



Antibacterial Activity of *Bambusa bambuse* L. against Multiple Drug Resistant (MDR) Bacteria Isolated from Clinical Specimen

Durgesh D. Wasnik, P. M. Tumane

Post Graduate Teaching Department of Microbiology, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur, Maharashtra, India.

*Corresponding author's E-mail: dw_6816@rediffmail.com

Accepted on: 07-01-2014; Finalized on: 28-02-2014.

ABSTRACT

The main objective of this study is antibacterial activity of *Bambusa bambuse* L. leaves extract against clinical isolates from wound infection. As microorganism are becoming resistant to present day antibiotics, our study focuses on antibacterial activity and future prophylactic potential of leaves of *Bambusa bambuse* L. The antibacterial activity of leaves extract of *Bambusa bambuse* L was evaluated on bacterial strains like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp., *Citrobacter* spp., *Acinetobacter* spp., *Streptococcus* spp., *Enterobacter* spp. and *Proteus mirabilis*. All bacteria were isolated from clinical samples are multiple drug resistant to many antibiotics. Antibacterial activity of five different solvent extracts (Methanol, acetone, ethanol, petroleum ether and n-Hexane) were prepared by using Soxhlet extractor. In-vitro antibacterial activity was performed by agar well diffusion method. The ethanolic and methanolic extract of *Bambusa bambuse* L. leaves showed excellent antibacterial activity against all tested multiple drug resistant bacteria. In the present investigation ethanol was the best solvent for the extraction of active component of plants. *Bambusa bambuse* L can be considered to be as equally potent as the antibiotics used now-a-days.

Keywords: Antibacterial activity, *Bambusa bambuse* L, Soxhlet Apparatus, Zone of inhibition.

INTRODUCTION

Bamboo is the longest grass as well as faster grower plant in the world. Bamboo, a group of large woody grasses belonging to the family Poaceae and subfamily Bambusoideae, is one of forest plants which has been used extensively, especially its shoot and wood. The shoot can be processed into various health foods and medicines^{1,2}, while the wood contributes mainly to housing and furniture.³ It consists of a hollow culm or stem, with nodes or joints between segments of the stem and oval leaves. The culm, branches and leaves stay green throughout the bamboo's life, even during winter. The bamboo's lifespan is not very long about 20 years, and it flowers once every 7 to 12 years, depending on the species. With tensile strength superior to mild steel, and a weight-to-strength ratio better than graphite, bamboo is the strongest growing woody plant on Earth. 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.⁴ 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.⁵ Bamboo shoots, both fresh and fermented, are a good source of phytosterols that are the precursors of many pharmaceutically active steroids found in plants and act as neutraceuticals.⁶ 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 the present investigation, the leaves extract of bamboo were subjected for antibacterial activity against multiple drug resistant strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp., *Citrobacter* spp., *Acinetobacter* spp., *Streptococcus* spp., *Enterobacter* spp. and *Proteus mirabilis* isolated from clinical specimen.

MATERIALS AND METHODS

Plant materials

The plant used in this study was *Bambusa bambuse* L. The leaves were collected from the local area of city. After collection, the leaves were shade dried at room temperature (30 - 35°C). 20 gm of leaves of *Bambusa bambuse* L. were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers for further use.

Preparation of extracts

Soxhlet extraction

The dried and powdered leaves materials (20 gms) were extracted with 200 ml of each solvent separately by using Soxhlet extractor for 2 to 5 h at a temperature not exceeding the boiling point of the Solvent [Lin *et al.*, (1999)]. The solvents used for the study were Methanol, Ethanol, Petroleum ether, Acetone and n-Hexane. The extracts were filtered and then concentrated to dryness. The extract were transferred to glass vials and kept at 4°C before use. The extracts were dissolved in 25% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml.



Phytochemical screening

Small quantity of aqueous, ethanol, acetone and methanol extracts of leaves of *Bambusa bambosa* L. was dissolved and used for detection of phytochemicals such as glycosides, phytosterols, proteins, alkaloids, flavonoids, tannins, saponins.⁷

Test for Glycosides

To the solution of extract in glacial acetic acid few drops of ferric chloride and conc. H_2SO_4 are added and observed for reddish brown coloration at the junction of 2 layers and bluish green color in upper layer.

Test for Terpenoids and Steroids

4 mg of extract is treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then conc. solution of H_2SO_4 was added slowly. Red violet color was observed for terpenoids and green bluish for steroids.

Test for flavonoids

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution is warmed and metal magnesium was added to this solution 5-6 drops of con. HCl was added. Red coloration was observed for flavonoids and orange for flavones.

Test for Tannins

To 0.5 ml of extract solution 1 ml water and 1-2 drops of ferric chloride solution was added. Blue color was observed for garlic tannins and greenish black for catecholic tannin.

Reducing sugar

To 0.5 ml of extract solution 1 ml of water and 5-8 drops of Fehling solution was added and observed for brick red colored ppt.

Test for Alkaloids

Most alkaloids are precipitate from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on boiling water bath with 2% HCl. After cooling the mixture was filtered and treated with few drops of Mayer's reagent. The sample then observed for turbidity or yellow ppt.

Test for Saponin

To 50 mg powder and add 20 ml distilled water shake for 15 minutes. Forming 2 cm foam was produced in measuring cylinder indicated positive results.

Test for Phenols

To 50 mg lead powder add 5 ml distilled water and add few drops of neutral $FeCl_3$. Dark green color was develops indicated positive results.

Test for Proteins

1 ml of plant extract add 2 ml of Ninhydrins. For positive results indicates forming purple color.

In vitro testing of extracts for antimicrobial activity

Bacterial Isolates

The bacteria were isolated from clinical sample such as urine, pus, sputum, blood etc. of patients. The antibacterial activity of leaves of *Bambusa bambosa* L. were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp., *Citrobacter* spp., *Acinetobacter* spp., *Streptococcus* spp., *Enterobacter* spp. and *Proteus mirabilis*. These isolate were stored in Nutrient agar slant at 4°C for further use.

Antibacterial assay

Prepare petri plates of sterile molten Mueller Hinton Agar (Hi-Media, Mumbai) at around 40°C and solidify the petri plates. After solidification 6 mm wells were prepared. In these wells solvent extracts of the leaves of *Bambusa bambosa* L. were added. The plate was incubated overnight at 37°C. After incubation the zones of inhibition were measured and recorded. These studies were performed in triplicate.

RESULTS AND DISCUSSION

Bamboo, belonging to the family Poaceae and subfamily Bambusoideae. Bamboo good source of phytosterols that are the precursors of many pharmaceutically active steroids found in plants and act as nutraceuticals. The major component of *Bambusa bambosa* L. contains flavonoids, lactone, phenolic acid. The flavonoids are mainly flavones, glycosides including orientin, homoorientin, isovintexin and vintexin. The role of leaves of *Bambusa bambosa* L. as an antioxidant, anti-inflammatory, anti-aging and anti-wrinkle factors. In this study, the phytochemical analysis of ethanolic extract of *Bambusa bambosa* L. showed the presence of Flavonoids, Phenols, Tannins, Terpenes, Saponins as well as carbohydrates and proteins [Table 1].

In the present study the antibacterial activities of the leaves of *Bambusa bambosa* L. has been investigated. The ethanolic and methanolic leaves extract of *Bambusa bambosa* L. showed excellent inhibitory action against all tested multiple drug resistant bacteria. These bacteria were resistant more than five antibiotics. The ethanolic extract showed highest inhibitory activity against *Proteus mirabilis*, *Streptococcus* spp. and *Citrobacter* spp. having 18 mm zone of inhibition. *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Acinetobacter* spp. showed 16 mm zone of inhibition. The least inhibitory activity found against *E. coli* (15mm), *S. aureus* (14 mm) and *Enterococcus* spp. (14 mm) [Table 2, Figure 1]. The study conducted by Vijay Kumar Singh et. al., (2010) carried out antibacterial activity of ethanolic extract of Bamboo leaves gave inhibitory activity against *S. aureus*, *E. coli* and *Pseudomonas* Spp. showed 2.1mm, 3.1mm and 1.2mm respectively.⁸ Comparing their results with our study with the same test organism and solvent shows that *Bambusa bambosa* L. extract gave excellent zone of inhibition shown in Table 2. However, this difference may be because of the difference in the phytochemical



composition in various part of the plant or may be also due to the extraction method used and/or environmental factors or difference in the genotypes of the Bamboo plant used.

Table 1: Analysis of phytochemical constituent of *Bambusa bambose* L. extract

Phytochemical components	Name of Test	Ethanollic Extract	Methanollic Extract
Alkaloids	Mayer's test	++	+
Flavonoids	Alkaline Reagent Test	++	+
Carbohydrates	Molisch's Test	++	-
Phenols	Ferric Chloride Test	++	+
Tannins	5% Ferric chloride	++	-
Steroids	Chloroform + Acetic acid + H ₂ SO ₄	+	+
Glycosides	Legal's Test	++	-
Proteins	Ninhydrin Test	++	+
Diterpenes	Copper acetate Test	+	+
Saponins	Distilled water + Heat	+	+

- = absent; ++ = Present in More Quantity; + = Present

Table 2: Antibacterial activity of *Bambusa bambose* L. extract against clinical isolates

Multiple Drug Resistant Bacteria	Zone of Inhibition on mm				
	<i>Bambusa bambose</i> L.				
	E	M	P	A	H
<i>E. coli</i>	15	10	10	NZ	11
<i>S. aureus</i>	14	10	08	10	11
<i>Pseudomonas aeruginosa</i>	16	12	08	NZ	NZ
<i>Proteus mirabilis</i>	18	14	NZ	11	08
<i>Streptococcus</i> spp.	18	16	NZ	12	NZ
<i>Klebsiella</i> spp.	15	12	NZ	08	NZ
<i>Enterococcus</i> spp.	14	12	NZ	NZ	08
<i>Enterobacter</i> spp.	16	12	NZ	NZ	NZ
<i>Acinetobacter</i> spp.	16	08	NZ	NZ	NZ
<i>Citrobacter</i> spp.	18	13	08	10	NZ

E – Ethanolic extract; M- Methanolic extract; P – Petroleum ether; A- Acetone extract; H- n-Hexane extract; NZ – No Zone

The methanolic extract of *Bambusa bambose* L. showed highest inhibitory activity against *Streptococcus* spp. (16 mm) followed by *Proteus mirabilis* (14 mm), *Citrobacter* spp. (13 mm), *Pseudomonas aeruginosa*, *Klebsiella* spp., *Enterococcus* spp. and *Enterobacter* spp. showed 12 mm zone of inhibition. The least zone of inhibition was found against *E. coli* (10 mm), *S. aureus* (10 mm) and *Acinetobacter* spp. (8 mm) [Table 2, Figure 1]. Petroleum ether and n – Hexane showed good inhibitory activity

against *E.coli*. The acetone extract showed highest inhibitory activity against *Streptococcus* spp. (12 mm), *Proteus mirabilis* (11 mm), *Citrobacter* spp. (10 mm) and *S. aureus* (10 mm) [Table 2, Figure 1]. The difference in the antibacterial activity with the same source when extracted with different solvent has proven that not all phytochemicals that are responsible for antibacterial activity are soluble in a single solvent. Hence solvents of different polarity should be employed as discussed in this study (polar: water, acetone, ethanol; non-polar: ethyl acetate, petroleum ether).⁹ The study has done by S. Anil Singh [2012] using methanolic extract of fermented *Bambusa balcooa* shoots was effective against *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* showed 11mm, 18mm and 13mm zone of inhibition.¹⁰ While comparing with our study with same tested organisms using methanolic leaves extract of Bamboo showed relative similar results. But in our study ethanolic extract showed highest yields as well as antibacterial activity compare to other solvent. Systematic screening of plants may lead to the discovery of novel active compounds. In the present study, the *Bambusa bambose* L. shows good inhibitory activity against all tested multiple drug resistant bacteria. This study helps in formulation of new drug for the treatment of infectious diseases caused by multiple drug resistant (MDR) bacteria.

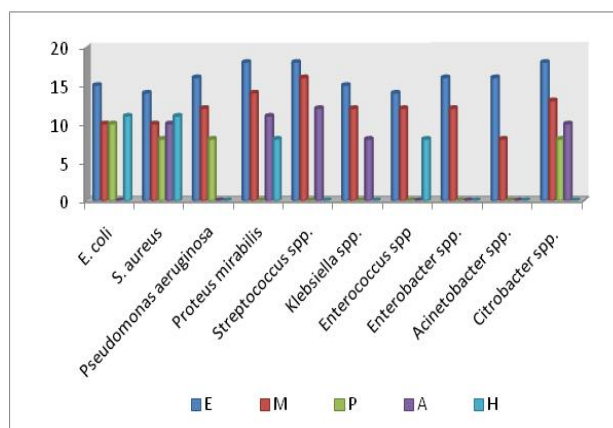


Figure 1: Antibacterial activity of *Bambusa bambose* L. extract against clinical isolates.

CONCLUSION

In conclusion, *Bambusa bambose* L. leaves extract possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds. Also further studies will be needed to purify the bioactive compounds of the ethanol extracts and characterize the aqueous fraction of the plants and their phytochemical mode of action should be further investigated.

REFERENCES

1. Choudhury D, Sahu JK, Sharma GD, Value addition to bamboo shoots: a review, J Food Sci Technol 2011, DOI 10.1007/s13197-011-0379-z.

2. Tanaka A, Hyo JK, Oda S, Shimizu K, Kondo R, Antibacterial activity of moso bamboo shoot skin (*Phyllostachys pubescens*) against *Staphylococcus aureus*, *J Wood Sci* 57, 2011, 542-544.
3. Satya S, Singhal P, Bal LM, Sudhakar P, Bamboo shoot: a potential source of food security, *Mediterr. J. Nutr. Metab*, 5, 2012, 1-10.
4. Singh SA, Singh HD, Robbarts N, Bora TC, Singh NR, Comparative Study of Chemical Properties of Soibum- A Traditional Fermented Bamboo Shoot Product and Its Biological Investigation, *Int. J. Biosci., Biochem and Bioinfo.*, 1(2), 2011, 114-118.
5. Lu BY, Wu X, Tie X, Zhang Y, Zhang Y, Toxicology and safety of antioxidant of bamboo leaves. Part I: acute and subchronic toxicity studies on antioxidant of bamboo leaves, *Food Chem Toxicol*, 43(5), 2005, 783–792.
6. Miettinen TA, Non-nutritive bioactive constituents of plants: phytosterols, *Int. J. Vit. Nut. Res*, 73, 2003, 127–134.
7. Sofowora H, Screening Plants for Bioactive Agents In: *Medicinal Plants and Traditional Medicine in Africa*, Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, 2, 1993, 134-156.
8. Vijay kumar Singh, Rahul Shukla, Satish V, Shankul Kumar, Sumit Gupta, Ashutosh Mishra, Antibacterial Activity of Leaves of Bamboo, *International Journal of Pharma and Bio Sciences*, 1(2), 2010, 1-5.
9. Ashok Kumar K, Narayani M, Subanthini A, Jayakumar M, Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels -Utilization of Fruit Waste, *International Journal of Engineering Science and Technology (IJEST)*, 6 June 2011 ISSN : 0975-5462 Vol. 3 No.
10. Anil Singh S, Bora TC, Rajmuhon Singh N, Preliminary Phytochemical Analysis and Antimicrobial Potential of Fermented Bambusa Balcooa Shoots, *The Bioscan*, 7(3), 2012, 391-394.

Source of Support: Nil, **Conflict of Interest:** None.

