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Phytochemical content and antioxidant capacity of ethyl acetate extracts from fifteen *Orthosiphon aristatus* leaves genotypes

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Abstract. *Bovani RP, Liwanda N, Batubara I, Ambarsari L, Nurcholis W. 2024. Phytochemical content and antioxidant capacity of ethyl acetate extracts from fifteen* Orthosiphon aristatus *leaves genotypes. Biodiversitas 25: 763-769. Orthosiphon aristatus* (Blume) Miq., also known as *kumis kucing* in Indonesia, is a medicinal plant belonging to the *Lamiaceae* family that contains various phytochemical compounds with antioxidant properties. This study investigated the phenolic content, flavonoid, rosmarinic acid, sinensetin, and antioxidant capacity of 15 genotypes of *O. aristatus* ethyl acetate extracts. Therefore, 15 genotypes were analyzed using quercetin as the standard for Total Phenolic Content (TPC) using a colorimetric method with gallic acid and Total Flavonoid Content (TFC). Sinensentin and rosmarinic acid were measured by High-Performance Liquid Chromatography (HPLC). Antioxidant capacity was determined using Trolox as a standard antioxidant by 2-Diphenyl Picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. The results revealed a diverse range of phytochemical compounds, with total phenolic content ranging from 3.39 to 13.80 mg GAE/g DW, total flavonoid content from 0.20 to 1.43 mg QE/g DW, rosmarinic acid from 0.02 to 0.19 mg/g DW, and sinensetin from 1.61 to 21.67 mg/g DW. Furthermore, the antioxidant assays demonstrated varying degrees of efficacy, with DPPH ranging from 2.09 to 13.98 µmol TE/g DW and FRAP from 0.07 to 2.96 µmol TE/g DW. Genotypes A10, A11, and A12 exhibited superior phytochemical contents and antioxidant capacities, highlighting their potential as valuable sources of medicinal compounds.

Keywords: Antioxidant, ethyl acetate, genotype, *Orthosiphon aristatus*, phytochemical

INTRODUCTION

Orthosiphon aristatus (Blume) Miq. is a member of the Lamiaceae family and commonly referred to as "*Kumis kucing*" in Indonesia. This medicinal plant, often called Java tea, is extensively cultivated in tropical countries and Southeast Asia, particularly in Indonesia, and is widely used in Southeast Asia and Europe (Ashraf et al. 2018; Jadid et al. 2020). It features three distinct flower variations: white, purple, and white flowers with purple patterns (Faramayuda et al. 2022). The leaves of this plant are commonly prepared as herbal tea to treat various ailments, including rheumatism, diabetes, urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, and hypertension (Hossain and Rahman 2015). Several studies have been conducted on the pharmacological activities of *O. aristatus*, including its antioxidant properties (Cai et al. 2018), inhibition of the α-glucosidase enzyme (Mohamed et al. 2015), antidiabetic properties (Lokman et al. 2019), antiobesity properties (Seyedan et al. 2017), anticancer properties (Movahedi et al. 2015), prevention of urinary system infections (Sarshar et al. 2017), antiosteoporotic properties (Bokhari et al. 2018), and antibacterial properties (Reshi et al. 2017).

Orthosiphon aristatus leaves contain secondary metabolites, such as alkaloids, coumarins, steroids, organic acids, isoprenoids, terpenoids, saponins, flavonoids, and phenolics (Maulana et al. 2022). The leaves *of O. aristatus* primarily contain rosmarinic acid and sinensetin as secondary metabolites. These compounds belong to the phenolic and flavonoid classes (Faramayuda et al. 2022). Rosmarinic acid and sinensetin have been reported to play a role in the antioxidant activities of *O. aristatus* (Juliani et al. 2016). To maintain the antioxidant activity of *O. aristatus*, selecting genotypes with a high concentration of phytochemicals is important. This is especially relevant for plant breeding programs, as the antioxidant activity of *O. aristatus* is closely linked to its phytochemical content.

The production of antioxidant compounds in medicinal plants, which are influenced by environmental factors and genetic makeup, was found to affect the levels of phytochemicals (Mannino et al. 2019; Yaldiz and Camlica 2019). Recently, breeders have relied on genetic and phenotypic traits to make crucial decisions in breeding programs. Developing varieties with the most suitable genotypes that can produce higher levels of phytochemicals and antioxidant activities under different environmental conditions is needed. Breeding programs should aim to produce plant varieties that demonstrate consistent yields under various environmental conditions (Carter et al. 2018). In a study conducted by Batubara et al. (2020), the genotypes of *O. aristatus* were evaluated based on their phytochemical content and pharmacological activities using an ethanol 70% extracted from 15 different genotypes. Chemometric analysis, including correlation and principal

component analysis, was also utilized to assess genetic variability within the *O. aristatus* genotypes. According to the findings, genotypes A1, A2, A7, A11, and A15 were superior due to their high phytochemical content and pharmacological activities, as determined by chemometric analysis. Therefore, it is essential to improve detailed comparative studies of *O. aristatus* genotypes using different solvents (ethyl acetate) based on the content and antioxidant capacity of *O. aristatus* chemicals. Ethyl acetate is widely utilized in industrial applications as a solvent. Aqueous ethyl acetate mixtures are commonly employed in various industrial processes (Bhoumick et al. 2023). Therefore, it is crucial to evaluate the phytochemical composition of *O. aristatus* to establish industrial-scale pharmacological development.

The primary objective of the present study was to explore the phytochemical makeup and antioxidant capabilities of the leaves obtained from the ethyl acetate extract of 15 distinct genotypes of *O. aristatus*. The analysis of phytochemical content included an assessment of phenolic, flavonoid, sinensetin, and rosmarinic acids. Since the *O. aristatus* genotypes were grown under identical soil conditions and environmental factors, the disparities in the outcomes were attributed to genetic variations among the different genotypes examined. Therefore, to identify superior genotypes for cultivating *O. aristatus* varieties on a large scale, the *O. aristatus* genotypes were classified based on their phytochemical composition and antioxidant capacity using regression and correlation analysis.

MATERIALS AND METHODS

Study area

This study was conducted in the Research Laboratory of the Department of Biochemistry, IPB University, Bogor, West Java, Indonesia. The materials used were 15 different *O. aristatus* genotypes cultivated in Tropical Biopharmaca Research Center, Bogor Agricultural University, West Java, Indonesia (6°32′25.47″ N, 106°42′53.22″ E, 142.60 m above sea level).

Plant material and sample preparation

The plant materials were composed of 15 distinct *O. aristatus* genotypes that varied according to their plant height (short plants being less than 30 cm tall, medium plants ranging from 30-60 cm tall, and tall plants exceeding 60 cm in height), flower color (either white or purple), and maturity group (early genotypes being 34 days or younger and late genotypes being 35 days or older). The 15 genotypes consist of A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14, and A15. The short plant group consisted of A1 and A10, the tall plant group consisted of A6 and A14, and the rest were medium plants. A2 had purple flowers, whereas the other genotypes had white flowers. A1, A2, A3, and A7 belong to the early maturity group, while the rest belong to the late maturity group. The 15 *O. aristatus* species were cultivated In a randomized complete block design with three replicates. Three months after planting, leaves were harvested to evaluate phytochemicals and antioxidant capacity. The leaves were washed and then dried in an oven for 48 hours at 50°C. The dried material was then ground to produce a 100-mesh powder used in the extraction process.

Extraction

The extraction process method use was modified slightly by Batubara et al. (2020) by mixing 15 g of *O. aristatus* leaf powder with 150 mL of ethyl acetate for 24 hours at room temperature on a rotating shaker set at 150 rpm (Eyela multi-shaker MMS-Germany). The solution was filtered through Whatman No. 4. Next, a rotary evaporator was used to evaporate the extracts and collected, weighed, and stored at 4°C for further analysis.

Quantification of total phenolic

The method described by Batubara et al. (2020) was used to determine the total phenolic content of each extract with a slight modification, which involves using the Folin-Ciocalteu reagent and gallic acid as a standard. Next, to do this, 10 µL of the extracted sample was mixed with 160 µL of distilled water, 10% Folin-Ciocalteu reagent (10 µL), and 10% Na2CO3 (20 µL). Next, the mixture was incubated for 30 minutes at room temperature, after which the absorbance was measured at 750 nm using a microplate reader (Epoch BioTek, USA). The total phenolic content was expressed as gallic acid equivalents (mg GAE/g DW)

Quantification of total flavonoid

The total flavonoid content of each extract was determined using a spectrophotometric method with a slight modification of Batubara et al. (2020). The total flavonoid content in each extract was measured using an aluminum chloride reagent, and quercetin was used as the standard. In a 96-well microplate, 10 µL of the extracted sample was mixed with 60 µL of methanol, 10 µL of 10% aluminum chloride, 10 µL of 1 M potassium acetate, and 120 µL of distilled water. The mixture was incubated at room temperature for 30 minutes, and a microplate reader (Epoch BioTek, USA) was used to measure the absorbance at 415 nm. Then, the absorbance results were expressed as quercetin equivalents (mg QE/g DW).

Rosmarinic acid and sinensetin quantification

The HPLC method used to measure the ethyl acetate extracts of *O. aristatus* genotypes was adapted from Batubara et al. (2020) with some modifications. Separate standard solutions of rosmarinic acid and sinensetin (ChromaDex) were prepared in methanol at concentrations ranging from 1-100 µg/mL and 0.3-24 µg/mL, respectively. The analysis was conducted by an HPLC system (Shimadzu, Kyoto, Japan) that was equipped with a degassing unit (DGU-20A5R), pump (LC-20AB), autosampler (SIL-20A HT), column oven (CTO-20AC), and UV-Vis detector (SPD-20A). The HPLC system was used with a Shim-Pack VP ODS C18 Shimadzu column (75×4.6 mm i.d., 3 µm pore size).

The mobile phases used for elution were an aqueous solution containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The gradient elution

system was as follows: from 0-7 minutes, the percentage of B increased from 2% to 20%; from 7-10 minutes, the percentage of B increased from 20% to 30%; from 10-18 minutes, the percentage of B increased from 30% to 50%; from 18-20.5 minutes, the percentage of B increased from 50% to 98%; from 20.5-23 minutes, the percentage of B remained at 98%; and from 23-26 minutes, the percentage of B decreased from 98% to 2%. The system was stabilized for 35 minutes before the next injection. For each genotype sample, 10 µL of injection volume was used, and the mobile phase flow rate was set at 1 mL/min, with a detector wavelength of 320 nm and a temperature of 30°C for 35 minutes. The rosmarinic acid and sinensetin concentrations were expressed in mg/g DW based on their respective calibration curves for standards.

Antioxidant capacity assay

2,2-Diphenyl-1-Picryl Hydrazyl (DPPH) assay

The scavenging activity of DPPH was assessed using the method described by Nurcholis et al. (2022) with modifications. The process involved adding 100 µL of *O. aristatus* ethyl acetate extract to 100 µL of 125 µM DPPH in methanol in a 96-well microplate (Costar, USA). The mixture was then incubated for 30 minutes in the dark at room temperature. Then, a microplate reader (BMG Labtech, Germany) measured the absorbance at 517 nm, and the outcomes were expressed as µmol Trolox equivalents (TE/g DW).

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay with adjustments was performed using the method outlined by Nurcholis et al. (2022). The FRAP reagent was then prepared by mixing 10 mM TPTZ in 40 mmol HCl, 300 mM acetate buffer (pH 3.6), and 20 mmol FeCl3 in a 10:1:1 (v/v/v) ratio. Approximately 100 µL of *O. aristatus* ethyl acetate extract was added to 300 µL of the FRAP reagent in a 96-well microplate (Costar, USA). The mixture was then incubated at 37°C, and the absorbance level was measured at 593 nm by a microplate reader (BMG Labtech, Germany). The antioxidant activity was expressed as µmol Trolox equivalents (TE/g DW).

Statistical analysis

The mean±Standard Deviation (SD) was determined from three repetitions. Furthermore, a quantitative analysis was conducted using a One-Way ANOVA with Duncan's test in IBM SPSS 25.0, and differences were considered significant at a P-value less than 0.05. Additionally, the relationships between phytochemical content and antioxidant capacity were examined using regression and correlation analysis in the Prism 8 program (Nurcholis et al. 2021).

RESULTS AND DISCUSSION

Extraction yield

The extraction yield of *O. aristatus* leaf genotypes was influenced by their genotype, as demonstrated by the results of the ethyl acetate extraction. The ethyl acetate extraction yields of the genotypes studied ranged from 5.11 to 14.99% DW (as shown in Table 1). Genotype A11 had the highest extraction yield, while Genotype A9 had the lowest.

Phytochemical content

The ethyl acetate extract phytochemical analysis of *O. aristatus* leaves presented in Table 1, including the Total Flavonoid Content (TFC), Rosmarinic Acid Content (RAC), Total Phenolic Content (TPC), and Sinensetin Content (SC) of 15 leaves. The study found that the results were statistically significant at a P value of less than 0.05. The TPC ranged from 1.79 to 13.8 mg GAE/g DW; the highest value was observed in genotype A10, while the lowest was in genotype A9. The TFC ranged from 0.20 to 1.46 mg QE/g DW among the *O. aristatus* genotypes, and genotype A12 had the highest TFC, which was not significantly different from A10, while genotype A9 had the lowest TFC. The highest RAC value (0.19 mg/g DW) was observed in genotype A8, while genotypes A9 and A15 exhibited the lowest RAC with the same value of 0.02 mg/g DW. The SC ranged from 1.61 to 21.67 mg/g DW, with the highest value recorded in A11 and the lowest in A1.

Antioxidant capacity

The antioxidant capacity of the studied genotypes was evaluated using the DPPH and FRAP assays (Table 1). The DPPH assay results revealed that the genotypes' antioxidant properties ranged from 2.09 (A9) to 13.98 (A11) µmol TE/g DW. It was observed that the DPPH value of A11 was not significantly different from that of A10 as the second highest (13.46 µmol TE/g DW) ($p<0.05$). In the FRAP assay, genotype A12 showed the highest antioxidant properties with a 2.96 mmol TE/g DW value. On the other hand, genotype A5 had the lowest capacity with a value of 0.049 mmol TE/g DW.

Relationship of antioxidant capacity to total phytochemical content

The correlation between the extraction yield, phytochemical compounds, and the phytochemical compounds with antioxidant ability was analyzed using Pearson's correlation coefficient for the ethyl acetate extracts of *O. aristatus* leaves. The Pearson's correlation results show significant positive correlations between the extraction yield and TPC (r = 0.5829), TFC (r = 0.8363), and SC (r = 0.5562), but not with RAC (r = 0.2596) (Figure 1). The relationship between DPPH and total phenolic content demonstrated a strong positive correlation (r = 0.6118), while a weaker positive correlation, but not statistically significant, was observed between total phenolic content and FRAP (r = 0.07753) (Figure 2A). Conversely, a significant positive correlation was noted between total flavonoid content and DPPH (r = 0.8494), and a positive correlation, albeit weaker, was evident between FRAP and total phenolic content (r = 0.6090) (Figure 2B). The ethyl acetate extracts of *O. aristatus* genotypes showed a positive correlation but not significant between rosmarinic acid and DPPH (r = 0.3257), while a negative correlation was observed between rosmarinic acid and FRAP (r = -0.2019) (Figure 3A). There was a

significant positive correlation between sinensetin content and DPPH (r = 0.5787), and a positive but not significant correlation between sinensetin content and FRAP (r = 0.1172) (Figure 3B).

Discussion

The phytochemical content and antioxidant capacity of medicinal plant species vary depending on the geographical conditions in which they grow, according to studies by Ahmadi et al. (2020) and Kabtni et al. (2020). Factors such as light intensity, water availability, carbon dioxide levels, stress conditions, and the composition of nutrients in the soil can all impact the phytochemical and antioxidant content of *O. aristatus*, according to various studies (Utami et al. 2016; Prinsloo and Nogemane 2018; Rajashekar 2018; Coelho et al. 2021; Shin et al. 2021). Additionally, the genetic makeup of plants, including the type of genotype, can influence the production of phytochemicals and antioxidants, both directly and indirectly (Nielsen et al. 2019). This is thought to be due to differences in the genes responsible for synthesizing compounds, which can result in variations in the quantity and number of phytochemicals produced (Samadi et al. 2022). Therefore, it is necessary to create controlled environmental conditions in plant breeding programs to select medicinal plants with superior performance (Batubara et al. 2020). This study examined 15 genotypes of *O. aristatus* collected from Indonesia for their phytochemical content and antioxidant capacity using ethyl acetate as a solvent. The extraction results described the level of diversity of the compounds present in the samples. The extraction results were also influenced by the plant's genotype, including that of *O. aristatus*. In addition, the type of solvent used greatly influences the quality of the extract (Wakeel et al. 2019).

The extraction results for *O. aristatus* using the ethyl acetate solvent in this study (Table 1) were lower than those reported by Batubara et al. (2020), who used ethanol as the solvent. These results indicate that using solvents during extraction affects sample extraction. In this study, the ethyl acetate solvent produced much lower TPC, TFC, RAC, and DPPH values than those obtained using ethanol (Batubara et al. 2020). According to Batubara et al. (2020), the ethanol extract from *O. aristatus* exhibited the highest extraction yield of 16.91% DW, with the highest phytochemical content for TPC of 36.08 mg GAE/g DW, TFC of 3.07 mg QE/g DW, RAC of 7.25 mg/g DW, and SC of 4.02 mg/g DW. Additionally, the study revealed that the antioxidant capacities of DPPH and FRAP were 15.55 µmol TE/g DW and 1.60 µmol TE/g DW, respectively. It is worth noting that the SC content and antioxidant capacity of FRAP in this study were significantly higher than those reported by Batubara et al. (2020), with a value of 21.67 mg/g DW for SC and 2.96 µmol TE/g DW for FRAP. Ethanol and ethyl acetate were the two chemical compounds with the most significant differences. The ethyl acetate solvent's lower extraction yield than the ethanol solvent in extracting phenolic compounds and flavonoids can be attributed to several factors. Ethyl acetate has lower solubility for polar compounds, including phenolic compounds and flavonoids, which tend to be polar (Thavamoney et al. 2018), resulting in a lower extraction efficiency and less-than-optimal recovery. As a more polar solvent, ethanol may be more specific for extracting polar compounds such as phenolics and flavonoids. In contrast, the less polar ethyl acetate may be more selective towards nonpolar compounds. In addition, the selectivity of the solvent toward the target component and the sustainability of the extraction may play key roles, as ethanol may have a better affinity for certain phenolic and flavonoid compounds (Laeliocattleya et al. 2018).

Phenolic compounds, known to possess antioxidant properties, are a group of metabolites that need to be quantified in plants (Nurcholis et al. 2018; Han et al. 2019). The Total Phenolic Compound contents (TPC) in all the extracts are presented in Table 1, with the highest level found in A10 (13.80 mg GAE/g DW). The leaves of *O. aristatus* contain caffeic and rosmarinic acids, which are phenolic compounds (Wahab and Chua 2023). This study found that the A8 genotype had the highest Rosmarinic Acid Content (RAC), measured at 0.19 mg/g DW. The leaves of *O. aristatus* contain eupatorin, sinensetin, 5-hydroxy-6,7,3',4'-tetramethoxyflavone, salvigenin, 6-hydroxy-5,7,4'-trimethoxyflavone, and 5,6,7,3'-tetramethoxy-4'-hydroxy-8-C-prenylflavone, which are flavonoids (Hossain and Rahman 2015). The flavonoid and sinensetin content of all the extracts analyzed in this study are presented in Table 1. The highest Total Flavonoid Content (TFC) was observed in A12, amounting to 1.46 mg QE/g DW. Additionally, the highest sinensetin content in *O. aristatus* leaves was found in A11, amounting to 21.67 mg/g DW. In comparison, the Pearson correlation analysis of phytochemical content (TPC, TFC, RAC, and SC) based on extraction yields illustrated the relationship between the extraction yields of TPC, TFC, and SC; however, this analysis does not illustrate the relationship between the extraction gain and RAC (Figure 1). These results indicate that using ethyl acetate as a solvent did not affect the rosmarinic acid content. This situation arises because of disparities in the polarity of the compounds and solvents, resulting in nonpolar solvents (ethyl acetate), which are suboptimal for extracting polar compounds (rosmarinic acid); rosmarinic acid was often extracted from the more polar solvents, such as a mixture of ethanol and water (Xie et al. 2017). Moreover, the results imply that genotype A10 is a promising candidate for developing *O. aristatus* varieties with high levels of phenolic productivity in plant breeding programs. Furthermore, genotypes A11 and A12 may be utilized to create high-yielding varieties breeding programs of *O. aristatus* with elevated levels of sinensetin and flavonoids.

Antioxidants are vital in preventing various human illnesses, as per Toghueo and Boyom (2019). Therefore, it is crucial to obtain plant genotypes with high antioxidant activity, as mentioned by Singh et al. (2018). This research assessed the ethyl acetate extracts of *O. aristatus* genotypes for their antioxidant capacity using DPPH and FRAP assays; the results revealed variations in DPPH and FRAP among the *O. aristatus* genotypes (as shown in Table 1). Genotypes A10, A11, and A12 on DPPH and FRAP assays values are higher than others (DPPH at 13.46, 13.98, and

12.01; FRAP at 0.65, 0.42, and 2.96). Environmental factors, including environmental stress, can directly impact the antioxidant capacity of plants. In the presence of stress, plants produce secondary metabolites, one of which can act as an antioxidant (Shin et al. 2021). However, in this study, the focus was on the genotype variation of *O. aristatus*, so all genotypes were grown under homogeneous environmental conditions. Genotypes A10, A11, and A12 have advantages over other genotypes, likely due to specific genes that enable them to produce antioxidant compounds more optimally (Samadi et al. 2022). These genotypes have the potential to be developed further as herbal medicines with antioxidant properties. To evaluate the antioxidant effects of *O. aristatus*, two different methods (DPPH and FRAP) were used to compare the plant's ability to fight against free radicals via different mechanisms (Nurcholis et al. 2022).

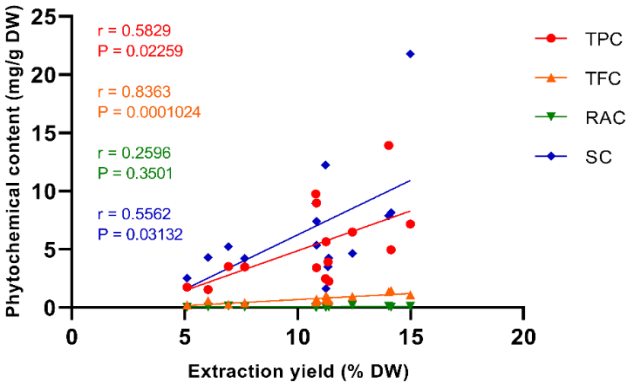

Figure 1. Simple linear correlation of extraction yield with Total Phenolic Content (TPC), Total Flavonoid Content (TFC), rosmarinic acid content, and Sinensetin Content (SC) in ethyl acetate extract. r (value Pearson's correlation coefficient), p-value indicates significance at $p<0.05$

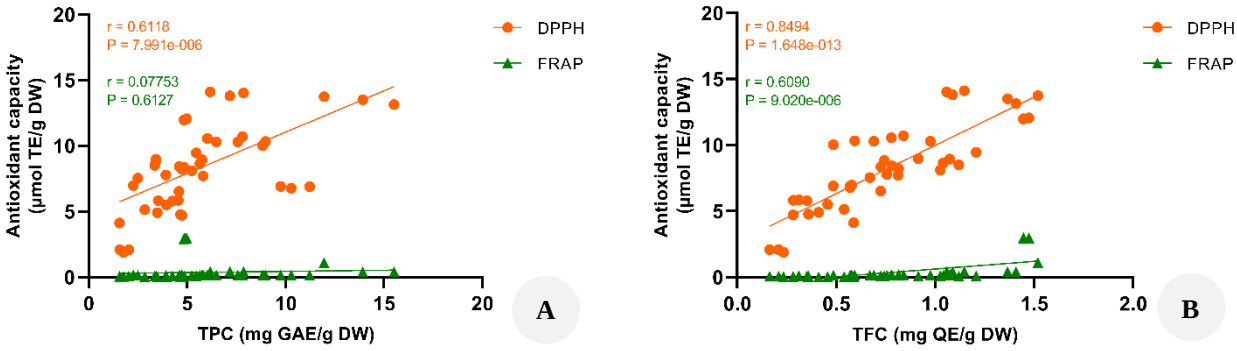

Figure 2. Simple linear correlation of: A. Total Phenolic Content (TPC) with antioxidant capacity DPPH and FRAP and B. Total Flavonoid Content (TFC) with antioxidant capacity DPPH and FRAP in ethyl acetate extract. r (value Pearson's correlation coefficient), p-value indicates significance at $p<0.05$

Table 1. Extraction yield, TPC, TFC, SC, and RAC of 15 *O. aristatus* genotypes in ethyl acetate leaf extracts

Genotypes	EY (% DW)	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	RAC (mg/g DW)	SC (mg/g DW)	DPPH (µmol TE/g DW)	FRAP (µmol TE/g DW)
A1	11.26	5.63de	1.1b	0.04h	1.61m	9.01d	0.15d
A2	11.23	3.98ef	0.73de	0.06f	12.32b	8.11e	0.14d
A3	10.84	3.39fg	0.93c	0.10e	7.46f	8.77d	0.09d
A4	11.35	4.99ef	0.86cd	0.15b	3.53k	7.87e	0.13d
A5	7.66	3.43fg	0.47ghi	0.05g	4.16j	5.18h	0.049d
A6	6.04	3.66f	0.41hi	0.05g	4.31j	4.54i	0.06d
A7	6.94	4.11ef	0.32ij	0.14c	5.27h	5.83g	0.09d
A8	12.43	6.78cd	0.87cd	**0.19a**	4.66i	10.52c	0.17d
A9	5.11	1.79g	0.20j	0.02i	2.50l	2.09j	0.07d
A10	14.04	**13.80a**	1.43a	0.11de	7.81e	13.46a	0.65b
A11	**14.99**	7.08cd	1.10b	0.11d	**21.67a**	**13.98a**	0.42c
A12	14.14	4.90ef	**1.46a**	0.03h	8.19d	12.01b	**2.96a**
A13	11.39	8.46c	0.71def	0.06f	4.31j	7.24f	0.19d
A14	10.84	3.86ef	0.59efg	0.03h	5.41g	10.21c	0.16d
A15	10.81	10.42b	0.54fgh	0.02i	8.95c	6.86f	0.16d

Note: EY: Extraction Yield, DW: Dried Weight, TPC: Total Phenolic Content, GAE: Gallic Acid Equivalent, TFC: Total Flavonoid Contents, QE: Quercetin Equivalent, RAC: Rosmarinic Acid Content, SC: Sinensetin Content, DPPH: 2,2-Diphenyl Picrylhydrazyl, FRAP: Ferric Reducing Antioxidant Power, TE: Trolox Equivalent; different letters in the same column represent the different results at $p<0.05$ statistically

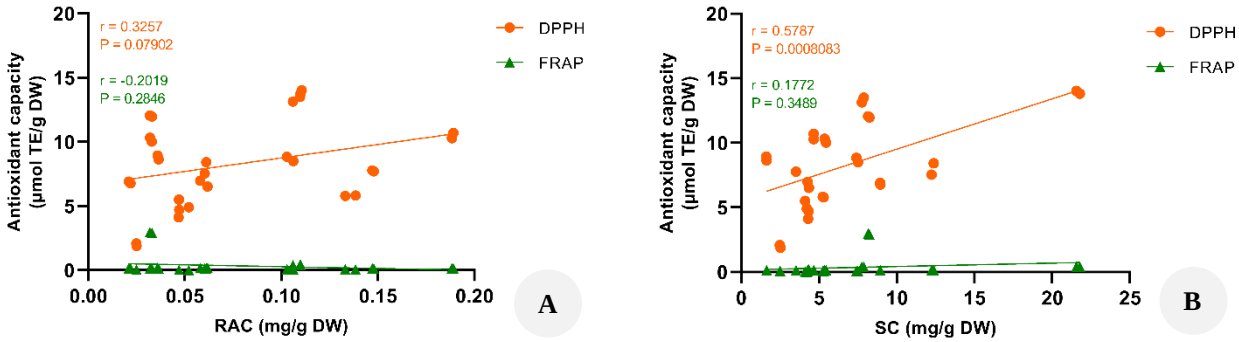

Figure 3. Simple linear correlation of: A. Rosmarinic Acid Content (RAC) with antioxidant capacity DPPH and FRAP and B. Sinensetin Content (SC) with antioxidant capacity DPPH and FRAP in ethyl acetate extract. r (value Pearson's correlation coefficient), p-value indicates significance at $p<0.05$

Pearson correlations between phytochemical compounds and antioxidant capacity were also analyzed as a further step. The Total Phenolic Content (TPC) of the ethyl acetate extract of *O. aristatus* was significantly correlated with the antioxidant capacity determined by the DPPH method but did not correlate with FRAP (Figure 2). These results indicate that the phenolic compounds extracted from *O. aristatus* mostly act as antioxidants using the DPPH method. The differences in the role of phenolic compounds as antioxidants in the DPPH (2,2-diphenyl-1-picrylhydrazyl) and Ferric Reducing Antioxidant Power (FRAP) tests can be explained by the basic principles behind each test. In the DPPH test, phenolic compounds act as electron donors and transfer electrons to DPPH free radicals, converting them into non-radical molecules. This reaction changes color from purple to yellow or white (Gulcin and Alwasel 2023). In contrast, in the FRAP test, phenolic compounds reduced iron ions (Fe^{3+}) to lower iron ions (Fe^{2+}). This property reflects the capacity of a compound to reduce transition metal ions via an electron-transfer mechanism (Sadeer et al. 2020). In contrast to TPC, Total Flavonoid Content (TFC) significantly correlated with both methods. These results proved that most flavonoid compounds can act as antioxidants using DPPH and FRAP methods (Figure 2). Similar to TPC, rosmarinic acid and sinensetin tended to act more as DPPH antioxidants than FRAP (Figure 3). Rosmarinic acid and sinensetin contain phenol groups that effectively respond to free radicals. Although both contain phenol groups, their reduction properties towards iron ions may not be as strong as their antioxidant properties in neutralizing free radicals. In *O. aristatus*, not only rosmarinic acid and sinensetin are present, but these two compounds are particularly noteworthy, and we aim to investigate their relationship with the antioxidant capacity produced. Several other studies have indicated that *O. aristatus* contains other phytochemical compounds, such as terpenoids, essential oils like ursolic acid and oleanolic acid, and so on (Hsu et al. 2010; Ashraf et al. 2018). Therefore, this research has significant implications for the potential development of *O. aristatus* as a medicinal plant by selecting the appropriate genotype to optimize the content of compounds and antioxidant capacity present in *O. aristatus.*

The ethyl acetate solvent used significantly impacted the extraction and phytochemical compounds obtained from *O. aristatus.* Ethyl acetate tends to be more useful for extracting nonpolar compounds from the phenolic and flavonoid groups. In addition, the use of *O. aristatus* plant genotypes also significantly influenced the compound content and antioxidant capacity, as evidenced by the significant differences between each plant genotype. Thus, the combination of ethyl acetate and *O. aristatus* genotypes significantly affected the test parameters in this study.

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