was added to the culture. Incubation

was done at room temperature for 30 minutes until the cell got lysed and color was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a microplate reader (ELISASCAN, ERBA).

% viability = (OD of Test/ OD of Control) X 100

DETERMINATION OF APOPTOSIS BY ACRIDINE ORANGE (AO) AND ETHIDIUM BROMIDE (EB) DOUBLE STAINING

DNA-binding dyes AO and EB (Sigma, USA) were used for the morphological detection of apoptotic and necrotic cells. AO is taken up by both viable and non-viable cells and emits green fluorescence if intercalated into double stranded nucleic acid (DNA). EB is taken up only by non-viable cells and emits red fluorescence by intercalation into DNA. The cells were cultured in Dulbecco's modified Eagles medium and grown to 60-70% confluency and treated with liver extracts at a final concentration of 100 mcg/ml for 24 h, the cells were washed with cold PBS and then stained with a mixture of AO (100 µg/ml) and EB (100 µg/ml) at room temperature for 10 minutes. The stained cells were washed twice with 1X PBS and observed by a fluorescence microscope in blue filter of fluorescent microscope (Olympus CKX41 with Optika Pro5 camera). The cells were divided into four categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation) and necrotic cells (uniformly orange-stained cell nuclei).

RESULTS AND DISCUSSION

Antimicrobial activity of acetone extracts of shoot of *Bambusa vulgaris*

The antimicrobial activity of acetone extracts of shoot of *Bambusa vulgaris* was investigated by agar disc diffusion method. The used parts of the plant showed antimicrobial activity against bacterial and fungal strains but in varying proportion. In Acetone shoot extract the highest zone of inhibition was exhibited by Enterococcus faecalis 21.67±2.08 (mm) and Klebsiella pneumonia 21.33±1.53 (mm). While the lowest inhibitory activity was showed against *Staphylococcus epidermidis*, 17.67±1.53 (mm). The extract showed activity against all the tested bacterial pathogens. A positive control (Gentamycin) showed activity ranging from 19.6±0.57 mm to

28.6±1.15 mm whereas negative control showed no activity against the bacteria.The results were displayed in the table no:1.

According to (Wang and Ng, 2003), the shoots of bamboo inhibit the growth of microorganism. Bamboo shoots have antimicrobial activity against pathogenic bacteria and fungi. A study found a distinctive antifungal protein from bamboo shoots called Dendrocin. Another research focused on the antimicrobial activity of two novel chitin-binding peptides (Pp-AMP 1 and Pp-AMP 2), from Japanese bamboo shoots (Fujimura *et al*., 2005). Besides, two similar studies found that the dichloromethane extract of bamboo shoots skin could inhibit the growth of *Staphylococcus aureus* (Tanaka *et al*., 2011 & 2013)

Antifungal activity

The antifungal activity of acetone extracts of *Bambusa vulgaris* was explained as follows:

In Acetone shoot extract the highest zone of inhibition was exhibited by *Aspergillus fumigates* 23.67±2.31 (mm) while the lowest inhibitory activity was showed against *Candida tropicalis* 15.67±3.06 (mm). The extract showed activity against all the tested fungal pathogens. A positive control (Nystatin) showed zone of inhibition in the range of 8.3±0.57 mm to17.5±0.86 mm whereas negative control showed no activity against the fungi. The results were displayed in the table no: 2. Manisha Vats *et al*., (2011) reported that petroleum ether and chloroform extracts exhibited prominent antibacterial and antifungal activity. The chloroform extract showed good inhibitory properties for *A. niger >P. Aeruginosa > C. albicans*. Zachariah *et al*., 2008 studied the antibacterial activity and antifungal activity of *Murraya koenigii* against *Staphylococcus aureus* and *Candida albicans* respectively. The maximum inhibitory zone of inhibition of coumarin was against (22 mm) *Bacillus subtilis* and *Candida albicans* (21 mm).

Table: No.1 Antibacterial activity of acetone extracts of *Bambusa vulgaris*

Sr. No	Bacterial Pathogens	Zone of inhibition mm mean ±SD	
		Shoot extract	Gentamycin (50 mcg/disc)
1	*Klebsiella pneumoniae*	21.33±1.53	28.6±1.15
2	*Staphylococcus epidermidis*	17.67±1.53	22.6±0.57
3	*Bacillus subtilis*	19.00±1.00	21.5±0.5
4	*Enterococcus faecalis*	21.67±2.08	22.8±1.04
5	*Enterobacter aerogenes*	20.00±0.00	19.6±0.57

Table: No.2 Antifungal activity of acetone extracts of Bambusa vulgaris

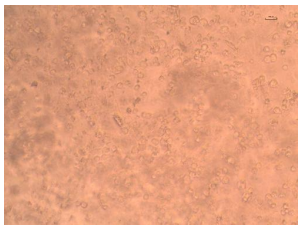
Sr. No	Fungal Pathogens	Zone of inhibition mm mean ±SD	
		Shoot extract	Nystatin (10µg/disc)
1	*Aspergillus fumigates*	23.67±2.31	14.5±0.5
2	*Aspergillus Niger*	23.00±1.73	8.3±0.57
3	*Candida albicans*	21.67±1.53	14.3±1.15
4	*Candida glabrata*	17.67±2.52	9.5±0.5
5	*Candida tropicalis*	15.67±3.06	17.1±0.76

***In vitro* Cytotoxic Activity of Acetone Shoot Extracts of Bamboo**

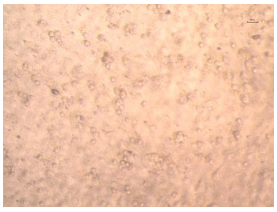
Cytotoxic activity was evaluated in the acetone shoot extracts of Bamboo by MTT assay using Hela cancer cell line. MTT assay confirmed the dose dependent antiproliferative effect of acetone extracts of Bamboo i.e. as dose of extract increases, number of viable cells decreases and the percentage inhibition increases (Fig.1). The absorbance directly correlates with the cell number (Mosmann, 1983). In the present work, the plant extracts showed the dose dependent MTT reduction. Shoot extracts showed low percentage of viability 48.95% at 100 µg/ml concentration.

Table No: 3 MTT assay of Bamboo Shoot

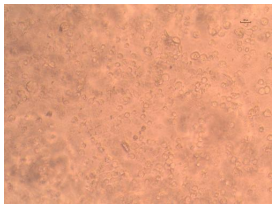
Sample Concentration (µg/ml)	Average Absorbance	Percentage Viability
6.25	0.1876	70.05228
12.5	0.1793	66.95295
25	0.1526	56.98282
50	0.1363	50.89619
100	0.1311	48.95444



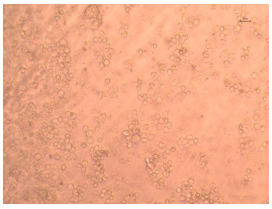
Anticancer activity of Bamboo shoot extract on Hela cell lines (6.25ug/ml)



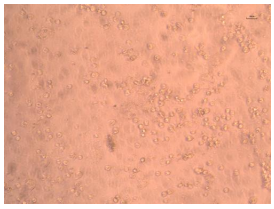
Anticancer activity of Bamboo shoot extract on Hela cell lines (12.5ug/ml)



Anticancer activity of Bamboo shoot extract on Hela cell lines (25ug/ml)



Anticancer activity of Bamboo shoot extract on Hela cell lines (50ug/ml)



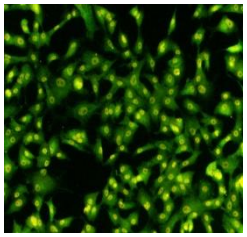
Anticancer activity of Bamboo shoot extract on Hela cell lines (100ug/ml)

Fig.1 Cytotoxic effect of acetone extract of Bamboo shoot

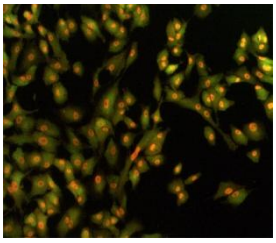
DETERMINATION OF APOPTOSIS BY ACRIDINE ORANGE (AO) AND ETHIDIUM BROMIDE (EB) DOUBLE STAINING.

AO\EB dual stain was employed to identify the apoptotic and necrotic cells. AO will stain the nuclei green by permitting into the cell membrane and EB will stain the nuclei orange when the cytoplasmic membrane integrity is lost. The A549 cells treated with extracts showed more apoptotic cells than control group.

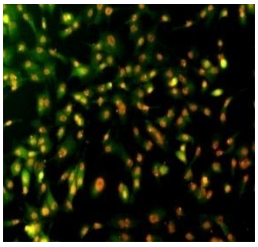
The cells were divided into four categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation) and necrotic cells (uniformly orange-stained cell nuclei)



Apoptosis activity of bambooshoot xtract(control)



Apoptosis activity of bamboo shoot extract (50µg/ml)



Apoptosis activity of bamboo shoot extract (100µg/ml)

Fig.2 Determination of Apoptosis

Apoptosis is important in embryological development, cell proliferation, cell differentiation, elimination of seriously damaged cells or tumor cells by chemopreventive or chemotherapeutic agents and many other physiological processes (Galati *et al* 2000). Apoptotic cells and bodies are rapidly recognized by macrophages before cell lysis, and then can be removed without inducing inflammation. Therefore, the induction of apoptosis is an important mechanism of chemoprevention and chemotherapy of cancer. To determine whether the inhibition of cell proliferation by acetone extract from bamboo shoot was due to the induction of apoptosis, we assessed the latter with the acridine orange/ethidium bromide method. Apoptotic activity of acetone extracts from bamboo shoot was investigated with respect to the morphological shape of cells by fluorescence microscopy. Fluorescence microscopy images clearly showed morphological changes such as reduction in size and cell volume, cell shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies of treated cells (Fig. 2) summarizes the results obtained with AO/EB double staining. Typical morphological changes after different time periods are

shown in Fig. 2. A time dependent increase in induction of apoptosis was also observed. Compared with spontaneous apoptosis observed in control cells, HeLa treated with 100 µg/mL acetone extracts of bamboo shoot showed increased percentages of early apoptotic cells.

The AO/EB and the MTT assays showed similar time-dependent effects on viability but the percent of viabilities were different. In MTT assays absorbance of control, cells were considered as 100% and percentages of viable cells were calculated as ratio to the control, while in AO/EB assay percentages of viable cells are real numbers, calculated as ratio to the total number of cells in treatment. If there is a difference in viability it is because mitochondrial function assays (MTT) detected cell death earlier than others, while apoptosis indicating assays (AO/EB) detected cell death later in the process, which is in correlation with other data (Oh *et al* 2004). According to obtained results, we can conclude that bamboo shoot has antiproliferative properties which increase with exposure time up to 24h when extracts have the best activity. The acetone bamboo shoot extract has better antiproliferative activity with lower IC50 values and induces a higher percentage of apoptotic cells after 12 h of exposure.

CONCLUSION

The antimicrobial study revealed that the extract is having broad spectrum of activity against the tested bacteria and fungi but in varying proportion. Because of this wide antimicrobial activity, the extract can be used in the treatment of various infections or inflammations. The mechanism of action should be identified by further works. Our findings demonstrated that acetone extract of *B. vulgaris* shoot has anti-proliferative effect on human Hela cancer cell line antiproliferative activity using human cancer cell lines may open ways in future *in vivo* studies on prevention and management of cancer.

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