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Research article

Antimicrobial activities of *Aloe vera* rind extracts against plant pathogenic bacteria and fungi

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Abstract

Leaves of *Aloe vera* contain useful substances. *Aloe vera* gel is used as a component of products, but the rind is discarded as waste despite having more phenol and flavonoid than the gel. The rind has substances with desirable functions, especially antimicrobial and antioxidant activities. The antimicrobial activities were examined of rind extracts against plant pathogenic bacteria: *Ralstonia solanacearum* and *Xanthomonas axonopodi* pv. *citri* and fungi: *Colletotrichum gloeosporioides* and *Fusarium oxysporum*. The antimicrobial activities of methanol, ethyl acetate and n-hexane of the rind extracts were tested against these bacteria and fungi. Values were determined for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC values of the methanol extract against *R. solanacearum* and *X. axonopodis* pv. *citri* were 25 mg/mL and 62.5 mg/mL, respectively, and the MBC values were 50 mg/mL and 125 mg/mL, respectively. The inhibition zones against the two bacteria were positively correlated with the concentration. At 15 mg/mL and 25 mg/mL, the methanol extracts had mean (± SD) values of mycelial growth inhibition against *F. oxysporum* of 30.85±1.46% and 54.70±1.64%, respectively, and 50.95±0.90% and 62.39±1.08%, respectively, against *C. gloeosporioides*. The inhibition percentage of mycelial growth increased with increasing concentration. The extracts had higher activity against *C. gloeosporioides* than *F. oxysporum.* At the same concentration, the methanol extract had the highest inhibition percentage of mycelial growth, followed by ethyl acetate and n-hexane. The results indicated that rind extracts could be utilized as phytochemical products to inhibit certain bacteria and fungi.

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Introduction

Aloe vera (L.) Burm. f. (*Aloe barbadensis* Miller) is a succulent, evergreen perennial medicinal plant belonging to the Asphodelaceae family that has been widely cultivated in tropical and subtropical climates (Añibarro-Ortega et al., 2019). The outer thick green layer is called the rind or leaf skin which has a protective role and synthesizes carbohydrates and proteins (Kar and Bera, 2018). *Aloe vera* has long been used in traditional medicine for preventing diseases and therapeutic use, with many studies revealing various pharmacological activities and therapeutic effects ascribed to *Aloe* species, such as anti-inflammatory, antimicrobial, wound healing, anti-tumor and antioxidant properties (Sharma et al., 2014; Kar and Bera, 2018). *Aloe vera* has been most cultivated in Prachuap Khiri Khan province, the West Region of Thailand (Prachuap Khiri Khan Provincial Agriculture and Cooperatives Office, 2021). At present, *Aloe vera* processing products are very popular, with it being used in many products such as fresh gel, food, pharmaceutical uses, cosmetics and toiletries (Ahlawat and Khatkar, 2011). *Aloe vera* rind, which weigh about 40% of the leaf weight (Añibarro-Ortega et al., 2019), is discarded as industrial waste and the expansion of the production of herbal processed products has resulted in increased quantities of agricultural waste every year. If these agricultural waste and residues are released to the environment without proper waste management procedures, they may cause environmental pollution and be harmful to human health. In addition, the management of these wastes may be costly. Studies conducted on fruit and vegetable waste have indicated it can be used to produce value-added compounds such as secondary metabolites, biopesticides, carbohydrates, phenolic compounds, flavonoids and carotenoids (Wadhwa et al., 2015; Kumar et al., 2020). Like *Aloe vera* rind, several kinds of fruit and peels are a major source of bioactive compounds which have potent antimicrobial and antibiotic properties (Vua et al., 2018), especially fruit peels such as pomegranate peels, lemon peels and banana peels that can be used as a source of antimicrobial agents (Mokbel and Hashinaga, 2005; Parashar et al., 2014). These agricultural wastes are mainly used as raw materials in pharmaceutical industries to manufacture value-added products (Saleem and Saeed, 2020).

The rind of the *Aloe vera* has a higher composition of various beneficial value-added compounds such as phenol and flavonoid than in the gel (Vidic et al., 2014). These most common substances include catechin, sinapic acid, ercetin, quercitrin, rutin, miricetin and epicatechin, which have antioxidant and antimicrobial activities (López et al., 2013; Skroza et al., 2019). Kwon et al. (2011) reported that *Aloe vera* rind extract in distilled water had an excellent antimicrobial effect against *Escherichia coli* and *Vibrio* spp. The zinc oxide nanoparticles from aqueous rind extract of *Aloe vera* had an antibacterial effect against *E. coli* (MTCC-41) and *Aspergillus niger* (MTCC-404) (Chaudhary et al., 2019). The pulp of *Aloe vera* had an inhibitory effect against *F. oxysporum* (Sahu et al., 2013). The liquid fraction reduced the rate of colony growth against *Rhizoctonia solani*, *F. oxysporum* and *Colletotrichum coccodes* (Sahu et al., 2013). In addition, the activities of methanol, ethyl acetate and hexane extracts of *Aloe vera* leaves against *Colletotrichum gloeosporioides, Colletotrichum capsici* and *Fusarium solani* were evaluated using a poisoned food technique. The extracts displayed higher activity against *Colletotrichum* species than *F. solani* (Nidiry et al., 2011). These pathogens can generate diseases and decrease both the quantity and quality of agricultural products, including the death of plants. *Aloe vera* rind is considered agricultural waste and is dumped into the environment instead of being used as antimicrobial agents or for pharmaceutical purposes. The *Aloe vera* rind and leaf extracts had antimicrobial activities against various plant pathogens such as *R solanacearum*, *X. axonopodis*, *C. gloeosporioides* and *F. oxysporum.* (Nidiry et al., 2011; Sahu et al., 2013) *R. solanacearum* generates bacterial wilt that is one of the most important and widespread bacterial diseases that devastates many economically important crops in Thailand such as ginger, pepper, tomato and potato (Bhunchoth et al., 2015). *R. solanacearum* is presumably the most destructive plant pathogenic bacterium (Genin, 2010; Kumar et al., 2017) and *X. axonopodis* pv. *citri* causes citrus canker, being considered one of the most serious bacterial diseases of all citrus varieties (Ference et al., 2018). Symptoms include necrotic lesions on fruit, leaves and stems and the infected fruits have low quality, causing the price to fall (Graham et al., 2004). Fungi are important pathogens of plants and cause more significant yield losses than bacteria or viruses (Sexton and Howlett, 2006). Symptoms of a disease caused by *C. gloeosporioides* are generally known as anthracnose. Outbreaks of this disease damage many crops in Thailand, which significantly reduces the quality and quantity of the crops products (Bordoh et al., 2020; Kongtragoul et al., 2020). *F. oxysporum* is a pervasive soil-borne pathogen that causes vascular wilt on a wide range of plants (Nefzi et al., 2016). It causes small wounds or natural openings in the roots, followed by discoloration and stem necrosis, causing blockage of xylem and the plant death (Chougule and Andoji, 2016).

The most effective method to protect crop plants against pathogens is chemical control. However, the unsuitable use of chemicals for a long time may cause resistance by pathogens. In addition, synthetic chemicals can be hazardous to human health and pollute the environment (Namsena et al., 2019). Consequently, restrictions on the use of chemical have been increasing and safe alternative control methods are necessary. Secondary metabolites in plants have several substances that are potent antimicrobrial agents and can protect the plant from pathogen damage (Gorlenko et al., 2020). The antimicrobial activities of plant extracts are environmentally safe, and the extracts can be degraded by natural soil microbes (Zaker, 2016). Thus, *Aloe vera* rind extracts could be a safe alternative way because the extracts contain potent biological substances, especially ones that are antibacterial and antifungal. Utilization of agro-industrial waste as raw material for extraction of active compound can reduce the waste from the industry. Additionally, the main benefits of using natural extracts are that they are safe for health and do not harm the environment, as well as reducing the budget to control pathogens. Since *R. solanacearum*, *X. axonopodis* pv. *citri, C. gloeosporioides* and *F. oxysporum can* cause extreme crop damage leading to economic loss, these bacteria and fungi were chosen in this study. Thus, the purpose of this research was to examine the antimicrobial activities of *Aloe vera* rind extracts against several plant pathogenic bacteria: *R. solanacearum* and *X. axonopodi* pv. c*itri* and fungi: *C. gloeosporioides* and *F. oxysporum*.

Materials and Methods

Location of Aloe vera cultivation in Thailand

Mature leaves of *Aloe vera* were collected from Pranburee district, Prachuap Khiri Khan province, Thailand in November, 2020. Prachuap Khiri Khan province is located in the southernmost area of the West Region. It has a latitudinal range of 10°54′N-12°39′N and a longitudinal range of 99°06′E-100°02′E. Almost all areas of the province are dry and have less soil-water in the dry season than other provinces in the same tropical savanna (tropical wet-dry) climate (*Aw*). The highest mean annual temperature is 39.4°C and the lowest mean annual temperature is 17.5°C (Prachuap Khiri Khan Provincial Agriculture and Cooperatives Office, 2021).

Plant material

The *Aloe vera* leaves were washed with tap water, followed by distilled water to remove dirt. Then, the surface was sterilized by soaking in 2% sodium hypochlorite solution for 30 min followed by rinsing with distilled water. The leaves were kept at room temperature until completely dried. The rind was peeled off and cut into small pieces that were dried in a hot air oven at 42°C for 72 hr. Then, the dried rind was ground to a fine powder and stored at 4°C prior to solvent extraction.

Preparation of plant crude extracts

Samples (20 g) of dried ground *Aloe vera* rind were extracted separately into 200 mL of methanol, ethyl acetate or n-hexane in a shaker at 150 revolutions per minute for 4 d at room temperature. The extracts were passed through Whatman No.1 filter paper and concentrated using a rotary evaporator. The concentrated extracts were lyophilized and weighed to calculate the yield using Equation 1:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of completely dried extract (g)}}{\text{Dry weight of } \textit{Aloe vera} \text{ rind powder (g)}} \times 100 \quad (1)$$

Test microorganisms

Strains of plant pathogenic bacteria and fungi were purchased from the Plant Protection Research and Development Office, Department of Agriculture, Thailand. Antibacterial activities were determined against two Gram negative phytopathogenic bacteria: *Ralstonia solanacearum* (DOA-BC1954) and *Xanthomonas axonopodis* pv. c*itri* (DOA-BC902). The phytopathogenic fungi selected for antifungal assay were *Colletotrichum gloeosporioides* (DOAC2537) and *Fusarium oxysporum* (DOAC2614). The bacteria were maintained on nutrient agar plates and the fungi on potato dextrose agar plates at 4°C until needed.

Antibacterial activity assay

Antibacterial test using agarwell diffusion method

The antibacterial activities of the methanol, ethyl acetate and n-hexane extracts were tested using agar well diffusion assay (Güven et al., 2005) with some modifications. Samples (20 mL) of sterilized nutrient agar (45°C) were poured into 9cm sterile Petri dishes and allowed to solidify. The plant pathogenic bacteria used in the test were *R. solanacearum* and *X. axonopodis* pv. *citri*. The bacteria were cultured on nutrient broth for 24 hr at 30°C; then, they were adjusted to 0.5 McFarland standard (1.5×10^8 colony forming units (CFU)/mL)

using 0.85% sterile NaCl. A 100 µL sample of nutrient broth culture of each inoculum was spread on nutrient agar plates. Wells (6 mm diameter) were punched in the agar plate using a sterile stainless steel cork borer and filled with 50 µL of one of the extracts at concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL and 25 mg/mL. Each concentration was filled in a well separately. The positive control was ampicillin (13.1 µg/disc) and the negative control was dimethylsulfoxide (DMSO). The plates were incubated at 30°C for 24 hr. The diameters of inhibition zones were measured in millimeters and expressed as mean ± SD values. The experiment was carried out in triplicate.

Determination of minimum inhibitory concentration of effective Aloe vera rind extracts

The effectiveness of the *Aloe vera* rind extracts on *R. solanacearum* and *X. axonopodis* pv. *citri* was analyzed by measuring the minimum inhibitory concentration (MIC) defined as the lowest concentration of an extract that inhibits the bacterial growth after 18−24 hr. The extracts providing a strongly positive result for the agar well diffusion method were used to determine MIC values. In this study, the MIC for the active *Aloe vera* rind extracts was performed using the broth microdilution method of Nayak et al. (2015) with some modifications. The stock solution of *Aloe vera* rind extract was prepared by dissolving in DMSO to a concentration of 100 mg/mL. Each bacterial suspension was standardized to a 0.5 McFarland standard. Concisely, 100 µL of Muller Hinton Broth (MHB) was added to sterile 96-well microplates. Then 100 µL of the extract was added to the first well and two-fold serial dilution was carried out. The concentrations of extracts used for the MIC analysis were 25 mg/mL, 50 mg/mL, 62.5 mg/mL, 100 mg/mL, 125 mg/mL and 250 mg/mL. Specifically, 100 µL of bacterial strains (1.5×10^8 CFU/mL) was added to each well. A negative control was prepared using DMSO while ampicillin (1 mg/mL) was used as a positive control. The microplate was incubated for 24 hr at 30°C. After incubating, 5 µL of 2,3,5-triphenyltetrazolium chloride (TTC) solution (0.5%) was added to each well of the microplate and incubated at 30°C for 4 hr. TTC is a colorless substance and the TTC method is considered a relatively fast method to examine the antibacterial activities of antimicrobial agents as the lowest concentration of the extract that does not produce a red color can be determined as the MIC.

Determination of minimum bactericidal concentration of effective Aloe vera rind extracts

To examine the MBC, 100 µL of the culture from each well of the broth micro-dilution assay after 24 hr of incubation was subcultured on Muller Hinton agar (MHA) plates. The MHA plates were further incubated at 30°C for 24 hr. The lowest concentration of crude extract without any bacterial growth on the agar plate was regarded as the MBC value. Each experiment was repeated three times.

Antifungal activity assay

C. gloeosporioides and *F. oxysporum* were grown on potato dextrose agar (PDA) medium and incubated at 25±2°C for 7 d before being used for antifungal bioassays. The antifungal efficacy of the *Aloe vera* rind extracts was examined using the poisoned food technique. Each extract was thoroughly mixed with the molten PDA medium and warmed to 45−50°C. Then, the extracts that had concentrations of 15 mg/mL or 25 mg/mL were poured into sterilized Petri dishes (9 cm in diameter) with a volume of 20 mL/dish. The fungicide Mancozeb at 1 mg/mL or 3 mg/mL concentration in the PDA medium was used as a positive control. A PDA medium without the extract was used as a negative control. A 6-mm diameter inoculum disc was punched out from the actively growing fungal mycelia using a sterilized cork borer. The discs were inoculated at the center of the Petri dishes and were incubated at 25±2°C for 7 d.

After incubation, the mycelial growth of phytopathogenic fungi was measured using a set of Vernier calipers and recorded in millimeters. Each experiment was performed in triplicate. The inhibition percentage of mycelial growth was calculated using Equation 2:

$$\text{Inhibition percentage of mycelial growth} = \frac{(R1 - R2)}{R1} \times 100 \quad (2)$$

where R1 = the average diameter (mm) of mycelial growth in control plate

R2 = the average diameter (mm) of mycelial growth in treatment plate

Statistical analysis

The results of the experiments were expressed as mean±SD values. All data were obtained in triplicate and analyzed using one-way analysis of variance in the IBM SPSS Statistics for Windows software package (IBM Corp. Released 2019. version 26.0. Armonk, NY: IBM Corp.). Values were considered

statistically significant at $p < 0.05$. Correlation analysis was carried out using the Pearson-product moment correlation.

Results

Yield of plant extracts

The yields of crude extracts from *Aloe vera* are shown in Table 1. The results showed that the methanol extraction of *Aloe vera* rind powder produced the highest amount of crude extract based on grams/100 g of dried *Aloe vera* (12.08%), followed by ethyl acetate extract (5.58%), and the lowest yield was n-hexane extract (3.17%). Significant differences were observed among these extraction yields.

Table 1 Extraction yields (percentage in grams of *Alo vera* rind; $n = 3$) obtained from different solvents

Crude extract	Percentage yield
Methanol	12.08±0.72[a]
Ethyl acetate	5.58±0.51[b]
n-Hexane	3.17±0.31[c]

Mean ± SD superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Antibacterial activities of crude extracts of Aloe vera rind

The antimicrobial activities of methanol, ethyl acetate and n-hexane extracts of *Aloe vera* rind against the growth of *R. solanacearum* and *X. axonopodis* pv. *citri* were determined primarily using the agar well diffusion method. The inhibition zone results are shown in Table 2 and show that the methanol and ethyl acetate extracts of *Aloe vera* rind had inhibitory potential against *R. solanacearum* and *X. axonopodis* pv. *citri* while the n-hexane extract did not show any inhibitory activity against these two bacterial strains. At 25 mg/mL of methanol and ethyl acetate extracts added to each well separately, the inhibition zones against *R. solanacearum* were 14.67±0.60 mm and 14.98±0.05 mm, respectively, whereas the diameters of the inhibition zones against *X. axonopodis* pv. c*itri* of the methanol and ethyl acetate extracts were 13.06±0.22 mm and 13.51±.08 mm, respectively. These results indicated that the *Aloe vera* rind extracts had a stronger inhibitory effect against *R. solanacearum* than against *X. axonopodis* pv. c*itri*. The correlation analysis showed that the diameters of inhibition zones against *R. solanacearum* and *X. axonopodis* pv. *citri* were highly positively correlated with the concentration of extracts at $p < 0.05$. That is, very high correlation coefficients (r)

Table 2 Antibacterial activity of *Aloe vera* rind extracts against *Ralstonia solanacearum* and *Xanthomonas axonopodis* pv. *citri* using well diffusion method

Solvent used for extraction	Concentration (mg/mL)	Inhibition zone diameter (mm)	
		R. solanacearum	*X. axonopodis* pv *citri*
Methanol	25	14.67±0.60[a]	13.06±0.22[b]
	20	10.80±0.50[b]	9.62±0.19[d]
	15	9.72±0.31[c]	8.92±0.06[e]
	10	8.63±0.42[d]	-
	5	-	-
Ethyl acetate	25	14.98±0.05[a]	13.50±0.08[a]
	20	11.12±0.17[b]	10.73±0.06[c]
	15	10.00±0.19[c]	9.52±0.13[d]
	10	8.76±0.16[d]	8.23±0.11[f]
	5	-	-
n-Hexane	25	-	-
	20	-	-
	15	-	-
	10	-	-
	5	-	-
Negative control (DMSO)		-	-

- = No inhibition zone;

Mean ± SD in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

between the concentration of methanol extract and the diameter of the inhibition zone against both bacteria (0.901 and 0.900, respectively). Furthermore, there were very high correlation coefficients between the concentration of ethyl acetate extract and the diameters of inhibition zones against *R. solanacearum* and *X. axonopodis* pv. *citri* (0.962 and 0.978, respectively). However, the percentage yield of methanol extract was about two-fold greater than that of the ethyl acetate extract (Table 1). Therefore, for further experimentation, the methanol extract of *Aloe vera* rind was chosen for MIC and MBC determination.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The MIC and MBC values of the methanol extract from the *Aloe vera* rind were evaluated for their antibacterial activity against *R. solanacearum* and *X. axonopodis* pv. *citri*. The MIC against *R. solanacearum* was 25 mg/mL while the MIC against *X. axonopodis* pv. *citri* was 62.5 mg/mL. The MBC of methanol extract against *R. solanacearum* was 50 mg/mL, and the MBC against *X. axonopodis* pv. *citri* was 125 mg/mL. The values are presented in Table 3.

Table 3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extract of *Aloe vera* rind against *Ralstonia solanacearum* and *Xanthomonas axonopodis* pv. *citri*

Test organism	Methanol extract of *Aloe vera* rind	
	MIC (mg/mL)	MBC (mg/mL)
R. solanacearum	25	50
X. axonopodis pv. *citri*	62.5	125

Antifungal activity assay

The effects of the methanol, ethyl acetate and n-hexane extracts from the *Aloe vera* rind were examined for their antifungal activities against *F. oxysporum* and *C. gloeosporioides*. The results of antifungal activity are shown in Table 4 and Figs. 1A–1B. The mycelial diameters and percentage inhibition of mycelial growth showed that *Aloe vera* rind extracts were effective in significantly reducing mycelial growth of *F. oxysporum* and *C. gloeosporioides* compared to the positive and negative controls after seven days of incubation. The methanol extract at 15 mg/mL and 25 mg/mL of *Aloe vera* rind produced 30.85±1.46% and 54.70±1.64%, respectively, mycelial growth inhibition against *F. oxysporum* and 50.95±0.90% and 62.39± 1.08%, respectively, against *C. gloeosporioides*. Furthermore, 25 mg/mL of the extracts had a significantly greater inhibitory effect than the 15 mg/mL concentration.

From Table 4, the effects of the concentrations at 15 mg/mL and 25 mg/mL of *Aloe vera* rind on mycelial growth against *F. oxysporum* and *C. gloeosporioides* demonstrated that the percentage of mycelial growth inhibition increased with increasing concentration of the *Aloe vera* rind extracts. Among the three types of extract at each concentration, the methanol extract produced the highest inhibition percentage of mycelial growth, followed by the ethyl acetate and n-hexane extracts, respectively. The correlation analysis indicated there was a moderately positive relationship between the concentration of the *Aloe vera* rind extracts and the percentage of mycelial growth inhibition against *C. gloeosporioides* ($r = 0.572$), while there was a slightly low, positive correlation between the concentration of the *Aloe vera* rind extracts and the percentage of mycelial growth inhibition against *F. oxysporum* ($r = 0.490$).

Table 4 Effects of different concentrations of *Aloe vera* rind extracts on percentage of mycelial growth inhibition of *Fusarium oxysporum* and *Colletotrichum gloeosporioides*

Treatment	Concentration (mg/mL)	*F. oxysporum*		*C. gloeosporioides*	
		Diameter of mycelial growth (mm)	% Inhibition	Diameter of mycelial growth (mm)	% Inhibition
Control	0	45.70±0.73^{a}	0^{i}	68.50±0.57^{a}	0^{h}
Methanol	15	31.60±0.82^{f}	30.85±1.46^{d}	33.60±0.62^{f}	50.95±0.90^{c}
	25	20.70±0.92^{i}	54.70±1.64^{a}	25.77±0.74^{h}	62.39±1.08^{a}
Ethyl acetate	15	34.60±0.85^{e}	24.29±1.86^{e}	42.80±0.83^{d}	37.52±1.21^{e}
	25	28.80±1.02^{g}	36.98±2.24^{c}	33.53±1.03^{f}	51.05±1.51^{c}
n-Hexane	15	42.63±0.83^{b}	6.71±0.33^{h}	58.40±0.78^{b}	14.74±1.34^{g}
	25	38.53±0.82^{c}	15.68±1.79^{g}	40.30±1.06^{e}	41.17±1.55^{d}
Mancozeb (positive control)	1	35.10±0.04^{d}	23.20±1.56^{f}	49.10±0.78^{c}	28.30±0.05^{f}
	3	27.00±0.05^{h}	40.92±1.43^{b}	30.20±0.85^{g}	55.90±0.06^{b}

% Inhibition = percentage of mycelial growth inhibition;
Mean±SD in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

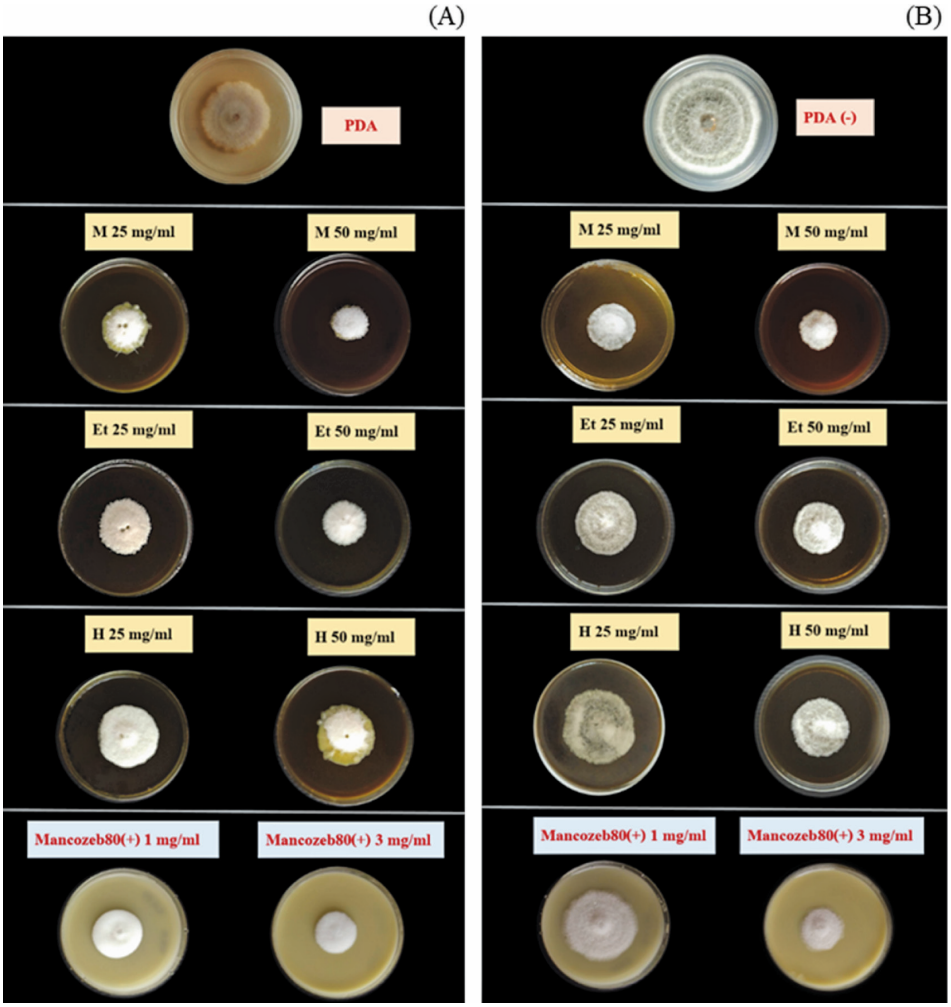

Fig. 1 Mycelial growth inhibition of *Aloe vera* rind extracts (M = methanol, Et = ethyl acetate, and H = n-hexane) at 15 mg/mL and 25 mg/mL concentrations against: (A) *Fusarium oxysporum*; (B) *Colletotrichum gloeosporioides*, where positive control = Mancozeb and negative control = non-treated potato dextrose agar (PDA)

Discussion

The current results illustrated that the methanol extract of *Aloe vera* rind had the highest amount of crude extract, followed by the ethyl acetate extract, with the lowest yield from the n-hexane extract. Various factors have been reported to affect the impact of *Aloe vera* rind extracts, particularly the extraction time, temperature and solvent polarity (Truong et al., 2019). The current results indicated that high polar solvents resulted in a high extraction yield. The extraction yield was relatively high using methanol (a polar solvent) compared to n-hexane (a nonpolar solvent). Similarly, the extraction of catechin, epicatechin and epigallocatechin from young ginger (Zingiber officinale Roscoe) using methanol produced a higher extraction yield than using acetone and chloroform (Ghasemzadeh et al., 2011).

The *Aloe vera* rind extracts using methanol or ethyl acetate as the solvent had potential against *R. solanacearum* and *X. axonopodis* pv. *citri* while the n-hexane extract did not produce any inhibitory activity against these two strains of bacteria. The increase in the size of the inhibition zone when the concentration of the extracts increased may have been due to the *Aloe vera* rind containing catechin, sinapic acid, ercetin, quercitrin, rutin, miricetin and epicatechin, which have a variety of biological activities (López et al., 2013). The results showed that there were very high correlations between the concentrations of *Aloe vera* rind extracts and the diameters of inhibition zones against *R. solanacearum* and *X. axonopodis* pv. *citri*. These results indicated that the *Aloe vera* rind extracts effectively inhibited the growth of *R. solanacearum* and *X. axonopodis* pv. *citri* and that the concentration of the extracts had an effect on the inhibitory efficacy. The *Aloe vera* rind

extracts contained major bioactive phytochemicals that play an important role in inhibiting the growth or killing bacteria. Therefore, the higher the concentration of the *Aloe vera* rind extract, the more the bacterial growth is inhibited. Similar to the findings of this research, Haq et.al (2020) reported that the diameter of the inhibition zone increased with an increased concentration of *Aloe vera* root and leaf extracts. Chaudhary et al. (2019) revealed that zinc oxide nanoparticles from aqueous peel extract were effective against *E. coli* and *A. niger*. Kwon et al. (2011) reported that the antimicrobial activity of *Aloe vera* rind extract in distilled water was strong against *E. coli* and *Vibrio* spp.

The results of the antifungal activity assay indicated that the methanol extract of the *Aloe vera* rind produced the highest inhibition percentage of mycelial growth, followed by the ethyl acetate and n-hexane extracts, respectively. The 25 mg/mL extract amounts were more effective against *C. gloeosporioides* and *F. oxysporum* than the 15 mg/mL extract amount. The activity of the extracts against *C. gloeosporioides* was higher than for *F. oxysporum*. The methanol extract of *Aloe vera* rind (25 mg/mL) had more than 50% inhibition activity against *C. gloeosporioides* and *F. oxysporum* mycelial growth. These results were supported by the work by Nidiry et al. (2011) that reported polar extracts had higher antifungal activity than non-polar extracts. Siva et al. (2008) identified that the antifungal activities of water, ethanol and acetone extracts of *Aloe vera* leaves at 50% concentration were effective in reducing the mycelial growth of *F. oxysporum* by 79%, 82% and 92%, respectively. Chougule and Andoji (2016) reported that methanol and ethyl acetate extracts of the *Aloe vera* stem had antifungal activity against *F. oxysporum*. Mohana Pradeep et al. (2020) found that the ethanol extract of *Aloe vera leaves* at 10% concentration produced 83% mycelial growth inhibition whereas at 3% and 5% concentrations there was mycelial growth inhibition of 7.7% and 40%, respectively, against *F. oxysporum* f.sp. *cubense*. The results of the current research may be valuable in the formulation of products to control plant pathogenic bacteria and fungi. Other main benefits of using *Aloe vera* rind extracts are that it is safe for human health and does not harm the environment, as well as producing value-added products from by-products, while reducing *Aloe vera* rind waste and the cost of management.

Conflict of Interests

The authors declare that there are no conflicts of interests.

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