EFFECT OF EXTRACTION ON THE ANTIBACTERIAL ACTIVITIES OF *DENDROCALAMUS ASPER* SHOOTS AGAINST *ESCHERICHIA COLI*, *STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA*, AND *VIBRIO PARAHAEMOLYTIUS*

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Abstract:

Dendrocalamus asper (D.asper) is one of the various species of bamboo found in Vietnam. Its wood is used to construct dwellings, and its shoot is nutritious. There is evidence of the antibacterial qualities of the bamboo shoot (*Dendrocalamus asper*). The main objective of this study is the antibacterial activity of *D.asper* shoots against bacteria. The antibacterial activity of shoot extract of *D.asper* was evaluated on bacterial strains like *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Vibrio parahaemolytius.* Using a Soxhlet extractor, the antibacterial activity of two distinct solvents (ethanol and hot water) was produced. All studied resistant bacteria were effectively inhibited by the active antibacterial activity of the shoots' ethanol and hot water extract. Ethanol proved to be the most effective solvent in this study for extracting the active ingredient from the shoot. Most hot water extracts demonstrated bactericidal activity in various zones of inhibition, ranging from 13,44±0,84 mm to 14,78±2,04 mm. Across various zones of inhibition (14.00±0.00 mm - 16.67±1.53 mm), most ethanol extracts exhibited antibacterial activity. The highest antibacterial efficacy against *Pseudomonas aeruginosa* was demonstrated by the ethanol extracts.

Keywords: *Dendrocalamus asper*; Antibacterial activity; Reflux extraction; Bamboo extracts; Bamboo shoots, *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa and Vibrio parahaemolytius.*

Introduction

The Poaceae family includes around 1,642 species of bamboo, the majority of which are found in Asia [1]. Bamboo is a family of large woody grasses in the Poaceae family and subfamily Bambusoideae. Both the stalk and the wood have been widely used as building materials. The wood is mostly used for housing and furnishings, while the shoot can be processed into a variety of healthy foods and medications [2] [3] [4]. The majority (85.63%) of a fresh bamboo shoot's composition is water. Bamboo shoots have the benefit of being low in fat and abundant in fiber, potassium, carbs, vitamins, and minerals [5]. The benefits of bamboo shoots include low fat, high fiber, potassium, carbs, vitamins, and minerals. Bamboo shoots are typically eaten in Indonesia by being cooked into a variety of meals like soup, sayur lodeh, spring rolls, and pickles. According to research done by [5], bamboo shoots can be included as a second element while creating nuggets. Bamboo shoots can be processed in a variety of ways, such as flour, in addition to being fresh. Similar to Haryani's investigation into the use of bamboo shoot flour as a doughnut replacement [6]. Fermentation is a method for processing bamboo stalks. Bamboo shoots can have their cellulose and fiber content increased through treatment for fermentation. Additionally, fermentation used in bamboo shoot treatment can boost the antibacterial activity of the plant [5]. *Dendrocalamus asper* bamboos are commercially exploited for making furniture, blinds, chopsticks, toothpicks, skewer sticks, handicrafts, and incense sticks [7]. Historically, both traditional medicine and food have used bamboo shoots [8]. Depending on the species and environmental variables, bamboo shoots have varying nutritional values [9] [10]. The bioactive substances found in bamboo shoots have biological effects such as antioxidant, antibacterial, and anti-allergy properties. Additionally, the extracts from bamboo shoots have been utilized to treat cancer and diabetes by lowering hyperglycemia [11] [12] [13] [14]. Additionally, they have been utilized in cosmetics, such as anti-aging goods [15]. Although bamboo shoots are full of biochemical substances and have nutritional advantages for human health, the young shoots contain cyanide-containing toxins. According to the species and amount of bamboo shoots, cyanogenic glycoside, a form of cyanide, is present in bamboo shoots [16]. Cyanide has immediate negative effects on human health at concentrations between 0.5 and 3.5 mg/kg of body

weight [17]. However, cyanide can be removed from food using cooking techniques such as boiling, steaming, heating, and frying [17]. Chongtham et al. (2011) [18] determined the following macronutrient contents of *Dendrocalamus asper* (g/100 g fresh weight): Amino acid, 3.12±0.07; protein, 3.59±0.06; carbohydrate, 4.90±0.11; starch, 0.39±0.08; fat, 0.40±0.06; dietary fiber, 3.54±0.07; vitamin C, 3.20±0.06; and vitamin E, 0.91±0.31. Bamboo shoots from *Dendrocalamus asper* contain a variety of nutritional advantages for human health. However, there are few reports of this species' biological activity.

Due to the dominance of the northeast and southeast winds, the country's climate resembles the monsoon conditions of Southeast Asia. Vietnam's climate varies greatly from region to region due to variations in latitude and topography, which result in an abundance of solar radiation and precipitation. These climatic elements are thought to be favorable for biodiversity in general and bamboo growth in particular [19]. Bamboo has always grown naturally in Vietnam, with the exception of the Red River and Mekong River delta regions, where it is only found now. Previously, several species with significant economic value grew in broad areas, but due to overexploitation, such areas have gradually shrunk [19]. In several regions of the nation, bamboo has also been widely cultivated for its shoot products, which has significantly increased farmers' revenue. However, certain common species with delectable shoots, such as *Dendrocalamus asper*, were foreign, and their management methods were imported [19].

Gram-negative bacilli called *Escherichia coli,* which are typically a part of the natural flora in the intestines, can accidentally cause illnesses like meningitis (the most common cause in children), sepsis, and urinary tract infections [20]. In most human situations, this bacteria can be found [21]. *Pseudomonas aeruginosa*, a type of gram-negative bacterium, can cause an eye infection, pneumonia-causing lung infections, mild external muscularity in swimmers, urinary tract infections, and wound and burn infections that produce bluish-green pus [22]. An indigenous flora of the skin, eyes, upper respiratory system, gastrointestinal tract, urethra, and, rarely, vagina is known to harbor Staphylococcus aureus, gram-positive spherical cocci. It has been linked to endocarditis, osteomyelitis, skin infections, and toxic shock syndrome [23]. Widely distributed in estuarine, marine, and coastal environments is the Gram-negative halophilic bacterium *Vibrio parahaemolytius*. When *V. parahaemolyticus* produces septicemia, ear infections, or wound infections in rare instances, it can be fatal for people who already have health issues [24].

Because of their flavones and glycosides, bamboo shoots have outstanding antioxidants, anti-free radicals, and anti-aging properties [25]. *Escherichia coli*, *Staphylococus aureus, Pseudomonas aeruginosa,* and *Vibrio parahaemolytius* have not been tested for the antibacterial activity of bamboo shoots. Thus, the goal of the current study was to determine whether the ethanol extracts and hot water extracts of *D. asper* bamboo shoots had any antibacterial properties.

Materials and Methods

Materials

The mature bamboo was found growing in the An Giang province of Southern Vietnam's Bay Nui region, where the *D. asper* bamboo shoots were harvested. Fresh *D. asper* samples were properly cleaned with tap water before being properly dried with the wind. After that, cut the mixture into pieces that are between 0.5 and 1.0 cm in size. The samples were subsequently processed into a coarse powder in a grinder mill. Chemicals utilized were ethanol 70^0, Tryptone Soya Agar, tryptophan casein soy broth (Merck, Germany).

Methods

Preparation D. asper shoot extracts

Hot water and ethanol were employed as solvents to remove the bamboo shoots. 25 g of dried *D. asper* shoot powders were dissolved in 100 ml of the solvent (1 g/4ml) in a round flask. For ethanol extraction, the mixture was then refluxed for around three hours while being treated with ethanol 70^0 for two hours at 60°C to 70°C [26] [27]. For hot water extraction, the mixture was submerged in a hot water bath between 80 and 90 degrees Celsius for two hours. The mixtures were directly filtered out of the hot mixture using Whatman filter paper. The *D. aspe*r extracts were split by centrifugation

at a speed of 60 rpm and a temperature of 45°C, and they were thereafter stored separately at a temperature of 4°C in bottles for use in the future.

Organoleptic evaluation

Organoleptic properties, such as color, appearance, flavor, texture, and taste, were assessed for the produced bamboo shoots and the extracts.

Evaluation of the antibacterial activity

Bacteria were activated on TSA disc (Tryptone Soya Agar) and incubated at 37ºC for 18 to 24 hours. Then take 3–5 groups of colonies with a diameter > 1 mm to make a suspension in TSB (tryptophan casein soy broth) and disperse the bacteria evenly on the agar disc. Incubate the suspension at 37°C for 4 to 6 hours for bacterial growth. Check the bacterial content in the suspension by spectroscopy at 625 nm and adjust the bacterial content to a concentration of $\mathbf{1-2\times10^{6}}$cells/ml. Prepare four Petri dishes, each with precisely 20 ml of media, to hold the four prepared test bacteria. The agar plate was then evenly covered with bacterial strains at a concentration of 10^6 CFU/ml, making bacterial strains and prepared agar plates. Drill 3 agar wells with a diameter of 6mm, equally spaced on Petri dishes and numbered from 1 to 3, respectively. To get a 100 mg/ml solution, combine 10 mg of each extract with 100 ml of water. The wells at positions 1, 2, and 3 were injected with 30 µl of each extract from bamboo shoots, respectively. The agar plate should be incubated for 24 hours at 37°C before the test results are examined. A sterile ring that stopped bacteria from growing was used to prove the extract's antibacterial effectiveness, with larger diameter rings demonstrating greater antibacterial activity. Using a ruler, check each agar plate for the presence of sterile rings and measure their diameter [28]**.**

Results and Discussion

Bamboo is a good source of phytosterols, which function as nutraceuticals and are the precursors of several pharmaceutically active steroids found in shoots. The antibacterial properties of *D. asper* shoots have been examined in this work. All tested multiple drug-resistant bacteria were effectively inhibited by the ethanol and hot water extracts of *D. asper*. Compared to certain antibiotics, these bacteria exhibited greater resistance.

Organoleptic evaluation

The characteristics of the extraction outcomes from this investigation were looked at in an organoleptic. There were differences found between the organoleptic characteristics of fresh shoots and the extracts, which are shown in Table 1. Acrid and white in color, fresh shoots were crunchy, whereas shoot extracts had a soft feel and a earthy flavor. The color of the shoot extract turned yellow-brown after extraction. Organoleptic testing for the current study's extract solution showed that it was yellow-brown in color and had a distinct earthy smell. The extracts with poor whiteness values may have brown coloration as a result of oxidation and reactivity between the fiber and oxygen in the air during the extraction process. However, the ethanol extract and hot water extract did not significantly differ in terms of color, taste, or scent. After extraction, the texture characteristics of the bamboo shoot underwent constant changes, including a decrease in resilience and hardness. These modifications decreased the shoot's ability to retain water by severing the link between the flesh and internal fibers. The interior nutrition contents were closely correlated with the color index. The sensory index and the storage time had a clear impact on these variables.

Table 1. Organoleptic evaluation of fresh shoots and extracts

Parameters	Shoots		
	Fresh	Ethanol extracts	Hot water extracts
Texture	Hard	Soft	Soft
Color	white	Yellow- Brown	Yellow-Brown
Taste	Acrid	Earthy smell	Earthy smell
Flavor	Nil	Nil	Nil

Evaluation of the antibacterial activity

Zones of bacterial inhibition were noted in the bamboo shoot extracts' eradication ability. The smaller the zone of colonization created by the test pathogens for the protectant test, the higher the potential for protection of the tested bamboo shoot extracts. In the ethanol extracts, the inhibition zone diameter was 14.00±0.00 mm in *Escherichia coli,* 15.67±1.53 mm in *Staphylococus aureus*, 16.67±1.53 mm in *Pseudomonas aeruginosa*, and 14.33±1.53 mm *Vibrio parahaemolytius.* All of the ethanol extracts experimental groups' zones of inhibition show activity against *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Vibrio parahaemolytius.* The zone of inhibition decreases from *E. coli* with 14.00 ± 0.00 mm and *V. parahaemolytius* with 14.33±1.53 mm to *S. aureus* with 15.67±1.53 mm and Pseudomonas *aeruginosa* with 16.67±1.53 mm at the same concentration. There is no a significant difference among the extracts on their zones of inhibition against *Escherichia coli* and *Vibrio parahaemolytius*. Among all (Figure 1), *Pseudomonas aeruginosa* is the most sensitive with the highest inhibition zones of 16.67±1.53 mm. The zone of inhibition for *Staphylococus aureus* is less than that of *Pseudomonas aeruginosa.* These studies were performed in triplicate.

Table 2. Antibacterial activity of ethanol extracts against bacteria

Bacteria	Zone of inhibition (mm)
Escherichia coli (E.coli)	14.00±0.00
Staphylococus aureus (S.aureus)	15.67±1.53
Pseudomonas aeruginosa (P.aeruginosa),	16.67±1.53
Vibrio parahaemolytius (V.parahaemolytius)	14.33±1.53

Interpretation:

<10 mm: inactive

10-13 mm: partially active

14-19 mm: active

>19 mm: very active

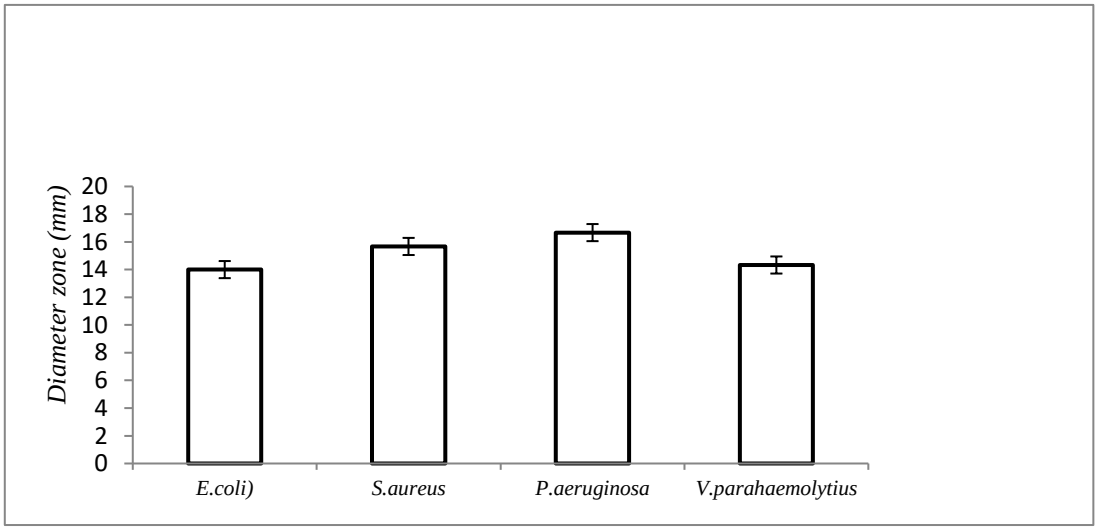

Figure 1. Anti-bacterial activities of ethanol from dendrocalamus asper shoots

The antibacterial activity of hot water extracts is shown in Table 3 and Fig.2. With regard to the hot water extract, every test organism is sensitive. With the maximum inhibition zones of 14,78±2,04 mm and 14,67±1,86 mm, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most sensitive of all. However, very less activity was observed in ethanol extract against *P.aeruginosa.* At

the same concentration, the zone decreases from 14,78±2,04 mm for *P.aeruginosa* and 14,67±1,86 mm for *S.aureus* to 13,56±0,51 mm for *E.coli* and 13,44±0,84 mm for *V.parahaemolytius*. In *E.coli* bacteria both ethanol extract and hot water extract has the same zone of inhibition. Significant inhibition was recorded with ethanol and aqueous extracts. It is important to note that the ethanol extracts can inhibit the growth of *P.aeruginosa* a kind of gram-negative bacteria that can cause urinary tract infections, burn and wound infections that result in bluish-green pus, eye infections, lung infections that cause pneumonia, and minor external muscularity in swimmers.

Table 3. Antibacterial activity of hot water extracts against bacteria

Bacteria	Zone of inhibition (mm)
Escherichia coli (E.coli)	13,56±0,51
Staphylococus aureus (S.aureus)	14,67±1,86
Pseudomonas aeruginosa (P.aeruginosa),	14,78±2,04
Vibrio parahaemolytius (V.parahaemolytius)	13,44±0,84

Interpretation:

<10 mm: inactive

10-13 mm: partially active

14-19 mm: active

>19 mm: very active

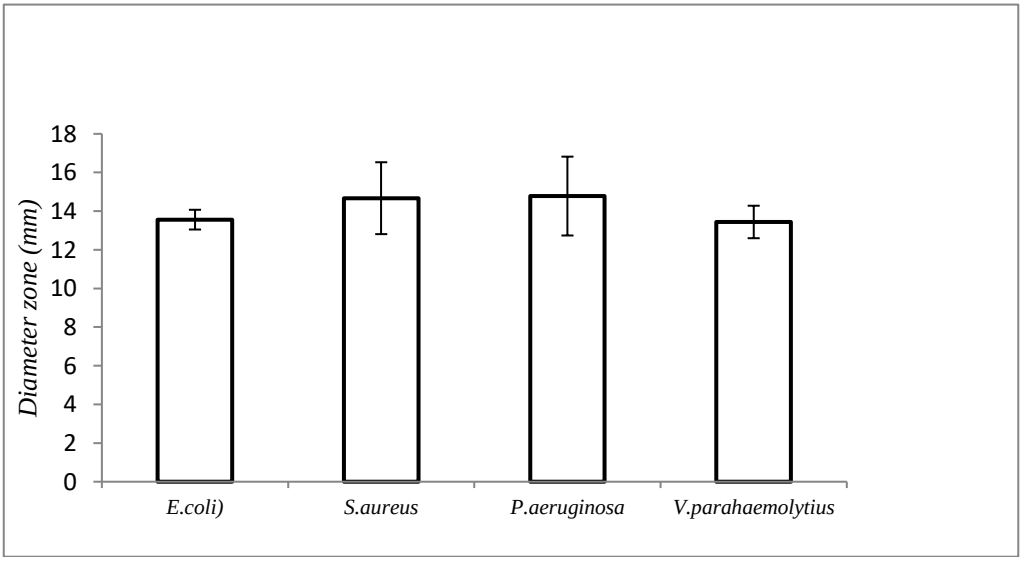

Figure 2. Anti-bacterial activities of hot water extracts from *Dendrocalamus asper* shoots

The antibacterial action of D.asper shoot extracts is primarily attributed to a multitude of bioactive components, including tannins, terpenoids, polyphenols, and flavonoids. It is found that all the test bacterial species i.e., *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Vibrio parahaemolytius* are sensitive to both ethanol and hot water extracts. This would suggest that the bamboo shoot extracts contain antimicrobial agents. Compared to hot water extracts, the ethanol extracts had more antibacterial activity. The majority of hot water extracts exhibited antibacterial activity across different zones of inhibition (from 13,44±0,84 mm to 14,78±2,04 mm). Antibacterial activity was demonstrated by the majority of ethanol extracts at different zones of inhibition (14.00±0.00 mm - 16.67±1.53 mm). For antibacterial activity against *Pseudomonas aeruginosa,* the ethanol extracts showed the strongest activities. It has been demonstrated that not all phytochemicals responsible for antibacterial activity are soluble in a single solvent due to variations in antibacterial activity with the same source when extracted with different solvents. Furthermore, one of the best solvents for antibacterial compounds found in plants is ethanol. Bamboo has

antimicrobial properties that could help maintain healthy skin. This suggests that bamboo shoots could be used as an alternative or source for synthetic medications used in medicine. Because natural sources like bamboo shoots have fewer negative effects than manufactured medications, using them is more important. It is essential to investigate the extracts' ability to inhibit the growth of other bacteria, test them in different solvents, and explore the D. asper shoots for further active chemicals.

Acknowledgment

This research is funded by Vietnam National University Hochiminh City (VNU-HCM) under grant number C2021-16-04.

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