Songklanakarin J. Sci. Technol.
40 (1), 87-92, Jan. - Feb. 2018

http://www.sjst.psu.ac.th

Original Article

Use of wood vinegar as fungus and malodor retarding agent for natural rubber products

Juntima Chungsiriporn[1]*, Prukraya Pongyeela[1], and Jutarut Iewkittayakorn[2]

[1] Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand

[2] Department of Molecular Biotechnology and Bioinformatics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand

Received: 22 November 2015; Revised: 14 October 2016; Accepted: 18 October 2016

Abstract

Natural rubber harvested from the rubber tree is one of the most economically important agricultural products for Thailand. Factories using primary rubber products especially rubber sheets and block rubber encounter problems associated with fungal growth and often receive public complaints about the malodor they generate as well as other environmental concerns. In this study, the use of wood vinegar produced from rubber wood to ameliorate these problems was investigated. Its efficiency as an antifungal agent was determined from the number of counts of fungal colonies formed per cm^2 and per gram of material, on rubber sheets and block rubber respectively. To analyze the effect of wood vinegar on malodor from block rubber processing, shredded rubber samples were prepared and the organic acids contained in the gas released during the rubber drying process were measured. The results showed that wood vinegar can significantly decrease the numbers of fungal colonies on rubber sheets and shredded rubber. A 5.0% application of wood vinegar which reduced fungal growth during the storage of shredded rubber also reduced the malodor from the drying process. The ability of wood vinegar to reduce fungi and the organic acid content of the air released during the rubber drying process is due to the presence of phenolic compounds and acetic acid. Thus soaking in wood vinegar is an effective way to deal with these problems and shows a very high potential to do so without any significant toxicity.

Keywords: wood vinegar, natural rubber, malodor, organic acid, antifungal

1. Introduction

Rubber industries make a significant contribution to the national economy of Thailand which stands tall as the world's No. 1 producer and exporter of natural rubber which produces about 3.82 million metric tons of rubber products annually (Industry Focus BOI, 2012). Fresh latex collected as a liquid can be processed into primary rubber products such as natural rubber (NR) sheet, block rubber, and ribbed smoked sheets, which can then be transported for subsequent processing into various final rubber products. In producing primary rubber products, especially rubber sheets there is often a problem with fungal growth and producers of block rubber often receive a high number of public complaints about malodors.

NR sheet is produced by adding a coagulant to fresh latex which is then compressed between two steel rollers, and dried in the sun. Because of the high moisture content of the NR sheets, fungal growth can occur during storage in farm buildings. This is a serious problem in NR production, since it may affect the quality of the final product and reduce the selling price of the rubber. Therefore, antifungal agents need to be added during the production of the NR sheets. Most commercial antifungal agents are highly toxic and not environmentally friendly (Mela *et al.,* 2013). There is a need

*Corresponding author
Email address: juntima.c@psu.ac.th

For research into non-toxic compounds that can act as fungicides which can be applied by farmers.

In block rubber processing factories, malodors originate from volatile organic compounds which are produced from microbial activity, including fungi, which break down non-rubber contaminants. Malodor is released during the storage of rubber scraps and cup lumps as well as thermal degradation during the process of drying shredded rubber. The components which have been reported to produce the smell include organic acids, ammonia, low molecular weight fatty acids (C2-C5), esters, hydrogen sulphide, and aromatic compounds and they become malodorous when they come into contact with water vapor (Hidayaty *et al.,* 2012). Malodor from factories has mainly been attributed to obnoxious volatile components, which are discharged into the atmosphere through a chimney during the drying stage of processing the block rubber.

Wood vinegar, or pyroligneous acid, which is produced during the production of wood charcoal, is a natural, environmentally friendly substance and, and its use a cost effective anti-fungal agent has been previously investigated. Raw wood vinegar is collected during the pyrolysis or carbonization of wood in airless conditions at temperatures over a range of 400–500 ^{o}C. The materials needed to produce wood vinegar, such as bamboo and wood, are readily available in Thailand. Acetic acid and phenolic compounds in wood vinegar have been reported to have fungicidal properties and can be used as an insect repellent, an odor remover, and as a wood preservative in place of other traditional chemicals (Oramahi & Yoshimura, 2013). The high concentrations of organic acids in wood vinegar exhibit high antimicrobial activity (Ma *et al.,* 2011). In recent years, there have been a number of attempts to apply wood vinegar to rubber production processes, for example, as a coagulating agent for natural rubber sheet production, as a preservative to treat rubber wood, and as an additive in rubber sheets. The use of wood vinegar as a coagulant and antifungal agent for NR sheet and block rubber also has been investigated and reported (Baimark & Niamsa, 2009; Ferreira *et al.,* 2005). However, none of these studies investigated the reduction of malodor emanating from the drying of shredded rubber during the production of block rubber. Further, because wood vinegar is readily available locally, it would be of interest for use as an additive and could become an important factor in the NR industry.

In this work, the effect of wood vinegar on the growth of fungi on NR sheets and malodors generated from the processing of block rubber were investigated. The study immersed NR sheets and block rubber in wood vinegar as an environmentally friendly antifungal agent with low toxicity. Wood vinegar is safe for human beings, land animals, and the environment but it is slightly toxic to fish and very toxic to plants in high doses though not in small amounts (Payamara, 2011). The antifungal efficiency of wood vinegar on NR sheet and shredded rubber was determined based on the reduction of the number of fungal colonies compared to untreated rubber. To analyze the malodor from block rubber processing, the organic acids contained in the gas released from shredded rubber during the drying process were measured.

2. Materials and Methods

2.1 Wood vinegar

Wood vinegar was produced from the pyrolysis of rubber wood and used without further purification. Smoke from the vents of charcoal kilns was condensed in stainless steel tubes and collected for use as raw distilled wood vinegar in a glass bottle as shown in Figure 1. The following is a summary of the key steps in producing wood vinegar adapted from Burnette (2013): (a) Arrange selected dry wood in a 200-liter drum kiln, and close the top with clay before setting the wood alight, (b) Approximately 30 minutes to 1 hour after ceasing to feed fuel into the kiln, if the smoke is yellowish and acrid, close off most of the outer vent, and (c) Collect the raw wood vinegar and leave it in a sealed bottle for approximately three months to allow sediments to settle. Thailand's Department of Agriculture reports that if wood is burned for 12 to 15 hours (or less, depending on the type and size of wood) in a 200-liter oil drum kiln, it should produce 2 to 7 liters of raw wood vinegar.

Figure 1. Distilled wood vinegar.

Fresh bananas (*Musa* ABB cv. Kluai 'Namwa', *Musa* ABB cv. Kluai 'Hom Thong', *Musa* ABB cv. Kluai 'Khai' and *Musa* ABB cv. Kluai 'Leb Mu Nang') at a maturity level of 6 (yellow peel) (Tapre & Jain, 2012) were purchased in January 2014 from the local market of Nonthaburi, Thailand. The peels were carefully removed without damaging them and were washed manually using tap water to remove dirt. The peels were reduced in size to pieces 2 cm × 2 cm and blanched in boiling water for 5 minutes, cooled quickly using cold water, and the water was drained. The pieces were dried at 65 °C in a hot-air oven (Memmert 100-800, Schwabach, Germany) for 24 hours, were then allowed to cool to room temperature, ground using a cross beater mill (SK100, Retsch, Haan, Germany), screened to select particles with a size between 37-1,000 μm and kept in zip lock polypropylene bags at room temperature until needed. The enzymes α-amylase, from *Bacillus subtilis,* and glucoamylase, from *Aspergillus niger,* were both present in Termamyl SC purchased from the Brenntag Company (Bangkok, Thailand).

Fresh wood vinegar produced from the effluent from a charcoal kiln has a temperature of 36-38 °C, a smoky odor, and a specific gravity between 1.005-1.050. Wood vinegar produced from rubber wood is composed of acetic acid (70.6 mg/mL), methanol (4.52 mg/mL), furfuraldehyde (0.67 mg/mL), phenol (0.46 mg/mL) as well as traces of neutral substances such as formaldehyde and acetone

(Theapparat *et al.,* 2014). It is reddish brown in color and by reason of its high volatile acid (10-30%) content it is acidic with a pH ranging from 2.0-3.0. and these acids contribute to its mild corrosive properties. The Wood vinegar is safe to human beings, animals, plants, and environment but slightly toxic to fish and very toxic to plants if too much is applied (Payamara, 2011). In this study, solutions of wood vinegar were prepared by dilution in distilled water at various concentrations to test for its ability to inhibit fungal growth on rubber sheets and to reduce the odor emanating from the drying of shredded rubber.

2.2 Preparation of natural rubber sheet samples

NR sheets were collected from a grower in Songkhla, Thailand. Sample sheets of size 10×10 cm were cut for the fungus-attack test. Wood vinegar solutions at concentrations of 0 (control), 10, 20, 50, and 100% by volume were prepared and used. The sample sheets were soaked in the wood vinegar solutions for 1 hour to test their effect in preventing fungal growth. The wood vinegar coated rubber sheets were then dried at room temperature, placed in four layers in trays and incubated over a 4-week storage period at room temperature (29±1 °C). The stored samples were examined for fungal growth after five days and at the end of the 4-week storage period. Samples of untreated rubber sheet were used as control samples.

2.3 Preparation of shredded rubber samples

Samples of shredded cup lumps of rubber were obtained from the Siam Indo Rubber Co., Ltd. Phattalung province, Thailand. For the antifungal test, 25 grams of the rubber samples were immersed in wood vinegar solution at different concentrations of 0 (control), 10, 20, 50, and 100% by volume for 1 hour, in a similar way to the rubber sheet samples. The odor reduction and antifungal properties of the wood vinegar on shredded rubber storage times before drying were also determined on shredded rubber samples of 600 grams soaked in wood vinegar solution at 5.0% v/v concentration for 1 hour and stored for 21 days. To study the malodor generated from the block rubber drying process, samples of the shredded rubber soaked in concentrations of 3%, 5% and 10% wood vinegar were dried in a drying test unit.

2.4 Testing for antifungal efficiency

The antifungal efficiency of the wood vinegar was studied by measuring the area of fungal growth on the surfaces of the rubber sheets and in the shredded rubber samples. Antifungal activity was also tested by carrying out microbial counts using the standard plate count method and the physicochemical properties were determined using FDA, 2001. Each of the NR sample sheets was cut into 2 x 2 cm samples and stacked in four layers, and 25 g shredded rubber samples were prepared. Both samples were soaked with 100 ml 0.1% peptone buffer and shaken for 30 minutes then cultured with potato dextrose agar (PDA) at 20-25 °C for five days. The antifungal efficiency of the wood vinegar on the NR sheet samples was determined by the reduction by fungal colony counts per cm^2 (CFU/cm^2) but for the shredded rubber samples this was reported by the formation of fungal colonies per gram (CFU/g).

2.5 Rubber drying and gas sampling

To analyze the effect of wood vinegar on malodor from the drying of shredded rubber, the organic acids containing in the gas released from the dryer were measured (Hidayaty *et al.,* 2012). The effect of storing times before drying of the shredded rubber on the generation of malodor was also studied. The rubber samples were placed in trays and left at the control drying time in the dryer at 130°C (Tham *et al.,* 2014). Figure 2 shows a schematic diagram of the shredded rubber drying experimental procedure. Odor in the air released from the dryer was measured by bubbling the gas released for three minutes through an impinger containing 50 ml NaOH solution. Concentrations of the organic acid contaminating the air released were absorbed and analyzed by the titration method every 10 minutes. Afterwards, analysis of the concentration of organic acids was performed by the titration of the NaOH solution with the equivalence point occurring when the solution changed color from colorless to soft pink.

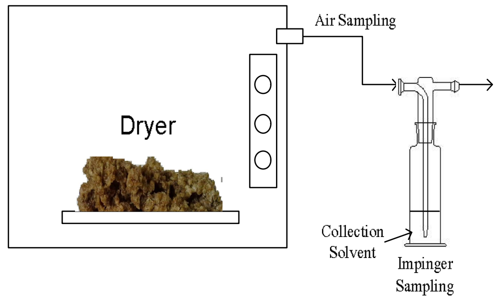

Figure 2. Schematic diagram of shredded rubber drying, air sampling, and gas analysis.

3. Results and Discussion

3.1 Antifungal efficiency of wood vinegar on natural rubber

Wood vinegar utilization previously was focused on pesticides in agriculture, forestry, or disinfectant in medicine. Recently, wood vinegar products have been developed for the human, animal, and plant which can be used as antibiotic diets to the performance, nutrient digestibility, and intestinal microflora in pigs (Choi *et al.,* 2009; Velmurugan *et al.,* 2009). The wood vinegar has clearly shown the positive result of fungi resistance (Lin and Shiah, 2006). Antimicrobial activity of wood vinegar (Oramahi & Yoshimura, 2013) has been investigated and attributed to the presence of compounds such as phenolic derivatives, carbonyls, and organic acids (Marumoto *et al.,* 2012).

3.1.1 Antifungal effect on NR sheet

The antifungal efficiency of the wood vinegar on the rubber sheet was measured based on CFU/cm^2 as described above in section 2.4. The sample was analyzed at the beginning of storage period and after five days of storage to investigating the performance of wood vinegar as an anti-fungal agent. The colony counts of the fungi appearing on the rubber sheets are presented in Table 1.

Table 1. Fungal count on rubber sheets after storage for 5 days.

Sample	Fungal Count (CFU/cm^2)
Initial Control	0.65×10^4
Control 5 days	0.95×10^4
10% v/v wood vinegar	0.75×10^4
20% v/v wood vinegar	0.55×10^4
50% v/v wood vinegar	$< 10^3$
100% v/v wood vinegar	$< 10^3$

The fungi initially present on the rubber sheets before soaking them in wood vinegar were measured and used as the control condition. After storage of the processed rubbers at room temperature (29±1 °C) for a period of five days, the wood vinegar at concentrations of 10 and 20% inhibited the growth of fungi on the rubber sheets by 21% and 42%, respectively. At concentrations of 50 and 100%, the wood vinegar almost completely inhibited the colonial growth of fungi (<10^3 CFU/cm^2) according to acidic condition occurred on the rubber surface with high concentration of acetic acid and phenolic compound. The fungal growth on the rubber sheets after soaking at various concentrations of wood vinegar after storage for five days and four weeks are presented in Figure 3 and 4, respectively.

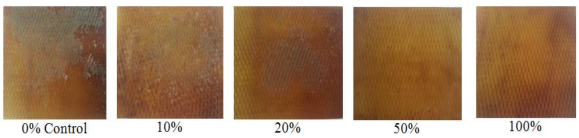

Figure 3. Antifungal effect of wood vinegar concentrations (%v/v) on rubber sheet after storage for 5 days.

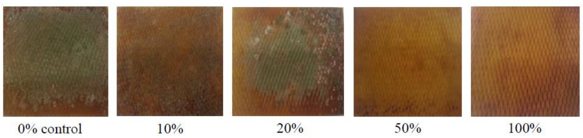

Figure 4. Antifungal effect of wood vinegar concentrations (%v/v) on rubber sheet after storage for 4 weeks.

After storage the NR sheets for four weeks, it was found that there were some colonies on the samples treated with 10% and 20% concentrations of wood vinegar but at 50% and 100% concentrations there was almost complete inhibition of the growth of fungal colonies. The wood vinegar has significantly high antifungal efficiency as it contains acetic acid and phenolic compound which are antimicrobial constituents. The previous research by Baimark and Niamsa (2009) investigated the use of wood vinegar as coagulating and antifungal agents to replace formic acid and acetic acid. The results supported this work in the view of wood vinegar was able to inhibit the fungi growth on NR sheets. Therefore this is an important guidance for rubber industry to inhibit fungi growth during NR sheets storage by soaking in wood vinegar. The wood vinegar containing phenolic compounds has significantly higher antifungal efficiency than formic acid and acetic acid. Phenolic compound in wood vinegar is a major antimicrobial constituent as it has a strong antifungal effect on microorganisms (Hwang *et al.,* 2005). In addition, the wood vinegar is cheaper than those chemicals.

3.1.2 Antifungal effect on shredded rubber

The fungus on the shredded rubber was analyzed for microbial counts using the standard plate count method in the same way as detailed above in section 2.4. Fungal growth was almost completely inhibited on the shredded rubber at wood vinegar concentrations of 50 and 100% in the same way as for the rubber sheets, as shown in Table 2. The antifungal efficiency of the wood vinegar on the shredded rubber samples was determined by the reduction by the formation of fungal colonies per gram (CFU/g).

Table 2. Fungal count on block rubber.

Sample	Fungal Count (CFU/g)
0% (Control)	1.1×10^6
10% v/v wood vinegar	1.9×10^5
20% v/v wood vinegar	3.7×10^4
50% v/v wood vinegar	150
100% v/v wood vinegar	50

The effect of soaking shredded rubber in wood vinegar solution at 5.0% concentration for 1 hour followed by storage for 21 days (WV Sample) was determined comparing with a sample not treated with wood vinegar (control). The results by measuring the fungal count (CFU/g) at 9, 13, 17, and 21-day storage. A fungal count of 2.0-3.5 x 10^7 CFU/g is shown in Figure 5.

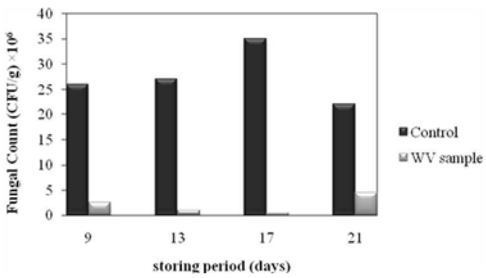

Figure 5. Effect of 5.0% v/v wood vinegar and storage period on fungal count (CFU/g) on block rubber.

It can be observed that a concentration of 5.0% wood vinegar exhibited good inhibiting properties on the growth of fungus on shredded rubber used for the production of block rubber. This is because of acetic acid and phenolic compounds containing in the wood vinegar. The use of wood vinegar at 5% can be effectively performed and recommended on shredded rubber for an economic even though higher concentrations of wood vinegar having more effects on reducing an amount of fungi on the rubber surface, 5.0%. However, wood vinegar was chosen due to its ability to meet the requirement of inhibiting properties.

3.2 Reducing the malodors produced in drying shredded rubber

Normally malodorous vapors from natural rubber processing factory can be removed by the scrubber system as the study conducted by Hidayaty *et al.* (2012). It does not solve the cause of problem. This experiment aimed to test the effectiveness of wood vinegar in reducing the odors produced during the drying of block rubber. These odors are mainly caused by fungus affecting the rubber, since most of the malodorous compounds are organic acids released during the drying of the fungal affected shredded rubber. Analysis of the organic acid in the gas stream was performed by the titration method. The experimental set-up simulated the drying of the rubber and was carried out at a temperature of 130°C with a heating rate of 10°C/min as described in section 2.5. The samples soaked in wood vinegar solution at concentrations of 0 (control), 3.0, 5.0, and 10.0% v/v were tested for their effect in reducing malodor as well as inhibiting fungal growth economically. The effects of wood vinegar at different concentrations in reducing malodor are detailed in the following section

3.2.1 Effect of wood vinegar concentrations

The shredded rubber samples were immersed in wood vinegar solution at concentrations of 0 (control), 3.0, 5.0, and 10.0% v/v Then the rubber was allowed to stand at room temperature for one day before being dried in a dryer. The effects on malodorous compounds release from the rubber drying were tested by collecting the releasing gas in chemical solution containing in impinger. The gas composition from the dryer was analyzed for organic acid concentrations as shown in Figure 6.

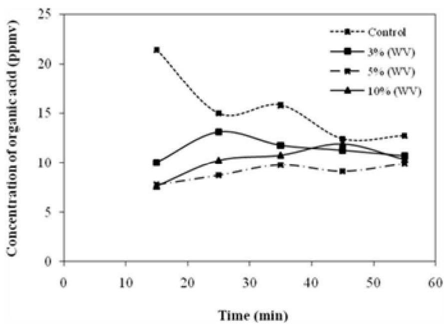

Figure 6. Organic acid concentrations in the air released from the drying of shredded rubber treated with different concentrations of wood vinegar.

The results indicated that the use of wood vinegar had a significant effect in reducing the organic acids released into the air during the drying process. The immersing of shredded rubber in wood vinegar at concentrations of 3.0-5.0% was able to reduce the release of malodor during the rubber drying process. A very low organic acids concentration released in the air was achieved identically to the use of 10.0%. As a result, the main constituent from 3.0-5.0% concentration can be effective in reducing of organic acids.

3.2.2 Effect of wood vinegar on rubber storage before drying

The effect of immersing the shredded rubber in wood vinegar at a concentration of 5% on the odor released during drying after different periods of storage at room temperature is shown in Figure 7. After one day of storage, the malodor from the sample soaked in wood vinegar was little different from that of the control sample. However, after storage for 17 days, the organic acid released from the dryer from the sample soaked in wood vinegar had a very low value of 5-10 ppmv compared to the untreated control sample which produced 25-30 ppmv organic acids. This clearly demonstrates the effectiveness of wood vinegar in reducing odor from stored block rubber. This is because a solution of wood vinegar increases the biological activity of various beneficial microbes (Zhai *et al.,* 2015) and decreases fungal formation which combats the bad odor. The wood vinegar solution subsequently diminishes the production of odor-causing organic acid in shredded rubber and decreases the odor releasing from dryer to atmosphere.

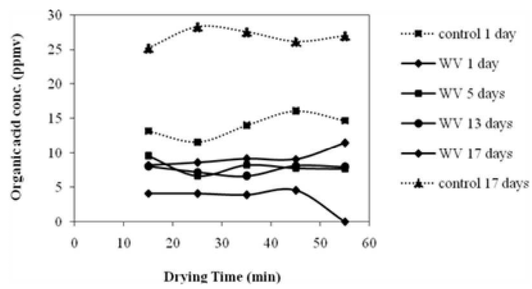

Figure 7. Organic acid concentration in gas samples released from shredded rubber during drying for various storage times using wood vinegar (WV) and without WV (control).

4. Conclusions

The results of this work demonstrate that wood vinegar produced from rubber wood show antifungal activity on the surface of rubber sheet as well as on shredded rubber. Therefore it has a high potential to solve the problem of complaints from the public about malodor and other environmental problems that occur in the rubber industry without the risk of introducing any significant toxicity. The effect is due to the presence of phenolic compounds and acetic acid in wood vinegar. Immersing the shredded rubber in a 5.0% wood vinegar solution can reduce fungal growth during storage which reduces malodor during the drying process. The management of fungi on the surface of rubber sheets and on shredded rubber used as a raw material for the production block rubber by soaking in wood vinegar is an effective way of eliminating malodor. However, the wood vinegar must be purified and appropriately diluted with water before use because the concentration of wood vinegar is an important factor to consider.

Acknowledgements

Authors are grateful to the PSU Research Fund for financial support and also appreciate kind support from the Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University.

References

Baimark, Y., & Niamsa, N. (2009). Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets. *Biomass and Bioenergy, 33*, 994–998.

Burnette, R. (2013). *An introduction to wood vinegar*. (Published as a Technical Note 2013).

Choi, J. Y., Shinde, P. L., Kwon, I. K., Song, Y. H., & Chae, B. J. 2009. Effect of wood vinegar on the performance, nutrient digestibility and intestinal microflora in weanling pigs. *Asian-Australasian Journal of Animal Sciences, 22*(2), 267–274.

Ferreira, V. S., Rêgo, I. N. C., Pastore, J. F., Mandai, M. M, Mendes, L. S, Santos, K. A. M., . . . Suarez, P. A. Z. (2005). The use of smoke acid as an alternative coagulating agent for natural rubber sheets' production. *Bioresource Technology, 96*, 605–609.

Hidayaty, K. N., Fadhilah, I. N., & Zairossani, M. N. (2012). Characteristics of Odour concentration from rubber processing factories via Olfactometry Technique. *Chemical Engineering Transactions, 30*, 121-126.

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

Industry Focus Board of Investment. (2012). Thailand continues to attract investment in rubber industry. *The Thailand Board of Investment (BOI), 22*, 11.

Lin, H. C. & Shiah, T. C. (2006). Evaluation of fungi resistance of moso Bamboo materials using bamboo vinegar with smoking process. *Quart Forest Research of Taiwan. Taiwan ROC, 28*(2), 51–66.

Marumoto, S., Yamamoto, S. P., Nishimura, H., Onomoto, K., Yatagai, M., Yazaki, K., . . . Watanabe, T. (2012). Identification of a germicidal compound against picornavirus in bamboo pyroligneous acid. *Agricultural and Food Chemistry, 60*, 9106–9111.

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

Mela, E., Arkeman, Y., Noor, E., & Achsani, N. A. (2013). Potential products of coconut shell wood vinegar. *Biological and Chemical Sciences, 4*, 1480-1493.

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

Payamara, J. (2011). Usage of wood vinegar as new organic substance. *International Journal of ChemTech Research, 3*(3), 1658-1662.

Tham, T. C., Hii, C. L., Ong, S. P., Chin, N. L., Abdullah, L. C., & Law, C. L. (2014). Technical review on crumb rubber drying process and the potential of advanced drying technique. *Agriculture and Agricultural Science Procedia, 2*, 26-32.

Theapparat, Y., Chandumpai1, A., Leelasuphakul, W., Laemsak, N., & Ponglimanont, C. (2014). Physicochemical characteristics of wood vinegars from carbonization of Leucaena leucocephala, Azadirachta indica, Eucalyptus camaldulensis, Hevea brasiliensis and Dendrocalamus asper. *Social Sciences, 48*, 916-928.

Velmurugan, N., Han, S. S., & Lee, Y. S. (2009). Antifungal activity of neutralized wood vinegar with water extracts of Pinus densiflora and Quercus serrata Saw Dusts. *Environmental Research, 3*(2), 167-176.

Zhai, M., Shi, G., Wang, Y., Mao, G., Wang, D., & Wang Z. (2015). Chemical compositions and biological activities of Pyroligneous acids from walnut shell. *Bioresources Technology, 10*(1), 1715-1729.