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Research Article

IN VITRO ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF *BAMBUSA ARUNDINACEA* (RETZ.) WILLD

SANDHIYA S[1,*], SUBHASREE N[1], SHIVAPRIYA SHIVAKUMAR[1], ARUNA AGRAWAL[2] AND G.P. DUBEY[2]

[1]Interdisciplinary School of Indian System of Medicine (ISISM), SRM University, Kattankulathur 603203, Tamilnadu, India, [2]National Facility for Tribal and Herbal Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, UP, India. Email sdhiya865@gmail.com.

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ABSTRACT

Objective: Aim of our study is to evaluate the total antioxidant capacity and antibacterial activity of leaf extracts of *Bambusa arundinacea* (Retz.) Willd.

Methods: *Bambusa arundinacea* leaves were collected, powdered and extracted with hexane, ethyl acetate, ethanol and hydroalcohol (70% ethanol) separately. The extracts were screened for their antibacterial activity by agar well diffusion method and antioxidant activity by hydroxyl radical scavenging activity, ferric thiocyanate method and DPPH assay.

Results: Antioxidant potential of hydroalcohol extract tested by DPPH free radical scavenging assay has showed a maximum of 51.41% of percentage inhibition at 100 µg/ml concentration.

Conclusion: The results revealed significant activity for hydroalcohol and ethanol extracts. This supports the folkloric usage of the plant and suggests it possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases.

Keywords: *Bambusa arundinacea*, Antioxidant activity, Antibacterial activity, DPPH, FTC, Hydroxyl radical.

INTRODUCTION

Bambusa arundinacea (*Mulmunkil* in Tamil and *Bams, Kantabams* in Hindi) is a tall thorny tree widely distributed in India. It belongs to the family Graminae, is an ayurvedic medicinal tree commonly known as Bamboo. Tall woody bamboo, stems are thorny and numerous, tufted and grow up to 40 m; branches numerous, internodes of 30–45 cm long, leaves thin, linear, up to 20 cm long and it flowers only once in its lifetime. The different parts of this plant contain silica, cholin, betain, cyanogenic glycosides, albuminoids, oxalic acid, reducing sugar, resins, waxes, benzoic acid, amino acids like lysine, methionine, arginine, cysteine, histidine, vitamins like niacin, riboflavin, thiamine, protein- glutelin, betaine, choline, enzymes like proteolytic enzyme, nuclease and urease. Various parts of this plant such as leaf, root, shoot and seed possess anti-inflammatory, antiulcer [1], anti-diabetic, anti-oxidant, antihelmintic, antifertility [2], antibacterial [3], astringent, emmenagogue activity [4]. In Ayurveda, leaves, stem and root are used as astringent, laxative and as diuretic.

Anti-arthritic activity of *B. arundinacea* in treating Rheumatoid Arthritis (RA) was proved using CFA-induced arthritis animal model. The possible mode of antiarthritic activity of methanolic extract of *B. arundinacea* was proved to be its anti–inflammatory property [5]. It is also mentioned that it has also been used to treat depression in premenstrual syndrome [6]. Tender shoot was used to treat bone fracture externally [7]. It was used as a component in Qalbeen, a herbomineral formulation in the treatment of ischaemic heart disease [8]. It was also a component of a polyherbal formulation, Reosto proved to be effective in the management of postmenopausal osteoporosis (PMO) [9]. Tribal women around Salem in Tamilnadu chew leaves of *B. arundinacea* Retz. in the morning and evening for 1-3 days to induce abortion of an early conception [10]. Leaf decoction is used to stimulate menstruation and as an antispasmodic to help relieve menstrual pain, in dysmenorrhoea and amenorrhea [11]. *Bambusa* leaf juice is given for strengthening the cartilage in osteoarthritis and osteoporosis. It plays a part in the integrity of the bones, arterial walls, skin, teeth, gums, hair and nails and has been used to alleviate eczema and psoriasis [2].

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body and to prevent the deterioration of fats and other constituents of foodstuffs. Oxidative stress causes serious cell damage leading to a variety of human diseases like Alzheimer's disease, Parkinson's disease, atheroscleorosis, cancer, arthritis, immunological incompetence and neurodegenerative disorders, etc. In these cases, there is a preference for antioxidants from natural rather than from synthetic sources [12,13]. Antioxidants are intimately involved in the prevention of cellular damage - the common pathway for cancer, aging, and a variety of diseases. The body relies on obtaining its antioxidants from food and other supplements. Epidemiological studies and intervention trials on prevention of cancer and cardiovascular disease in people taking antioxidant supplements are suggestive that dietary intake of antioxidants can help scavenge free radicals and oxidants and protect the body against diseases [14]. In view of the immense medicinal importance for the antioxidants we aim at evaluating the antioxidant potential of the extracts of *B. arundinacea*.

The aim of the study is to determine the total antioxidant capacity and antibacterial activities of leaves of *B. arundinacea*. To measure the total antioxidant capacity of leaf extracts we chose hydroxyl radical scavenging assay, FTC assay (inhibition of lipid peroxidation) and DPPH assay.

MATERIALS AND METHODS

Extraction of plant materials

Leaves were collected from Kattankulathur, Tamilnadu, India and authenticated at the Department of Botany, Faculty of Science, Banaras Hindu University, Varanasi, India. The plant materials were washed, air-dried for 15 days and milled into coarse powders using cutter mill.

Extraction and determination of extractive value

Five hundred grams of the powdered plant material was cold-macerated with hexane, ethylacetate, ethanol and hydroalcohol (70% ethanol) separately over 48 h. The extracts were filtered through Whatman filter paper No.1 and concentrated under reduced pressure with a rotary evaporator. They were then dried in a hot air oven to constant weights at 40°C and extractive values were calculated on dry weight basis using the equation:

% extractive value (yield %) = (Weight of dry extract/weight taken for extraction) × 100

Antibacterial activity

Agar well diffusion method

The agar well diffusion method was followed to evaluate the antibacterial activity of the extracts [15,16]. The bacterial culture - *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 7443), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 2295) were activated by inoculating a loopful of the strain in the nutrient broth (25 ml) and incubated at room temperature on a rotary shaker. Then 0.2 ml of inoculum (inoculum size was 10^8 cells/ml as per McFarland standard) was poured and spread on to the solidified Mueller Hinton agar media. A well was prepared in the plates with a cup-borer (0.7 cm) and 100 µl containing 100 µg of the test compound was pipetted directly into the well. The plates were incubated overnight at 37°C. For each bacterial strain, controls were included that comprised pure solvents instead of the extract. The experiment was performed thrice under strict aseptic conditions. Antibacterial activity was determined by measuring the diameter of the zone of inhibition.

Antioxidant activity

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the method of Changxing *et al* with some modifications [17]. 1 mL sample solution with different concentrations (0, 0.4, 0.8, 1.6, 3.2 and 4.0 mg/mL) was added to 2 mL of sodium phosphate buffer (150 mM, pH 7.4) containing 10 mM $FeSO_4$, 2 mM sodium salicylate and 6 mM H_2O_2. Incubated at 37°C for 30 min. Absorbance of the hydroxylated salicylate complex was detected at 510 nm using uv spectrophotometer (UV 3200 from Labindia)

Inhibition (%) = $1-(A_0-A_1/A_2) \times 100$

Where A_0 is absorbance of sample with sodium salicylate solution, A_1 is absorbance of tested sample without sodium salicylate solution and A_2 is absorbance of the reagent.

Ferric thiocyanate (FTC) method

A screw-cap vial containing a mixture of 4 mg (4 ml) of a sample (final concentration, 0.02%) in 99.5% ethanol, 4.1 ml of 2.5% linoleic acid in 99.5% ethanol, 8.0 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was incubated at 40°C in the dark. To 0.1 ml of this mixture, 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% ammonium thiocyanate was added. Three minutes after the addition of 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm. This step was repeated every 24 hours until the control reached its maximum absorbance value [18,19,20].

DPPH assay

The DPPH assay was used to measure the free radical scavenging capacity of the plant extracts [21,22]. 200 µL of 0.004% DPPH methanolic solution was pipetted into each well of a 96-well plate followed by 20 µL of sample or standard or solvent for the blank. The mixture was incubated at 30°C for 1 h, and the absorbance at 515 nm was measured with a microplate reader. The inhibition percentage of the radical scavenging activity was calculated using the following formula:

Inhibition (%) = $100 - 100\ (A_S \div A_0)$

Where A_0 is absorbance of the blank and A_S is absorbance of the sample at 515 nm. All assays were conducted in triplicate.

Statistical analysis

Results were expressed as mean (standard deviation SD) of three measurements. Statistical analysis was performed using Student's *t*-test and $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Successful prediction or identification of compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent and we found that plant extracts in ethanol and hydroalcohol (alcohol in combination with water) provided more consistent antimicrobial activity compared to the extracts of hexane and ethyl acetate. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity by their ability to dissolve or diffuse in the different media used in the assay. Ethyl acetate and hexane extracts were not active against all the microorganisms tested. Hydroalcohol as well as ethanol extracts were active against all bacterial strains screened. *E. coli* was the most susceptible bacteria amongst all the bacterial strains investigated in the present work (Table 1).

Table 1: Antibacterial activity of extracts of *Bambusa arundinacea*

Plant Extracts	Zone of inhibition (mm)				
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*K. pneumoniae*
Hexane	-	-	-	-	-
Ethyl acetate	-	-	-	-	-
Ethanol	19 ± 0.5	17 ± 0.2	16 ± 0.6	13 ± 0.1	-
Hydroalcohol	21 ± 0.2	18 ± 0.7	18 ± 0.3	17 ± 0.5	-

- No inhibition

The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipids and proteins. These radicals are highly strong reactive oxygen species and there is no specific enzyme to defense against them in human body [23,24]. Therefore it is necessary to discover some molecules with good scavenging capacity on these. In this study, this capacity of all the extracts of *B. arundinacea* was compared with ascorbic acid. Hydroalcohol and ethanol extracts have showed better activity than those of other two extracts (Fig. 1).

The FTC method was used to measure the amount of peroxide at the beginning of the lipid peroxidation, in which peroxide reacts with ferrous chloride and form ferric ion. The ferric ion then combines with ammonium thiocyanate and produce ferric thiocyanate which is red in colour. Higher the absorbance indicates lower the activity. The absorbance values of none of the extracts exceeded the negative control (without extract) at the end point indicating the presence of antioxidant activity. Except hexane extract all others showed lower absorbance which indicates the strong antioxidant activity of extracts (Fig. 2).

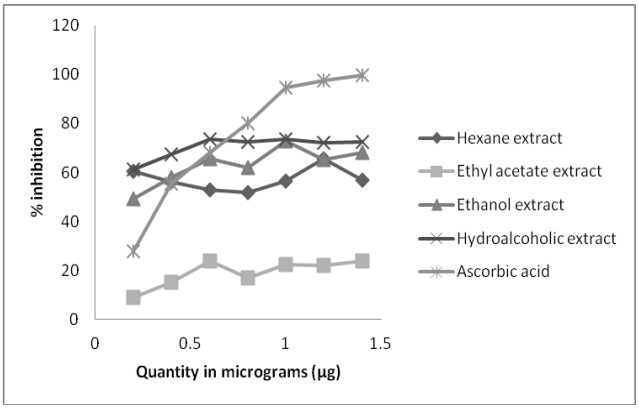

Fig. 1: Percentage inhibition of hydroxyl radical scavenging activity *in vitro* by leaf extracts of *Bambusa arundinacea.*

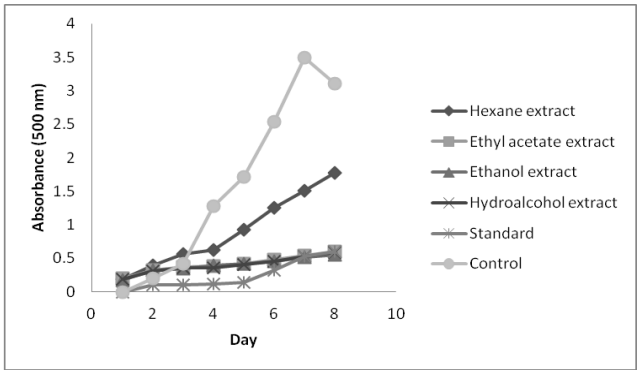

Fig. 2: Antioxidant activity of *Bambusa arundinacea* by Ferric thiocyanate method.

There are many different experimental methods by which the free radical scavenging activity can be estimated. One such method, by which total free radical scavenging activity can be evaluated, is by determining their efficiency to scavenge DPPH radicals. This method is based on the reduction of DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption. Because of its odd electron, DPPH gives a strong absorption maximum at 517 nm by visible spectroscopy (purple colour). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, that is, a free radical scavenging antioxidant, the resulting decolorization is stoichiometric with respect to the number of electrons captured. When the above extracts were tested for the DPPH free radical scavenging ability, the hydroalcohol extract at 100µg/ml showed strong radical scavenging activity with percentage inhibition of 51.41% and hexane, ethyl acetate and ethanol extracts showed relatively poor free radical scavenging activity of 21.75%, 12.96% and 4.31% respectively. The order of scavenging activity was maximum in hydroalcohol extract followed by hexane, ethyl acetate and ethanol extracts. The values are also comparable with commercial antioxidant L-ascorbic acid (60.27%) at the same concentration (Fig. 3).

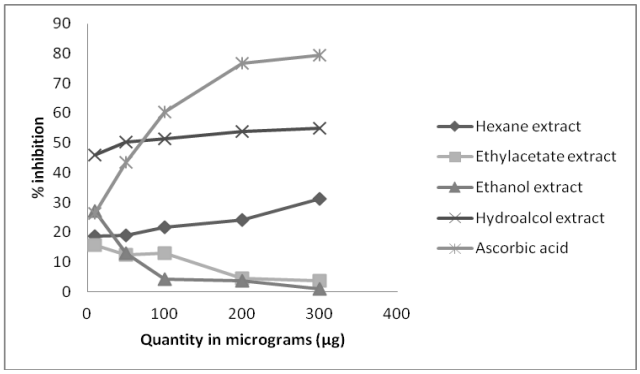

Fig. 3: Percentage inhibition of DPPH radical scavenging activity *in vitro* by leaf extracts of *Bambusa arundinacea.*

From our investigation of screening leaf extracts of *B. arundinacea*, the results obtained confirm the therapeutic potency of the plant used in traditional medicine. In addition, these results form a good basis for selection of candidate plant extract for further phytochemical and pharmacological investigation. The results of the present study support the folkloric usage of the studied plant and suggest it possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extract can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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