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Bambusa blumeana var. *Luzonensis* Schultes AND *Schizostachy brachycladum* Kurz SHOOT EXTRACTS AS POTENTIAL NATURAL SOURCES OF ANTI BIOTICS AND ANTI-OXIDANT

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ABSTRACT

B. blumeana var. *luzonensis* and *S. brachycladum* shoot ethanol and hot water extracts as potential natural sources of antibiotics and anti-oxidantand profiling of their phytochemical constituents was carried out. Cardiac glycosides, flavonoids, saponins, tannins and terpenoids were present in all bamboo shoots extracts while steroid was also detected in the ethanol extracts of *B. blumeana* var. *luzonensis* and *S. brachycladum* shoots.*S. brachycladum* shoot ethanol extract formed the biggest zone of inhibition at 12 and 24 hours of incubation with 8.68 mm and 8.36 mm while *S. brachycladum* shoot hot water extract produced the widest zone of incubation against *S. aureus* with 13. 40 mm and 15.32 mm. Meanwhile, for the protectant test, *E. Coli* produced the smallest zone of colonization in *B. blumeana* var. *luzonensis* ethanol extract with 7.14 mm and 9.97 mm while *S. aureus* formed the smallest zone of colonization in *S. brachycladum* shoot ethanol extract of 6.28 mm after 12 hours of incubation. Furthermore, all bamboo shoot extracts possessed DPPH radical scavenging activity. *B. blumeana* var. *luzonensis* shoot ethanol extract had the highest yield with 64. 80 % radical scavenging activity and 27.59 mg AAE/g sample phenolic content.

Keywords: *Bambusa blumeana* var. *Luzonensis*, *Schizostachy brachycladum*,anti biotics, anti-oxidant

INTRODUCTION

Herbal medicine has been a part of culture around the world and it represents one of the most important fields of traditional medicine [1]. According to Singh and Das [2], the medicinal applications of bamboo in the traditional medicine system were first mentioned around 500 AD. Based from previous studies, the phytochemicals present in bamboo shoots are effective antioxidants. Additionally, they possess anti-bacterial, anticancer, and anti-fungal properties. Several researches also 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 and anti-cancer property [2, 3, 4, 5, 6, 7, 8, 9, 10]. Furthermore, bamboo shoots also contain high proteins, amino acids, carbohydrates, many important minerals, and vitamins (thiamine, niacin, vitamin A, vitamin B6, and vitamin E)[11, 12].

Many studies on the pharmacological potentials of different parts of bamboo of various species had already been conducted but none or little focused on *B. Blumeana var luzonensis* and *S.brachycladum.* These are two of the endemic species of bamboo in the Philippines, which are known for their many uses. However, their medicinal importance has never been explored. Thus, the present study aimed to determine their potential biological activities and to identify the phytochemical constituents of the aforementioned bamboo shoots extracts.

MATERIALS AND METHODS

Preparation of Bamboo shoots extracts

Young shoots of *B. Blumeana* var. *luzonensis* and *S. Brachycladum* were obtained in Digdig Caranglan, Nueva Ecija and Isabela, Philippines. The bamboo shoots were cut into small pieces, air-dried and were grinded into powdered form. For the extraction, hot water and 80% ethanol were used as solvents.

Screening of Phytochemical Composition of Bamboo Shoots

Table 1, presents the procedure used in the screening of phytochemical constituent of bamboo shoots.

Table 1: Phytochemical Screening of Bamboo Shoots

PHYTOCHEMICAL TESTED	PROCEDURE	POSITIVE RESULTS
ALKALOIDS	5 ml extract + 10% (HCH_3CO2) in C_2H_5OH + NH_4OH	Formation of white precipitate or turbidity
CARDIAC GLYCOSIDES	1 ml H_2SO_4 + (5ml extracts + 2 ml of glacial HCH_3CO_2+ $FECl_3$)	Formation of brown ring
FLAVONOIDS	5 ml extract + 5 drops NH_3	Yellow coloration
SAPONINS	of 0.5 ml of the extract + 10ml H_2O	Persistent frothing
STEROIDS	(5 ml extract +2 ml of H_2SO_4) + 2ml acetic anhydride	Violet to blue or green precipitate
TANNINS	0.5 ml extract+ 10ml H_2O+ 2ml 5% FeCl	Brownish green to blue-black coloration
TERPENOIDS	(5 ml extract +2 ml $CHCl_3$) +3 ml of H_2SO_4	Formation of the reddish brown at the interface

***Laboratory Manual for the UNESCO [13]**

Evaluation of the Antibacterial Activity of Bamboo Shoot Extracts

The anti-bacterial activity of the bamboo shoots extracts against *E. coli* and *S. aureus* were evaluated using the discs diffusion method.

Eradicant test

The eradicant test was adapted to the work of Valentino et al. [14]. The paper discs were soaked in the shoot extracts. Then, sterile plates were poured with 0.1 ml of the bacterial suspension. The discs were soaked in different treatments and then it was seeded equidistantly in the sterile MH agar plates. The plates were incubated at room temperature. The zones of inhibition were measured using a vernier caliper at 12 and 24 hours of incubation. The greater the zone of inhibition formed, the greater the eradicating activity/ potential of the extracts being tested.

Protectant test

The paper discs were soaked in the bacterial suspension. The sterile plates were poured with 0.1 ml of the shoot extracts. The paper discs were seeded equidistantly in the sterile MH agar plates. The plates were stored at room temperature and zones of bacterial colonization were measured using vernier caliper after 12 and 24 hours of incubation. Absence of bacterial growth or the smaller the zone of bacterial colonization formed the greater the protectant potential of the extracts being tested

Evaluation for Antioxidant Activity

The DPPH radical scavenging activity and the total phenolic content of hot water and ethanol extracts that were extracted from the shoots of *B. Blumeana* var. *luzonensis* and *S. brachycladum* were analyzed at the Chemistry Laboratory of Center for Natural Sciences at St. Mary's Univeristy, Bayombong, Nueva Vizcaya. DPPH radical scavenging activity was done

as following Kolak et al. [15]. The total phenolic content of the hot water and ethanol extracts were determined using the Folin-Ciocalteu method as described by Hodzic et al.[16].

RESULTS AND DISCUSSION

Phytochemical Constituents of Bamboo Shoots

Green medicine or plant-derived drugs are of current trends since they are regarded as safe, costless, readily available and side effect-free[17]. Accordingly, phytochemicals such as carotenoids, phenolics and dietary fibers are continuing increased in attention because of their antioxidant, antimutagenic, anticancer property and other health promoting properties [18].

Phytochemical constituents of the bamboo shoot extracts is shown in the Table 2. Results revealed that cardiac glycosides, flavonoids, saponins, tannins and terpenoids were present in both ethanol and hot water extracts of *B. blumeana* var *luzonensis* and *S. brachycladum*. Meanwhile, steroids were present only in the ethanol extracts of the bamboo shoots. The presence of various phytochemicals coincides with the findings of Singh et al. [19], Coffie et al [20] and Valentino et al. [14].

Phytochemicals such as alkaloids, flavonoids, steroids, terponoids, tannins, saponins, and cardiac glycosides present in different extracts exhibit a number of biological activities [21, 22]. Flavonoids have been classified as flavonols, flavones, flavanols (catechins), flavanones, anthocyanidins, and isoflavonoids (can be used as antioxidant, antibacterial, cytotoxicity, anti-inflammatory, oestrogenic activity, anti-allergic activity, vascular activity and antitumor activity [23, 24, 25]. Whereas, saponins provide defense against fungal and bacterial pathogens and slows the growth of cancer cells [26, 27, 28].

Antibacterial Activity

Eradicant and Protectant potentials of Bamboo shoots extracts against *E. coli*

Zone of inhibition and zone of colonization of *E.coli* are presented in Table 3. As eradicant, among all the bamboo shoot extracts used, only the paper discs with *S. brachycladum* shoot ethanol extracts formed zone of bacterial inhibition of 8.81 mm after 12 hours of incubation. Meanwhile, after 24 hours of incubation, zones of inhibition were already observed in discs treated with bamboo shoot extracts. The widest zone of bacterial inhibition was formed in discs with *S. brachycladum* shoot ethanol extract with 8.36 mm followed with 7.68 mm in *S.*

brachycladum shoot hot water extract and *B. blumeana* var. *luzonensis* ethanol extract with 7.63 mm. As protectant, the smallest zone of colonization of *E. coli* was observed in plates treated with *B. blumeana* var. *luzonensis* ethanol extracts with 7.14 mm followed by *S. brachycladum* ethanol extract with 7.67 mm. While at 24 hours of incubation, increased in bacterial colonization of *E. coli* were observed in all plates treated with bamboo shoot extracts, wherein the smallest zone of colonization wasrecorded in plates treated with *B. blumeana* var. *luzonensis*ethanol extract with 9.97mm.

Statistical analysis showed that *B. Bluemana* var *luzonensis* and *S. brachycladum* shoot ethanol extracts have comparable effects with the streptomycin sulfate (commercial antibiotics) whereas they lack the eradicating activity against *E. coli*.

Eradicantand Protectant activity of bamboo shoots extracts against *S. aureus*

Eradicantand protectant activity of the bamboo shoots extracts against *S. aureus* are shown in Table 4. As eradicant, at 12hrs of incubation, discs treated with *S. brachycladum* shoot hot water extract had the widest zone of bacterial inhibition with 13.40 mm followed by 9.34mm in *S. brachycladum* shoot ethanol extract. At 24 hrs of incubation, increased in zones of inhibition were observed wherein *S. brachycladum* shoot hot water extract produced the widest zone of inhibition (15.32 mm) followed by *S. brachycladum* shoot ethanol extract (9.21 mm). For the protectant test against *S. aureus*, the smallest zone of colonization of *S. aureus* was recorded in plates treated with *S. brachycladum* shoot ethanol extract of 6.28 mm, followed by 6.48 mm in *B. blumeana* var. *luzonensis* ethanol extract. At 24 hours of incubation, the smallest zone of colonization of *S. aureus* was observed in plates treated with *B. blumeana* var. *luzonensis* shoot ethanol extract with 6.65 mm followed by *S. brachycladum* shoot ethanol extract with 9.33 mm.

Statistically, only *S. brachycladum* hot water extract are potent eradicating agent while ethanol extracts of both *B. Blumeana* var *luzonensis* and *S. brachycladum* are potent protectant agents against *S. aureus.*

Table 2: Phytochemical composition of *B. blumeana* var. *luzonensis* and *S. brachycladum* shoots extracts

Phytochemical composition	*B. blumeana* var. *luzonensis* Hot water extract (HWE)	*B. blumeana* var. *luzonensis* Ethanol extract (EE)	*S. brachycladum* HWE	*S. brachycladum* EE
Alkaloids	-	-	-	-
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+

Saponins	+	+	+	+
Steroids	-	+	-	+
Tannins	+	+	+	+
Terpenoids	+	+	+	+

(+) = present (-) = absent

Table 3: Diameter zone of inhibition of *B. blumeana* var. *luzonensis* and *S. brachycladum* shoot extracts against *E.coli* at 12 and 24 hrs of incubation

Treatments	Zone of inhibition		Zone of colonization	
	12 hrs	24 hrs	12 hrs	24 hrs
***B. blumeana*var*luzonensis* EE**	6.00^{b}	7.59^{b}	7.14^{bc}	9.97^{b}
***S. brachycladum* EE**	8.81^{b}	8.36^{b}	7.67^{bc}	10.93^{b}
***S. brachycladum* HWE**	6.00^{b}	7.68^{b}	20.59^{a}	26.19^{a}
***B. blumeana* var. *luzonensis* HWE**	6.00^{b}	7.63^{b}	17.82^{a}	21.44^{a}
Streptomycin Sulfate (+)	19.75^{a}	19.96^{a}	6.00^{c}	6.00^{b}
Sterile distilled water (-)	6.00^{b}	6.00^{b}	23.80^{a}	20.97^{a}

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

Table 4: Diameter zone of inhibition and zone of colonization of *B. blumeana* var. *luzonensis* and *S. brachycladum* shoots extracts against *S. aureus* at 12 and 24 hrs of incubation

Treatments	Zone of inhibition Zone of colonization			
	12 hrs	24 hrs	12 hrs	24 hrs
***B. blumeana* var. *luzonensis* EE**	6.00^{c}	8.72^{c}	6.48^{b}	6.65^{bc}
***S. brachycladum* EE**	9.34^{bc}	9.21^{c}	6.28^{b}	9.33^{bc}
***S. brachycladum* HWE**	13.40^{b}	15.32^{b}	11.96^{a}	12.72^{ab}
***B. blumeana* var. *luzonensis* HWE**	6.00^{c}	8.60^{c}	11.09^{a}	12.18^{ab}
Streptomycin sulphate (+)	34.36^{a}	36.15^{a}	6.00^{b}	6.00^{c}
Sterile distilled water (-)	6.00^{c}	6.00^{c}	15.18^{a}	17.01^{a}

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

Antibacterial activity of bamboo shoots extracts can be due to the presence of phytochemicals as elucidated in Table 2. Flavonoids and saponins are reported to exhibit a wide range of biological activities like antimicrobial, anti-inflammatory, analgesic, anti-allergic, cytostatic and antioxidant properties. Additionally, saponins arephytoanticipins or phytoprotectants [29, 30]. Furthermore, presence of fatty acids, esters, long chain alcohols and aldehydes could also enhance the antibacterial property These metabolites form synergistic effects that inhibit *E. coli* and *Staphylococcus aureus* [31, 32].

Based from the results, ethanol extracts of bamboo shoots tested had the potential anti-bactericidal effect towards the test pathogens, which can be due to the nature of biologically active constituents whose activity, can be increased in the presence of the ethanol and due to the stronger extraction capacity of ethanol [33, 34, 35]. Meanwhile, lack or inactive bacterial activity of bamboo shoots extracts can be

attributed to the absence of alkaloids in the tested bamboo shoots extracts [36].

Antioxidant Activity

DPPH Radical scavenging assay

Antioxidant is a substance that delays, prevents or removes oxidative damage to a target molecule by producing free radicals [37].

Shown in Table 5 is the antioxidant property of *B. blumeana* var. *luzonensis* and *S. brachycladum* shoots extracts. Among the four extracts tested, ethanol extract of *B. blumeana* var. *luzonensis* had the highest scavenging activity with 64.80% followed by *S. brachycladum* ethanol extract 63.69 %, *B. blumeana* var. *luzonensis* hot water extract with 50.28% while *S. brachycladum* hot water extract had the lowest free radical scavenging activity with 44.13 %.

For the total phenolic content, *B. blumeana* var. *luzonensis* ethanol shoot extracts had the highest value of 27.59 AAE/g, followed by *B. blumeana* var. *luzonensis*hot water shoot extracts of 24.40 AAE/g while *S. brachycladum* hot water shoot extracts had the lowest total phenolic content of 16.07 AAE/g. This results implied that *B. blumeana* var. *Luzonensis* shoot extracts is superior *to S. brachycladum* shoot extracts with regards to their phenolic content. Meanwhile, the superiority of could be due change in temperature used in hot water extraction. As revealed by Zhang et al [38], heat causes the decomposition of phenolic compounds.

Table 5: Antioxidant activity of the *B. blumeana*var. *luzonensis*and *S. brachycladum* shoots extract

Treatments	Radical Scavenging Activity (%)	Total phenolic content (mg AAE/g sample)
***S.brachycladum* EE**	**63.69**	**21.87**
***S.brachycladum* HWE**	**44.13**	**16.05**
***B. blumeana*var*luzonensis* EE**	**64.80**	**27.59**
***B. blumeana*var*luzonensis* HWE**	**50.28**	**24.40**
Catechin (control)	**87.71**	

Phenolic compounds present in plants are important bioactive compounds as they exhibit strong natural anti-oxidative and anti-inflammatory properties and sometimes antimicrobial activities as well [39, 40, 41, 42]. The phenolic compounds are represented firstly by the flavonoids. Flavonoids are a large group of naturally occurring polyphenols processing a wide range of pharmacological activities [43]. Results also coincides with the findings of Chongtham et al. [44] wherein shoots have antioxidant capacity due to the presence of flavonoids.

CONCLUSION

Bambusa blumeana var *luzonensis* and *Schizostachy brachycladum* are natural

sources of antibacterial and anti-oxidant agents which can be attributed to its phytochemical constituents.

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