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The *in-vitro* Antioxidant Properties of Crude Drugs Traditionally Used for Diabetes Management in Northern Banyumas

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ABSTRACT

Chinaberry (*Melia azedarach* L.) leaves, Malayan cherry (*Muntingia calabura* L.) fruits, and Yacon (*Smallanthus sonchifolius* (Poepp.) H.Rob.) leaves are used for traditional diabetes treatment by Banyumas (Central Java, Indonesia) people. This study characterized selected quality parameters, evaluated the *in-vitro* antioxidant activity as the preliminary assay for its antidiabetic activity, and calculated the total flavonoid content (TFC) and total phenolic content (TPC) of those crude drugs. The plant materials of each species were collected from three different areas in Banyumas and dried into crude drugs. The quality parameters were determined according to the standard method in the Indonesian Herbal Pharmacopeia (IHP) 2017. The antioxidant activity was evaluated by the standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and ferric reducing antioxidant power (FRAP) assays, while TFC and TPC were analyzed following standard methods in IHP 2017. The correlations between antioxidant activities and the TFC-TPC were analyzed by Pearson's correlation test. The values for acid-insoluble ash, loss on drying, total ash, ethanol extractable, and water-extractable of all crude drugs were quantified, with TPC and TFC proposed as the chemical content parameter for Malayan cherry fruits and Yacon leaves. Yacon leaves and Malayan cherry fruits contained the highest TFC and TPC at 8.01±0.72 mg Quercetin equivalent (QE)/g dry weight (DW) and 11.54±1.44 mg Gallic acid equivalent (GAE)/g DW, respectively. Malayan cherry fruits exerted the strongest DPPH scavenging activity (366.13±17.65 mM Trolox equivalent (TE)/g DW) and FRAP (1025.33±50.47 mM TE/g DW). Moderate to strong correlations were observed between DPPH radical scavenging activity – FRAP and TFC-TPC of Malayan cherry fruits and Yacon leaves. Hence, flavonoids and phenolic compounds of both crude drugs contributed to their antioxidant activity.

Keywords: Antioxidant; Flavonoids and phenolic compounds; Quality parameters; Traditional antidiabetic plants

INTRODUCTION

People in northern Banyumas, Central Java, utilized 11 plant species for the traditional management of diabetes. Most medicinal plants have been well characterized as antidiabetic plants with a long history of ethnopharmacological uses elsewhere (Utaminingrum et al., 2020). Also, the seven plants' monograph is included in the recent edition of Indonesian Herbal Pharmacopeia (IHP) (Indonesian MoH, 2017). However, the standard quality for crude drugs of Chinaberry (*Melia azedarach* L., Meliaceae) leaves, Malayan cherry (*Muntingia calabura* L., Muntingiaceae) fruits, and Yacon (*Smallanthus sonchifolius* (Poepp.) H.Rob., Asteraceae) leaves are not available to date.

The chinaberry tree is commonly used for natural nematocide, herbicide, and pesticide. It is traditionally utilized for anthelmintic, diuretic, emmenagogue, expectorant, vermifuge, and the treatment of inflammatory-related diseases. The plant contains diterpenoids, triterpenoids, sterols, flavonoids, and glycosides, with limonoid terpenoids as the major constituents. It exerts *in-vitro* anthelmintic, antifertility, antihyperglycemic, antimalarial, antimicrobial, antioxidant, antipyretic, cytotoxic, and hepatoprotective activities (Sultana et al., 2014). The Malayan cherry tree has been used in many traditional medicine systems for various purposes, from treating inflammatory diseases to health tonics. At least 87 secondary metabolites have been isolated from this plant. Antidiabetic, antihypertensive, antimicrobial, antiinflammation, antinociceptive, antioxidant, antipyretic, antiulcer, cardioprotective, cytotoxic, and insecticidal activity assays have been subjected to various extracts with a wide array of efficacies (Mahmood et al., 2014).

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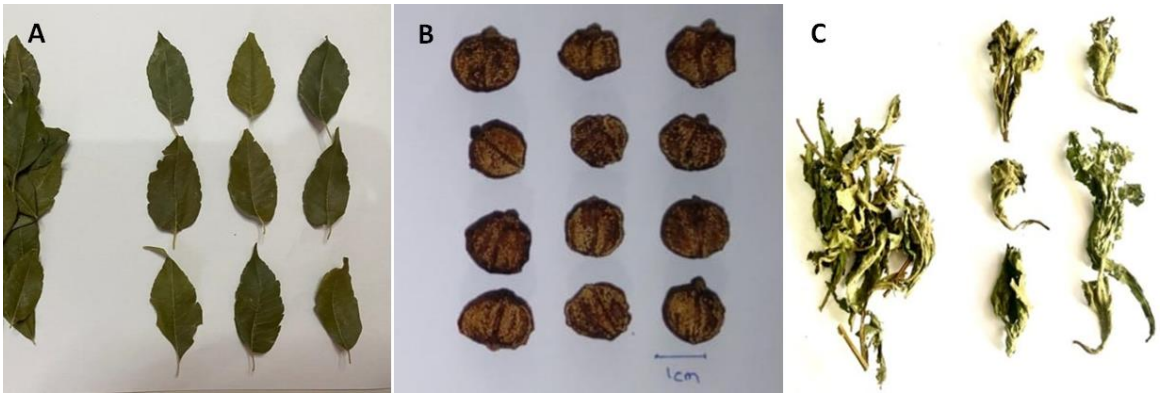

Figure 1. Crude drugs of Chinaberry leaves (A), Malayan cherry fruits (B), and Yacon leaves (C)

On the other hand, Yacon tubers are commonly consumed in Southern America as a functional food with high oligosaccharides content. It is also traditionally used in the treatment of degenerative diseases. This plant is rich in terpenoids, particularly sesquiterpenes and diterpenes. It showed the antihyperglycemic and ameliorated the oxidative status in animal models (De-Almeida-Paula et al., 2015).

The uncontrolled reactive oxygen species (ROS) generation and deficient expression of cellular antioxidant enzymes to handle those radicals commonly occur during the diabetic condition. This phenomenon potentially led to oxidative stress-related pancreatic cell damage and diminished peripheral tissues' sensitivity toward insulin. The external antioxidants might significantly minimize the damage and delay the harmful effects on the body. The intake of antioxidants could be beneficial in diabetic management, particularly in preventing diabetic complications (Bandeira et al., 2013). Hence, an antioxidant activity assay can be performed as the initial evaluation of the antidiabetic capacity of phytopharmaceuticals. This study characterized the basic quality standard and evaluated the antioxidant properties of Chinaberry leaf, Malayan cherry fruit, and Yacon leaf crude drugs collected from three areas in Northern Banyumas to validate their traditional use as antidiabetic treatment in the area.

METHODOLOGY

Reagents and instruments

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), Folin-Ciocalteau reagent, Gallic acid, Quercetin, and Trolox were obtained from Sigma (United States). Acetic acid, aluminum chloride, chloroform, deionized water, ethanol, hydrochloric acid, methanol, sodium acetate, sodium carbonate, and sodium hydroxide were also used in this study. The instruments used in this study were analytical balance (Ohaus, United States), furnace (Carbolite, United Kingdom), oven (Memmert, Germany), and spectrophotometer UV-Vis (Shimadzu, Japan).

Plant materials

The dark green-colored leaves, ten stalks from the apical shoots and below, of the Chinaberry tree were collected in Ketenger, Gandatapa, and Sumbang, while the reddish-colored and somewhat softened fruits of Jamaican berry were obtained from Karangsalam, Banteran, and Tambaksogra. Yacon leaves in a darker green shade located on seven stalks of the apex and below were collected in Kemutug Lor and Bantarwuni. For comparison purposes, Yacon leaves were also collected from Selandaka of Sub-district Sumpiuh (Figure 1). The location of plant material collections is shown in Figure 2. The identity of all plants used in this study was determined in the Laboratory of Pharmaceutical Biology, Universitas Muhammadiyah Purwokerto, Indonesia, with the reference ID of 273-DAP, 253-WA, and 272-RDS, respectively. The crude drugs of each plant material were obtained by rack-drying at 40^{o}C.

Determination of selected quality parameters of the crude drugs

The crude drugs were evaluated for their value of acid-insoluble ash, ethanol extractable, loss on drying, total ash, and water-extractable parameters. The determination of those parameters followed the general methods in the IHP (Indonesian MoH, 2017).

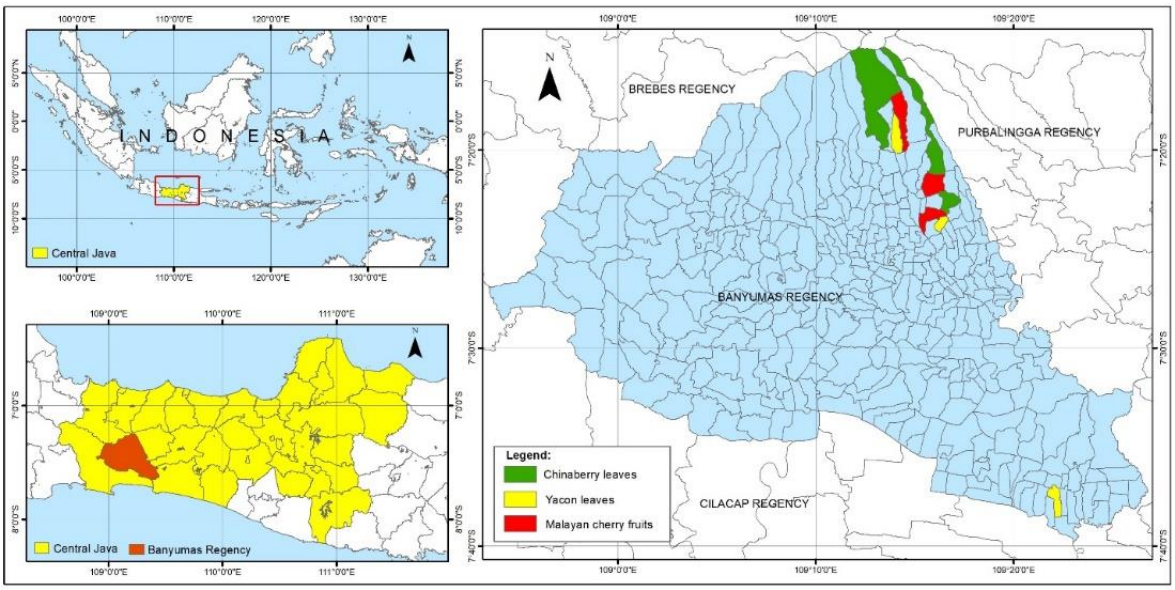

Figure 2. Location of plant materials collection

Determination of antioxidant content of the crude drugs

The determination of antioxidant contents of all crude drugs followed the standard TFC and TPC method in the IHP using aluminum chloride and Folin-Ciocalteau reagents, respectively (Indonesian MoH, 2017). Ethanol was used to extract each crude drug under sonication for 60 min, at a ratio of 1:25 w/v. The extracted sample or the standard solution was mixed with ethanol, 10% aluminum chloride, 1M sodium acetate, and deionized water at a ratio of 5:15:1:1:28. The mixture reaction was stood at room temperature for 30 min and read at 370 nm. The standard solutions were Quercetin in ethanol at 6.25-100 µg/ml, and TFC in each crude drug was reported as mg Quercetin equivalents (QE)/g dry weight (DW).

For the TPC determination, the extracted sample or the standard solution was mixed with 7.5% Folin-Ciocalteu reagent in a ratio of 1:5 at room temperature for 8 min. A total of four parts of 1% sodium hydroxide were added, and after standing for 60 min, the mixture's absorbance was read at 730 nm. Gallic acid in ethanol at 6.25-100 µg/ml was used as the standard, and TPC was reported as mg Gallic acid equivalent (GAE)/g DW.

Determination of antioxidant activities of the crude drugs

The extract was prepared the same way described in the determination of antioxidant content section. The analysis of DPPH scavenging activity and FRAP followed the standard methods with minor modifications (Thaipong et al., 2006). The extracted sample or the standard solution was reacted with 0.025 mg/ml DPPH in methanol in a ratio of 1:10, protected from light at room temperature, for 30 min. The mixture's absorbance was read at 517 nm. Trolox solution at 0-400 µM was used for the standard calibration curve equation, and each crude drug's free radical scavenging activity was reported as mM Trolox equivalent (TE)/g DW.

For the FRAP analysis, the extracted sample or the standard solution was mixed with FRAP reagent (a freshly prepared mixture of 300 mM acetate buffer, 10 mM TPTZ in HCl, and 20 mM ferric chloride in a ratio of 10:1:1, final pH 3.6) in a balance of 1:19 at room temperature for 30 min. The mixture's absorbance was measured at 594 nm. Trolox solution at 0-225 µM was used for the standard curve equation, and the FRAP of extracts was reported as mM TE/g DW extract.

Statistical analysis

The effect of the location of plant material collection on the loss of drying, total ash, acid-insoluble ash, water extractable, and ethanol extractable and their mean separation within the same crude drugs, as well as mean separation of each crude drug toward TFC, TPC, DPPH scavenging activity, and FRAP, were evaluated by one way ANOVA and Duncan's test. The different values were considered significant at $p < 0.05$, and the analysis was conducted by standard procedures in SPSS ver. 13 (IBM, US).

RESULT AND DISCUSSION

The location of plant specimen collection did not affect the loss on drying of Chinaberry leaves (p = 0.621) and Malayan cherry fruits (p = 0.105). However, the loss on drying of Yacon leaf crude drugs collected from different areas was widely varied (p = 0.000). The crude drugs collected in Kemutug Lor contained the highest moisture content, followed by those from Selandaka and Bantarwuni. Loss on drying represented the purity aspect of the crude drug's quality and was related to the microbial contamination risk. The excess moisture in crude drugs enabled microbial growth that might lead to pathogenic contamination or decreasing bioactive metabolite contents during storage (Agarwal et al., 2014). IHP 2017 set the standard for loss on drying of crude drugs at not more than 10% (Indonesian MoH, 2017). Hence, all crude drugs but Yacon leaves collected in Kemutug Lor should meet the official requirement. However, the data on the loss drying of Chinaberry and Yacon leaves and Malayan cherry fruits are still limited, so we don't have any comparison for the values obtained in our study. As the crude drugs were just prepared and had not undergone storage, the excess moisture of one from Kemutug Lor likely originated from improper drying. Also, the relatively similar geographical conditions where plant material is collected might be responsible for the comparable loss on drying in all crude drugs.

Ash contents represented inorganic impurities and indicated the purity aspects of the crude drug's quality. The total ash of crude drugs of the Chinaberry tree (p = 0.001), Malayan cherry fruits (p = 0.002), and Yacon leaves (p = 0.001) were significantly affected by the growing location, with the lowest value observed in those collected at Sumbang, Tambaksogra, and Selandaka, respectively. The location of plant collections also statistically determined the crude drug's acid-insoluble ash of Chinaberry leaves (p = 0.001), Malayan cherry fruits (p = 0.000), and Yacon leaves (p = 0.000). The lowest acid-insoluble ash content of each crude drug was those obtained from Ketenger, Tambak Sogra, and Kemutug Lor. The ash in the crude drugs might be originated from the soil where the producing plant grows or from preparation processing, i.e., the addition of minerals (Tauheed et al., 2017). As crude drugs in this study did not undergo any processing but drying, the first-mentioned reason is more likely applicable. Slamet Mountain is an active volcano and has sent minerals to the surface over many eruptions since ancient times. Our study suggested that the plant materials collected from places at different distances from the crater of Slamet Mountain contained different values of total and acid-insoluble ash. The overall total ash of Chinaberry leaves in this study (6.63±0.79%), however, was quite similar to those obtained from Batu Materia Medica, Indonesia (6.77±0.28%) (Ervina et al., 2020). Also, the total ash of Malayan cherry fruits from Andhra Pradesh, India, was much higher (5.47±0.77%) than our result (1.02±0.27%) (Peter et al., 2020). No data on the ash content of Yacon leaves is available yet.

The solvent-soluble extractable value is the primary method to determine the content aspects of the crude drug's quality, mainly when a suitable chemical or biological assay is unavailable (Alamgir, 2017). The water extractable of the crude drugs of the Chinaberry tree (p = 0.000), Malayan cherry tree (p = 0.000), and Yacon leaves (p = 0.000) were significantly defined by the location of plant collection. The highest water extractable was found in those collected in Sumbang, Karangsalam, and Kemutug Lor, respectively. Similarly, the ethanol extractable of crude drugs was also statistically affected by the plant's growing location. The crude drugs of Chinaberry leaves, Malayan cherry fruits, and Yacon leaves collected in Ketenger, Tambaksogra, and Selandaka contained the highest ethanol extractable (Table I). Hence, the constituents of all three crude drugs varied with the plant growing location. It's not uncommon that the metabolites of the plant are widely varied. It is considered the intraspecific metabolite's variations, and the phenomenon has been observed in Chinaberry and Malayan cherry trees (Dougnon and Ito, 2022; Zolkeflee et al., 2021). Our results suggested that Chinaberry and Yacon leave contained more polar compounds, while Malayan cherry fruits were rich with semi to non-polar ones. Isolation of three flavonoid glycosides with antiangiogenic activity from Chinaberry leaves collected in Tokushima Prefecture, Japan, supported our finding (Kumazawa et al., 2013). Nevertheless, Chinaberry leaves from Batu Materia Medica contained much higher ethanol extractable (27.28±1.30%) than that was observed in our study (17.33±4.52%) (Ervina et al., 2020). Similarly, Peruvian and Ecuadorian-originated Yacon leaves contained caffeoylquinic acids, and water-soluble phenolic acids (Daniela Russo et al., 2015). In line with our result, Malayan cherry fruits originated from Campinas-SP, Brazil, and contained water-insoluble unfree and esterified phenolic compounds that contributed to a higher proportion of ethanol-soluble extractable (Pereira et al., 2018).

TFC of the same crude drugs collected from different places was comparable. Plant species

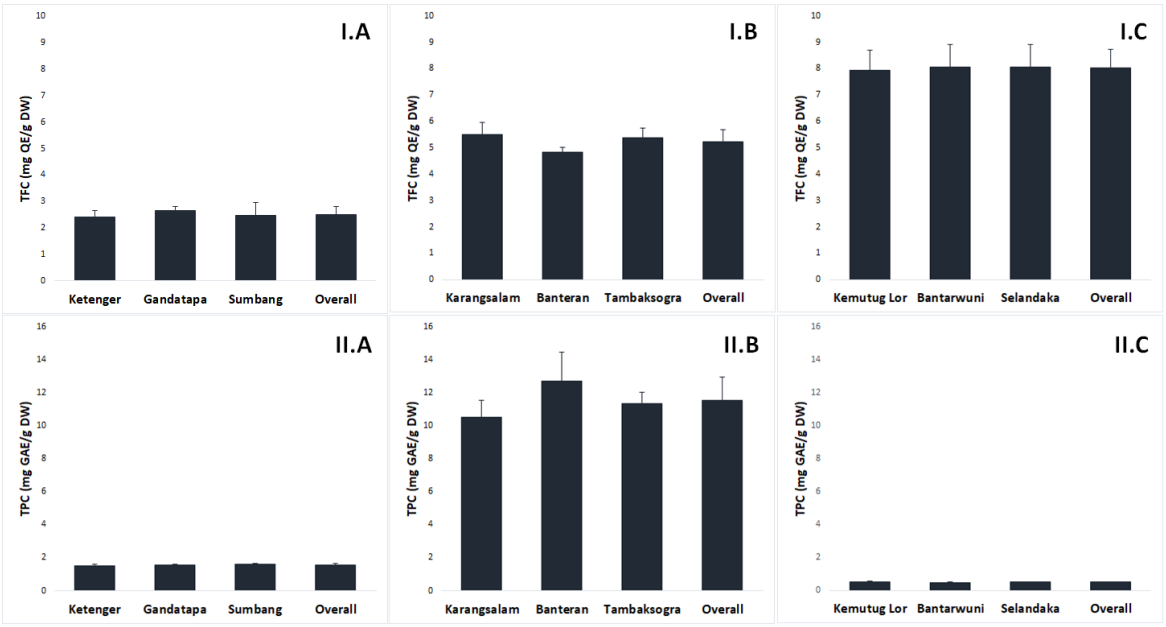

Figure 3. TFC (I) and TPC (II) of crude drugs of Chinaberry leaves (A), Malayan cherry fruits (B), and Yacon leaves (C)

Table I. The value of physicochemical quality parameters of the crude drugs

Crude drugs	Origin	Parameter value (%)				
		Loss on drying	Total ash	Acid-insoluble ash	Water extractable	Ethanol extractable
Chinaberry leaves	Ketenger	9.30±0.10	7.58±0.26^{b}	0.12±0.02^{a}	12.33±1.76^{a}	22.33±1.89^{c}
	Gandatapa	9.10±0.66	6.40±0.43^{b}	0.28±0.02^{c}	23.50±1.00^{b}	12.50±1.80^{a}
	Sumbang	8.77±0.91	5.92±0.13^{a}	0.23±0.02^{b}	26.67±1.26^{c}	17.17±1.53^{b}
	Overall	9.06±0.61	6.63±0.79	0.21±0.07	20.83±6.63	17.33±4.52
Malayan cherry fruits	Karangsalam	9.98±0.36	8.44±0.15^{b}	1.37±0.11^{c}	31.38±0.31^{c}	31.23±0.15^{a}
	Banteran	9.40±0.23	7.89±0.33^{b}	0.92±0.04^{b}	27.08±0.76^{b}	34.35±0.32^{b}
	Tambaksogra	8.98±0.71	6.42±0.59^{a}	0.78±0.03^{a}	25.34±0.17^{a}	36.93±0.38^{c}
	Overall	9.45±0.60	7.58±0.97	1.02±0.27	27.93±2.72	34.17±2.49
Yacon leaves	Kemutug Lor	15.29±0.81^{c}	4.68±0.19^{c}	0.13±0.01^{a}	33.50±1.32^{c}	18.33±1.26^{b}
	Bantarwuni	7.93±0.20^{a}	4.15±0.10^{b}	0.22±0.01^{c}	27.50±1.32^{b}	12.67±1.89^{a}
	Selandaka	9.37±0.32^{b}	3.68±0.15^{a}	0.17±0.02^{b}	23.33±1.26^{a}	22.33±1.61^{c}
	Overall	10.86±3.41	4.17±0.45	0.18±0.04	28.11±4.57	17.78±4.43

The superscripted alphabet within the same columns indicated the different parameter values between the same crude drugs collected from different places

significantly affected the TFC of crude drugs (p = 0.000). Yacon leaves showed the highest flavonoid contents (8.01±0.72 mg QE/g DW), while the Chinaberry leaves were the lowest (2.52±0.30 mg QE/g DW) (Figure 3). The available data suggested that the TFC of crude drugs was widely varied according to many factors. TFC of Chinaberry leaves in our study was much higher than one collected in Karonga, Malawi (0.532 mg QE/g DW) but considerably lower than that collected in Kavre, Nepal (41.07±1.53 mg QE/g DW) (Kharel and Sharma, 2020; Mwamatope et al., 2020). Although Chinaberry leaves contained a low level of TFC in this study, some flavonoids, i.e., astragalin, kaempferol glycosides, Quercetin, and rutin, have been isolated from Chinaberry leaves (Kumazawa et al., 2013; Zeng et al., 2019). On the other hand, the TFC of Malayan cherry fruits collected from Jember, Indonesia (3.30 mg QE/g DW) was lower than our result (5.24±0.43 mg QE/g DW) (Simamora et al., 2020). Similar to our finding, Indonesian-originated Yacon leaves

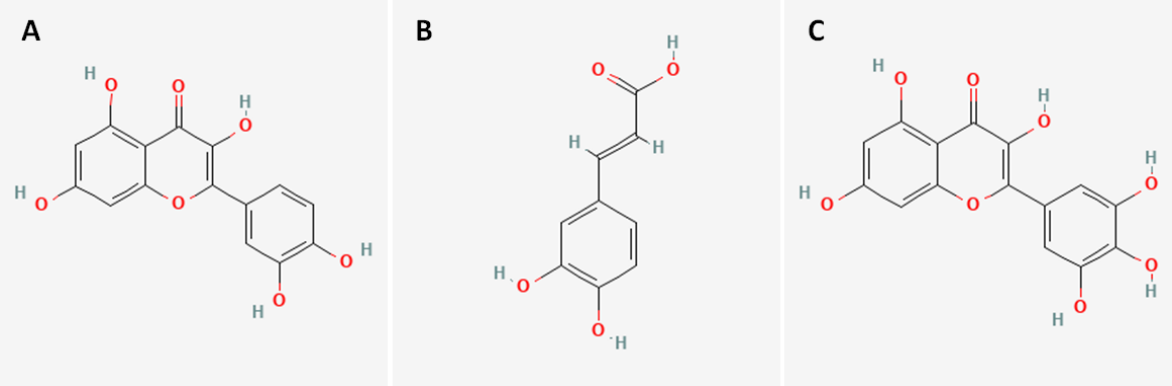

Figure 4. The primary constituents of the crude drugs showed quercetin of Chinaberry leaves (A), caffeic acid of Malayan cherry fruits (B), and myricetin of Yacon leaves (Pubchem, 2022)

showed a high level of TFC (66,19±13,34 mg QE/g DW), while those collected in Peru and Ecuador ranged from 110.34±4.68-199.29±58.75 mg QE/g DW according to cultivars and leaf maturity (Khajehei et al., 2017; Ramonah et al., 2020). Numbers of free and glycosylated flavonoids, particularly gossypetin, kaempferol, Quercetin, and rutin derivates, were putatively detected in Yacon leaves (Padilla-González et al., 2020). Myricetin and rutin were the flavonoids with the highest concentration among others (Khajehei et al., 2017).

Similarly, an equal value of TPC was observed in Chinaberry leaves, Malayan cherry fruits, and Yacon leaves collected from different places. The plant species statistically affected the TPC of the crude drugs (p = 0.000). Malayan cherry fruits contained the highest phenolic compounds, and Yacon leaves showed the lowest level of phenolic compounds among other crude drugs (Figure 3). Previous reports mentioned that the TPC of Chinaberry leaves collected in Malawi and Nepal was 7.83 and 13.96±2.99 mg GAE/g, respectively (Kharel and Sharma, 2020; Mwamatope et al., 2020). Hence, they were much higher than observed in this study (1.56±0.07 mg GAE/ g DW). In addition to flavonoids, at least three phenolic compounds have been isolated from the Chinaberry tree, i.e., 1,7,8-trihydroxy-2-naphtaldehyde; 2-methoxy-4-(2-propenyl)phenyl β-D-glucoside; and benzyl 3-O-β-D-glucopyranosyl-7-hydroxybenzoate (Zeng et al., 2019). TPC of Malayan cherry fruits in this study (11.54±1.44 mg GAE/g DW) was higher than those collected in Thailand and Jember (1.11-5.19 and 10.85 mg GAE/g, respectively) but lower than those from South America (46.51±0.79-76.67±21.67 mg GAE/g) (Khajehei et al., 2017; Nasution et al., 2022; Simamora et al., 2020). Various phenolic compounds, i.e., caffeic acid, cyanidin-3-O-glucoside, gallic acid, gentisic acid, gallocatechin, and protocatechuic acid, were characterized in the Brazilian-originated Malayan cherry fruits (Pereira et al., 2018). On the other hand, our result regarding TPC (0.51±0.03 mg GAE/g DW) was much lower than Yacon leaves collected in Medan (6.74±0.15 mg GAE/g DW) (Ramonah et al., 2020). Putative analysis by Liquid Chromatography-Mass Spectrometry (LC-MS) showed that Yacon was rich in phenolic compounds, with significant constituents of anthocyanins, *trans*-cinnamic acid derivatives, and flavonoids (Padilla-González et al., 2020). The compounds found in high content were caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, myricetin, and rutin (Khajehei et al., 2017). The structure of the main flavonoids and/or phenolic compounds of Chinaberry leaves, Malayan cherry fruits, and Yacon leaves are presented in Figure 4.

The DPPH scavenging activity was significantly influenced by the crude drug-producing plants (p = 0.000), while it is equal between crude drugs of different origins. Malayan cherry fruits were the crude drugs with the highest DPPH scavenging activity at 366.13±17.65 mM TE/g DW (Figure 5). The available data suggested that the DPPH scavenging activity of the plants evaluated in this study was widely varied. For example, Malawian Chinaberry leaves methanolic extract weakly scavenged DPPH with % radical scavenging activity of 42.72±0.18%, while the IC_{50} of Indonesian ethanolic extract was 232.00±11.00 µg/ml (Ervina et al., 2020; Mwamatope et al., 2020). Also, the IC_{50} of DPPH scavenging activity of Taiwanese Malayan berry fruit methanolic and Malaysian-grown ethanolic extract was

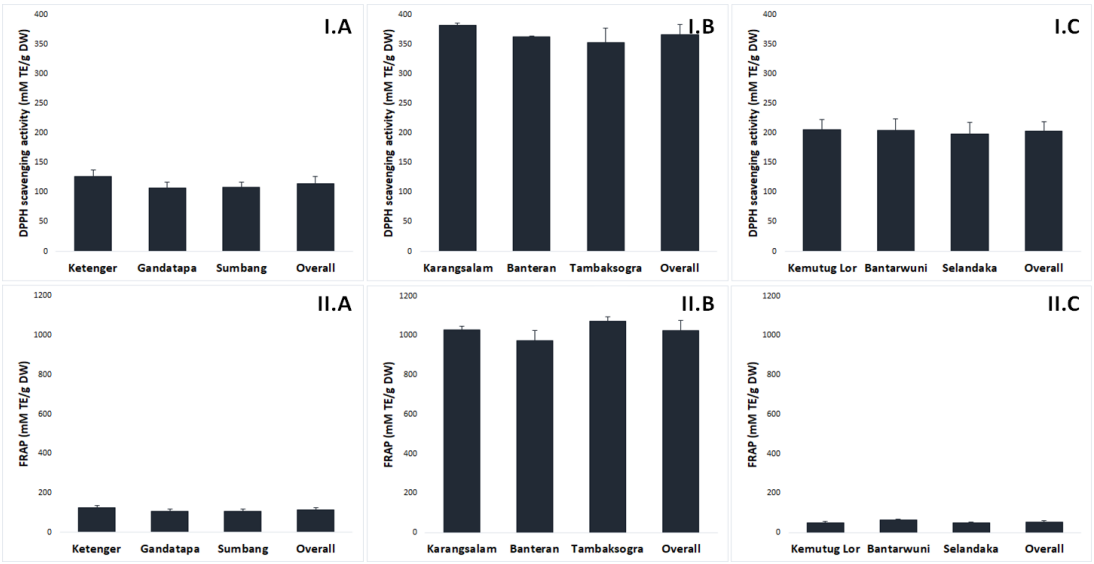

Figure 5. Antioxidant activity of crude drugs of Chinaberry leaves (A), Malayan cherry fruits (B), and Yacon leaves (C) evaluated by DPPH scavenging activity (I) and FRAP (II) mechanisms

350,00±20.00 and 8.49±0.24 µg/ml, respectively (Lin et al., 2017; Yunus et al., 2021). Yacon leaf extracts also showed a wide range of DPPH scavenging activity. Samples collected from different places in Ecuador and Peru showed IC_{50} values of 2.08±0.99-4.39±0.32 mg/ml, Japanese Yacon leaf water extracts strongly scavenged DPPH with an EC_{50} value of 28000 mg/ml (Daniela Russo et al., 2015; Sugahara et al., 2015).

Plant species affected the FRAP of the crude drugs, with a p-value of 0.000. Similar to the free radical scavenging activity, Malayan cherry fruits (1025.33±50.47 mM TE/g DW) showed the highest FRAP and subsequently followed by Chinaberry leaves (114.05±13.07 mM TE/g DW) and Yacon leaves (54.22±7.99 mM TE/g DW) (Figure 5). Previous reports presented that the FRAP of all three samples evaluated in this study ranged greatly according to geographical, genetic, and processing aspects. For example, the FRAP of Chinaberry leaf ethanolic extract from Batu showed a ferric reducing capacity of 106.20±1.53 Rutin equivalent/sample (Ervina et al., 2020). On the other hand, the methanolic extract of the leaves collected in Bhaktapur, Nepal, was 65.02±3.09 mg TE/g DW (Shrestha et al., 2021). The methanolic extract of the fruits from Paraná, Brazil, showed much lower FRAP (of around 100 mM TE/g DW) than our result (Rotta et al., 2017). The ferric-reducing activity of Southern American Yacon leaf methanolic extract was 31.55±0.96- 66.80±1.90 mg TE/g DW, while those cultivated in Stuttgart, Germany, showed FRAP ranging from 798.11±52.23- 994.55±83.38 mM Fe^{2+}/g DW (Khajehei et al., 2017; Daniela Russo et al., 2015).

DPPH scavenging assay evaluated the sample's ability to transfer single electrons and Hydrogen atoms to stabilize the radical structure. In contrast, the FRAP assay quantified the sample's capacity in transferring single-electron for redox reaction. The double bonds and aromatic rings of flavonoids and phenolic compounds likely contributed to both mechanisms of antioxidant actions. The double bonds of phenolic compounds might transfer a single electron, probably quantified in both DPPH scavenging activity and FRAP assays. In addition, the transfer of hydrogen atoms from the hydroxyl group to stabilize the radicals might be quantified in the DPPH assay (Pisoschi et al., 2016; Santos-Sánchez et al., 2019). The TFC of Chinaberry leaves was moderately correlated to DPPH and weakly correlated to its FRAP, while the TPC showed the opposite trend (Table II). Our result was consistent with the previous report on the moderate correlation between TPC and FRAP in Batu-originated Chinaberry leaves (Ervina et al., 2020). Hence, flavonoids and phenolic compounds were likely not the antioxidant compounds in the Banyumas-originated Chinaberry leaves. Several limonoid triterpenoids isolated from Chinaberry fruits exerted substantial *in-vitro* antioxidant activities in astrocytes and lipopolysaccharide (LPS)-activated

Table II. Correlation between antioxidant contents and antioxidant activities of the crude drugs

Crude drugs	Parameters	Pearson's correlation coefficient toward	
		DPPH scavenging activity	FRAP
Chinaberry leaves	TFC	0.392	-0.213
	TPC	-0.018	-0.345
Malayan cherry fruits	TFC	0.489	0.725
	TPC	-0.029	0.032
Yacon leaves	TFC	0.511	-0.020
	TPC	0.385	-0.312

RAW 264.7 macrophages supported our thesis (Park et al., 2020; Qiu et al., 2019).

On the other hand, the TFC of Malayan cherry fruits was moderately correlated to its free radical scavenging activity and strongly correlated to the FRAP. However, the fruit's TPC was weakly correlated to both antioxidant activity parameters (Table II). The same positive correlations between TPC-TFC and their antioxidant activity have also been reported in the fruits collected in Kolhapur, India (Kolar et al., 2011). Hence, DPPH scavenging activity and FRAP of Malayan cherry fruits from Banyumas were attributable to their content of flavonoids and phenolic compounds. Ultimately, the TFC correlation of Yacon leaves showed a similar pattern to that of Chinaberry leaves, with a moderate correlation between TPC and DPPH scavenging activity and FRAP (Table II). Our result was slightly different from a report from Southern America that a strong correlation between TPC and FRAP was reported in the Yacon leaf landraces, with a moderate correlation toward radical scavenging activity (Daniela Russo et al., 2015). Also, polyphenolic compounds were primarily detected in water extracts of the leaves with a potent antioxidant activity (D. Russo et al., 2015). However, other secondary metabolite groups could be the major contributor to the antioxidant activity of the Yacon leaves collected in Banyumas. The melampolide sesquiterpene lactones isolated from Yacon leaves, which showed considerably *in-vitro* antioxidant effects in the LPS-activated RAW 264.7 macrophages, might contribute to the said activity (Hong et al., 2008).

Our results supported the traditional use of Chinaberry leaves, Malayan cherry fruits, and Yacon leaves for antidiabetic management in Northern Banyumas. At least, the antidiabetic activity of those plants was mediated through indirect antioxidative mechanisms. Our findings complete the available information on the antidiabetic mechanisms of the Chinaberry tree, i.e., decreasing the gastrointestinal tract's insulin demand, increasing peripheral cells' insulin sensitivity, and slowing-down gastric emptying rate (Seifu et al., 2017). On the other hand, the antidiabetic mechanisms of the Malayan cherry tree involved blood glucose level reduction, insulin sensitivity improvement, and pancreatic β-cells regeneration, while those of Yacon were via glucoregulation improvement and oxidative stress and inflammatory reaction amelioration (Aligita et al., 2018; Santos et al., 2017). As the flavonoids and phenolic compounds are the main contributors to the antioxidant properties in Malayan cherry fruits and Yacon leaves, TFC and TPC are proposed as the chemical content parameters for the standardization of both crude drugs. It also rules out those metabolite groups from being the antioxidant compounds in Chinaberry leaves.

CONCLUSION

This current study characterized the physicochemical properties of Chinaberry leaf, Malayan cherry fruit, and Yacon leaf crude drugs as the initial parameters for their quality. The crude drugs with the highest TFC and TPC were Yacon leaves and Malayan cherry fruits, respectively, while Malayan cherry fruits showed the most potent antioxidant activities. The antioxidant activity of both crude drugs was attributable to their flavonoid and phenolic compound contents, but other compounds might be the primary antioxidants in Chinaberry leaves.

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