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Antibacterial and Antioxidant Activities of Proteins Extracted from *Annona squamosa* Seeds

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Abstract
Annona squamosa are important medicinal plants in Malaysia which have been used in traditional medicine. The purpose of the study was to determine antibacterial and antioxidant activities of protein extracted of the seeds of *A. squamosa*. Well diffusion assay, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were used to test antibacterial activity against four pathogenic bacteria namely *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA). DPPH assay and Superoxide dismutase activity SOD assay used to antioxidant activity. Protein extracts from *A. squamosa* have antibacterial and antioxidant activities. It could be concluded that the protein extracted of this plant had a good antibacterial and antioxidant effects. The results suggest that these plants can be a new source of antimicrobials against pathogenic bacteria.

1. Introduction

Natural products from different sources such as plants and microorganism have played an important role in the prevention of infectious diseases and are of general use in health care [1]. Antibacterial peptides and protein are important compounds for plants as they are a part of the defence against of infections by a range of harmful pathogens [2, 3]. They display antibacterial activity towards bacteria at low concentrations compared to other bioactive compounds from natural sources [4]. The first plant source from where antibacterial proteins were isolated is wheat flour (*Triticum aestivum*) which has the ability to inhibit the growth of *Pseudomonas solanacearum, Xanthomonas campestris* and *Corynebacterium michiganese* [5].

Mandal, Dey *et al.* [6] reported that three purified proteins (Cn-AMP1, Cn-AMP2, Cn-AMP3) extracted from green coconut water demonstrated antibacterial activity towards *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa*.

The genus *Annona* comprises 120 species. An economically significant species is *A. squamosa* which belongs to the Annonaceae family. Its specific native range is indefinite because of widespread commercial cultivation but is generally deemed to originate from the Caribbean region [7]. Common names for this plant are *Nona*, sugar apple, ata, gishta and sweet sop [8; 9]. It is a small semi-evergreen tree/shrub, 3-7 m tall, with irregular or crown branches. The leaves are oblong-lanceolate and pale green on both surfaces. The flowers are greenish-yellow and produced in single or short lateral clusters [10]. The petioles are green and 0.6-1.3 cm in length. The fruit of this plant is round, heart shaped, ovate or conical. It is green-yellow in colour initially, but

the ripe fruit is white with the sweetly aromatic pulp also white [8]. The seeds are shiny, numerous, and blackish or dark brown in colour [11].

A. squamosa extracts from several solvents have antibacterial activity against many strains of bacteria. Padhi, Panda *et al.* [12] used different solvents for leaf extracts of *A. squamosa* to inhibit Gram-positive bacteria such as *S. aureus, B. subtilis, S. epidermidis* and Gram-negative bacteria including *E. coli, P. aeruginosa, S. typhi, Vibrio alginolyticus* and *V. cholera*. The silver nanoparticles of aqueous extract from the leaf of sweet apple exhibited antibacterial activity against *B. cereus* (NCIM 2703), *B. subtilis* (NCIM 2635), *S. typhimurium* (NCIM 2501), *S. aureus* (NCIM 2654), *P. aeruginosa* (NCIM 5032) and *Proteus vulgaris* (NCIM 2813) [13].

The aim of this study is to determine the antibacterial and antioxidant activities proteins extract of from *A. squamosa*.

2. Materials and Methods

2.1. Plant Collection

A. squamosa leaves were collected in November, 2010, from Juasseh, Kuala Pilah. This plant was identified at the herbarium and given the registration numbers KLU 047368. All samples were washed under tap water and dried in an oven at 40°C for 3 days. The plant materials were then put through a grinder with a mesh size of 2 mm.

2.2. Proteins Extract From *A. squamosa* Seeds

This method was based on [14] with some modification. The dried powders of the seeds were added to 100 ml of 20 mM sodium acetate (pH 5). This mixture was filtered and centrifuged at 5343 g for 30 min at 4°C. The supernatant was precipitated overnight using 65% ammonium sulphate at 4°C. The supernatant was centrifuged under the same conditions. The pellet was collected and analysed against pathogenic bacteria and antioxidant assays.

2.3. Determination of Antimicrobial Activities

For this study, four species of bacteria were used. *S. aureus* (RF 122), *E. coli* (UT 181), *B. cereus* (ATCC 14579), and *P. aeruginosa* (PA 7) were procured from cultures maintained at the Fermentation Technology Laboratory in the Microbiology Division, Institute of Biological Sciences, University of Malaya, Malaysia. Other strains used in this study included methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC BA-43) and *Helicobacter pylori* ATCC 43504. Antibacterial activities were measured using well diffusion assay. The positive control used was 10 mg/ml of tetracycline, while the negative control was 5% DMSO. All extracts were checked for their respective MIC values using a standard protocol [15]. MBC values were determined by sub-culturing the MIC assay tubes onto Muller-Hinton agar (Difco, Detroit, MI, USA), and represent the dilution at which growth was detected.

2.4. Determination of Antioxidant Activities of Plants

2.4.1. DPPH Radical Scavenging Assay

Free radical scavenging activity was determined using the method of Bozin, Mimica-Dukic *et al.* [16] The reagent of the assay is 2, 2- diphenyl-1- picrylhydrazyl solution (Sigma Aldrich GmbdH, Germany). The percentage of DPPH radical scavenging activity of the resulting solutions was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Ascorbic acid (10 mg/ml) was used as a positive control of the assay.

IC_{50} was calculated using linear regression plots. The IC_{50} values represent the concentrations of samples that are required to scavenge for 50% of DPPH free radicals.

2.4.2. Superoxide Dismutase Activity Assay

SOD activity was determined using a SOD Assay Kit-WST (Dojindo Molecular Technologies, Gaithersburg). The protocol used in this study was modified from [17]. The positive control was ascorbic acid (10 mg/ ml).

2.5. Statistical Analysis

Data is expressed as mean ± SD. Statistical analyses were carried out using SPSS version 17. One-way ANOVA followed by Duncan's multiple comparison were used to compare the values of samples with the control. A P value < 0.05 was deemed as indicating significant differences. Each treatment was duplicated thrice and each experiment was repeated at least twice.

3. Results

3.1. Antibacterial Activity

In a well diffusion assay, proteins extracts from *A. squamosa* showed inhibition zones against selected test microorganisms. Figure 1 shows the zones of inhibition for the proteins extract from the seeds of *A. squamosa* as 6.33 mm for *S. aureus*, 8.66 mm for *B. cereus*, 6.33 mm for *P. aeruginosa*, 6.33 mm for MRSA and no inhibition for *E. coli* and *H. pylori* at a high concentration of this extract (100 mg/ml).

MIC and MBC values Proteins extracted from *A. squamosa* seeds at a concentration of 1.67 mg/ml displayed inhibition against *S. aureus*, *B. cereus* and MRSA *P. aeruginosa* while was inhibited at a lower concentration of 0. 83 mg/ml (Table 1).

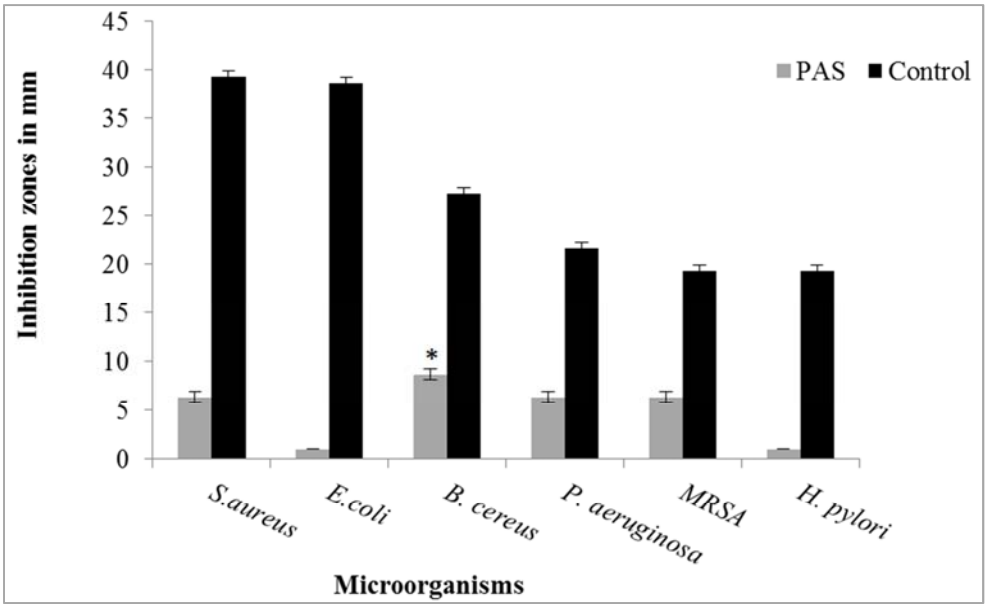

Figure 1. *Inhibition zones of proteins extract of A. squamosa (PAS) on the test microorganisms. All experiments were done in triplicates and values represent means ± SD. * significant different at p > 0.05.*

Table 1. *MIC and MBC of proteins extract of A. squamosa (PAS) on the test microorganisms.*

Bacteria	Plant extracts (mg/ml)	
	MIC	MBC
S. aureus	1.67*	>1.67*
E. coli	0.00	0.00
B. cereus	1. 67*	>1.67*
P. aeruginosa	0.83	0.83
MRSA	0.83	0.83
H. pylori	0.00	0.00

Na–non active at high concentration. * Significant different at p > 0.05.

3.2. Antioxidant Activity

Figure 2 presents the free radical scavenging activity of the proteins extracted from *A. squamosa* seeds as 49.62% (IC_{50} 10.08 mg/ml) compared to ascorbic acid as a positive control at 96.59% (IC_{50} 5.18 mg/ml).

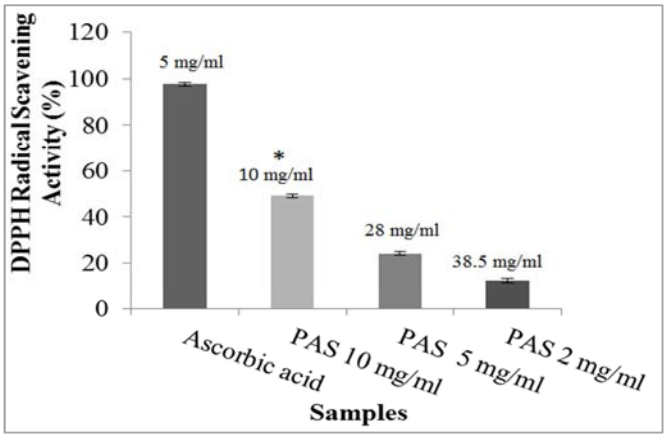

Figure 2. *Free radical scavenging activity with IC_{50} values of proteins extracts of A. squamosa seeds (PAS). All experiments were done in triplicates and values represent means ± SD. * significant different at p > 0.05.*

The of SOD-like activity for proteins extracted from *A. squamosa* seeds have moderate antioxidant activity at 70.13% compare with ascorbic acid at 96.97% for ascorbic acid (Figure 3).

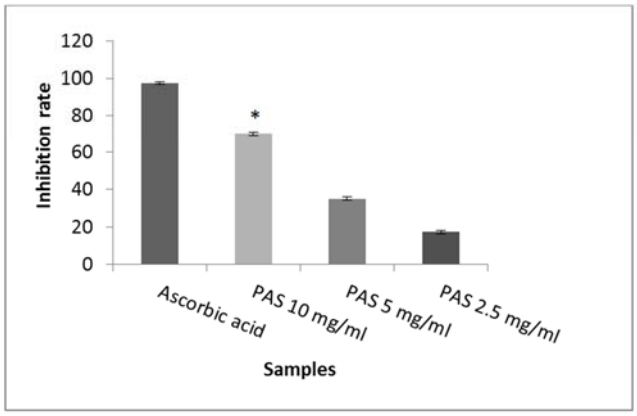

Figure 3. *The rate of inhibition of SOD–like activity of proteins extracts of A. squamosa seeds (PAS). All experiments were done in triplicates and values represent means ± SD. * significant different at p > 0.05.*

4. Discussion

Proteins extracts from the fruits of *A. squamosa* seeds have antibacterial properties against *S. aureus, B. cereus*, *P. aeruginosa* and MRSA but not against *E. coli* and *H. pylori.* Antibacterial proteins generally have cysteine or glycine residues which can play a role in their activities against bacteria [18-20]. MIC and MBC values are significant against *B. cereus,* S. *aureus*, *P. aeruginosa* and MRSA. The seeds of this plant are known to be rich in amino acids such as tryptophan, threonine, tyrosine, serine and alanine. These amino acids in proteins can play a role in antibacterial activities against bacteria while some bacteria show

resistance to proteins [21].

Proteins extracts of *A. squamosa* seeds have antioxidant activities based on the DPPH and SOD assays with significant values of IC_{50} comparable with ascorbic acid. Proteins extracts from the seeds have comparatively higher levels of antioxidant. This plant has been shown to be rich in certain amino acids which may contribute to the antioxidant activity [21].

5. Conclusions

In conclusion, this is the first report that studied antibacterial activity antioxidant capacity in proteins extracts from *A. squamosa* seeds. These proteins extracts of *A. squamosa* seeds had antibacterial activity against resistant strains of test bacteria including MRSA and *H. pylori* have resistance against proteins extracts. Proteins extract from *A. squamosa* seeds have antioxidant activity. Proteins have important compounds which may be used to develop biopharmaceuticals against infectious diseases and antioxidants source in future.

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