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Potent antibacterial activity of wood vinegar from Matang Mangroves, Malaysia

Background

In Matang Mangroves, Perak, Malaysia, wood vinegar or pyroligneous acid is a by-product of charcoal making from the billets of *Rhizophora*. Smoke from the vents of charcoal kilns is condensed in steel tubes and collected in plastic drums as raw distillate (Fig. 1).

Fig. 1. Smoke from the mangrove charcoal kiln is condensed and collected as wood vinegar

Freshly collected wood vinegar from the charcoal kiln has a temperature of 36-38°C and a smoked aroma (Loo, 2008). When fractionated, wood vinegar consists of 5.5% acetic acid, 3.4% methanol and 6.5% wood tar. Due to its high amount of volatile acids (8-10%), wood vinegar is acidic with pH ranging from 2-3. These acids contribute to its mild corrosive properties.

Wood vinegar has been traditionally used as deodorizer, fertilizer, sterilizer and antimicrobial agent (Loo, 2008). It has a wide range of applications such as industrial, agricultural, medicinal and home uses. In Thailand, wood vinegar is used to treat skin infections and dandruff (Rakmai, 2009).

Mangrove wood vinegar from Matang has been studied for its phenolic content and antioxidant properties (Loo *et al*., 2007). Three antioxidative compounds of syringol, catechol and 3-methoxycatechol were isolated (Loo *et al*., 2008). This report represents the first study on the antibacterial activity of Matang wood vinegar.

Materials and methods

Two bottles of wood vinegar produced by a factory in Matang Mangroves were tested for antibacterial activity. Non-distilled wood vinegar is dark brown in colour, resembling coffee (Fig. 2). After distillation, it becomes golden brown, resembling tea.

Fig. 2. Bottles of non-distilled (left) and distilled (right) mangrove wood vinegar

The agar well diffusion technique reviewed by Ncube *et al.* (2008) was adopted with slight modifications. Bacteria tested were Gram-positive *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*, and Gram-negative *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Inoculums (100 µl) were spread evenly onto 20 ml Mueller-Hinton agar set in 90 mm Petri dishes using a sterile cotton swab. Wells were punched into the inoculated agar with the help of a sterilised borer (6 mm).

To obtain the diameter of inhibitory zone (DIZ), the distilled and non-distilled wood vinegar was diluted 1:1 with ultra-pure water, and 50 µl were transferred into each well and the plates were incubated overnight. Antibacterial inhibition was also determined based on the minimum inhibition concentration (MIC). The wood vinegar was serially diluted two fold each time to determine the lowest concentration to show a zone of inhibition. Serial dilution (1:1) was made using ultra-pure water.

Results and discussion

Results showed that distilled and non-distilled mangrove wood vinegar from Matang inhibited all six Gram-positive and Gram-negative bacteria tested. Generally, Gram-positive bacteria were more susceptible than Gram-negative bacteria.

Against Gram-positive bacteria (Fig. 3), DIZ ranged from 15 ± 1 mm (distilled and non-distilled wood vinegar for *B. cereus*) to 28 ± 2 mm (non-distilled wood vinegar for *M. luteus*) (Table 1). In terms of DIZ, there was no significant difference at $p < 0.05$ between distilled and non-distilled wood vinegar. MIC was 6.25% for *B. cereus*, and 12.5% for *M. luteus* and *S. aureus*.

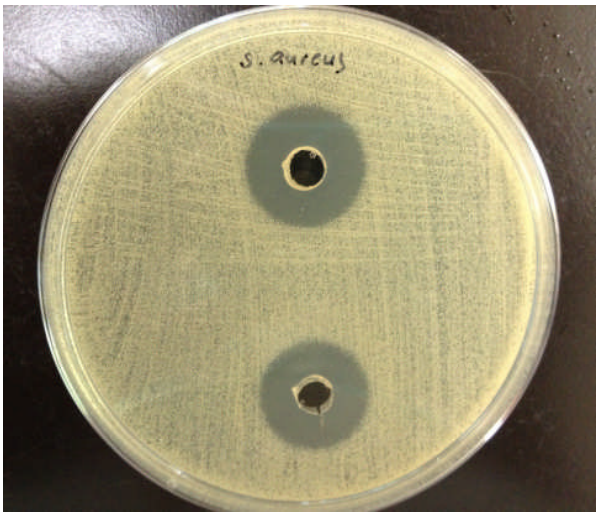

Fig. 3. Inhibitory zones of non-distilled (top) and distilled (bottom) wood vinegar against Gram-positive *S. aureus*

Against Gram-negative bacteria (Fig. 4), DIZ ranged from 9 ± 2 mm (distilled wood vinegar for *S. typhi*) to 17 ± 1 mm (non-distilled wood vinegar for *P. aeruginosa*) (Table 2). Based on DIZ, values of non-distilled wood vinegar were significantly higher than distilled wood vinegar for *P. aeruginosa* and *S. typhi*. DIZ values for *E. coli* were comparable. MIC was 25.0% for *E. coli* and *S. typhi*, and 12.5% for *P. aeruginosa*.

Based on DIZ, the overall ranking of susceptibility of Gram-positive and Gram-negative bacteria to distilled and non-distilled wood vinegar was *M. luteus* > *S. aureus* > *P. aeruginosa* ~ *B. cereus* > *E. coli* ~ *S. typhi*. Based on MIC, the overall ranking of susceptibility was *B. cereus* > *M. luteus* ~ *S. aureus* ~ *P. aeruginosa* > *E. coli* ~ *S. typhi*. Gram-positive *B. cereus* and *M. luteus* were the most sensitive, while Gram-positive *E. coli* and *S. typhi* were the least susceptible.

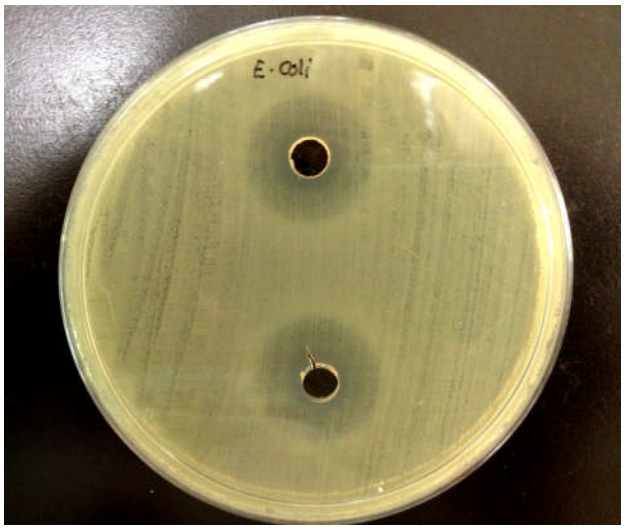

Fig. 4. Inhibitory zones of non-distilled (top) and distilled (bottom) wood vinegar against Gram-negative *E. coli*

Antimicrobial activity of non-mangrove wood vinegar (bamboo, eucalyptus and rubber) against dermatitis bacteria and fungi has been reported by Rakmai (2009). Gram-negative bacteria have an outer membrane of lipoprotein and lipopolysaccharide, which is selectively permeable, and can regulate access of antimicrobials into the underlying cell structures (Chopra & Greenwood, 2001; Cabeen & Jacobs-Wagner, 2005). This renders them generally less susceptible to plant extracts than Gram-positive bacteria.

With potent and broad-spectrum antibacterial activity, mangrove wood vinegar from Matang can be developed into useful household products such as antiseptic cream, lotion, shampoo and toothpaste. Through proper food safety analysis, mangrove wood vinegar may also be used as a preservative for meat because of its distinctive smoked aroma.

Acknowledgement

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Table 1. Diameter of inhibitory zone and minimum inhibition concentration of distilled and non-distilled wood vinegar against Gram-positive bacteria

Wood vinegar	*Bacillus cereus*		*Micrococcus luteus*		*Staphylococcus aureus*	
	DIZ (mm)	MIC (%)	DIZ (mm)	MIC (%)	DIZ (mm)	MIC (%)
Distilled	15 ± 1^{a}	6.25	27 ± 2^{a}	12.5	20 ± 1^{a}	12.5
Non-distilled	15 ± 1^{a}	6.25	28 ± 2^{a}	12.5	21 ± 1^{a}	12.5

Analysis of variance of DIZ, based on 1:1 dilution of wood vinegar, was done using the Student's t-test. Columns with the same superscript letter (a) are not significant at $p < 0.05$. Abbreviations: DIZ = diameter of inhibitory zone and MIC = minimum inhibition concentration.

Table 2. Diameter of inhibitory zone and minimum inhibition concentration of distilled and non-distilled wood vinegar against Gram-negative bacteria

Wood vinegar	*Escherichia coli*		*Pseudomonas aeruginosa*		*Salmonella typhi*	
	DIZ (mm)	MIC (%)	DIZ (mm)	MIC (%)	DIZ (mm)	MIC (%)
Distilled	10 ± 2^{a}	25.0	15 ± 0^{b}	12.5	9 ± 2^{b}	25.0
Non-distilled	13 ± 2^{a}	25.0	17 ± 1^{a}	12.5	13 ± 1^{a}	25.0

Analysis of variance of DIZ, based on 1:1 dilution of wood vinegar, was done using the Student's t-test. Columns with different superscript letters (a-b) are significant at $p < 0.05$. Abbreviations: DIZ = diameter of inhibitory zone and MIC = minimum inhibition concentration.