Jurnal Ilmiah Ibnu Sina, 10(1), Maret 2025, 116-127
p-ISSN: 2502-647X; e-ISSN: 2503-1902

QUALITY CHARACTERIZATION AND ANTIOXIDANT PROPERTIES OF ROSE PERIWINKLE CRUDE DRUGS

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Artikel submitted: December 7th, 2024; Accepted: March 25th, 2025
DOI: https://doi.org/10.36387/jiis.v10i1.2320

ABSTRAK

Daun dan bunga tapak dara (Catharanthus roseus (L.) G. Don) digunakan untuk pengobatan tradisional diabetes di Banyumas, Indonesia. Penelitian ini mengevaluasi karakter mutu, kadar flavonoid total, kadar fenolik total, dan aktivitas antioksidan dari simplisia daun dan bunga tapak dara. Bahan tumbuhan dikumpulkan dari tiga lokasi di Kecamatan Baturraden dan Sumbang, yang kemudian dikeringkan menggunakan lemari pengering. Karakter mutu simplisia, kadar flavonoid total, kadar fenolik total, dan aktivitas antioksidan dievaluasi menggunakan metode standar. Karakter mutu simplisia adalah sebagai berikut: susut pengeringan (9,12±0,50%), abu total (8,20±0,71%), abu tidak larut asam (0,24±0,06%), sari larut air (28,82±6,77%), dan sari larut etanol (35,17±1,79%). Profil kromatografi lapis tipis diperoleh dari pemisahan dengan fase gerak kloroform-metanol (8:2) dan fase gerak silika GF254. Kadar flavonoid total dan kadar fenolik total simplisia masing-masing sebesar 7,66±0,48 mg setara kuersetin/g dan 7,27±1,07 mg setara asam galat/g. Aktivitas antioksidan metode penangkal radikal DPPH sebesar 148,95±12,00 setara Trolox/g dan metode FRAP sebesar 651,14±77,91 mM setara Trolox/g. Aktivitas penangkal radikal DPPH dan FRAP berkorelasi lemah-sedang dengan kadar flavonoid total dan kadar fenolik total. Dengan demikian, kandungan senyawa fenolik dan flavonoid dari simplisia tapak dara yang terkarakterisasi sifat fisika kimianya tidak berkorelasi signifikan dengan aktivitas antioksidannya.

Kata kunci: *Antioksidan, Tapak dara, Simplisia, Flavonoid dan senyawa fenolik, Karakter mutu*

ABSTRACT

Leaves and flowers of rose periwinkle (*Catharanthus roseus* (L.) G. Don) are used for traditional diabetes management in Banyumas, Indonesia. This study evaluated the selected quality characters, total flavonoid content (TFC), total phenolic content (TPC), and the antioxidant activities of rose periwinkle crude

drugs. Plant materials were collected from three locations in the Baturraden and Sumbang subdistricts and dried using an artificial heat drying rack to obtain crude drugs. The crude drugs' quality characters, TFC, TPC, and antioxidant activities were evaluated using standard methods. The quality characters of the crude drugs were specified as follows: loss on drying (9.12±0.50%), total ash (8.20±0.71%), acid-insoluble ash (0.24±0.06%), water extractable (28.82±6.77%), and ethanol extractable (35.17±1.79%). The chromatographic profile was generated from chloroform-methanol (8:2) separation over silica GF254. The TFC and TPC were 7.66±0.48 mg Quercetin equivalent (QE)/g and 7.27±1.07 mg Gallic acid equivalent (GAE)/g, respectively. The antioxidant activities were 148.95±12.00 Trolox equivalent (TE)/g for DPPH radical scavenging activity and 651.14±77.91 mM TE/g for FRAP. The DPPH radical scavenging activity and FRAP were weakly to moderately correlated to TPC and TFC. The phenolic compound and flavonoid contents of physicochemically-characterized rose periwinkle were not significantly correlated to their antioxidant activities.

Keywords: Antioxidant, *Catharanthus roseus*, Crude drugs, Flavonoids and phenolic compounds, Quality characters

INTRODUCTION

People in Sumbang and Baturraden, Banyumas, traditionally use various plants to maintain their health and to treat diseases. There are 11 plant species utilized for the traditional management of diabetes, and one of those plants is rose periwinkle (*Catharanthus roseus* (L.) G.Don, *tapak dara* in Bahasa Indonesia, Apocynaceae) (Utaminingrum et al., 2020). This plant is utilized for treating and preventing cancer and diabetes in multiple places in Indonesia (Hartanti & Budipramana, 2020). As of 2021, at least 344 secondary metabolites of alkaloid, flavonoid, phenolic acid, and terpenoid groups were reported. In addition to the antihyperglycemic effect, rose periwinkle showed prominent antibacterial, antifungal, cytotoxic, and larvicidal activities (Kumar et al., 2022).

The quality of herbal medicines varies widely, depending on the botanical origin and preparation process. Only crude drugs with an acceptable quality would result in satisfactory safety and efficacy. Hence, absolute qualitative and quantitative values, i.e., morphology, fingerprinting profile, and physicochemical properties, should be set as standards

for quality (Noviana et al., 2022). Those parameters are set to ensure the identity, purity, and quality of a given crude drug. In Indonesia, the quality standards of crude drugs are formalized in the form of Indonesian Herbal Pharmacopeia (IHP). The second edition describes 253 crude drugs and extracts monographs (Kemenkes RI, 2017). As the monograph of rose periwinkle has not been included in the IHP, it is essential to characterize its quality.

Antioxidants might be beneficial in minimizing oxidative stress and further delaying the progression and complications of diabetes (Bandeira et al., 2013). Certain extracts or other materials derived from plants with prominent antioxidant activity had the potential to be studied for their role in the prevention of diabetes. This article reported crude drug characters and evaluated the antioxidant contents and activities of rose periwinkle crude drugs collected from Baturraden and Sumbang, Banyumas. It served as the preliminary study to validate its traditional use as an anti-diabetes in the area.

METHOD

Instruments and Materials

Instruments used in this study include analytical balance (Ohaus PX224/E, United States), furnace (Carbolite Gero, United Kingdom), oven (Memmert UF55, Germany), and spectrophotometer UV-Vis (Shimadzu UV-1900i Plus, Japan). Solvents and reagents, i.e., 2,2-diphenyl-1-picrylhydrazyl (DPPH), $AlCl_3$, HCl, $NaCH_3COO$, Na_2CO_3, NaOH, chloroform, ethanol, methanol, Folin-Ciocalteau reagent, FRAP reagents, Gallic acid, Quercetin 95%, and Trolox were obtained from Sigma-Aldrich (St. Louis, United States). The plant was authenticated by the in-house botanist of the Department of Pharmaceutical Biology, University Muhammadiyah Purwokerto (reference number JR-254).

Crude drug processing

The rose periwinkle flowers and leaves were collected from the same bushes in three collection sites with different altitudes: Pamijen (Sub-district of Baturraden, 270 m above sea level, asl), Banteran (150 m asl) and Sumbang (Sub-district of

Sumbang, 750 m asl). The leaf and flower mixture was dried in a rack dryer at 40°C.

Characterization of quality profile

The evaluation of quality parameter value, i.e., acid-insoluble ash, ethanol extractable, loss on drying, total ash, and water-extractable, was conducted by their respective standard method (Indonesian Ministry of Health, 2017).

Determination of thin layer chromatography (TLC) profile

The sample extraction method and stationary phase for TLC were conducted as per our previous method (Hartanti, Charisma, Fitri, et al., 2022). The sample was separated with the mobile phase of chloroform-methanol (8:2). The separated spots were visualized under a long-wave UV lamp.

Determination of TFC and TPC

TFC and TPC of all crude drugs were determined as a previously described method (Hartanti, Charisma, Agustina, et al., 2022).

Analysis of antioxidant activities

The determination of DPPH scavenging activity and FRAP assays followed a previous report (Hartanti, Charisma, Agustina, et al., 2022).

Statistical Analysis

The normally distributed quality parameter values, TFC, TPC, DPPH radical scavenging activity, and FRAP data were individually compared by one-way ANOVA and Duncan's multiple range test. The correlation between antioxidant activities and antioxidant compounds was evaluated by the Pearson correlation test. A significant difference or correlation was established at a sig. <0.05.

RESULTS AND DISCUSSION

All crude drugs from Pamijen, Banteran, and Sumbang were macroscopically similar (Figure 1A). The proposed water extractable for rose periwinkle crude drugs was 28.82±6.77%. Crude drugs of Sumbang showed the highest water extractable, followed by those from Pamijen and Banteran. The proposed loss on drying for rose periwinkle crude drugs was 9.12±0.50%, and all three crude drugs showed a comparable value. The same pattern

was observed in the total ash parameter, with the proposed value of 8.20±0.71%. Conversely, statistically different acid-insoluble ash values were observed for crude drugs collected in those places. Nevertheless, the proposed acid-insoluble ash value was 0.24±0.06% (Figure 1B, 1C, and 1D). On the other hand, the crude drugs from all places showed comparable ethanol extractable, with the proposed value of 35.17±1.79% (Figure 1E and 1F).

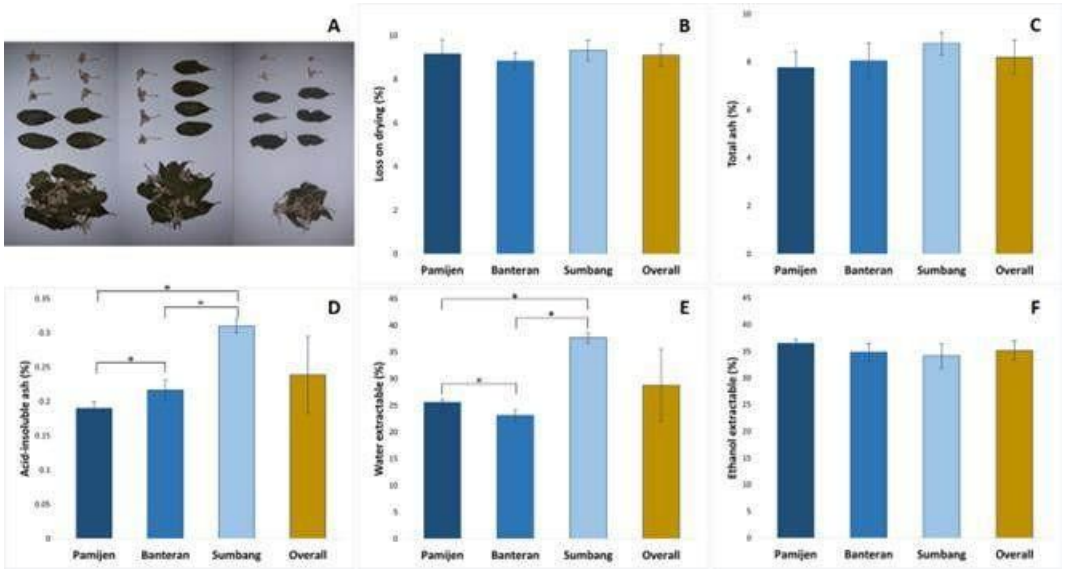

Figure 1. The crude drugs (A) and the values of loss on drying (B), total ash (C), acid-insoluble ash (D), water extractable (E), and ethanol extractable (F) of the rose periwinkle. The bridge and asterisk indicated significantly different values between bars (n=3).

A crude drug's quality is defined by identity, purity, and content aspects (Alamgir, 2017). In this study, the purity aspects were represented by loss on drying, total ash, and acid-insoluble ash. The loss on drying represented a high amount of water and significantly defined the microbial spoilage risks, i.e., loss of bioactivity and generation of toxic effects, during storage (Agarwal et al., 2014; Chandra et al., 2019). The IHP set the loss on drying standard as not more than 10% for any crude drugs. Our result was within the specified official requirement (Kemenkes RI, 2017). On the other hand, the ash values represented the presence of inorganic matter on the crude drugs. The ashes might occur

as the contaminant from the environment where the plant is grown or acquired from processing crude drugs (Tauheed et al., 2017).

The solvent-soluble extractive value represented the content aspect of crude drugs. It is imperative when a specific chemical or biological assay for a given crude drug is unavailable (WHO, 2011). Our result suggested that rose periwinkles contain more semipolar compounds than their polar counterparts.

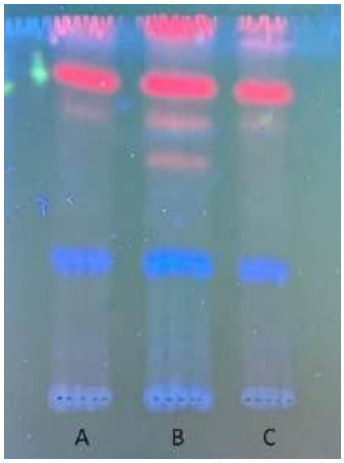

Figure 2. The TLC chromatogram of rose periwinkle obtained from Pamijen (A), Banteran (B), and Sumbang (C), visualized under UV366.

The TLC profile showed three common major spots in all crude drugs, with Rf values of 0.32, 0.73, and 0.80. Under UV light at 366 nm, those spots generated blue, red, and red colors, respectively. Interestingly, rose periwinkle collected in Banteran showed an additional spot at Rf of 0.62 with red color under UV light (Figure 2).

The TLC profile represents crude drug quality's identity, purity, and content. It is the most commonly used, rapid, and simple chromatographic technique in the routine crude drug quality control process (Mukhi et al., 2016). In this study, TLC profile was utilized as the mean to specify the identity aspects of the crude drugs. The different profile shown by Banteran-originated crude drugs likely represented the intraspecific phytochemical variation. In rose periwinkle, such variation has been reported among tissues derived from various stages of cultured cells and the normal plant (Lee et al., 2020). Further study is needed to determine the compounds that might be utilized as markers for crude drugs.

The TFC and TPC of all rose periwinkle crude drugs were low, with an overall average value of 7.66±0.48 mg QE/g DW and 7.27±1.07 mg GAE/g DW, respectively. The crude drugs from

Pamijen, Baturraden, and Sumbang contained comparable total flavonoid and phenolic contents (Figures 3A and 3B). All crude drugs showed a strong DPPH radical scavenged activity with an overall average value of 148.95±12.00 mM TE/g DW. The activity displayed by all crude drugs was statistically equal. The dried flowers and leaves were also highly reduced ferric into ferrous with a FRAP value of 651.14±77.91 mM TE/g DW. Interestingly, crude drugs from Banteran showed a significantly lower FRAP than those from Pamijen and Sumbang (Figure 3C and 3D).

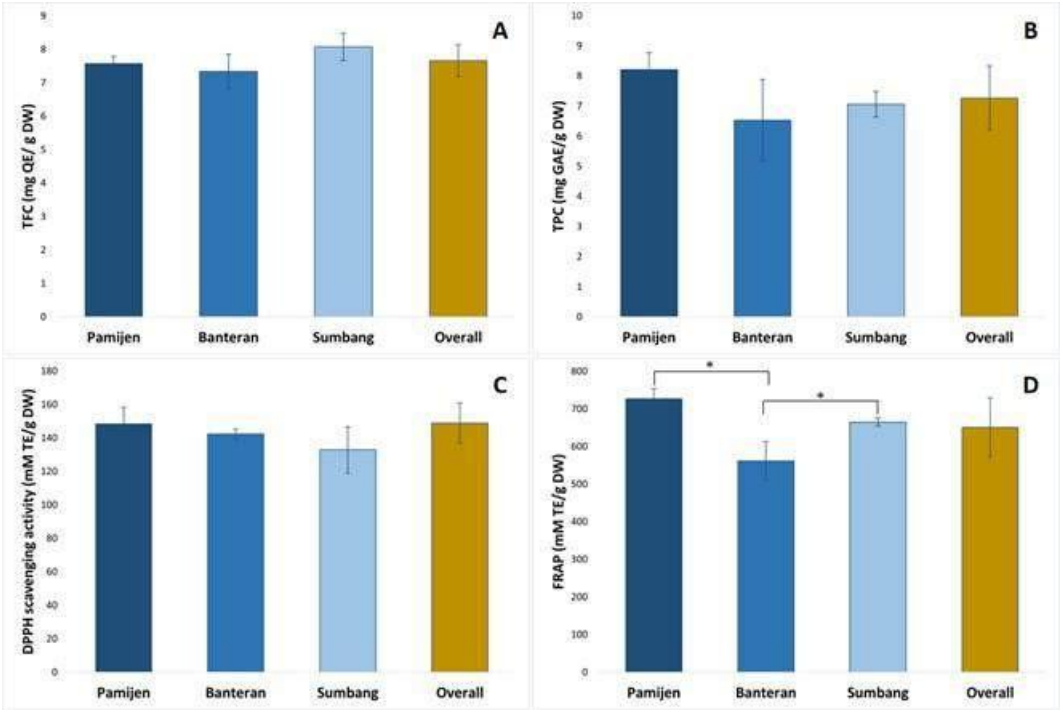

Figure 3. TFC (A), TPC (B), DPPH radical scavenging activity (C), and FRAP (D) of rose periwinkle crude drugs. The bridge and asterisk indicated significantly different values between bars (n=3)

The alkaloids, particularly from indole and bisindole groups, are the main constituents of rose periwinkle (Kumar et al., 2022). Some phenolic compounds were identified in the alkaloid-free fraction; however, they were not flavonoids and belonged to the phenolic acid group (Espejel-Nava et al., 2018). It might explain the low concentration of flavonoids in the extracts. Similar results were

reported in Punjab, India, that the TPC of rose periwinkle leaf ethanol extract collected in Punjab was also insignificant (Rani et al., 2017). The leaf aqueous and stem methanolic extracts of crude drugs from Cape Peninsula and Vietnamese rose periwinkle contained moderately high phenolic compounds (Goboza et al., 2020). Similar to this study, the leaf water extract at 200 μg/ml scavenged DPPH by 87.7% (Syeda & Riazunnisa, 2020). However, rose periwinkle stem methanolic extract collected in Vietnam showed lower antioxidant activity, with DPPH scavenging activity and FRAP of 71.53±2.64 and 83.46±3.61 mg TE/g, respectively (Pham et al., 2018).

TFC was weakly correlated to DPPH scavenging activity, as that of TFC to FRAP. The same pattern was also shown between TPC and both FRAP and DPPH scavenging activity of rose periwinkle crude drugs (Table 1). Hence, the antioxidant activity of rose periwinkle was not attributable to phenolic compounds and flavonoids. Previous reports mentioned that alkaloids were the antioxidant metabolites of the plant. At 0.05 mM and 0.15 mg/mL, the FRAP and DPPH scavenging activity of the vindoline were comparable to those of standard ascorbic acid (Goboza et al., 2020). Other than alkaloids, the antioxidant activities of the plant were also likely attributable to saponins (Pham et al., 2019).

Table 1. Correlation between TFC – TPC and their antioxidant activity Parameters Pearson's correlation coefficient (interpretation)

	DPPH	**FRAP**
TFC	0.253 (weak)	0.674 (moderate)
TPC	0.263 (weak)	0.566 (moderate)

Our finding supported the traditional use of rose periwinkle as an antidiabetic treatment in Baturraden and Sumbang, which might be done through an indirect antioxidative mechanism. A study reported that the rose periwinkle extract intake reduced the blood glucose level in rats and modulated insulin-dependent glucose transport gene translocation to the cell membrane (Al-Shaqha et al., 2015). Hence, the direct antihyperglycemic effect of rose periwinkle was mediated by the enhancement of glucose uptake in the peripheral

tissues. An effective herbal medicine should exert the main, indicated activity and several supporting ones, i.e., analgesic, anti-inflammatory, antimicrobial, antioxidant, and immunomodulator activities (Afendi et al., 2016). From this point of view, the antioxidant activity of rose periwinkle supports the antidiabetic effect.

However, the results of this study should not be used and applied in a rush. More studies are needed to support using rose periwinkle as an herbal medicine. This plant is included in the prohibited and restricted herbal medicine ingredient list, so it should not be formulated into herbal medicines and commercially sold for therapeutic indications (Peraturan Badan Pengawas Obat Dan Makanan Nomor 25 Tahun 2023 Tentang Kriteria Dan Tata Laksana Registrasi Obat Bahan Alam, 2023). A more detailed standardization process is needed to obtain a good-quality crude drug with a satisfying efficacy and safety profile. The fractionation process optimization is required to help eliminate the highly toxic vinca alkaloids so the fraction is safe for use (Kumar et al., 2022).

CONCLUSION

The loss on drying, total ash, acid-insoluble ash, water extractable, and ethanol extractable values of rose periwinkle crude drugs collected from Baturraden and Sumbang were 9.12±0.50, 8.20±0.71, 0.24±0.06, 28.82±6.77, and 35.17±1.79%, respectively. The crude drugs exerted a considerably high DPPH radical scavenging activity (148.95±12.00 mM TE/g DW) and FRAP (651.14±77.91 mM TE/g dry DW) but showed a low level of TFC (7.66±0.48 mg QE/g DW) and TPC 7.27±1.07 mg GAE/g DW). According to the weak to moderate positive correlations between antioxidant activities and TFC-TPC, the antioxidant capacity of rose periwinkle might not be attributable to its flavonoids and phenolic compounds.

ACKNOWLEDGEMENT

The authors would like to thank Lembaga Penelitian dan Pengabdian kepada Masyarakat

(LPPM) Universitas Muhammadiyah Purwokerto for funding this study through Hibah Penelitian Fundamental II 2022.

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