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Research Article

Antibacterial Effects of Condensed Tannin Extracted from *Acacia mangium*

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Abstract

The awareness of overused of antibiotics in livestock has been arisen recently. This is due to the emergence of antibiotic-resistance strains which make them more difficult to treat infections caused by the resistant bacteria. Therefore, this article will briefly discussed about the potential of using condensed tannins isolated from *Acacia mangium* to inhibit the growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella* sp. From the study, condensed tannin shows a good inhibitory activity against tested strains at the concentration of 100 mg/mL which comparable with Vancomycin (30 µg). In future, this compound could be a used as antibacterial agent to treat bacterial infection, especially for livestock animal.

Keywords: *Condensed Tannin; Acacia; Antibacterial; Plant Compounds; Antibiotic-Resistant*

Introduction

Bacterial infections in livestock has a great importance worldwide in terms of economic and health. There are several important diseases caused by the bacteria. One of them are mastitis, a disease which commonly seen in dairy cows and goats. Mastitis is usually caused by bacterial infection of the mammary gland and subclinical mastitis is the most common form of mastitis where no gross inflammation of the udder and no gross changes in the milk are seen [1]. There is decreased in the production and quality of milk. Primary cause of mastitis in cattle are well-recognized groups of microorganisms such as *Streptococcus*, *Staphylococcus aureus*, *Mycoplasma* and *Pasteurella*. Some coliforms such as *Pseudomonas*, also been found frequently infecting the udder, which known as Environmental Mastitis. Also, *Staphylococcus aureus* mastitis in heifers often occurs before calving. The germ of this cattle disease's spreads through the damping house, milking with dirty hands, injury in mammary glands or udder and coagulation of milk in the mammary glands. The milk becomes sieve like curd, has yellow colored sediment, sometimes with blood. When the mammary glands and udder get damaged, farmers/producer will stop the milk production. The infected cows are commonly treated with antibiotics, usually the beta lactam antibiotics of the penicillin family [2].

Salmonellosis also is one of the common diseases in livestock that caused by *Salmonella* infections. The infected animals will show the clinical signs of coughing, dyspnea, and later on may caught by pneumonia disease [3]. Usually, the veterinarian will give antibiotics for the treatment of those infections. However, nowadays, the emergence of antibiotic-resistant bacteria has become a problem to successful treatment for bacterial infections [4]. Therefore, many scientists start to find other ways to replace the antibiotics. Recently, many studies have been done to use biologically active compounds (also known as secondary compounds) extracted from plants to treat bacterial infections [5], in livestock. They include tannins, phenols, steroids and alkaloids. Naturally, these compounds are found in various parts of plants includes leaves, stem, barks, and pods/seed [6].

Tannins are high molecular weight phenolic compounds present in many plants [7]. There are two groups of tannins; hydrolysable tannins and condensed tannins. Condensed tannins (CT) were known to be able to interact with biological systems through the induction of some physiological effects, such as antioxidant, anti-allergy, anti-hypertensive, and antimicrobial activities [8,9].

Acacia is a widespread genus of tropical-subtropical trees and shrubs found in Central/South America through Africa to Southeast Asia and Australia [10]. The predominant Acacia species in Malaysia is *Acacia mangium.* It possesses many advantageous growth characteristics such as a high leaf yield, drought tolerance, adaptation to acidic infertile soils and large temperature variations [11]. This plant also was shown to contain tannins. However, the reactivity of these tannins was dependent on the structure and their chemical nature to associate with the substrate [12].

Therefore, this study was conducted to assess the antibacterial activity of condensed tannins extracted from *Acacia mangium* against three types of common pathogen which are *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella sp.* The activity was determined based on data of diameter zone of inhibitions and minimum inhibition concentration (MIC).

Materials and Methods

Condensed tannin extraction

Crude tannins were extracted from the dried parts of *Acacia mangium* using aqueous acetone/diethyl ether as described by Terrill., *et al.* (1992) [13]. The crude tannins were extracted from 2g of dried samples in 50 ml centrifuge tubes with 40 ml extraction solvent (70% (v/v) aqueous acetone containing 0.1% (w/v) ascorbic acid). The supernatant was filtered under vacuum, to remove any particulate plant residues. The filtrate was then washed with an equal volume of diethyl ether in a separation funnel to remove chlorophyll, pigments and low molecular weight phenolic acids. Traces of acetone and diethyl ether in the condensed tannin extracts were further evaporated under vacuum in a rotary evaporator at 40°C and the CT were lyophilized at -56°C for 48 hours.

The concentration of condensed tannin in each fraction was measured using modified vanillin assay. Catechin and vanillin were purchased from Sigma Gmbh, Germany and all other reagent was analytical grade. A 20 µL of fractions were seeded into 96-wells plate and 120µL of Vanillin reagent and 60µL of concentrated hydrochloric acid were added into each well. The plate was incubated for 15 minutes in dark and absorbance was recorded at wavelength 500nm using Epoch Elisa reader (BioTek Instruments, USA). Results were calculated using the standard curve of (+)-catechin (SigmaAldrich, USA).

Bacterial cultures and preservation

The microorganisms, were supplied by Bioassay Laboratory of Livestock Science Research Centre, MARDI, Malaysia. The bacterial strains used are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* sp. These bacteria were cultured and maintained in Nutrient broth and Mueller-Hinton agar (Bacton-Dickinson, BD, USA) for 24 hours at 37°C under aerobic conditions. The purity of bacterial species was monitored using gram staining method. These bacteria were preserved frozen under -40°C in glycerol (15%) and freeze dried in sterilized skim milk (10%).

Antibacterial activity assay

The antibacterial activity of the CT was carried out by disc diffusion test (Kim, 1995). In this study, polyethylene glycol (PEG), a CT inhibitor was used as one of the treatment groups to observe the CT-inhibitory activity against tested strains. The inhibitory activity of CT was compared with Gentamycin (10 µg), Metronidazole 5 µg and Vancomycin (30 µg). 100 mg/ml of extracted condensed tannins and CT-PEG were used against 0.5 McFarland of tested bacteria. After 24 hours incubation at 37°C, the zones of inhibition were formed. The diameter of the zones of inhibited growth was measured to the nearest whole millimetre, including the diameter of 6mm disk as its antimicrobial activity. The endpoint was taken as the area showing no obvious growth that could be detected with the visibility, not including a faint of growth or tiny colonies which can be detected with difficulty at the edge of the zone of inhibited growth.

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after an overnight incubation [14]. The procedures were in accordance with the recommendations provided by the Clinical and Laboratory Standards Institute (CLSI). Mueller Hinton broth was inoculated with a standardized inoculum of the test bacteria (approximately 10^5 CFU/mL) in 96-wells plate. Several dilutions of CT extracts were added into each well. After 20 hours incubation, the MIC was recorder as the lowest concentration of antibacterial with no visible growth of the bacteria [15]. Each test was run in triplicates.

Statistical analysis

The zone of inhibitions and MIC (mg/mL) values after treated with condensed tannins were calculated based on the sample means of triplicates of the diameter zone (in mm) and the concentration of CT extracts (in mg/mL).

Results and Discussion

The concentration of condensed tannin extracts was determined using modified vanillin assay based on catechin values. The CT extracts were then diluted into 100 mg/mL and tested against three types of bacterial strains and the data were presented in table 1.

Description	Diameter zone of inhibition (mm)*		
	S. aureus	***P. aeruginosa***	***Salmonella sp.***
Condensed tannins	17.83 ± 0.82	11.67 ± 1.03	10.33 ± 1.03
Condensed tannins + PEG	11.75 ± 0.52	NI	NI
Metronidazole (5µg)**	NI	NI	NI
Vancomycin (30 µg)	16.83 ± 0.52	NI	16.83 ± 0.75
Gentamycin (10 µg)	21.75 ± 0.88	21.75 ± 0.88	19.33 ± 1.03
Minimum inhibitory concentration			
CT concentration (mg/mL)	4.0	4.0	16.0

Table 1: *Inhibitory activity of condensed tannins on Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella sp.*
PEG (1:1): Polyethylene glycol (CT-inhibitor) at 1 to 1 ratio with CT, CT: condensed tannins at concentration of 100 mg/ml, NI: no inhibition.
**mean ± standard deviation.*
***Negative control.*

Based on the results, all strains were found to be susceptible to condensed tannins at the concentration of 100 mg/mL. This could be due to the characteristics of CT which were able to bind with proteins (on bacterial cell wall of Gram-positive bacteria, or plasma membrane of Gram-Negative bacteria), where it causes cell damage and death [16,17]. However, based on the diameter zone, it was found that CT react more stronger to the Gram-positive bacteria (*S. aureus*) compared to Gram-negative bacteria (*P. aeruginosa* and *Salmonella sp*.). The Gram-negative bacteria was less susceptible to the action of condensed tannin due to the lipopolysaccharide outer membrane surrounding the bacterial cell wall [18], which restricts diffusion of hydrophobic compounds. Gram-negative bacteria were also reported by other previous studies to be more resistant to the plant-origin antimicrobials and even show no effect, compared to the Gram-positive bacteria [19,20].

The minimum concentration of condensed tannins to inhibit the growth of *S. aureus* and *P. aeruginosa* were recorded as low as 4 mg/mL, however higher concentration at least 16 mg/mL of CT required to inhibit the growth of *Salmonella* sp. These results showed that CT act more effective towards *S. aureus* and *P. aeruginosa*, compared to *Salmonella sp*. Theoretically, there are several mechanisms of action involves in the inhibition process. They include the destabilization of cytoplasmic and plasma membranes, inhibition of extracellular microbial enzymes and metabolisms, and deprivation of the substrate required for microbial growth. However, the mode of antimicrobial

action of plant tannin may be related to their ability to inactivate microbial adhesion, enzymes and cell envelope transport proteins, and mineral uptake or polymerization through oxidation reactions. Hence, the disintegrative ability of tannin compounds on the bacterial colonies probably resulted from their interference with the bacterial cell wall to inhibit their growth. while the degree of toxicity resulting from their polymerization may be responsible for the degree of the bactericidal activity of the extract over a short period of time in the MIC test [21].

The antimicrobial activities of condensed tannin against pathogen microbes were also reported by Suraya (2011) [22]. The report showed that the extracted CT from *Rhizophora apiculata* bark inhibited *S. aureus* at 100 mg/mL of CT extracts with 11.0 mm diameter of inhibition zone. The diameter of inhibition zone also was found to be fair for Gram-positive and Gram-negative bacteria, plus there was no inhibition of fungi in CT extract observed.

Conclusion

Currently, microbial infections were significantly associated with morbidity and mortality due to the development of microbial resistance to antibiotics. The data obtained in this study show condensed tannins have a potency to be considered as a preservative agent for antibacterial activities against *S. aureus, Pseudomonas aeruginosa* and *Salmonella sp*. The CT extract was able to inhibit the growth of the tested bacteria at concentration of 16 mg/mL. This study provided important baseline information for the use of condensed tannins isolated from *A. mangium* as the antibacterial agent for some types of bacteria. However, more group of pathogenic bacteria from animal, including yeast and fungi should be tested to enhance the scientific findings.

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Conflicts of Interest

There is no any conflict of interest.

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