

In Vitro Antibacterial Activity of *Psidium guajava* Linn. Leaf Extracts against Pathogenic Bacteria in Pigs

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ABSTRACT

Guava, Psidium guajava Linn., is an important medicinal plant in tropical and sub-tropical regions. Previous studies of the in vitro and in vivo antibacterial activities of Guava extracts have raised expectations for the potential of developing medicinal compounds for human use. In addition, with increased antibiotic-resistant bacteria and drug residues observed in pork, medicinal plants, such as guava, might serve as an alternative source of medicinal compounds for veterinary medicine. In the present study, crude extracts from the leaves of *P. guajava* were obtained using three different extraction methods. The extracts were tested for antibacterial activities against pathogenic bacteria in pigs, namely, *Streptococcus suis*, *Pasteurella multocida*, *Escherichia coli* and *Salmonella typhimurium*. The results showed that leaf extracts of *P. guajava* in water and methanol potentially inhibited growth of all tested bacteria, while extraction with acetone exhibited inhibition zones only in colonies of *Streptococcus suis* and *Pasteurella multocida*. The water and methanol extracts of guava leaves showed the same minimum inhibitory concentration (MIC) values against *Pasteurella multocida* (0.156 mg/ml), *Escherichia coli* (5 mg/ml) and *Salmonella typhimurium* (5 mg/ml), while the acetone extract showed the highest antibacterial activity against *Streptococcus suis* and *Pasteurella multocida* at a MIC of 0.312 mg/ml. In summary, this investigation provides preliminary information for using guava extracts to control bacterial diseases in pigs. Given this finding, the crude extracts of guava leaves show promise as an alternative antibacterial source for use in veterinary medicine.

Keywords: *Psidium guajava* Linn., Leaf extract, MIC, Antibacterial activity, Pig

INTRODUCTION

Many investigations have studied the use of plants as an alternative, and natural source of medicinal products for humans. Guava (*Psidium guajava* Linn.)

is among the most commonly studied medicinal plants (Chiwororo and Ojewole, 2008; Gutierrez et al., 2008). *P. guajava* is widely known for its antibacterial, antiviral and antioxidant properties (Jaiarj et al., 1999; Goncalves et al., 2005; Tachakittirungrod et al., 2007). The antimicrobial activity of *P. guajava* against several bacteria species is commonly applied in human medicine in southern Africa and parts of Asia (Begum et al., 2002a; Rahim et al., 2010). A recent report has demonstrated effective antibacterial activity of *P. guajava* leaf and bark extracts against multidrug-resistant *Vibrio cholerae* in humans (Rahim et al., 2010). This study, together with other reports, shows the potential of *P. guajava* as an alternative, indigenous herbal medicine for controlling bacterial infections in humans (Lutterodt, 1989; Mahfuzul Hoque et al., 2007; Anas et al., 2008; Rahim et al., 2010). However, few studies have examined the potential for using *P. guajava* crude extract against pathogenic bacteria in farm animals, especially pigs.

Generally, bacterial infection in pigs is controlled by antibiotics (Kernodle and Kaiser, 1994; Lee et al., 2001; Bywater, 2004). The use of antibiotics in pig production has attracted considerable attention because their misuse can lead to the development of antibiotic-resistant bacteria (Bywater, 2004). Additionally, the accumulation of antibiotics in pork is risky to consumers and may become a major public health problem (Lautner, 1997; Duong et al., 2006). Thus, natural products have been extensively considered as an alternative source to control bacterial diseases in animal production (Shan et al., 2007; Pachanawan et al., 2008).

In the present study, *in vitro* antibacterial activity of *P. guajava* leaf extract on pathogenic bacteria in pigs was investigated. Four different bacterial pathogens, namely, *Streptococcus suis*, *Pasteurella multocida*, *Escherichia coli* and *Salmonella typhimurium* were used. The antibacterial activity and the minimum inhibitory concentration (MIC) of *P. guajava* leaf extracts from different extraction methods against certain pathogenic bacteria in pigs are described. The results of this study shed new light on the application of herbal medicine to control bacterial diseases in pigs.

MATERIALS AND METHODS

Plant materials and extractions

Fresh leaves of mature plants of *P. guajava* from Hang Dong District, Chiang Mai Province were collected. Crude leaf extracts were obtained by using the decoction and maceration methods as previously described (Jaiarj et al., 1999). For the decoction method, one kg of fresh *P. guajava* leaves was boiled in 3 liters of water for 2 h. They were then wrapped in filter paper (Whatman No. 1) and the filtrates were collected. The extract solution was subsequently heat-dried and finally pulverized, producing a powder of *P. guajava* leaf extract. The extract was kept at 4°C until use. For the maceration method, *P. guajava* leaves were washed, dried and milled into coarse powder by mechanical grinder. Then, 280 g of dried *P. guajava* leaf powder was suspended in either acetone or 75% methanol for 10 days. The extract solutions were then filtered with filter paper

(Whatman No.1), concentrated under a rotary evaporator and finally pulverized until a powdery crude *P. guajava* leaf extract was obtained. The extracts were kept at 4°C until use.

Microorganisms and preparation of culture media

Mueller Hinton agar (MHA, Pronadisa®, Barcelona, Spain) was used for the direct sensitivity test. The medium was prepared and treated according to the manufacturer's instruction. Briefly, 35 g of medium was mixed with one liter of distilled water, enclosed in a screw cap container and autoclaved at 121°C for 15 min. Thereafter, the medium was dispensed into 90-mm sterile agar plates and left to set. The agar plates were incubated at 37°C for 24 h to verify their sterility.

Four pathogenic bacteria isolated from pigs, *S. suis*, *P. multocida*, *E. coli* and *S. typhimurium* were grown and maintained in blood agar at 37°C. Before use, they were diluted in sterile normal saline and the turbidity was adjusted equivalent to a 0.5 McFarland standard, creating 10⁸ colony forming units (CFU)/ml (Lorian, 2005).

Antibacterial susceptibility test

The agar disc diffusion method was employed to determine the antibacterial activity of *P. guajava* leaf extracts from different extraction methods. Petri dishes of MHA were inoculated with bacterial suspension. Subsequently, sterile filter discs (9 mm in diameter) integrated with 40 mg/disc of each *P. guajava* leaf extract were placed and incubated at 37°C for 24 h. Acetone, distilled water and kanamycin were used as the control. Inhibition of bacterial growth was determined by the presence and size of clear zone around the discs. These inhibition zones were interpreted according to the standard of the antibiotic sensitivity test manual (Lorian, 2005). The growth inhibition diameter was an average of three measurements. Experiments were done in triplicate. Data were analyzed by using mean and standard deviation.

Minimum inhibitory concentration test

The MIC of *P. guajava* leaf extracts from different extraction methods was determined by using the agar dilution method as previously described with minor modification (Zampini et al., 2005). Briefly, extracts were diluted serially two-fold with their solvents, and mixed with MHA in the ratio of 1:10, dispensed into the 90 mm plates and left to set at room temperature. Plates were inoculated with an inoculum of bacteria (10⁴ CFU/ml) and incubated under standard conditions (5% CO₂, 37°C) for 24 h. Distilled water and acetone were used as the control with tested organisms. The least concentration of the samples with no visible growth of bacteria was determined as the MIC.

RESULTS

The *P. guajava* leaf extracts from water, methanol and acetone extractions displayed viscous, brownish-green color and yielded 22.6%, 7.3% and

7.0% w/w of dried leaves, respectively. To determine the antibacterial activity of *P. guajava* leaf extracts against certain pathogenic bacteria in pigs, discs integrated with crude extracts of *P. guajava* leaves from three different extraction methods were tested in colonies of *S. suis*, *P. multocida*, *E. coli* and *S. typhimurium*. The antibacterial activity was measured by the presence of an inhibition zone around the discs. *P. guajava* leaf extracts in water and methanol exhibited a moderate, clear inhibition zone in all colonies of tested bacteria while extraction in acetone produced an inhibition zone only in colonies of *S. suis* and *P. multocida* (Table 1). The water and methanol extracts produced a clear and large inhibition zone against *P. multocida* with a zone size of 29.7 ± 2.2 and 30.6 ± 1.2 mm in diameter, respectively. The inhibition zones of both extracts against *P. multocida* were larger than that of kanamycin (Table 1), while those against *S. suis*, *E. coli* and *S. typhimurium* were slightly smaller than that of kanamycin.

Table 1. Antibacterial activity of *P. guajava* leaf extracts from three different solvents against pathogenic bacteria in pigs as determined by disc diffusion method.

Bacteria	Inhibition zone (mm) ^a			
	Water	75% Methanol	Acetone	Kanamycin ^b
<i>S. suis</i>	15.5±3.1	12.6±0.3	11.6±0.7	21.6±0.5
<i>P. multocida</i>	29.7±2.2	30.6±1.2	23.3±1.5	25.1±1.7
<i>E. coli</i>	13.3±0.8	13.9±2.0	0	23.4±1.1
<i>S. typhimurium</i>	13.3±0.3	12.3±0.2	0	23.4±0.8

Note: a = solvent control (water, acetone, methanol) gave no zone. b = antibiotic control

The MIC values for the various preparations of *P. guajava* leaf extracts were also investigated. The results showed that *P. guajava* leaf extracts from water, methanol and acetone had the lowest MIC against *P. multocida* at 0.16, 0.16 and 0.31 mg/ml, respectively (Table 2). The extract of *P. guajava* leaves with acetone, which previously showed no inhibition zone against *E. coli* and *S. typhimurium* (Table 1), exhibited antibacterial activity at high MIC values of 20 mg/ml against these two pathogenic bacteria. In contrast, the *P. guajava* leaf extract with acetone had low MIC values against *S. suis* and *P. multocida* (0.31 mg/ml; Table 2).

Table 2. MIC of *P. guajava* leaf extracts from three different solvents against pathogenic bacteria in pigs.

Bacteria	MIC (mg/ml)		
	Water	75% Methanol	Acetone
<i>S. suis</i>	10.00	5.00	0.31
<i>P. multocida</i>	0.16	0.16	0.31
<i>E. coli</i>	5.00	5.00	20.00
<i>S. typhimurium</i>	5.00	5.00	20.00

DISCUSSION

An increased prevalence of antibiotic-resistant bacteria in animals as well as observations indicating high proportions of drug residues in pork and chicken meat have raised potential public health concerns (Duong et al., 2006; Kang et al., 2006; Hong et al., 2007). This has created a growing need to develop alternative compounds to control bacterial diseases in farm animals. Historically, medicinal plants have been used in humans in many regions of the world as an important natural source for controlling bacterial infections (Moore and Atkins, 1977; Wat et al., 1980). As a result, medicinal plants, including *P. guajava*, have been widely investigated as an alternative therapeutic antimicrobial source (Inuma et al., 1994; Cimanga et al., 1996; Palombo, 2009; Rahman et al., 2009). However, few studies so far have indicated the antibacterial activity of *P. guajava* on pathogenic bacteria in pigs (Kanbutra et al., 2003).

In the present study, leaves of *P. guajava* were extracted in various solvents differing in polarity and were tested against several pathogenic bacteria in pigs. *S. suis* and *P. multocida* represented bacteria typically causing systemic and respiratory tract infections whereas *E. coli* and *S. typhimurium* represented bacteria causing intestinal bacterial diseases. The results of the agar disc diffusion test and measurements of MIC values showed that *P. guajava* leaf extracts inhibited growth of *S. suis*, *P. multocida*, *E. coli* and *S. typhimurium* *in vitro*. Although *P. guajava* crude extract from acetone exhibited no inhibition zone in the agar plate tests of *E. coli* and *S. typhimurium*, the data obtained from the MIC test indicated that this extract exhibited antibacterial activity at high MIC values. The current findings also indicated that *P. multocida* was most sensitive to *P. guajava* leaf extracts.

The observations from this study are consistent with previous reports describing the antibacterial activity of *P. guajava* (Jaiarj et al., 1999; Abdelrahim et al., 2002; Anas et al., 2008; Rahim et al., 2010). It is well accepted that chemical compounds present in *P. guajava*, such as anthocyanins, alkaloids, flavonoids, tannins and triterpenoids, play an important role in its antimicrobial activity *in vitro* (Jaiarj et al., 1999; Begum et al., 2004; Belemtougri et al., 2006; Ghosh et al., 2010). Recently, Ghosh et al. (2010) reported the effects of two triterpenoids, betulinic and lupeol, extracted from *P. guajava* leaves against fungi and bacterial pathogens. In addition to their antimicrobial activities, flavonoids and triterpenoids from the leaves of this plant also mediate spasmolytic action of the rat jejunum through calcium-antagonist activity (Morales et al., 1994; Begum et al., 2002b). The results from the present study indicated that guava extract possessed high activity against several pathogenic bacteria in pigs. These findings, coupled with knowledge from previous studies, suggest that the antibacterial activity of guava leaf extracts might be due to the flavonoids and triterpenoids existing in the extracts. The antidiarrheal activity of the extracts may be due to the combination of its antimicrobial activity and an increased spasmolytic activity of the intestine.

In summary, since *P. guajava* is readily found and easily grown throughout Thailand, this study's findings suggest that this herb is promising for practical use as a natural antibiotic agent to replace the use of traditional antibiotics on pig farms. The results of this study show the potential for developing a new and

feasible application of using *P. guajava* as an alternative antimicrobial source for farm animals.

ACKNOWLEDGEMENTS

The authors would like to thank W. Benchasuwon, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, for her technical assistance. This work was partly supported by the Research Fund Unit, Faculty of Veterinary Medicine, Chiang Mai University, Thailand. The National Institute of Animal Health, Thailand kindly provided the pathogenic bacteria.

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