**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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**Novelty statement**

The scope and benefit of this study is to evaluate the feasibility utilization of non-edible parts of *M. esculenta* (L.) Crantz for being applied as functional food or cosmetic active ingredients. The phenolic and polysaccharide crude extracts of *M. esculenta* (L.) Crantz were extracted by different solvents, for which the potential of the extracts was evaluated biological properties including antioxidant, tyrosinase inhibitory properties as well as the estimated amounts of bioactive compounds; phenolics, flavonoids, and carbohydrates. This manuscript could provide the scientific data about the feasibility of using these extracts as active ingredients in food, and cosmetic industries. In my point of view, this manuscript could fulfil the utilization of residual agricultural wastes for reducing environmental impact.

**Abstract**

The aim of this study was to evaluate the biological properties of cassava (*Manihot esculenta* (L.)Crantz) extracts. The crude phenolic extracts and polysaccharide fractions from various parts of two cassava cultivars (Huaybong 60 and Rayong 72) were determined for the amount of phenolic compounds and carbohydrates and biological activities including; antioxidant, and tyrosinase inhibition properties.Various parts of cassava were successively extracted by soxhlet extraction using *n*-hexane, ethyl acetate, and ethanol. Water soluble and insoluble polysaccharides were extracted with aqueous and aqueous alkaline extraction, respectively. The highest extractive yield was Cassava peel ethanolic extract (Rayong 72) (11.97%) while the highest yield of polysaccharides extract was insoluble polysaccharides from cassava (Rayong 72) (9.19%). Cassava leaves extracts contained higher phenolic content (39.54 to 83.98 mg GAE/g extract), flavonoid contents (110.38 to 287.78 mg QE/g extract). The carbohydrate contents of the root extracts were higher than other parts. Leaves extracts provide high potential antioxidant activities based on three assays; DPPH radical scavenging activity (54.64 to 107.65 mg TE/g extract), ferric reducing capacity (507.86 to 966.00 mg TE/g extract), and ABTS cation radical scavenging activity (478.37 to 989.49 mg TE/g extract). Most of 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 alternative antioxidant active ingredient in food and cosmetics due to their antioxidant activities whereas polysaccharide fractions, as tyrosinase promoting agent, could be possibly applied as one mechanism for preventing hair greying. These findings indicate feasible utilization of cassava for valued-added.

**Keywords**: Antioxidant; Anti-tyrosinase; *Manihot esculenta* (L.)Crantz; Phenolic compounds; Polysaccharides

**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 concerned due to their toxic as carcinogen or cell toxicity. Natural compounds to which could be obtained from renewable source are interesting accompanying with their less effect to human health, therefore, many studies focus on exploring new sources of natural compounds (Lobo *et al.*, 2010).

Plants contain various natural substances possessing antioxidants property, such as tetraterpenes, carotenoids, ascorbic acid, glutathione, and phenolics. Phenolic compounds are the predominant secondary metabolites in plants and the major abundance in nature are flavonoids, and phenolic acids (Kasote *et al*., 2015). These exert many pharmacological properties including microbial inhibition, anti-inflammation, anti-diabetic as well as anti-aging by scavenging reactive oxygen species (ROS) (Rahman *et al.*, 2022). Polysaccharides, one of plants’ metabolites, could function as many applications including moisturizer, stabilizer in foods and cosmetics, film former, and antioxidants. In addition, seaweed polysaccharides are capable in inhibiting tyrosinase enzyme (Jesumani et al., 2020). Extraction of bioactive compounds from agricultural residuals has become interesting in the past decade for not only reducing in environmental impact but obtaining valuable natural extracts. Vast amounts of agricultural wastes, such as citrus peels, maize cob, grape pomace, potato peels, have been investigated for their feasibility as natural sources of bioactive compounds possessing anti-inflammatory, antimicrobial, anti-diabetic, 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 large production areas. Among cassava cultivars, Huay bong 60 and Rayong 72 are generally grown in Northeastern of Thailand (Fu *et al*., 2014). Its root is source of starch while the other portions, such as leaves, stem, and tuber peel are normally used as animal feeds or discarded. Recently, these materials have been studied for its feasibility to be used as functional food, nutraceutical and cosmetic purposes. Various pharmacological functions, including antimicrobial, anti-inflammatory, hepatoprotective, anti-melanogenesis and antioxidant properties have been observed in cassava leaves (Jampa *et al*., 2022; Mustarichie *et al.*, 2020; Dewi and Normasari, 2021) as well as their capability to increase antioxidant enzymes *in vivo* (Baheker 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 be observed in cassava stems (Yi *et al.*, 2011). Phenolic compounds, such as quercetin-3-*o*-rutinoside (rutin), kaempferol-3-*o*-rutinoside, myricetin-3-*o*-rutinoside, have been isolated and identified from cassava leaves and roots (Blagbrough *et al.*, 2010). However, there is scarce scientific data in the comparison among all parts of cassavaas well as polysaccharides and their biological properties including antioxidant and tyrosinase inhibitory potential. Therefore, the feasibility of various parts of cassava extract and polysaccharide fraction were evaluated for phenolic and flavonoid contents, antioxidant, and tyrosinase inhibition properties.

**Materials and Methods**

**Material and chemicals**

Two cultivars of cassava (Huaybong 60 and Rayong 72) were collected from Nakorn Ratchasima province of Thailand in June 2015. The collected plant materials were separated into 4 parts (peels, roots, leaves, and stems), rinsed with distilled water, and dried with a circulating hot air oven at 50±2°C. The dried samples were powdered and sieved through 250-mesh to obtain a sample powder which stored at 4°C for further extraction preparation. Gallic acid, Folin Ciocalteu’s reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ), 2,2'-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid) (ABTS), kojic acid, and L-3,4-dihydroxyphenylalanine (L-DOPA) were obtained from Sigma-Aldrich (Steinheim, Germany).

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

The extraction procedure of phenolic, water-soluble, and water-insoluble polysaccharide were conducted according to the slightly modification of the methods described by Hadzri *et al*. (2014), Fan *et al.* (2011), and Peng *et al.* (2005), respectively. Briefly, sample (10 g) was successively extracted by 200 mL of hexane, ethyl acetate, and ethanol by soxhlet apparatus for 6 h of each solvent. The extract was filtered through Whatman® filter paper no. 1, evaporated and lyophilized to obtain a crude phenolic extract.

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

**Determination of total phenolic content**

The total phenolic content was determined based on Folin-Ciocalteu’s assay described by Waterman and Mole (1994). Briefly, sample solutions (0.5 mL) were mixed with deionized water (7.5 mL), Folin-Ciocalteu’s reagent (0.5 mL), finally with 200 g/L sodium carbonate solution (1.5 mL). The mixtures were incubated at ambient temperature for 1 h and the absorbance was then measured at 760 nm. The results were expressed as gallic acid equivalents (mg GAE/g extract).

**Determination of total flavonoid content**

The total flavonoid content was determined based on aluminium colorimetric assay (Sathishkumar *et al.*, 2008). Briefly, the sample solutions (3.7 mL) were mixed with 50 g/L sodium nitrite (0.15 mL), 100 g/L aluminium chloride solution (0.15 mL), finally with 40 g/L sodium hydroxide (1.0 mL). The mixtures were incubated at ambient temperature for 5 min and the absorbance was then measured at 510 nm. The results were expressed as quercetin equivalents (mg QE/g extract).

**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 50 g/L phenol solution (6 mL). The mixtures were incubated at 90°C for 5 min and the reaction was stopped by cooling down in ice bath. The absorbance was measured at 490 nm and the results were expressed as glucose equivalents (mg GE/ g extract).

**DPPH**• **scavenging activity**

The DPPH• scavenging activity was determined according to the method previously described by Gülçin *et al*. (2003). Briefly, the sample solution (1 mL) was mixed with 0.1 mmol/L DPPH• solution (3 mL). The mixtures were incubated in dark condition at ambient temperature for 30 min and the absorbance was measured at 517 nm. A control containing 1 mL of ethanol and 3 mL of DPPH• solution was prepared. The percent inhibition was calculated as the following equation:

% DPPH• scavenging activity = [(A0 – A1)/A0] × 100

A0 was the absorbance of the control (without sample) and A1 was the absorbance of the mixture containing the sample. Trolox was used for comparison to the samples, and the results were expressed as trolox equivalent (mg TE/g extract).

**Ferric reducing antioxidant power (FRAP) assay**

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

**ABTS**•+ **scavenging activity**

The ABTS•+ scavenging activity was determined according to the method described by Re *et al.* (1999). Briefly, the ABTS•+ solution was prepared by the reaction between 7mM ABTS solution and 2.45 mM potassium persulphate (100 mL) in the dark condition at ambient temperature for 16 h. The ABTS•+ solution was diluted with potassium phosphate buffer (50 mM, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. The sample solutions (0.1 mL) were with ABTS•+ solution (3 mL). The mixtures were incubated at ambient temperature for 30 min ant the absorbance was measured at 734 nm. The results were expressed as trolox equivalent (mg TE/g extract).

**Tyrosinase inhibition assay**

The tyrosinase inhibition activity was determined based on L-DOPA substrate assay (Vardhan and Pandey, 2014). Briefly, a 0.5 mg/mL of sample (20 µL) or kojic acid, as standard comparative compound, was mixed with potassium phosphate buffer (50 mM, pH 6.8, 110 µL), and 1000 U/mL mushroom tyrosinase solution in potassium phosphate buffer (20 µL). After incubation at 37°C for 10 min, 10 mM L-DOPA (50 µL) was added prior be incubated at 37°C for 10 min and the absorbance was measured at 475 nm. The mixture contained no sample was used as control. The tyrosinase inhibition was calculated as the following equation:

% Tyrosinase inhibition = [(A0 – A1)/A0] × 100

A0 was the absorbance of the control (without sample) and A1 was the absorbance of the mixture containing the sample.

**Statistical analysis**

All measurement were conducted in triplicate and the results were expressed as mean ± S.D. SPSS program version 21 (SPSS Inc, Chicago, IL, USA) was used for all statistical analyzes. One Way Analysis of Variance (ANOVA) through Duncan’s multiple range-test was used to determine significant difference among samples. The results with *P* < 0.05 were regarded as statistical differences.

**Results**

Various parts of cassavawere successively extracted to obtain crude phenolic extracts and polysaccharide fractions that the amount of phenolics and polysaccharides as well as antioxidant and anti-tyrosinase activities were then evaluated. The extractive yield of crude phenolic and polysaccharides 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 different in extractive yield among the plant parts (P < 0.05) that high extractive yield was obtained from cassava leaves. Secondary metabolites synthesized by plants could be found in all parts, however, vary in their amount and types. In addition, bioactive compounds’ solubility depended on their chemical structures that relate to polarity. This phenomenon, therefore, could influence on 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 aceatate, ethanolic extracts, and water-insoluble polysaccharide extractwere selected to be futher analyzed. evaluated for phenolic content, flavonoid content, antioxidant activities and tyrosinase inhibition property.

**Determination of phenolic, flavonoid and carbohydrate contents**

The phenolic and flavonoid contents in the cassava crude phenolic and polysaccharide extracts were quantified based on Folin-Ciocalteau’s, and aluminium chloride colorimetric methods, respectively, as shown in Table 3. The high amount of flavonoid compounds in the crude phenolic extracts was found in the LEo60, LEc60, and PEo60 extracts. The LEo60 extract also contained the highest amount of phenolic compounds (P < 0.05). This finding indicated that cassava cultivar Huaybong 60 could be considered as potential source of phenolics and flavonoids, especially its leave and stem which 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 carbohydrate content of the crude phenolic and polysaccharide extracts was estimated according to phenol-sulfuric acid assay as 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 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 potential source of polysaccharides when considering carbohydrate content incorporate with extractive yield.

**Antioxidant activities**

The DPPH radical scavenging assay is based on hydrogen atom donation in the presence of antioxidants to scavenge and convert DPPH• to non-radical form of DPPH. The crude phenolic extracts exhibited DPPH• 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 results were in agreement with the phenolic and flavonoid contents of the crude phenolic extracts that cassava leaves were the most potential compared with the other parts (P< 0.05). Low potential antioxidant activity was observed in the polysaccharide extracts (4.76-26.52 mg TE/g extract) except cassava leave polysaccharide extract of Huay Bong 60 cultivar (53.22 mg TE/g extract).

Ferric reducing antioxidant power (FRAP) is used to evaluate capability of the antioxidant to reduce ferric to ferrous ion. The crude phenolic extracts exhibited potential reducing property which were in the range from 40.47 to 966.00 mg TE/g extract. The LEo60 extract exhibited 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 on scavenging cation radical. 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 those phenolic content, ethanolic extracts of cassava leaves, especially Huaybong 60 cultivar were potential as 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 due to 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. High relationship between phenolic content with FRAP value and ABTS cation radical scavenging activity of crude phenolic extracts (*R2* = 0.938 and 0.920, respectively) and polysaccharide extracts (*R2* = 0.783 and 0.957, respectively) were observed. These findings indicate that antioxidant property determined in the extracts could be attributed to electron donor mechanism. However, less correlation between phenolic content and DPPH• scavenging activity of crude phenolic extract was observed (*R2* = 0.346) while there was moderate correlation between phenolic content and DPPH• scavenging activity of polysaccharide extracts (*R2* = 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, however, 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 leave polysaccharides was greater than cassava peel polysaccharides.

**Determination of anti-tyrosinase activity**

Tyrosinase, a key enzyme in the melanogenesis, is used to evaluate the potential of plant extract to suppress melanin synthesis through enzyme inhibition mechanism that could provide lightening effect (Park *et al*., 2015). Therefore, crude phenolic and polysaccharide extracts of cassava were evaluated for their capability to inhibit tyrosinase enzyme. 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, the PW72 and SW72 could not be dissolved in the buffer solution used in the condition of this assay but 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 limitation of pH for tyrosinase function (Naidja *et al*., 1997). As the 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 melanogenesis process which might be applied in the product aimed to increase melanin pigment, such as, anti-grey hair product.

**Discussion**

Aging are process in living organisms caused from intrinsic and extrinsic factors. Excessive oxidative condition is largely attributed to imbalance between free radicals and antioxidant system which leads many degradations of biological molecules. Many research focus 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. Different types of solvent used for the phenolic extraction is one factor affecting extraction’s efficiency in terms of various profiles of natural compounds and biological activity (Borges *et al*., 2020). Solubility of natural compounds depended on their polar compatibility with solvent according to “like dissolve like”. Alcohols, as extraction solvents, provides potential in obtaining extracts with high antioxidant properties compared with lower polar solvents such as acetone, petroleum ether, etc. (Nawaz *et al*., 2020). Ethanol is also considered as biobased-solvent for green extraction that is suitable for cosmetic, pharmaceutical, and food ingredients industries (Chemat, 2019).

Various parts of plant comprised different profile of phenolics in terms of both quality and quantity (Arumugam *et al*., 2019). Many studies found that leaves are the plant part rich in phenolic compounds while stem and roots contain less amount. It has been reported that leaves stalk of cassava contains high amount of phenolics and flavonoids (Suresh *et al*., 2011). Many plant metabolites are synthesized to defense against insect, arthropod herbivores, and pathogens (Silva *et al*., 2006). Cassava root and leaves has 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 quercetin-3-*O*-rutinoside, myricetin-3-*O*-rutinoside, hyperoside, nicotiflorin, and narcissin (Tao, 2019). These coumarins and flavonoids exhibit potential antioxidant properties (Lee *et al*., 2013). Esculin has been reported that exerts superoxide radicals through 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 the number and position of the active hydroxyl group (-OH) (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 couldfunction 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 high content of uronic acid, glucuronic acid, and galacturonic acid in acidic polysaccharide could exert potential antioxidants from their ability in hydrogen atom donor (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 an important role in the rate-limiting step to convert L-tyrosine, starting material for melanin biosynthesis, to L-DOPA. Inhibition of tyrosinase enzyme through competitor or act as hindrance at enzyme active site could lower rate of melanogenesis. Phenolic compounds are one of tyrosinase inhibitor according to the presence of hydroxyl functional group in their chemical structures. It plays an important role to inhibit tyrosinase by entering the hydrophobic activity cavity of tyrosinase which affect the catalytic activity. Higher number of hydroxyl group could indicate more potential in tyrosinase inhibition (Zuo *et al.*, 2018). In this study, low level of anti-tyrosinase property observed in the crude phenolic extracts might be due to the fact that many of phenolics in the extracts had low amount of hydroxyl group. However, the ethanolic extract of cassava leaves have been reported that could significantly reduce melanin content in the cultured B16 melanoma cell at the concentration of 250 μL/mL or higher (Jampa *et al*., 2022). In contrast, tyrosinase enzyme could be enhanced its activity to promote melanogenesis for being applied in hypopigmentation disorders. There are some phenolic compounds that could improve tyrosinase activity which lead to increase in melanin biosynthesis (Niu and Aisa, 2017). In this study, polysaccharide fractions of cassava promoted tyrosinase activity indicating feasibility on using cassava polysaccharides for re-pigmentation in melanogenesis process. However, mechanism on enhancing tyrosinase activity of polysaccharides has not been elucidated yet as far as literature is ascertained.

**Acknowledgements**

The feasibility of utilizing various parts of cassava (*M. esculenta* (L.) Crantz), Huaybong 60 and Rayong 72 cultivars, as anti-aging and cosmetic ingredients were evaluated. The amounts of phenolic, flavonoid, and polysaccharide accompanying with antioxidant properties and tyrosinase inhibitory effect of the crude phenolic and polysaccharide extracts obtained from various parts of cassava. Ethanol was appropriate solvent for the extraction of cassava compared to the other solvents while cassava leaves provide high extractive yield with 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, has the potential for being used as antioxidant agents while its polysaccharide 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 study and elucidated for its mechanism.

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

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Plant part Cultivars | | Extraction solvent | | | | |
| Crude extract | | | Polysaccharides | |
| *n*- 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 |

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

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

Values are expressed in mean ± S.D from triplicate

A-C Means the column followed by different letters are significantly different in percent extraction yield of *Manihot esculenta* (L.) Crantz extracts (*P*<0.05)

a-d Means the extracts of each plant part followed by different letters are significantly different in percent extraction yield of *Manihot esculenta* (L.) Crantz extracts (*P*<0.05)

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

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Extract | Total phenolics1 | Total flovanoids2 | Total  carbohydrate3 | FRAP4 | DPPH radical scavenging activity4 | ABTS  radical scavenging activity4 | % Inhibition tyrosinase enzyme | % Activation tyrosinase enzyme |
| PEc60 | 50.77±3.32DE | 224.71±11.83B | NA | 520.18±2.52F | 64.98±1.42E | 483.83±3.12EF | 12.18±2.73CD | - |
| PEo60 | 66.16±0.65C | 279.90±6.83A | NA | 940.32±15.98B | 57.95±5.06F | 825.03±18.76C | 19.87±0.45A | - |
| REc60 | 47.18±1.30F | 216.82±18.07B | NA | 531.48±5.24F | 39.25±4.76H | 376.01±5.15H | 5.56±1.44E | - |
| REo60 | 6.68±0.43L | 189.23±0.00C | NA | 40.47±5.81L | 16.90±0.70I | 83.94±0.00M | ND | - |
| LEc60 | 42.78±0.59G | 287.78±6.83A | NA | 507.86±15.31F | 92.84±2BC | 478.37±14.38F | ND | - |
| LEo60 | 83.98±0.75A | 275.95±6.83A | NA | 966±27.60A | 90.17±0.43C | 989.49±15.90A | 14.10±0.45BC | - |
| SEc60 | 51.81±0.82D | 201.05±0.00B | NA | 637.29±8.72D | 63.12±2.53E | 535.01±3.13D | 2.78±1.34EF | - |
| SEo60 | 42.20±0.59G | 201.05±0.00B | NA | 438.01±2.91G | 72.00±1.32D | 415.59±7.38G | 18.16±1.18A | - |
| PEc72 | 25.31±0.28J | 90.67±6.83EF | NA | 294.20±8.09J | 48.14±1.24G | 260.00±5.42K | 17.95±1.05A | - |
| PEo72 | 22.65±0.59K | 110.38±27.31E | NA | 348.64±10.17I | 95.86±1.96B | 324.15±8.52I | 9.51±0.2D | - |
| REc72 | 27.85±0.33I | 74.90±18.07F | NA | 408.22±5.24H | 39.66±0.79H | 280.47±7.38J | 10.58±1.98D | - |
| REo72 | 6.91±0.28L | 67.02±24.62F | NA | 65.13±1.45K | 18.94±0.65I | 112.60±0.00L | 17.09±2.00AB | - |
| LEc72 | 39.54±1.02H | 157.69±13.66D | NA | 586.95±18.20E | 54.64±0.51F | 496.79±13.00E | ND | - |
| LEo72 | 69.98±0.16B | 110.38±6.83E | NA | 806.78±6.66C | 107.65±2.42A | 878.25±0.00B | 14.53±2.75BC | - |
| SEc72 | 48.68±0.71EF | 86.73±27.31EF | NA | 564.36±11.62E | 37.28±0.50H | 479.05±13.42F | ND | - |
| SEo72 | 49.61±0.28DE | 90.671±18.07EF | NA | 562.30±8.84E | 48.14±0.59G | 475.64±5.15F | 5.77±1.14E | - |
| PW60 | 24.96±0.49a | ND | 1341.74±984.42bc | 386.65±8.09a | 53.22±0.22a | 358.±1.93a | - | 52.14±3.69b |
| RW60 | 6.10±0.16g | ND | 1990.81±167.81ab | 106.21±5.24e | 6.25±0.13g | 73.71±0.97f | - | 44.12±7.61b |
| LW60 | 15.82±0.33bb | ND | 981.61±42.64c | 273.65±9.07b | 15.23±0.26e | 208.82±0.97c | - | 65.70±1.28a |
| SW60 | 11.42±0.00e | ND | 1044.68±76.95c | 129.84±1.45d | 4.76±0.07h | 184.25±0.97d | - | 41.56±10.74b |
| PW72 | 13.39±0.16d | ND | 1257.56±47.80c | 218.18±21.94c | 26.52±0.15b | NA | - | NA |
| RW72 | 8.18±0.16f | ND | 2294.36±62.58a | 122.65±0.00de | 8.66±0.19f | 112.60±0.00e | - | 44.76±6.02b |
| LW72 | 14.66±0.16c | ND | 205.00±13.66d | 262.35±1.45b | 18.46±0.26d | 245.67±3.86b | - | 43.70±9.83b |
| SW72 | 8.30±0.57f | ND | 1537.46±112.82bc | 235.64±5.24c | 19.39±0.07c | NA | - | NA |
| Kojic acid | NA | NA | NA | NA | NA | NA | 46.30±1.98 | - |

Values are expressed in mean ± S.D. from triplicate

NA (Not analyzed)

ND (Not determined)

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

A-D Means the column followed by different letters are significant difference in part used of crude phenolic extracts (*P*<0.05)

a-d Means the column followed by different letters are significant difference in part used of polysaccharide extracts (*P*<0.05)

\* Means the column followed are significant difference in part used of crude extracts (*P*<0.05)