

Feasibility study on using phenolic and polysaccharide extracts from different parts of *Manihot esculenta* (L.) Crantz as food and cosmetic active ingredients

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Abstract

Cassava (*Manihot esculenta* (L.) Crantz) is extensively used as food in tropical and subtropical regions. This study aimed to evaluate the biological properties of various parts of cassava. The crude phenolic extracts and polysaccharide fractions of two cassava cultivars (Huaybong 60 and Rayong 72) were determined for the total phenolic and carbohydrate contents as well as biological activities, including antioxidant and tyrosinase inhibition properties. Various parts of cassava were successively extracted by using *n*-hexane, ethyl acetate, and ethanol, respectively, through Soxhlet extraction. Water-soluble and insoluble polysaccharides were extracted with aqueous and aqueous alkaline extraction, respectively. The extraction solvents influenced the extractive yield of crude extracts from various parts of cassava ($P < 0.05$). The cassava peel (Rayong 72) ethanolic extract was the highest extractive yield (11.97%), while the highest extractive yield of polysaccharides was insoluble polysaccharides from cassava stem (Rayong 72) (9.19%). Cassava leaves extracts contained higher phenolic content (39.54 to 83.98 mg GAE/g extract) and flavonoid contents (110.38 to 287.78 mg QE/g extract). The total carbohydrate content in the root extracts was higher than in other parts. Cassava leaves extracts provide high potential antioxidant activities based on three assays: ferric reducing capacity (507.86 to 966.00 mg TE/g extract), DPPH radical scavenging activity (54.64 to 107.65 mg TE/g extract), and the scavenging activity of ABTS cation radical (478.37 to 989.49 mg TE/g extract). Most of the cassava crude extracts slightly inhibited tyrosinase enzyme activity. In contrast, cassava polysaccharide enhanced tyrosinase activity. The overall results proved that ethanolic extracts of cassava leaves could be considered as an alternative antioxidant active ingredient in food and cosmetics due to their antioxidant activities, whereas polysaccharide fractions, as a tyrosinase-promoting agent, could possibly be applied as a mechanism for preventing hair greying. These findings indicate feasible utilization of cassava for value-added products.

1. Introduction

Numerous synthetic compounds have been used for various approaches, including agriculture, medicine, food, and cosmetics. Considering such synthetic antioxidant and skin lightening agents, e.g., butylated hydroxytoluene (BHT), and hydroquinone, commonly used in the past, any risks or side effects in the long term have been of concern due to their toxicity as carcinogens or cell toxicity. Natural compounds that can be obtained from renewable sources are interesting, accompanied by their less effect on human health; therefore, many studies

focus on exploring new sources of natural compounds (Lobo *et al.*, 2010).

Plants contain various natural substances possessing antioxidant properties, such as tetraterpenes, carotenoids, ascorbic acid, glutathione, and phenolics. Plant phenolics are the most predominant secondary metabolites, and the major abundance in nature is flavonoids and phenolic acids (Kasote *et al.*, 2015). These exert many biological properties, including anti-inflammation, anti-diabetic, microbial inhibition, as well as anti-aging by scavenging

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reactive oxygen species (ROS) (Rahman *et al.*, 2022). Polysaccharides, one of plants' metabolites, could function in many applications, including moisturizer, stabilizer in foods and cosmetics, film former, and antioxidants. In addition, seaweed polysaccharides are capable of inhibiting tyrosinase enzyme (Jesumani *et al.*, 2020). Extraction of bioactive compounds from agricultural residuals has become interesting in the past decade, not only reducing environmental impact but also obtaining valuable natural extracts. Vast amounts of agricultural wastes, such as citrus peels, maize cob, grape pomace, and potato peels, have been investigated for their feasibility as natural sources of bioactive compounds possessing anti-diabetic, antimicrobial, anti-inflammatory, and antioxidant activities (Oleszek *et al.*, 2023).

Manihot esculenta (L.) Crantz (cassava), belonging to the family *Euphorbiaceae*, is a commercial tuber plant in Thailand, which is one of the largest production areas. Among cassava cultivars, Huay bong 60 and Rayong 72 are generally grown in the Northeastern part of Thailand (Fu *et al.*, 2014). Its root is a source of starch, while the other portions, such as leaves, stem, and tuber peel, are normally used as animal feeds or abandoned. Recently, for their feasibility to be used as functional food, nutraceutical and cosmetic purposes have been studied. Various biological functions, including hepatoprotective, antimicrobial, anti-inflammatory, anti-melanogenesis and antioxidant properties, have been observed in cassava leaves (Mustarichie *et al.*, 2020; Dewi and Normasari, 2021; Jampa *et al.*, 2022) as well as their capability to increase antioxidant enzymes *in vivo* (Bahekar and Kale, 2016). Cassava leaves could be possible to be applied in cosmetics as sunscreen, lightening and anti-aging by enhancing collagen synthesis (Jampa *et al.*, 2022). Antioxidant properties are also observed in cassava stems (Yi *et al.*, 2011). Phenolic compounds, such as kaempferol-3-*o*-rutinoside, myricetin-3-*o*-rutinoside, quercetin-3-*o*-rutinoside (rutin) from cassava roots and leaves have been isolated and identified (Blagbrough *et al.*, 2010). However, there is scarce scientific data in comparison among all parts of cassava as well as polysaccharides and their biological properties, including antioxidant and tyrosinase inhibitory potential. Therefore, phenolic and flavonoid contents, as well as biological functions including antioxidant and anti-tyrosinase properties of various parts of cassava extracts and polysaccharide fractions, were evaluated for their feasibility to be applied in food and cosmetic purposes. This study aimed to comparatively investigate and identify the variations in bioactive composition and biological activities among different cassava parts and their polysaccharide fractions.

2. Materials and methods

2.1 Materials and chemicals

Two cultivars of cassava (Huaybong 60 and Rayong 72) were collected in June 2015 from Nakorn Ratchasima province, Thailand. The collected plant materials were separated into 4 parts (peels, roots, leaves, and stems), washed with distilled water, and then dried at $50\pm 2^\circ\text{C}$ by a circulating hot air oven. Dried samples were powdered and sieved (250-mesh), obtaining a sample powder which was then stored (4°C) before being used for the preparation of the extracts. 2,2'-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid) (ABTS), L-3,4-dihydroxyphenylalanine (L-DOPA), 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]), Folin Ciocalteu's reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), kojic acid, and 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), were obtained from Sigma-Aldrich (Steinheim, Germany).

2.2 Preparation of phenolic and polysaccharide extracts from *Manihot esculenta*

The extraction procedure of phenolic, water-soluble, and water-insoluble polysaccharides was conducted according to the slight modification of the methods described by Hadzri *et al.* (2014), Fan *et al.* (2011), and Peng *et al.* (2005), respectively. Briefly, a sample (10 g) was successively extracted using a Soxhlet apparatus for 6 h with each solvent (200 mL) as follows: hexane, ethyl acetate, and ethanol. The crude phenolic extracts were obtained after each mixture was filtered through filter paper (Whatman® no. 1), evaporated and finally lyophilized.

After phenolic extraction, polysaccharide fractions were obtained from each cassava residue. In brief, distilled water was added to cassava residue from phenolic extraction with an LS ratio of 15:1 (mL:g), stirred, and then boiled for 2 h. After filtration, the original volume of the filtrate was concentrated to 1/5. Consequently, the obtained solution was added with cold 95% ethanol (4 ×) and the mixture was stored at 4°C in a dark place overnight. To obtain a water-soluble polysaccharide extract, the precipitate was filtered, washed with cold 95% ethanol, and finally lyophilized. Water-insoluble polysaccharides were extracted with alkaline extraction by using the residue from the extraction of water-soluble polysaccharides. Dried residues (10 g) were mixed with 1N sodium hydroxide (LS ratio of 50:1) for 2 h at ambient temperature. After that, the solution was filtered and neutralized with acetic acid. After centrifugation (4000 rpm, 20 min). The precipitate was collected and lyophilized to obtain water-insoluble polysaccharide extracts. All abbreviations of the extracts are shown in Table 1.

Table 1. Abbreviations of the extracts used in this study.

Plant part	Cultivars	Extraction solvent				
		Crude extract			Polysaccharides	
		<i>n</i> -Hexane	Ethyl acetate	Ethanol	Water soluble	Water insoluble
Peels	Huay Bong 60	PH60	PEc60	PEo60	PW60	PN60
	Rayong 72	PH72	PEc72	PEo72	PW72	PN72
Roots	Huay Bong 60	RH60	REc60	REo60	RW60	RN60
	Rayong 72	RH72	REc72	REo72	RW72	RN72
Leaves	Huay Bong 60	LH60	LEc60	LEo60	LW60	LN60
	Rayong 72	LH72	LEc72	LEo72	LW72	LN72
Stems	Huay Bong 60	SH60	SEc60	SEo60	SW60	SN60
	Rayong 72	SH72	SEc72	SEo72	SW72	SN72

2.3 Determination of total phenolic content

The total phenolic content was determined based on Folin-Ciocalteu's assay as previously described by Waterman and Mole (1994). Briefly, 0.5 mL of Folin-Ciocalteu's reagent was added to the solution containing 7.5 mL of deionized water and 0.5 mL of the sample solutions. After that, 1.5 mL of 200 g/L sodium carbonate solution, and the mixtures were incubated at an ambient temperature (1 h). The absorbance measurement for the estimation of total phenolic content was conducted at 760 nm, and the results are stated as gallic acid equivalents (mg GAE/g extract).

2.4 Determination of total flavonoid content

The aluminium colorimetric assay described by Sathishkumar *et al.* (2008) was used to determine the total flavonoid content. Briefly, 3.7 mL of the sample solution was mixed with 0.15 mL of 50 g/L sodium nitrite solution, and then 0.15 mL of 100 g/L aluminium chloride solution was added. and 1.0 mL of 40 g/L sodium hydroxide. The absorbance at 510 nm was measured after the mixtures were incubated at an ambient temperature (5 min), and the results are stated as quercetin equivalents (mg QE/g extract).

2.5 Determination of total carbohydrate content

The amount of polysaccharide was determined based on phenol-sulfuric acid carbohydrate assay (Masuko *et al.*, 2005) with slight modifications. Briefly, the sample solutions (1 mL) were hydrolyzed to monosaccharide by the addition of concentrated sulfuric acid (3 mL), and then immediately reacted with 6 mL of 50 g/L phenol solution. After the mixture was incubated at 90°C (5 min), the reaction was stopped by cooling down in an ice bath. The absorbance at 490 nm was measured, and the results are stated as glucose equivalents (mg GE/g extract).

2.6 Antioxidant assays

2.6.1 DPPH scavenging activity

The DPPH scavenging activity was evaluated by

using the method previously described by Gülçin *et al.* (2003). Briefly, 1 mL of sample solution was mixed with 3 mL of 0.1 mmol/L DPPH solution. The absorbance at 517 nm was measured after the mixtures were incubated in dark conditions at an ambient temperature (30 min). A control containing 3 mL of DPPH solution mixed with 1 mL of ethanol was prepared. The following equation was used to calculate the percent inhibition:

$$\% \text{ DPPH scavenging activity} = [(A_c - A_s)/A_c] \times 100$$

Where A_c : the absorbance of the control solution without sample; A_s : the absorbance of the test solution with the sample. Trolox was used as comparative compound, and the results are stated as trolox equivalents (mg TE/g extract).

2.6.2 Ferric reducing antioxidant power assay

The ferric reducing capacity as an antioxidant power was determined based on the method previously described by Benzie and Strain (1996). Briefly, ferric reducing antioxidant power (FRAP) reagent containing 100 mL of 10 mM TPTZ solution in 40 mM hydrochloric acid, 100 mL of 20 mM ferric (III) chloride and 25 mL of 300 mM acetate buffer (pH 3.6) was prepared once used. An aliquot of sample solution (0.1 mL) was mixed with 1.5 mL of FRAP reagent (37°C) and 1.4 mL of 300 mM acetate buffer (pH 3.6). The absorbance at 593 nm was measured after the incubation of the mixture at ambient temperature (30 min). The results are stated as trolox equivalent (mg TE/g extract).

2.6.3 ABTS⁺ scavenging activity

The determination of ABTS⁺ scavenging activity was conducted based on the method described by Re *et al.* (1999). Briefly, the solution of ABTS⁺ was prepared through the reaction between 2.45 mM potassium persulphate and 7mM ABTS solution with an equal volume in dark conditions at ambient temperature (16 h). The 3 mL of ABTS⁺ solution (diluted with 50 mM potassium phosphate buffer (pH 7.4) to an absorbance at 734 nm of 0.70±0.02) was mixed with the 0.1 mL of

sample solutions. The absorbance at 734 nm was measured after the mixture was incubated at ambient temperature (30 min). The results are stated as trolox equivalent (mg TE/g extract).

2.7 Tyrosinase inhibition assay

The determination of tyrosinase inhibition activity was conducted based on the L-DOPA substrate assay (Vardhan et al., 2014). Briefly, a sample solution was mixed with 110 μ L of 50 mM potassium phosphate buffer (pH 6.8) and 20 μ L of 1000 U/mL mushroom tyrosinase solution in 50 mM potassium phosphate buffer (pH 6.8). A 50 μ L of 10 mM L-DOPA was added after the mixture was incubated at 37°C (10 min). Finally, the mixture was incubated at 37°C (10 min), and the absorbance at 475 nm was measured. The mixture without the sample was used as a control. The following equation was used to calculate tyrosinase inhibition.

$$\% \text{ Tyrosinase inhibition} = [(A_c - A_s)/A_c] \times 100$$

Where A_c : the absorbance of the control solution (without sample); A_s : the absorbance of the test solution with the sample. Kojic acid was used as a comparative compound, and the results are stated as kojic acid equivalents (mg KE/g extract).

2.8 Statistical analysis

All measurements were determined in triplicate, and the results are shown as mean \pm S.D. All statistical analyses were conducted by the SPSS program version 21 (SPSS Inc, USA). One-way analysis of Variance (ANOVA) (Duncan's multiple range test) was applied to determine significant differences ($P < 0.05$).

3. Results

Various parts of cassava were successively extracted to obtain crude phenolic extracts and polysaccharide fractions, and the amount of phenolics and

polysaccharides, as well as antioxidant and anti-tyrosinase activities, were then evaluated. The crude phenolic and polysaccharides from the extraction of various cassava parts had extractive yields that varied from 0.31-11.97% (w/w), as shown in Table 2. The extraction yields of crude extracts and polysaccharides were in the range from 0.31 (REc72) to 11.97 (PEo72) and 0.32 (PW60) to 5.42 (SW72), respectively. In comparison, there were differences in extractive yields among the plant parts ($P < 0.05$), with a high extractive yield obtained from cassava leaves. Secondary metabolites synthesized by plants could be found in all parts; however, they vary in their amount and types. In addition, bioactive compounds' solubility depended on their chemical structures, which relate to polarity. This phenomenon, therefore, could influence the extraction efficiency of plant bioactive compounds by using different solvents. The obtained phenolic and polysaccharide extracts were tested for their solubility in various solvents. From observation, hexane and water-insoluble polysaccharide extracts were less soluble to all solvents tested in the study. Therefore, ethyl acetate, ethanolic extracts, and water-soluble polysaccharide extracts were selected to be further analyzed. evaluated for phenolic, flavonoid, and carbohydrate (for water-insoluble polysaccharide) contents, antioxidant activities and tyrosinase inhibitory property.

3.1 Determination of phenolic, flavonoid and carbohydrate contents

Folin-Ciocalteu's and aluminium chloride colorimetric methods were used to estimate the amount of phenolic and flavonoid contents in cassava extracts, respectively. As observed in Table 3, the crude extracts of LEO60 contained the highest total flavonoid content, followed by the LEC60, and PEO60 extracts ($P < 0.05$). This finding indicated that cassava cultivar Huaybong 60 could be considered as a potential source of phenolics and flavonoids, especially its leaves and stem, which

Table 2. Extraction yield of *Manihot esculenta* (L.) Crantz in various cultivars, parts used, and extraction solvents.

Plant part	Cultivars	Extraction solvent				
		Crude extract			Polysaccharides	
		<i>n</i> -Hexane	Ethyl acetate	Ethanol	Water soluble	Water insoluble
Peels	Huay Bong 60	1.80 \pm 0.80 ^{Bbcd}	1.76 \pm 1.16 ^{BCbcd}	4.11 \pm 1.64 ^{Bb}	0.32 \pm 0.05 ^{Bd}	3.61 \pm 0.49 ^{Abc}
	Rayong 72	1.99 \pm 0.09 ^{Bbcd}	3.71 \pm 1.29 ^{Bbc}	11.97 \pm 3.03 ^{Aa}	1.19 \pm 0.6 ^{Bcd}	4.13 \pm 1.51 ^{Ab}
Roots	Huay Bong 60	0.86 \pm 0.05 ^{Bb}	0.50 \pm 0.3 ^{BCb}	4.32 \pm 1.63 ^{Bab}	1.09 \pm 0.18 ^{Bb}	7.43 \pm 5.63 ^{Aa}
	Rayong 72	0.47 \pm 0.16 ^{Bb}	0.31 \pm 0.22 ^{Cb}	4.69 \pm 3.92 ^{Bab}	0.91 \pm 0.23 ^{Bb}	1.88 \pm 1.26 ^{Ab}
Leaves	Huay Bong 60	10.54 \pm 4.36 ^{Aab}	9.04 \pm 4.32 ^{Aab}	5.61 \pm 2.63 ^{Bab}	5.09 \pm 2.09 ^{Aab}	9.01 \pm 3.04 ^{Aab}
	Rayong 72	11.33 \pm 6.94 ^{Aa}	3.62 \pm 0.33 ^{Bb}	4.71 \pm 1.06 ^{Bab}	3.67 \pm 1.44 ^{ABb}	7.42 \pm 2.74 ^{Aab}
Stems	Huay Bong 60	1.23 \pm 0.07 ^{Bb}	1.20 \pm 0.15 ^{BCb}	2.25 \pm 0.42 ^{Bb}	0.75 \pm 0.03 ^{Bb}	3.08 \pm 2.67 ^{Ab}
	Rayong 72	1.70 \pm 0.43 ^{Bb}	1.65 \pm 0.63 ^{BCb}	4.62 \pm 2.01 ^{Bab}	5.42 \pm 3.13 ^{Aab}	9.19 \pm 4.89 ^{Aa}

Values are presented in mean \pm SD of triplicate. Values with different uppercase superscripts in the same column are statistically significantly different ($P < 0.05$) while values with different lowercase superscripts are statistically significantly different among the different plant parts of each cultivar ($P < 0.05$).

Table 3. Total phenolic, flavonoid, and carbohydrate contents, FRAP, DPPH and ABTS radical scavenging activity, percent inhibition of tyrosinase enzyme, and percent activation of tyrosinase enzyme of *Manihot esculenta* (L.) Crantz extracts.

Extract	Total phenolics ¹	Total flavonoids ²	Total carbohydrate ³	FRAP ⁴	DPPH radical scavenging activity ⁴	ABTS radical scavenging activity ⁴	% Inhibition tyrosinase enzyme	% Activation tyrosinase enzyme
PEc60	50.77±3.32 ^{DE}	224.71±11.83 ^B	NA	520.18±2.52 ^F	64.98±1.42 ^E	483.83±3.12 ^{EF}	12.18±2.73 ^{CD}	-
PEo60	66.16±0.65 ^C	279.90±6.83 ^A	NA	940.32±15.98 ^B	57.95±5.06 ^F	825.03±18.76 ^C	19.87±0.45 ^A	-
REc60	47.18±1.30 ^F	216.82±18.07 ^B	NA	531.48±5.24 ^F	39.25±4.76 ^H	376.01±5.15 ^H	5.56±1.44 ^E	-
REo60	6.68±0.43 ^L	189.23±0.00 ^C	NA	40.47±5.81 ^L	16.90±0.70 ^I	83.94±0.00 ^M	ND	-
LEc60	42.78±0.59 ^G	287.78±6.83 ^A	NA	507.86±15.31 ^F	92.84±2 ^{BC}	478.37±14.38 ^F	ND	-
LEo60	83.98±0.75 ^A	275.95±6.83 ^A	NA	966±27.60 ^A	90.17±0.43 ^C	989.49±15.90 ^A	14.10±0.45 ^{BC}	-
SEc60	51.81±0.82 ^D	201.05±0.00 ^B	NA	637.29±8.72 ^D	63.12±2.53 ^E	535.01±3.13 ^D	2.78±1.34 ^{EF}	-
SEo60	42.20±0.59 ^G	201.05±0.00 ^B	NA	438.01±2.91 ^G	72.00±1.32 ^D	415.59±7.38 ^G	18.16±1.18 ^A	-
PEc72	25.31±0.28 ^I	90.67±6.83 ^{EF}	NA	294.20±8.09 ^J	48.14±1.24 ^G	260.00±5.42 ^K	17.95±1.05 ^A	-
PEo72	22.65±0.59 ^K	110.38±27.31 ^E	NA	348.64±10.17 ^I	95.86±1.96 ^B	324.15±8.52 ^I	9.51±0.2 ^D	-
REc72	27.85±0.33 ^I	74.90±18.07 ^F	NA	408.22±5.24 ^H	39.66±0.79 ^H	280.47±7.38 ^J	10.58±1.98 ^D	-
REo72	6.91±0.28 ^L	67.02±24.62 ^F	NA	65.13±1.45 ^K	18.94±0.65 ^I	112.60±0.00 ^L	17.09±2.00 ^{AB}	-
LEc72	39.54±1.02 ^H	157.69±13.66 ^D	NA	586.95±18.20 ^E	54.64±0.51 ^F	496.79±13.00 ^E	ND	-
LEo72	69.98±0.16 ^B	110.38±6.83 ^E	NA	806.78±6.66 ^C	107.65±2.42 ^A	878.25±0.00 ^B	14.53±2.75 ^{BC}	-
SEc72	48.68±0.71 ^{EF}	86.73±27.31 ^{EF}	NA	564.36±11.62 ^E	37.28±0.50 ^H	479.05±13.42 ^F	ND	-
SEo72	49.61±0.28 ^{DE}	90.67±18.07 ^{EF}	NA	562.30±8.84 ^E	48.14±0.59 ^G	475.64±5.15 ^F	5.77±1.14 ^F	-
PW60	24.96±0.49 ^a	ND	1341.74±984.42 ^{bc}	386.65±8.09 ^a	53.22±0.22 ^a	358.±1.93 ^a	-	52.14±3.69 ^b
RW60	6.10±0.16 ^b	ND	1990.81±167.81 ^{ab}	106.21±5.24 ^c	6.25±0.13 ^b	73.71±0.97 ^f	-	44.12±7.61 ^b
LW60	15.82±0.33 ^b	ND	981.61±42.64 ^c	273.65±9.07 ^b	15.23±0.26 ^c	208.82±0.97 ^c	-	65.70±1.28 ^a
SW60	11.42±0.00 ^c	ND	1044.68±76.95 ^c	129.84±1.45 ^d	4.76±0.07 ^h	184.25±0.97 ^d	-	41.56±10.74 ^b
PW72	13.39±0.16 ^d	ND	1257.56±47.80 ^c	218.18±21.94 ^c	26.52±0.15 ^b	NA	-	NA
RW72	8.18±0.16 ^f	ND	2294.36±62.58 ^a	122.65±0.00 ^{de}	8.66±0.19 ^f	112.60±0.00 ^e	-	44.76±6.02 ^b
LW72	14.66±0.16 ^c	ND	205.00±13.66 ^d	262.35±1.45 ^b	18.46±0.26 ^d	245.67±3.86 ^b	-	43.70±9.83 ^b
SW72	8.30±0.57 ^f	ND	1537.46±112.82 ^{bc}	235.64±5.24 ^c	19.39±0.07 ^c	NA	-	NA
Kojic acid	NA	NA	NA	NA	NA	NA	46.30±1.98	-

¹ mg GAE/g extract, ² QE/g extract, ³ mg GE/g extract, ⁴ mg TE/g extract

Values are presented in mean±SD of triplicate. Values with different uppercase superscripts in the same column are statistically significantly different ($P<0.05$) among the different parts used of crude phenolic extracts while values with different lowercase superscripts in the same column are statistically significantly different among the different parts used of polysaccharide extracts ($P<0.05$). NA: not analyzed, ND: not determined.

were rich in flavonoids (201.05-287.78 mg QE/g extract). In comparison between cultivars, Huay Bong 60 crude extracts contained higher phenolic compounds than Rayong 70 extracts ($P < 0.05$). Among polysaccharide extracts, the PW60 possessed the highest phenolic compounds, followed by the LW60 and LW72 extracts ($P < 0.05$). However, all polysaccharide extracts contained no flavonoid compounds.

The crude phenolic and polysaccharide extracts were estimated for total carbohydrate content according to phenol-sulfuric acid assay, and the results are shown in Table 3. The results showed that all crude phenolic compounds contained no carbohydrate. The highest amount of carbohydrate was found in the RW72 extract, followed by the RW60 extract ($P < 0.05$), indicating that cassava root extract was rich in polysaccharides. However, cassava leaves (Huay Bong 60) and stems (Rayong 72) could be considered as a potential source of polysaccharides when considering carbohydrate content incorporated with extractive yield.

3.2 Antioxidant activities

The DPPH radical scavenging assay measures the capability of hydrogen atom donation of antioxidants to scavenge and convert DPPH \cdot to a non-radical form of DPPH. The crude phenolic extracts exhibited DPPH \cdot scavenging activity, ranging from 16.90 to 107.65 mg TE/g extract and the highest potential was LEO72 followed by PEO72, LEC60 and LEO60 extracts. These findings were in accordance with the phenolic and flavonoid contents of the crude phenolic extracts, which showed that cassava leaves were the most potent compared with the other parts ($P < 0.05$). Less potential antioxidant activity was found in the polysaccharide extracts (4.76-26.52 mg TE/g extract) except the cassava leaves polysaccharide extract of Huay Bong 60 cultivar (53.22 mg TE/g extract).

The evaluation of antioxidant capability to reduce ferric to ferrous ion is a mechanism used in the ferric reducing antioxidant power (FRAP) assay. The crude phenolic extracts exhibited potential reducing property in a broad range between 40.47 and 966.00 mg TE/g extract. The LEO60 extract exhibited the highest potential, followed by the PEO60, LEO72 and SEc60 extracts. Surprisingly, the polysaccharide extracts also possessed high FRAP value, ranging from 106.21 to 386.65 mg TE/g extract, especially cassava peel and leaves polysaccharide extracts of Huaybong 60 cultivar. These findings were in agreement with the phenolic contents of polysaccharide extracts.

ABTS $^{+}$ antioxidant assay is used to evaluate the capability of the extract to scavenge cation radicals. The

crude phenolic extracts possessed potential ABTS $^{+}$ scavenging activity which was in the range from 112.60 to 989.49 mg TE/g extract. Similar to the phenolic content, ethanolic extracts of cassava leaves, especially Huaybong 60 cultivar, were potentially effective as a cation radical scavenger ($P < 0.05$). Similar to reducing ability based on FRAP assay of polysaccharide extracts, cassava peel and leaves polysaccharides exhibited high potential ABTS $^{+}$ scavenging activity (208.82-358.27 mg TE/g extract). The PW72 and SW72 were not available to be evaluated because they could not be dissolved in water, which is the solvent system of this assay.

The correlations between phenolic content and antioxidant properties of crude and polysaccharide extracts were also evaluated. A high relationship between phenolic content and FRAP value and ABTS $^{+}$ scavenging activity of crude phenolic extracts ($R^2 = 0.938$ and 0.920 , respectively) and polysaccharide extracts ($R^2 = 0.783$ and 0.957 , respectively) was observed. These findings indicate that the antioxidant property evaluated in the extracts may be attributed to an electron donor mechanism. However, the DPPH scavenging activity of crude phenolic extract was less relative to phenolic content ($R^2 = 0.346$), which was in contrast to the relationship with polysaccharide extracts ($R^2 = 0.730$).

In comparison, the crude phenolic extracts of cassava leaves were rich in phenolic and flavonoid compounds with potential antioxidant properties ($P < 0.05$) compared with other cassava parts. Although polysaccharides of cassava peel (Huaybong 60) exhibited the highest antioxidant activity, cassava leaves in both cultivars could be considered as potential sources of antioxidant polysaccharides due to the fact that the extractive yield of the cassava leaf polysaccharides was greater than that of cassava peel polysaccharides.

3.3 Determination of anti-tyrosinase activity

Tyrosinase, a key enzyme in melanogenesis, is used to evaluate the potential of plant extract to suppress melanin synthesis through an enzyme inhibition mechanism that could provide a lightening effect (Park *et al.*, 2015). Therefore, the capability to inhibit tyrosinase enzyme of crude phenolic and polysaccharide extracts of cassava was evaluated. As the results shown in Table 3, the tyrosinase inhibition properties of crude phenolic extracts were found in the range between 5.56 to 19.87%, except REo60, LEC60, LEC72, and SEc72 extracts that showed no tyrosinase inhibitory effect. From the solubility test, PW72 and SW72 could not be dissolved in the buffer solution used in the condition of this assay, but were suitably dissolved by 1% (v/v) acetic acid. However, this condition could not be used to

evaluate the inhibitory effect of tyrosinase enzyme due to the limitation of pH for tyrosinase function (Naidja *et al.*, 1997). As a result, polysaccharide extracts did not inhibit tyrosinase enzyme; however, they enhanced tyrosinase activity in the range between 41.56 to 65.70%, indicating that cassava polysaccharides could increase the melanogenesis process, which might be applied to the product aimed to increase melanin pigment, such as an anti-grey hair product.

4. Discussion

Aging process in living organisms is caused by intrinsic and extrinsic factors. Excessive oxidative conditions are the main cause of the antioxidant-free radical imbalance, which accelerates the rate of biological molecule degradation. Any research focuses on evaluating agricultural residuals to be utilized as sources of antioxidants or bioactive compounds. This study successively extracted crude phenolic extracts using different solvents and polysaccharide fractions from two cultivars of cassava, including Huay Bong 60 and Rayong 72, which are local and hybrid cultivars, respectively. The total phenolic, total flavonoids, and carbohydrate were estimated, and antioxidant activity as well as tyrosinase inhibition were also evaluated. Solvent used for the extraction of phenolics is one factor affecting extraction's efficiency in terms of various profiles of natural compounds and biological activity (Borges *et al.*, 2020). The solubility of natural compounds depended on their polar compatibility with the solvent according to "like dissolves like". Alcohols, as extraction solvents, provide potential in obtaining extracts with high antioxidant properties compared with lower polar solvents such as acetone and petroleum ether. (Nawaz *et al.*, 2020). Ethanol is also considered a biobased solvent for green extraction that is suitable for cosmetic, pharmaceutical, and food ingredients industries (Chemat, 2019).

Various parts of the plant comprised different profiles of phenolics in terms of both quality and quantity (Arumugam *et al.*, 2019). Many studies found that plant leaves are rich in phenolic compounds, while stems and roots contain less. It has been reported that the leaf stalk of cassava contains a high amount of phenolics and flavonoids (Suresh *et al.*, 2011). Many plant metabolites are synthesized to defend against insect, arthropod herbivores, and pathogens (Silva *et al.*, 2006). Cassava root and leaves have been reported from phytochemical analysis that contain glycosides, hydroxycoumarins, triterpenoids, alkaloids, steroids, flavonoids, and tannins (Blagbrough *et al.*, 2010; Chinnadurai *et al.*, 2019). Two major classes of phenolic compounds, flavonoids and coumarins, are identified in

cassava. The identified phenolic compounds in cassava stem are isovanillin, 6-deoxygacareubin, pinoresinol, *p*-coumaric acid, etc., while coumarins, such as scopoletin, esculetin, scopolin, and esculin, are found in cassava stem and leaves. Cassava leaves also contain various flavonoids, including hyperoside, myricetin-3-*O*-rutoside, nicotiflorin, narcissin, and quercetin-3-*O*-rutoside (Tao, 2019). These coumarins and flavonoids exhibit potential antioxidant properties (Lee *et al.*, 2013). Esculin has been reported to exert a scavenging effect as well as prevent lipid peroxidation reaction (Biljali *et al.*, 2012). Antioxidant properties could be attributed to phenolic compounds according to their chemical structure, such as active hydroxyl group (-OH) in terms of its number and position (Bendary *et al.*, 2013).

Polysaccharides in cassava are normally found in its tuber in the form of starch containing amylose and amylopectin as major compositions (Sánchez *et al.*, 2009). However, polysaccharides from different parts of cassava are less studied. Various forms of polysaccharides, such as conjugate polysaccharides, polysaccharide mixture, polysaccharide chelating ion, could function through various mechanisms of antioxidant properties, which include ROS/RNS scavenger, regulation through the endogenous antioxidant stress Nrf2/ARE pathway, and inhibition of the iNOS expression (Mu *et al.*, 2021). Reducing ability observed in the polysaccharides of cassava extracts might be explained according to previous studies that a high content of uronic acid, glucuronic acid, and galacturonic acid in acidic polysaccharides could exert potential antioxidants from their ability as hydrogen atom donors (Yan *et al.*, 2019).

The melanogenesis is the process with complex mechanisms to synthesize eumelanin and pheomelanin in the cells called melanocytes. Tyrosinase is a key enzyme functioning in an important role in the rate-limiting step to convert L-tyrosine, the starting material for melanin biosynthesis, to L-DOPA. Inhibition of the tyrosinase enzyme through a competitor or acting as a hindrance at an enzyme active site could lower the rate of melanogenesis. Many phenolic compounds are tyrosinase inhibitors according to the presence of a hydroxyl functional group in their chemical structures. It plays an important role in inhibiting tyrosinase by entering the hydrophobic activity cavity of the tyrosinase enzyme, which affects the catalytic activity. A higher number of hydroxyl groups could indicate more potential in tyrosinase inhibition (Zuo *et al.*, 2018). In this study, a low level of anti-tyrosinase property observed in the crude phenolic extracts might be due to the fact that many phenolic compounds in the extracts had a low amount of hydroxyl group. However, the ethanolic

extract of cassava leaves has been reported to significantly reduce melanin content in the cultured B16 melanoma cells at a concentration of 250 mL/mL or higher (Jampa *et al.*, 2022). In contrast, tyrosinase enzyme could enhance its activity to promote melanogenesis for being applied in hypopigmentation disorders. There are some phenolic compounds that could improve tyrosinase activity, which leads to an increase in melanin biosynthesis (Niu and Aisa, 2017). In this study, polysaccharide fractions of cassava promoted tyrosinase activity, indicating the feasibility of using cassava polysaccharides for re-pigmentation in the melanogenesis process. However, a mechanism for enhancing tyrosinase activity of polysaccharides has not been elucidated yet, as far as the literature is ascertained.

5. Conclusion

In summary, the feasibility of utilizing various parts of cassava (*M. esculenta* (L.) Crantz), Huaybong 60 and Rayong 72 cultivars, as anti-aging and cosmetic ingredients has been conducted. The amounts of phenolic, flavonoid, and polysaccharide, accompanied by antioxidant properties and tyrosinase inhibitory effect, of the crude phenolic and polysaccharide extracts obtained from various parts of cassava. Ethanol was an appropriate solvent for the extraction of cassava compared to the other solvents, while cassava leaves provide a high extractive yield with a high amount of phenolics and flavonoids, as well as potential antioxidant properties. Cassava peel and leave polysaccharides also possess antioxidant properties and could be considered as tyrosinase activation, especially Huaybong 60 cassava leaves polysaccharide. This study suggests that cassava leaves, Huaybong 60 cultivar, have the potential for being used as antioxidant agents, while its polysaccharides could be utilized as anti-hypopigmentation agents. Based on these observations, the evaluation of anti-aging and anti-hypopigmentation effects of cassava extracts on cellular levels should be further studied and elucidated for its mechanism.

Conflict of interest

The authors declare no conflict of interest.

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