Malaysian Journal of Microbiology, Vol 16(6) 2020, pp. 438-445
DOI: http://dx.doi.org/10.21161/mjm.190652

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Chemical composition and antimicrobial activities of wood vinegars from carambola, coconut shells and mango against selected plant pathogenic microorganisms

Nur Adliza Baharom[1]*, Mohammad Hariz Abdul Rahman[2], Mohammad Shahid Shahrun[3], Farah Huda Sjafni Suherman[1] and Siti Nur Hafizah Masdar[1]

[1]Horticulture Research Center, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Agrobiodiversity and Environments Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[3]Soil Science, Water, and Fertilizer Research Center, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
Email: nuradliza@mardi.gov.my

Received 27 November 2019; Received in revised form 16 March 2020; Accepted 18 June 2020

ABSTRACT

Aims: This work aimed to evaluate the antimicrobial activities of three different sources of wood vinegars obtained from pyrolysis of carambola (*Averrhoa carambola*), coconut shells (*Cocos nucifera*) and mango (*Mangifera indica*) and to identify their chemical composition.
Methodology and results: Agar well diffusion technique was employed to assay the antifungal activity of the wood vinegars against *Fusarium oxysporum*, *Colletotrichum gleosporoides,* and *Pestalotiopsis microspora* and disc diffusion technique for antibacterial screening against *Ralstonia solanacearum*. The chemical compositions of these wood vinegars were also analyzed using GC-MS by employing the headspace technique. All wood vinegars exhibited antimicrobial activity against tested pathogens. Wood vinegar from carambola exhibited the most promising antimicrobial effect followed by coconut shells and mango. The GC-MS analysis revealed the wood vinegars from coconut shells, carambola, and mango are different in the chemical composition and active compounds. Major compounds identified in coconut shells are furfural, phenol, benzofuran, acetic acid, hexanal, ethanone, and formic acid. In carambola, the main compounds are furfural, imidazole, 3-pyridinecarboxaldehyde, benzaldehyde, phenol, benzofuran, indene, acetic acid, indazole, naphthalene, cyclohexanecarboxylic acid, palmitamide, palmitic acid, heptadecanenitril, and sterylamide. Meanwhile, the main chemical compounds in the pruning of mango-based vinegar consist of toluene, furfural, imidazole, annulene, benzaldehyde, phenol, carbamic acid, acetic acid, naphthalene, heptadecanenitril, and stearylamide.
Conclusion, significance and impact of study: It is suggested that wood vinegar from carambola, coconut shells, and mango is a promising antimicrobial agent in plant disease control, showing good potential for inhibition of selected plant pathogenic microorganisms.

Keywords: wood vinegar, pyrolysis, plant disease, antimicrobial, antifungal

INTRODUCTION

Plant disease is one of the major limiting factors in plant cultivation. Major plant diseases such as anthracnose caused by *Colletotrichum gleosporoides,* Fusarium wilt caused by *Fusarium oxysporum*, leaf spot caused by *Pestalotiopsis microspora* and bacterial wilt caused by *Ralstonia solanacearum* have been reported to cause more than 50-100% yield losses to several types of crops such as chili, tomato, and brinjal. These pathogens have been identified as significant plant pathogens in many countries including Thailand, China, and India (Than *et al.*, 2008; Shen *et al.*, 2014; Jiang *et al.*, 2017; Mostert *et al.*, 2017). However, the management of these diseases usually relies on the intensive use of chemical pesticides which will lead to a detrimental effect on the environment.

Wood vinegar, also called pyroligneous acid (PA), is a brown, flavorful liquid produced by distillation of wood in the absence of air (Yang *et al.*, 2016). It condenses into the liquid when the gas generated from combustion is cooled (Jeong *et al.*, 2007). Production of wood vinegars was made by the carbonization of several types of wood. There are several studies conducted to use wood vinegars in agriculture as it can improve plant growth, soil quality, and control pest and diseases (Mahmud *et al.*, 2016). However, the use of wood vinegar is relatively new

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(6) 2020, pp. 438-445
DOI: http://dx.doi.org/10.21161/mjm.190652

in Malaysia. Several active chemical compounds in wood vinegars were reported to be associated with properties to control plant disease (Hwang *et al.*, 2005; Jeong *et al.*, 2007). Previous studies have stated that wood vinegars showed promising antimicrobial effects against pathogenic microorganisms due to the presence of several major compounds such as organic acids, alcohol, furfural and furan, phenol, and also methoxyphenol derivatives (de Souza Araújo *et al.*, 2018). Acetic acids, which are the common compounds found in wood vinegars have been reported to exhibit high antimicrobial effect due to its high acidity. The presence of phenolic compounds in wood vinegars also has been reported to have the ability to inhibit the pathogenic fungi and bacteria (Hwang *et al.*, 2005; Jeong *et al.*, 2007; Ma *et al.*, 2011). The wood vinegars produced from several biomasses such as palm kernel, timbre, pineapple, and bamboo has been studied for its antimicrobial activities (MeiZhi *et al.*, 2010; Oramahi and Yoshimura, 2013; Mahmud *et al.*, 2016). Nevertheless, wood vinegar from different plants differs in their composition. Studies have described that wood vinegar produced using a different source of plant materials might present different levels of bioactivity and different quantities of bioactive constituents (Yang *et al.*, 2016). Chemical composition and concentration of compounds intrinsically depend on which plant material is charred.

There is a limited study available on the antimicrobial activities of wood vinegars obtained from carambola, mango, and coconut shells and their chemical constituents. Therefore, this study was conducted to evaluate the chemical composition and antimicrobial activities of three sources of wood vinegars obtained from pyrolysis of carambola, mango, and coconut shells against selected plant pathogenic microorganisms which are *F. oxysporum*, *C. gleosporoides*, *P. microspora* and *R. solanacearum*.

MATERIALS AND METHODS

Preparation of wood vinegars

Wood vinegars used in this study were prepared from coconut shells (*Cocos nucifera*), carambola pruning (*Averrhoa carambola*), and mango pruning (*Mangifera indica*). It was obtained from Agrobiodiversity and Environment Research Centre, MARDI Headquarters, Serdang, Selangor. Samples were undergoing the process of pyrolyzation using vertical kiln at approximately >400 °C for 2 h. Samples were stored in room temperature in a closed container at 0, 6, and 12 months and analyzed using Gas Chromatography Mass Spectrometry (GC-MS) to determine the phenolic compound and other active ingredients present.

GC-MS analysis of wood vinegars

The chemical compositions of these wood vinegars were also analyzed using GC-MS by employing the headspace technique. A 10 mL of wood vinegars sample was placed in a 30 mL glass vial (5 cm ID × 10.0 cm H). A 250 µL gastight microsyringe (Hamilton® GASTIGHT® syringe 1825) was rinsed at least five times with acetone and another 5 times with air. The needle tip was then inserted into the vial followed by drawing the plunger repeatedly for 5 times and final volume at 250 µL. All the samples were quickly introduced in the injector port at GC-MS (PerkinElmer Clarus 600, USA). Samples were injected into a single taper liner with an internal volume of 1200 µL and analyzed using a DB-5ms capillary column (Agilent Technologies, NEW Castle, DE, US)(30 m × 0.25 mm, 0.25 µm film thickness) with the constant flow at 1 mL/min with inlet temperature at 200 °C, mass spectrometry (MS) transfer line temperature at 300 °C, MS source temperature at 200 °C, the initial temperature of 50 °C held for 1 min, then 10 °C /min ramp to 250 °C and held for 10 min. The mass spectrometer was operated in full scan mode at 50-450 m/z. Data were analyzed using TurboMass Software by PerkinElmer and were identified by comparing their mass spectra against the database of National Institute Standard and Technology (NIST MS 14.0, Gaithersburg, MD, USA) with matches of greater than or equal to 90% (Yang *et al.*, 2016).

Preparation of cultures

Pure cultures of *F. oxysporum*, *C. gleosporoides, P. microspora*, and *R. solanacearum* were all locally isolated from vegetable research plot in MARDI Serdang, Selangor. All isolates were identified using morphological and molecular analysis. For fungal identification, it was confirmed by molecular approaches based on the gene sequencing of the internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA). The ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) regions of the rDNA were amplified by using polymerase chain reaction (PCR) according to White *et al.* (1990). The multiple sequence alignments were developed from the sequence data and ITS sequences of 3 fungal isolates were compared with sequences from the GenBank (National Centre for Biotechnology Information; http://www.ncbi.nlm.nih.gov) through BLAST searches. Molecular identification for bacteria was performed by 16S rRNA gene sequence analysis. PCR amplication of the 16S rRNA gene was carried out using universal primers 758f (5′-CAAACAGGATTAGATACC-3′) and 970r (5'-TCGCAGAAGTTCGTCTTTCA-3'). Raw sequence files were manually edited using BioEdit. Similarity searches were conducted using the Ezbiocloud database (www.ezbiocloud.net) with validated type strains. All isolates were periodically subcultured and maintained on potato dextrose agar (PDA) (DIFCO, USA) and nutrient agar (DIFCO, USA) for further use.

Antifungal screening

Agar well diffusion technique was employed to screen the antifungal activity of the wood vinegars against *F.*

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(6) 2020, pp. 438-445
DOI: http://dx.doi.org/10.21161/mjm.190652

oxysporum, *C. gleosporoides*, and *P. microspore* using 0, 6, and 12-month old wood vinegars. Each PDA plate was inoculated with a 6 mm disc of 7 days old isolates in the center. Four wells of 4 mm diameter were made, at a distance of 1 cm surrounding the fungal disc. Then, 50 µL of respective treatments such as wood vinegars, sterile distilled water (negative control), and commercial biofungicide with botanical extracts (positive control) were added into each well. The plates were then left undisturbed to allow diffusion of the wood vinegars into the agar and incubated at 25 °C until the control fungal growth reached the plate edge. Each experiment was carried out in triplicate and the mean diameter of the fungal colony was measured in millimetre at 7 and 20 days after incubation as proposed by Jackson *et al.* (1991) with slight modification. The percentage of inhibition reduction was measured using the following equation proposed by Ncube *et al.* (2008).

$$Rr = \frac{(R1 - R2) \times 100}{R1}$$

Where, Rr = percentage of inhibition reduction in colony diameter; R1 = colony diameter on the untreated medium (mm) (negative control); and R2 = colony diameter on the treated medium (mm).

Antibacterial screening

The screening of the wood vinegars for antibacterial activities was conducted by the disc diffusion method. Agar plates containing 25 mL nutrient agar was prepared and then inoculated with 50 µL of bacterial suspensions (10^5 CFU/mL). After that, 6 mm Whatman® Antibiotic Assay Discs (Whatman, USA) were impregnated with 50 µL of wood vinegar and placed on the inoculated plates, then incubated at 37 °C for 72 h. Distilled water was used as a negative control. All discs were fully dried before applying on the bacterial lawn. Each experiment was carried out in triplicate and the antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs and expressed as the mean zone of inhibition diameters (mm) ± standard deviation (Yang *et al.*, 2016).

Data analysis

Data collected were subjected to analysis of variance (ANOVA) by Statistical Analysis System (SAS 9.0). Means were compared using Least Significance Difference (LSD) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition of wood vinegars

GC-MS analysis conducted on coconut shells, carambola, and mango pruning used in this study was identified that the vinegar products are different in the composition of the chemicals and active compounds (Table 1). However, there are also similarities in certain characteristics. Major compounds identified in coconut shells are furfural, phenol, benzofuran, acetic acid, hexanal, ethanone, and formic acid. In carambola, the main compounds are furfural, imidazole, 3-pyridinecarboxaldehyde, benzaldehyde, phenol, benzofuran, indene, acetic acid, indazole, naphthalene, cyclohexanecarboxylic acid, palmitamide, palmitic acid, heptadecanenitril, and sterylamide. Meanwhile, the main chemical compounds in the pruning of mango-based vinegar consist of toluene, furfural, imidazole, annulene, benzaldehyde, phenol, carbamic acid, acetic acid, naphthalene, heptadecanenitril, and stearylamide. The variation in the chemical composition of wood vinegar is influenced by temperature and several parameters, such as species of wood, moisture content of wood, and time of combustion (Mohan *et al.*, 2008; Omulo *et al.*, 2017). In addition, the antimicrobial effect of wood vinegars varied depending on the material source used as discussed in the below paragraph.

Identification of microbial cultures

All fungal isolates obtained were identified based on their morphological characteristics and reconfirmation by ITS sequences of the isolate. They were successfully amplified by ITS 1 and ITS 4 primers and the BLAST result through the GeneBank showed the regions of local similarity between sequences. The fungal isolates were identified as *C. gleosporoides*, *F. oxysporum,* and *P. microspora*. *Ralstonia solanacearum* was identified using the universal PCR primers 758f and 970r and generated a 1,423-bp 16S rRNA gene fragment. The similarity search conducted using Ezbiocloud revealed that they shared 99.23% identity with *R. solanacearum* strain LMG 2299(T) (GenBank accession no. EF016361) (Table 2).

Antimicrobial effect of wood vinegars

The antifungal activity of three different sources of wood vinegar which were coconut shells, carambola, and mango was observed on PDA using agar well diffusion technique. In general, after 7 days of incubation, the mycelia were significantly inhibited by the wood vinegars at 50 to 100 % inhibition indicating that wood vinegars from carambola, coconut shells, and mango have promising antifungal properties against plant pathogenic fungi (Table 3). Prolonged inhibition of wood vinegars against pathogenic fungi until 20 days of incubation can be seen where wood vinegar from carambola significantly inhibited mycelia growth of *F. oxysporum*, *C. gleosporoides* and *P. microspora* followed by coconut shells, mango and commercial biofungicide. However, it can also be seen that the performance of wood vinegars differs significantly between the source of plant materials and incubation days. After 20 days of incubation with wood vinegar, the inhibition activity of some wood vinegars against pathogenic fungi was decreased. Wood vinegars from carambola showed constant inhibition reduction against all fungal pathogens

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(6) 2020, pp. 438-445
DOI: http://dx.doi.org/10.21161/mjm.190652

Table 1: Chemical composition of wood vinegars derived from coconut shells, carambola, and mango analyzed by GC-MS and its characteristic.

No.	Retention time (min)	Source of wood vinegars			Characteristic	Reference
		Coconut shells	Carambola	Mango		
1	3.12	nd	nd	toluene	Aromatic, antibacterial	Kobayashi *et al.*, 2000
2	4.04	furfural	furfural	furfural	Antifungal	Suresh *et al.*, 2019
3	4.10	nd	imidazole	imidazole	Antifungal	Rani *et al.*, 2013
4	4.88	nd	nd	annulene	Aromatic	Simpson, 2012
5	4.91	nd	3-Pyridinecarboxaldehyde	nd	Electrophilic	Klumpp and Lau, 1999
6	6.11	nd	benzaldehyde	benzaldehyde	Antifungal	Kim *et al.*, 2011
7	6.21	nd	nd	carbamic acid	Antifungal	Gupta, 2017
8	6.22	phenol	phenol	phenol	Antibacterial	Jeong *et al.*, 2007; Hwang *et al.*, 2005
9	6.58	benzofuran	benzofuran	nd	Antifungal	Khodarahmi *et al.*, 2015
10	7.34	nd	indene	nd	Antifungal	Akhter *et al.*, 2019
11	7.48	acetic acid	acetic acid	acetic acid	Antifungal, antibacterial	Hwang *et al.*, 2005; Jeong *et al.*, 2007; Suresh *et al.*, 2019
12	7.59	formic acid	nd	nd	Antifungal, antibacterial	Brütsch *et al.*, 2017
13	7.967	ethanone	nd	nd	Antifungal, antibacterial	Erol *et al.*, 1996
14	8.14	hexanal	nd	nd	Antifungal, antibacterial	Fadida *et al.*, 2015
15	8.25	nd	indazole	nd	Antifungal, antibacterial, aromatic	Li *et al.*, 2003
16	9.62	nd	naphthalene	naphthalene	Antifungal	Ryu and Chae, 2005
17	12.69	nd	cyclohexanecarboxylic acid	nd	Aromatic	Simpson, 2012
18	18.16	nd	palmitic acid	nd	Antifungal	Liu *et al.*, 2008
19	20.17	nd	heptadecanenitril	heptadecanenitril	Antibacterial	Gouda *et al.*, 2017
20	20.91	nd	palmitamide	nd	Antibacterial	Ahmed *et al.*, 2017
21	23.10	nd	stearylamide	stearylamide	Antibacterial	Ahmed *et al.*, 2017

nd: not detected.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(6) 2020, pp. 438-445
DOI: http://dx.doi.org/10.21161/mjm.190652

Table 2: List of microbial isolates used and their accession numbers.

Isolate name	Host	Sequence overlap (bp)	Similarity (%)	Genbank accession no.
C. gleosporoides	chili	630	99.23	HQ645078.1
F. oxysporum	tomato	543	100.00	KJ774041.1
P. microspora	brinjal	551	100.00	KT459350.1
R. solanacearum	chili	1423	100.00	EF016361

Table 3: Inhibition of pathogenic fungi against wood vinegars at different shelf life.

Pathogenic fungi	Treatments / Shelf life	Percentage of inhibition reduction (%)					
		After 7 days of incubation*			After 20 days of incubation*		
		0	6	12	0	6	12
F. oxysporum	Coconut shells	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00^{a} ± 0.00^{a}	nt	nt	91.97 ± 15.24^{a}
	Carambola	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	nt	nt	100.00 ± 0.00^{a}
	Mango	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	nt	nt	40.93 ± 9.15^{b}
	Commercial biofungicide	47.34 ± 32.73^{b}	41.74 ± 14.28^{b}	64.23 ± 31.43^{b}	nt	nt	54.2 ± 26.83^{b}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	nt	nt	0.00 ± 0.00^{c}
C. gleosporoides	Coconut shells	57.19 ± 21.98^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	nt	nt	73.55 ± 45.18^{b}
	Carambola	75.45 ± 2.92^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	nt	nt	100.00 ± 0.00^{a}
	Mango	75.02 ± 12.97^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	nt	nt	26.74 ± 18.98^{b}
	Commercial biofungicide	67.47 ± 0.86^{a}	62.07 ± 5.35^{b}	67.73 ± 2.73^{b}	nt	nt	57.45 ± 32.72^{b}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	nt	nt	0.00 ± 0.00^{c}
P. microspora	Coconut shells	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	nt	nt	100.00 ± 0.00^{a}
	Carambola	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	nt	nt	100.00 ± 0.00^{a}
	Mango	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	nt	nt	83.90 ± 1.15^{b}
	Commercial biofungicide	68.99 ± 2.03^{b}	57.12 ± 5.35^{b}	85.72 ± 3.54^{b}	nt	nt	65.42 ± 4.28^{c}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	nt	nt	0.00 ± 0.00^{d}

*Values are the mean of three replications and standard deviation. Means in the same column followed by the same letter are not significantly different at $p \leq 0.05$ according to the Least Significance Difference (LSD) test.
nt: Not tested.

at 100%, whereas the inhibition activity of wood vinegars from coconut shells remained constant against *P. microspora* only. Wood vinegars from mango significantly decreased in inhibition activity compared with carambola and coconut shells for *C. gleosporoides*, *F. oxysporum* and *P. microspora*. The decreased in inhibition reduction percentage may due to the losses of antimicrobial properties of wood vinegars through the time. Wood vinegars is highly acidic with pH below 3.00 and include several families of volatile organic compounds such as organic acids, phenols, aldehydes, phenols, alcohols, ketones, pyranfuran derivatives, and polyphenolic compounds (Suresh *et al.*, 2019). Volatization of chemical compounds found in wood vinegars may occur during 20 days of incubation and lead to losses of compounds to inhibit the pathogens. Application timing, method, and the rate were reported to affect fungicide efficacy (Woodward *et al.*, 2015). Fungicides or biofungicide application commonly recommended being re-apply after 7 or 14 days of application depending on the manufacturer's recommendation (DOA, 2020). From the results, the frequency of wood vinegars application can be proposed to be repeated before or at 20 days after the first application. Therefore, the results can serve as baseline data in the frequency of wood vinegars application. The different shelf life of wood vinegars ranging from freshly made wood vinegars (0 month old), 6 months old, and 12 months old wood vinegars were also subjected to antimicrobial testing using *F. oxysporum*, *C. gleosporoides* and *P. microspora* (Table 3). The effectiveness of the wood vinegar against tested fungi showed greater inhibition activity when the longer shelf life of wood vinegars was used. The results showed that 12 months old wood vinegars showed the highest

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(6) 2020, pp. 438-445
DOI: http://dx.doi.org/10.21161/mjm.190652

inhibition activity as compared with freshly made wood vinegars. From the results, there is variation in the inhibition activity between the source of wood vinegars and the duration of the wood vinegars have been stored. This is because the active compounds in the wood vinegars increased as the storage time increased (Suresh *et al.*, 2019). Due to that, in the antibacterial efficacy test, only 12 months old wood vinegars were used.

The diameter of the inhibition zone of different wood vinegars source against bacteria *R. solanacearum* can be seen in Figure 1. Clear inhibition zones with brownish pigmentation of the agar were observed around the filter paper discs impregnated with wood vinegars compared with the negative control which shows zero rates of inhibition against *R. solanacearum*. The antibacterial effect of 12 months old wood vinegars was presented in Table 4. All the wood vinegars have an inhibitory effect on *R. solanacearum* with a range of disc inhibition diameter from 19-23 mm, indicating that the wood vinegars possess an antibacterial effect against pathogenic bacteria. Wood vinegar from carambola exhibited the highest inhibition zone (22.5 mm) compared to wood vinegars from coconut shells (20.4 mm) and mango (19.0 mm). Although in above paragraph, we speculate that the antimicrobial properties of wood vinegars were loss through the time and therefore lead to decrease in inhibition reduction of fungal growth, however, in contrast, the diameter of zone of inhibition of bacteria remains constant until 20 days of incubation. The differences in wood vinegars performance on fungi and bacteria growth may due to the acidic character of the wood vinegars. A previous study reported that the growth of bacteria is suppressed in lower pH conditions while the growth of fungi increased (Rousk *et al.*, 2010). This result is consistent with the previous study by Rousk *et al.* (2010). Therefore, wood vinegars consistently suppressed the growth of *R. solanacearum* through time as the lower pH of wood vinegars affect the growth of bacteria. This is an interesting finding as wood vinegars from coconut shells, carambola and mango may have the potential to be applied as antibacterial agents for *R. solanacearum* (Yang *et al*., 2016).

The finding in this study indicated that wood vinegars have good antifungal and antibacterial properties in accordance to the other reported studies (Hwang *et al.*, 2005; Jeong *et al.*, 2007; MeiZhi *et al.*, 2010; Mahmud *et al.*, 2016; de Souza Araújo *et al.*, 2018). Antimicrobial effects shown by wood vinegars were due to high acidity and the presence of several phenolic compounds such as phenol, furfural, and acetic acid that have been reported to play the main role in the antimicrobial activity (de Souza Araújo *et al.*, 2018). Based on our GC-MS analysis, all three wood vinegars contain acetic acid, phenol, and furfural. These compounds were reported to exhibit the antimicrobial properties against pathogenic fungi and bacteria (Hwang *et al.*, 2005; Ryu and Chae, 2005; Jeong *et al.*, 2007; Liu *et al.*, 2008; Ma *et al.*, 2011; Rani *et al.*, 2013; Khodarahmi *et al.*, 2015). Besides, organic acids such as acetic acid and aldehyde such as furfural and benzaldehyde were reported to be microbial inhibitors. The synergistic activity of the various compounds found in wood vinegars is believed to be accountable for the high antimicrobial efficacy as proposed by Suresh *et al.* (2019).

Table 4: Antibacterial activity of 12 months old wood vinegars against *R. solanacearum*.

Wood vinegars	Diameter of zone of inhibition after 3 days (mm)	Diameter of zone of inhibition after 20 days (mm)
Coconut shell	20.40 ± 1.38^{b}	20.40 ± 1.38^{b}
Carambola	22.50 ± 1.17^{a}	22.50 ± 1.17^{a}
Mango	19.00 ± 1.41^{b}	19.00 ± 1.41^{b}
Negative control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

*Values are the mean of three replications and standard deviation. Means in the same column followed by the same letter are not significantly different at $p \leq 0.05$ according to the Least Significance Difference (LSD) test.

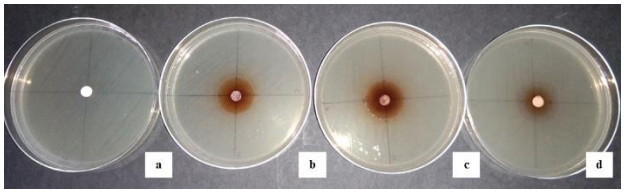

Figure 1: Inhibition zone of various wood vinegar sources against *R. solanacearum* after 20 days incubation on NA. a) Negative control (distilled water) b) Coconut shells c) Carambola d) Mango

It will also difficult for the pathogen to develop resistance against the wood vinegars due to the multiple sites of action of each of these compounds. Wood vinegar from carambola revealed to have more antimicrobial compounds as compared to wood vinegars produce from coconut shells and mango as discussed in the earlier paragraph. Therefore, wood vinegar from carambola exhibited better antimicrobial activity. From here, we can conclude that wood vinegar from carambola exhibited a better antimicrobial effect compared to coconut shells and mango.

CONCLUSION

In conclusion, all three wood vinegars produced from coconut shells, carambola, and mango showed good antimicrobial activity against plant pathogenic fungi (*F. oxysporum*, *C. gleosporoides*, *P. microspora*) and bacterium (*R. solanacearum*). This is the first study of the chemical composition and antimicrobial activities of wood vinegars produced from coconut shells, carambola, and mango. Due to its promising potential in inhibition of pathogenic microorganisms, wood vinegars can be proposed as a solution for disease management. However, further studies on chemical constituent in wood vinegars and *in vivo* studies are required to validate the relationship between bioactivity and the constituents.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(6) 2020, pp. 438-445
DOI: http://dx.doi.org/10.21161/mjm.190652

ACKNOWLEDGEMENTS

This research was funded by Malaysian Agricultural Research and Development Institute (MARDI) under RMK11 Development Fund (PRH403).

REFERENCES

Ahmed, S., Liu, H., Ahmad, A., Akram, W., Abdelrahman, E. K. N., Ran, F. and Hu, X. (2017). Characterization of anti-bacterial compounds from the seed coat of chinese windmill palm tree (*Trachycarpus fortunei*). *Frontiers in Microbiology* **8, 1894.**

Akhter, N., Pan, C., Liu, Y., Shi, Y. and Wu, B. (2019). Isolation and structure determination of a new indene derivative from endophytic fungus *Aspergillus flavipes* Y-62. *Natural Product Research* **33(20), 2939-2944.**

Brütsch, T., Jaffuel, G., Vallat, A., Turlings, T. C. J. and Chapuisat, M. (2017). Wood ants produce a potent antimicrobial agent by applying formic acid on tree-collected resin. *Ecology and Evolution* **7(7), 2249-2254.**

Department of Agriculture. (2020). *Pesticide Information*. Department of Agriculture: http://www.doa.gov.my/index.php/pages/view/302. [Retrieved on 11 June 2020].

de Souza Araújo, E., Pimenta, A. S., Feijó, F. M. C., Castro, R. V. O., Fasciotti, M., Monteiro, T. V. C. and de Lima, K. M. G. (2018). Antibacterial and antifungal activities of pyroligneous acid from wood of *Eucalyptus urograndis* and *Mimosa tenuiflora. Journal of Applied Microbiology* **124(1), 85-96.**

Erol, D. D., Calis, U. and Yuluğ, N. (1996). Synthesis of ethanone derived from 2-benzoxazolinones and their antimicrobial activities. *Arzneimittel-Forschung* **46(2), 205-206.**

Fadida, T., Selilat-Weiss, A. and Poverenov, E. (2015). N-hexylimine-chitosan, a biodegradable and covalently stabilized source of volatile, antimicrobial hexanal. Next generation controlled-release system. *Food Hydrocolloids* **48, 213-219.**

Gouda, N., Singh, R. K., Meher, S. N. and Panda, A. K. (2017). Production and characterization of bio oil and bio char from flax seed residue obtained from supercritical fluid extraction industry. *Journal of the Energy Institute* **90(2), 265-275.**

Gupta, P. K. (2017). Chapter 37 — Herbicides and fungicides. *In:* Reproductive and Developmental Toxicology. 2nd edn. Gupta, R. C. (eds.). Academic Press, United States. **pp. 657-679.**

Hwang, Y. H., Matsushita, Y. I., Sugamoto, K. and Matsui, T. (2005). Antimicrobial effect of the wood vinegar from *Cryptomeria japonica* sapwood on plant pathogenic microorganisms. *Journal of Microbiology and Biotechnology* **15(5), 1106-1109.**

Jackson, A. M., Whipps, J. M. and Lynch, J. M. (1991). *In vitro* screening for the identification of potential biocontrol agents of Allium white rot. *Mycological Research* **95(4), 430-434.**

Jeong, J. H., Jeong, D. E., Lee, S. J., Seul, K. J., Ryu, C. M., Park, S. H. and Ghim, S. Y. (2007). The effects of wood vinegar on growth and resistance of peppers. *Korean Journal of Microbiology and Biotechnology* **35(1), 41-44.**

Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X. and Liao, B. (2017). Bacterial wilt in China: History, current status, and future perspectives. *Frontiers in Plant Science* **8, 1549.**

Khodarahmi, G., Asadi, P., Hassanzadeh, F. and Khodarahmi, E. (2015). Benzofuran as a promising scaffold for the synthesis of antimicrobial and antibreast cancer agents: A review. *Journal of Research in Medical Sciences* **20(11), 1094-1104.**

Kim, J. H., Chan, K. L., Mahoney, N. and Campbell, B. C. (2011). Antifungal activity of redox-active benzaldehydes that target cellular antioxidation. *Annals of Clinical Microbiology and Antimicrobials* **10, 22-23.**

Klumpp, D. A. and Lau, S. (1999). 3-Pyridinecarboxaldehyde: A model system for superelectrophilic activation and the observation of a diprotonated electrophile. *The Journal of Organic Chemistry* **64(20), 7309-7311.**

Kobayashi, H., Uematsu, K., Hirayama, H. and Horikoshi, K. (2000). Novel toluene elimination system in a toluene-tolerant microorganism. *Journal of Bacteriology* **182(22), 6451-6455.**

Li, X., Chu, S., Feher, V. A., Khalili, M., Nie, Z., Margosiak, S. and Yager, K. M. (2003). Structure-based design, synthesis, and antimicrobial activity of indazole-derived SAH/MTA nucleosidase inhibitors. *Journal of Medicinal Chemistry* **46(26), 5663-5673.**

Liu, S., Ruan, W. and Li, J. (2008). Biological control of phytopathogenic fungi by fatty acids. *Mycopathologia* **166, 93-102.**

Ma, X. H., Wei, Q., Zhang, S. S., Shi, L. and Zhao, Z. (2011). Isolation and bioactivities of organic acids and phenols from walnut shell pyroligneous acid. *Journal of Analytical and Applied Pyrolysis* **91, 338-343.**

Mahmud, K. N., Yahayu, M., Sarip, S. H. M., Rizan, N. H., Min, C. B., Mustafa, N. F. and Zakaria, Z. A. (2016). Evaluation on efficiency of pyroligneous acid from palm kernel shell as antifungal and solid pineapple biomass as antibacterial and plant growth promoter. *Sains Malaysiana* **45(10), 1423-1434.**

MeiZhi, Z., WenJun, H., Lei, W. and JingLi, G. (2010). Chemical compositions and antimicrobial activities of three wood vinegars. *Acta Botanica Boreali-Occidentalia Sinica* **30(6), 1247-1252.**

Mohan, D., Shi, J., Nicholas, D. D., Pittman, Jr C. U., Steele, P. H. and Cooper, J. E. (2008). Fungicidal values of bio-oil and their lignin-rich fractions obtained from wood/bark fast pyrolysis. *Chemosphere* **71, 456-465.**

Mostert, D., Molina, A. B., Daniells, J., Fourie, G., Hermanto, C., Chao, C. P. and Viljoen, A. (2017). The distribution and host range of the banana Fusarium wilt fungus, *Fusarium oxysporum* f. sp. *Cubense*, in Asia. *PLoS ONE* **12(7), e0181630.**

Ncube N. S., Afolayan A. J. and Okoh A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *African Journal of Biotechnology* **7(12), 1797-1806.**

Omulo, G., Willett, S., Seay, J., Banadda, N., Kabenge, I., Zziwa, A. and Kiggundu, N. (2017). Characterization of slow pyrolysis wood vinegar and tar from banana wastes biomass as potential organic pesticides. *Journal of Sustainable Development* **10(3), 81-92.**

Oramahi, H. A. and Yoshimura, T. (2013). Antifungal and antitermitic activities of wood vinegar from *Vitex pubescens* Vahl. *Journal of Wood Science* **59(4), 344-350.**

Rani, N., Sharma, A. and Singh, R. (2013). Imidazoles as promising scaffolds for antibacterial activity: A review. *Mini-Reviews in Medicinal Chemistry* **13(12), 1812-1825.**

Rousk J., Brookes P. C. and Bååth E. (2010). Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. *Soil Biology and Biochemistry* **42, 926-934.**

Ryu, C.-K. and Chae, M. J. (2005). Synthesis and antifungal activity of naphthalene-1,4-diones modified at positions 2, 3, and 5. *Archives of Pharmacal Research* **28(7), 750-755.**

Shen, H. F., Zhang, J. X., Lin, B. R., Pu, X. M., Zheng, L., Qin, X. D. and Xie, C. P. (2014). First report of *Pestalotiopsis microspora* causing leaf spot of oil palm (*Elaeis guineensis*) in China. *Plant Disease* **98(10), 1429.**

Simpson, J. H. (2012). Chapter 4 - 1H and 13C chemical shifts. *In:* Organic Structure Determination Using 2-D NMR Spectroscopy: A Problem Based Approach. 2nd edn. E. Simpson (eds.). Academic Press, United States. **pp. 101-112.**

Suresh, G., Pakdel, H., Rouissi, T., Brar, S. K., Fliss, I. and Roy, C. (2019). *In vitro* evaluation of antimicrobial efficacy of pyroligneous acid from softwood mixture. *Biotechnology Research and Innovation* **3, 47-53.**

Than, P. P., Prihastuti, H., Phoulivong, S., Taylor, P. W. J. and Hyde, K. D. (2008). Chilli anthracnose disease caused by *Colletotrichum* species. *Journal of Zhejiang University: Science B* **9(10), 764-778.**

White, T. J., Bruns, T. D., Lee, S. and Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* PCR Protocol: A Guide to Methods and Application. Innes, M., Gelfand, D. H., Sninsky, J. J. and White, T. J. (eds.). Academic Press, United States. **pp. 315-322.**

Woodward, J. E., Russell, S. A., Baring, M. R., Cason, J. M. and Baughman, T. A. (2015). Effects of fungicides, time of application, and application method on control of *Sclerotinia* blight in peanut. *International Journal of Agronomy* **2015, Article ID 323465.**

Yang, J. F., Ho, C. H., Liang, M. T., Gao, Z. J., Wu, Y. W. and Chuang, L. Y. (2016). Chemical composition, antioxidant, and antibacterial activity of wood vinegar from *Litchi chinensis*. *Molecules* **21, 1150-1160.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538