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Antimicrobial Activity of Wood Vinegar from *Dimocarpus longan*

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Abstract

The antimicrobial activity of wood vinegar from *Dimocarpus longan* was determined in this study. For this, the antimicrobial activity was evaluated against 14 bacterial and 6 fungal strains using an agar well diffusion assay. It was observed that the longan wood vinegar exhibited antibacterial activity against all bacterial strains tested. However, the wood vinegar only showed an inhibitory activity against one fungus which was the yeast *Candida albicans*. In addition, a preliminary characterisation of the chemical compositions of the longan wood vinegar was made by gas chromatography-mass spectrometry (GC-MS). A total of 6 chemical compounds were identified, representing ca. 60% of the compositions in the wood vinegar. Three major components including 9-octadecenoic acid (oleic acid, 24.56%), n-hexadecanoic acid (palmitic acid, 24.33%), and tetradecanoic acid (myristic acid, 7.16%), were found in the wood vinegar.

Keywords: Antibacterial activity; *Dimocarpus longan;* Longan; Pyroligneous acid; Wood vinegar

1. Introduction

Wood vinegar, also scientifically known as pyroligneous acid, is a brown, condensed acidic liquid with smoky odour, produced by charcoal burning process (Yaman, 2004). During the charcoal production (with high temperature and oxygen absence), smoke from the burning wood flows into a long pipe to allow condensation. The distillate obtained is typically stored in the container for three months. The three layers are then developed: i) light oil on top, ii) translucent brown wood vinegar at the middle, and iii) the thick wood tar at the bottom. Only the translucent brown liquid is used as raw wood vinegar (Ogawa and Okimori, 2010; Lehmann and Joseph, 2015). Many different sources of wood can be used to produce various wood vinegars; these include bamboo (Cui and Wu, 2010), coconut shell (Wititsiri, 2011), eucalyptus (Tarasin, 2013), litchi (Yang *et al.,* 2016), oak (Kim *et al.,* 2000), and walnut shell (Zhai *et al.,* 2015). In general, wood vinegar, albeit prepared from different wood types, have been considered as safe and natural. It has been reported that

wood vinegar displays various biological activities including antimicrobial activity (Hwang *et al.,* 2005), antioxidant activity (Loo *et al.,* 2007), and termiticidal activity (Oramahi and Yoshimura, 2013). Due to these distinct characteristics, many applications of wood vinegar have been introduced; these include the areas of agriculture (Mungkunkamchaoa *et al.,* 2013), food (Yamauchi *et al.,* 2016), environment (Liu *et al.,* 2018), and medicine (Lee *et al.,* 2011).

Longan (*Dimocarpus longan* Lour.), an evergreen tree belonging to the Sapindaceae family, is indigenous to many Asian countries (China, India, Myanmar, Thailand, and Vietnam). The longan fruit in Thailand is economically important considering that its export value increases annually. It is estimated that the export value of all types of longan fruit (i.e., fresh, frozen, and dried products) is worth than 400 million USD in 2015 (DITP, 2015). In this study, we further explore an additional benefit of longan tree if its wood vinegar possesses antimicrobial activity. This would greatly promote an alternative use of longan considering that the trees must be trimmed after each harvest and typically discarded as agricultural waste.

2. Materials and Methods

2.1 Longan wood vinegar sample

Raw wood vinegar sample prepared from *D. longan* was kindly provided by Mr. Chan Wisitwanichakul (Ban Thi, Lamphun). The production process was carried out as shown in Figure 1, and the liquefied products obtained were brown and transparent with smoky odour. The longan wood vinegar samples (LWV) were aliquot and kept in the dark at 4°C until required.

Figure 1. Production process of longan wood vinegar. A) longan tree; B) longan trimming; C) carbonisation process; D) longan wood vinegar.

2.2 Antimicrobial activity assay

Fourteen of bacterial strains used were *Bacillus cereus* TISTR 687, *B. subtilis* TISTR 008, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* DMST 17303, *Micrococcus luteus* TISTR 884, *Pseudomonas aeruginosa* ATCC 15442, *P. fluorescens* TISTR 358, *Salmonella* Typhimurium TISTR 292, *Serratia marcescens* TISTR 1354, *Staphylococcus aureus* TISTR 1466, *S. epidermidis* ATCC 14990, *Streptococcus mutans* DMST 26094, and two methicillin-resistant *Staphylococcus aureus* strains (CRP41 and CR010). Six fungal strains were used as follows: two strains of *Colletotrichum acutatum* (NJ01 and NJ02), *C. gloeosporioides, Lasiodiplodia theobromae, Trichoderma reesei TISTR* 3080, and yeast *Candida albicans* TISTR 5779.

For antibacterial activity assay, agar well diffusion technique was adopted with detail as follows (Boyanova et al., 2005). All bacteria were cultured on nutrient broth and incubated at 37°C for 24 h. A bacterial lawn was then prepared by transferring an overnight bacterial culture (with an OD600 value of 0.6) onto the surface of the nutrient agar plates using swabbing technique. The wells were prepared in the swabbing plates using a sterile cork-borer (6 mm diameter). A volume of 20 µl of the LWV sample was transferred into the well carefully and the plates were then incubated for 24h at 37°C. The zone of inhibition was measured and recorded. For antifungal activity assay, a mycelial agar plug (5 mm in diameter) of each fungal strain was transferred to the centre of the PDA plate. The wells were then prepared at 2 cm surrounding the fungus using a sterile cork-borer. 20 µl of the LWV sample was transferred into the well and the plates were incubated for 24h at 30°C. Antifungal activity was then observed by the inhibition zone of the mycelial growth. The minimum inhibitory concentration (MIC) of the LWV sample was also determined. For this, the LWV sample was prepared in serial two-fold dilutions considering that its initial concentration as 1 Unit (U). Using a serial two-fold dilution, the LWV sample was diluted in sterile distilled water yielding the diluted LWV sample ranging in the concentration of 0.500, 0.250, and 0.125 U. Determination of the MIC value was then performed using the agar well diffusion assay as previously described. The MIC value was defined as the lowest concentration level in which the zone of inhibition could be observed. Each experiment was carried out in three replicates. The data, recorded by measuring the zone of growth inhibition around the discs (in mm), were expressed as means ± SD.

2.3 Chemical analysis of wood vinegar

The compounds of the wood vinegar sample were determined using GC/MS. The system was comprised of the Agilent 6890 N gas chromatograph and the Agilent 5973 N mass spectrometer (Agilent Technologies, USA). Purified helium gas at a flow rate of 1.0 mL/min, was passed through the Agilent HD-5MS (5% phenyl-polymethylsiloxane) capillary column (30m x 0.25mm internal diameter, 0.25 µm in film thickness). The oven temperature profile was established as follows: initial 60°C hold time of 5 min, and then heated to 250°C at 3°C / min. The mass spectra were recorded at ionisation energy of 70 eV. Components were identified by comparing their mass spectra with those in the National Institute of Standards and Technology. The results were accepted when constituents with matched percentage of > 90% were identified.

3. Results and Discussion

3.1 Antimicrobial activity of the LWV

Wood vinegars, widely used in agriculture with various applications, can be obtained from many different sources of wood (Ogawa and Okimori, 2010; Lehmann and Joseph, 2015). The products have been recognized as safe natural inhibitors, in which they have various bioactivities such as antifungal, termiticidal, and insect-repelling activities (Kartal *et al.,* 2004; Kiarie-Makara *et al.,* 2010). In this report, we further present the data for the in *vitro* antimicrobial properties and chemical constituents of the wood vinegar prepared from longan (*D. longan* Lour.). The product obtained locally in the process of producing longan wood charcoal, is a condensed acidic liquid and its appearance is brown and transparent with smoky odour (Figure 1).

Table 1. Antimicrobial activity of longan wood vinegar, assessed by agar well diffusion method. Data shown were mean and SD of zone of inhibition recorded in millimeter.

Testing microorganisms	Zone of inhibition (mm)
Gram-positive bacteria	
Bacillus cereus TISTR 687	18.00 ± 1.08
Bacillus subtilis TISTR 008	17.83 ± 2.04
Listeria monocytogenes DMST 17303	15.06 ± 2.04
Micrococcus luteus TISTR 884	15.94 ± 1.39
Staphylococcus aureus TISTR 1466	19.56 ± 1.69
MRSA CRO10	10.44 ± 0.97
MRSA CRP41	15.39 ± 0.98
Staphylococcus epidermidis ATCC 14990	16.72 ± 1.23
Streptococcus mutans DMST 26094	14.11 ± 1.31
Gram-negative bacteria	
Escherichia coli ATCC 25922	19.28 ± 1.45
Pseudomonas aeruginosa ATCC 15442	13.39 ± 1.42
Pseudomonas fluorescens TISTR 358	16.11 ± 1.23
Salmonella Typhimurium TISTR 292	13.56 ± 0.70
Serratia marcescens TISTR 1354	14.39 ± 1.20
Fungi	
Candida albicans TISTR 5779	17.56 ± 0.01
Colletotrichum acutatum NJ01	0
Colletotrichum gloeosporioides	0
Lasiodiplodia theobromae	0
Trichoderma reesei TISTR 3080	0

MRSA = methicillin resistant *Staphylococcus aureus*.

Table 2. Minimum inhibitory concentrations (MICs) of longan wood vinegar against pathogenic bacteria. Data shown were relative unit considering that the concentration of the crude wood vinegar sample is 1.0 Unit. Data in the parentheses were the diameter of the zone of inhibition (mm).

Testing bacteria	MICs (Unit)
Gram-positive bacteria	
Bacillus cereus TISTR 687	0.250 (4.83 ± 0.24)
Bacillus subtilis TISTR 008	0.125 (2.99 ± 0.47)
Listeria monocytogenes DMST 17303	0.125 (2.33 ± 0)
Micrococcus luteus TISTR 884	0.250 (7.33 ± 4.70)
Staphylococcus aureus TISTR 1466	0.250 (4.33 ± 0.94)
MRSA CRO10	0.250 (3.99 ± 0.94)
MRSA CRP41	0.250 (3.99 ± 0.94)
Staphylococcus epidermidis ATCC 14990	0.250 (6.16 ± 0.70)
Streptococcus mutans DMST 26094	0.125 (0.66 ± 0.61)
Gram-negative bacteria	
Escherichia coli ATCC 25922	0.250 (6.49 ± 4.39)
Pseudomonas aeruginosa ATCC 15442	0.125 (1.50 ± 2.12)
Pseudomonas fluorescens TISTR 358	0.250 (4.33 ± 0.23)
Salmonella Typhimurium TISTR 292	0.250 (4.99 ± 1.41)
Serratia marcescens TISTR 1354	0.250 (7.00 ± 0)

MRSA = methicillin resistant *Staphylococcus aureus*.

The results for antimicrobial activity of the LWV sample are shown in Table 1. Based on the agar well diffusion assay, the LWV product appeared to be potent showing a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria with an inhibition zone ranging from 10.44 to 19.56 mm (Table 1). It is interesting to note that the LWV sample also exhibited its activity against two clinical isolates of *S. aureus* (MRSA CRO10 and CRP41). However, for antifungal activity, the LWV sample only showed an inhibitory activity against the *C. albicans* yeast. The results of minimum inhibitory concentration (MIC) determinations also showed that the LWV sample remained active when testing against all testing bacteria with MIC values of 0.125 and 0.250 U (Table 2).

Antimicrobial activity is one of the key topics in which the researchers have studied for the bioactivities of wood vinegar. It has been shown that wood vinegar displays a wide range of antimicrobial property. Chan *et al.* (2012) and Yang *et al.* (2016) reported that wood vinegar had a strong antibacterial effect on Gram-positive (i.e., *Bacillus cereus, Micrococcus luteus*, and *Staphylococcus aureus*), and Gram-negative bacteria (i.e., *Escherichia coli. Pseudomonas aeruginosa*, and *Salmonella typhi*). Our findings are in agreement with their data especially for *S. aureus* which was the most susceptible. Based

on our data, overall ranking of susceptibility was as follows: *S. aureus* > *E. coli* > *B. cereus* > *B. subtilis* > *S. epidermidis*. It has been suggested that Gram-positive bacteria are generally more sensitive to metabolites (i.e., antibiotics or plant extracts) than Gram-negative bacteria because of the absence of outer membrane (Trombetta *et al.,* 2005). It should also be noted that the LWV sample used was active against the two clinical strains, albeit at different level. Besides, the LWV product used at a diluted concentration of 0.125 and 0.250 U remains active highlighting an appropriate use in the field considering that a use of the LWV sample at high concentration may cause a side effect on plant including leaf burning. In contrast, many reports have shown that wood vinegar also inhibits the pathogenic fungi (Hwang *et al.,* 2005; Jung, 2007; Oramahi and Yoshimura, 2013). Our finding is different considering that only the yeast *C. albicans* was affected by the LWV product. Considering that bacteria and fungi are different microbial type (the former is prokaryotic and the latter is eukaryotic), it is therefore not a surprise to see a different inhibitory effect of the LWV product. The cell wall of yeast and fungi is glucan- and chitin-based which is totally different from that of the bacteria (peptidoglycan-based), and this discrepancy may thus cause the fungi (including yeast) to be more resistant.

Chemical profiles of the LWV

The components of the LWV sample were characterised by GC-MS. The typical chromatogram and chemical contents of the LWV sample were shown in Fig. 2 and Table 3. In total, there were 13 constituents in which the GC-MS analysis revealed six compounds as listed in Table 3. The remaining seven compounds could not be identified because their identity did not match the mass spectra available in the database. Three major components representing 56.05% were fatty acid type; these include tetradecanoic acid (myristic), n-Hexadecanoic acid (palmitic), and 9-octadecenoic acid (oleic) (Table 3). Other identified compounds were minor being alkane and aldehyde groups (less than 0.9%). Wood vinegar is a complex substance consisting of many organic compounds namely aldehydes, alcohols, esters, derivatives of furan and pyran, heterocyclic compounds, hydrocarbons, ketones, nitrogen compounds, organic acids and phenolics in which the major ones are organic acids and phenolics (Hwang *et al.,* 2005; Souza *et al.,* 2012). To date, more than 200 chemicals have been identified in wood vinegar obtained from different resources (Guillen and Manzanos, 2002; Wei *et al.,* 2010; Yang *et al.,* 2016). Our data confirm that organic acids are predominant although their biological activities related to antibacterial must be further explored. This is also in agreement with an acidic nature of the LWV (pH 3.2). An antimicrobial activity detected is also possibly derived from the extreme acidic characteristics of the LWV product as well as the presence of a wide variety of organic acid compounds.

Table 3. Chemical compounds of the longan wood vinegar analysed by GC-MS.

Peak no.	Compound	Formula	MW	% Area
1	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.38	7.16
2	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	24.33
4	9-octadecenoic acid	$C_{18}H_{34}O_2$	282.47	24.56
7	9-Oxabicyclo[6.1.0]nonane	$C_{18}H_{14}O$	126.20	0.08
8	9,17-Octadecadienal	$C_{18}H_{34}O$	264.45	0.51
9	9-Octadecenal	$C_{18}H_{34}O$	266.47	0.29

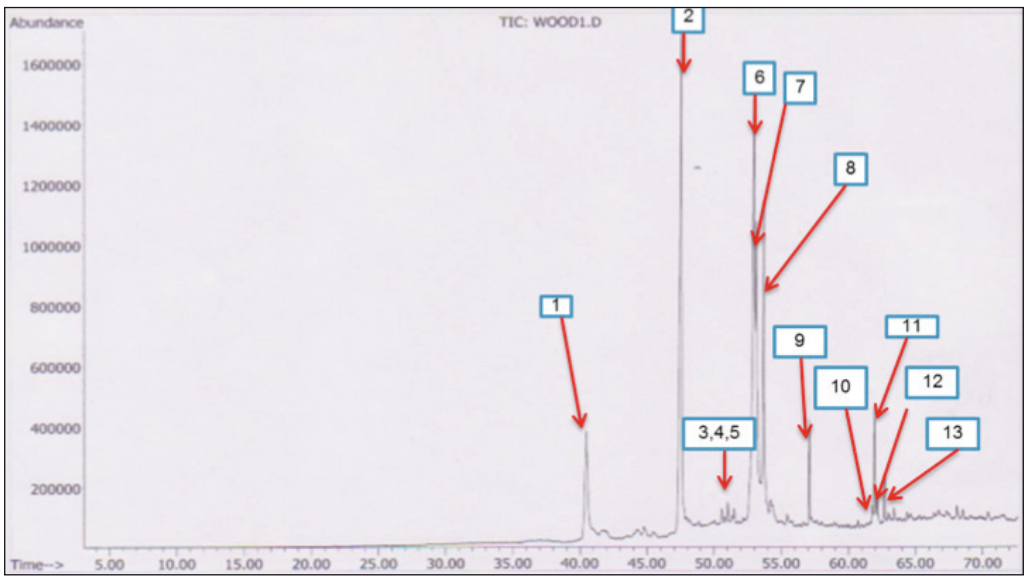

Figure 3. GC-MS analysis of the chemical constituents of the longan wood vinegar.

4. Conclusions

Our result shows that the wood vinegar prepared from *Dimocarpus longan* Lour., is able to suppress the growth of pathogenic bacteria and thus can be used as an antibacterial agent. Although this result is preliminary, the data obtained are promising for future application in various approaches (i.e., clinical treatment or food industry). Furthermore, it is important to further conduct the detailed analysis of the chemical compositions present in the wood vinegar from *D. longan*.

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