

## Evaluation of the antibacterial effects of tannins extracted from *Dichrostachys cinerea*

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### ABSTRACT

**Background:** For an extensive duration, indigenous and therapeutic plants have demonstrated their essentiality in promoting human health. *Dichrostachys cinerea* is a botanical species that has been historically employed in African and Indian traditional medicine for the therapeutic management of diverse human ailments.

**Material and Methods:** The plant's root was obtained from the authorized vendor. The plant underwent proper authentication by a laboratory that is officially recognized by the government, confirming its classification as *Dichrotachys cinerea*.

**Results:** Tannins, which are compounds with pharmacological significance, were extracted from *Dichrostachys cinerea* and subjected to analysis against several bacterial strains including *Staphylococcus aureus*, *Shigella boydii*, *Shigella flexneri*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The agar diffusion method was employed for the assessment of their antimicrobial activity. The tannins shown antibacterial properties against all of the microbes tested. *Shigella flexneri* exhibited the highest level of resistance to tannins derived from the plant material, followed by *Shigella boydii*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* in descending order.

**Conclusion:** The investigation demonstrated that the tannins exhibited a minimum inhibitory concentration ranging from 4.1 to 5.3 mg/ml, and a minimum bactericidal concentration ranging from 4.6 to 6.1 mg/ml.

**Keywords:** Tannins, *dichrostachys cinerea*, antimicrobial activity, bactericidal.

### INTRODUCTION

Green plants are a rich source of a wide variety of useful compounds due to their high levels of synthetic activity, which may be observed in a wide spectrum. The antibacterial qualities of higher plants and shrubs, such as those found in particular vegetables, were at first recognized [1]. As an illustration, the phenolic compound known as thymol, which may be found in the essential oil of plants like *Thymus vulgaris* and *Monarda punctata*, possesses properties that are both antibacterial and antiviral. *Acalypha indica* contains a chemical called acalyphine, which is used in the medical treatment of gum

inflammation. The chemical in question has both expectorant and emetic properties [2-4].

It is known that the *Sida acuta* plant contains alkaloids. The plant is used in the region of Central America for the aim of managing a variety of different health concerns, including asthma, kidney inflammation, cold symptoms, fever, and headaches. The extract of the plant has high antibacterial activity, particularly against pathogenic pathogens. Tannins have been isolated and characterized from numerous plant families. Tannins were the focus of an investigation that was carried out on *Euphorbia hirta* as

part of a larger study. Antibacterial activity against *Salmonella typhi* was observed when the plant was grown in vitro. Tannins are a broad group of polymeric phenolic compounds that have the ability to "tan" leather as well as "precipitate" gelatin from a solution. Tannins can also be used to treat wounds [5-7].

Tannin is toxic to filamentous fungi, yeast, and bacteria. Tannin is also toxic to yeast. The botanical specimen under discussion is known as a shrub and can generally grow to a height of between 5 and 10 meters on its vertical dimension. The arrangement of the leaves, known as pinnate, demonstrates that the foliage has a composite quality. A pendulous spike is the distinguishing feature of the floral arrangement. The lower half of each bloom is tinted a pinkish white, while the upper half is colored yellow. This gives the blossoms a two-toned appearance. Tannins produced from *D. cinerea* were investigated for their potential to inhibit the growth of microorganisms [8-11].

The primary purpose of this study was to investigate the antibacterial properties of the *D. cinerea* plant against various microorganisms. The objective was to provide evidence supporting its antimicrobial activity.

## MATERIAL AND METHOD

### The origin of plant

The origin of the plant was obtained by collecting the root from the approved dealer. The laboratory that was authorized by the government determined that the plant in question was in fact *Dichrotachys cinerea*.

### Microorganisms

The clinical isolates of *Staphylococcus aureus*, *Shigella boydii*, *Shigella flexneri*, *Escherichia coli*, and *Pseudomonas aeruginosa* utilized in this study were procured from recently published research conducted by a research organization in the same field.

### Preparation of plant extract

The preparation of the ethanolic extract from the plant's root was conducted in accordance with the established method, with minor adjustments. The plant root sample, weighing 50 g, was subjected to air-drying and afterwards pulverized into a fine powder using an electric blender.

The composite substance was carefully poured into a glass container, often known as a beaker, and afterwards combined with 10 milliliters of pure ethanol at a temperature consistent with the surrounding environment, specifically  $28 \pm 2^\circ\text{C}$ . The mixture underwent extraction through agitation on a rotary shaker. The extraction process was permitted to continue for a duration of 48 hours. The extract was separated and the solvent was eliminated using evaporation at room temperature ( $28 \pm 2^\circ\text{C}$ ) in order to produce the extract [12, 13].

### Extraction of tannins

A 3 g sample of the powdered root was subjected to boiling in 5 ml of distilled water for duration of 3 minutes using a hot plate. The hot mixture underwent filtration, and the resultant filtrate was utilized for the ferric chloride test. The filter sample, weighing 1.0 g, was carefully transferred into a beaker, followed by the addition of 10 mL of distilled water. The substance underwent a boiling process for duration of 5 minutes. Subsequently, a total of two droplets of a 5% solution of ferric chloride ( $\text{FeCl}_2$ ) were introduced. The observation of the formation of a greenish precipitate served as an indication of the existence of tannins [14, 15].

### Antimicrobial test

The agar diffusion method was utilized to perform the antibacterial assay. The bacteria under investigation were inoculated onto nutrient agar plates and uniformly dispersed throughout the surface using a sterilized glass spreader. Aseptic technique was employed to utilize a sterile cork borer in order to make wells with a diameter of 5 mm on the nutritional agar medium. The agar disks that had been excised were carefully retrieved using forceps that had undergone sterilization through the process of incineration. Different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, or 3.5 mg/ml) of tannins obtained from the plant extract were administered into individual wells. Control trials were conducted wherein plant extracts were not included. The plates were allowed to stay undisturbed for a period of one hour at room temperature in order to promote the dispersion of chemicals before the commencement of microbial growth. The plates underwent incubation at a temperature of  $37^\circ\text{C}$  for a period of 24 hours. Following that, the areas of inhibition were recorded [16, 17].

**Table 1: Antibacterial activity of extract**

Concentration (mg/ml)	Zone of inhibition (Diameter)				
	<b>Staph. aureus</b>	<b>Sh. boydii</b>	<b>Sh. flexneri</b>	<b>E. coli</b>	<b>P. aeruginosa</b>
0.5	0.00	0.00	0.00	0.00	0.00
1.0	0.00	0.00	0.00	0.00	0.00
1.5	8.6 ± 0.02	8.8 ± 0.01	13.0 ± 0.2	8.7 ± 0.2	7.1 ± 0.3
2.0	9.1 ± 0.03	10.0 ± 0.04	16.0 ± 0.3	11.0 ± 0.2	9.0 ± 0.01
2.5	12.5 ± 0.4	13.0 ± 0.3	18.0 ± 0.02	13.1 ± 0.2	10.0 ± 0.02
3.0	14.0 ± 0.02	16.0 ± 0.2	21.0 ± 0.03	15.3 ± 0.3	12.4 ± 0.3
3.5	17.0 ± 0.03	20.0 ± 0.4	23.0 ± 0.06	17.4 ± 0.2	15.0 ± 0.2

#### Determination of MIC

Various concentrations of tannins extracted from *D. cinerea*, spanning from 4.0 to 6.0 mg/ml, were introduced into individual test tubes. Subsequently, an overnight culture of *Staphylococcus aureus*, *Shigella boydii*, *Shigella flexneri*, *Escherichia coli*, and *Pseudomonas aeruginosa* was injected into individual test tubes. The cultures were appropriately diluted to attain a final concentration of  $10^6$  cells per milliliter. The tubes were incubated at a temperature of 37°C for a period of 24 hours. The minimum inhibitory concentration (MIC) was established in each instance by identifying the lowest concentration of tannin that exhibited no visible growth of the inoculated test organism in the broth culture. [18, 19].

#### Minimum bactericidal concentration determination

After independently cultivating the test organisms in nutritious broth enriched with varying concentrations of the active components, the resulting broth was then injected onto freshly prepared agar plates to assess the bactericidal effect. The culture underwent incubation at a temperature of 37°C for a period of 24 hours. The minimal bactericidal concentration (MBC) was operationally defined as the concentration of alkaloid that exhibited no observable colony growth on the solid medium after the designated incubation period [20, 21].

#### RESULTS AND DISCUSSION

The present study involved the extraction and analysis of tannins, which exhibited antibacterial activity against *Staphylococcus aureus*, *Shigella boydii*, *Shigella flexneri*,

*Escherichia coli*, and *Pseudomonas aeruginosa*, as evidenced by the data presented in Table 1. The inhibitory activities identified in tannins are consistent with previous research indicating that the antibacterial properties of plants may be attributable to the presence of tannins, alkaloids, flavonoids, terpenoids, or essential oils. The results of this study are consistent with the research conducted by Kurosaki and Nishi, since they also observed a favorable link between the concentration of tannins and their antibacterial efficacy [22, 23]. According to Kurosaki and Nishi, higher concentrations of antimicrobial substances demonstrated a notable suppression in microbial growth. Several plant species with elevated tannin levels have exhibited antimicrobial effects against a range of microorganisms. The antibacterial activities of leaf extract obtained from *Eugenia uniflora* were investigated in a study conducted by Adebayo et al. The presence of tannins, alkaloids, and glycosides in the extract was revealed by the researchers. The antibacterial action of African medicinal herbs has been observed in vivo, resulting in the identification of several active components. Two instances of substances that have been extracted include muziyadial, which is acquired from *Warburgia salutaris*, and vemodalin, which is derived from *Vernonia coloratus* [23-25]. The range of minimum inhibitory concentration (MIC) values for the tannins identified in this specific study against the test organisms ranged from 4.1 to 5.3 mg/ml, as presented in Table 2 and figure 1. Furthermore, the range of the minimum bactericidal concentration (MBC) was observed to be between 4.6 and 6.1 mg/ml.

**Table 2: Tannins from *Dichrostachys cinerea* have MICs and MBCs**

Organism	MIC (mg/ml)	MBC (mg/ml)
Staph. aureus	5.3	6.1
Sh. boydii	4.4	5.2
Sh. flexneri	4.1	4.6
E. coli	5.3	5.7
P. aeruginosa	5.5	5.5

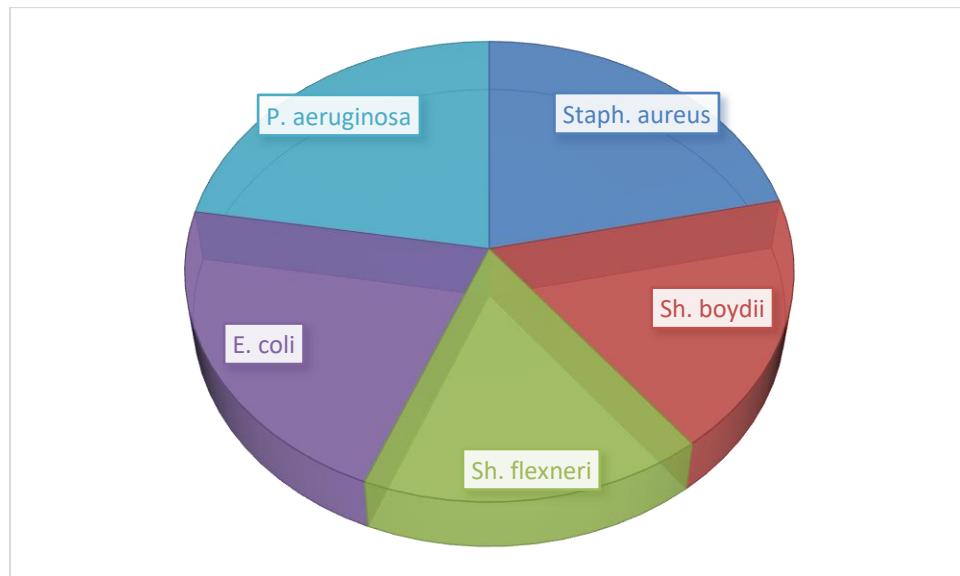


Figure 1: Tannins MICs and MBCs

Antimicrobial agents exhibiting limited efficacy against a particular organism are associated with a higher minimum inhibitory concentration (MIC), whereas antimicrobial medicines demonstrating strong efficacy yield a lower MIC. The findings of the current study provide empirical evidence that corroborates the long-standing utilization of *D. cinerea* in traditional medicine. The current investigation proposes that the tannins extracted from the experimental plant exhibit noteworthy antibacterial properties. Hence, there exists the potential for the cultivation of this plant as a viable source of antibacterial agents [26-28].

## CONCLUSION

The utilization of indigenous and therapeutic plants has demonstrated significant importance in promoting human health over an extended period. *Dichrostachys cinerea* is a botanical species that has been traditionally employed in Indian traditional medicine for the management of diverse human ailments. This study aims to examine the ethnomedicinal applications, phytochemical ingredients, pharmacology, and toxicity of *D. cinerea*, with the objective of establishing a scientific consensus that can guide future research and utilization of the therapeutic properties associated with this plant. The present study's results offer empirical support for the historical use of *D. cinerea* in traditional medicine. The present study posits that the tannins derived from the experimental plant demonstrate significant antibacterial activities. Therefore, it is possible to cultivate this plant as a promising reservoir of antibacterial compounds.

## Funding

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## Conflict of Interest

None

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