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Karakter Mutu Simplisia dan Ekstrak Tumbuhan Antidiabetes Lokal dari Banyumas

Selected Quality Characters of Crude Drug and Extract of Traditional Antidiabetic Plants from Banyumas

Dwi Hartanti[1*], Shintia Lintang Charisma[2], Hidayah Anisa Fitri[3], Fitriani[4], Denia Awanda Putri[5], Jeri Rinawati[6], Widya Agustina[7], Alwani Hamad[8]

[1,2,3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto
[4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto
[5,6,7]Study Program of Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto
[8]Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Muhammadiyah Purwokerto

*email: dwihartanti@ump.ac.id

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ABSTRAK

Daun mindi (*Melia azedarach* L.), buah kersen (*Muntingia calabura* L.), serta daun dan bunga tapakdara (*Catharanthus roseus* (L.) G.Don) digunakan masyarakat Baturraden dan Sumbang (Banyumas) untuk pengobatan tradisional diabetes. Penelitian ini mengkarakterisasi beberapa parameter mutu simplisia dan ekstrak ketiga tumbuhan tersebut. Simplisia dibuat dari bahan tumbuhan yang dikumpulkan dari tiga desa di wilayah kecamata Baturraden dan Sumbang. Morfologi makroskopik dan mikroskopik, profil kromatografi lapis tipis (KLT), kadar fenolik total, dan kadar flavonoid total simplisia, serta kadar air, kadar abu total, dan kadar abu tidak larut asam ekstrak dianalisis dengan metode standar dalam Farmakope Herbal Indonesia (FHI) 2017. Karakter makroskopik, fragmen diagnostik, dan profil KLT dari simplisia hasil penelitian ini diusulkan untuk menjadi standar dalam memastikan kebenaran identitas simplisia. Kadar fenolik total dan kadar flavonoid total simplisia daun mindi, buah kersen, serta daun dan bunga tapakdara masing-masing sebesar 0,74±0,07 dan 0,99±0,31; 1,62±0,36 dan 1,39±0,11; dan 0,94±0,02 dan 1,98±0,04%. Nilai tersebut diusulkan sebagai standar untuk aspek kandungan masing-masing simplisia. Data kadar air, kadar abu total, dan kadar abu tidak larut asam diusulkan sebagai standar aspek kemurnian dari masing-masing ekstrak.

Kata Kunci: *Catharanthus roseus,* Kadar Fenolik Total, Kadar Flavonoid Total*, Melia azedarach, Muntingia calabura*, Parameter Mutu

ABSTRACT

Chinaberry (Melia azedarach L.) leaves, Malayan cherry (Muntingia calabura L.) fruits, and rose periwinkle (Catharanthus roseus (L.) G.Don) leaves and flowers are traditionally used for diabetes treatment by Baturraden and Sumbang (Banyumas) people. This study characterized selected quality parameters of their crude drugs and extracts. The crude drugs were prepared from the plant materials collected from three villages in those two subdistricts. Macroscopic and microscopic morphology, thin layer chromatography (TLC) profile, total flavonoid content (TFC), and total phenolic content (TPC) of the crude drugs, as well as moisture content, total ash, and acid-insoluble ash of the extracts, were determined according to the standard method

in the Indonesian Herbal Pharmacopeia (IHP) 2017. The macroscopic characters, diagnostic fragments, and TLC profile of the crude drugs presented in this study are proposed for the identity aspect of their quality standard. TFC and TPC of Chinaberry leaves, Malayan cherry fruits, and rose periwinkle flowers-leaves were 0.74±0.07 and 0.99±0.31; 1.62±0.36 and 1.39±0.11; and 0.94±0.02 and 1.98±0.04%, respectively. Those values were proposed as the content standard for the respective crude drug. The value of the purity aspects of the quality parameters of the extracts is reported and proposed.

***Keywords**: Catharanthus roseus, Melia azedarach, Muntingia calabura, quality parameters, total flavonoid content, total phenolic content*

1. INTRODUCTION

Baturaden and Sumbang (Banyumas, Central Java) people used 11 plant species to prevent and treat diabetes. They utilized Chinaberry (*Melia azedarach* L., Meliaceae) leaves, Malayan cherry (*Muntingia calabura* L., Muntingiaceae) fruits, and rose periwinkle (*Catharanthus roseus* (L.) G.Don, Apocynaceae) flowers and leaves, among other medicinal plants. The official standard quality for crude drugs and extracts of these three plants is unavailable as their monograph has not been included in any editions of Indonesian Herbal Pharmacopeia (IHP) (Indonesian MoH, 2017; Utaminingrum et al., 2020). IHP is the official monograph for herbal material and herbal preparation in Indonesia. Hence, all crude drugs and extracts intended for medicinal uses should comply with the standard set in the recent edition of IHP. The quality of crude drugs and extracts highly affected the safety and efficacy profile. Standards, constant parameters, and absolute qualitative and quantitative values of crude drugs and extracts of a given medicinal plant should be characterized to assure their quality (Das et al., 2019; Mukhi et al., 2016).

The chinaberry tree is a natural herbicide, nematocide, and pesticide plant. Anthelmintic, emmenagogue, expectorant, and vermifuge are the reported traditional uses of this plant. It showed major secondary metabolites of terpenoids, sterols, flavonoids, and glycosides. The *in-vitro* anthelmintic, antihyperglycemic, antimalarial, antimicrobial, antioxidant, antipyretic, and cytotoxic activities have been evaluated with a wide range of effectiveness (Sultana et al., 2014). The Malayan cherry tree is used for various health purposes in many countries, including Indonesia. It contains more than 80 compounds, all of which have been isolated and characterized. Numerous pharmacological activities, from antidiabetic to cytotoxic activities, of this plant have been evaluated (Mahmood et al., 2014). Rose periwinkle is a typical plant used to traditionally manage cancer and diabetes. There are more than 300 compounds characterized in this plant. Further, this shrub's cytotoxic, antihyperglycemic, antimicrobial, antioxidant, and larvicidal activities have been confirmed (Kumar et al., 2022).

This study characterized selected quality standards of crude drugs and extracts of Chinaberry leaf, Malayan cherry fruit, and rose periwinkle flower-leaf collected from different areas in Baturaden and Sumbang to provide their respective initial quality standard profile.

2. MATERIALS AND METHODS

2.1 Materials

Chemicals and reagents were purchased from Sigma (US), i.e., $AlCl_3$, chloralhydrate, Folin-Ciocalteau reagent, formic acid, gallic acid, hydrochloric acid, CH_3COONa, NaOH, and quercetin. Solvents used were chloroform, ethanol, ethyl acetate, n-hexane, methanol, and water. Plant materials for the experiments were collected from three different villages of Baturraden and Sumbang area, and their identity was determined in the Laboratory of Pharmaceutical Biology, Universitas Muhammadiyah Purwokerto (Table 1).

2.2 Crude drug preparation

Malayan cherry fruits were halved, while Chinaberry leaves and rose periwinkle flowers-leaves were wholly dried. The plant materials were dried at 40^{o}C in a cabinet drying rack.

2.3 Observation of crude drug morphological characters

The macroscopical characters of the crude drugs were organoleptically described. The diagnostic fragments as microscopical characters were observed in the powdered crude drugs under a light microscope (Olympus, Japan) connected to a camera (OptiLab, Indonesia) with chloralhydrate as the mounting agent.

Table 1. Details of plant materials used in the study

Plant name	Plant parts	Origin	Authentication reference
Chinaberry tree	Leaves	Karangtengah, Gandatapa, and Sumbang	273-DAP
Malayan cherry tree	Fruits	Karangsalam, Banteran, and Tambaksogra	253-WA
Rose periwinkle	Flowers and leaves	Pamijen, Banteran, and Sumbang	254-JR

2.4 Thin layer chromatography (TLC) profile of crude drugs

The powdered crude drugs were extracted in ethanol (in a ratio of 1:10) by sonication-assisted maceration for 15 min. The extract was collected after filtration and used as the sample for analysis. Silica gel 60 F_{254} was used as the stationary phase. The mobile phase for separation of Chinaberry leaves, Malayan cherry fruits, and rose periwinkle was chloroform-methanol (9:1), chloroform-methanol-formic acid (7:2.5:0.5), and n-hexane-ethyl acetate (8:2), respectively. The detection was conducted under UV light at 366 nm.

2.5 Determination of the chemical content of the crude drugs

Phenolic compounds and flavonoids were chosen as the chemical content of the crude drugs, and their quantitative enumeration was reported as total phenolic content (TPC) and total flavonoid content (TFC). TPC and TFC were determined by following the standard method in the IHP (Indonesian MoH, 2017). 400 mg of the powdered crude drugs were extracted in 10 ml of ethanol by sonication-assisted maceration for 60 min. The filtrate was directly used, with proper dilution, for TPC and TFC analysis. Quercetin solutions were used as the standard at 50-250 µg/ml. A reaction mixture of 0.5 ml of extract or standard, 1.5 ml of ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M CH_3COONa, and 2.8 ml of water was homogenized. After 30 min, the absorbance was read at 370 nm in a UV-Vis spectrophotometer (UV-Vis (Shimadzu, Japan). TFC was reported in % and calculated by Formula 1.

$$TFC = \frac{CxVxf}{W}\ x\ 100\% \quad \text{.....(1)}$$

With C = concentration of flavonoid total obtained from standard curve equation in mg Quercetin equivalent/g crude drugs, V = volume of the first prepared solution, f = dilution factor, and W = weight of the crude drugs.

Gallic acid solutions at 6.25-100 µg/ml were used as the standard for TPC determination. The reaction mixture for TPC consisted of 1 ml of extract or standard, and 5 ml of 7.5% Folin-Ciocalteu reagent was stood for 8 min. Upon addition with 4 ml of 1% sodium hydroxide for 60 min, the absorbance was read at 730 nm. TPC was reported in % and calculated by Formula 2.

$$TPC = \frac{CxVxf}{W}\ x\ 100\% \quad \text{.....(2)}$$

With C = concentration of phenolic content obtained from standard curve equation in mg gallic acid equivalent/g crude drugs, V = volume of the first prepared solution, f = dilution factor, and W = weight of the crude drugs.

Extraction of crude drugs

A total of 100 g of each crude drug was extracted with 1000 ml of 70% ethanol by maceration method. The yield of extraction was calculated accordingly (Indonesian MoH, 2017).

2.6 Determination of selected quality parameters of the crude drugs

Moisture content, total ash, and acid-insoluble ash of each crude drug were analyzed by the gravimetric method according to the official method in the IHP (Indonesian MoH, 2017)

2.7 Statistical Analysis

The effect and mean separation of the plant material origin on the TFC, TPC, moisture content, total ash, and acid-insoluble within each crude drug were evaluated by one-way ANOVA and Duncan's test at $p < 0.05$. The analysis was conducted by standard procedures in SPSS ver. 26 (IBM, US).

Figure 1. Macroscopic morphology of crude drugs of Chinaberry leaf (upper) collected from Karangtengah (A), Gandatapa (B), and Sumbang (C); Malayan cherry fruit (middle) collected from Karangsalam (D), Banteran (E), and Tambaksogra (F); and rose periwinkle (lower) collected from Pamijen (G), Banteran (H), and Sumbang (I)

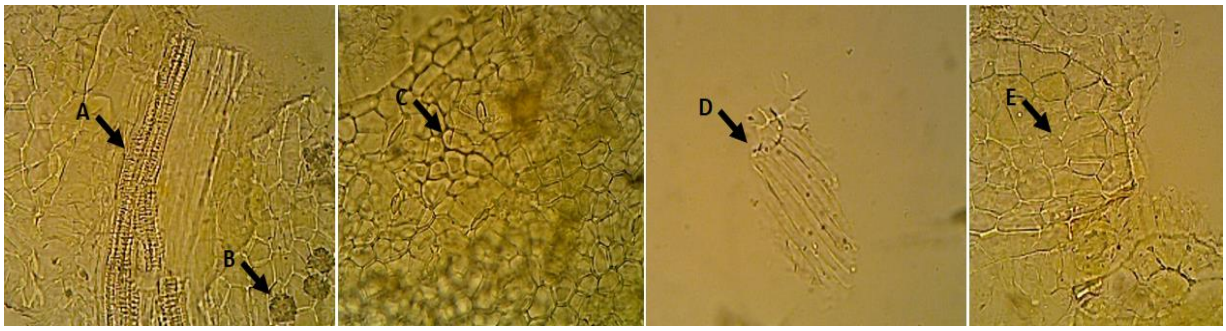

Figure 2. Diagnostic fragments of Chinaberry leaf crude drugs showed spiral vessel (A), rosette-shaped calcium oxalate crystal (B), upper epidermis with underlying parenchyma (C), palisade parenchyma (D), and upper epidermis (E)

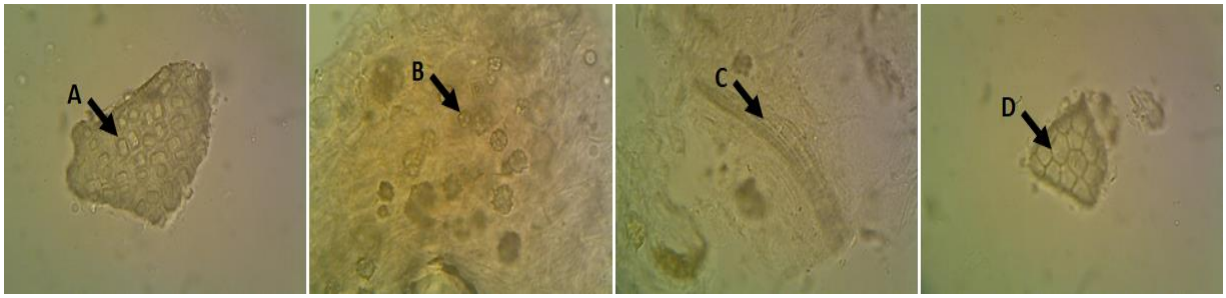

Figure 3. Diagnostic fragments of Malayan cherry fruit crude drugs showed prismatic-shaped (A) and rosette-shaped (B) calcium oxalate crystals, spiral vessels (C), and exocarps (D)

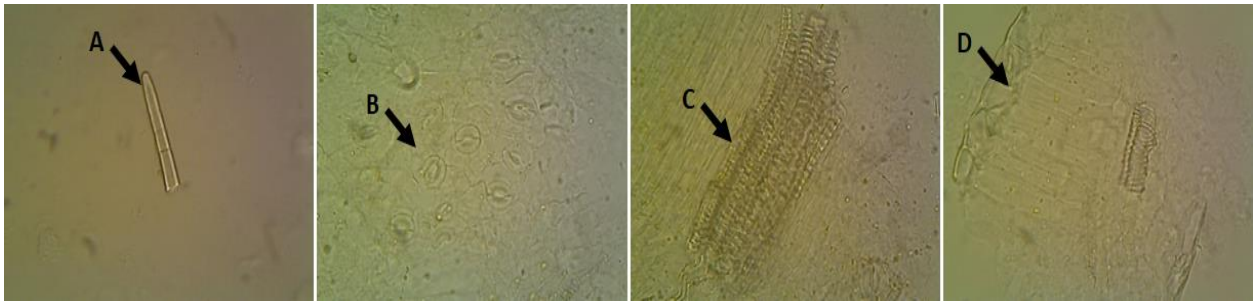

Figure 4. Diagnostic fragments of rose periwinkle flower and leaf crude drugs showed covering trichome (A), lower epidermis with stomata (B), spiral vessels (C), and mesophyll with palisade parenchyma (D)

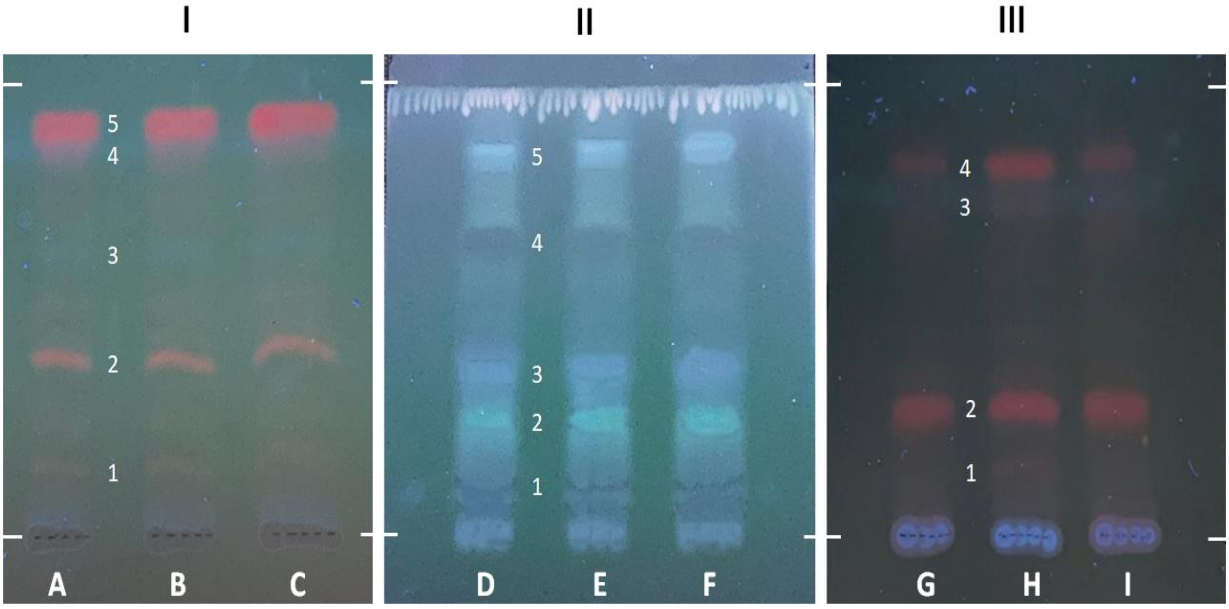

Figure 5. TLC profile of crude drugs of Chinaberry leaf (I) collected from Karangtengah (A), Gandatapa (B), and Sumbang (C); Malayan cherry fruit (II) gathered from Karangsalam (D), Banteran (E), and Tambaksogra (F); and rose periwinkle (III) collected from Pamijen (G), Banteran (H), and Sumbang (I)

Table 2. TLC profile of the crude drugs

Crude drugs	Separated bands		
	Band number	Rf value	Color
Chinaberry leaves	1	0.15	Pale red
	2	0.40	Red
	3	0.63	Blue
	4	0.85	Pink
	5	0.91	Dark red
Malayan cherry fruits	1	0.09	Dark blue
	2	0.25	Teal
	3	0.35	Light blue
	4	0.65	Dark blue
	5	0.86	Light blue
Rose periwinkle flowers and leaves	1	0.13	Pink
	2	0.31	Red
	3	0.74	White
	4	0.81	Red

3. RESULTS AND DISCUSSIONS

The morphology of the crude drugs collected from different places is macroscopically similar (**Figure 1**). Chinaberry leaf crude drugs are dried, whole, broken, serrate-edged, elliptic-shaped, dark green leaves with an aromatic scent and bitter taste. Malayan cherry ones are dried, asymmetrically halved, evenly edged, dark brown, small fruits with an aromatic odor and somewhat sweet taste. On the other hand, rose periwinkle crude drugs are a mixture of dried, whole and broken, entire-edged, lanceolate-shaped, dark green leaves and funnel-shaped, five-meriated, pale pink flowers with the aromatic scent and bitter taste.

The prominent diagnostic fragments of crude drugs are presented in **Figure 2-4.** Chinaberry leaves show spiral vessels, rosette-shaped calcium oxalate crystals, palisade, spongy parenchyma, and somewhat sharply-angled epidermis. The shape of the epidermis observed in this study differs from one grown in Pakistan, which was more curved-edged with numerous stomata (Sultana et al., 2011). Calcium oxalate crystals, spiral vessels, and exocarps are observed in the Malayan cherry fruits. There are no data available on the pharmacognostic characteristics of this fruit. However, some fruits described in IHP showed diagnostic fragments similar to those in this study. Calcium oxalate crystal is a diagnostic fragment of nutmeg and lily of the valley fruits, while exocarp is a diagnostic fragment for mangosteen pericarps (Indonesian MoH, 2017). Rose periwinkle flower-leaf crude drugs contain fragments of covering trichomes, epidermis with stomata, spiral vessels, and palisade parenchyma.

Similarly, there is no report available on the morphological aspects of this crude drug. For comparison, covering trichome of torch ginger in IHP is in a similar shape as in this study. Also, the epidermis, vessels, and parenchyma are commonly used as diagnostic fragments in many leaf-originated crude drugs described in IHP (Indonesian MoH, 2017).

Each crude drug of different origins showed a similar TLC profile (**Figure 5**). 5, 5, and 4 distinctive bands separated from Chinaberry leaf, Malayan cherry fruit, and rose periwinkle flower-leaf crude drug extracts (Table 2). Hence, chromatographic systems utilized in this study can effectively separate the extract.

A given crude drug is of high quality when the aspects of identity, purity, and content are all within the specified standard value in their official monograph (Alamgir, 2017). The morphological characters, both macroscopic and microscopic, defined the identity aspect altogether with the TLC profile. The diagnostic fragments and the TLC condition of each crude drug reported in this study might be used for the rationale of future monograph production.

Phenolic compounds and flavonoids are proposed as the chemical content of all crude drugs. TPC and TFC are commonly reported in gallic acid equivalent (GAE) and Quercetin equivalent (QE). However, in this study, they are written in the percentage of crude drugs as in the official monograph. The origin of plant materials does not affect the TPC and TFC of all three crude drugs evaluated in this study. TPC is superior to TFC in the leaf-containing crude drugs, while the opposite trend is observed in fruit-based. The highest TPC is shown by rose periwinkle flowers and leaves, followed by Malayan cherry fruits and Chinaberry leaves. On the other hand, Malayan cherry fruits and Chinaberry leaves exert the highest and lowest TFC, respectively (**Table 3**).

TPC of a given plant species is widely varied according to the botanical source and geographical origin. For example, Malawian and Nepalese Chinaberry leaves showed TPC of 7.83 and 13.96±2.99 mg GAE/g, respectively (Kharel & Sharma, 2020; Mwamatope et al., 2020). The major phenolic compounds in this plant are flavonoids and 4-hydroxybenzoate-derivates (Zeng et al., 2019). Similarly, Thai and Indonesian Malayan cherry fruits exerted a TPC of 1.11-10.85 mg GAE/g (Nasution et al., 2022; Simamora et al., 2020). The major phenolic compounds in this fruit are caffeic acid, gallic acid, gentisic acid, gallocatechin, and protocatechuic acid (Pereira et al., 2018). TPC of Indian and South African rose periwinkle leaf extracts is 23.20±0.72 mg tannic acid equivalent (TAE)/g and 44.51 mg GAE/g, respectively (Goboza et al., 2020; Rani et al., 2017). The wide variation of TFC in a given plant material is also commonly reported. TFC of Malawian and Nepalese Chinaberry leaves ranged from 0.532-41.07 mg QE/g DW, with astragalin, kaempferol, quercetin, and rutin as the major compounds (Kharel & Sharma, 2020; Kumazawa et al., 2013; Mwamatope et al., 2020; Zeng et al., 2019). As for Malayan cherry fruits, one collected in Jember showed a TFC of 3.30 mg QE/g DW, while that of Indian rose periwinkle was 12.00±0.59 mg QE/g (Rani et al., 2017; Simamora et al., 2020). Chinaberry leaves showed the lowest TPC and TFC among other crude drugs. The available data reported that phenolic compounds and flavonoids are not the dominant constituents of this plant. The principal secondary metabolites in the Chinaberry tree are limonoid terpenoids (Sultana et al., 2014).

Each crude drug results in a different extraction yield, with Malayan cherry fruits are the highest, followed by Chinaberry leaves and

rose periwinkle flowers-leaves (**Table 4**). Our Chinaberry leaves yield is higher than the one collected in Malang (30.10±0.42%) (Ervina et al., 2020). On the other hand, that of Malayan cherry fruits is much higher than one of Jember and Makassar, with a yield of 21.15 and 29.01%, respectively (Nur et al., 2020; Simamora et al., 2020). Similarly, the extraction yield of rose periwinkle is also higher than that of various plant parts of the shrub collected in India, which ranged from 3.21-9.31% (Kumar et al., 2017).

The plant material origin did not affect moisture content but statistically defined total ash and acid-insoluble ash of Chinaberry leaves. The highest total and acid-insoluble ash are shown by one collected in Karangtengah and Sumbang, respectively **(Table 4**). There is no data available for the value of these quality parameters of the ethanol extract of the plant leaf to date. The plant material origin significantly affected all moisture content, total ash, and acid-insoluble ash of Malayan cherry fruits. The highest moisture and ash content was observed in one collected from Tambaksogra (**Table 4**). As in Chinaberry leaves, there is no quality parameter available for the ethanol extract of this fruit. However, for comparison purposes, the value of these three parameters of Malayan cherry leaf ethanol extract collected in Banjarbaru was 8.88, 2.27±0.15, and 0.05±0,04%, respectively. The plant material origin of rose periwinkle flowers and leaves significantly affected acid-insoluble ash but did not define the other two parameters. Crude drugs with the lowest acid-insoluble ash content were collected in Banteran (**Table 4**). Similar to the other two crude drugs, the data on these plant extract quality parameters were also unavailable.

Moisture content, total ash, and acid-insoluble ash represented the purity aspects of the extracts. Moisture content is commonly associated with the risk of microbial spoilage, while ash contents indicate inorganic impurities in the extracts. The moisture in an extract is originated from the improper solvent evaporation process and moisture absorption during storage. As the extracts were freshly prepared, the first mentioned cause is likely more applicable to our result. On the other hand, ash in the extract might be gained from plant cultivation and crude drug processing. Contamination from the soil might be the source of the ash detected in the extracts (Agarwal et al., 2014; Tauheed et al., 2017).

Table 3. Chemical content of the crude drugs

Crude drugs	Origin	TFC (%)	TPC (%)
Chinaberry leaves	Karangtengah	0.72± 0.07	1.02±0.03
	Gandatapa	0.77±0.09	0.99±0.01
	Sumbang	0.73±0.03	0.96±0.90
	Overall	0.74±0.07	0.99±0.31
Malayan cherry fruits	Karangsalam	1.47±0.24	1.47±0.10
	Banteran	1.60±0.26	1.30±0.04
	Tambaksogra	1.80±0.56	1.40±0.14
	Overall	1.62±0.36	1.39±0.11
Rose periwinkle flowers and leaves	Pamijen	1.04±0.06	1.96±0.05
	Banteran	0.90±0.05	1.90±0.13
	Sumbang	0.89±0.09	2.08±0.10
	Overall	0.94±0.02	1.98±0.04

Table 4. Quality characters of the extracts

Crude drugs	Origin	Parameter value (%)			
		Yield	Moisture content	Total ash	Acid-insoluble ash
Chinaberry leaves	Karangtengah	36.49	4.85±0.60	7.13±0.18^{C}	0.17±0.01^{A}
	Gandatapa	32.15	5.09±0.19	6.33±0.07^{A}	0.22±0.01^{B}
	Sumbang	39.21	5.12±0.20	6.70±0.13^{B}	0.27±0.01^{C}
	Overall	35.95±3.56	5.02±0.33	6.72±0.13	0.23±0.01
Malayan cherry fruits	Karangsalam	46.33	5.83±0.28^{A}	1.74±0.34^{A}	0.25±0.01^{A}
	Banteran	41.33	6.82±0.30^{A}	3.01±0.24^{B}	0.41±0.01^{B}
	Tambaksogra	38.32	7.09±0.45^{B}	3.34±0.10^{B}	0.54±0.08^{C}
	Overall	41.99±4.05	6.58±0.65	2.69±0.76	0.40±0.13
Rose periwinkle flowers and leaves	Pamijen	27.35	4.14±0.22	8.77±0.46	0.34±0.06^{B}
	Banteran	28.93	4.04±0.23	8.05±0.74	0.19±0.01^{A}
	Sumbang	36.35	3.77±0.56	7.77±0.68	0.25±0.04^{B}
	Overall	30.88±4.81	3.98±0.19	8.20±0.14	0.26±0.02

Different alphabets within the same parameter of a given crude drug represented statistically different values at $p<0.05$.

4. CONCLUSION

This current study characterized the identity and content aspects of quality of crude drugs and purity aspects of the extracts of Chinaberry leaves, Malayan cherry fruits, and rose periwinkle flowers-leaves as the initial parameters for their quality. The macroscopic characters, diagnostic fragments, and TLC profile of the crude drugs are proposed. The crude drugs with the highest TFC and TPC were Malayan cherry fruits and rose periwinkle flowers-leaves, respectively. The standard value for the extracts' moisture content, total ash, and acid-insoluble ash are also proposed.

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