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PHYTOCHEMICAL SCREENING AND DETERMINATION OF THE BIOLOGICAL ACTIVITIES OF *Bambusa vulgaris* var. *striata* AND *Dendrocalamus asper* SHOOT EXTRACTS

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ABSTRACT

This paper dealt with the phytochemical constituents, anti-oxidant potential and bioassay of the anti-bacterial activity of *B. vulgaris* var. *striata* and *D. asper* shoot extracts. Cardiac glycosides, flavonoids, terpenoids, and saponins in all tested extracts. In addition, steroids were also present in ethanol extract of *B. vulgaris* var. striata and *D. asper* shoot while tannins were only detected in ethanol extract of *B. vulgaris* var. *striata*shoots. For the antibacterial activity, results exhibit the potential of the ethanol extract of *B. vulgaris* and *D. asper* shoots as protectant and eradicant against *E.coli* and *S. aureus* at 12 and 24 hours of incubation. Whereas, antioxidant activity of ethanol extract of *D. asper* showed the highest radical scavenging activity with a mean of 64. 80%.And the highest total phenolic content was recorded in *B. vulgaris* var.*striata* ethanol extract with 49.90 mg AAE/g sample.

Keywords: **Phytochemical, bamboo shoots, anti-oxidant, anti-bacterial**

INTRODUCTION

Bamboo is known as one of the most economically important plant in the world since time immemorial due its known benefits and uses [1]. In recent times, scientists focused on its potentiality as ingredients for modern functional foods and

nutraceuticals. Every part of the bamboo such as leaves, shoots, culm sheath and culms are used either as food or for medicine and health benefits [2].Based on several researches, they contain proteins, carbohydrates, vitamins, fibers, phytosterols, quantities of minerals and less cholesterol contents which are essential for our body [2, 3].

Phytochemicals are naturally present in the plants and are biologically significant playing an important role in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms [4]. They are proven as antidiarrheal, anti-aging agent and antitumor and reducing the risk of cancer, control blood pressure, hypertension, obesity, and also protects our body from coronary diseases and potential carcinogens [1, 5, 6, 7, 8].

In the Philippines, little information on the phytochemical constituents well as the biological potentials of bamboo species is available. Thus, the conduct of the study to determine the phytochemical constituents and biological activity which include the antibacterial and anti oxidant properties ofthe two species of bamboo shoots specifically *Dendrocalamus asper* and *Bambusa vulgaris* var. *striata* using ethanol and hot water extract.

MATERIALS AND METHODS

Collection of Plant Materials

Young shoots of *B. vulgaris* var. *striata* and *D. asper* were collected at Lingap Kalikasan, Central Luzon State University, Science City of Munoz, Digdig, Caranglan, and Guimba, Nueva Ecija. The bamboo shoots were washed and cut into small pieces, air-dried then were ground into powdered form.

Preparation Bamboo Shoots Extracts

Ethanol and hot water were used as solvents for the extraction of the bamboo shoots. Twenty-five grams of the powdered bamboo shoots were mixed with 100 ml of solvents. For ethanol extraction, powdered bamboo shoots were mixed 80% ethanol in a sterile flask for 48 hours, filtered and were subjected to rotary evaporator set at 60 rev/min at 45°C.). For hot water extraction, the mixture was placed in a hot water bath with 80-90 °C for 2 hours. Then, the mixture was filtered with Whatman filter paper No.1 and the filtrate was poured in a sterile amber bottle.

Screening of Phytochemical Composition of Bamboo Shoot Extracts

Screening of phytochemical constituent of bamboo shoots were carried

out by following the standard methods described in Laboratory Manual for the UNESCO [9]. The presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, and terpenoids were determined.

Determination of Biological Activity of Bamboo Shoot Extracts

Evaluation of the Antibacterial Activity

Antibacterial potentials (as eradicant and protectant) of the bamboo shoot extracts were evaluated using disc diffusion method at 12 and 24 hrs of incubation. Pure cultures of *E.coli*and *S. aureus* were grown in Nutrient Agar for 24 hours and were transferred to Nutrient Broth and were standardized to 1.5 X 10^8 cells/ml using McFarland standards.

For the eradicant test, paper discs were soaked in the shoot extracts. Then, 0.1 mL of the bacterial suspension was poured and spreadin the sterile plates with MH agar. Then, the paper discs were soaked in different treatments (bamboo shoots extracts) and were seeded equidistantly in the plates. For protectant test, the paper discs were soaked in the bacterial suspension and 0.1 ml of the bamboo shoot extracts were poured in plates with MH agar. Then, the paper discs were seeded equidistantly in the plates. Zones of bacterial inhibition (for eradicant test) and zones of bacterial colonization (for protectant test) were measured using a vernier caliper after 12 and 24 hours of incubation.

DPPH radical scavenging assay

The DPPH scavenging activity of the ethanol and hot water extracts from the shoots of B. *vulgaris* var. *Striata* and *D. asper* using DPPH were assayed following the procedure described by Kolak et al. [10].

Total phenolic content

The total phenolic content of the hot water and ethanol extract was determined using the Folin-Ciocalteu method as described by Hodzic et al. [11].

RESULTS AND DISCUSSION

Phytochemical composition of bamboo shoot extracts

Screening of phytochemical constituents of ethanol and hot water shoot extracts of *B. vulgaris* var. *striata* and *D. asper* were carried out to evaluate the presence or absence of phytochemicals namely: alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids. Table 1 shows the results of phytochemical screening of the hot water and ethanol extract of *B. vulgaris* var. *striata* and *D. asper* shoots. Cardiac glycosides, flavonoids, saponins and terpenoids were detected in both hot water and ethanol

extracts of *B. vulgaris* var. *striata* and *D. asper*. On the other hand, traces of steroids were observed only in ethanol extracts of *B. vulgaris* var. *striata* and *D. asper* while tannins were present only in *B. vulgaris* var. *Striata* ethanol extract.

These coincides with the findings of Girriand Janmejoy [12] ; Bhatt et al. [13] ; Jeyaram et al.[14], Chongtham et al.[2], wherein majority of bamboo species were found to be good sources of secondary metabolites. Phytochemicals were found in fermented bamboo shoot extracts of *B. Balcooa* and leaf extracts of *B. vulgaris* [1, 8, and 15.]. The presence of alkaloids, tannins, phenolic, glycosides, saponins, flavonoids nuclease, urease, proteolytic enzyme, cyanogenic glycogen and alkaloid and anthraquinones, phytosterols, and phenols *B. vulgaris* specieswere also reported in previous studies [16, 17, and 18].

Bamboo shoot extracts as eradicant of *S. aureus* and *E.coli*

Presented in table 2 are the results of the eradicant test. Among all the bamboo shoot extracts tested, *B. vulgaris* var. *striata* ethanol extract registered the widest zone of inhibition at 12 and 24 hrs of incubation against *S. aureus* (with mean 13.75mm and 12.54mm) and *E. coli* (with a mean of 8.64 mm and 8.86 mm) whereas, *B. vulgaris* var. *striata* hot water extract had the smallest zones of inhibition with 7.32 mm against *S. aureus,* and 7.26 mm and 7.24 mm against *E. coli* at 12 and 24 hrs of incubation.

Bamboo shoot extracts as protectant against *S. aureus* and *E.coli*

For the protectant test, zone of colonization of the test pathogens were recorded, the smallest zone of colonization formed the greater the protectant potential of the bamboo shoot extracts tested. In Table 3, the smallest zone of colonization of *E. coli* was observed in plates treated with *B. vulgaris* var. *Striata* ethanol extract with a mean zone of colonization of 8.94 mm and 9.36 mm at 12 and 24 hrs of incubation. The same effect of *B. vulgaris* var. *Striata* towards *S. aureus* were recorded with 6.00 mm and 6.96 mm at 12 and 24 hrs of incubation, respectively. Statistically, zones of colonization produced by *E. coli* and *S. aureus* in ethanol extract of *B. vulgaris* var. *striata*at 12 hrs and 24 hrs of incubation were comparable to streptomycin sulfate. Thus, the antimicrobial potential of *B. vulgaris* var. *striata* shoot extracts.

Antimicrobial potential of the bamboo shoot extracts can be attributed to the presence of various phytochemicals of all the bamboo shoot extracts tested. The presence of tannins and steroids in ethanol

extract of *B. vulgaris* var. *striata* were considered as essential secondary metabolites responsible in fighting against microbial pathogens [19]. In addition, the active ingredients responsible for the antibacterial activity are best extracted with ethanol and methanol [20, 21].Moreover, studies have already proven that phytochemical of bamboo extracts including leaf and bamboo shoots are capable of being antibacterial, antifungal, that can fight against pathogenic disease [22].

Meanwhile, the differences on the sensitivity of the test pathogens towards the bamboo shoot extracts is due to the structural composition of the test pathogens. The bioactive compounds present in the bamboo extracts can easily penetrate the cell of *S. aureus* due to the absence of outer membrane in gram-positive bacteria [8]. Whereas, *E. coli* which is a Gram-negative bacteria are reportedly to be highly resistant to many known antibiotics due to the thicker peptidoglycan layer of the bacteria cell that protect them from the action of antibiotics [23].

Antioxidant Activity of Bamboo Shoot Extracts

DPPH radical scavenging activity and Total Phenolic Content

Plant derived antioxidant compounds such as polyphenols and flavonoids which possess the ability to protect the body from damage caused by free radicals which are much safer than synthetic anti-oxidants [24, 25]. In this present study, the DPPH radical scavenging activity of hot water and ethanol shoot extracts of *B. vulgaris* var. *striata* and *D. asper* were determined and the data are presented in Table 4. Among all the bamboo shoot extracts tested, ethanol extract of *D. asper* had the highest radical scavenging activity of 64.80% followed by hot water extract of *D. asper* with 55.87 %.Meanwhile, *B. vulgaris* var. *striata* showed radical scavenging activity of 48.04 % for ethanol extract and 21.23 % hot water extract. Whereas for the total phenolic content, *B. vulgaris var striata* ethanol extracts had the highest of 49.90 mg AAE/g followed by it hot water extracts of 32.98 and *D. asper* hot water extracts of 28.14. These values clearly showed that hot water and ethanol shoot extracts of *B. vulgaris* var. *striata* and *D. asper* have the ability to scavenge free radicals as a promising source of antioxidant.

Similar results were obtained by Soesanto [26] and Goyal et al., [25].As reported by Nakajima et al [27] and Zhang et al [28], bioactive compounds with anti-oxidative activities are phenolic compounds

represented by the flavonoids represented by oreintin, homoorientin, vitexin and secondary phenolic acids represented by p-coumaric acid, chlorogenic acid, caffeic acid and ferrulic acid. Moreover, several studies have identified the oxidants from different species of bamboo such as *Phyllostachy sedulis, Flageria robusta,* and *Sasa borealis* [29, 30, 31].

Meanwhile, total phenolic content is a good indicator for antioxidant activity. Antioxidantproperty of plant is strongly correlated with the total phenolic content [32, 33]. In particular, bamboo shoots can be a good dietary source of natural phenolic oxidants. The antioxidant action of polyphenol compounds depends on their free radical scavenging capacity and its ability to reduce iron [34].

In this study, the result in the total phenolic content was not correlated with the antioxidant activity of bamboo shoot extract wherein *D. asper* hot water and ethanol extract had higher antioxidant activity but its total phenolic content is relatively lower to the phenolic content of *B. vulgaris* var. *striata* ethanol and hot water shoot extract. Thus, the anti-oxidant activity of the bamboo shoot extracts tested could be attributed to the presence of phytochemicals such as cardiac glycosides, flavonoids, saponins and terpenoids which is detected in this study (Table 2). According to Omuyuri et al. [35], the phytochemicals such as flavonoids, tannins and terpenoids are secondary metabolites rich in antioxidant and free radical scavenging properties.

CONCLUSION

Findings of the study revealed the anti-bacterial activity of *B. vulgaris var striata* as protectant and eradicant against *E. coli* and *S. aureus*. While both *D. asper* and *B. vulgaris var striata* ethanolic and hot water extracts possess anti-oxidant activity which can also be attributed the presence of phytochemicals.

Table 1: Phytochemical composition of hot water and ethanol shoot extracts of *B.vulgaris* var. *striata* and *D. asper*

Phytochemicals	*B. vulgaris* var. *striata*		*D. asper*	
	Hot water extracts	Ethanol extracts	Hot water extracts	Ethanol extracts
Cardiac Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	-	+	-	+
Tannins	-	+	-	-
Terpenoids	+	+	+	+
Alkaloids	-	-	-	-

Legends: (+) = presence of phytochemical; (-) absence of phytochemical

Table 2: Diameter zone of inhibition of *B. vulgaris* var. *striata*and *D. asper* shoot extracts against *S. aureus* at 12 and 24 hrs of incubation

Treatments	Zone of Bacterial inhibition (mm) Against S. aureus		*Against E. coli*	
	12 hours	24 hours	12 hours	24 hours
B. vulgaris var. *striata*hot water extract	7.32[c]	7.32[d]	7.26[c]	7.24[d]
B. vulgaris var. *striata* ethanol extract	13.75[b]	12.54[b]	8.64[b]	8.86[b]
D. asper hot water extract	7.56[c]	7.47[d]	7.27[c]	7.25[d]
D. asper ethanol extract	12.52[b]	11.02[c]	8.00[bc]	8.28[c]
Streptomycin sulfate	29.24[a]	30.13[a]	25.76[a]	25.46[a]
Sterile distilled water	6.00[c]	6.00[e]	6.00[d]	6.00[e]

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

Table 3: Diameter zone of colonization of *B. vulgaris* var. *striata*and *D. asper* shoot extracts against *S. aureus* at 12 and 24 hrs of incubation

Treatments	Zone of Bacterial colonization (mm) Against *S. aureus*		Against *E. coli*	
	12 hours	24 hours	12 hours	24 hours
B. vulgaris var. *striata*hot water extract	33.67[a]	42.49[a]	41.51[a]	50.96[b]
B. vulgaris var. *striata* ethanol extract	6.00[d]	6.96[c]	8.94[d]	9.36[e]
D. asper hot water extract	36.59[a]	45.58[a]	45.92[a]	56.41[a]
D. asper ethanol extract	9.64[c]	14.89[b]	14.56[b]	31.56[d]
Streptomycin sulfate	6.00[d]	6.00[c]	6.00[d]	6.00[f]
Sterile distilled water	14.53[b]	19.03[b]	34.10[c]	38.86[c]

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

Table 4: Radical scavenging activity and total phenolic content of bamboo shoots hot water and ethanol extract

Bamboo Shoot Extracts	Radical Scavenging Activity (%)	Total Phenolics (mg AAE/g sample)
B. vulgaris var. *striata* Hot Water	21.23	32.98
B. vulgaris var. *striata* Ethanol	48.04	49.90
D. asper Hot Water	55.87	28.14
D. asper Ethanol	64.80	31.00

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

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