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Determination of secondary metabolites and antibacterial property of extract from the leaves and bark of *Syzygium lineatum (DC.) Merr. & L.M. Perry* (Malibado)

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Abstract
Phytochemical screening using the standard qualitative technique revealed the presence of phenols, terpenoids, tannins, glycosides and anthraquinones in the leaves extract and saponins, alkaloids, phenols, steroids, tannins, flavonoids, anthraquinones, glycosides and sugar in the bark extract. Paper disc diffusion method was applied in evaluating the antibacterial activity of the crude extract against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. The bark extract was active against S. aureus and E. coli measuring clear zones of inhibition of 21mm and 22.66mm respectively. This indicate the presence of these bioactive components in *S. lineatum*, which proves its potency in the treatment of some bacterial infections.

Keywords: phytochemical analysis, indigenous tree, paper disc diffusion method, antibacterial activity, *Escherichia coli*, *Staphylococcus aureus*

1. Introduction
Malibado (*Syzygium lineatum (DC.) Merr & L.M. Perry*) is one of the fruits that the Philippines can call its very own as it is native to Luzon where it originated from primary forests at low medium elevation [1]. *S. lineatum* (malibado) berries are believed to contain antioxidants which give fruits their vibrant color and it is also a good source of Vitamin C, dietary fiber and essential dietary mineral and it contains phytonutrient that brings varieties of health benefits [8].
As it true with many indigenous trees, *S. lineatum* (malibado), a Philippine berry is now rarely found in its natural habitat, a fact that that drives the need to implement conservation measures. The best way to do this is to promote its usefulness as a highly nutritious food source which could be provided by data on its functionality as well as the phytochemicals or secondary metabolites it contains [6]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-merging infectious diseases [5]. This study is an attempt to determine the secondary metabolites present and evaluate the antibacterial activity of the leaves and bark extract of *S. lineatum* (malibado) against the pathogenic bacteria.

2. Materials and Methods
Plant Materials
S. lineatum (malibado) plant were collected at Annafunan, Echague, Isabela, Philippines. These were selected because of their availability of plant species in the town that could be tapped for the food supplementing and nutritional values. The identity of the plant was authenticated by a forester from the Community Environment and Natural Resources Office, San Isidro, Isabela, Philippines.

Extraction of Bioactive Constituents
The dried leaves and bark of *S. lineatum* were cut into piece and air dries for three days resulting to coarse particles. A 500 grams of leaves and 650 grams of bark of air-dried samples was macerated enough quantity of ethanol. The sample was soaked undisturbed at room temperature for 72 hours and then filtered. Extraction was repeated using 1.0L of ethanol for another 48 hours. The filtrates were combined and concentrated under reduced pressure using a rotary evaporator at room temperature below 50^{O}C. The crude extract was further dried to a constant weight of 338.6 grams for the leaves and 146.6 grams for the bark extract.

Phytochemical Evaluation of the Crude Extract
Chemical classes of secondary metabolites were investigated in the *S. lineatum* crude extracts (leaves and bark) through identification reactions using the standard procedures [2, 9, 7]. The constituents evaluated includes: alkaloids, flavonoids, saponins, tannins, steroids, reducing sugars, glycosides, coumarins, terpenoids, phenolics and anthraquinones.

Detection of Antibacterial Activity
The antibacterial activity of *S. lineatum* leaves and bark extract was evaluated against two strains, *S. aureus* (gram-positive bacteria) and *E. coli* (gram-negative bacteria). The disc diffusion method [3] was used in the microbial evaluation. Bacterial cultures maintained on nutrient agar slants were taken and ascetically inoculated into 10ml sterile broth. Then broth containing the bacteria were incubated at 37^{O}C at 24 hours and designated as working stocks for antibacterial studies. *S. lineatum* leaves and bark extract was screened over the range of 0.5 to 10 mg/ml concentration using paper disc diffusion method. About 1 ml of the bacterial suspension was taken and diluted in 10ml autoclaved water and this suspension was inoculated on semi-solidified nutrient agar medium. Small autoclaved discs about 6mm diameter size of Whatman filter paper (No. 41) were impregnated with 1 ml of the extract then these saturated paper discs were inoculated equidistantly. These set up were incubated at 37^{O}C for 24 hours. In the whole investigation, paper disc impregnated with 85% ethanol was

taken as control. The zone of inhibition (ZOI) around each disc indicative of the sensitivity at that extract was observed and measured using sterilized caliper. Activity of the extract was compared with the corresponding references [4].
ZOI value of < 10mm, maybe expressed as inactive
ZOI value of 10-13mm, partially active
ZOI value of 14-19mm, active
ZOI value of >19mm, very active

3. Results and Discussions

Phytochemical Analysis

The preliminary phytochemical screening of the leave and bark extract of *S. lineatum* indicates that the plants is quite rich in phytochemicals (Table 1). Results showed that the leaves extract contains phenols, terpenoids, tannins, glycosides and anthraquinones. Analysis of the bark extract revealed the presence of saponins, alkaloids, phenols, steroids, tannins, flavonoids, anthraquinones, glycosides and sugar.

Table 1: Phytochemical Screening of *S. Lineatum* Leaves and Bark Extract

Qualitative Test	Results	
	Leaf Extract	Bark Extract
Saponin Test	--	+
Alkaloid Test	--	+
Flavonoid Test	--	+
Phenol Test	+	+
Tannin Test	+	+
Steroid Test	--	+
Terpenoid Test	+	--
Glycoside Test	+	+
Anthraquinone Test	+	+
Coumarin Test	--	--
Sugar Test	--	+

+ = presence of the active constituents
- = absence of active constituents

These phytochemical compounds present in the plants were found to be interesting because they inhibit bacteria growth by mechanisms different from presently used treatment regimens, and could therefore be of clinical value in the treatment of resistant bacteria.

Antibacterial Activity

The results of the antibacterial activity of *S. lineatum* leaves and bark extract are presented in Table 2 and Figures 1a to Figure 3. As indicated by the results, the *S. lineatum* bark extract exhibits a good antimicrobial activity against the pathogens with a zone of inhibition of 22.66 mm against *E. coli* and 21 mm against *S. aureus*. The disc impregnated with the bark extract gave the highest zone of inhibition considered to be very active when compared with the standard. The result is similar to the data obtained by Umaru [10] when they evaluated the antibacterial activity of the bark extract of Leptadenia hastata and upon analysis showed that the bark extract contained various bioactive constituents that might possible for the antibacterial activity of the plant.

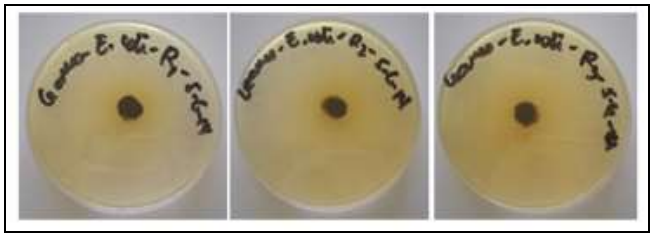

Fig 1a: Result of Antibacterial Activity of S. Lineatum leaves against *E. coli*

Fig 1b: Result of Antibacterial Activity of S. Lineatum leaves against *S. aureus*

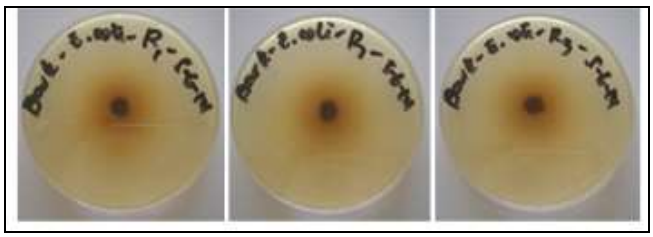

Fig 2a: Result of Antibacterial Activity of *S. Lineatum* bark against *E. coli*

Fig 2a: Result of Antibacterial Activity of *S. Lineatum* bark against *S. aureus*

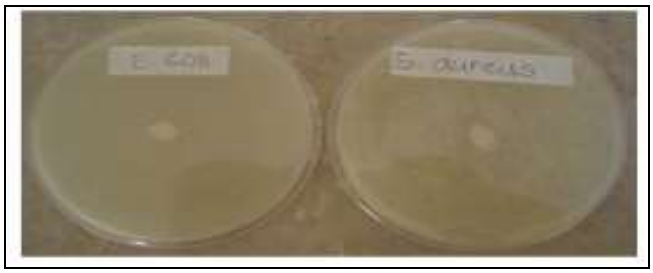

Fig 3: Result of Antibacterial Activity of Ethanol that serve as the control

Table 2: Inhibition of S. *Lineatum* Leaves and bark Extract Against *E. Coli* and *S. Aureus*

S. *Lineatum* Extract	Zone of Inhibition (mm)	
	S. aureus	E. coli
Control (Ethanol)	18	15
Leaves	5	8
Bark	21*	22.66*

4. Conclusion

The continuous emergence of diseases and the increasing prices of medicine call for the discovery of new less expensive plant-based medicines. The study revealed that *S. lineatum* bark extract have a high potential as antibacterial agents. The antibacterial property can be attributed to the secondary metabolites present in the plants. Results from the present study can provide baseline information about the antibacterial potential and phytochemical constituents of the plants.

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