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Antidermatophytic Activity of Wood Vinegars Prepared from *Fraxinus formosana* at Different Collection Temperatures

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Wood vinegars were collected from the chimney outflow of earthen kiln at 5 different temperatures (73–80, 90–92, 100–108, 120–125 and 145–162°C) during the charcoal preparation from branches and tree tops of *Fraxinus formosana*. The antidermatophytic activities of the vinegars were evaluated by the broth microdilution method, the minimum inhibitory concentration (MIC), against *Epidermophyton floccosum* (EF), *Trichophyton mentagrophytes* (TM) and *Trichophyton rubrum* (TR). The ether stratification of wood vinegars for three fractions (acidic, neutral and phenolic) and the ether fraction was also investigated. The active fractions in the vinegars were analyzed and identified by GC–MS. All vinegars, and the ether fraction and the phenolic fraction for neutral and phenolic materials had the inhibitory activities with MICs at 0.63–2.50, and 0.08–0.31 mg/mL against EF, TM, and TR. 2,6–dimethoxyl–phenol and 3β,11α–dihydroxy–5β–androstan–17–one were found to be the major compounds in the phenolic material and the neutral material of phenolic fraction that demonstrated strong antidermatophytic activities due to the higher contents. The wood vinegars and their fractions exhibit antidermatophytic activities, and might be considered for application on treating skin fungal infections after appropriate processing.

Key words: Wood Vinegar, *Epidermophyton floccosum* (EF), *Trichophyton mentagrophytes* (TM), *Trichophyton rubrum* (TR), Minimum Inhibitory Concentration (MIC)

INTRODUCTION

After joining the World Trade Organization (WTO), the "Landscape Agri–Forestation and Greening Scheme" has been promoted at 2002 in Taiwan with the purpose to assist farmers and agricultural enterprises in long–term non–cropping and to implement agri–forestation. *Fraxinus formosana* Hayata is one of the main board-leaf forestation species in the plain area of Taiwan. Taiwan Forestry Bureau (2009) reports that *Fraxinus formosana*, one of the popular species, in the "Green Forestation Project" is planted on the forest phase change in southern and eastern Taiwan. The planting density of agri–forestation is high, regular thinning is required for view of urban forest landscape and type growth of the tree itself. The results are small–diameter logs (SDLs) and branches and tree tops (BTW). SDLs and BTW must be removed, such as managing the stand species, obtain a more desirable landscape, provide a better habitat in urban (Chen *et al*., 2000; Kim H. G. and Kim K. T., 2000; Levan–Green and Livingston, 2001; Lin *et al*., 2005), and use for biomass energy used (Demirbas, 2005; Onay and Kockar, 2003; Stamatov *et al*., 2006).

For SDLs its use such for composting or laminating, but BTW is generally discarded and is under–utilized. To increase the value–added of BTW, wood charcoal plays an important role to reduce climate change because it signifies the reduction of carbon in the atmosphere. Using BTW to make wood charcoal as a valued–added product that accumulates CO_2 without spreading it into the atmosphere and at the same time provides jobs for the local population is a good deal (Ogawa *et al*., 2006; Okimori *et al*., 2006; Hwang *et al*., 2013). It directly creates a new way to produce wood charcoal, and indirectly plays an important role to increase the efficiency of the urban forests (Hwang *et al*., 2008). Besides, wood vinegar, a by–product of the wood charcoal manufacturing process using BTW, is under–utilized in Taiwan.

In the preparation of wood into wood charcoal, the smoke and steam at the chimney outflow are collected by the cooling system to obtain a yellow brown supernatant liquid, which is wood vinegar that has a particular smoky flavour; 80–90% of the main constituents are water, and 10–20% are organic constituents; mostly they are acetic acid, and the rest are about 200 kinds of organic coompounds (Hwang *et al*., 2006; Hwang *et al*., 2008; Lin *et al*., 2009). Wood vinegar can be used as a pesticide disinfector, compost fermentation accelerant, plant growth regulator, and insect repellant and deodorant (Ikimoto and Ikeshima, 2000; Yatagai, 2002; Mu *et al*., 2003; Lin *et al*., 2008), but there is not yet any systematic *in vitro* study of the effect of wood vinegar on antidermatophytes. Lin *et al*. (2009) suggests that wood

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vinegar is useful to restrain molds and prevents microbiological deterioration. The bamboo vinegars, collected at different temperatures, and the ether fraction and the acidic, neutral and phenolic fractions, divided by ether stratification, have antidermatophytic activity for *Epidermophyton floccosum* (EF), *Trichophyton mentagrophytes* (TM) and *Trichophyton rubrum* (TR) (Lin *et al*., 2018).

The composition of wood vinegar is the same as bamboo vinegar, includung more than 200 kinds of organic compounds, is much more complex than that of bamboo vinegar (Chen *et al*., 2006; Hwang *et al*., 2006; Hwang *et al*., 2008; Lin *et al*., 2009; Lin *et al*., 2018), and can prevent termites and disease germ of plants, fungi resistance of wood/bamboo materials, improve the soil, promote crop or prevent worm growth, reduce the use of agricultural chemicals, and sterilization (Ishihara, 1996; Nishimiya *et al*., 1998; Yataga, 2002; Hwang *et al*., 2005; Lin *et al*., 2006a; Lin and Shiah, 2006b). However, the effect of wood vinegar collected at different temperatures during the production process has never been investigated to determine the fungal infections of the skin and nails, which are common in humans and are caused mostly by dermatophytes. Dermatophytosis, which is caused mainly by genera of Trichophyton, Epidermophyton, and Microsporum, is a frequent dermatological problem in tropical and subtropical countries. A microbiological surveillance demonstrated that molds actually account for a quarter of skin fungal pathogens in hospitalized fever patients in central Taiwan (Huang and Wang, 2003). While the causative agents vary in species geographically, EF, TM and TR, are the most common anthropophilic dermatophytes in Taiwan (Chuang *et al*., 2007; Han *et al*., 2014).

Investigations; therefore, in this study were carried out to evaluate the antidermatophytic activity. The following experimentals prepared the BTW of *Fraxinus formosana* in Taiwan into wood charcoal from the earthen kiln and collected the wood vinegars at different temperatures from the chimney outflow of earthen kiln. The basic properties of wood vinegars, including pH value, specific gravity, acidic material content, dissolved tar content, and color number, were investigated. The antidermatophytic activities of EF, TM and TR from the wood vinegars, and the ether fraction and three fractions (acidic, neutral and phenolic), divided by ether stratification, were evaluated by the minimum inhibitory concentration (MIC), and the possible active compounds were further resolved by gas–chromatography (GC) coupled with mass spectrometry (MS).

MATERIALS AND METHOD

Experimental materials

Wood vinegars

The wood vinegars, collected at different temperatures during the manufacturing process of charcoal from BTW of *Fraxinus formosana*, were provided by the Division of Forest Utilization, Taiwan Forestry Research Institute (TFRI), Taipei, Taiwan (Hwang *et al*., 2006; Hwang *et al*., 2008). The vinegars were collected at temperatures ranging from 80 to 159˚C (Furuda, 1987; Nishimiya *et al*., 1998). The temperature was measured by a thermocouple at the chimney outflow of the earthen kiln during the charcoal manufacturing process (pyrolysis). The five different temperature ranges of wood vinegars were collected at 73–80, 90–92, 100–108, 120–125 and 145–162˚C.

Fungal Strains

The dermatophytes used were *Epidermophyton floccosum* (EF, BCRC 30531), *Trichophyton mentagrophytes* (TM, BCRC 32066), and *Trichophyton rubrum* (TR, BCRC 32805) of dermatophytes.

Culture medium

Sabouraud dextrose agar (SDA) was bought from Difco Laboratories Inc. Roswell Park Memorial Institute medium–3–(N–morpholino) propanesulfonic acid (RPMI–1640) and MOPS were bought from U.S. SIGMA.

Experimental methods

Basic properties of wood vinegars

The pH value of wood vinegars collected at different temperatures was measured by a pen type pH meter (SUNTEX TS–1). The specific gravity of wood vinegars collected at different temperatures was measured by a hydrometer when they were put in a 200 mL measuring cylinder. The experimental steps and calculated formula of acidic material content and dissolved tar content of wood vinegars refer to (Lin *et al*., 2018). The color number of the vinegars was measured by color number meter (Orbeco hellige 705–V2); the color disc was used for checking the determination according to the ASTM D–1544 Gardner method. The above tests for the basic properties of wood vinegars were all repeated three times.

Ether stratification and Identification of compounds

The ether fractions of wood vinegars, including acidic, neutral and phenolic fractions were stratificated, and the experimental steps refer to (Sugiruma, 1995; Lin *et al*., 2018). To analyze the compounds in the wood vinegars, the ether fraction, and the phenolic fraction, GC–MS (Agilent model 6890 GC with model 5973 MSD) was applied. The experimental steps refer to (Lin *et al*., 2018). Volatile compounds were identified by comparing the mass spectra with the data system libraries (Wiley/NBS and Nation Institute of Standard and Technology) and by Kovats indices (KI) estimated in accordance with modified Kovats method (Cheng *et al*., 2009). The relative percentages of individual compounds were calculated from the corresponding peak areas (Lin *et al*., 2008; Lin *et al*., 2016; Lin *et al*., 2018).

Fungal Strains and Culture Conditions

The dermatophytes used for experimental controls were obtained from the Taiwan Bioresource Collection and Research Center in Taiwan. The EF, TM, and TR were regularly cultured on Sabouraud dextrose agars

(SDA; Difco) at 25˚C. RPMI–1640 (Sigma) buffered to pH 7.0 with MOPS was used for the broth microdilution susceptibility assay, to determine the minimum inhibitory concentration (MIC). All the works were performed in biosafety level–2 (P2) facility. The protocol and the procedures of the study were approved by the National Chiayi University Biosafety Committee.

Susceptibility Assay by Broth Microdilution

MIC of the vinegars, and the ether fraction and three fractions (acidic, neutral and phenolic) was determined alternatively by the broth microdilution method using serially diluted wood vinegars. In brief, each microdilution well that contained 100 μL of the MOPS–buffered RPMI–diluted extract received an equal volume of 50–fold diluted conidia stock. The well–containing plates were incubated at 25˚C for 48 h. After incubation, the plates were examined visually, and the MIC for the assayed each diluted vinegar or fraction was the lowest concentration showing no apparent growth of the target microorganism. The experimental steps refer to (Lin *et al*., 2018).

Statistical analysis

The test results were represented by a mean (standard deviation). Statistical analysis was performed using one–way analysis of variance followed by Duncan's multiple comparison test using Statistical Product and Service Solutions (SPSS). Differences were considered statistically significant when $\rho < 0.05$.

RESULTS AND DISCUSSION

Basic properties of wood vinegars

The basic properties of the wood vinegars obtained were pH value of 2.45 to 3.49, specific gravity ranging from 1.005 to 1.008, acidic material content ranged between 3.84 and 7.62%, dissolved tar contents was from 0.38 to 0.59%, and the color number ranged from 8 to 12, and the color was clarified deep yellow brown. The thermal degradation turns into pyrolysis during vinegar collection, the pH value is relatively acidic, and the compositions of wood turn into water, CO_2, acetic acid, propanoic acid, and many different organic acid compounds (Hwang *et al*., 2006; Lin *et al*., 2008). Moreover, the wood is composed of cellulose, hemicellulose, and lignin, the thermal decomposition temperature of hemicellulose is lower than 260˚C, and the chimney outflow temperature is 80–102˚C; that of cellulose is 260–310˚C, and the chimney outflow temperature is 105–123˚C; that of lignin is 310–450˚C, and the chimney outflow temperature is 125–159˚C (Hwang *et al*., 2013).

Antidermatophytic activity of wood vinegar

There was an obvious antidermatophytic activity on EF, TM and TR, and the MICs were 1.25–2.50, 0.63–1.25 and 0.63–1.25 mg/mL (Table 2). The above results approach the antidermatophytic activity of bamboo vinegars, collected from the chimney outflow of earthen kiln at the different temperatures (80, 90–93, 100, 120–125 and 140–145˚C) during the bamboo charcoal preparation from Moso bamboo (Lin *et al*., 2018). Table 2 was also showed that the wood vinegars collected at temperature 73–80 and 145–162˚C had the best antidermatophytic activity, 0.63–1.25 mg/mL. Lin *et al*. (2008) report that the antidermatophytic activity of wood vinegars is not directly related to the pH value (Table 1), but related to its compounds.

Table 2. Minimum inhibitory concentrations against dermatophytesof wood vinegars collected at different temperatures
Unit: mg/mL

Collected temperature (˚C)	EF [1)]	TM	TR
73–80	1.25	0.63	1.25
90–92	2.50	0.63	1.25
100–108	2.50	0.63	1.25
120–125	2.50	1.25	1.25
145–162	1.25	0.63	0.63

[1)] EF: *Epidermophytonfloccosu*;
TM: *Trichophytonmentagrophytes*; TR: *Trichophytonrubrum*

The antidermatophytic activity obviously increased after ether stratification (Table 3); the antidermatophytic activity of the ether fraction (EE), and the neutral (NC), phenolic (PC) fractions was significantly, and that of the acidic fraction (AC) was worse. The MICs of NC had similar results for EF, TM and TR, which were 0.08–0.31 mg/mL. The EE at 90–92˚C and 120–125˚C had better antidermatophytic activity for three of them, and the lowest MICs were 0.16 mg/mL. Table 3 was also showed that the MICs of PC was lower than the others, the MICs for EF, TM and TR, which were 0.08–0.31 mg/

Table 1. Basic properties of wood vinegars collected at different temperatures

Collected temperature (˚C)	pH value	Specific gravity	Acidic material content (%)	Dissolved tar content (%)	Color number
73–80	$2.45 \pm 0.11^{a1)}$	1.005 ± 0.00^{a}	3.84 ± 0.10^{a}	0.38 ± 0.01^{a}	8 ± 0.08^{a}
90–92	2.68 ± 0.05^{ab}	1.006 ± 0.00^{a}	4.20 ± 0.10^{b}	0.49 ± 0.02^{b}	7 ± 0.14^{a}
100–108	3.27 ± 0.54^{b}	1.005 ± 0.00^{a}	3.93 ± 0.05^{a}	0.49 ± 0.17^{b}	8 ± 0.05^{a}
120–125	3.41 ± 0.06^{c}	1.008 ± 0.00^{b}	4.95 ± 0.15^{c}	0.39 ± 0.03^{a}	10 ± 0.11^{b}
145–162	3.49 ± 0.05^{c}	1.008 ± 0.00^{b}	7.62 ± 0.20^{d}	0.59 ± 0.03^{c}	12 ± 0.08^{c}

[1)] Mean ±standard deviation with the different superscripts is significantly different ($\rho < 0.05$) by Duncan's multiple range tests

mL, the temperatures 120–125˚C had the best 0.08 mg/m for EF. According to the temperatures at which the ether stratification fractions of wood vinegar had the best antidermatophytic activity for three dermatophytes, the antidermatophytic activities for TR was the best when the temperature was 73–80˚C, and the MICs of EE, and NC, PC were 0.16, and 0.08, 0.16 mg/mL, respectively. When the temperature was 90–92˚C, the best antidermatophytic activity for EF and the MICs of EE, and NC, PC were 0.31, and 0.31, 0.08 mg/mL. The antidermatophytic activity for TM was still the best at 90–92˚C, and the MICs was 0.16, and 0.31, 0.08 mg/mL for the EE, and NC, PC.

From the above results, the antidermatophytic activity of PC is better than others because the phenolic compounds can damage the cell membrane, the entocyte flows out, leading to cytolysis, or because of the antidermatophytic mechanism that kills cells gradually (Chan and Yang, 2001). Hwang *et al*. (2005) indicates that the phenol, 3–methyl–phenol (m–cresol), and 4–methyl–phenol (p–cresol) have antidermatophytic activity for *Pythium splendens*, *Fusarium oxysporum*, *Phytophthora capsici*, and *Ralstonia solanacearum*.

Table 3. Minimum inhibitory concentrations against dermatophytes of ether stratification fraction of wood vinegars collected at different temperatures Unit: mg/mL

Collected Temperature (˚C)	EF[1]				TM				TR			
	EE[2]	AC	NC	PC	EE	AC	NC	PC	EE	AC	NC	PC
73–80	0.63	>2.50	0.08	0.16	0.31	>2.50	0.08	0.16	0.16	>2.50	0.08	0.16
90–92	0.31	>2.50	0.31	0.08	0.16	>2.50	0.31	0.08	0.31	>2.50	0.16	0.08
100–108	0.63	>2.50	0.31	0.16	0.16	>2.50	0.31	0.16	0.16	>2.50	0.16	0.31
120–125	0.63	>2.50	0.16	0.08	0.31	>2.50	0.16	0.31	0.16	>2.50	0.16	0.31
145–162	0.63	>2.50	0.16	0.16	0.31	>2.50	0.16	0.16	0.16	>2.50	0.16	0.16

[1] Same as Table 2
[2] EE: Ether fraction; AC: Acidic fraction; NC: Neutral fraction; PC: Phenolic fraction

Table 4. Acidic and phenolic compounds of ether fraction from wood vinegars collected at different temperatures Unit: %

Time	Identified compound	Collected temperature (˚C)				
		73–80	90–92	100–108	120–125	145–162
Acidic compounds						
2.43	Propanoic acid	8.52	5.31	4.29	4.28	8.95
3.59	Butanoic acid	2.47	2.81	1.82	1.36	1.51
9.77	Dehydroacetic Acid	2.55	–[1]	–	–	–
11.70	4–hydroxy–3–methoxy–benzoic acid	–	–	–	0.93	–
	Total	13.54	8.13	6.10	6.57	10.46
Phenolic compounds						
5.96	Phenol	4.81	4.89	5.04	5.17	5.09
6.72	2–methyl–phenol	2.82	2.83	2.75	2.78	2.53
6.92	3–methyl–phenol (m–Cresol)	5.89	5.31	5.90	5.64	13.48
6.98	4–methyl–phenol (p–Cresol)	–	–	–	–	4.83
7.04	2–methoxy–phenol	20.32	15.51	11.47	9.50	–
7.16	2–ethyl–phenol	–	–	0.89	–	–
7.56	2,4–dimethyl–phenol	1.87	1.63	2.22	1.83	1.93
7.72	4–ethyl–phenol	2.37	1.97	4.64	4.60	–
7.77	3–ethyl–phenol	–	–	–	–	4.05
7.94	2–methoxy–4–methyl–phenol (Creosol)	6.42	6.74	6.05	5.01	7.06
8.08	2–ethoxy–phenol	–	1.29	–	–	–
8.25	4–ethyl–3–methyl–phenol	–	–	–	–	0.85
8.34	2,6–dimethoxy–phenol	16.50	15.28	17.00	15.86	21.67
8.60	4–ethyl–2–methoxy–phenol	6.39	5.47	6.01	4.80	6.31
	Total	67.39	60.93	61.97	55.20	66.77

[1] –: Trace amount (cannot be measured)

Compounds of the stratified fractions of wood vinegars

The acidic compounds, including propanoic, butanoic, dehydroacetic acid, and 4–hydroxy–3–methoxy–benzoic acid were 6.10–13.54% after the wood vinegar was stratified by ether. The acidic compounds of ether fraction was the lowest, likely because some of acidic compounds were dissolved in the water fraction; the phenolic compounds of ether fraction had the highest content (55.20–67.39%), whereby the 2,6–dimethoxy–phenol content was the highest, then 2–methoxy–phenol, 3–methyl–phenol (m–Cresol), and 4–ethyl–2–methoxy–phenol (Table 4). There were 14 types of compounds, but the content of phenolic compounds was the highest at 73–80 and 145–162˚C of collected temperature. The compounds of 2–methoxy–phenol (20.32%) was highest at 73–80˚C, and then 2,6–dimethoxy–phenol (16.50%), 3–methyl–phenol (m–Cresol) (5.89%) and Phonel (4.81%); the compounds of 2,6–dimethoxy–phenol (21.67%) was highest at 145–162˚C, and then 3–methyl–phenol (m–Cresol) (13.48%), 2–methoxy–4–methyl–phenol (Creosol) (7.06%) and 4–ethyl–2–methoxy–phenol (6.31%).

The neutral compounds of ether fraction were 19.07–38.23%, and the major compounds in each collected temperature were Furfural and 1,2,5–trimethoxy–3–methyl–benzene (Table 5). The neutral substances are generated in the pyrolysis stage, such as aldehyde, ketone, alcohols, and derivants (Kuriyama, 1979). There were 27 types of neutral compounds, but the content of compounds was the highest at 90–92, 100–108 and 120–145˚C of collected temperature. The highest compounds of 3β,11α–dihydroxy–5β–androstan–17–one (24.34%) was at 120–125˚C, and Cholesterol (8.66%) at 100–108˚C and 3β,11α–dihydroxy–5β–androstan–17–one (4.59%) at 90–92oC; as well as then 1,2,5–trimethoxy–3–methyl–benzene (1.99%) was at 120–125˚C, and 1,2,3–trimethoxybenzene

Table 5. Neutral compounds of ether fraction from wood vinegars collected at different temperatures Unit: %

Time	Identified compound	Collected temperature (˚C)				
		73–80	90–92	100–108	120–125	145–162
Neutral compounds						
4.02	Furfural	3.45	2.24	2.79	1.94	5.28
5.07	2–methyl–2–cyclopenten–1–one	0.96	1.38	0.83	0.56	0.68
5.14	1–(2–furanyl)–ethanone	1.95	2.65	1.49	0.89	1.14
5.18	Butyrolactone	–[1)]	–	–	–	1.09
5.80	3–methyl–2–cyclopenten–1–one	–	2.01	1.53	–	2.40
6.48	2–hydroxy–3–methyl–2–cyclopenten–1–one	1.43	1.64	0.86	–	–
6.61	2,3–dimethyl–2–cyclopenten–1–one	–	3.91	3.07	1.49	1.86
6.80	2,3,4–trimethyl–2–cyclopenten–1–one	–	–	0.47	–	0.58
7.17	4,6–Dimethyl–2–pyrimidone	–	1.16	–	–	–
7.36	3–hydroxy–2–methyl–4H–pyran–4–one (Maltol)	–	–	–	1.17	3.42
7.81	1,4–dimethoxybenzene	–	1.55	–	–	–
8.04	1,2–benzenediol	–	–	–	1.50	–
8.19	Benzenethiol	–	–	–	1.63	–
8.53	2–methoxy–1,4–benzenediol	–	1.10	–	–	–
8.72	2,3–dihydro–1H–inden–1–one	–	–	–	–	0.97
8.87	1,2,3–trimethoxybenzene	–	1.18	4.60	0.34	0.44
9.49	1,2,3–trimethoxy–5–methyl–benzene	0.59	–	0.90	0.53	0.54
10.07	Apocynin	–	–	2.01	0.87	–
10.18	Butylated hydroxytoluene	1.17	0.35	–	0.34	–
10.26	1,2,5–trimethoxy–3–methyl–benzene	5.15	4.36	3.72	1.99	3.53
10.52	N–(phenylmethylene)–benzenamine	–	–	0.53	–	–
11.12	4–hydroxy–3,5–dimethoxy–benzaldehyde	–	0.83	–	–	–
11.53	1–(4–hydroxy–3,5–dimethoxyphenyl)–ethanone	1.01	0.78	0.46	0.65	–
14.36	Cholesterol	–	–	8.66	–	–
17.36	Epiandrosterone	1.58	1.22	–	–	–
19.69	5,11–diethyl–5,6,11,12–tetrahydro–2,8–dimethoxy–,(5R,11S)–rel–chrysene	1.78	–	–	–	1.09
20.01	3β,11α–dihydroxy–5β–androstan–17–one	–	4.59	–	24.34	–
	Total	19.07	30.94	31.93	38.23	22.78

[1)] Same as Table 4

(4.60%) at 100–108˚C and 1,2,5–trimethoxy–3–methyl–benzene (4.36%) at 90–92oC

Antidermatophytic activity of the main compounds

Table 6 showed the phenolic and neutral materials results of phenolic compounds of ether fraction from wood vinegars collected at different temperatures. The phenolic materials were about 24.40–74.85%, in which 2,6–dimethoxyl–phenol (22.35–65.03%) was the majority, and then 4,5–dimethoxy–2–methyl–phenol (2.05–9.82%), and there was about same amount of neutral material (25.16–75.60%), in which 3β,11α–dihydroxy–5β–androstan–17–one (10.63–52.51%). In the scope of this study, the study suggested that the antidermatophytic activity was determined by using the broth microdilution method to obtain the MIC resultes. The antidermatophytic activity of phenolic fraction for EF, TM, and TR was related to phenolic and neutral compounds of ether fraction (Tables 4 and 5). The MICs of NC and PC for three dermatophytes were 0.08–031 mg/mL (Table 3). It indicates that the wood vinegars, collected at 73–80, 90–92, 100–108, 120–125 and 145–162˚C, after ether stratification are all with the antidermatophytic activity for three dermatophytes because of the contents of the the phenolic and neutral materials (Table 6) in phenolic fraction.

CONCLUSION

The wood vinegars used were collected at different temperatures from *Fraxinus formosana* to investigate the antidermatophytic activity of the vinegars and the ether stratification fractions. The pH value of wood vinegars was 2.45–3.49, the specific gravity was 1.005–1.008, the acidic material content was 3.84–7.62%, the dissolved tar content was 0.38–0.59%, and the color number was 8–12. The gas chromatography showed that the acidic compounds of ether fraction were 6.10–13.54%, where the main compound was Propanoic acid; the phenolic compounds of ether fraction were 55.20–67.39%, and the content of 2,6–dimethoxy–phenol was the highest; the neutral compounds were 19.07–38.23%. The phenolic material of phenolic fraction was 24.40–74.85% and the neutral material of phenolic fraction was 25.16–75.60% after ether stratification, and the 2,6–dimethoxyl–phenol and 3β,11α–dihydroxy–5β–androstan–17–one were the majority. The antidermatophytic activity of phenolic compounds of ether fraction for EF, TM, and TR was related to phenolic and neutral materials of phenolic fraction, and the MICs of NC and PC for three dermatophytes were 0.08–031 mg/mL. Therefore, the wood vinegars, such like bamboo vinegars, were collected from different temperatures exhibited strong antidermatophytic activities. The vinegars application on treating skin fungal infection into shampoo or soap may be practical and scientifically sounding.

AUTHOR CONTRIBUTION

Han Chien LIN designed this paper and wrote the paper. Shao–Hung WANG performed the MIC experiments and supervised the data analysis. Yi–Han HUANG analyzed the data with statistical processes and carried out GC–MS experiments. Gwo–Shyong HWANG supported the wood vinegars obtained. Noboru FUJIMOTO supervised the work and provided reference resources.

Table 6. Compounds of phenolic and neutral materials of phenolic fraction from wood vinegars collected at different temperatures

Unit: %

Time	Identified compound	Collected temperature (˚C)				
		73–80	90–92	100–108	120–125	145–162
Phenolic material						
7.07	2–methoxy–phenol	–[1)]	–	–	–	3.16
7.94	2–methoxy–4–methyl–phenol (Creosol)	–	–	–	–	2.34
8.61	4–ethyl–2–methoxy–phenol	–	7.47	–	–	2.93
9.13	2,6–dimethoxy–phenol	65.03	50.32	30.58	22.35	32.09
9.77	4,5–dimethoxy–2–methyl–phenol	9.82	9.36	4.37	2.05	4.72
	Total	74.85	67.15	34.95	24.40	45.24
Neutral material						
10.18	Butylated hydroxytoluene	–	1.79	–	–	–
10.27	1,2,5–trimethoxy–3–methyl–benzene					
17.03	12.17	5.57	2.48	5.44		
16.72	Bis(2–ethylhexyl) phthalate	8.13	–	5.32	4.85	1.87
17.36	1,2,3,4,5,6,7,8–octamethyl–anthracene	–	–	6.28	–	–
19.33	1,3a,8,8a–tetrahydro–2–methyl–1–methylene–3,8a–diphenyl–cyclopent–indene	–	8.26	14.42	15.76	9.80
20.01	3β,11α–dihydroxy–5β–androstan–17–one	–	10.63	33.46	52.51	37.66
	Total	25.16	32.85	65.05	75.60	54.77

[1)] Same as Table 4

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