JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(3), 277-286

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Recycling of sawdust waste as biodegradable active gelatin films against *Aspergillus flavus,* a field-borne pathogen in garlics (*Allium sativum* Linn.)

Pornanan Boonkorn [1,*]

[1]*Faculty of Science, Lampang Rajabhat University, 52100, Lampang, Thailand*

ARTICLE INFO

Original Article

Article history:
Received 27 March 2024
Revised 15 May 2024
Accepted 18 May 2024

Keywords:
Biodegradable films
Garlic postharvest
Microwave-assisted extraction
Rain tree sawdust
Tannin extraction

DOI: 10.22077/jhpr.2024.7451.1371
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Faculty of Science, Lampang Rajabhat University, 52100, Lampang, Thailand.

Email: pornanan.b@g.lpru.ac.th

ABSTRACT

Purpose: Sawdust, a by-product of wood workplaces, poses environmental contamination and reduces workspace efficiency. This research aimed at recycling sawdust from rain tree by incorporating its extracts into gelatin films to create active films with antifungal properties against *Aspergillus flavus*. **Research method:** Sawdust was extracted by microwave with various solvents and electrical powers. The extract (0, 0.25, 0.5, 1, and 2%) were then tested for *A. flavus* inhibition. The extract was also incorporated with gelatin for making wrapped films and tested for inhibition potential on garlic inoculated with *A. flavus*. **Findings:** The optimal microwave extraction condition utilized a solvent mixture comprising distilled water and 95% ethanol in a 1:1 v/v ratio, applying 100 watts of electrical power for 30 seconds, repeated 5 times. This method yielded 23.26 mg/g of tannin. Furthermore, the 2% concentration of the extract significantly inhibited both mycelium growth and spore germination of *A. flavus* ($P \leq 0.05$) when tested on a petri dish. Additionally, incorporating 2% of the crude extract into gelatin film resulted in the most favorable outcome. This treatment demonstrated the capability to prolong the shelf life of wounded-inoculated garlic for more than 12 days. **Research limitations:** No limitations were found. **Originality/Value:** Sawdust originating from a rain tree can be recycled biodegrable actine gelatin films against *A. flavus*, a field-bomepathogen in garlic.

INTRODUCTION

The volume of sawdust from wood carving areas continues to increase due to a rise in demand for wood products. Ban Luk Tai Village is a wood handicraft village in Lampang province, the northern part of Thailand. Most of the villagers have a career in wood carving. Many items, like mortars and pestles, tables, home decoration accessories and wood games, are made from rain tree wood. The villager has not fully exploited the potential for recycling these waste materials, and therefore they are accumulating, causing less work space and starting to rot. Sustainable and applicable methods for enhancing sawdust usage must be looked for.

Several studies have shown that various parts of the rain tree have phytochemical substances that show antioxidant, antibacterial, insecticidal, antifungal and cytotoxic effects (Prasad et al. 2008; Ukoha et al., 2011; Vinodhini & Rajeswari, 2018; Boonkorn et al., 2020). Ukoha et al. (2011) suggest that ground pods of *S. saman* could be a significant source of natural antimicrobials and antifungals, such as tannins. The incorporation of extracted from rain tree sawdust with wrapping film is interesting because plastic films are highly concerned as they are harmful to the environment. Gelatin, a product made from animal protein, is a kind of edible-biodegradable polymer that has potential for the manufacturing of food packaging applications (Chen et al., 2019). Wang et al. (2017) added different concentrations of tannins to the gelatin solution and found that the tannin-gelatin film showed potential development value in the field of food packaging.

Garlic (*Allium sativum* L.) is classified within the Alliaceae botanical family and ranks as the second most commonly utilized *Allium* variety after onion (Amerian et al., 2024). Garlic serves as both a culinary ingredient and a medicinal herb (Ayed et al., 2019), commonly used in various Thai dishes. Presently, garlic sold in stores is often prepared by cutting into bulbs and packaging in mesh bags, which typically have a short shelf life due to being susceptible to destruction by the *A. flavus* fungus present on the garlic skin since cultivation. Therefore, peeling the garlic and packaging it in trays covered with easily biodegradable plastic, containing natural extract compounds capable of inhibiting the growth and germination of fungal spores, can be a method that helps extend shelf life for storage and distribution without the use of chemical substances.

For all the reasons mentioned above, the developed natural biodegradable films with rain tree extracts can release phytochemicals into the agricultural products or food surfaces. This can increase the stability of the products or prolonging the storage life of crops. It is also friendly to the environment. During this study, the microwave-assist extraction of sawdust waste was optimized, and the inhibition efficiency of field-borne pathogen in garlic, *A. flavus*, was investigated *in vitro*. The crude extract was also incorporated into the gelatin film to form an active gelatin film, and *in vivo* testing with garlic was performed.

MATERIALS AND METHODS

Microwave-assist extraction of the plant materials

Sawdust waste from rain tree (*Samanea saman* (Jacq.) Merr.) was obtained from Ban Luk Tai Wood Handicraft Village in Lampang province, Thailand. Dried, finely ground sawdust (10 g) was taken in a glass flask of approximately 250 ml capacity. A hundred ml of three extraction solvents, including distillate water, distillate water: 95% ethanol (1:1), or 95% ethanol was added and compared. The flasks were then subjected to microwave (Model MS20A3010A, Sumsung, Korea) and each of the three electrical power treatments was applied, including 100, 300, or 450 watts. Plant materials were irradiated in a microwave for

30 s through five consecutive extraction cycles. For each cycle, the extract was allowed to cool to room temperature for 2 min before the next cycle begin. The temperature of the finishing cycle was recorded and then the content of the glass flask was subjected to centrifugation for 10 min at 3000g at 4°C. The supernatant was dried at 50°C until weight stability, and then the crude extract was collected and weighted.

The basic chemical characteristics of tannin in the crude extracts were determined by the interaction with 1% Iron chloride ($FeCl_3$), 1%bovine serum albumin (BSA), and 1% lead acetate ($Pb(C_2H_3O_2)_2$) following the methods of Moosophin et al. (2010) and Elgailani and Ishak (2016). The presence of tannin in the extract made white precipitation with BSA, dark red precipitation with $Pb(C_2H_3O_2)_2$ and black precipitation with $FeCl_3$ which considered positive. Quantifying tannin in the crude extracts was performed using the Folin-Ciocalteu method, followed by the methods of Ukoha et al. (2011). Absorbance was measured with a UV/Visible spectrophotometer (Model Velocity 18R, Dynamica Scientific Ltd., UK) at 700 nm. The tannin content was expressed in terms of mg/g of tannic acid. All the determinations of tannin in the solutions were carried out in triplicate. The best extraction condition will be chosen for the inhibition efficiency test on the food-borne pathogen in garlic, *A. flavus*, both *in vitro* and *in vivo*

Inhibition efficiency test of crude extracts on *A. flavus* (*in vitro*)

A. flavus strain was provided by the microbial laboratory of the Science Faculty, Lampang Rajabhat University. The microbial were inoculated on potato dextrose agar (PDA) solid medium for 7 days at 25°C, and then their mycelium was cut and cultured on a newly prepared PDA petri-dish every 3 days for 21 days. To conduct the *in vitro* inhibitory test on mycelium growth, we followed the protocol of Masiello et al. (2019) with slight modifications. The crude extract was incorporated into warm liquid PDA medium to achieve concentrations of 0, 0.25, 0.5, 1, and 2% (w/v). As a positive control, we employed 1% benomyl fungicide in PDA. After solidification of the tested medium, the same age mycelium discs along the edge of *A. flavus* colony were cut with a sterile cork borer (0.4 cm diameter), and each one was placed on the surface of a PDA plate, 5 plates per treatment. The mycelium was incubated in dark at 25 °C for 7 days. Mycelium diameter was determined every day for 7 days. For the *in vitro* inhibitory test on spore germination, we followed the protocol of Hu et al. (2013) with slight modifications. Spores of *A. flavus* were washed from a 7-day-old *A. flavus* growth on a PDA plate and suspended in sterile distilled water to produce a final concentration of 1×10^6 spore's ml^{-1}. Twenty microliters of the spore suspension was poured and spread on the surface of the PDA plate containing crude extract of each treatment as described above, 5 plates per treatment. The number of colony-forming units (cfu) ml^{-1} was determined at 0, 24, 48, and 72 h after the incubation in dark at 25°C.

The incorporation of crude extracts into gelatin films

The active gelatin films were prepared by casting process as described by Etxabide et al. (2022) with slight modifications. A hundred ml of distilled water was mixed with 3.5 g of beef skin gelatin (Gelatin 160 Bloom, Australia) under continuous stirring at 2000 rpm, 50°C until completely dissolved. Then, 4 ml of glycerol was added and stirred for 5 min. Finally, the crude extract was added to the film solution to obtain the concentration of 0, 0.25, 0.5, 1, and 2%. From the preliminary study, adding crude extract of more than 2% caused obvious uneven porous on the film surface. For these reasons, 0-2% of the crude extract was chosen for this study. The positive control was 1% benomyl fungicide. The mixtures were stirred for 15 min to obtain a homogeneous film solution. The 10 mL of each film solution treatment

was then applied on a 170×120×5 mm glass plate and kept in a desiccator until dried. Film thickness was measured by a hand-held micrometer (RS PRO, Thailand).

Film color was measured by colorimeter (Model F50, FLUXANAR, Germany) and expressed as L* a* and b* values. The morphology of the film was examined by light microscope (Model CX22, OLYMPUS, Japan). For all determinations, five measurements were taken at random positions on each film sheet, 5 sheets per treatment. For testing the mechanical properties of the tested films, the tensile strength and elongation at break for each film were measured by using a digital push-pull gauge (HF-2 Model, ABALLTECHNO Co., Ltd., China). Ten specimen samples for each tested film were subjected to the analysis. The time to the decomposition of the film was also determining, all treated films were placed on dry soil in a ventilated room at room temperature (35±2°C).

Inhibition efficiency test of the gelatin films on *A. flavus* in garlics

Garlic bulbs (*Allium sativum*) grown under standard cultural practices were harvested at the commercial maturity stage from an orchard in Mae Hong Son, Thailand in March 2021. The uniform and non-damage bulbs were selected, and their peel was gently unwrapped, surface sterile with 75% ethanol, and dried for 30 min at room temperature. They were separated into 2 groups: wounded inoculated and non-wounded inoculated with *A. flavus* spores. For the wounded-inoculated group, the bulbs were artificially wounded with a sterile needle (1 mm depth), and then a droplet (5 µl) of the spore suspension at a concentration of 2.2×10^6 spores ml^{-1} was placed on that needle-made wounds and then dried for 30 min at room temperature.

The non-wounded inoculated group, a droplet of the spore suspensions was placed directly on the bulb surface. Five pieces of garlic bulb were placed in each paper tray, 3 trays per treatment, and then tightly covered with gelatin films with varying crude extract concentrations. All samples were stored at room temperature (35±2°C). The disease incidence was inspected every two days. Bulbs showing brownish spots on the surface with white mycelium and greenish spores were considered infected pieces.

Statistical analysis

The experimental setup was arranged in a completely randomized design (CRD). Data were subjected to two-way analysis of variance (ANOVA) using SPSS software (Trial version). Duncan's multiple range tests were used to determine significant differences between treatments at a 95% confidence interval.

RESULTS AND DISCUSSION

The microwave-assist extraction method was used in this study. This novel green technology is non-thermal, quick, give high extraction yield, low solvent and time consuming, do not use any hazardous chemicals, and ensures the stability of thermolabile components in contrast to other extraction technique (Bagade & Patil, 2021; Kayahan & Saloglu, 2021; Antony & Farid, 2022). Das et al. (2020) stated that tannin in plant materials can be extracted with water alone or water with other solvents, and several advanced technologies such as microwave or ultrasonication had shown to extract tannins efficiently.

From the study, the appropriate solvent for extracting sawdust was the mixture of distillate water: 95% ethanol (1:1) at electrical power of 100, 300, and 450 watts (Table 1). The condition gave statistically ($P\leq0.05$) higher extraction yield and tannin concentration than other conditions. No difference was found between the three electrical powers of the same solvent. All tested extraction conditions gave a final temperature of the extracts not more than 60 °C which is all in good ranges because at high temperatures, thermal degradation of

important substances in the plant materials may occur. From basic chemical tests of the crude extract, the interaction with metal ions such as $FeCl_3$ and $Pb(C_2H_3O_2)_2$ and the ability to precipitate BSA indicated the presence of tannin (Moosophin et al., 2010; Elgailani & Ishak, 2016).

The finding was in accordance with Moosophin et al. (2010) who found that a solvent mixture of water and 95% ethanol (1:1 v/v) gave the highest yield of tannin from mangosteen peel extract. Meanwhile, the yield of tannin reported in this study was about two times higher than reported by Boonkorn et al. (2020) who extracted tannin from rain tree sawdust by using 95% ethanol at 80°C. Das et al. (2020) report the success of various kinds of solvent used in tannin extraction from plants such as water, acetone, methanol, ethanol, sodium sulfite, and NaOH. The different results of extraction yield might be related to the processing parameters such as plant species, raw materials, particle size, temperature, and time.

For the next antifungal test of crude extracts against *A. flavus*, the appropriate condition to extract sawdust was used. As a result, solvents of distillate water: 95% ethanol (1:1) and electrical power at 100 watts were selected. Crude extract from rain tree resulted in significantly reduced mycelium disc diameter of *A. flavus* (Table 2). Increasing the concentration of the crude extracts in the PDA plate resulted in a significant reduction in the mycelium disc diameter of *A. flavus.* The concentration of 1 and 2% could obviously suppress, although could not totally inhibit, the mycelium growth when compared to the control, 0.25 and 0.5%. While 1% benomyl can completely inhibit the growth of mycelium as it is a highly effective fungicide.

Incorporated crude extract in the PDA plate also significantly inhibited the spore germination of *A. flavus* compared to the control. The number of colony-forming units of the fungi on the PDA plate was considerably reduced after exposures from 0.25 up to 2% of the crude extract. At 72 h after incubation, germinated spores were visually noticeable in the control, 0.25%, and 0.5% treatment with the recorded fungal concentrations of 8.82×10^5, 6.31×10^5, and 7.11×10^5 cfu ml^{-1}, respectively. Meanwhile, a significantly reduced of spore germination was found in the 1 and 2% treatments with the average value of 4.68×10^5 and 1.32×10^5 cfu ml^{-1}, respectively. The results were in accordance with various works that found highly significant antifungal activity from the rain tree extract (Prasad et al., 2008; Vinodhini & Rajeswari, 2018; Boonkorn et al., 2020).

Table 1. Percent yield, tannin content, and basic chemical tests of the extract from sawdust those extracted with various conditions.

Solvents	Electrical power (watts)	Final temperature (°C)	Percent yield (%)	Chemical tests			Tannin (mg g^{-1})
				1% $FeCl_3$	1% BSA	1% $Pb(C_2H_3O_2)_2$	
distillate water	100	38^{c}	1.12^{b}	+	+	+	10.78^{c}
	300	47^{bc}	1.40^{b}	+	+	+	19.29^{ab}
	450	51^{b}	1.27^{b}	+	+	+	20.19^{ab}
distillate water: 95% ethanol (1:1)	100	39^{c}	2.83^{a}	+	+	+	23.26^{a}
	300	49^{bc}	3.11^{a}	+	+	+	22.96^{a}
	450	58^{a}	3.17^{a}	+	+	+	23.50^{a}
95% ethanol	100	39^{c}	1.48^{b}	+	+	+	16.57^{b}
	300	54^{ab}	1.78^{b}	+	+	+	21.93^{ab}
	450	58^{a}	1.72^{b}	+	+	+	21.49^{ab}

Note: Different superscripts within the same column indicate statistically significant different values ($P \leq 0.05$), and the symbol "+" means "positive" in chemical tests (i.e. presence of tannin in the extract).

Germination of fungal spores is a vital step in the pathogenesis of *A. flavus* and any effect on their germination may have a corresponding effect on the disease severity. It appeared that direct contact of fungal spores to the PDA plate with crude extract of rain tree at high concentration (1-2%) could partly inhibit the fungal spore germination, possibly due to lethal action on the spores. The ability of tannins to disrupting the fungal cell membrane structures is well known. Two mechanisms of fungicidal effects by tannin were described by Carvalho *et al.* (2018) including (i) tannin binds to ergosterol in fungal membrane and then make pores in the structure or (ii) tannin inhibits enzymes involved in the ergosterol synthesis. Moreover, the tannin in the rain tree extracts can precipitate protein in the microbial cell, according to the ability to precipitate BSA protein as was shown in this experiment. Denature of the cell membranes and protein precipitating might be the reasons for the inhibition of mycelium growth and spore germination in *A. flavus* in this study.

Active gelatin films incorporated with various concentrations of sawdust crude extract were made by casting technique. The color measurement (L*, a* and b*) of the treated films was shown in Table 3. Higher concentrations of the crude extract tended to decrease L* and increased a* and b* values than lower concentrations when compared to control or benomyl treatments. This indicated that when the extract concentration ranging from 0.25 to 2% was used, the film was darker and more reddish and yellowish. These were most probably due to the brownish color of the sawdust extracts. Accordance to Peña et al. (2010) who found that gelatin films incorporated with tannin turned from light yellow to brownish color as tannin content increased.

Film thickness tended to increase with increasing the extracts or benomyl, but no statistical difference. The increasing solids content in the polymer matrix of the films enhances the film thickness layer (Said & Sarbon, 2022). Tensile strength values of the treated gelatin films were also examined, it was 19.36 MPa in non-treated gelatin films (0% crude extract) and was gradually decreased to 13.11 MPa by incorporating sawdust extracts up to 2%. The free volume of the film matrix weakens its structural stability, thus may be the reason for the lowering of the film's tensile strength. The elongation at break values of the treated films also decreased with an increase in the sawdust extract concentration, reflecting the decreased flexibility and elongation potential of the films. The gelatin films in this study showed higher values of tensile strength and elongation at break than those reported by Jirukkakul (2022). The difference in gelatin source and other ingredients that corporations use in the films may be the reasons.

Table 2. Inhibition efficiency on *A. flavus* of the crude extracts from rain tree sawdust at the various concentrations compared to benomyl.

Treatment	Mycelium diameter (cm)	Spore germination ($\times 10^5$ cfu ml^{-1})
0%	7.60^{c}	8.82^{a}
benomyl	0.00^{d}	0.00^{e}
0.25%	5.80^{a}	6.31^{b}
0.50%	5.58^{a}	7.11^{b}
1%	3.60^{ab}	4.68^{c}
2%	3.30^{b}	1.32^{d}

Note: Different superscripts within the same column indicate statistically significant different values ($P \leq 0.05$).

Table 3. Property determinations of gelatin films incorporated with various concentrations of the crude extracts or benomyl.

Treatment	Color measurements			Thickness (mm)	Mechanical properties	
	L*	a*	b*		Tensile strength (Mpa)	Elongation at break (%)
0%	91.85^{a}	-0.83^{d}	5.11^{d}	0.04^{a}	19.36^{a}	105.20^{a}
Benomyl	85.03^{c}	-0.36^{c}	7.10^{b}	0.08^{a}	10.15^{c}	58.77^{b}
0.25%	90.51^{a}	0.04^{b}	5.50^{cd}	0.04^{a}	15.23^{b}	82.80^{ab}
0.50%	90.18^{ab}	0.05^{b}	5.88^{bcd}	0.04^{a}	15.88^{b}	83.13^{ab}
1%	89.87^{ab}	0.31^{b}	6.81^{bc}	0.06^{a}	14.32^{b}	83.02^{ab}
2%	88.03^{b}	1.08^{a}	10.34^{a}	0.07^{a}	13.11^{b}	61.57^{b}

Note: Different superscripts within the same column indicate statistically significant different values ($P \leq 0.05$).

The surface of the active gelatin films incorporated with varying concentrations of the extract was evaluated using a transmitted light microscope, which demonstrated a uniformity matrix only in the control. Increasing the extracts or benomyl showed partial granules in the film matrix with a particle size of approximately 10–50 μm. The small granules in the film matrix lead to a decrease in tensile strength and elongation at break values, as mentioned above. The incomplete dissolution of the materials in the gelatin film matrix was found, in accordance with the finding of Hanani et al. (2019) who found the same characteristics of the films when pomegranate peel was incorporated into gelatin films.

After wounded inoculating garlic with *A. flavus* and then wrapped with the tested films, disease incidence on garlic first appeared on the fifth day of storage at 35±2°C. Symptom of the fungal attack was first showed brownish spots on the flesh with white mycelium (which was judged as "infected"). A longer storage period increased the development of white mycelium and powdery masses of green spores. Incorporated extracts in the wrapped films significantly reduced disease incidence in garlic, with the impacts of the treatment depending on the extract concentrations (Table 4). The incidence of disease on wounded inoculated garlic contacted to 2% crude extract or benomyl films was significantly lower than on garlic exposed to 0, 0.25, 0.5, or 1%.

At twelve days after storage, the wounded inoculated garlic exposed to treated gelatin films at the concentrations 0, 0.25, 0.5, 1, and 2 % had disease incidence of 100, 100, 86.67, 80, and 33.33%, respectively. The benomyl film also could not totally inhibit the fungal spore germination, with 6.67% of disease incidence on the 12th day of storage. The inability of 2% crude extract and fungicide to control the incidence of *A. flavus* may be attributed to the growth of fungal spores that growth inside the flesh of garlic tissues, which were not directly in contact with the tested films. The non-wounded inoculated group stored at the same temperature, on the other hand, they did not show any disease incidence throughout 12 days. The reason may be that *A. flavus* is a fungal pathogen that only attacks plants or tissues that have been damaged by various stresses, like artificial wounds in this study. They do not attack healthy tissues.

As a whole, the study showed the efficiency of gelatin films incorporated with 2% crude extracts from rain tree sawdust in retarding the growth of *A. flavus*, thus resulting in prolonged storage life of garlic. Gelatin film has been proven to increase the shelf life of fresh products by delaying the microbial spoilage and providing moisture and gas barrier properties (Said & Sarbon, 2022; Moradinezhad & Ranjbar, 2023). The addition of active ingredients into the gelatin films to maintain the quality and lengthen the shelf life of food, the films must have the ability to capture and release active ingredients into the products or the environment. Wang et al. (2017) added different concentrations of tannins to the gelatin solution by the casting method and their analysis showed a physical crosslinking effect between tannin and

gelatin, dominated by a hydrogen bond and hydrophobic bond. This finding proved that the incorporation of plant tannin into gelatin is practical. The hygroscopic nature of gelatin film allowed closed attachment between the film and the product's surface, active ingredients inside the film could be slowly released from the film to the moist surface of the products. Etxabide et al. (2022) incorporated grape tannin extracts into active gelatin films and discovered that the films may release tannins by up to 20%, resulting in antioxidant inhibition levels of up to 13%. According to Valdés et al. (2020), the active gelatin film's ability to limit microbial growth cannot be attributed to a single mechanism, but rather to a variety of reactions that take place on the entire microbial cell.

In the decomposition test of the film, at 36±2 °C and 41% RH, the 0% film treatment started to decompose within a week. The 0.25, 0.5, and 1% treatments were followed by a decomposition process that began within 2-3 weeks. Said and Sarbon (2022) stated that the biodegradation rate of single gelatin films was 18-25 % after 3 days. The higher moisture makes the films become more susceptible to degradation towards microorganism attacks. The incorporation of additional substances in the film matrix will lower moisture content, thus lowering the biodegradation rates. After 1 month of the degradation test, the stable structure of the film was found in the benomyl and the 2% treatment; both treatments started a little bit distorted but still degraded less than 25% (Fig. 1). As a result, the 2% film treatment could be naturally decomposed and had a lifetime of more than 1 month, and after that, it started to gradually deformation. In this sense, the treated films in this work can degrade naturally through biodegradation. The potential use of gelatin films as substitute materials for packaging applications is highlighted by their excellent functional qualities and favorable environmental impact (Etxabide et al., 2016).

Table 4. Percent of disease incidence on wounded inoculating garlic with *A. flavus* after being wrapped by gelatin films incorporated with various concentrations of crude extracts from rain tree sawdust or benomyl and then kept at 35±2°C for 12 days.

Treatment	Percent of disease incidence (%)
0%	100.00^{a}
benomyl	6.67^{d}
0.25%	100.00^{a}
0.50%	86.67^{ab}
1 %	80.00^{b}
2%	33.33^{c}

Different superscripts within the same column indicate statistically significant different values ($P \leq 0.05$).

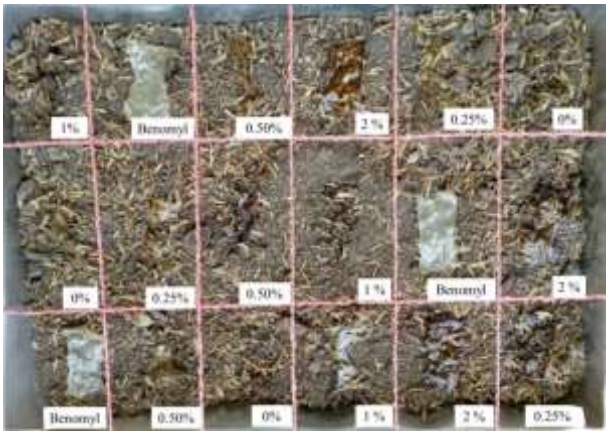

Fig. 1. The appearance of the gelatin films incorporated with various concentrations of crude extracts from rain tree sawdust, after 1 month of the degradation test at 35±2°C, 41% RH.
Note: The tested films with three replications were arranged randomly, each row had all treatments.

CONCLUSION

In conclusion, the optimum condition in extracting the sawdust from the raintree was 100 watts of the microwave with the mixed solvent of distilled water: 95% ethanol (1:1 v/v) which gave the highest yield of extract and tannin content. The extracts could inhibit mycelium growth and spore germination of *A. flavus*, especially at 2% of the concentration used. The incorporated 2% extracts into gelatin films also prolonged the storage life of wounded inoculating garlic with *A. flavus* for more than 12 days. All treated films in this study could be naturally gradual decomposed within a month.

Conflict of interest

The author has no conflict of interest to report.

Acknowledgements

I would like to thank the Faculty of Sciences, Lampang Rajabhat University, Lampang, Thailand, for grant and generous support. In addition, all villagers in Luk Tai village are gratefully acknowledged for providing research sites and sawdust wastes for the study. Thanks to Chitnarong Sirisathitkul and Yaowarat Sirisathitkul for their helpful suggestions.

REFERENCES

Amerian, M., Khoramivafa, M., Palangi, A., Gohari, G., & Ntatsi, G. (2024). The effect of nitrogen and selenium on some phytochemical characteristics and allicin of garlic leaf. *Journal of Horticulture and Postharvest Research*, *7*(Special Issue - Postharvest Technologies), 77-92. https://doi.org 10.22077/jhpr.2024.7162.1354

Antony, A., & Farid, M. (2022). Effect of temperatures on polyphenols during extraction. *Applied Sciences, 12*(4), 1-14. https://doi.org/10.3390/app12042107

Ayed, C., Mezghani, N., Rhimi, A., & AL Mohandes Dridi, B. (2019). Morphological evaluation of Tunisian garlic (*Allium sativum* L.) landraces for growth and yield traits. *Journal of Horticulture and Postharvest Research*, *2*(Issue 1), 43-52. https://doi.org/10.22077/jhpr.2018.1838.1033

Bagade, S. B., & Patil, M. (2021). Recent advances in microwave assisted extraction of bioactive compounds from complex herbal samples: A Review. *Critical Reviews in Analytical Chemistry, 51*(2), 138-149. https://doi.org/10.1080/10408347.2019.1686966

Boonkorn, P., Chuajedton, A., & Karuehanon, W. (2020). The crude tannin extraction from wood scrap wastes for prolonging the shelf life of litchi fruits. *International Journal of GEOMATE, 18*(67), 208-213. https://doi.org/10.21660/2020.67.5562

Carvalho, R. S., Carollo, C. A., de Magalhães, J. C., Palumbo, J. M. C., Boaretto, A. G., Nunes e Sá, I. C., Ferraz, A. C., Lima, W. G., de Siqueira, J. M., & Ferreira, J. M. S. (2018). Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from *Cochlospermum regium* (mart. Et. Schr.) Pilger roots: Mechanisms of action and synergism with tannin and gallic acid. *South African Journal of Botany*, 114, 181-187. https://doi.org/10.1016/j.sajb.2017.11.010

Chen, H., Chen, H., Wang, J., Cheng, Y., Wang, C., Liu, H., Bian, H., Pan, Y., Sun, J., & Han, W. (2019). Application of protein-based films and coatings for food packaging: A review. *Polymers*, *11*(12), 1-32. https://doi.org/10.3390/polym11122039

Das, A. K., Islamb, Md. N., Faruk, Md. O., Ashaduzzaman, Md., & Dungani, R. (2020). Review on tannins: Extraction processes, applications and possibilities. *South African Journal of Botany*, 135, 58-70. https://doi.org/10.1016/j.sajb.2020.08.008

Elgailani, I. E. H., & Ishak, C. Y. (2016). Methods for extraction and characterization of tannins from some acacia species of Sudan. *Pakistan Journal of Analytical and Environmental Chemistry, 17*(1), 43-49. http://dx.doi.org/10.21743/pjaec/2016.06.007

Etxabide, A., Leceta, I., Cabezudo, S. Guerrero, P., & de la Caba, K. (2016). Sustainable fish gelatin films: From food processing waste to compost. *ACS Sustainable Chemistry and Engineering*, *4*(9), 4626-4634. https://doi.org/10.1021/acssuschemeng.6b00750

Etxabide, A., Yang, Y., Mate, J. I., de la Caba, K., & Kilmartin, P. A. (2022). Developing active and intelligent films through the incorporation of grape skin and seed tannin extracts into gelatin. *Food Packaging and Shelf Life*, 33, 1-12. https://doi.org/10.1016/j.fpsl.2022.100896

Hanani, Z. A. N., Yee, F. C., & Nor-Khaizura, M. A. R. (2019). Effect of pomegranate (*Punica granatum* L.) peel powder on the antioxidant and antimicrobial properties of fish gelatin films as active packaging. *Food Hydrocolloids*, 89, 253-259. https://doi.org/10.1016/j.foodhyd.2018.10.007

Hu, X. G., Liu, L., Hu, K., Yang, X. L. & Wang, G. X. (2013). *In Vitro* screening of fungicidal chemicals for antifungal activity against *Saprolegnia*. *Journal of the World Aquaculture Society*, *44*, 528-535. https://doi.org/10.1111/jwas.12052

Jirukkakul, N. (2022). Physical and antioxidant properties of gelatine film added with sesame, rice bran, and coconut oil. *International Food Research Journal*, *29*(5), 1020-1031. https://doi.org/10.47836/ifrj.29.5.05

Kayahan, S., & Saloglu, D. (2021). Microwave-assisted extraction of antioxidant phenolic compounds from artichoke (*Cynara scolymus* L. cv Bayrampasa): optimisation and kinetic modeling. *International Food Research Journal*, *28*(4), 704-715. http://dx.doi.org/10.47836/ifrj.28.4.07

Masiello, M., Somma, S., Ghionna, V., Logrieco, A. F. & Moretti, A. (2019). *In Vitro* and in field response of different fungicides against *Aspergillus flavus* and *Fusarium* species causing ear rot disease of maize. *Toxins*, *11*(11), 1-18. http://dx.doi.org/10.3390/toxins11010011

Moosophin, K., Wetthaisong, T., Seeratchakot, L., & Kokluecha, W. (2010). Tannin extraction from mangosteen peel for protein precipitation in wine. *KKU Research Journal*, *15*(5), 377-385.

Moradinezhad, F., & Ranjbar, A. (2023). Advances in postharvest diseases management of fruits and vegetables: A review. *Horticulturae*, *9*(10), 1099. https://doi.org/10.3390/horticulturae9101099

Prasad, R. N., Viswanathan, S., Devi J. R., Nayak, V., Swetha, V. C., Archana, B. R., Parathasarathy, N., & Rajkumar, J. (2008). Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. *Journal of Medicinal Plants Research*, *2*(10), 268-270. https://doi.org/10.5897/JMPR.9001040

Peña, C., de la Caba, K., Eceiza, A., Ruseckaite, R., & Mondragon, I. (2010). Enhancing water repellence and mechanical properties of gelatin films by tannin addition. *Bioresource Technology*, *101*(17), 6836-6842. https://doi.org/10.1016/j.biortech.2010.03.112

Said, N. S., & Sarbon, N. M. (2022). Physical and mechanical characteristics of gelatin-based films as a potential food packaging material: A Review. *Membranes*, *12*(5), 442. https://doi.org/10.3390/membranes12050442

Ukoha, P. O., Cemaluk, E. A. C., Nnamdi, O. L., & Madus E. P. (2011). Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. *African Journal of Pure and Applied Chemistry*, *5*(8), 237-244. https://dx.doi.org/10.2139/ssrn.3965366

Valdés, A., Garcia-Serna, E., Martínez-Abad, A., Vilaplana, F., Jimenez, A., & Garrigós, M. C. (2020). Gelatin-based antimicrobial films incorporating pomegranate (*Punica granatum* L.) seed juice by-product. *Molecules*, *25*(1), 1-20. https://doi.org/10.3390/molecules25010166

Vinodhini, S., & Rajeswari V. D. (2018). Review on ethnomedical uses, pharmacological activity and phytochemical constituents of *Samanea saman* (jacq.) Merr. rain tree. *Pharmacognosy Journal*, *10*(2), 202-209. https://doi.org/10.5530/pj.2018.2.35

Wang, K., Wang, W. H., Zhang, Y., & Liu, A. J. (2017). Impact of tannin on the properties of gelatin edible film. *Modern Food Science and Technology*, *33*(3), 251-256. http://doi.org/10.13982/j.mfst.1673-9078.2017.3.038