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THE CRUDE TANNIN EXTRACTION FROM WOOD SCRAP WASTES FOR PROLONGING THE SHELF LIFE OF LITCHI FRUITS

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ABSTRACT: Waste from wood scraps is an important problem in areas of wood handicraft performed. The purposes of this research were to study the optimum condition for crude tannin extraction from those wastes and usage them for prolonging shelf life of litchi fruits. Various chemicals used for the extraction included distilled water, 95% ethanol, 8% sodium carbonate and their combinations. The proportion between wood scrap per solvent were 1:10 or 1:20 $g.mL^{-1}$ and extraction temperature were 70, 80 or 90°C. The basic chemical characteristics of the extracted tannin were tested. Quantification of tannin was performed by spectrophotometer. The results found that optimum solvent was 95% ethanol with proportion at 1:10 $g.mL^{-1}$ and 80°C extracted temperature, which gave the maximum amount of tannin (12.34 $mg.g^{-1}$ dry weight). Then, crude tannin was tested for the *in vitro* fungicidal effect to pathogenic fungi isolated from litchi fruits. The PDA medium containing crude tannin at different concentrations including; 0, 0.1, 0.2 and 0.4 % were tested in comparing to 0.1% benomyl fungicide. The result was found that PDA containing 0.2 and 0.4% of extracted tannin significantly ($P \leq 0.05$) inhibited growth of fungal mycelium better than 0 and 0.1 % but still lower than benomyl fungicide. The *in vivo* test with litchi fruits by soaking the fruits in various concentrations of crude tannin; 0, 0.1, 0.2 and 0.4%, compared to 0.1% benomyl fungicide was found that 0.2% tannin could extend the shelf life of litchi fruits better than other concentrations but its efficiency was still lower than benomyl fungicide.

Keywords: Tannin, Wood scrap, Shelf life, Litchi

1. INTRODUCTION

Tannins are polyphenolic compounds found in almost plants. The compounds are widely used in tanning leather industries, clarifying wine and beer, or used as mordant in dyeing and astringents in medicine. Mangrove is well known as tannin resources [1]. The increasing demand for the raw material in extracting natural tannin along with the conservation of mangrove as a coastal protection and cultivated area for various marine lives, new resources of tannin are needed. Wooden handicraft, such as carvings, mortars and mobiles, is one of important products of Northern Thailand. Waste from wood scrap is a problem in the area. Villagers re-use them as mushroom cultivation medium and charcoal briquette. Tannin extracted from these wood scraps is one of an interesting way in reducing wooden waste. There are several studies on antimicrobial effects of tannin extracts from various plants such as rain tree pods [2], coconut mesocarp [3], guava leaves [4] or chestnut and quabracho [5]. A number of tannin extraction solvents and conditions have also been tested and reported.

Litchi (*Litchi chinensis* Sonn. cv. Hong Huay) is an economically important fruit of Thailand. Postharvest loss of the fruit is a big problem for both domestic and foreign markets. Postharvest deterioration of the fruit is mainly due to fungal decay. The high incidence of postharvest disease in litchi is primarily caused by green mold, *Penicillium digitatum*. The fungi preferentially penetrate through wounds that occur during harvesting and postharvest handling. Fungicide is widely used to reduce pathogenic fungi and extend shelf life of litchi fruits. However, it is becoming less effective because of pathogenic resistance, along with consumer concerns about possible risk associated with the use of chemicals. Among a number of new strategies being investigated to control postharvest decay, natural plant extract may be an alternative to traditional postharvest fungicide practices. The purposes of this research was to study the optimum condition for crude tannin extraction from wood scrap wastes and usage them as an antifungal agent for prolonging shelf life of litchi fruits.

2. MATERIALS AND METHODS

Wood scrap wastes was collected from Look village, Lampang province, Thailand in May, 2018. They were dried, cut into small pieces and milled to a fined powder. Tannin extraction was conducted with various solvents included distilled water, 95% ethanol, 8% sodium carbonate, 95% ethanol : distilled water (1:1 v/v) and 8% sodium carbonate : distilled water (1:1 v/v). The proportion between wood scrap per solvent were 1:10 or 1:20 $g.mL^{-1}$ and

extraction temperature were 70, 80 or 90°C. Each experiment was performed in triplicate.

Wood scrap and solvent at the tested ratio were added into a 1000-ml flask and then incubated in a water bath at the tested temperature with the shaking speed of 100 rpm for 2 hours.Crude extracted tannin from all treatments were then evaporated to dryness in hot air oven at 50°C to constant weight. Quantification of tannin was performed by spectrophotometer at 700 nm, serial dilutions of standard tannic acid were used to obtain the calibration curve [2].The basic chemical characteristics of the extracted tannin were tested comparing to standard tannic acid by the interaction with ferric chloride (1% $FeCl_3$), 1% gelatin, and lead acetate (1% $Pb(C_2H_3O_2)_2$) followed the methods of Moosophin et al. [6] and Elgailani and Ishak [7].

2.1 In Vitro and in Vivo Antifungal Activity Screening Test

A 7-day-old of *Penicillium digitatum* on potato dextrose agar (PDA) plate, obtained from the Department of Biology, Faculty of Science, Lampang Rajabhat University, Lampang, Thailand, was used for the study.

For the *in vitro* test, crude extracted tannin with the optimum extraction condition from the previous experiment was used. The crude extract was suspended in a newly prepared PDA medium, before the agar hardened, to make a concentration of 0, 0.1, 0 . 2 and 0.4 percent. Positive control was 0.1% benomyl. Mycelium discs of *P. digitatum* were cut with a sterile cork-borer (0.4 cm diameter) and each one was placed on the surface of a treatment PDA plate, 5 plates per treatment. All the dishes were then incubated at 25ºC. Mycelium diameter was measured after72 hours of the incubation

For the *in vivo* test, 'Hong Huay' litchi fruits that grown under standard cultural practices were harvested at commercial maturity stage from an orchard in Amphoe Mae-rim, Chiang Mai, Thailand in May, 2018. Before any commercial postharvest treatment was applied, the fruits were selected by hand and transported to the laboratory at Lampang Rajabhat University within 5 hours. The uniform and non-damage fruits were selected. The pedicels of the fruit were cut, 0.5 mm left intact on fruit, and divided into 5 groups. Crude extracted tannin was suspended in distilled water to make a concentration of 0, 0.1, 0.2 and 0.4 percent. Positive control was 0.1% benomyl fungicide. All the fruits were then soaked in the prepared tannin treatment suspensions or fungicide suspension for 20 minutes with softly shaking fruits in the suspensions every 2 minutes. After soaking, fruits were dried at 25ºC for 1 hour and then kept in foam tray, 10 fruits per tray, and wrapped with polyvinylchloride plastic. All treatments were stored at 15$\pm$1ºC, samples were taken every 2 days. Fruit samples in each treatment, three replicates of ten fruits, were inspected for disease incidence. Fruits showing white mycelium on the peels were considered as infected fruits. Disease incidence was expressed as percentage of infected fruits from three replicates of ten fruits. Shelf lives of the fruits were considered acceptable only if disease incidence was less than 50%.

2.2 Statistical Analysis

All analyses were run in triplicate or more. Data analysis was done by One-Way ANOVA and mean differences were analyzed by least significant differences at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Wood scrap waste samples of rain tree (*Samanea saman* (Jacq.) Merr.), from mortars handicraft, was collected from Look village, Lampang province, Thailand in May, 2018 as shown in Fig. 1.Woods of rain tree were left outdoor until making mortars and left a lot of wood scrap waste under the machine.

Fig. 1 Woods of rain tree and their scrap waste from mortars handicraft

Maceration method was used for tannin extraction in this study. It is a simple extraction method, done by immersing plant sample in an organic solvent. Organic solvents will penetrate the

cell wall into the cavity of the cell that contains the active substances so the active substances will dissolve [4]. In this study, the appropriate condition in extracting tannin from wood waste was 95% ethanol with a proportion of wood scrap per solvent at 1:10 or 1:20 g.mL^{-1}, 80 or 90°C extracted temperature, which gave the maximum amount of tannin (Table 1).The color of all crude extracts were present in Fig.2 which shown that the color of the extract were not accordance to tannin content. Various researchers tried to find an appropriate solvent in extracting tannin from plants. Various results of that were found, such as acetone for *Galium tunetanum* extraction [8], acetone-water mixtures for grape skin extraction [9], 8% sodium carbonate for *Pinus oocarpa* extraction [10], mixture of water and 95% ethanol for mangosteen peel extraction [6] and n-hexane for pods of rain tree extraction [2]. These different results may come from the different in plant species, part of the plants or conditions and methods of the extraction. In this study, we found that extraction ratio of wood scrap per solvent at 1:10 or 1:20 and extraction temperature at 80ºC or 90ºC gave the similar content of crude tannin (not statistically difference at P≤ 0.05). So, for an economically reason, the low solvent and temperature usage would be an ideal extraction procedure for feasible usage. As a reason, the ratio at 1:10 and temperature at 80ºC were suitable in tannin extraction in this study. Dried brownish crude extract from the optimum extraction condition was shown in Fig. 3.

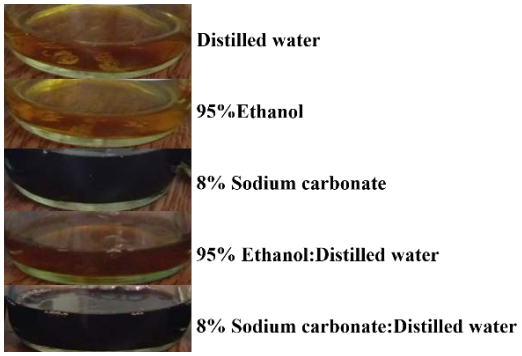

Fig. 2 Color of the crude extract after extraction with various solvents

Tannin extracted from wood scrap of rain tree with 95% ethanol at 80ºC extraction temperature and 1:10 g.mL^{-1} proportion of wood scrap per solvents was then tested for antifungal effect against mycelium disc of *P. digitatum*, which is an important pathogenic fungi of litchi fruits after harvest, and the ability for prolonged the storage life of litchi fruits at 15±1ºC. The basic chemical characteristics of the crude tannin extract in comparison with standard tannin were shown in Table 2. The ability to interact with metal ions such as $FeCl_3$ and $Pb(C_2H_3O_2)_2$ and the ability to precipitate protein such as gelatin in comparison to standard tannin indicated the presence of tannin in the crude extract as described by Moosophin, Wetthaisong, Seeratchakot and Kokluecha [6] and Elgailani and Ishak [7].

Table 1 Tannin content from wood scrap extracted with various conditions

Solvents	Extraction ratio (g.mL^{-1})	Extraction temperature (ºC)	Tannin content (mg. g^{-1})
Distilled water	1:10	70	2.05^{c}
		80	2.46^{c}
		90	2.54^{c}
	1:20	70	2.18^{c}
		80	2.62^{c}
		90	2.76^{c}
95% ethanol	1:10	70	7.26^{b}
		80	12.34^{a}
		90	12.55^{a}
	1:20	70	7.65^{b}
		80	12.12^{a}
		90	12.47^{a}
8% sodium carbonate	1:10	70	4.06^{b}
		80	5.11^{b}
		90	6.03^{b}
	1:20	70	4.51^{b}
		80	6.14^{b}
		90	7.03^{b}
95% ethanol: distilled water	1:10	70	5.11^{b}
		80	6.10^{b}
		90	6.55^{b}
	1:20	70	6.02^{b}
		80	6.54^{b}
		90	7.11^{b}
8% sodium carbonate: distilled water	1:10	70	2.06^{c}
		80	2.63^{c}
		90	2.65^{c}
	1:20	70	2.42^{c}
		80	2.47^{c}
		90	2.83^{c}

Fig.3 Crude extract of tannin extracted with 95% ethanol, 80°C at 1:10 g.mL^{-1}proportion

Table 2 Test of basic chemical characteristics of the extracted tannin from wood scrap in comparison with standard tannin

Tannin type	Chemical tests		
	1% $FeCl_3$	1% gelatin	1% $Pb(C_2H_3O_2)_2$
Standard tannic acid	Black precipitate	White precipitate	Dark red precipitate
Extracted tannin*	Greenish black precipitate	White precipitate	Dark red precipitate

Note:*Tannin was extracted with an optimum extraction condition (95% ethanol, 80°C and 1:10 $g.mL^{-1}$ proportion)

Tannin treatment *in vitro* resulted in significantly reduced mycelium disc diameter of *P. digitatum* when compared to control (Table 3 and Fig.4). The mycelium growth inhibition was numerically but not significantly ($P \leq 0.05$) enhanced with increasing tannin concentration from 0.1 to 0.4 percent. The mycelium diameters after growth on PDA with tannin at concentrations 0, 0.1, 0.2 and 0.4% for 72 hours were 9.00, 3.18, 2.68 and 2.66 cm, respectively. The results confirm with the findings of Mailoa, Mahendradatta, Laga and Djide [4] who found a significant inhibition of five different pathogen microbial growth after treated with tannin extracted from guava leaves with 30% ethanol. They estimated that tannin in an extract can inhibit the synthesis of 1,3 β-glucan, the main component of the fungal cell wall, by inhibiting the enzyme responsible for building that polymer. Although tannin treatment could obviously suppress the mycelium growth when compared to the control, but the efficacy was less than 0.1%benomyl fungicide. Hoque, Akanda, Miah, Bhuiyan, Miah and Begum [5] also found that Chestnut tannin and Quabracho tannin did not show satisfactory inhibition of mycelium growth of six fungal pathogens when compared to fungicides.

The incidence of disease from *in vivo* test on litchi fruit peels first appeared on the 8th day of storage at 15±1°C in all groups, except for the 0.2% tannin and 0.1%benomyl treatment (data not shown). Symptoms of fungal attack first appeared with white mycelium on fruit peel (which was judged as infected fruit). Severity increased with storage time with the development of mass white mycelia and powdery masses of olive-green spores. The disease incidence increased to 100, 100, 73.33, 100 and 46.66% in 0, 0.1, 0.2 and 0.4% tannin or 0.1% benomyl, respectively after 14 days of storage at 15±1°C (Table 4 and Fig. 5).

The 0.1% benomyl and 0.2% tannin treatments could delay the disease incidence after storage. Interestingly that litchi fruits in 0.4% tannin treatment had more severely disease incidence than the 0.2% treated fruits. The fruit peel damage from acidic suspension of 0.4% tannin may be the reason for this situation, because pH of the 0.4% tannin suspension were 3.21 (data not shown) which may destroy litchi fruit peel compared to the 0.2% tannin treatment which had higher pH value (4.56). Damage on fruit peel led the fungal easily penetrated through wounds and growth rapidly.

Although 0.2% tannin and fungicide treatment could delay disease incidence on litchi fruits, however, it should be noted that the treatments did not totally control the fruit decay at the end of storage period. The inability of tannin and fungicide to control final incidence of mold may be attributed to the growth of survival fungal spores after treatments or fungal spores in ambient air penetrated into natural micro-cracking on the fruit peel tissues as described by Underhill and Critchley [11].

Table 3 Mycelium disc diameter of *P. digitatum* after placed on PDA plate with various concentrations of tannin or 0.1% benomyl fungicide for 72 hours

Treatment	Diameter (cm)
Control	9.00 [a]
0.1% tannin	3.18 [b]
0.2% tannin	2.68 [b]
0.4% tannin	2.66 [b]
0.1% benomyl	0.00*[c]

Note: *The fungal was died after 48 hours of the incubation in PDA with benomyl fungicide

Table 4 Percent of disease incidence of litchi fruits after soaked in various concentrations of tannin or 0.1% benomyl fungicide and then kept at 15±1°C for 14 days

Treatment	Percent of disease incidence (%)
Control	100.00 [a]
0.1% tannin	100.00 [a]
0.2% tannin	73.33 [b]
0.4% tannin	100.00 [a]
0.1% benomyl	46.66 [c]

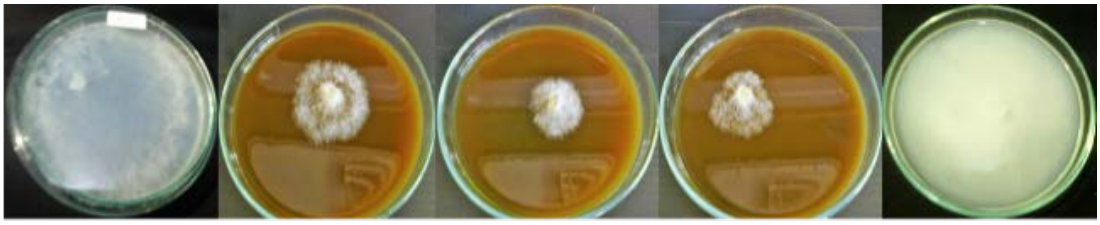

Fig. 4 Mycelium diameter of *P. digitatum* after placed on PDA plate with various concentrations of tannin or 0.1% benomyl fungicide for 72 hours

Fig.5 Disease incidence of litchi fruits after soaked in various concentrations of tannin or 0.1% benomyl fungicide and then kept at 15±1°C for 14 days

4. CONCLUSIONS

1. The optimum solvent to obtain tannin extract of wood scrap waste of rain tree was 95% ethanol with proportion of samples per solvent at 1:10 $g.mL^{-1}$ and 80°C extracted temperature, which gave the maximum amount of tannin at 12.34 $mg.g^{-1}$ dry weight
2. Tannin extract from wood scrap waste of rain tree had antifungal activity against *Penicillium digitatum* (*in vitro*) and could prolonged the storage life of litchi fruits

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