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ANTI-OXIDANT AND ANTIBACTERIAL POTENTIALS OF *Bambusa blumeana* J. A. and J. H. Schultes AND *Bambusa vulgaris* Schrad.ex Wendl. SHOOT EXTRACTS

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ABSTRACT

Antioxidant, antibacterial potential and the phytochemical constituents of *Bambusa blumeana* and *Bambusa vulgaris* shoots extracts were explored. As eradicant against *E. coli*, at 12 and 24 hours of incubation the widest zone of inhibition was observed in paper discs treated with *B. blumeana* ethanol extract with 10.05 mm while in *S. aureus* the widest zone of inhibition was observed in paper discs treated with *B. vulgaris* with 14.63 mm and 13.76 mm, respectively. Moreover, protectant test showed that the smallest zone of bacterial colonization of *E. coli* and *S. aureus*, were observed in plates treated with *B. vulgaris* ethanol extract with 7.54 mm, 6 mm, 14.25 mm and 8.89 mm respectively, at 12 and 24 hours of incubation. DPPH radical scavenging assay and total phenolic content revealed the antioxidant potential of bamboo shoots. Among the tested bamboo shoot, *B. Blumeana* ethanol extract had the highest radical scavenging activity while *B. vulgaris* had the highest total phenolic content. These pharmacological activities can be due to the presence of phytochemicals such as cardiac glycosides, saponins, tannins, steroids and terpenoids.

Keywords: Anti-oxidant, Antibacterial Potentials, *Bambusa blumeana*, *Bambusa vulgaris*

INTRODUCTION

In the Philippines, bamboo shoots are locally known as nutritious and healthy food which is usually abundant during rainy seasons. Whereas in China and South Asian

countries, bamboo shoots have been regarded as one of the traditional medicines. It is believed that bamboo extract may have antioxidant activities, antimicrobial and anti-inflammatory effects [1, 2, 3, 4, 5, and 6]. Several studies revealed the presence of phytochemicals in different parts of bamboo plant. Phytochemicals are considered as non-essential nutrients and non-nutritive plant chemicals that have protective or disease preventive properties [7]. Phytochemicals in bamboo includes flavonoids and their glucosides, active polysaccharides, special amino acids and their peptides, aromatic elements as well as microelements such as manganese, zinc and selenium which was already been proven to have biological functions in the production of novel drugs [8, 9].

In the present study, antibacterial, antioxidant and phytochemical constituents of *Bambusa vulgaris* and *B. bluemana* were elucidated.

MATERIALS AND METHODS

The study was divided into three sub-studies. Sub- Study 1 dealt with evaluation of the antibacterial potential of the bamboo shoots extracts as protectant and eradicant against *E. coli* and *S.aureus*; Sub-Study 2 determined the anti-oxidant activity through DPPH free radicals scavenging activity and total phenolic content and the screening of the phytochemical constituents of the bamboo shoot extracts were performed in Sub-Study 3.

Preparation of Bamboo Shoots Extracts

Young shoots samples of *B. blumeana* and *B. vulgaris* were collected at Brgy. Gabaldon, Science City of Munoz, Nueva Ecijaand Brgy. Macarse, Zaragosa, Nueva Ecija. The bamboo shoots were cut into small pieces, air dried and grinded into powdered form. The powdered bamboo shoots were extracted using 80% ethanol and hot water as solvents in a ratio of 1:4.

Sub-study 2. Evaluation of the Antibacterial Activity of Bamboo Shoot Extracts

Preparation of bacterial culture

Disc diffusion method at 12 to 24 hours of incubation was used to determine the antibacterial activity of the bamboo shoots extracts against *E.coli* and *S. aureus.* The bacterial cultures were grown in Nutrient Agar and were transferred in Nutrient Broth for 24 hours and were standardized to 1.5 x 10^8 cells/ml using McFarland standards.

Preparation of the assay plates and Paper discs

In sterile Erlenmeyer flask, 19 g of Mueller Hinton Agar II was mixed with 500 ml of distilled water. The mixture was placed

in a hot plate stirrer to dissolve. Then, it was sterilized at 121°C (15psi) for 15 minutes. After the sterilization, the agar was distributed in the assay plates with approximately 20ml each. Whatman Filter paper No. 1 circular discs with a diameter of 5mm were used as paper discs. The paper discs were sterilized in an autoclave at 121°C (15psi) for 15 minutes.

Eradicant test

The paper discs were soaked in the shoot extracts and were allowed to dry for 30 minutes. Then, 0.1 ml of the bacterial suspension was poured in the previously plated plates and was spread using T-rod. The discs were soaked in different treatments and were seeded equidistantly in the plates. The zone of inhibition was measured using a digital Vernier caliper at 12 and 24 hours of incubation.

Protectant test

The paper discs were soaked in the bacterial suspension. Previously platedplates were poured with 0.1 ml of the shoot extracts. The paper discs were seeded equidistantly in the plates and the plates were stored at room temperature and zone of bacterial colonization was measured using a digital vernier caliper after 12 and 24 hours of incubation.

Sub-study 2. Antioxidant Activity and Total Phenolic Content of Bamboo Shoots Extracts

The DPPH radical scavenging activity and the total phenolic content of hot water and ethanol extracts of *B. blumeana* and *B. vulgaris* shoots were analyzed at the Chemistry Laboratory of Center for Natural Sciences at St. Mary's University, Bayombong, NuevaVizcaya.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the ethanol and hot water extracts from the shoots of *B. Blumeana* and *B. vulgaris* using DPPH were assayed following the procedure described by Kolak et al.[11]. The extracts were dissolved in methanol to a final concentration of 500 ppm. A 0.1 mM DDPH in methanol were freshly prepared by diluting 1 mL DPPH stock solution (3.49 mg DPPH in 10 ml methanol) to 100 mL methanol. Then, 1 ml of the extract and 4 mL of DPPH solution were mixed and incubated in the dark at 37° C for 30 minutes. Triplicate test was done in each extracts. The absorbance reading was monitored at 517 nm using UV- Vis spectrophotometer (APEL-100) and the ability to scavenge the DPPH radical was calculated using the equation below:

% DPPH scavenging effect = [($A_{control}$- A_{sample})/ $A_{control}$] x l00

Where $A_{control}$was the absorbance of the control, which is the DPPH solution without the extract, the $A_{sample}$was the absorbance of the test sample containing the mixture of DPPH and the ethanol and crude extract. The synthetic antioxidant cathechin was used as positive control.

Total phenolic content

The total phenolic content of the ethanol and hot water extractswere determined using the Folin-Ciocalteu method as described by Hodzic et al [12].At first, a calibration curve was made at different concentrations (0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml) ascorbic acid using APEL-100 UV-vis spectrophotometer (PD-303UV). These ascorbic acid solutions (volume of 200 microliter/mcL) were placed in vials. To each vial, 200 mcL of Folin-Ciocalteu reagents were added and the vials were incubated at room temperature for 5 minutes. For this test, 20 ml of sodium carbonate with the concentration of 1 mg/ml was prepared. Then, 1 ml sodium carbonate was added to the mixture of ascorbic acid and FC reagent was transferred to cuvettes and read using UV-Vis spectrophotometer at 680 nm wavelength. After standardizing the calibration curve, 3 mg of the extract was dissolved in a 3 ml distilled water. From each of the extract, 200 mcL was transferred to vials (in triplicates) and incubated at room temperature for 5 minutes. Then, 1 ml sodium carbonate was added to the mixture of the extracts and FC reagent and was read using the APEL-100 UV-vis spectrophotometer (PD-303UV) at 680 nm wavelength. Absorbance values of the extracts were compared with the calibration curve using the ascorbic acid. The total phenolic was calculated based on the standard curve of the ascorbic acid and its linear regression using the equation below:

Y=mx+b

Where y represented the OD, m represented the slope of ascorbic acid, and b represented the y-intercept. Ascorbic Acid Equivalent (AAE) was expressed as AAE/gram sample.

Sub-study 3. Screening of Phytochemical Composition of Bamboo Shoots

Screening of phytochemical constituent of bamboo shoots were carried out by following the standard methods described in Laboratory Manual for the UNESCO [13, 14]. The various phytochemical constituents were alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, and terpenoids.

Test for alkaloids

Five milliliters of the extracts were prepared in a beaker and 200 ml of 10% HCH_3CO2 in C_2H_5OH was added. The mixture was filtered and the filtered extract was allowed to become concentrated in a water bath until it reached one fourth of the original volume. NH_4OH was added and the changes were then observed. Observed formation of the white precipitate or turbidity indicated the presence of alkaloids.

Test for cardiac glycosides

One milliliter of concentrated sulphuric acid (H_2SO_4) was prepared in the test tube. Five milliliters of bamboo shoot extract was mixed with 2 ml of glacial HCH_3CO_2 containing one drop of $FECl_3$. The mixture was added carefully to 1 ml of concentrated H_2SO_4 that the concentrated H_2SO_4 underneath the mixture. The appearance of brown ring was observed for the presence of cardiac glycosides.

Test for flavonoids

Five (5) ml extract of bamboo shoot and a5 drops of 1% ammonium (NH_3) solution were mixed in the test tube and yellow coloration is an indication for the presence of flavonoids.

Test for saponins

A volume of 0.5 ml of the extract was added to 10 ml of sterile distilled water and was shaken vigorously to obtain a stable persistent froth.

Test for steroids

Two milliliters of acetic anhydride was added to a 5 ml extract of plant sample with 2 mL of H_2SO_4, formation of violet to blue or green precipitate was observed for the presence of steroids.

Test for tannins

In a 10 ml of distilled water, 0.5 ml of extract and two (2) ml of 5% FeCl was added, brownish green to blue black coloration signifies the presence of tannins.

Test for terpenoids

Five milliliters of extracts was added with 2 ml $CHCl_3$and then, three (3) ml of $H_2SO_4$was added, formation of the reddish brown of the interface was observed for the presence of terpenoids.

RESULTS AND DISCUSSION

Sub-study 1. Antibacterial Activity of Bamboo Shoots

In this study the antibacterial activity(as eradicant and protectant) of *B. blumeana* and *B. vulgaris* shoots extracts were evaluated using disc diffusion method against *E. coli* and *S. aureus* at 12 and 24 hrs of incubation.

Bamboo shoot extracts as Eradicant

Zones of bacterial inhibition were observed for the eradicating potential of the

bamboo shoots extracts. Eradicants inhibit and prevent infections caused by the pathogenic microorganisms. Among the four extracts used against *E. coli*, *B. blumeana* ethanol extract had the widest zone of inhibition at 12 hours of incubation of 10.05 mm, followed by *B. vulgaris* ethanol extract with 9.09 mm. Furthermore, at 24 hours of incubation reduction in the zone of inhibition was observed. The widest one of inhibition was recorded in *B. vulgaris* ethanol extract of 8.66 mm followed by 8.50 mm in *B. blumeana* ethanol extract. Mean while, against *S. aureus,* ethanol extract of *B. vulgaris* had the largest zone of inhibition after 12 and 24 hours of incubation with 14.63 mm and 13.76 mm, respectively. Followed by the ethanol extract of *B. blumeana* with 10.76 mm and 8.86 mm. Statistical analysis revealed that the zone of inhibition produced by the ethanol extract of *B. vulgaris* and *B. blumeana* shoots. These suggest the potential antibacterial activity *B. blumeana* and *B. vulgaris* ethanol extracts as eradicant against *E. coli* and *S. aureus*(Table1).

Table 1: Diameter of zones of inhibition of bamboo extracts against *E. coli* at 12 and 24 hours of incubation

Treatments	Diameter Zone of Inhibition(mm)			
	E. coli		*S. aureus*	
	12 hours	24 hours	12 hours	24 hours
***B. vulgaris* ethanol extract**	**9.09^{b}**	**8.66^{b}**	**14.63^{b}**	**13.76^{b}**
***B. blumeana*ethanol extract**	**10.05^{b}**	**8.50^{b}**	**10.76^{c}**	**8.86^{c}**
***B. vulgaris* Hot water extract**	**7.31^{bc}**	**7.38^{bc}**	**7.79^{d}**	**7.82^{c}**
***B. blumeana* Hot water extract**	**8.56^{bc}**	**7.51^{bc}**	**7.82^{d}**	**7.33^{cd}**
Streptomycin sulfate	**25.67^{a}**	**26.38^{a}**	**25.89^{a}**	**27.82^{a}**
Sterile distilled water	**6.00^{c}**	**6.00^{c}**	**6.00^{d}**	**6.00^{d}**

***Means with the same letter superscript are not significantly different at 5% level of significance by Duncan Multiple Range Test (DMRT).**

Bamboo shoot extracts as protectant

For the protectant test, the smaller the zone of bacterial colonization the more effective the extract will be, since the action of the protectant is to prevent or to hinder bacterial growth. The result of protectant test of ethanol and hot water extracts of *B. blumeana* and *B. vulgaris* against *E. coli* and *S. aureus* are presented in Table 2. At 12 hours of incubation, the smallest zone of *E. coli* colonization was obtained in plates treated with *B. vulgaris* ethanol extracts with 7.54 mm followed by hot water extract of *B. blumeana* with 12.94 mm. After 24 hours of incubation, zone of *E. coli* colonization in *B. vulgaris* ethanol extract treated plates have increased to 14.25 mm. Meanwhile for *S. aureus*, no zone of *S. aureus* colonization was observed in plates treated with *B. vulgaris* ethanol extract. While small zones of bacterial colonization were observed in plates treated with ethanol and hot water

extract of *B. blumeana* with 8.97 mm and 11.27 mm, respectively. While at 24 hours of incubation, zones of *S. aureus* colonization in plates treated with ethanol extract of *B. vulgaris* was 8.89 mm, followed by *B. blumeana* hot water extract with 12.34 mm. The results signified that the ethanolic bamboo shoot extract are potent protectant against *S. aureus* at all incubation period.

The results of study showed that *B. blumeana* and *B. vulgaris* ethanol extracts are more effective that the hot water extracts as eradicant and protectant against *S. aureus* and *E. coli* at all incubation period. Similar results were demonstrated in the study of Singh et al. [10] and Vijay et al. [15] wherein the ethanolic extract showed better antibactericidal activity. Also, ethanol is one of the best solvent for antimicrobial substances in plants[16, 17].Additionally, in most cases plant extract are much active against gram-positive bacteria than gram-negative bacteria[18].

Antioxidant Activity and Total Phenolic Content of *B. blumeana* and *B. vulgaris* shoot extracts

In this study the antioxidant property of two selected bamboo species were evaluated using DPPH radical scavenging assay. Antioxidants are compounds that prevent or act against oxidative process of free radicals [19, 20, 21, and 22].

Table 3 showed that all of the extracts exhibited radical scavenging activity. Ethanol extract of *B. blumeana* exhibited the highest radical scavenging activity of 58.66% followed by *B. vulgaris* ethanol extract, *B. blumeana* hot water extract and lastly *B. vulgaris* hot water extract with 58.10%, 54.19% and 52.51% respectively. In addition, the total phenolic content of shoot extract of *B. vulgaris* and *B. blumeana* were also determined. *B. vulgaris* ethanol extract has the highest total phenolic content of 30.45mg AAE/g followed by 28.36 mg AAE/g by ethanol extracts and the lowest is *B.blumeana* hot water extract with 20.12mg AAE/g of sample. Thus, the results proved that *B. blumeana* and *B. vulgaris* possess anti-oxidant properties.

Detection of the anti-oxidant and phenols in bamboo shoots is in accordance with previous studies [1, 2]. Also, phenols are reported to contain high level of antioxidant activities due to the presence of hydroxyl groups which can eliminate free radicals[23, 24, 25, and 26].

Phytochemical analysis of the extracts

Phytochemicals exhibit a wide range of beneficial biological effect and could neutralize oxidation of biological molecules

by scavenging free radicals and chelating free catalytic metals. These phytochemicals possess antioxidant activity [27].

As presented in Table 4, cardiac glycosides, saponins, and tannins were detected in both ethanolic and hot water extracts of *B. blumeana* and *B. Vulgaris* shoots. However, flavonoids were only found in ethanol and hot water extracts of *B. vulgaris*, while steroids and terpenoids were only present in ethanol extract of the two bamboo shoots.

Table 2: Diameter zone of colonization of *E. coli andS, aureus* in different bamboo shoots extracts

Treatments	Diameter Zone of Colonization(mm)			
	E. coli		*S. aureus*	
	12 hours	24 hours	12 hours	24 hours
***B. vulgaris* ethanol extract**	7.54^{c}	14.25^{b}	6.00^{d}	8.89^{bc}
***B. blumeana*ethanol extract**	19.20^{bc}	28.52^{a}	8.97^{cd}	18.10^{b}
***B. vulgaris* Hot water extract**	18.13^{b}	22.88^{b}	13.65^{ab}	22.88^{a}
***B. blumeana* Hot water extract**	12.94^{ab}	17.00^{b}	11.27^{bc}	12.34^{b}
Streptomycin sulfate	6.00^{c}	6.00^{c}	6.00^{d}	6.00^{c}
Sterile distilled water	20.75^{a}	29.23^{a}	15.35^{a}	21.65^{a}

*Means with the same letter superscript are not significantly different at 5% level of significance by Duncan Multiple Range Test (DMRT).

Table 3: Radical scavenging activity of bamboo extract against DPPH radical and total phenolic content

Treatment	Radical Scavenging Activity (%)	Total Phenolic Content (mg AAE/g of sample)
***B. vulgaris* ethanol extract**	**58.10%**	**30.45mg AAE/g of sample**
***B. blumeana* ethanol extract**	**58.66%**	**23.19mg AAE/g of sample**
***B. vulgaris* hot water extract**	**52.51%**	**28.36mg AAE/g of sample**
***B. blumeana* hot water extract**	**54.19%**	**20.12mg AAE/g of sample**

Table 4: Phytochemical analysis of *B. blumeana* and *B. vulgaris* shootextracts

Phytochemical Composition	*Bambusablumeana*		*Bambusa vulgaris*	
	Ethanol extract	Hot water extract	Ethanol extract	Hot water extract
Alkaloids	-	-	-	-
Cardiac glycosides	+	+	+	+
Flavonoids	-	-	+	+
Steroids	+	-	+	-
Saponins	+	+	+	+
Tannins	+	+	+	+
Terpenoids	+	-	+	-

(+) present; (-) absent

In previous study of Chandrawaliet al. [28], alkaloids, steroids, tannins terpenoids and saponins have been isolated from the shoots of *B. vulgaris*.Similarly, Nazreen et al.[29], Zhang et al.[30], and Zhang et al [31], revealed that extract of bamboo leaf mainly contains flavone glycosides, phenolic acids, coumarin lactones, flavonoids, polysaccharides and peptides. In addition, phytochemical analysis of the aqueous leaf extract of *B. vulgaris* revealed the presence of alkaloids, tannins, phenolics, glycosides, saponins, flavonoids andanthraquinones [32].

In totality, the superiority of the ethanolic extracts of *B. vulgaris* and *B.*

blumeana as anti-microbial agents and sources of anti-oxidant can be correlated and attributed to the presence of phytochemicals. As previously reported, the benefits of bamboo shoots can be attributed to the presence of phytochemicals such as tannins and flavonoids [33, 34]. Studies have shown that there are several types of flavonoids that present biological activity, which produce significant anti-inflammatory and antimicrobial action which is due to its capacity in complexing extracellular and soluble proteins such as structures from bacterial cell walls [35, 36, 37, and 38].Furthermore, flavonoids is another constituent of bamboo shoots that exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, analgesic, anti-allergic, cytostatic and antioxidant properties [39]. Also, alkaloids are toxic to microbes that effectively kill bacteria and viruses, fungi and they also possess antifungal and anti-inflammatory and anti-hypertensive agents [40, 41, and 42].

Phytochemical screening of bamboo shoots revealed that flavonoids were only present in *B. vulgaris* ethanol and hot water extracts, which could attributed to high total phenolic content of *B. vulgaris.* Likewise, antioxidant activity of the bamboo shoots can be due to the presence of different phytochemicals such as cardiac glycosides, saponins, steroids, tannins and terpenoids. Bamboo shoots are also reported to have anticancer, antibacterial, and antiviral activity due to the presence of lignans that are an important component of fiber [43].

CONCLUSION

Bambusa vulgaris and *Bambusablumeana* shoots extracts are potent sources of anti-oxidant compound. In addition, ethanolic shoot extracts showed antibactericidal activity against *E.coli* and *S. aureus*.

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