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Polyphenolics and Antioxidant Potential of Five Medicinal Plants Found in East Kalimantan, Indonesia

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Abstract: Following the 1998 forest fire disaster, the Taman Borneo–Mulawarman University Park (KRUS) in East Kalimantan, Indonesia, has served as an important source of germplasm to preserve the plant biodiversity in tropical rain forests, including under-utilized medicinal species. The present study quantified polyphenolics in the bark, leaves, and fruits of *Artocarpus dadah* (AD), *Clerodendrum disparifolium* (CD), *Spatholobus ferrugineus* (SF), *Pternandra azurea* (PA), and *Psychotria carthagenensis* (PC) found in the KRUS to validate the potential antioxidant activity of these species. Phenolics were quantified spectrophotometrically using gallic acid, tannic acid, and catechin as the standards. Antioxidant activity was measured in terms of the half maximal inhibitory concentration (IC₅₀) for 2,2-diphenyl-1-picrylhydrazyl scavenging activity. Among the five test species, PA exhibited the highest total phenolic content (692.51 ± 110.67 ppm gallic acid equivalent), PC leaves exhibited the highest tannin content (22.12 ± 5.34 ppm tannic acid equivalent), and CD bark exhibited the highest flavonoid content (35.06 ± 7.25 ppm catechin equivalent). Moreover, CD bark exhibited the highest antioxidant activity (IC₅₀ = 21.8, R = 0.938). In conclusion, the bark, leaves, and fruits of AD, CD, SF, PA, and PC collected from the KRUS are the potential sources of antioxidants, including phenolics, tannins, and flavonoids. The novelty of the present short communication is the quantification of polyphenolics and their antioxidant potential in five medicinal plants, namely AD, CD, SF, PA, and PC, found in East Kalimantan, Indonesia.

Keywords: *Artocarpus dadah*, *Clerodendrum disparifolium*, polyphenolics, *Pternandra azurea*, *Psychotria carthagenensis*, *Spatholobus ferrugineus*.

印度尼西亚东加里曼丹发现的五种药用植物的多酚和抗氧化潜力

摘要: 1998 年森林火灾之后, 印度尼西亚东加里曼丹的婆罗洲穆拉瓦曼公园大学公园(克鲁斯)成为保护热带雨林植物生物多样性的一个重要种质来源, 包括未充分利用的药用物种。本研究对克鲁斯中发现的蒿属药物(广告)、金银花(光盘)、铁锈病菌(顺丰)、紫薇(公共广播)和迦太基精神病(个人电脑)的树皮、叶子和果实中的多酚进行了量化, 以进行验证这些物种的潜在抗氧化活性。酚类物质使用没食子酸、单宁酸和儿茶素作为标准进行分光光度法定量。根据 2,2-二苯基-1-

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苦基胼清除活性的半数最大抑制浓度(我知道了50)测量抗氧化活性。在五个测试物种中，公共广播的总酚含量最高（ 692.51 ± 110.67 百万分之一没食子酸当量），个人电脑叶的单宁含量最高（ 22.12 ± 5.34 百万分之一单宁酸当量），光盘树皮的黄酮含量最高（ 35.06 ± 7.25 百万分之一儿茶素当量）。此外，光盘树皮表现出最高的抗氧化活性（我知道了 $50=21.8$ ，电阻 = 0.938 ）。总之，从克鲁斯收集的广告、光盘、顺丰、公共广播和个人电脑的树皮、叶子和果实是抗氧化剂的潜在来源，包括酚类、单宁和类黄酮。本短文的新颖之处在于对印度尼西亚东加里曼丹发现的五种药用植物（即广告、光盘、顺丰、公共广播和个人电脑）中的多酚类物质及其抗氧化潜力进行了量化。

关键词：蒿属植物、杜鹃花、多酚、紫檀、迦太基精神病、铁锈病菌。

1. Introduction

Prior to the massive fire disaster in 1998 that destroyed much of the forest cover at the heart of East Kalimantan, this rain forest was home to over 280 plant species that found numerous applications for industrial, food, and medicinal purposes [1, 2]. Polyphenolics in various indigenous plants, including *Macaranga* species [3], *Syzygium leucoxylon* [4], and some Zingiberaceae plants [5], have been explored to promote the use of non-wood forest products while simultaneously conserving biodiversity. In a study in Borneo, polyphenolics in wild edible plants, including *Helminthostachys zeylanica*, *Schismatoglottis ahmadii*, *Heckeria umbellatum*, *Lasia spinosa*, *Gonostegia hirta*, and *Aniseia martinicensis*, were measured to assess their antioxidant properties [6]. Furthermore, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *Willughbeia coriacea*, *Phyllanthus urinaria*, *Eleutherine palmifolia*, *Eusideroxylon zwageri*, *Dendrophthoe pentandra*, *Passiflora foetida*, and *Vitex pinnata* has been documented [7].

Secondary succession following the 1998 fire disaster revealed several surviving plant species; therefore, a germplasm of those species has been established [8]. Recently, the Taman Borneo–Mulawarman University Park (KRUS) in East Kalimantan, Indonesia, was explored as the source of germplasm for tropical rain forest species, and these efforts have prompted further investigations on the potential applications of this germplasm.

The Kutai and Dayak communities in East Kalimantan, in addition to other Indonesian tribes, use *Artocarpus dadah* Miq. (AD), *Clerodendrum disparifolium* Blume (CD), *Spatholobus ferrugineus* (Zoll. & Moritz) Benth. (SF), *Pternandra azurea* (DC.) Burkill (PA), and *Psychotria carthagenensis* Jacq. (PC) as herbal medicines [9, 10]. The medicinal properties of these plants have primarily been attributed to their phytochemical components, implicating antioxidant activity. However, the potential

antioxidant activity of the bark, leaves, and fruits of AD, CD, SF, PA, and PC has been scarcely validated.

The bark of AD contains cycloarthobioxanthone—a derivative of prenylated flavonoids in the C-3 group [11]. This compound has been reported to exhibit potent cytotoxicity against P-288 murine leukemia cells [12]. Further, a previous study has identified phenolic compounds with potential antioxidant properties in the stem epidermis and young stem of CD [13]. Additionally, alkaloids, flavonoids, polyphenols, and terpenoids in the stem of SF have been qualitatively analyzed [14]. The leaves of PC contain hallucinogenic alkaloids, which are often used by the Ayahuasca tribe [15]. However, the phytochemical properties of PA have been poorly documented.

To this end, the present study quantified polyphenolics in the bark, leaves, and fruits of AD, CD, SF, PA, and PC collected from the KRUS in East Kalimantan, Indonesia, and explored their antioxidant potential.

2. Materials and Methods

2.1. Materials

The raw materials used in the present study included the samples of fruits, leaves, and bark of AD, CD, SF, PA, and PC, which are used in traditional medicine, collected from the germplasm collection of the KRUS, East Kalimantan, Indonesia.

2.2. Species Identification

The species identity was confirmed following conventional taxonomic methods, as verified by Dr. Medi Hendra of the Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University.

2.3. Quantification of Total Phenolics

Total phenolics were quantified using the methodology described by Mu'nisa *et al.* [16], with

some modifications. Briefly, the plant parts (i.e., bark, leaves, or fruits) were accurately weighed to 0.3 g and dissolved in 10 mL of absolute ethanol (SmartLab, Indonesia) and distilled water (Soil Laboratory, Mulawarman University) at 1:1 ratio. Next, approximately 0.2 mL of the solution was added to 15.8 mL of distilled water, and 1 mL of 50% (v/v) aqueous Folin–Ciocalteu (Sigma-Aldrich, Germany) reagent was added to the sample. The sample was incubated for ± 8 min at $28 \pm 2^\circ\text{C}$. Then, 3 mL of 5% (w/v) aqueous Na_2CO_3 (Sigma-Aldrich, Germany) was added, and the sample was incubated for additional ± 2 h in the dark at $28 \pm 2^\circ\text{C}$. Finally, each sample was transferred to a cuvette, and absorbance was measured at 725 nm (UV/Vis BioSpectrophotometer, Eppendorf, Germany). The measured value of absorbance was extrapolated on the standard curve of gallic acid, which was prepared using the same protocol. Total phenolic content was expressed in parts per million of gallic acid equivalent (GAE).

2.4. Quantification of Total Tannins

Total tannins were quantified using the methodology described elsewhere [17], with some modifications. Briefly, the plant parts (0.5 g) were macerated in 10 mL of diethyl ether (Merck, Singapore) for 20 h at $28 \pm 2^\circ\text{C}$, and the samples were filtered through a Whatman filter paper. The obtained residue was boiled in 100 mL of distilled water for 2 h at 100°C , cooled to room temperature ($28 \pm 2^\circ\text{C}$), and re-filtered. The extracts obtained were diluted 100 times. About 0.1 mL of the extract was added with 0.1 mL of 50% (v/v) aqueous Folin–Ciocalteu reagents and was homogenized with a vortex mixer. The sample was added to 2 mL of 2% (w/v) aqueous Na_2CO_3 and was homogenized. Each of the solutions was incubated for 30 min in a dark place at room temperature. The absorbance was measured at 760 nm (UV/Vis BioSpectrophotometer, Eppendorf, Germany). The readings were interpolated on the standard curve graph of tannic acid (Sigma-Aldrich, Germany) that was prepared in the same fashion. The total tannins were expressed in ppm of tannic acid equivalent (TAE).

2.5. Total Flavonoids

Total flavonoids were analyzed using specified modifications from previous research [18]. About 1 mg of each plant part was weighed and dissolved in 10 mL of absolute ethanol (SmartLab, Indonesia), and 0.7 mL of distilled water was added to the solution. To each sample, 0.1 mL of 5% aqueous NaNO_2 (Sigma-Aldrich, Germany) was added. The samples were allowed to stand for 5 minutes at room temperature ($28 \pm 2^\circ\text{C}$). The sample was added to 0.1 mL of 10% aqueous AlCl_3 (Sigma-Aldrich, Germany). After being incubated for 6 minutes at room temperature, about 0.5 mL of 1 M NaOH (Merck, USA) was added. All ingredients were mixed evenly and allowed to stand for

10 minutes at room temperature. Each of the samples was prepared 1:1 (v/v) in absolute ethanol (SmartLab, Indonesia), and the absorbance was measured at 510 nm. The results obtained were interpolated on the standard curve graph of catechin (Sigma-Aldrich, Germany) prepared in the same way. Total flavonoids were expressed in ppm of the catechin equivalent (CAE).

2.6. Antioxidant Activity

The antioxidant activity test was performed by calculating the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) reduction [19]. A total of 1 mL diluted plant part in ethanol was added to 1 mL of DPPH (0.15 mM in ethanol), and at the same time, a control consisting of 1 mL DPPH with 1 mL of ethanol was prepared. The solution was homogenized and then incubated in a dark place at room temperature for 30 minutes. Each solution was prepared in a cuvette, and the absorbance was measured at 517 nm. Vitamin C was used as a positive control, and ethanol was used as a blank. The DPPH radical scavenging activity of the extract was calculated using equation 1:

$$\% \text{ antioxidant activity} = \frac{\text{control absorbance} - \text{sampel absorbance}}{\text{control absorbance}} \times 100 \quad (1)$$

The absorbance of the control was the absorbance of DPPH + ethanol.

The absorbance of the sample was the absorbance of the DPPH + sample.

The obtained data was presented with the IC_{50} value, and the value was obtained from nonlinear regressing %–DPPH reduction inhibition by sample at concentrations of 31.25; 62.5; 125; 250; and 500 ppm. The nonlinear regression analysis of the One Phase Decay curve fitting was completed with GraphPad Prism 6.0.

3. Results and Discussion

3.1. Total Phenolics

In light of the previous research, we have collected five species, namely AD, CD, SF, PA, and PC. The highest total phenolics were established in the bark of *Pternandra azurea*. XBased on the results (Table 1), total phenolics of barks in four species were higher than that of leaves. The phenolics of AD fruit was relatively the same as its leaf. The content of phenolics depends on species and the solvent used to dilute the samples, as indicated in previous research [19, 20].

3.2. Total Tannins

Tannins may have a positive association to human health or the opposite, depending upon its concentration [21]. Tannins were distributed in parts of plants, i.e., leaf, bark, fruit, and root. In contrast to total phenolics that were higher in barks of four species of

samples, tannin contents were varied in the observed parts of plants. Leaves of PC and PA had higher tannins than the bark. Tannins in bark and leaf of CD and SF were not significantly different. The same condition occurred with AD leaf and fruit (Table 1). However, tannins in the observed species should be further investigated to allow isolation of the pure compound in order to investigate its true nature.

3.3. Total Flavonoids

Flavonoid antioxidants are associated with the existence of 2- or 3-phenylchroman structures. These structures have been observed in pure constituents of plants and fruits [22], i.e., quercetin. For the five observed species, the highest contents of flavonoids were found in bark of CD, followed by bark of SF and

PA. The other samples had flavonoid contents between 3.92 ± 0.20 and 5.44 ± 1.47 ppm (Table 1).

3.4. Antioxidant Activity

Wild plants in Borneo may contain high antioxidant activity. For example, more than 70% of DPPH radical was successfully scavenged by the extracts of six medicinal plants from Borneo [7]. In this research, antioxidant activity was measured with IC_{50} of DPPH radical scavenging activity. The lowest IC_{50} value was observed in SF leaf, followed by SF bark. All samples had higher scavenging activity than vitamin C. It was deduced that polyphenols, tannins, and flavonoids of the five observed species were associated with DPPH radical scavenging activity (Table 1).

Table 1 Recapitulation of total phenolics, tannins, flavonoids, and antioxidant activity of bark, leaf, and fruit of AD, CD, SF, PA, and PC

Sample	Code	TPC	HTC	TFC	IC ₅₀	R-square
		GAE (ppm)	TAE (ppm)	CAE (ppm)	Value (ppm)	
Fruit of <i>Artocarpus dadah</i>	AD fruit	288.26 ± 34.18	136.01 ± 30.99	4.17 ± 0.13	116.4	0.759
Leaf of <i>Artocarpus dadah</i>	AD leaf	269.57 ± 53.20	120.13 ± 61.90	4.65 ± 0.73	54.5	0.941
Bark of <i>Spatholobus ferrugineus</i>	SF bark	607.14 ± 78.80	164.06 ± 10.64	18.92 ± 7.28	25.0	0.938
Leaf of <i>Spatholobus ferrugineus</i>	SF leaf	507.82 ± 28.90	134.03 ± 44.19	4.51 ± 0.28	21.8	0.938
Bark of <i>Clerodendrum disparifolium</i>	CD bark	575.34 ± 22.99	196.07 ± 27.04	35.06 ± 7.25	92.5	0.994
Leaf of <i>Clerodendrum disparifolium</i>	CD leaf	466.53 ± 191.40	191.85 ± 10.14	5.44 ± 1.47	52.7	0.986
The bark of <i>Pternandra azurea</i>	PA bark	692.51 ± 110.67	126.58 ± 13.68	14.81 ± 4.71	45.4	0.797
Leaf of <i>Pternandra azurea</i>	PA leaf	628.62 ± 60.51	152.39 ± 8.69	4.06 ± 0.20	63.4	0.986
Bark of <i>Psychotria carthagenensis</i>	PC bark	581.19 ± 33.61	181.92 ± 32.41	4.10 ± 0.21	44.8	0.971
Leaf of <i>Psychotria carthagenensis</i>	PC leaf	283.24 ± 88.48	222.12 ± 5.34	3.92 ± 0.20	37.3	0.938

4. Conclusion

Phenolics, tannins, flavonoids, and DPPH scavenging activity were successfully measured from bark, leaf, and fruit of AD, CD, SF, PA, and PC collected from Taman Borneo - Mulawarman University Park (KRUS), East Kalimantan, Indonesia. The highest total phenolics was observed in bark of PA with a value of 692.51 ± 110.67 ppm GAE. PC leaf had the highest total tannins of 222.12 ± 5.34 ppm TAE. Flavonoids were densely populated in bark of CD with a value of 35.06 ± 7.25 ppm CAE. From the five observed species, the highest antioxidant activity was found in bark of CD, with IC_{50} of DPPH scavenging activity of 21.8 and an R-value of 0.938. The limitation of this study is related to the initial finding, that it should be further followed up by thorough research on the possibility of using AD, CD, SF, PA, and PC as potential sources of traditional medicinal plants from Indonesian native tropical rain forests. Our findings further confirm the use of *Artocarpus dadah* (AD), *Clerodendrum disparifolium* (CD), *Spatholobus ferrugineus* (SF), *Pternandra azurea* (PA), and *Psychotria carthagenensis* (PC) as a potential germ

plasm bank that can be further characterized as an effective and potential source of antioxidants.

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