A Preliminary Study on the Antimicrobial Activities of Asiaticoside and Asiatic Acid against Selected Gram Positive and Gram Negative Bacteria

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ABSTRACT: *Centella asiatica* is a medicinal plant traditionally used for the treatment of various disorders including infections. In this study, two bioactive compounds of *Centella asiatica* namely asiatic acid and asiaticoside were tested for antibacterial activity against two Gram negative and three Gram positive bacteria using disc diffusion method. They were *Helicobacter pylori*, *Escherichia coli*, *Streptococcus pneumoniae, Staphilococcus aureus* and *Pseudomonas aeroginosa*. The antibacterial activities were assessed by the presence or absence of inhibition zones. The result of this preliminary study demonstrated that asiatic acid has antibacterial activity against all the bacteria except *Pseudomonas aeruginosa* with inhibition zones ranged from 7–12 mm. No inhibition activity was observed for asiaticoside against all the bacteria. This data revealed that asiatic acid is responsible for the antibacterial activity of *Centella asiatica.*

Keywords: Asiatic acid, anti-bacteria, asiaticoside, *Centella asiatica*

Introduction

The use of medicinal plants and their derivatives as source for antimicrobial drugs has become more important nowadays due to the growing incidences of drug-resistant pathogens (Hammer et al., 1999; Martin and Ernst, 2003; Samy and Gopalakrishnakone, 2010). Various plants including *Centella asiatica* (*C. asiatica*) and their derivatives have been used as therapeutic agents for various human diseases since prehistoric times (Henkel et al., 1999; Nostro et al., 2000; Taemchuay et al., 2008). *C. asiatica*, popularly known in Malaysia as pegaga, is a small herbaceous annual plant with small-sized leaves and short petiole stem. It grows in damp swampy areas in tropical and sub-tropical regions including Malaysia (Verma et al., 1999). This plant has been used to cure various conditions including headache, asthma, eczemas, ulcers and wound healing, and has been commercialized for various medicinal and cosmetic purposes (Ullah, 2009). Furthermore, it is claimed to possess a wide range of pharmacological effects such as anti-oxidant, anti-ulcer, anti-stress, anti-cancer, anti-microbial and wound healing effect.

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C. asiatica is also believed to increase energy and sexual potency as well as maintains and prolongs health life, and has been used as tonic in Ayurvedic medication (Chakraborty et al., 1996; Sharma et al., 1996; Kimura et al., 2008).

C. asiatica contains four main bioactive compounds known as asiatic acid, asiaticoside, madecassicacid and madecassoside (Matsuda et al., 2001). Among them, asiaticoside and asiatic acid are the most important bioactive compounds which are responsible for the medicinal values of this plant (Hausen, 1993; Lu et al., 2004; Zheng and Qin, 2007; Pittella et al., 2009). Thus, the extraction and isolation of these compounds have drawn public interest. Many researchers around the world have been conducted extensive study to investigate other potential values of these compounds for the benefit of human's health. Though the therapeutic values of asiaticoside and asiatic acid have been shown in various fields, knowledge with regard to their potential as antibacterial agent is still lacking. Most studies on antibacterial effects of this plant mainly focus on plant extracts. Thus, this study was conducted to evaluate the antibacterial activity of asiaticoside and asiatic acid against selected Gram negative and Gram positive bacteria including *Helicobacter pylori (H. pylori)*, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*).

Material and Methods

Bacteria culture

Two Gram negative bacteria (*Escherichia coli* ATCC 29952, *Helicobacter pylori* ATCC 45903, *Pseudomonas aeruginosa*) and three Gram positive bacteria (*Streptococcus pneumoniae*, *Staphylococcus aureus*) obtained from the Culture Laboratory of the School of Health Sciences, Universiti Sains Malaysia were used in this study. *H. pylori* was cultured onto Colombia blood agar or Muller Hinton agar (BBL USA) with 5% sheep blood in an anaerobic jar supplemented with microaerobic gas pack BR 0056A (Oxoid, UK) at 37^{o}C for 48 to 72 hours. *S. pneumoniae* was cultured on Colombia blood agar or Muller Hinton agar with 5% sheep blood and incubated in a tin jar provided with candle for one to two days. The colony was preserved in Tryptic Soy Broth (Oxoid, UK) with 20% glyserol in -80^{o}C prior to use. *E. coli*, *S. aureus* and *P. aeruginosa* were cultured onto Muller Hinton agar overnight at 37^{o}C.

Preparation of bacterial suspensions

H. pylori was inoculated in Tryptic Soy Broth with 5% fetal bovine serum and incubated in microaerobic environment at 37^{o}C for 48-78 hours. The inoculum was adjusted to a 0.5 McFarland turbidity standard (Uyub et al., 2010). *S. pneumoniae, E. coli, S. aureus* and *P. aeruginosa* were inoculated in Muller Hinton broth and incubated at 37^{o}C for 14 to 16 hours, the inocula were then adjusted to a 0.5 McFarland standard of turbidity.

Disk diffusion assay

The disk diffusion assay suggested by national committee for clinical laboratory standard (NCCLS) was used for antimicrobial test with slight modifications. Six mm sterilized filter paper disks (Whatmann, UK) were impregnated with 20 µl of asiaticoside and asiatic acid (Sigma, USA) diluted to a concentration of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml thus each disc contained 20 µg, 10 µg, 5 µg, 2.5 µg, respectively. DMSO was used as the negative control and standard antibiotics were used as the positive control. Amoxicillin (10 µg) was used as the positive control for *E. coli*, gentamycin (5 µg) for *S. pneumoniae, S. aureus* and *P. aeruginosa,* while clarithromycin (10 ug) was used as the positive standard for *H. pylori*. The experiment was performed in triplicate and the mean diameters of the inhibition zones were measured based on the nearest millimeter of the clear zone surrounding the discs.

Results

The antibacterial activities of asiaticoside and asiatic acid against five different bacteria are presented in **TABLE 1**.

TABLE 1- Antibacterial activities of asiaticoside and asiatic acid of *Centella asiatica* against 5 different bacteria

Bacteria strain	Asiaticoside	Asiatic acid	+ve control
Helicobacter pylori ATCC 45903	NZ	12 (20)	NZ (10)
		8 (10)	
Escherechia coli ATCC 29952	NZ	7 (20)	20.5 (10)
Staphilococcus aureus	NZ	8 (20)	24 (5)
Streptococcus pneumoniae	NZ	7 (20)	42 (5)
Pseudomonas aeruginosa	NZ	NZ	15 (5)

Inhibition zone (mm) (Concentration, µg /disc)
*NZ –no inhibition zone

Asiatic acid inhibited the growth of all the bacteria except *P. aeruginosa* with various inhibition effects ranged from 7-12 mm at 20 µg. The compound also inhibited the growth of *H. pylori* at 10 µg with an inhibition zone of 8 mm. However, no inhibition effects were detected for all the bacteria against asiaticoside. No inhibitory zones were observed for all the bacteria against asiatic acid at concentration less than 20 µg and for *H. pylori* at concentration less than 10 µg. Inhibition zones were observed for standard antiobiotics except for clarithromycin.

Discussion

Asiaticoside and asiatic acid are the most important bioactive compounds presence in *C. asiatica* which responsible for its medicinal values (Hausen, 1993; Lu et al., 2004; Zheng and Qin, 2007; Pittella et al., 2009).

In this preliminary study, we evaluated the inhibition effects of asiaticoside and asiatic acid against selected Gram negative and Gram positive bacteria. Although asiatic acid, an active metabolite of

asiaticoside, has not been reported in the scientific literature as an antibacterial agent, the result presented in this preliminary study shows that this bioactive compound capable of inhibiting the growth of all the bacteria except *P. aeruginosa* with inhibition zones ranged from 7-12 mm at 20 µg. Inhibitory effect was also observed for *H. pylori* against 10 µg asiatic acid with diameter of the inhibition zone at 8 mm. Surprisingly, no inhibition zone was detected for all the bacteria against asiaticoside. This data is not in agreement with previous report showing that asiaticoside has antibacterial and fungicidal effects against various pathogens and fungi (Hausen et al., 1993; Taemchuay et al., 2008). We believed that this phenomenon might be the result of the extraction method used to extract or synthesis the compound. The extraction of bioactive compounds from medicinal plants is influenced by various factors including the method and solvent used for the extraction (Kim et al., 2009). Furthermore, in this study, the inhibitory effects of these compounds were only evaluated by disc diffusion method. This method seems to be limited and influenced by other factors such as concentration of extract, duration of exposure and the bacteria tested (Okoli and Iroegbu 2005). Thus, further study should be carried out to confirm this data with other method such as minimum inhibition concentration (MIC) and minimum bacterial concentration (MBC).

Even though asiatic acid is capable of inhibiting the growth of 4 out of 5 bacteria tested in the present study, the inhibition zones were less than the reference antibiotics and at higher concentration (20 µg), thus indicating that asiatic acid only possess weak antibacterial property against all the tested bacteria in comparison with the standard antibiotics. For *H. pylori*, no inhibition zone was detected against standard antibiotics, clarithromycin at 10 µg. No inhibition was also observed for *H. pylori* against 10 µg amoxicillin or 5 µg gentamycin (data not shown). We assumed that this bacterium might be resistant to those antibiotics.

In conclusion, the results of this preliminary study indicate that asiatic acid has antibacterial effects against *H. pylori*, *E. coli*, *S. aureus* and *S. pneumonia*. Further research with other bacteria and method of evaluation is needed to support this finding.

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