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Original Research Article

Potential of *Pennisetum purpureum* Weed Extract on Delaying of Anthracnose Pathogen Disease Development on Mango Fruits

Eh Teet Sudau[1]* and Tse Seng Chuah[2]

[1]Department of Agrotechnology and Bio-Industry, Politeknik Jeli, Jalan Raya Timur-Barat, 16200 Tumpat, Kelantan, sudau@pjk.edu.my

[2]Faculty of Plantation and Agrotechnology, UiTM Cawangan Perlis, Kampus Arau, 02600 Arau, Perlis, chuahts@uitm.edu.my

*Corresponding author: Eh Teet Sudau; Department of Agrotechnology and Bio-Industry, Politeknik Jeli, Jalan Raya Timur-Barat, 16200 Tumpat, Kelantan, sudau@pjk.edu.my

Abstract: Anthracnose is the primary pre-and postharvest disease of mango, which is caused by *Colletotrichum gloeosporioides*. *In-vitro* and *in-vivo* studies were done to evaluate the antifungal activity of *Pennisetum purpureum* weed extract on inhibition of *C. gloeosporioides*. The *in-vitro* result showed that methanol crude extract of *P. purpureum* exhibited the best antifungal activity against *C. gloeosprioides* with an average minimum and maximum inhibition concentration value of 3.13 mg/ml and 5.28 ml/g on *C. gloeosprioides*, respectively. Meanwhile, the *in-vivo* result demonstrated that the fruits coated with sodium alginate (polysaccharides / edible coating) incorporated crude extract at 18 mg/ml concentration were most effective in retaining fruit firmness. They had a minor size lesion in diameter (0.34 cm) compared to other treatments. Then, the phytochemical assay was done on methanolic crude extract to identify the phytochemical compounds contained in *P. purpureum*. The study has revealed the presence of phenolic compounds (tannins), alkaloids,s and flavonoids in the *P. purpureum* methanolic crude extract. This study has demonstrated that alginate-based coating incorporated with methanolic crude extract of *P. purpureum* could be used to inhibit the anthracnose pathogen of *C. gloeosporioides* due to the presence of several phytochemical compounds, thereby extending the shelf life of mango fruits.

Keywords: anthracnose, coating, compound, plant extract, mango

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1. Introduction

Mango (*Mangifera indica* L.) is one of the essential crops cultivated in Malaysia and is often referred as the 'King of fruits in the tropical; famously due to its excellent eating quality, bright color, sweet taste, and luscious flavor (Krishna & Singh, 2007; Buniamin *et al*., 2020). However, anthracnose is the primary disease that limits mango production in all countries that grow mangoes, especially where high humidity prevails during cropping (Akem, 2006; Mo *et al*., 2018). Typically, *Colletotrichum gloeosporioides* (Penz.) Sacc is a causative agent for anthracnose disease which directly reduces mango fruit quality and quantity, in which the disease incidence can reach up to 100% (Santiago *et al*., 2006; Martinez *et al*., 2020). The post-harvest phase is the most damaging and economically significant phase of the disease worldwide (Akem, 2006). The most common method for controlling post-harvest anthracnose diseases is synthetic fungicides (Uddin *et al*., 2018). However, they have been found unacceptable for consumers due to the presence of fungicide residues, environmental risks, and the payment of fungicide resistance cases worldwide (Nasir *et al*., 2017).

Plants produce diverse secondary metabolites such as terpenoids, alkaloids, polyacetylenes, flavonoids, unusual amino acids, and sugars. They can be a significant source of new agricultural chemicals (Choi *et al*., 2008). Recently, a new concept of incorporating antimicrobial compounds from plants into coating films to maintain and improve the storage stability of fruits has been developed (Fan *et al*., 2009). Sodium alginate is a type of polysaccharide that has been used as an edible coating film for fresh products since it can reduce gas exchange between food products and the environment, which may decrease respiration rates and thereby delay ripening and senescence of fresh produce in a similar way to storage under modified or controlled atmosphere (Aloui & Khwaldia, 2016). Recent studies also have proved that polysaccharides–based coatings have the ability to carry different natural antimicrobial agents to preserve the quality of such as mango, grape, blueberry, minimally processed papaya and fresh-cut pineapples (Aloui & Khwaldia, 2016). In a different study done by Avramescu *et al.* (2020), the author demonstrated that edible coating incorporated with plant extracts containing polyphenols could improve the organoleptic qualities of embedded food due to the extract coating is able to protect the food from different aggressive pathogens. Chen *et al.* (2019) studied the effect of clove ethanol extract on "Newhall" navel orange. The immersion of fruit in 100 mg/mL clove ethanol extract for 3 minutes can significantly reduce the rot rate and weight loss of "Newhall". The rate of decay on "Newhall" was lower if compared to the control group with readings of 5.9% and 10%, respectively after 100 days of storage. In another study, Tesfay *et al*. (2017) also had proved the ability of plant extract as a bio-fungicide on postharvest fruit. The author had investigated the antifungal activity of methanolic and ethanolic moringa plant extract by coating them on avocado. This study has revealed that methanolic and ethanolic moringa plant extract coatings had lower mass loss, ethylene

production and respiration rate compared to the uncoated fruit. Ethanolic leaf extract had inhibition of 43.6% and 42.9% against *C. gloeosporioides* and *A. alternata*, respectively.

Pennisetum purpureum (Schumach), commonly known as elephant grass, Uganda grass, or Napier grass, is a perennial grass that belongs to the Poaceae family (Negawo *et al*., 2017). *P. purpureum* is reported as common weed species along roadsides and wastelands, which can be found in many countries (Henderson, 2001). It is commonly used as animal food (Okaraonye & Ikewuchi, 2009. It is one of the major grasses adopted by Kenyan small scale dairy farmers due to easy adaptability, high herbage yield, easy establishment, rapid regeneration, high quality for utilization and tolerance to drought (Negawo *et al*., 2017). Negawo *et al*. (2017) also has highlighted on characterizing disease resistance of Napier grass. In different study, Napier grass contains phytochemicals such as alkaloids, flavonoids, terpenoids, cardiac glycosides and saponins that significantly contributed to the observed antimicrobial and antioxidant abilities which could be used as a potential source for the development of novel therapeutic drugs (Jack *et al.*, 2020). However, there was limited finding on exploitation of *P. purpereum* extract in coating agent for retaining postharvest shelf life's of fruits. Therefore, the purpose of present investigation was to explore the potential of *P. purpureum* crude extract against fungal pathogens that could be useful for the expansion postharvest technology for the control of infectious pathogenic disease such *C. gloeosporioides*.

2. Materials and Methods

2.1. Plant Sample

The *P. purpureum* sample was morphologically identified before sampling. Then, the aerial part tissues of *P. purpureum*, such as stem and leaves, were collected around wastelands of Gong Badak and Tok Jembal, Kuala Terengganu. Meanwhile, mango fruits were purchased from a local market in Batu Enam, Kuala Terengganu. The fruits at index 2 (light green in color) were selected for size uniformity. The fruits with apparent injuries were discarded.

2.2 Preparation of Crude Extract

The aerial part tissues of *P. purpureum* were cleaned and cut into small pieces within 1 to 3 cm. The tissues were dried in a vacuum freeze dryer and ground until they became powder. The amounts of 1.5 kg powder of *P. purpureum* were undergone an exhaustive serial extraction to obtain methanolic crude extract. The extraction process was carried out using organic solvents in increased polarity, which began with hexane, followed by ethyl acetate and methanol (Nduagu *et al*., 2008). Then, the extract was dried using a rotary evaporator. Plant extracts were diluted in dimethyl sulphoxide (DMSO) in a two-fold serial dilution starting from 100 mg/ml (10%) until 0.78 mg/ml (0.078%).

2.3 Source of Fungal Isolate

Pure culture of *C. gleosporioides* was purchased from the CBS Fungal Biodiversity Centre, The Netherlands, then cultured on the sabaoroud dextrose agar (SDA). The culture of *C. gleosporioides* was incubated at room temperature ranging from 22^{o}C to 25^{o}C. After seven days, sterilized distilled water was poured into Petri dishes to remove the spores using a sterilized loop. The fungal spore suspensions of *C. gleosporioides* were adjusted with sterilized distilled water to obtain 0.5 McFarland turbidity standards (approximate cell density of 1 to 2 x 10^{8} CFU/ml). The spore solution of *C. gleosporioides* was inoculated on an SDA medium to verify the validity of the inoculums.

2.4 In-vitro Assessment of Antifungal Activity

The antifungal assay was performed by agar well diffusion method (modified from Pokharkar *et al*., 2008) by tested a serial dilution (078 mg/ml, 1.56 mg/ml, 3.13 mg/ml, 6.25 mg/ml 12.50 mg/ml. 25.00 mg/ml, 50.00 mg/ml, and 100.00 mg/ml) of all fractions of *P. purpureum* crude extracts (hexane fraction, ethyl acetate fraction and methanol fraction) against *C. gleosporioides*. Nutrient agar (SDA) was inoculated with the fungi suspensions (adjusted to 0.5 McFarland standard) by spreading the fungal inocula on the media using the sterile swab. Wells were prepared in the plates with the help of a sterilized cork borer (5 mm), and then 40 µl of the crude extract was pipetted into the well. Plates were then incubated at room temperature 25±2^{o}C for 48 hours. After the incubation period, the diameters of inhibition zones were measured and expressed in mm. The minimum inhibitory concentration (MIC) was recorded as the lowest extract concentration that inhibited the fungal pathogens' visible growth. The total activity of the crude extract was determined and recorded in ml/g using the formula below (Mdee *et al*., 2009). Mancozeb (at concentration of 0.0156 mg/ml, 0.0313 mg/ml, 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.50 mg/ml, 1.00 mg/ml, and 2.00 mg ml) were used as positive control while 100% of DMSO was used as a negative control. All treatments were carried out in triplicate with 25 fruits per replicate.

$$\text{Total activity (ml/g)} = \frac{\text{quantity of material extracted from 1 gram of dried plant material}}{\text{MIC value}}$$

2.5 Preparation of Coating Film

The coating solution was prepared by dissolving 2 g sodium alginate into 100ml of distilled water (2% w/v). The sodium alginate was heated at 70^{o}C and stirred until the solution became clear (modified from Alharaty and Ramaswamy, 2020). Then, 1.5% (w/w) glycerol was added to the coating solution. The methanolic crude extract was then incorporated into the coating solution at 9 mg/ml (3 times the MIC value) and 18 mg/ml (6 times the MIC value). These solutions were homogenized for 3 min at 12,500 rpm using a

homogenizer (Ultra Turrax T25 IKA). Two percent (w/v) calcium chloride bath was prepared in different containers.

2.6 In-vivo Treatment

Mango fruits at index two were washed, sanitized by immersion in 10% sodium hypochlorite for 2 minutes, rinsed, and air-dried. The fruits were then injured by inflicting a deep rind wound using sterilized needles. One hundred twenty mangoes were randomly divided into four groups, each group containing 30 mangoes. Three groups of mangoes were dipped into the coating solution; fruit coating without *P. purpureum* extracts incorporated (CWE), fruit coating with *P. purpureum* extracts incorporated at a concentration of 9mg/ml (CE1). Fruits coated with *P. purpureum* extracts were incorporated at a concentration of 18 mg/ml (CE2) for 2 min. The excess coating material was allowed to drip off for 1 min before submerging the fruits again for 2 min in the calcium chloride solution. One group of fruits was dipped into distilled water and acted as a control. All fruits were left to dry overnight at the ambient temperature of 25^{o}C and then inoculated by dipping into the spore suspension for 3 min. Treated and non-treated fruits were placed into a plastic container, respectively. Then, a beaker of distilled water was placed into the plastic container to achieve the condition of ±85% relative humidity. The fruit was stored for 16 days at 25^{o}C in sealed containers. For each treatment, fruits were inspected for physical analysis such as skin color, size of the lesion, decay incidence, fruit weight loss, fruit firmness and chemical analysis, including total soluble solids (TSS), total titratable acidity (TTA), pH value and Vitamin C content.

2.6.1 Size of lesion

The diameter size of the lesion on the surface of the fruit developed by *C. gleosporioides* was observed and recorded every two days of incubation.

2.6.2 Firmness

The firmness values of each mango were measured at three points of the equatorial region using the TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK). The texture analyzer fitted a P/2N probe with a load range set at 5 kg. The probe descended toward the sample with a constant force to a depth of 5 mm at a rate of 0.5 mms^{-1} and automatic return. The vital force (g) to puncture the skin was recorded.

2.6.3 Weight loss

The weight loss was calculated by deducing the initial weight from the final weight. The initial weight of fruits was obtained by weight the fruits by using an analytical balance (Shimadzu, B x 12KH) on day 1 of the experiment period. Meanwhile, the final weight of fruits was obtained on day 12 and day 16 of the storage period.

2.6.4 Skin color

Chromameter (Konica Minolta, CR-400) was used to determine skin color changes on fruits at the end of the storage period. The instrument was calibrated using a standard white reflector plate. The colour measurements were recorded in terms of lightness L* (L*=0 for black and L*=100 for white), and the chromaticity parameters a* (green [−] to red [+]) and b* (blue [−] to yellow [+]). From these parameters, the Chroma (color saturation, C = [a*2 + b*2]1/2) and the hue angle (H= tan−1 [b*/a*]) were calculated. The measurement was done in triplicate.

2.6.5 Total soluble solids (TSS)

TSS was determined with a hand-held digital refractometer (Alago). Results were expressed as a percentage of Brix.

2.6.6 Total titratable acidity (TTA)

TTA was determined by titrating 10 g of homogenate mango with 0.1 M NaOH to the end point of pH 8.1. Results were expressed as a percentage of acid.

2.6.7 pH value

The pH values of individual samples were recorded at 25^{o}C using a pH meter (Mettler Toledo AG).

2.6.8 Vitamin C content

Vitamin C (ascorbic acid) content was determined by titrating 10 g of homogenate mango with 2,6 dichlorophenol Indophenol dye.

2.7 Phytochemical Assays

The methanolic extract was subjected to the phytochemical test using the methods of Nduagu *et al*. (2008) and Raaman (2006). The phytochemical screening of methanolic crude extract was carried out to evaluate the presence of alkaloids, flavonoids, saponins, steroids, tannins, amino acids, and fixed oils.

2.8 Statistical Analysis

The experiments for the *in-vitro, in-vivo,* and phytochemical tests were laid out in a completely randomized design (CRD) with three replications. The values for *in-vitro* data were expressed as mean. General Linear Model was performed using the statistical software program (one-way ANOVA) to compare means across treatments for all data of *in-vivo*. Log10 (x+1) and x2 transformation were performed on pH and chroma data on day 12,

respectively. The formula computed standard errors of difference between means (SED) by inputting the appropriate sample size and error term values.

3. Results and Discussions

3.1 In-vitro Assessment of Antifungal Activity

3.1.1 Yield of crude extracts

Figure 1 shows the different fractions of total yield crude extracts obtained from 150 g dried tissue powder of *P. purpureum*. The extracted yield gained from the hexane fraction gave the lowest amount of section (2.66 g). Meanwhile, methanol could remove more amounts of crude extracts from *P. purpureum* than other solvents and yielded 16.5 g of crude extracts (Figure 1). The natural extract fraction yield was 4.13 g for the ethyl acetate fraction. The extract yields gained from three organic solvents were varies in amounts. This study agreed with Jack *et al*. (2020), who reported the percentage yield of extracts to be in the order, hexane < ethyl acetate < methanol. This is because the extract yields of the plant materials are strongly dependent on the nature of extracting solvent due to the presence of different compounds of varied chemical characteristics and polarities, which may or may not be soluble in a particular solvent (Sultana *et al*., 2009). This study indicated that most of the chemical compounds contained in P. purpureum extract were similar in polarities with methanol solvent since it could extract the highest amount of crude extract from *P. purpureum*.

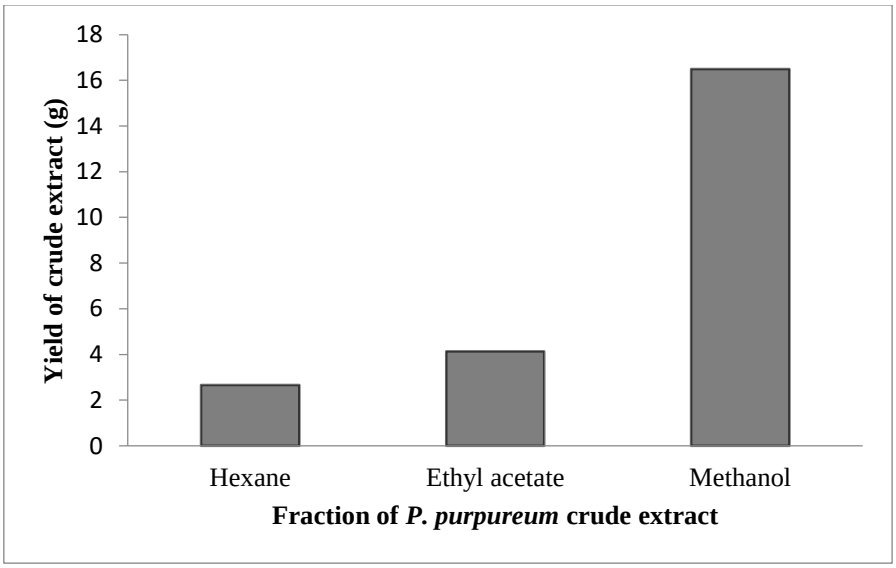

Figure 1. Effect of organic solvents on yields of *P. purpureum* crude extracts.

3.1.2 *In-vitro* assessment of antifungal activity

Three fractions of *P. purpureum* extracts showed varied responses in their antifungal potentials when tested on *C. gleosprioides* (Table 1). The extracts inhibited the

germination of *C. gloeosporoides* at concentrations ranging from 3 to 100 mg/ml, depending on the extractant used. The methanolic crude extract exhibited the most effective antifungal activity, with an average minimum inhibition concentration (MIC) value of 3.13 mg/ml (Figure 2). The crude extract from the ethyl acetate fraction had a lower antifungal effect with a MIC value of 50 mg/ml. It is suggested that crude extract of *P. purpureum* from methanol fraction consists of more active compounds that may possess antifungal activity than crude extract from ethyl acetate fraction. No inhibition of germination of *C. gloeosporoides* was observed when the corresponding spores were treated with the extracts of hexane fractions and DMSO (Table 1).

According to Ogbebor and Adekunle (2008), this difference is expected because plants vary in their chemical constituents. In a different study, hexanolic, ethanolic, and methanolic *P. purpureum* extracts had exhibited antifungal activity on the *Aspergillus niger* isolate (Jack *et al*., 2020).) Meanwhile, Ahmad and Akram (2019) observed that methanolic extract and ethyl acetate extract of *Calligonum polygonoides* exhibit antifungal properties against *Aspergillus niger* by giving the MIC value of 6.5 µg and 9.8 µg, respectively. In contrast, crude extract from hexane and aqueous fractions of *Calligonum polygonoides* had no antifungal effect on the inhibition of *A. niger.* The past investigation has proved that the antifungal potential of plant extracts from various solvents depends on biochemical compounds present in the extracts. Akbar *et al.* (2018) had found that ethyl acetate leaf fraction of *Amaranthus viridus* L. exhibited the best antifungal activity if compared to other extract fractions (*n*-hexane and chloroform) and reduced the fungal growth up to 44% in *Alternaria alternata,* 39% in *Aspergillus flavus*, 48% in *Drechslera australiensis*, 48% in *Fusarium oxysporum* and 45% in *M.acrophomina phaseolina*. The author had mentioned that 1,2- Benzenedicarboxylic acid, mono (2-ethylhexyl) ester) being the highest concentration in the ethyl acetate leaf fraction of *A. viridis,* which may be responsible for antifungal activity (Akbar *et al*., 2018).

Meanwhile, mancozeb, a positive control, exhibited excellent antifungal activity at a concentration as low as 0.0313 mg/ml (Figure 2). This is because mancozeb is an active ingredient of fungicide and very concentrated, while methanolic crude extract contains a blend of various chemical constituents.

Total activity indicates the volume at which extract can be diluted while still having the ability to kill microorganisms. The methanolic fraction of *P. purpereum* exhibited the highest total activity for inhibition of *C. gleosprioides* spore germination. On the other hand, a one-gram crude extract of *P. purpereum* can be diluted to 5.28 ml with DMSO and still inhibit the germination of *C. gleosprioides* (Figure 3). The total activity of the ethyl acetate fraction was 0.0826 ml/g, while the hexane fraction of crude extract did not inhibit spore germination of *C. gleosprioides*. However, it had lower total activity compared to the study by Mdee *et al*. (2009). The total activity of acetone extracts of *Solanum mauritianum*

and *Lantana camara* against *Trichoderma harzianum* and *Aspergillus parasiticus* were eight ml/g and 11 ml/g, respectively (Mdee *et al*. 2009).

Table 1. Antifungal activity of *P. purpureum* crude extract by using agar diffusion method.

Inhibition zone *						
Concentration (mg/ml)	Hexane Fraction	Ethyl acetate Fraction	Methanol Fraction	DMSO	Concentration (mg/ml)	Mancozeb
0.78	-	-	-	-	0.0156	-
1.56	-	-	-	-	0.0313	+
3.13	-	-	+	-	0.0625	++
6.25	-	-	+	-	0.1250	++
12.50	-	-	+	-	0.2500	++
25.00	-	-	+	-	0.5000	++
50.00	-	+	+	-	1.0000	++
100.00	-	+	+	-	2.0000	++

* - : No inhibition zone, + : Inhibition zone ranged from 6 to 10mm, ++ : Inhibition zone ranged from 11 to 16mm

Data for MIC were not subjected to ANOVA due to no variance among replicates.

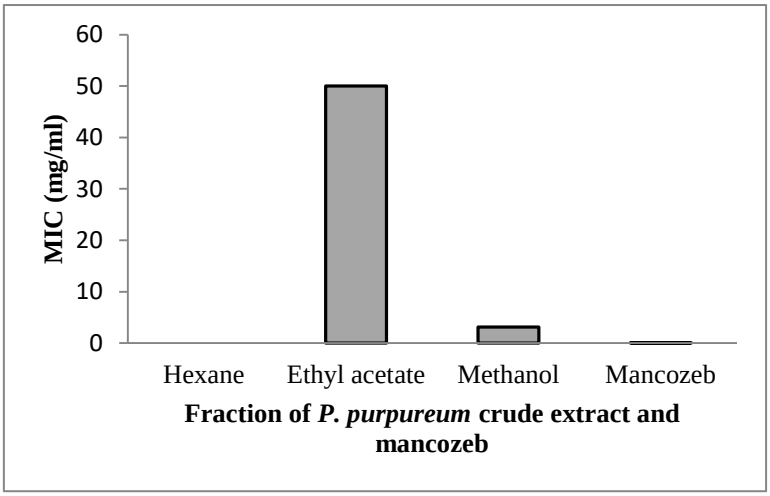

Figure 2. Effects of different fractions of crude extracts and fungicide (mancozeb) on minimum inhibitory concentration (MIC) values of *Colletotrichum gloeosporoides*.

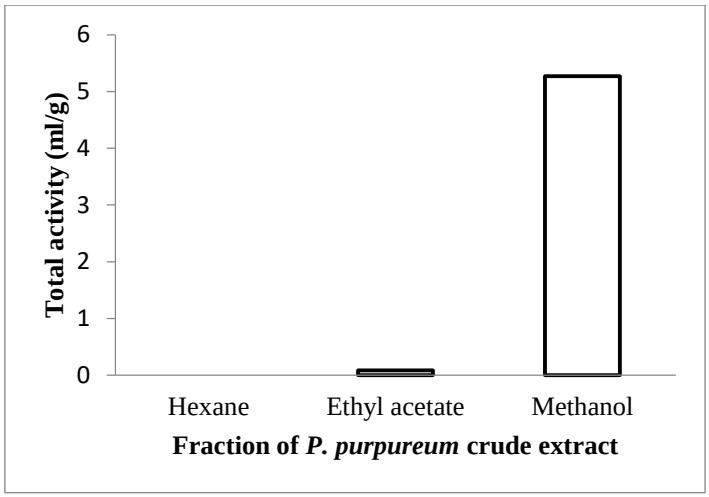

Figure 3. Total activity of crude extract fractions of *P. purpereum* against germination of *Colletotrichum gleosprioides.*

3.2 *In vivo Treatment*

Post-harvest application of methanolic *P. purpureum* crude extract to control anthracnose infection on mango caused by *C. gloeosporioides* was evaluated in the *in-vivo* study. The uncoated mangoes had significantly lower values in TTA (0.22%) and vitamin C (13.47%) content than the coated mangoes. In addition, the uncoated mangoes exhibited significantly higher values in TSS (19.20%), pH (4.27), chroma (52.29 C*), hue angle (81.88 H^o), weight loss (18.89 g), and size lesion (>1.1 cm) as compared to all treatments of coated mangoes after 12 days of storage at room temperature of 25^{o}C (Table 2). Chill *et al.* (2007) mentioned that the physicochemical changes happened when fruits started to ripen during incubation (development of anthracnose on mangoes) and produced the ethylene gas. Thus, this will speed up the senescence process and accelerates the ripening process. As a result, the TSS and pH values of mango in the present study were increased, but TTA and vitamin C content were decreased in uncoated fruits after the storage period. On the other hand, the coated fruits (CWE, CE1, and CE2) exhibited delayed ripening. The result demonstrated that all coated fruits had low value of TSS (14%), pH (< 3.60), weight loss (<16.46g), chroma (<29.64 C*), hue angle (<-81.07 H^o) and size lesion (< 0.30cm). Besides, all coated fruits also possess a higher value of TTA (>0.45%) and vitamin C (>15.90%) content compared to control or uncoated fruits. The coating film on the fruit surface acted as a water vapour barrier, reducing water evaporation from the fruit surface. Besides, it modifies the atmosphere inside the fruit, where internal oxygen becomes lower and internal carbon dioxide increases. This may inhibit respiration and limit the availability of energy deteriorative processes such as color changes and moisture losses (Banks *et al*., 1997; Fan *et al*., 2009).

Upon subsequent storage, all coated fruits showed a progressive weight loss after 16 days of storage compared to those stored for 12 days (Table 2). However, no significant

effects were demonstrated in TSS, TTA, pH, vitamin C content, chroma, and hue angle of mangoes regardless of coating treatments on day 16 after storage. Meanwhile, there was no data for uncoated fruits since they were discarded on day 12 of the experiment period due to severe infection of anthracnose pathogens. Incorporating P. purpureum crude extract into the coating significantly positively affected the firmness of mangoes. Fruits coated with CE2 achieved the highest firmness value (209.87 g) as compared to those of fruit coated with CWE (202.31 g) or CE1 (206.27 g) on day 16 after storage.

Table 2. Effects of different coating treatments with or without incorporated *P. purpureum* extracts on physical and chemical parameters of mango fruits on day 12 after storage at room temperature of 25^{o}C.

	Coating treatments			
Physico-chemical parameters	Uncoated	CWE	CE1	CE2
TSS (Brix %)	19.20 ± 0.72 ^{b}	14.97 ± 0.45^{a}	14.53 ± 0.12^{a}	14.95 ± 0.26^{a}
TTA (%)	0.22 ± 0.02 ^{a}	0.45 ± 0.03^{b}	0.51 ± 0.03^{b}	0.45 ± 0.03^{b}
pH	4.27 ± 0.17 ^{b}	3.60 ± 0.07^{a}	3.58 ± 0.00^{a}	3.55 ± 0.13^{a}
Vitamin C (%)	13.47 ± 0.22^{a}	15.97 ± 0.13^{b}	15.90 ± 0.05^{b}	16.01 ± 0.39^{b}
Weight loss(g)	18.89 ± 0.74^{b}	16.46 ± 0.60^{a}	15.76 ± 0.25^{a}	15.75 ± 0.35^{a}
Chroma (C*)	52.29 ± 0.52^{b}	29.64 ± 3.68^{a}	29.15 ± 0.75^{a}	29.49 ± 1.00^{a}
Hue angle (H^{o})	81.88 ± 0.27^{b}	-81.07 ± 1.65^{a}	-83.02 ± 0.48^{a}	-83.02 ± 0.53^{a}

CWE denotes coating without *P. purpureum* extract incorporated, CE1 denotes coating with *P. purpureum* extract incorporated at a concentration of 9g/L, and CE2 denotes coating with *P. purpureum* extract incorporated at 18 g/L.

Mean within the same row followed by a similar letter has no significant difference at $P<0.05$ after the Tukey test.

In general, anthracnose diameter lesions on mangoes inoculated with *C. gloeosporioides* increased significantly ($P<0.05$) with days of storage (Figure 5). However, this study can prove that methanolic crude extract of *P. purpureum* could delay the spreading of the disease based on the lesion size presented in Figure 5. The lesion size on mango coated with CE1 and CE2 show the slowest effects of the disease, where *C. gloeosporioides* caused the black spot symptom on mango at day 10 of the experiment period, with the reading of both lesion sizes were <0.05 cm and then, the sizes were increased to <0.65 cm and <0.40 cm, respectively on day 16 of the experiment period. In contrast, the lesion size on uncoated mango and mango coated without crude extract of *P. purpureum* (CWE) started to spread on day 4 of the experiment period. The uncoated mango showed the largest lesion on day 12 of the experiment with the reading almost to 1.20 cm in diameter long and was discarded from the experiment. Meanwhile, the lesion on CWE mango was increased to >0.8 cm on day 16 of the experiment.

The results also suggest that mangoes coated with CE2 treatment had the most active antifungal activity by inhibiting the spread of anthracnose pathogen (Figure 6).

Furthermore, the phytochemical analysis has revealed that methanolic crude of *P. purpureum* extract contained the phenolic compound (tannins), alkaloids, and flavonoids (Table 4) which might be responsible for the antifungal activity in this study. Research done by Okaraonye and Ikewuchi (2009) has supported this finding where *P. purpureum* is rich in tannins, alkaloids, and flavonoids. These compounds might play essential roles in exhibiting antifungal properties on mango pathogen. The researchers demonstrated that some plant chemical compounds, such as polyphenols, tannins, flavonoids, and alkaloids, are known to have antimicrobial activity (Akinpelu *et al*., 2008; Tiwari *et al*., 2011). These compounds react by forming a complex with the cell wall, binding to adhesins and substrate deprivation (Tiwari *et al*., 2011). Flavonoids exhibit a wide range of biological activities, one of which is their ability to scavenge hydroxyl and superoxide anion radicals, thus health-promoting in action. Tannins exert antimicrobial activities by iron deprivation, hydrogen binding, or specific interactions with vital proteins, such as enzymes in microbial cells (Akinpelu *et al*., 2008).

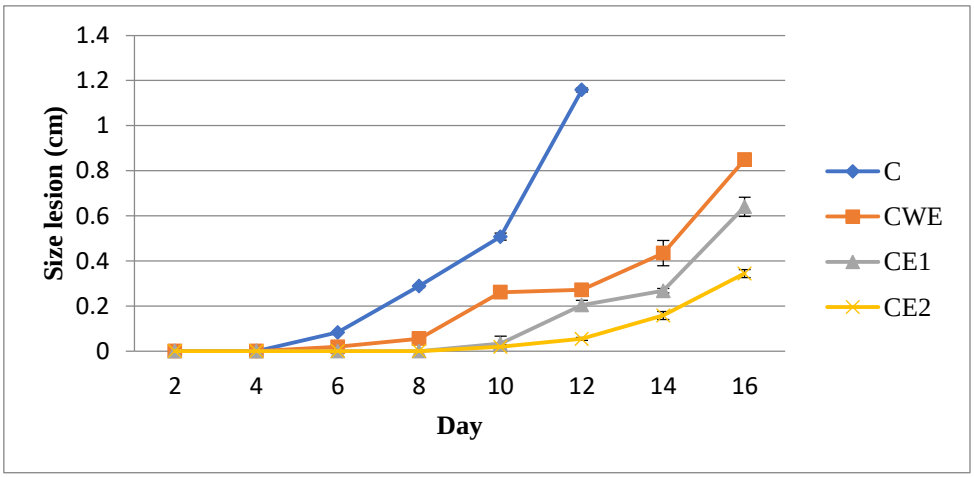

Figure 5. Effects of different coating treatments on lesion size of mango fruits due to *Colletotrichum gloeosporioides.* C denotes uncoated mango, CWE denotes coating without *Pennisetum purpureum* extract incorporated, CE1 denotes coating with *P. purpureum* extract incorporated at a concentration of 9 mg/ml, and CE2 denotes coating with *P. purpureum* extract incorporated at a concentration of 18 mg/ml. Data are means of three replicates ± SE.

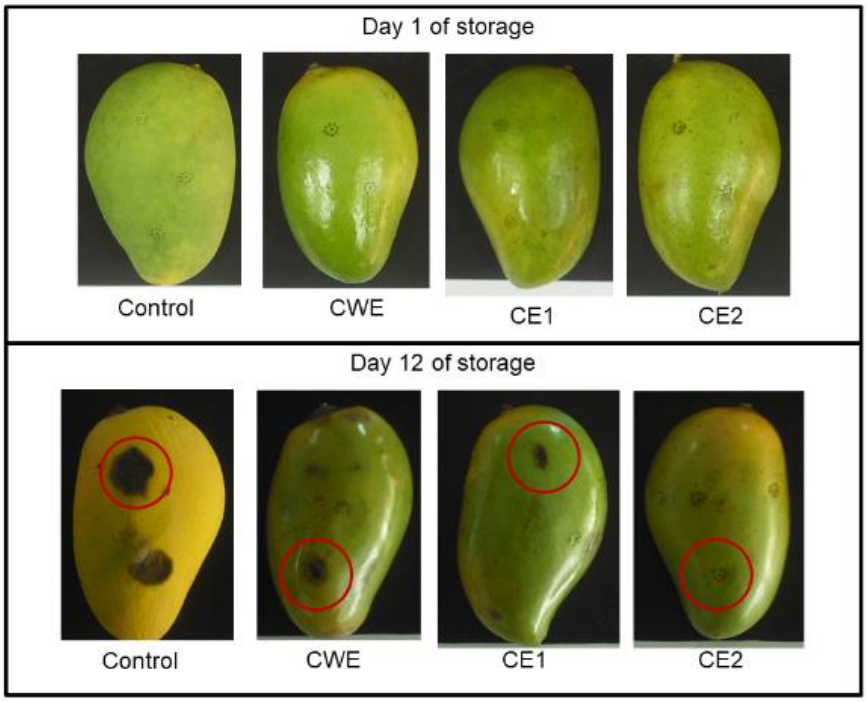

Figure 6. Effects of different coating treatments on *Colletotrichum gloeosporioides* of mango after 12 days of storage. The red circle indicates a lesion due to infection of *C. gloeosporioides*. Control denotes uncoated mango, CWE denotes coating without *Pennisetum purpureum* extract incorporated, CE1 denotes coating with *P. purpureum* extract incorporated at a concentration of 9 mg/ml, and CE2 denotes coating with *P. purpureum* extract incorporated at a concentration of 18 mg/ml.

Table 4. Phytochemical screening of *P. purpureum* methanolic crude extract

Phytochemical Test	Corresponding Constituent	Result
Frothing Test	Saponins	-
Liebermann-Burchard's Test	Steroids	-
Ferric chloride Test	Tannins	+
Shinoda Test	Flavonoids	+
Mayer Test Dragendorff Test	Alkaloids	+
Ninhydrin Test	Amino acids	-
Spot Test	Fixed	-

(-) indicates the absence of a constituent, (+) indicates the presence of a constituent

4. Conclusions

The alginate coating incorporated with *P. purpureum* extract has suitable antifungal properties for delaying anthracnose symptom expression caused by *C. gloeosporioides* on

mango fruits until day 16 after storage at room temperature 25^0C. This study also revealed that phytochemical analysis of *P. purpureum* extract consists of a blend of phenolic compounds (tannins), alkaloids, and flavonoids, which might be responsible for suppressing the growth of *C. gloeosporioides* on mango fruit. The coating treatments can also delay the ripening process of mango fruits, reducing weight loss and slowing down the decrease of firmness. Further investigation on the pure fraction of biochemical compounds of *P. purpureum* could be done to develop a new environmentally bio fungicide for postharvest application.

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