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Original Article

STANDARDIZATION OF *ORTHOSIPHON ARISTATUS,* BLUME MIQ

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ABSTRACT

Objective: The main compounds in *O. aristatus* are rosmarinic acid, sinensetin, and eupatorin. Sinensetin and rosmarinic acid compounds have the potential as antiviral agents. The focus of this research is *O. aristatus* purple and white-purple varieties. This study aimed to determine the levels of three main secondary metabolites of *O. aristatus*, one of the specific standardizations.

Methods: The standardization parameters to be tested were to determine the main compound levels by using thin-layer chromatography densitometry on two varieties of *O. aristatus*.

Results: The highest value levels of sinensetin and rosmarinic acid in purple variety *O. aristatus* were 0.53 and 1.32% w/w, respectively. The highest level of eupatorin was 0.88% w/w in the ethanol extract of white-purple varieties of *O. aristatus.* The main secondary metabolites in the two varieties of *O. aristatus* were more significant in the leaves than in the stems. Meanwhile, the sinensetin and rosmarinic acid levels in the ethanol extract of leaves and stems of the purple variety *O. aristatus* were higher and significantly different than in the white-purple ones. However, the levels of eupatorin were higher and significantly ($p<0.05$) different in the white-purple variety compared to the purple variety.

Conclusion: The purple variety is due to greater sinensetin and rosmarinic acid levels in the purple variety than in the white-purple ones.

Keywords: *O. aristatus,* White-purple varieties, Purple varieties, Active compounds, TLC-densitometry

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INTRODUCTION

The active compounds in *O. aristatus* have diuretic, hypoglycemic, and antihypertensive effects [1]. The extract of *O. aristatus* causes the diuretic effect and contains flavonoids (sinensetin and tetramethoxyflavone) [1]. The active isolates of *O. aristatus* (methylripariochromene A, acetovanillochromone, orthochoromen A) has an antihypertensive effect [1]. In addition, the flavonoid compound (sinensetin) in *O. aristatus* has activity in lowering blood sugar levels [2, 3]. Several studies about the extract of *O. aristatus* conducted *in vitro* show anti-tumor and anti-microbial effects [1]. There is a report of *in vitro* study on sinensetin and tetramethilscutellarein about the capability of anti-tumor activity in Ehrlich ascites tumor cells [1]. It has also been proven that the liquid extract from *O. aristatus* shows anti-bacterial activity against two serotypes of *Streptococcus mutants*. *O. aristatus* extract also inhibits the germination of six test fungal species [1]. Other pharmacological activity reports of the *O. aristatus* plant are antihyperglycemic [2], anti-epilepsy [4], analgesics antipyretic [5], rheumatoid treatment [6], and osteoarthritis arthritis [6], treatment to overcome gastric disorders [7, 8], hepatoprotective effect [9, 10], antioxidants [11, 12], enhancing memory [13], treating cardiovascular disorders [14], antiviral [15], and immunomodulators [16-22].

During the COVID-19 pandemic, the *O. aristatus* is a potential plant to be developed. Based on *silico* studies, several compounds have potential as COVID-19 inhibitors, including rosmarinic acid [23-25], sinensetin [26], cirsimaritin [27], caffeic acid derivatives [28], sagerinic acids [29], *β*-caryophyllene [30], and 1,8-cineole [31]. In addition to this inhibitive character, the *O. aristatus* plant's active compound has potential as an antiherpetic [32] (caffeic acid [33], N-transferulolyl tyramine [34], *β*-caryophyllene [35], limonene [36], eugenol [37, 38], p-cymene) [39], as anti-influenza virus (sinensetin [40], caffeic acid [41], limonene [42], 1,8-cineol [43], linalool [44], eugenol [45], aurantiamide) [46], as anti-viral hepatitis (rosmarinic acid [47], ladanein [48], oleanolic acid [49], ursolic acid [50], danshensu) [51], as anti-Japanese viral encephalitis (rosmarinic acid) [52], as anti-enterovirus 71 (rosmarinic acid) [53], and as anti-human immunodeficiency virus (HIV) (lithospermic acid [54], chicoric acid [55], 2,3-dicaffeoyltartaric acid [56], thansione ⅡA [57], oleanolic acid [58, 59], maslinic acid) [60].

There are three *O. aristatus* in Indonesia, namely, *O. aristatus* with purple, white-purple and white varieties [61]. Meanwhile, in Malaysia, there are some reports of two varieties, such as purple-flowered and white flowers of *O. aristatus* [62]. According to Padua *et al.*, old leaves of *O. aristatus* with purple flowers had sinensetin up to 0.4% [63]. Similar data reports by Lee stated that those purple varieties had higher bioactive compounds than the white varieties [64]. White-flowered *O. aristatus* has many leaves, branches, stems, and roots. Meanwhile, the purple and white-flowered *O. aristatus* had the most extensive leaf area indices [65]. Hence, the results of this study are expected to provide levels of the active compounds of the two varieties of *O. aristatus,* so that it can be used as a specific parameter to ensure the consistency of traditional medicinal products.

MATERIALS AND METHODS

Preparation of plant material

O. aristatus were collected from the Manoko Experimental Garden, West Bandung, Indonesia. The processes carried out on plant samples were sorting, drying in an oven at 50 °C and reducing particle size.

Extraction of plant material

The extraction was done through maceration, in which there were four macerators prepared, and each macerator was added 100 g of purple leaves (LP), purple stems (SP), white-purple leaves (LWP) and white-purple stems (SWP) raw material. After that, each macerator was added 1.5 l of ethanol solvent. Meanwhile, the filtrate was concentrated using a water bath to form a thick extract.

Preparation of the standard and samples solutions

Standard solutions of rosmarinic acid, sinensetin, and eupatorin were prepared at a concentration of 1000 mg/l in methanol. The stock

solution was diluted with methanol up to five concentrations ranging. Furthermore, the acetone, ethyl acetate, and ethanol extracts from two varieties of *O. aristatus* were prepared by dissolving 15 mg of extract in 1 ml of methanol and sonicated for 45 min.

Chromatographic conditions

Standard solutions and samples were applied on TLC plates, and the standard solution and sample were applied using a micropipette with a volume of 5 ml. The mobile phase of toluene consisting of ethyl acetate: formic acid: water (3: 3: 1: 0.2) [66] was pre-saturated in the chamber. Observing the area of each spot with a densitometry CAMAG analyzer, monitoring was carried out at a wavelength of 254 nm. Data analysis was performed using the win CATS software.

Data analysis

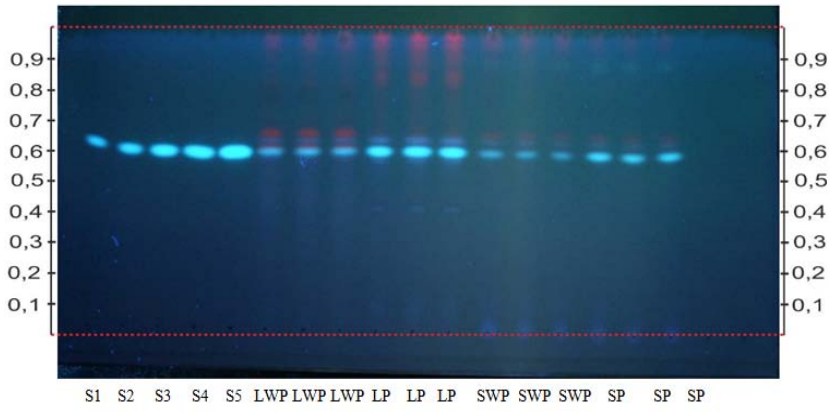
Data processing was performed by one-way ANOVA using SPSS 22 software P values <0.05.

RESULTS

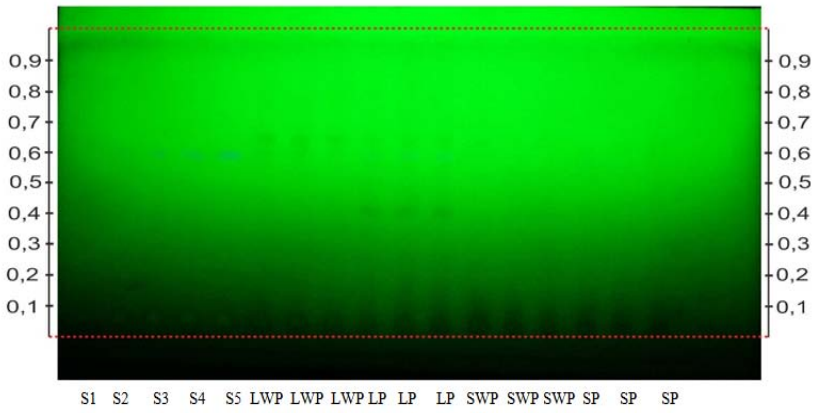
The previous research reported that the differences in morphology of white-purple and purple *O. aristatus* were sighted in leaf colour, petal colour, crown colour, the colour of the stems of the pistil, and colour of stamens (fig. 1) [67].

Determination of the main secondary metabolites levels in two varieties of *O. aristatus* using TLC-Spectrodensitometry referred to the research conducted by Hossain [72] with the development and modification of the mobile phase, standard concentrations, and determined compounds, not only sinensetin. The linearity correlation coefficient (R^2) of determining the levels of the main secondary metabolites of two varieties of *O. aristatus* is presented in table 1.

Fig. 1: *O. aristatus* plant. A: the flower (white-purple) b: the flower (purple variety) c: the leaf of (white-purple), d: the leaf (purple variety)



A



B

Fig. 2: The TLC profiling of the ethanol extract of two varieties *O. aristatus* and the standard sinensetin in UV light at 365 (A) and 254 nm (B). S1 = sinensetin (60 ppm), S2 = sinensetin (70 ppm), S3 = sinensetin (80 ppm), S4 sinensetin (90 ppm), S5 = sinensetin (100 ppm), LWP = leaves ethanol extract (white-purple), LP = leaves ethanol extract (purple), SWP = stem ethanol extract (white-purple), SP = stem ethanol extract (purple)

Table 1: The regression equation of determining the levels of secondary metabolites of two varieties of *O. aristatus* using TLC-densitometry

Compound	Linearity range (µg/ml)	Regression equation	R^2(*n*= 3)
Sinensetin	60-100	y = 86.872x-4438.5	0.9954
Eupatorin	60-100	y = 28.509x-458.66	0.9986
Rosmarinic acid	60-100	y = 30.043x-1365	0.9961

The results of TLC showed the presence of a sinensetin compound in two varieties of *O. aristatus* because there were spots with the same retention factor (Rf = 0.60) as the sinensetin standard with bright blue fluorescence. TLC profiles and 3D chromatogram displays are illustrated in fig. 2 and fig. 3. The levels of sinensetin in leaves of purple varieties were 0.55% w/w, leaves of white-purple varieties were 0.43% w/w, stems of purple varieties were 0.39% w/w, and stems of white-purple varieties were 0.38% w/w respectively.

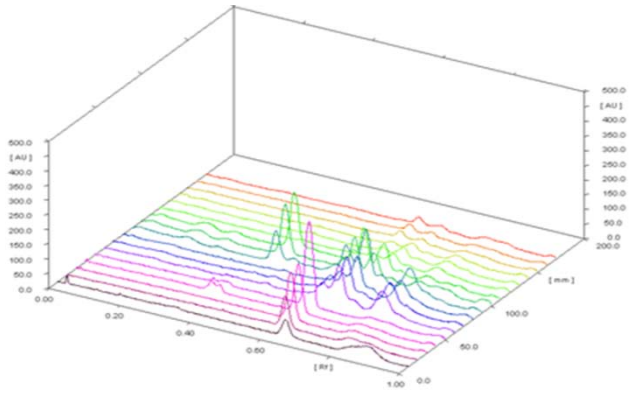
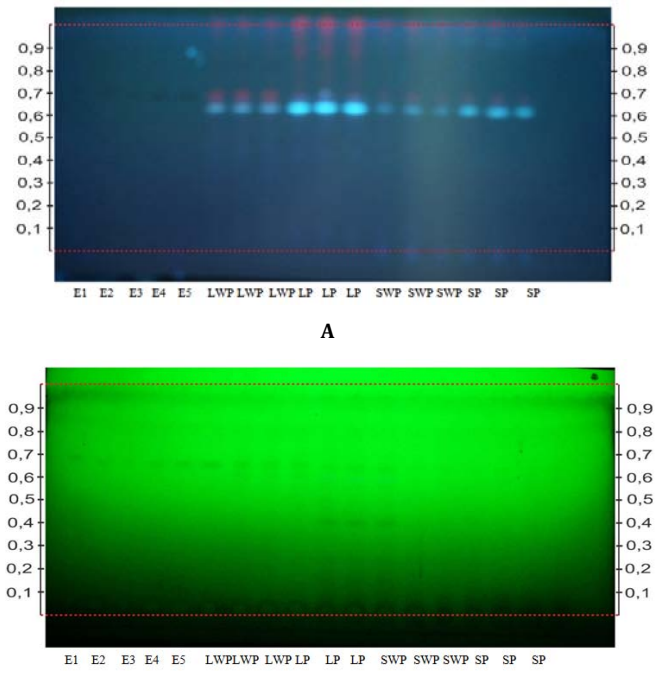


Fig. 3: Chromatogram of the ethanol extract of two varieties, *O. aristatus* and the standard sinensetin (3D-TLC). pink chromatogram = sinensetin standard, blue chromatogram = leaves ethanol extract (white-purple), green chromatogram = leaves ethanol extract (purple), brown chromatogram = stem ethanol extract (white-purple), red chromatogram = stem ethanol extract (purple)

Eupatorin was detected in leaves and stems of white-purple varieties, whereas in purple varieties, it was only detected in leaf parts with Rf = 0.67 (fig. 4 and fig. 5). The level of eupatorin in the ethanol extract of purple varieties *O. aristatus* leaves was 0.45% w/w, while the stem was not detected. The ethanol extract of leaves and stems of white-purple varieties contained eupatorin with 1.09% w/w and 0.18% w/w, respectively.

Rosmarinic acid was detected at Rf 0.43 on a UV-254 nm observation lamp (fig. 6 and 7). The levels of rosmarinic acid in the ethanol extract of leaves and stems of purple varieties were 1.36% w/w and 0.46% w/w, respectively, while those in white-purple varieties were 0.43% w/w and 0.38% w/w, respectively.

The comparison of sinensetin, eupatorin, and rosmarinic acid levels is presented in table 2 and fig. 8.



A

B

Fig. 4: The TLC profiling of the ethanol extract of two varieties O. aristaus and the standard eupatorin in UV light at 365 (A) and 254 nm (B). E1 = eupatorin (60 ppm), E2 = eupatorin (70 ppm), E3 = eupatorin (80 ppm), E4 = eupatorin (90 ppm), E5 = eupatorin (100 ppm), LWP = leaves ethanol extract (white-purple), LP = leaves ethanol extract (purple), SWP = Stem ethanol extract (white-purple), SP = Stem ethanol extract (purple)

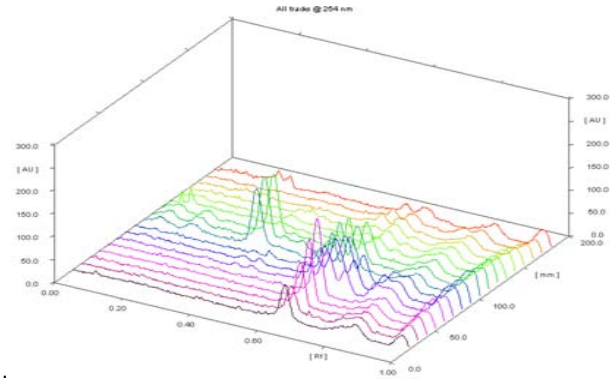


Fig. 5: Chromatogram of the ethanol extract of two varieties, *O. aristatus* and the standard eupatorin (3D-TLC). pink chromatogram = eupatorin standard, blue chromatogram = leaves ethanol extract (white-purple), green chromatogram = leaves ethanol extract (purple), brown chromatogram = stem ethanol extract (white-purple), red chromatogram = stem ethanol extract (purple)

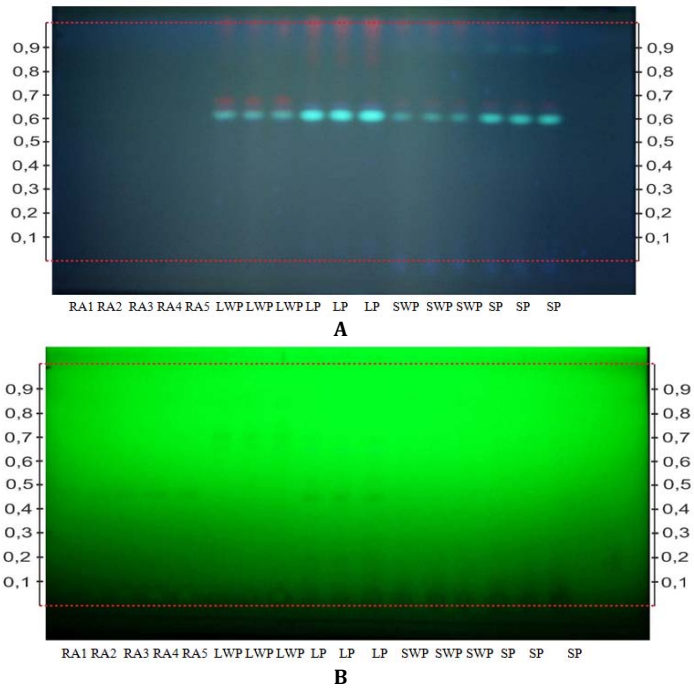


Fig. 6: The TLC profiling of the ethanol extract of two varieties O. *aristatus* and the standard rosmarinic acid in UV light at 365 (A) and 254 nm (B). RA 1 = rosmarinic acid (60 ppm), RA 2 = rosmarinic acid (70 ppm), RA 3 = rosmarinic acid (80 ppm), RA 4 = rosmarinic acid (90 ppm), RA 5 = rosmarinic acid (100 ppm), LWP = leaves ethanol extract (white-purple), LP = leaves ethanol extract (purple), SWP = Stem ethanol extract (white-purple), SP = Stem ethanol extract (purple)

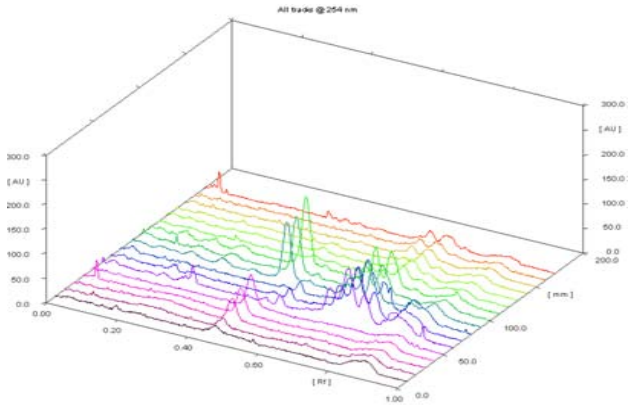


Fig. 7: Chromatogram of the ethanol extract of two varieties, *O. aristatus* and the standard eupatorin (3D-TLC). pink chromatogram = sinensetin standard, blue chromatogram = leaves ethanol extract (white-purple), green chromatogram = leaves ethanol extract (purple), brown chromatogram = stem ethanol extract (white-purple), red chromatogram = stem ethanol extract (purple)

Table 2: Levels of rosmarinic acid, sinensetin, eupatorin from ethanol extracts of two varieties of *O. aristatus* with TLC-densitometry

Sample	Sinensetin (% w/w)±SD (n = 3)	Eupatorin (% w/w)±SD (n = 3)	Rosmarinic acid (% w/w)±SD (n = 3)
LWP	0.43±0.00[a]	1.09±0.07[a]	0.43±0.01[a]
LP	0.55±0.02[b]	0.44±0.07[b]	1.36±0.08[b]
SWP	0.38±0.01[c]	0.18±0.01[c]	0.38±0.00[c]
SP	0.39±0.00[c]	ND	0.46±0.01[a]

Mean values with different superscript letters were significantly different (p<.05), ND: Not detected

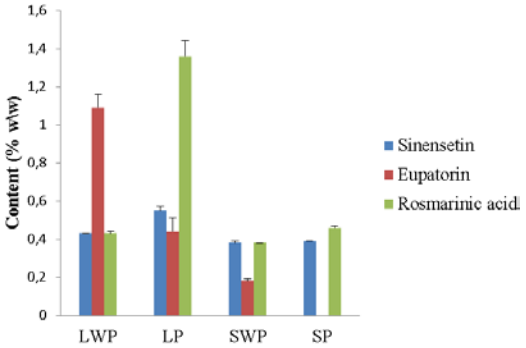


Fig. 8: The comparison of sinensetin, eupatorin, and rosmarinic acid in ethanol extracts of two varieties of *O. aristatus*. LWP = leaves ethanol extract (white-purple), LP = leaves ethanol extract (purple), SWP = Stem ethanol extract (white-purple), SP = Stem ethanol extract (purple)

DISCUSSION

In purple varieties, the colour of the stems of the pistil (purple) and colour of stamens (purple). Meanwhile, the colour of the stems of the pistil (white-purple), and colour of stamens (white-purple) [67]. Observing the plant morphology or sources of traditional medicinal ingredients was essential to ensure the correctness and validity of the plants to be used. Thus, morphological studies were carried out in this analysis to differentiate purple and white-purple varieties to verify the possibility of variations in the levels of secondary metabolites of both varieties. Febijslami *et al.*, Keng and Siongand Almatar *et al.* have also conducted studies comparing the morphology of purple, white-purple, and white varieties [61,62,68]. Their studies showed that the purple variety *O. aristatus* were higher than those of the white varieties [63,64], but there was no comparison of secondary metabolite levels reported between the purple and white-purple varieties.

The leaf shape was rhombus in the purple and white-purple varieties; this result was consistent with the one recorded by Keng and Siong [62]. The colour of the two *O. aristatus* varieties' leaves was not different from the report of Almater *et al.* [68]. Green-purple was the colour of purple flower petals, which was consistent with a report by Keng and Siong [62], while white was the colour of petals of the white-purple type. The purple variety's crown colour was purplish, and the white-purple variety was white. The morphology of flowers was the most fundamental distinction between these two varieties. There were some variations and similarities in the morphology of roots, leaves, and flowers in the genus *Lamiaceae* [69].

According to Faramayuda *et al.*, there were no differences in phytochemical content between purple and white-purple varieties. The crude drugs and ethanol extracts of both varieties contained secondary metabolites of alkaloids, flavonoids, tannins, polyphenolic, saponin, steroid and triterpenoid, monoterpenoid and sesquiterpenoid [67]. The research at the genetic level on *O. aristatus*, can be used to distinguish between white and purple varieties [70]. Both varieties were reported to have different bioactive compounds, mostly purple varieties that produced higher levels of bioactive compounds than white varieties [64]. Morphological studies showed that the morphology of flowers and leaves could identify both varieties [62, 71].

Several previous studies reported that purple varieties had higher levels of sinensetin than white ones [64]. The results of a study reported by Febjislami reported that the levels of sinensetin in the methanol extract of *O. aristatus* growing in Indonesia were higher in purple varieties than in white varieties [73]. White varieties of O. aristatus, which had the characteristics of a plant with medium height and had strong anthocyanin colouration on the stem [74]. These results indicated that in addition to the influence of varieties, plant age factors could affect sinensetin levels because they were related to anthocyanin concentrations and maturity levels. In addition, flower colour had a strong correlation with sinensetin levels. Flower colour could be used for initial estimates of *O. aristatus,* which tend to have high sinensetin levels when found in the field.

Hossain and Ismail recorded monitoring of the TLC profile of the *O. aristatus* variety with mobile phase chloroform–ethyl acetate (60:40), where sinensetin was observed at Rf 0.49 and reported that sinensetin levels with TLC-densitometry on the extract of *O. aristatus* that grew in Penang Malaysia with acetone: water (70:30) solvent was 0.32% w/w, methanol: water (1:1) 0.15% [72]. The determination of sinensetin levels in *O. aristatus* growing in Fujian Zhangzhou, Guangxi Yulin, and Yunnan Kunming, averaged 0.057 mg/g [75]. The levels of sinensetin in the stems, and roots of *O. aristatus* obtained from the Yulin Chinese herbal medicine market in Yulin China using HPLC-MS, were 0.097 mg/g, 0.103 mg/g, and 2.719 mg/g, respectively [76]. This report aligned with this study's results, where the levels of sinensetin in the leaves were more significant than in the stems.

In general, eupatorin levels were higher in white-purple varieties than in purple varieties. The result was inversely proportional to sinensetin levels, where purple varieties were higher than white-purple varieties. The results of previous studies reported that eupatorin levels in the leaves of one *O. aristatus* variety were 0.209 mg/g and 4.73 mg/g, while those in the roots and stems were 0.184 mg/g and 0.285 mg/g [76]. Rosmarinic acid levels in the leaves of the two varieties of O. aristatus were more significant (p<0.05) than in stems. In other studies, it was reported that the rosmarinic acid levels of water-ethanol extract in the roots, stems and leaves were 0.018 g/g, 0.008 g/g, and 0.020 g/g raw material [76]. Methanol-water extract of *O. aristatus* contained rosmarinic acid as much as 2.826 mg/g [75]. The morphological observations of the purple variety *O. aristatus* had a purple tinge to the crown and pistil stalk, while the white-purple variety had a purple tinge.

The results of this morphological observation aligned with what has been reported by Faramayuda [79, 80] stating that the levels of sinensetin and rosmarinic acid compounds were higher in the purple variety. The result of previous studies also reported higher flavonoid levels in the purple variety than in the white-purple variety [81]. Sinensetin and rosmarinic acid compounds had the potential as antiviral [82], antihypertensive [83, 84] and antidiabetic [85-87]. Moreover, sinensetin concentrations were lower in non-polar solvents such as hexane [77]. Rosmarinic acid was more soluble in polar solvents and had four hydroxyl groups and one carboxyl group (water) [78]. Compounds of rosmarinic acid were more drawn to ethanol solvents than ethyl acetate solvents [77].

CONCLUSION

The morphological difference between the purple and white-purple varieties of *O. aristatus* lies in the colour of the crown and pistil stalk. Sinensetin and rosmarinic acid levels are higher in purple varieties than in white-purple varieties, while eupatorin compounds are

higher in white-purple varieties than purple ones. This study has proved differences in the levels of active compounds of the two varieties of *O. aristatus.* Therefore, the findings of this study are expected to become a recommendation for herbal medicine developers in choosing the varieties of *O. aristatus.*

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AUTHORS CONTRIBUTIONS

Fahrauk Faramayuda, Soraya Riyanti, Suryani Akhirul Kahfi Syam, Elfahmi, Totik Sri Mariani, Sukrasno experimented and wrote the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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