JJBS

Volume 15, Number 5,December 2022
ISSN 1995-6673
Pages 771 – 777
https://doi.org/10.54319/jjbs/150505

Jordan Journal of Biological Sciences

DPPH Free Radical Scavenging Activity of *Citrus aurantifolia* Swingle Peel Extracts and their Impact in Inhibiting the Browning of *Musa Paradisiaca* L. Var. Kepok Tanjung Explants

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Received: January 25, 2022; Revised: April 28, 2022; Accepted: June 6, 2022

Abstract

Musa paradisiaca var. Kepok Tanjung is a popular banana cultivar that is widely consumed by the majority of the Indonesia's population. However, the browning of explants caused by the polyphenol oxidase (PPO) enzyme is becoming a major constraint for its production which can lead to the low productivity of seedlings. Therefore, this research aims to determine the antioxidant activity of Citrus aurantifolia peel extracts and their ability in inhibiting the browning of Kepok Tanjung bananas explants at the shoot initiation stage under in vitro condition. The extracts were obtained through the maceration of C. aurantifolia peel residue using n-hexane, ethyl acetate, and ethanol solvents which were added to the culture medium. Furthermore, the 50% inhibitory concentration (IC_{50}) was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, while enzyme activity was assessed by spectrophotometric method using catechol substrate. The results showed that the ethyl acetate extract of C. aurantifolia peel from day 6 at concentration of 0.3 mg/mL can inhibit oxidation, as shown by the reduced browning index of the explants. Meanwhile, the highest antioxidant activity was discovered at day 9 with PPO enzyme activity of 80.261 U/mL at the same concentration.

Keywords: DPPH, antioxidant, C. aurantifolia, browning, M. paradisiaca

1. Introduction

Musa paradisiaca var. Kepok Tanjung is a popular banana cultivar that is widely used as an ingredient in flour production due to its sweet taste and distinctive aroma (Putri *et al.,* 2015). The increase in demand for Kepok Tanjung bananas has become an obstacle for farmers, especially for propagation using seeds. Meanwhile, one of the best alternatives for producing its seedlings is through *in vitro* plant propagation techniques (Mose *et al.,* 2020; El-Sayed *et al.,* 2021). This method is able to produce high quality banana explants in large quantities, uniformly, within a short time, and also support vigorous plants' development during their subsequent growth cycles(Ferdous *et al.,* 2015).However, the browning of the explants becomes a major constraint that often occurs when they are injured due to the high polyphenols exposure (Puspitasari and Syauqy, 2014).

Research indicated that the browning of the explants was caused by the activity of residual peroxidase (POD), polyphenol oxidase (PPO) (Xu *etal.,* 2015;Wu *et al.,* 2018), and phenylalanine ammonia lyase (PAL) (Sun *et al.,* 2015).PPO is a group of oxidoreductase enzymes containing two Cu atoms, and it is located in the chloroplast of the plants. This enzyme catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones with molecular oxygen as a co-substrate (Oliveira *et al.,* 2011; Al-Gabbiesh *et al*., 2015). O-quinone is a highly reactive molecule that passes through non-enzymatic secondary reactions to form a brown complex polymer known as melanin and a crosslinked polymer with protein functional groups (Taranto *et al.,* 2017). Meanwhile, loss of subcellular compartmentalization that is due to aging, injury, interaction with pests or pathogens, mishandling during postharvest processing, and storage gave contact between PPO and vacuolar phenolic substrates (Taranto *et al.,* 2017). The presence of reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$) also play an important role in causing the browning of explants. Moreover, research also showed that proteins, phospholipids, and pigments are degraded by free radicals during the browning process (Misra *etal.,* 2010). When the browning of explants was not properly treated, it can cause a decrease in regenerative ability, reduction of callus

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growth, inhibition of adventitious shoot growth, and death of culture tissue (Xu *et al.,* 2015).

Previous studies showed that browning in explants can be reduced by graphene oxide (GO) for Chinese orchid (Wu *et al.,* 2018), pretreatment using potassium citrate antioxidant solution of plantain (Onuoha *et al.,* 2011), and application of ascorbic acid, citric acid, and activated charcoal in the media to banana explants (*Musa spp*. cv. Grand Naine) (Safwat *et al.,* 2015).

Antioxidants compounds can delay oxidation reaction by capturing excess of free radicals and preventing chain reactions. Chemical compounds such as carotenoids, ascorbic acid, tocopherols, glutathione, cysteine, phenolics, and flavonoids, as well as several other enzymes, namely superoxide dismutase (SOD), guiacol peroxidase (GPX), catalase-peroxidase (APX), catalase (CAT), and glutathione reductase (GR) may become paramount importance in catalyzing the cleavage of O_2 into H_2O_2 and O_2(Zou *et al.,* 2016). The conversion of H_2O_2 to water is carried out in the cytosol and chloroplast by the glutathione ascorbate cycle, which involves APX and GR enzymes (Misra *et al.,* 2010).

Citrus aurantifolia peel contains various chemical compounds such as molasses, pectin, polyphenols, vitamins, minerals, essential oils, and dietary fiber(Rafiq *et al.,* 2018), which can be used for applications in industrial or agriculture. The presence of phenol and flavonoid makes the peels to pose the ability as a natural antioxidant where they can produce semipolar and polar compounds that can be extracted with various solvents such as ethyl acetate and ethanol (Okubgo and Oriakhi, 2015).

Loizzo *et al.* (2012) reported that antioxidant activity of *C. aurantifolia* peel that was extracted with polar (methanol) and non-polar (n-hexane) solvents indicated that methanol extract was more active than n-hexane when using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. *C. aurantifolia* contains polyphenolic compounds possessing antioxidant properties that can capture free radicals, so that the oxidation process will be reduced by the addition of *C. aurantifolia* extract. The presence of polyphenolic compounds was traced by extracting *C. aurantifolia* peel using a variety of non-polar, semi-polar, and polar solvents such as n-hexane, ethyl acetate and ethanol.

Antioxidant has been used for inhibiting the browning of explant in tissue culture, as discussed above. So far, efforts to inhibit browning of explant were conducted by the application of synthetic antioxidants (e.g., Wu *et al.,*2018; Safwat *et al.,* 2015;Onuoha *et al.,* 2011). Research using natural antioxidants from *C. aurantifolia* peel extract in tissue culture has not yet been reported. Therefore, this current research is carried out to determine the presence of antioxidants and their suspected active compounds for reducing the browning of *M. paradisiaca* var. Kepok Tanjung explants. This research will become an important contribution in sustainable banana productions.

2. Materials and Methods

2.1. Materials

The materials used in this research include lime *(C. aurantifolia)* from Cibodas Village, West Bandung Regency, Indonesia, while explants of *M. paradisiaca* var. Kepok Tanjung was taken from the Indonesian Tropical Fruits Research Institute (IP2TP), Ministry of Agriculture, Subang Regency, Indonesia.

2.2. Preparation of non-volatile extract of C. aurantifolia peel

A 400 g of *C. aurantifolia* peel chopped into pieces of about 2 cm in size and put into a distillation flask, soaked with distilled water for 3 hours to separate the essential oils. The residue was extracted by maceration in succession using n-hexane, ethyl acetate, and ethanol solvents. Extraction was carried out for 3 days, where each extract was collected and concentrated with a rotary evaporator to remove the solvent. Subsequently, the concentrated extract was used for further purposes. The*C. aurantifolia* extraction preparation step was done by three replications.

2.3. DPPH free radical scavenging activity of C. aurantifolia peel extract

The antioxidant activity test was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, as a highly reactive free radical chromogen. The concentration of the tested substance varied based on the ability to scavenge DPPH free radicals,

A total of 0.1 mL of n-hexane extract, ethyl acetate, and ethanol with a concentration of 100-4000 ppm was added to 2 mL of DPPH (0.21 mM in 95% ethanol). The mixture was shaken and kept for 60 minutes in a dark room and ambient temperature. Subsequently, light absorbance was measured with a UV-Vis spectrometer at 517 nm and the DPPH inhibition was calculated using the equation by (Safdar *et al.,* 2016).

$$\text{Inhibition } (\%) = \frac{A_c - A_s}{A_c} \times 100\%$$

Description: A_c and A_s are control and sample absorbance, respectively

IC_{50} was measured with,

$$IC_{50} = (50 - b)/a$$

Description: IC_{50} is obtained from regression equation of inhibition percentage against extract concentrations.

2.4. Liquid vacuum column chromatography (KVC)

The stationary phase used was silica gel G60 with a size of ± 200 mesh, while the mobile phase was graded between the ratio of n-hexane: ethyl acetate: ethanol. Before the separation process with KCV, the sample was impregnated using silica gel with a size of 50-100 mesh. The sample was inserted at the top of the column which is spread evenly and was covered with filter paper. Furthermore, it was eluted from low polarity, increased by 10%, and the column was sucked dry at each fraction collection (Maro *et al.,* 2015). The combination of the same fraction was monitored by thin-layer chromatography (TLC) using a silica gel plate GF254, n-hexane: ethyl acetate in a ratio of 7:3, and the eluent was observed at 254 nm and 366 nm UV lamps, respectively.

2.5. Identification of active compound of extract

Identification of compounds using liquid chromatography-mass spectrometry (LC-MS) was carried out on the fraction with the highest antioxidant activity. A total of 0.5 g of extract was dissolved in 50 mL of methanol p.a. and the solution was filtered using a 0.22-micron syringe filter, put into a 2 mL vial, and injected into the LC-MS system.

2.6. Treatment and maintenance of explants

C. aurantifolia peel extract with the highest activity obtained based on the antioxidant activity test was treated with three levels of concentration of 0.1, 0.2, and 0.3 mg/mL *C. aurantifolia* peel extracts. As positive controls, the 0.1 mg/mL ascorbic acid and PVP were used. Each extract concentration was added to Murashige and Skoog (MS) culture media with 2.5 ppm benzyl amino purine (BAP) and 0.1 ppm thidiazuron (TDZ). The banana explants were incubated in a room with a light intensity of 1,000 – 4,000 lux, a temperature of 25±2°C, 80% humidity with 16 hours of irradiation for 15 days of shoot initiation stage.

2.7. Browning index

Visual observation of browning was carried out every 3 days for 15 days of the shoot initiation stage. Browning level was recorded as follows: level 0: no browning, level 1: browning only in the explant incision, level 2: browning appeared in culture media with an area of fewer than 0.5 cm^2 where the explant was the center, level 3: browning appeared in culture media with an area of more than 0.5 cm^2, where the explant was the center (Wu *et al.,* 2018). Meanwhile, the browning index was calculated using formula below:

$$\text{Browning Index} = \sum \frac{(a) \times (b)}{(A) \times (b')} \times 100\%$$

Description: a is the number of browning explants, A is the total number of explants, b is the browning level, and b' is the highest level of browning.

2.8. Extraction of polyphenol oxidase enzymes (PPO)

M. paradisiaca explants were taken randomly on days 0, 3, 6, 9, and 12 for each treatment and kept at frozen temperature of -18°C. All treatments were replicated three times. The explants (5 g) were cut into small pieces, dissolved in 5 mL 50 mM citrate buffer, which contain 0.5% (w/v) tritone X-100, 2 mM EDTA, 1 M NaCl, and 0.5% (w/ v) PVP, and homogenized for 10 minutes at 4°C and pH 7.0. Subsequently, the supernatant was filtered using cheesecloth and centrifuged at 12,000 rpm for 20 minutes. The supernatant that contained PPO was used as a crude extract of PPO, which was incubated for 30 minutes at 17°C (Murniati *et al.,* 2018).

2.9. Polyphenol oxidase enzyme (PPO) activity test

The PPO activity was observed using the spectrophotometric method with catechol substrate and modified according to the Sigma procedure. The units were measured at initial rates using a UV-Vis-nir scanning double beam spectrophotometer (UV-Vis 1800, Shimadzu).

Subsequently, 0.1 mL of crude extract of PPO was mixed with the substrate at various concentrations of 0.20 - 0.40 mM and dissolved in 3 mL of 50 mM citrate buffer, which contained 2.1 mM ascorbic acid and 0.65 mM EDTA. The optimal PPO activity was determined by varying the pH from 6.8 to 7.2 and incubation temperature from 10 to 30°C. The PPO activity (U/mL) obtained in the assay was estimated by the initial reaction rate and expressed as absorbance curve (A) vs time (minutes) (Gauillard *et al.,* 1993). Meanwhile, the total activity (U) was obtained by multiplying the activity (U/mL) and the total volume (mL) (Dennison, 2002). The calculation of PPO activity is explained as follows:

$$\text{PPO Activity} = \frac{1}{sb} \cdot \frac{V_{total}}{V_{enzyme}} \cdot \frac{d\,[substart]}{dt}$$

Description:

$\frac{1}{sb}$ = absorbance slope vs [substrate] substrate standard curve

$\frac{d\,[substart]}{dt}$ = absorbance slope vs time (minute) from initial speed

2.10. Data analysis

The obtained data were analyzed using SPSS 25.0, and the Shapiro-Wilk test was conducted to evaluate the data normality. When the analysis of variance (ANOVA) with a 95% confidence level (P= 0.05) has a significant effect, it was continued with Duncan's test at a 95% confidence level (P= 0.05).

3. Results and Discussion

3.1. C. aurantifolia peel extract

The yield percentage of *C. aurantifolia* peel extract through different solvent polarity levels reveals that the highest extraction yield was obtained with nonpolar solvent of ethanol (4.76%) followed by semi polar solvent of ethyl acetate (2.28%) and nonpolar solvent of hexane (0.37%). This result can be explained partly due to that the semi-polar and polar chemical components of *C. aurantifolia* peel such as polyphenols and flavonoids are more in abundance than non-polar components. Differences in polarity and concentration among the extraction solvents may influence the variation in the extraction yield. Hegazy and Ibrahium (2012) investigated various organic solvents for extracting flavonoid and polyphenol compounds from orange peel and found that the extraction yields ranged from 8.27% to 28.32%, with hexane and methanol extraction, respectively. Likewise, Safdar *et al.* (2016) who extracted *C. retuculate* peel using different techniques and solvents found that ethanol extraction yielded more extract (18.46%) than ethyl acetate extraction (5.12%).

3.2. DPPH free radical scavenging activity of C. aurantifolia peel

In measuring antioxidant activity, IC_{50} was defined as the amount of substance concentration that can inhibit 50% of the oxidation reaction by DPPH. Figure 1 shows the percentage of inhibition as a function of the substance concentration. Antioxidant activity test showed that each extract had a different percentage of inhibition (IC_{50}). It showed a positive correlation between the concentration of the extract and the percentage of inhibition of 2.78 mg/mL with a correlation value of 0.992 for n-hexane, 0.19 mg/mL with a correlation value of 0.991 for ethyl acetate,

and 2.45 mg/mL with a correlation value of 0.994 for ethanol.

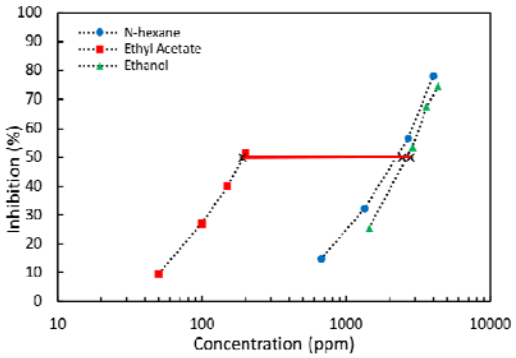

Figure 1. The inhibition percentage of n-hexane, ethyl-acetate, and ethanol extract of *C. aurantifolia* peel in various concentration. The value of IC_{50} is shown by the red horizontal line. The values of line regression approximation for each extract denoted by black cross.

Positive correlation between the concentrations of all extracts and the percentage of inhibition (0.992 for n-hexane, 0.991 for ethyl acetate, and 0.994 for ethanol) indicates the effectiveness of the inhibition caused by the increase in the number of polyphenols contained in the extract. Furthermore, the highest inhibitory activity was shown by ethyl acetate extract by giving the highest percentage of inhibition. It is indicated by the smallest IC_{50}value of ethyl acetate extract of 0.19 mg/mL, followed by n-hexane extract of 2.45 mg/mL, and ethanol extract of 2.78 mg/mL. The result is in an agreement with previous research, which recorded that the antioxidant activity of flavonoids is contained in the ethyl acetate extract of *C. aurantium* (Shen *et al.,* 2019).

3.3. Ethyl acetate extract fractionation

Fractionation of the ethyl acetate extract by vacuum liquid chromatography was carried out to trace the most active fraction and obtain six combined fractions (A-F fraction), with antioxidant activity as shown in Table 1.

Table 1. Antioxidant activity of six fractions, from ethyl acetate extract

Combined Fraction	Concentration (ppm)	Inhibition (%)	IC_{50} (mg/mL)
	0	0.00 □ 0.00	
	1377	9.50 □ 0.18	
A	4590	21.07 □ 0.32	13.42 □ 0.001[a]
	9180	34.96 □ 0.49	
	18360	66.38 □ 0.86	
	0	0.00 □ 0.00	
	463	24.84 □ 0.27	
B	1158	46.45 □ 0.23	1.30 □ 0.001[b]
	1853	69.01 □ 0.96	
	2317	84.35 □ 0.46	
	0	0.00 □ 0.00	
	118	20.82 □ 0.23	
C	157	35.43 □ 1.95	0.23 □ 0.002[c]
	235	51.14 □ 1.44	
	313	69.02 □ 0.95	
	0	0.00 □ 0.00	
	20	9.94 □ 0.60	
D	39	15.45 □ 0.99	0.15 □ 0.005[e]
	78	22.79 □ 4.86	
	156	52.96 □ 1.41	
	0	0.00 □ 0.00	
	11	13.03 □ 0.18	
E	33	28.97 □ 1.13	0.05 □ 0.001[f]
	55	51.42 □ 0.62	
	66	62.71 □ 0.22	
	0	0.00 □ 0.00	
	117	36.43 □ 0.67	
F	176	50.73 □ 0.60	0.18 □ 0.005[d]
	234	62.75 □ 1.87	
	293	76.62 □ 2.06	

*Statistical test of significant difference with 95% confidence level. The mean value followed by the same letters showed no significant difference (P <0.05).

Based on the Table1, fraction E gave the highest activity with an IC_{50} value of 0.05 mg/mL, which was included in the strong category, followed by fractions D and F with moderate categories of 1.5 and 0.18 mg/mL, respectively. Meanwhile, the other fractions were in the weak category according to Molyneux (2004).

3.4. Identification of active compounds

The LC chromatogram and its mass fraction were analyzed to predict the compounds with a role in the E fraction, as shown in Figures 2, 3, and 4.

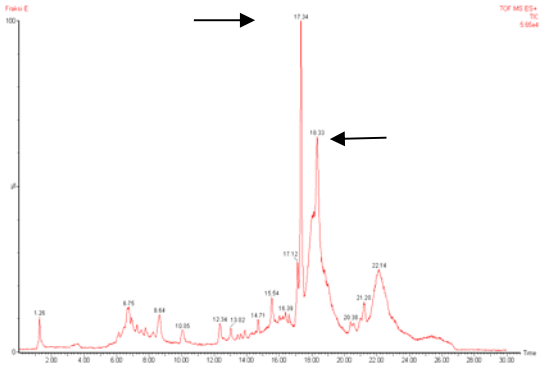

Figure 2. LC chromatogram of fraction E inverted phase with C-18 column, methanol solvent: water gradient

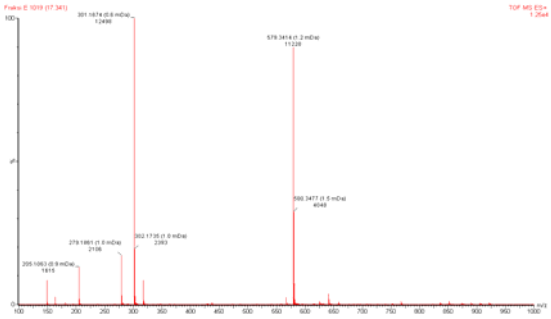

Figure 3. Peak MS experience pattern with a retention time of 17.34 minutes

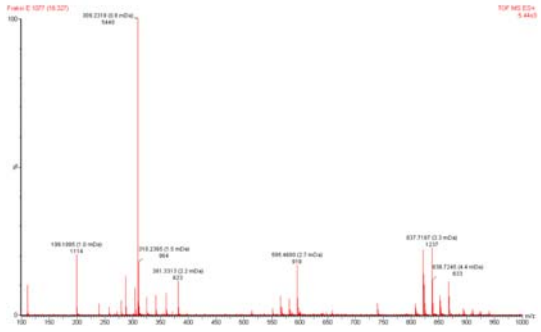

Figure 4. Peak MS experience pattern with a retention time of 18.33 minutes

Figure 2 shows the LC-MS chromatogram profile of fraction E at several peaks. The main component was estimated to play an important role in antioxidant activity with retention times of 17.34 and 18.33 minutes. According to the mass spectrum on the TOP MS ES+ technique, the m/z molecular ion (M+) for the main peak (tr 17.34 min) was 580.3477, while the second peak (tr 18.33 min) was 838.7245. Based on the molbase.com database approach, m/z 580.3477 was derived from the mass of the formula $C_{39}H_{50}O_{20}$, which is assumed to be a naringin compound (Figure 5). Meanwhile, m/z 838.7245 was derived from the mass of the formula $C_{39}H_{50}O_{20}$ which is an epimedin A1 compound (Figure 6). These compounds are effective antioxidants and are often present in citrus peels, naringin (Chen *et al.,* 2008; Chen *et al.,* 2017; Safdar *et al.,* 2016; Xu *et al.,* 2015).

The main flavonoid components in the *Citrus* genus, such as naringin, naringenin, hesperidin, quercetin, and rutin are also discovered in the *C. aurantifolia* peel (Loizzo *et al.,* 2012). Meanwhile, flavonoids function directly in capturing ROS and reducing the formation of hydrogen peroxide (H_2O_2) (Nakao *et al.,* 2011). It was also reported that naringin increases the activity of antioxidant enzymes, such as superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase-peroxidase (APX), catalase (CAT), and glutathione reductase (GR). Furthermore, hesperidin reduced free radicals by inhibiting XO, as a key mechanism of antioxidant action, while other flavonoids such as naringenin, quercetin, and rutin capture hydroxyl radicals (·OH) directly (Zou *et al.,* 2016).

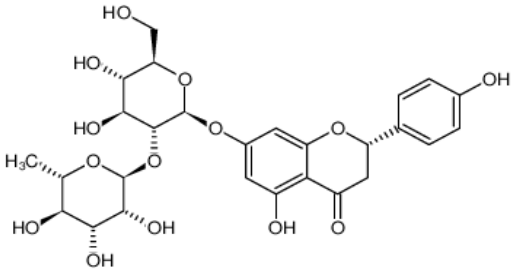

Figure 5. The chemical structure of naringin

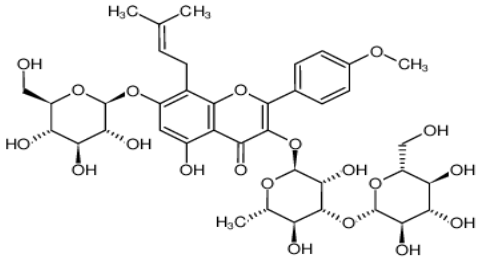

Figure 6. Chemical structure of epimedin A1

Naringin and epimedin A1 are polyphenolic compounds, meanwhile, the large number of hydroxyl groups bound to the compounds made them function as antioxidants by donating hydrogen atoms bonded to oxygen atoms in DPPH reagents. The activity of the E fraction was stronger (0.05 mg/mL) than the ethyl acetate extract (0.46 mg/mL), which indicated the antagonistic action of other compounds in the ethyl acetate extract.

3.5. Browning index

The increase in incubation time was followed by a higher browning index in all samples (Figure 7). Meanwhile, the additional treatment on day 3 had no significant effect on the browning index of *C. aurantifolia* peel extract compared to the negative control (ethyl acetate solvent). After 6 days, the browning index was significantly lower than the control because the positive control ascorbic acid and PVP were pure compounds at the same concentration (0.1 mg/mL). Similarly, the active substance in the ethyl acetate extracts was mixed with other chemical compounds contained in the extract. After 6 days, the browning index of 0.3 mg/mL of *C. aurantifolia* peel extract was slightly lower than 0.1

mg/mL and 0.2 mg/mL, respectively. It showed that the application of antioxidant extract can significantly reduce the browning index. Specifically, the 0.3 mg/mL treatment which has a better effect at the shoot initiation stage after 6 days as visualized in Figure 8a and 8b that contrasted to the control in Figure 8c and 8d.

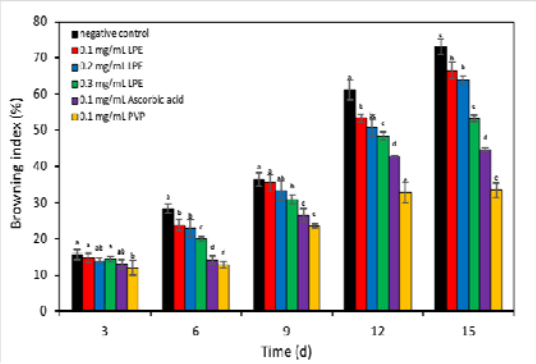

Figure 7. Effect of *C. aurantifolia* peel extract concentration on the browning index (%) of *M. paradisiaca* explants at the shoot initiation stage. Each value is expressed as mean ± SD (n=7).*The mean value followed by the same letters showed no significant difference (P <0.05).

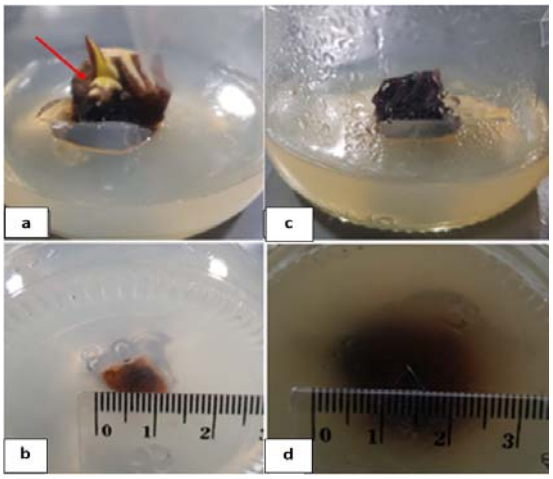

Figure 8. Shoot initiation stage of *M. paradisiaca* tissue culture.

a. The upper view of shoot initiation (red arrow) of explant that had slightly browned on culture treated with *C. aurantifolia* extract. b. The bottom view of explant that have slightly browned in culture treated with *C. aurantifolia* extract. c. The upper view of the explant that shows large browning and did not grow buds in the culture without *C. aurantifolia* extract treatment. d. The bottom view of the explant that shows large browning in the culture without *C. aurantifolia* extract treatment.

The treatment of *C. aurantifolia* peel extract can reduce browning in *M. paradisiaca* explants as indicated by a lower browning index and shoot growth initiation (Figure 8a,b). Whilst the treatments without *C. aurantifolia* peel extract showed a higher browning index, stunted growth, and no shoots appeared as shown in Figure 8c,d. It assumes that oxidation process which leads to a cell death is inhibited by polyphenols contained in the peel extract of *C. aurantifolia* (Taranto *et al.*, 2017).

3.6. The activity of polyphenol oxidase enzyme (PPO)

After 3 and 6 days, there was no significant change in the PPO activity in explants cultured compared to controls. However, the PPO activity of the sample was significantly lower than the control. This occurred because a longer incubation time increases the interaction between the active substances contained in the extract of *C. aurantifolia* peel with banana explants. The PPO activity with 0.3 mg/mL peel extract of *C. aurantifolia* was slightly lower than 0.1 mg/mL and 0.2 mg/mL after 3 days. Therefore, the administration of antioxidants significantly inhibited PPO activity, specifically treatment 0.3 mg/mL which had a better effect on inhibiting PPO activity after 9 days (Figure 9).

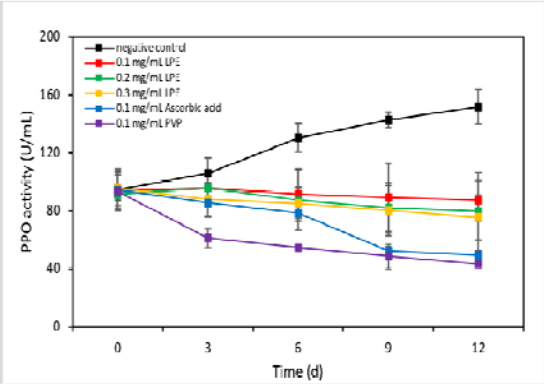

Figure 9. Effect of *C. aurantifolia* peel extract concentration on the PPO enzyme activity (U/mL) of *M. paradisiaca* explants at the shoot initiation stage. Each value is expressed as mean ± SD (n = 3).

The antioxidant compounds extracted from the *C. aurantifolia* peel extracts play a salient role in scavenging the ROS produced when *M. paradisiaca* explants are injured. Furthermore, the presence of ROS explosion affected membrane integrity and led to a loss of cellular compartmentalization, which caused the accumulation and oxidation of phenolic compounds (Xu *et al.*, 2015). The released phenolic acts as a signaling molecule and promotes an increase in PPO levels through feedback regulation. Similarly, bioactive compounds contents also can inhibit the function of oxidant enzymes such as nitric oxide synthase (NOS), lipoxygenase (LOX), xanthine oxidase (XO), cyclooxygenase (COX), NADPH oxidase (NOX), and myeloperoxidase (MPO), which are the main promoters of ROS formation (López-Alarcón and Denicola, 2013).

4. Conclusion

This research shows that ethanol solvent produces the highest extract yield for *C. aurantifolia*. However, the highest yield quantity obtained does not have the highest antioxidant activity. The best IC_{50} value (0.19 mg/mL) obtained in the ethyl acetate extract was higher compared to ethanol and n-hexane extracts. It is assumed that naringin ($C_{27}H_{32}O_{14}$) and epimedin A1 ($C_{39}H_{50}O_{20}$) are the compounds that contribute to the antioxidant activity of ethyl acetate extract. Ethyl acetate extract of *C. aurantifolia* peel with various concentrations affected the browning index inhibition and PPO enzyme activity. The treatment that gave the best response to the inhibition of

the browning index and PPO enzyme activity was 0.3 mg/mL ethyl acetate extract concentration. Therefore, *C. aurantifolia* peel can be used as an antioxidant for reducing the browning in banana propagation.

Acknowledgements

The authors are grateful to the Ministry of Research and Technology/National Research and Innovation Agency for funding through the Basic Research Grants for Higher Education (PDUPT) scheme (No. 1207/UN6.3.1/PT.00/2021) and the Universitas Padjadjaran for ALG of Prof. Tati Herlina (No. 1959/UN6.3.1/PT.00/2021) and Prof. Euis Julaeha (No: 2203/UN6.3.1/PT.00/2022).

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