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Antioxidant Activity of Cellulosic Waste Extracts

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Several cellulosic wastes have a good potential as the bioactive substances. The cellulosic waste means part of organic products being waste or well-known as by-products. They are commonly found either in peel, seed, or husk part. This study aimed to determine the potential antioxidant of cellulosic waste from selected fifteen fruiting peel extracts. Among them, the methanol extract of coconut husk (*Cocos nucifera* L.) had the highest antioxidant activity then followed by methanol extracts of avocado (*Persea americana*) and peanut (*Arachis hypogaea*) peels. The extracts showed DPPH inhibitory activity with IC_{50} values of 8.74, 24.85, and 43.64 µg/mL, respectively. In addition, the ABTS inhibitory activity showed that the methanol extract of *C. nucifera* husk was the highest activity as well with the IC_{50} value of 3.29 µg/mL compared with trolox (IC_{50} 3.11 µg/mL) as a positive control. Therefore, this study reported that the cellulosic waste of *C. nucifera* husk extract should be served as antioxidant source.

Keywords: antioxidant; cellulosic waste; peel extracts; IC_{50}; DPPH; ABTS

I. INTRODUCTION

Each year, there are about 1.3 billion tons of food which are wasted around the world (Gustavsson *et al.*, 2011). *Food and Agriculture Organization* (FAO) reported that both food loss and waste are to be a serious global issue because one-third of all food is specially produced for daily human food. Among any food products, fruits and vegetables are the highest wastage rates to 45 %. In addition, roots, and tubers at percentage of 45 % are wasted after human consumed them as well. It should be noted that food waste is dominated with organic by-products. From these reasons, FAO makes "save food" as an initiative program to decreasing food loss and waste problems. For supporting this program, making organic by-products to be more useful is such a brilliant solution.

We have reported that natural organic products have good biological activities as antidiabetic (Fatmawati *et. al.*, 2010; Fatmawati *et. al.*, 2011a; Fatmawati *et. al.*, 2011b; Fatmawati *et. al.*, 2014a; Fatmawati *et al.*, 2014b), anticancer (Sukandar *et. al.*, 2016; Sukandar *et al.*, 2018), antibacterial (Ramadhania *et. al.,* 2018; Ramadhania *et al.,* 2019) as well as antioxidant (Putri *et. al.,* 2018; Putri & Fatmawati, 2019). Nowadays, the usage of natural products related to traditional medicine is growing rapidly. The traditional medicines of natural products have a small side effect. They are commonly found in some plants. In addition, they have active compounds namely the secondary metabolites which are needed as an immunomodulatory for a good lifestyle (Izzo & Ernst, 2009). Moreover, phytochemical compounds obtained from plants can be used as single therapeutic agents or combined formulations in drug development.

World Health Organization (WHO) estimates that 80 % of people around the world depend on traditional medicine for health and they are used to consuming the herbal medicines as their health supplements. In this decade, we reported that many of traditional herbal medicines based on ethnobotanical studies are recommended as antioxidant agents. Recently, *Ananas comosus* peel extract was reported as antioxidant against DPPH and ABTS radicals (Putri *et al.,* 2018). As we know, *A. comosus* peel is one of the food wastes

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from the fruiting body. However, it exhibited a significant antioxidant effect. These results implied that either natural organic products or by-products have the bioactive substances which can inhibit free radical.

Free radical or called as reactive oxygen species (ROS) gives any negative effects for the human body. This ROS is a very reactive electron specimen which is caused by several factors such as pollution, lifestyle, or UV radiation. As a result, that reactive radical attacks some of cell tissues in the body. From these evidence, ROS can cause any metabolic disorder or chronic diseases including diabetes, cancer, infection, cardiovascular disease, etc. However, the herbal medicines will donor a proton to deactivating the radical effects. Hence, anti-free radical is urgently needed to protect the human body.

This present study aimed to determine the potential antioxidant of cellulosic waste from selected fifteen plants. They were some of the common organic by-products such as peanut peel (*Arachis hypogaea* L.), soybean peel (*Glycine max* L. Merr), coconut palm peel (*Elaeis guineensis*), coconut husk (*Cocos nucifera* L.), palmyra peel (*Borassus flabellifer*), areca nut peel (*Areca catechu*), corn husk and cob (*Zea mays* L.), tanduk banana peel (*Musa balbisiana*), kepok banana peel (*Musa paradisiaca*), langsat peel (*Lansium parasiticum*), avocado peel (*Persea americana* Mill.), pumpkin peel (*Cucurbita moschata*), sweet potato peel (*Ipomoea batatas*), and bagasse (*Saccharum officinarum* L.). As we know, the cellulosic waste of fruiting body is commonly used to being a waste and some of them has been processed into animal feed ingredients. Thus, this research provided the information about the more valuable of them and further research can be carried out to determine their bioactive compounds.

II. MATERIALS AND METHOD

A. Materials

The selected fifteen cellulosic wastes in this study were peel of peanut peel (*A. hypogaea* L.), soybean peel (*G. max* L. Merr), coconut palm peel (*E. guineensis*), coconut husk (*C. nucifera* L.), palmyra peel (*B. flabellifer*), areca nut peel (*A. catechu*), corn husk and cob (*Z. mays* L.), tanduk banana peel (*M. balbisiana*), kepok banana peel (*M. paradisiaca*), langsat peel (*L. parasiticum*), avocado peel (*P. americana* Mill.), pumpkin peel (*C. moschata*), sweet potato peel (*I. batatas*), and bagasse (*S. officinarum* L.). The samples were collected from the cellulosic waste in Keputih traditional market, Surabaya, Indonesia. The used solvents for extraction were methanol, ethyl acetate, dichloromethane, and *n*-hexane. The chemicals used in determining antioxidant activity were DPPH solution, ABTS solution, dimethyl sulfoxide (DMSO), Potassium persulfate ($K_2S_2O_8$), ethanol and distilled water. The used tools included glassware, shaker, rotary evaporator, UV-vis spectrophotometer, laminar flow, autoclave, vortex, analytical scale, and incubator.

B. Extraction

Each of the selected fifteen fruiting peels or wastes was washed and dried at room temperature. Then the sample was milled in small pieces to facilitate the absorption of solvents in the process. The sample was weighed (25 g) and macerated using methanol, ethyl acetate, dichloromethane, and *n*-hexane for 24 hours. After that, the sample was filtered with Whatman No 1 filter paper and then the extract was obtained by using a rotary evaporator. The extract was weighed to determine the yields of each extract.

C. DPPH Radical Scavenging Assay

Evaluation of DPPH radical scavenging activity was assayed according to our previous reported method (Putri and Fatmawati, 2019). Each of crude extracts (10 mg) was dissolved in 1 mL methanol. The solution of crude extracts was taken of 33.33 µL and mixed with 1 mL of DPPH radical solution (6×10^{-5} M). The solutions were mixed with vortex for 10 seconds. After 20 min incubation for 37°C, absorbance of the reaction mixtures was measured at 515 nm by spectrophotometer (UV-Vis) as A_s value. A blank sample was prepared with methanol of 33.33 µL and 1 mL DPPH radical solution as A_b value. Trolox was used as a positive control. The experiment was assayed in triplicate. Then, the antioxidant activity was determined based on the Equation (1).

$$\text{Inhibition (\%)} = [(A_b - A_s)/A_b] \; x \; 100 \qquad (1)$$

Where A_s: Sample absorbance and A_b: Blank absorbance.

D. ABTS Radical Cation Scavenging Assay

ABTS activity was assayed by previous method (Putri and Fatmawati, 2019). The solution was made from ABTS solutions (5 mL, 7 mM) and $K_2S_2O_8$ solutions (88 µL, 140 mM). The solution was allowed for 12-16 hours at dark place room temperature. The mixture was added by ethanol of 99.5% to give an absorbance of 0.7 ± 0.02 at 734 nm. Each of crude extracts (10 mg) was dissolved in 1 mL DMSO. The solution of crude extracts was taken of 10 µL and mixed with 1 mL ABTS. The solutions were mixed with vortex for 10 seconds. After 4 min incubation for 37°C, the absorbance value (A_s) was measured at 734 nm by spectrophotometer (UV-Vis). A blank sample (A_b) was prepared with methanol of 10 µL and 1 mL ABTS. Trolox was used as a positive control. The experiment was carried out in triplicate. Antioxidant activity was calculated using the Equation (1).

E. Statistical Analysis

All the results were expressed as mean ± SD of triplicate measurements. The data is analysed with two independent variables (IC_{50} values of DPPH and ABTS) by using a single-factor ANOVA. The result showed that there were significant differences ($p > 0.05$) of the extracts. The significant value was set at $\alpha = 0.05$ and the graphical representations were designed by Microsoft Excel 2016.

III. RESULT AND DISCUSSION

A. Extraction of the Fifteen Cellulosic Wastes

The sixty crude extracts from the selected fifteen fruiting peels as cellulosic waste had been obtained. Several organic solvents had been used to obtain the crude extracts, such as *n*-hexane, dichloromethane, ethyl acetate, and methanol. Among these extracts, the methanol extracts showed the highest yield. The percentage of methanol extracts yield was showed in Table 1. The results showed that methanol extract of *L. parasiticum* peel has the highest yield of all methanol extracts with yield of 23.0 % from weigh *L. parasiticum* peel of 25.0 g.

Table 1. The yield of methanol extracts of the selected fifteen fruiting peels

No.	Methanol extract samples	Yield (%)
1	*A. hypogaea* peel	2.1
2	*G. max* peel	8.5
3	*E. guineensis* peel	20.3
4	*C. nucifera* husk	4.0
5	*B. flabellifer* peel	7.4
6	*A. catechu* peel	7.0
7	*Z. mays* husk	7.5
8	*Z. mays* cob	9.5
9	*M. balbisiana* peel	4.3
10	*M. paradisiaca* peel	11.4
11	*L. parasiticum* peel	23.0
12	*P. americana* peel	11.0
13	*C. moschata* peel	1.0
14	*I. batatas* peel	2.5
15	*S. officinarum* waste	16.8

B. Extraction of the Fifteen Cellulosic Wastes

Antioxidant activity of the fifteen fruiting peel extracts was evaluated by DPPH and ABTS assays at concentration of 100 µg/mL. The results showed that the methanol extracts presented approximately the highest inhibitory activity against DPPH radical of all extracts. As presented in Figure 1, the methanol extracts exhibited inhibitory activity at percentage value of 21.25 – 77.52 %. Other extracts such as *n*-hexane, dichloromethane, and ethyl acetate extracts showed inhibitory activities at percentage values of 0.8 – 68.71, 0.19 – 71.05, and 2.53 – 77.92 %. Among percentage values of the fifteen methanol extracts, methanol extract of *C. nucifera* husk showed the lowest inhibitory concentration with IC_{50} value of 8.74 µg/mL as presented in Figure 2(a). In addition, methanol extract of *C. nucifera* husk also showed the highest inhibitory activity against ABTS radical cation with IC_{50} value of 3.29 µg/mL as presented in Figure 2(b) and summarised in Table 2.

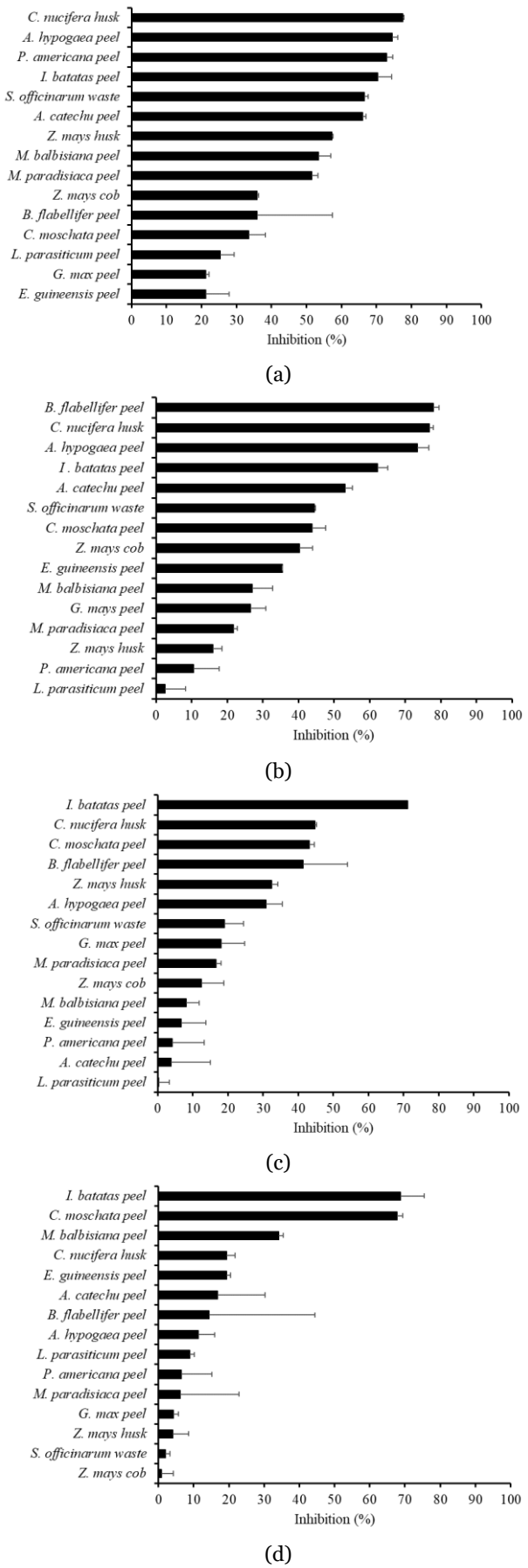


Figure 1. Inhibitory activity of (a) methanol, (b) ethyl acetate, (c) dichloromethane, and (d) *n*-hexane extracts against DPPH at concentration of 319.46 µg/mL.

C. Discussion

The extracts of 15 cellulosic wastes were assayed for their inhibitory activities on both DPPH and ABTS. In this study, the antioxidant activity was determined by using DPPH and ABTS methods. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical test is the most used method in antioxidant testing system. DPPH is a stable radical that reacts with compound donating a hydrogen atom. This method is based on DPPH scavenging through the addition of antiradical species or an antioxidant which causes discolouration of the DPPH solution. Moreover, DPPH assay is performed by adding the sample solution with DPPH working solution in methanol. Then, the mixed solution is measured absorbance value at wavelength 515 nm. Thus, decreasing the absorbance value of the reaction mixture indicates radical scavenging activity (Krishnaiah *et al.,* 2011).

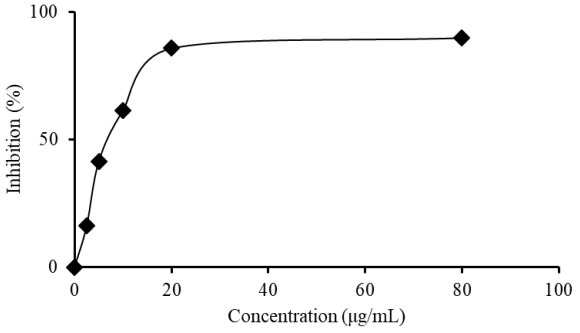
ABTS or known as 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid is also familiar in antioxidant assay because of simple and very fast. It was the first method recognised by Rice-Evans and Miller (1994) and was developed by Re *et al.* (1999). This method is based on activation of metmyoglobin and hydrogen peroxide with ABTS radical to produce ABTS radical cation. Then, this radical solution shows a blue colour through the reaction of ABTS radical cation with potassium persulfate because ABTS cation radicals are produced by the oxidation of ABTS with potassium persulfate. Thus, the antioxidant side donating a hydrogen was measured by spectrophotometry at wavelength 734 nm (Re *et al.,* 1999). Moreover, ABTS radical cation is soluble in water and organic solvents. It is not affected by ionic strength so that it can be used in several media to determine the antioxidant capacity of extracts that both of hydrophilic and lipophilic. Thermodynamically, a compound can inhibit ABTS radical cation. If the compound has a low redox potential of ABTS about 0.68 V so that it can react with ABTS easily, the phenolic compounds for example (Prior *et al.,* 2005). Therefore, it can be assumed that the test extract contained phenolic compounds is able to inhibit the activity of ABTS radical cation. Both DPPH and ABTS methods are recommended to determine antioxidant activity spectrophotometrically.

Table 2. IC_{50} values of antioxidant activity by using DPPH and ABTS

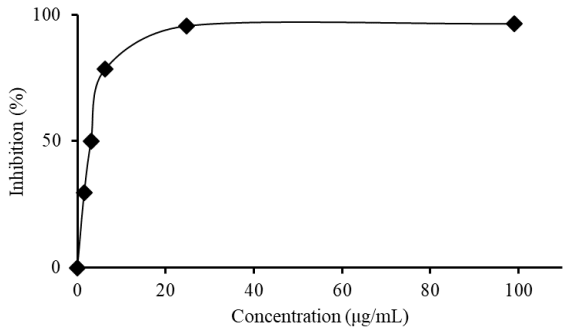
Extracts	IC_{50} (µg/mL) ± SD	
	DPPH	ABTS
Methanol *C. nucifera* husk	8.74 ± 2.24	3.29 ± 0.63
Methanol *P. Americana* peel	24.85 ± 1.29	33.08 ± 0.73
Methanol *A. hypogaea* peel	43.64 ± 1.04	27.28 ± 0.23
Ethyl acetate *A. hypogaea* peel	58.06 ± 1.45	33.34 ± 0.33
Ethyl acetate *B. flabellifer* peel	70.25 ± 0.56	48.41 ± 0.11
n-Hexane *C. moschate* peel	74.73 ± 2.61	79.20 ± 1.16

As the results in Figure 1 and 2, the DPPH inhibitory effects of various extracts such as methanol, ethyl acetate, dichloromethane, and *n*-hexane had been demonstrated. Here, we focus on the top three of the lowest IC_{50} value which is implied as antioxidant sources from the cellulosic waste based on our results. First is about the best antioxidant activity of fifteen cellulosic waste namely *C. nucifera* husk. The results showed that methanol extract of *C. nucifera* husk presented the highest inhibitory effect of 77.52 % compared with those of other extracts. Then, the methanol extract of *C. nucifera* husk was determined its IC_{50} value. Interestingly, the IC_{50} value of *C. nucifera* husk was almost the same as that of a positive control, trolox. These results meaned that *C. nucifera* husk was strongly recommended for antioxidant source. In addition, a good antioxidant activity had been showed by ethyl acetate extract of *C. nucifera* husk as well. These results implied that most of *C. nucifera* husk might be consisted by bioactive polar compounds. Recently, two novel lignans have been isolated from ethyl acetate fraction of endocarp of *C. nucifera* (Elsbaey *et al.,* 2019). Based on the chemical structure of lignan, it has a lot of hydroxyl groups which are possible for donor proton to stabilise the radical effect. Furthermore, some previous studies also reported that *C. nucifera* extracts had showed inhibitory activity against DPPH (Arivalagan *et. al.,* 2018; Thebo *et. al.,* 2016; Chakraborty & Mitra, 2008). Thus, it could be inferred that coconut husk is potential as a health supplement.

Next was about the inhibitory effect of the cellulosic waste of *P. americana*. As we know, the fruiting body of *P. americana* is one of dietary foods consisting of low fat. Lately, there was a report about the characterisation of *P. americana*. That study demonstrated that *P. americana* contains perseitol (Bonvehi *et al.,* 2019), a sugar alcohol founded only in *P. americana*. In this present study, *P. americana* peel as a cellulosic waste was reported its inhibitory activity against DPPH and ABTS. These results showed that the methanol extract of *P. americana* peel presented inhibitory activity against DPPH and ABTS significantly. Rodriguez-Carpena *et al.* (2011) reported that ethyl acetate extract of avocado peel has a good antioxidant activity (Rodriguez-Carpena *et al.,* 2011). However, methanol extract of *P. americana* peel was higher inhibitory activity than that of the ethyl acetate extract in our present studies. Furthermore, these results were supported by some previous studies, they reported that the antioxidant activity of *P. americana* peel was better than that of *P. americana* fruit or seed (Alkhalf *et. al.,* 2018; Melgar *et. al.,* 2018; Calderon-Oliver *et al.,* 2016). In addition, *P. americana* peel from Indonesia was also reported a significant inhibitory effect against both DPPH and ABTS (Antasionasti *et al.*, 2017). Hence, from these present studies, *P. americana* peel, known as high nutritional and functional properties of avocado by-products (Araujo *et al.,* 2018), should be used as antioxidant.



(a)



(b)

Figure 2. Antioxidant activity of *C. nucifera* husk against (a) DPPH, and (b) ABTS

Furthermore, *A. hypogaea* peel also showed a significant inhibitory effect against ABTS with lower IC_{50} value. *A. hypogaea* peel contains a high total phenolic compound as well as three potentially antioxidant compounds including 5,7-dihydroxychromone, eriodictyol, and luteolin. Among them, 5,7-dihydroxychromone showed the strongest antioxidant activity to inhibiting DPPH free radicals (Qiu *et al.,* 2012) but the radical inhibitory activity of *A. hypogaea* showed not significant percentage value (Adhikari *et al.,* 2018). According to Yu *et al.* (2005) that the higher antiradical activity, the higher total phenolic compounds (Yu *et al.,* 2005). The results indicated that the ability of an extract to inhibit free radical activity depends on the concentration of total phenolic and types of phenolic compounds in the extract. Therefore, this study assumed that methanol extract of *A. hypogaea* contained the highest phenolic compounds level of others. In addition, the previous study about antioxidant activity of *A. hypogaea* also was reported in seed, seed peel, and peel parts. Among them, the highest antioxidant activity was in the seed peel with inhibition value of 89.97 %, followed by peel (61.09 %) and seed (11.00 %) (Taha *et al.,* 2012). Those result implied that *A. hypogaea* peel is a potential source as antioxidant agent.

IV. CONCLUSION

The cellulosic waste from the fruiting peels and waste is recommended as antioxidant agents. Among them, methanol extract of *C. nucifera* husk was highly recommended because it showed the highest antioxidant activity both in DPPH and ABTS assay with low IC_{50} value. Thus, this research can be used as a new finding for further investigation to identify the active compound of cellulosic waste of those fruiting peel extracts.

V. ACKNOWLEDGEMENT

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