Research Article

Identification of Secondary Metabolites from Callus *Orthosiphon aristatus* (Blume) Miq by Thin Layer Chromatography

Fahrauk Faramayuda[1,2*], Totik Sri Mariani[3], Elfahmi[1,4] and Sukrasno[1]

[1]School of Pharmacy, Institut Teknologi Bandung (ITB), Bandung, West Java, Indonesia, 40132; [2]Faculty of Pharmacy, Universitas Jenderal Achmad Yani (UNJANI), Cimahi, West Java, Indonesia, 40532; [3]School of Life Sciences and Technology, Institut Teknologi Bandung (ITB), Bandung, West Java, Indonesia, 40132; [4]Biosciences and Biotechnology Research Center, Institut Teknologi Bandung (ITB), Bandung, West Java, Indonesia, 40132.

Abstract | The flowers *of Orthosiphon aristatus* are purple, white-purple, and white. The main chemical compounds of *O. aristatus* are rosmarinic acid, eupatorin and sinensetin. The *O. aristatus* plant's potential as traditional medicine is established, so it is necessary to produce its active compounds abundantly and to propagate purple and white-purple varieties of *O. aristatus*. A strategy that can be adopted to achieve plant tissue culture (callus induction). This research aim is to identify secondary metabolite in callus using thin layer chromatography (TLC). The profiling of ethanol extracts of callus of two varieties *O. aristatus* were carried out using solvent system toluene-ethyl acetate- formic acid-water (3:3:1:0.2). The results of monitoring TLC suggest that callus obtained from Schenk and Hildebrandt (SH) media can proceed to the suspension culture stage because it showed brighter fluorescence spot than other callus and plant extracts, especially for rosmarinic acid. This research provides new information about secondary metabolites in callus derived from two varieties of *O. aristatus* on various growth media.

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***Correspondence** | Fahrauk Faramayuda, School of Pharmacy, Bandung Institute of Technology (ITB), Bandung, West Java, Indonesia; **Email:** ramayuda.f@gmail.com
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Keywords | *In-vitro* culture, White-purple, Purple variety *O. aristatus*, Growth media, Phytochemistry of callus

Introduction

The potential of the *O. aristatus* plant as a medicinal plant has been widely studied, including as diuretics (Arafat *et al*., 2008), treatment of gastric disorders (Yuniarto *et al*., 2017), antidiabetic (Mohamed *et al*., 2011), antihypertensive (Matsubara *et al*., 1999), hepatoprotective (Yam *et al*., 2007; Maheswari *et al*., 2008), antimicrobial (Ho *et al*., 2010; Hossain *et al*., 2008), antioxidants (Alshawsh *et al*., 2012; Akowuah *et al*., 2004), anti-epilepsy (Kar *et al*., 2018), memory enhancer (George *et al*., 2015), treatment of cardiovascular disorders (Abraika *et al*., 2012), rheumatoid treatment and osteoarthritis (Adawiyah *et al*., 2018), anticancer (Pauzi *et al*., 2018; Halim *et al*., 2017), antiviral (Ripim *et al*., 2018; Abdelwahed *et al*., 2020; Li *et al*., 2020; Sarkar and Das, 2020; Wondmkun and Mohammed, 2020; Lin *et al*., 2019; Hsieh *et al*., 2020; Faramayuda *et al*., 2021b) and immunomodulatory (Harun *et al*., 2015; Woottisin *et al*., 2011; Friedman, 2015; Kim *et al*., 2008; Takano *et al*., 2004; Sanbongi *et al*., 2004; Youn *et al*., 2003). The pharmacological activity of *O. aristatus* is inseparable from its main compounds, namely rosmarinic acid, sinensetin, and eupatorin (Guo *et al*., 2019). Not only these three compounds,

but *O. aristatus* also contains rhamnazin (Malterud *et al*., 1989), danshensu (Nuengchamnong *et al*., 2011) and Orthosiphol D (Takeda *et al*., 1993; Awale *et al*., 2002).

According to Febjislami *et al*. (2019), three varieties of *O. aristatus* grow in Indonesia, namely purple, white and white purple. The levels of sinensetin are more significant in purple varieties' than in white varieties (Lee, 2004; Anggraeni, 1992). Recently, the white-purple and purple *O. aristatus* decreased, especially in Indonesia (Febjislami *et al*., 2019). Therefore it is necessary to produce active compounds without directly processing them from the original plant and efforts to micropropagate *O. aristatus* using plant tissue culture technique. Several studies on tissue culture of *O. aristatus* have previously been reported, including induction of the callus from *O. aristatus,* which can grow on MS medium with 0.4 ppm 2,4-D;1 ppm NAA + 1 ppm 2,4-D; 2 ppm 2, 4-D; 1.0 ppm Kinetin + IAA 1.0 ppm (Faramayuda *et al*., 2020; Wai and Lai, 2004; Bordbar *et al*., 2015; Sheena and Jothi, 2015; Ali *et al*., 2017; Faramayuda *et al*., 2021a). However, these studies did not report identification of active compounds in the callus of the *O. aristatus*, which were derived using Gamborg, Schenk, and Hildebrandt (SH) and N6 media. The findings of this research are expected to provide new knowledge on the secondary metabolites content of callus derived from two varieties of *O. aristatus.*

Materials and Methods

Chemicals and reagents

Rosmarinic acid, sinensetin, eupatorin, silica gel pre-coated plate 60 F254 were purchased from Sigma. Acetone, ethanol, ethyl acetate, toluene, formic acid were purchased from Loba Chemie (Mumbai, India).

Collection of plants

White-purple and purple *O. aristatus* leaves and stems (wild type) were obtained from the Manoko experimental garden in West Bandung, Indonesia. The plants were taxonomically identified with letter number 6115/I1.CO2.2/PL/2019. Callus of two varieties of *O. aristatus* was obtained from the Laboratory of Plant Tissue Culture, Center for Research and Innovation, ITB.

Callus and plant (wild type) extraction

The callus was removed from the medium and dried in a 70°C oven (Memmert, Germany). The dry callus was then crushed. Callus powder was extracted separately by maceration using n-hexane, acetone, ethyl acetate, and ethanol as solvent. Crude extract from of 1 g of *O. aristatus* (wild type) was extracted with acetone, ethyl acetate, and ethanol (the amount of each solvent was 15 mL).

Identification of secondary metabolites

On silica gel GF 254 plates, thick extracts from two varieties of *O. aristatus* were applied. The mobile phase used was ethyl acetate-toluene-formic acid and water (3:3:1:0.2) (Craciun *et al*., 2014). The plate was then inserted for the development. TLC profile analysis was performed under UV light 366 nm.

Data analysis

For each compound, the Rf value was calculated by dividing the distance traveled by the component with the distance traveled by the eluent (mobile phase).

Results and Discussion

Morphological identification and characterization

The results of the identification of two plant varieties of *O. aristatus* explained that two plant samples were identified as white purple and purple varieties of *O. aristatus*. The most striking difference among the two varieties is the flowers morphology (Keng and Siong, 2006; Almatar *et al*., 2013). The purple variety has a purple crown color, while the white-purple variety is white-purple. These results were in line with the studies reported (Febjislami *et al*., 2019; Batubara *et al*., 2020; Faramayuda *et al*., 2020).

Analysis of secondary metabolite content in callus of two varieties of O. aristatus

The callus of two varieties of *O. aristatus* came from MS, Gamborg, SH, and N6 media with growth regulators 2.4 D 0.4 ppm. Callus derived from purple varieties of *O. aristatus* on all media had a soft texture and greenish-white. The callus was white-brown using white-purple variety, while callus induced on the N6 and SH media were white-green (Figures 1, 2).

Regeneration of *O. aristatus* has been carried out using nodal segment, where some shoots (6.1 shoots per explant) were observed from explants nodal stems on MS medium with added plant growth regulators BAP 6.7 µM and four weeks culture period (Lee and Chan, 2004).

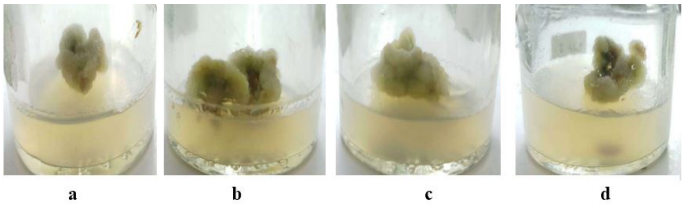

Figure 1: *Callus O. aristatus purple varieties. (a) MS media, (b) Gamborg media, (c) N6 media and (d) SH media.*

Figure 2: *Callus O. aristatus white-purple varieties. (a) MS medium, (b) Gamborg media, (c) N6 media, and (d) SH media.*

When leaf explants were cultured in MS supplemented with 2,4-D 1.0 ppm and NAA 1.0 ppm as growth regulators, callus development was observed. Callus cells in the MS liquid medium supplemented with 2,4-D 1.0 ppm, proved to be the best conditions *for O. aristatus* cell culture suspension (Lee and Chan, 2004). Research conducted by Faramayuda *et al.* (2020) reported that callus of two varieties of *O. aristatus* grown on MS + 2.4 D 0.4 ppm resulted in optimal growth. This research is a continuation of Faramayuda's research reported in 2020, where callus induction of white purple, and purple varieties of *O. aristatus* was carried out on media other than MS and secondary metabolites compounds were identified. This research contributes to understanding of the phytochemical content of the callus derived from two varieties of *O. aristatus* using in Gamborg, SH, and N6 media.

The concentration for each standard compound and the test sample are 100 mg/L and 1500 mg/L, respectively. The volume of bottling on TLC for standard and sample is 10 µL. In the ethanol, ethyl acetate, and callus acetone extracts of purple varieties derived from MS, Gamborg, N6, and SH media, the presence of sinensetin compounds was detected at Rf 0.5 (Figure 3). Sinensetin fluorescence was brighter in callus growing on N6 media (NU1; NU2; NU3) (Figure 3). Rosmarinic acid was detected in MU2, NU1, NU3, SU1, SU2, and SU3. Eupatorin compounds were detected in ethanol and ethyl acetate extracts of white-purple variety (wild type) *O. aristatus* under the observation of a 254 nm UV lamp. Eupatorin was not detected in all callus extracts (Figure 4).

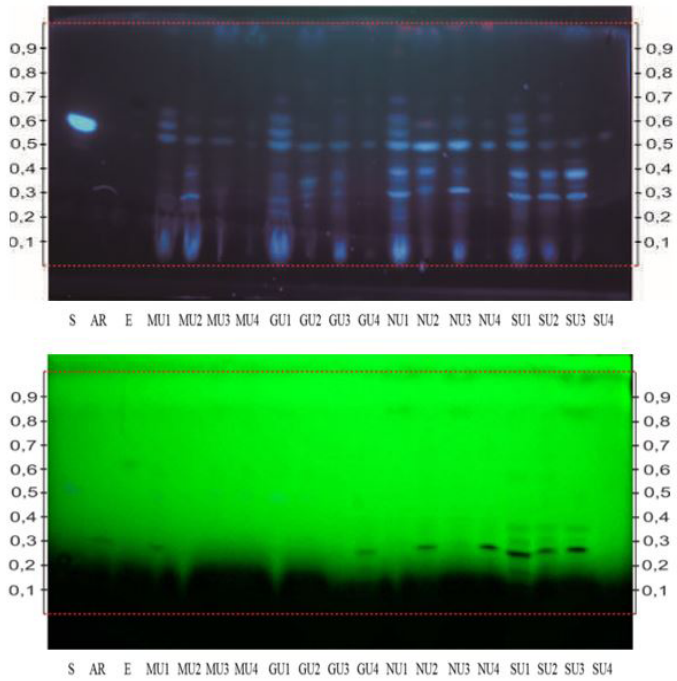

Figure 3: *TLC profile of purple variety callus extract. Stationary phase: Silica GF254 Mobile phase: Toluene: Ethyl Acetate: Formic Acid: Water (3: 3: 1: 0.2).*
S: Sinensetin; AR: Rosmarinic Acid; E: Eupatorin; MU1: Callus purple varieties ethanol extract. (MS medium); MU2: Callus purple varieties ethyl acetate extract. purple (MS medium); MU3: Callus purple varieties acetone extract. (MS medium); MU4: Callus purple varieties n-hexane extract (MS medium); GU1: Callus purple varieties ethanol extract (Gamborg medium); GU2: Callus purple varieties ethyl acetate extract. purple (Gamborg medium); GU3: Callus purple varieties acetone extract. (Gamborg medium); GU4: Callus purple varieties n-hexane extract (Gamborg medium); NU1: Callus purple varieties ethanol extract (N6 medium); NU2: Callus purple varieties ethyl acetate extract. purple (N6 medium); NU3: Callus purple varieties Acetone extract (N6 medium); NU4: Callus purple varieties n-hexane extract (N6 medium); SU1: Callus purple varieties ethanol extract (SH medium); SU2: Callus purple varieties ethyl acetate extract. purple (SH medium); SU3: Callus purple varieties acetone extract (SH medium); SU4: Callus purple varieties n-hexane extract (SH medium).

Observation of TLC profiles wild-type leaf and *O. aristatus* extract and callus of purple variety showed that sinensetin was detected in ethanol, ethyl acetate, and acetone extracts of *O. aristatus* leaves and stems with bright blue fluorescence. Rosmarinic acid in purple variety callus looks clear and brighter than wild type (Figure 5). There were some spots in the callus of purple variety but not in wild types (Figure 5). Sinensetin was detected in all extracts on leaves and stems of *O. aristatus*, but the bands for stems were fainter than the leaves. Sinensetin in white-purple variety callus from SH medium appeared more dim than the wild type, but for rosmarinic acid, the fluorescence appeared brighter than the wild type (Figure 6).

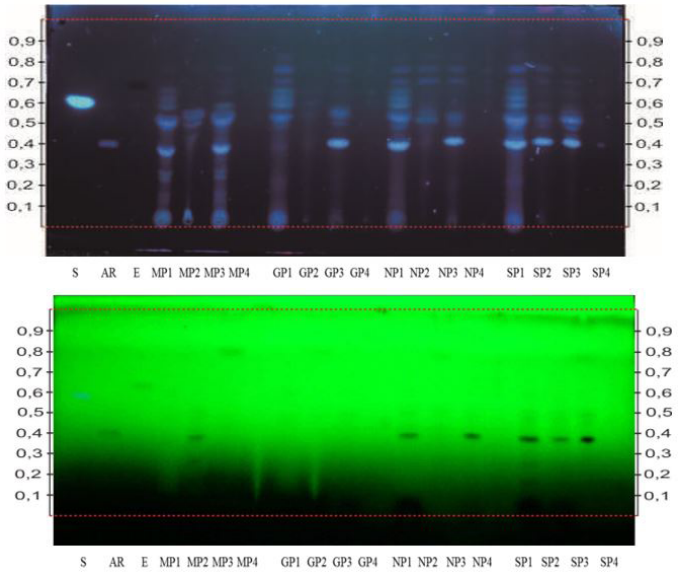

Figure 4: *TLC profile of callus extract from white-purple variety. Stationary phase: Silica GF254 Mobile phase: Toluene: Ethyl Acetate: Formic Acid: Water (3: 3: 1: 0.2).*
MP1: Callus white-purple varieties ethanol extract. (MS medium); MP2: Callus white-purple ethyl acetate extract. purple (MS medium); MP3: Callus white-purple acetone extract. (MS medium); MP4: Callus white-purple n-hexane extract (MS medium); GP1: Callus white-purple ethanol extract (Gamborg medium); GP2: Callus white-purple ethyl acetate extract. purple (Gamborg medium); GP3: Callus white-purple acetone extract. (Gamborg medium); GP4: Callus white-purple n-hexane extract (Gamborg medium); NP1: Callus white-purple ethanol extract (N6 medium); NP2: Callus white-purple ethyl acetate extract. purple (N6 medium); NP3: Callus white-purple acetone extract (N6 medium); NP4: Callus white-purple n-hexane extract (N6 medium); SP1: Callus white-purple ethanol extract (SH medium); SP2: Callus white-purple ethyl acetate extract. purple (SH medium); SP3: Callus white-purple acetone extract (SH medium); SP4: Callus white-purple n-hexane extract (SH medium).

Several hypotheses clarify how secondary metabolites in callus can be detected. Callus is totipotent, which means that each cell has the same complete genetic information and is capable of producing the same secondary metabolites (Efferth, 2019). Plant tissue culture can affect plant cells biosynthetic pathways to produce secondary metabolites derivatives (Sarfaraj *et al.*, 2012). Several studies have reported that there are active compounds in callus, including resveratrol (Nandagopal *et al.*, 2017), shikonin (Hao *et al.*, 2014), hypericin (Walker *et al.*, 2002), and justicidin b (Ionkova *et al.*, 2013).

In research conducted by Faramayuda *et al.* (2020), the content of sinensetin and rosmarinic acid was observed in the acetone extract of callus from purple and white-purple varieties of *O. aristatus* using MS medium supplemented with 2, 4-D 0.4 ppm. From the comparison of TLC profiles between wild type and callus from two varieties of *O. aristatus*, there are several compound spots in the callus that can be further identified and characterised.

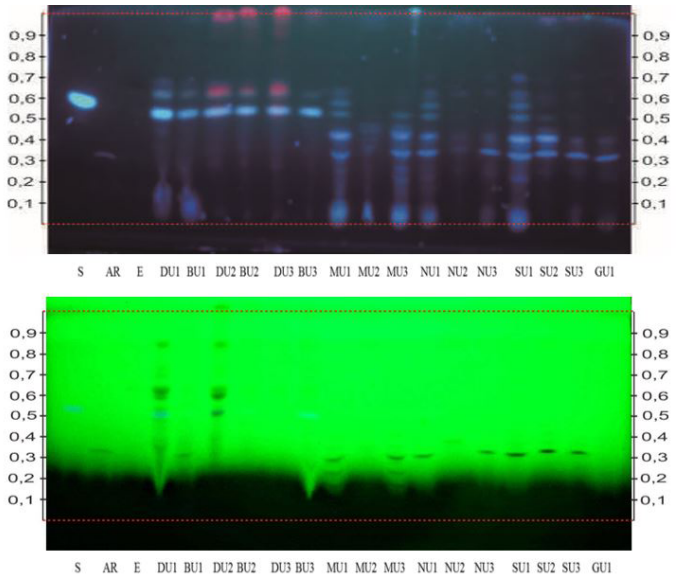

Figure 5: *TLC profile of wild type extract and callus varieties of purple. Stationary phase: Silica GF254 Mobile phase: Toluene: Ethyl Acetate: Formic Acid: Water (3: 3: 1: 0.2).*
DU1: leaf ethanol extract of purple varieties (wild type); BU1: stem ethanol extract of purple varieties (wild type); DU2: ethyl acetate extract of purple varieties (wild type); BU2: stem ethyl acetate extract of purple varieties (wild type); DU3: acetone extract of purple varieties (wild type); BU3: stem acetone extract of purple varieties (wild type).

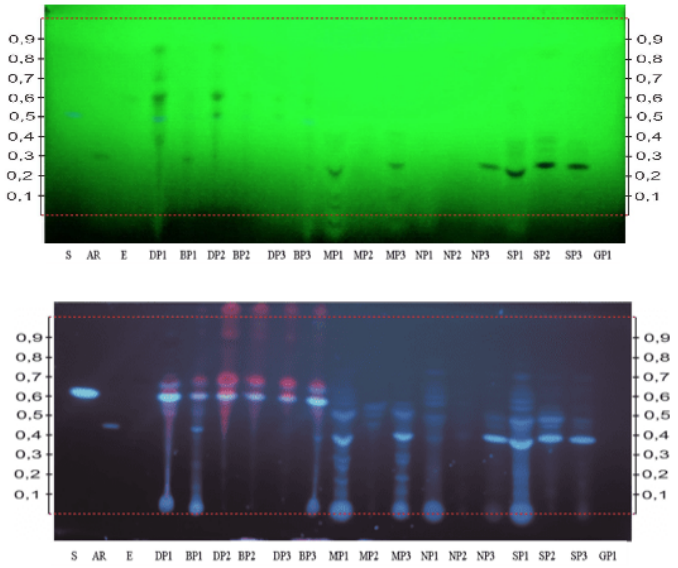

Figure 6: *TLC profile of wild type extract and callus white-purple varieties. Stationary phase: Silica GF254 Mobile phase: Toluene: Ethyl Acetate: Formic Acid: Water (3: 3: 1: 0.2).*
DP1: leaf ethanol extract of white-purple varieties (wild type); BP1: stem ethanol extract of white-purple varieties (wild type); DP2: ethyl acetate extract of white-purple varieties (wild type); BP2: stem ethyl acetate extract of white-purple varieties (wild type); DP3: acetone extract of white-purple varieties (wild type); BP3: stem acetone extract of white-purple varieties (wild type).

Conclusions and Recommendations

The callus derived from two varieties of *O. aristatus* contain rosmarinic acid and sinensetin. In the callus originating from N6 and SH media, rosmarinic acid spots were visible with blue fluorescence.

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Novelty Statement

This study provides new information regarding the secondary metabolite content of callus from two varieties of *O. aristatus* grown on Gamborg, N6, and SH media. So far, research on callus induction from *O. aristatus* was limited to MS media.

Author's Contribution

Fahrauk Faramayuda performed the experiment and wrote the manuscript with assistance and supervision from Prof. Sukrasno, Dr. Elfahmi, and Dr. Totik Sri Mariani.

Conflict of interest

The authors have declared no conflict of interest.

References

Abdelwahed, W., S. Falah and R. Hasan. 2020. Antiviral activity of different misai kucing extracts against herpes simplex virus type 1. Eurasia J. Biosci., 14(1): 1003-1012.

Abraika, O.S.S., I.J. Atangwho, A. Sadikun, M.Z. Asmawi and E.A. Hussain. 2012. *In vitro* activity-guided vasodilatory effect of *Orthosiphon stamineus* leaves. Exp. Integ. Med., 2(3): 255–261. https://doi.org/10.5455/jeim.080612.or.033

Adawiyah, R., B. Nur, A. Che, A. Tantowi, S. Fong and L. Suhaila. 2018. Java Tea (*Orthosiphon stamineus*) protected against osteoarthritis by mitigating inflammation and cartilage degradation: A preclinical study. Inflammopharmacology, https://doi.org/10.1007/s10787-017-0432-2

Akowuah, G., Z. Ismail, I. Norhayati, A. Sadikun and K. Mohd. 2004. Sinensetin, eupatorin, 3′-hydroxy-5, 6, 7, 4′-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia. Food Chem., 87: 559–566. https://doi.org/10.1016/j.foodchem.2004.01.008

Ali, H., S.A. Karsani, R. Othman and J.S. Yaacob. 2017. Production of coloured callus in *Orthosiphon stamineus* Benth and antioxidant properties of the extracted pigments. Pigment Resin Technol., https://doi.org/10.1108/PRT-01-2017-0009

Almatar, M., Z. Rahmat and F. Salleh. 2013. Preliminary morphological and anatomical study of *Orthosiphon stamineus*. Indian J. Pharm. Biol. Res. 1: 1–6. https://doi.org/10.30750/ijpbr.1.4.1

Alshawsh, M., M. Abdulla, S. Ismail, Z. Amin, S. Qader, A.H. Hadi and N. Harmal. 2012. Free radical scavenging, antimicrobial and immunomodulatory activities of *Orthosiphon stamineus*. Mol. (Basel, Switzerland). 17: 5385–5395. https://doi.org/10.3390/molecules17055385

Anggraeni, T., 1992. Kandungan utama daun kumis kucing. Prosiding Forum Komunikasi Ilmiah, Pusat Penelitian dan Pengembangan Tanaman Industri., Bogor.

Arafat, O.M., S.Y. Tham, A. Sadikun, I. Zhari, P.J. Haughton and M.Z. Asmawi. 2008. Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats. J. Ethnopharmacol., 118(3): 354–360. https://doi.org/10.1016/j.jep.2008.04.015

Awale, S., Y. Tezuka, A.H. Banskota, K. Kouda, M.T. Kyaw and S. Kadota. 2002. Four highly oxygenated isopimarane-type diterpenes of *Orthosiphon stamineus*. Planta Med., 68(3): 286–288. https://doi.org/10.1055/s-2002-23137

Batubara, I., K. Komariah, A. Sandrawati and W. Nurcholis. 2020. Genotype selection for phytochemical content and pharmacological activities in ethanol extracts of fifteen types of *Orthosiphon aristatus* (Blume) Miq. leaves using chemometric analysis. Sci. Rep., 10(1): 1–11. https://doi.org/10.1038/s41598-020-77991-2

Bordbar, L., S. Subramaniam, N.B. Jelodar and L.K. Chan. 2015. Effects of abiotic factors on cell biomass and rosmarinic acid production in cell suspension cultures of *Orthosiphon stamineus*

benth. Emir. J. Food Agric., 27(10): 756–762. https://doi.org/10.9755/ejfa.2015-04-018

Craciun, M., G. Cretu, M. Miricioiu, A. Birloiu, D-D. Clej and A. Nechifor. 2014. Identification, separation and quantification of rosmarinic acid from extract of Orthosiphon by HPTLC. Rev. Chimie., 65: 621–626.

Efferth, T., 2019. Biotechnology applications of plant callus cultures. Engineering, 5(1): 50–59. https://doi.org/10.1016/j.eng.2018.11.006

Faramayuda, F., T.S. Mariani, Elfahmi and Sukrasno. 2020. Short Communication: Callus induction in purple and white-purple varieties of *Orthosiphon aristatus* (Blume) Miq. Biodiversitas, 21(10): 4967–4972. https://doi.org/10.13057/biodiv/d211063

Faramayuda, F., Mariani TS., Elfahmi and Sukrasno. 2021a. Phytochemical analysis of callus two varieties *Orthosiphon aristatus* (Blume) Miq on Murashige And Skoog media : A strategic step of secondary metabolite production. Int. J. Appl. Pharm., 13: 71-77. http://dx.doi.org/10.22159/ijap.2021.v13s2.14

Faramayuda, F., Mariani, T. S., Elfahmi And Sukrasno. 2021b. Potential of *Orthosiphon aristatus* Blume Miq as Antiviral : A review. Trop. J. Nat. Prod. Res., 5(3): 410-419. http://dx.doi.org/org/10.26538/tjnpr/v5i3.1

Febjislami, S., A. Kurniawati, M. Melati and Y. Wahyu. 2019. Morphological characters, flowering and seed germination of the Indonesian medicinal plant *Orthosiphon aristatus*. Biodiversitas, 20: 328–337. https://doi.org/10.13057/biodiv/d200204

Febjislami, S., M. Melati, A. Kurniawati and Y. Wahyu. 2019. Karakter Agronomi dan Kadar Sinensetin Beberapa Aksesi Tanaman Kumis Kucing (*Orthosiphon stamineus*). J. Hortic. Indonesia, 9(3): 206–215. https://doi.org/10.29244/jhi.9.3.206-215

Friedman, T., 2015. The effect of rosmarinic acid on immunological and neurological systems: A basic science and clinical review. J. Rest. Med., 4: 50–59. https://doi.org/10.14200/jrm.2015.4.0105

George, A., S. Chinnappan, Y. Choudhary, V.K. Choudhary, P. Bommu and H.J. Wong. 2015. Effects of a proprietary standardized *Orthosiphon stamineus* ethanolic leaf extract on enhancing memory in sprague dawley rats possibly via blockade of adenosine A 2A Receptors. Evid. Based Complemen. Altern. Med., https://doi.org/10.1155/2015/375837

Guo, Z., B. Li, J. Gu, P. Zhu, F. Su, R. Bai, X. Liang and Y. Xie. 2019. Simultaneous quantification and pharmacokinetic study of nine bioactive components of *Orthosiphon stamineus* Benth. Extract in Rat Plasma by UHPLC-MS/MS. Molecules (Basel, Switzerland). 24(17). https://doi.org/10.3390/molecules24173057

Halim, N.H., N. Pauzi, S.H.R. Hamil, A. Shafaei, Z. Ismail and K.S. Mohd. 2017. Standardization of *Orthoshiphon stamineus* raw material and extracts for anti-uterine. fibroid. Int. J. Pharm. Phytochem. Res., 9(4): 512–515.

Hao, H., C. Lei, Q. Dong, Y. Shen, J. Chi, https://doi.org/10.25258/phyto.v9i4.8123 H. Ye and H. Wang. 2014. Effects of exogenous methyl jasmonate on the biosynthesis of shikonin derivatives in callus tissues of arnebia euchroma. Appl. Biochem. Biotechnol., 173. https://doi.org/10.1007/s12010-014-1025-9

Harun, N.H., A.W. Septama and I. Jantan. 2015. Immunomodulatory effects of selected Malaysian plants on the CD18/11a expression and phagocytosis activities of leukocytes. Asian Pac. J. Trop. Biomed., 5(1): 48–53. https://doi.org/10.1016/S2221-1691(15)30170-2

Ho, C., I. Noryati, S. Sulaiman and A. Rosma. 2010. In vitro antibacterial and antioxidant activities of *Orthosiphon stamineus* Benth . extracts against food-borne bacteria. Food Chem., 122(4): 1168–1172. https://doi.org/10.1016/j.foodchem.2010.03.110

Hossain, M.A., Z. Ismail, A. Rahman and S.C. Kang. 2008. Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth. Ind. Crops Prod., 27(3): 328–334. https://doi.org/10.1016/j.indcrop.2007.11.008

Hsieh, C.F., J.R. Jheng, G.H. Lin, Y.L. Chen, J.Y. Ho, C.J. Liu, K.Y. Hsu, Y.S. Chen, Y.F. Chan, H.M. Yu, P.W. Hsieh, J.H. Chern and J.T. Horng. 2020. Rosmarinic acid exhibits broad anti-enterovirus A71 activity by inhibiting the interaction between the five-fold axis of capsid VP1 and cognate sulfated receptors. Emerg. Microb. Infect., 9(1): 1194–1205. https://doi.org/10.1080/22221751.2020.1767512

Ionkova, I., P. Sasheva, T. Ionkov and G. Momekov. 2013. Linum narbonense: A new valuable tool for biotechnological production of a potent

anticancer lignan Justicidine B. Pharm. Mag., 9: 39–44. https://doi.org/10.4103/0973-1296.108138

Kar, B., M. Choo, U.P. Kundap, Y. Kumari, S. Hue, I. Othman and M.F. Shaikh. 2018. *Orthosiphon stamineus* leaf extract affects TNF- α and seizures in a zebrafish model. Front. Pharmacol., 9. https://doi.org/10.3389/fphar.2018.00139

Keng, C.L. and L.P. Siong. 2006. Morphological similarities and differences between the two varieties of cat's whiskers (*Orthosiphon stamineus* Benth.) grown in Malaysia. Int. J. Bot., https://doi.org/10.3923/ijb.2006.1.6

Kim, H.K., J. Lee, J.S. Lee, Y.M. Park and T. Yoon. 2008. Rosmarinic acid down-regulates the LPS-induced production of monocyte chemoattractant protein-1 (MCP-1) and Macrophage Inflammatory Protein-1 alpha (MIP-1 alpha) via the MAPK Pathway in Bone-Marrow Derived Dendritic Cells. Mol. Cells, 26: 583–589.

Lee, W.-L. and L. Chan. 2004. Plant regeneration from stem nodal segments of *Orthosiphon stamineus* Benth., a medicinal plant with diuretic activity. *In Vitro* Cell. Dev. Biol. Plant, 40: 115–118. https://doi.org/10.1079/IVP2003500

Lee, W., 2004. Micropropagation and cell culture of misai kucing (*Orthosiphon stamineus* Benth.) and detection of rosmarinic acid in the *In vitro* Cultures. Thesis. Universiti Sains Malaysia.

Li, J., X. Jie, X. Liang, Z. Chen, P. Xie, X. Pan, B. Zhou and J. Li. 2020. Sinensetin suppresses influenza a virus-triggered inflammation through inhibition of NF-κB and MAPKs signalings. BMC Complement. Med. Ther., 20(1): 135. https://doi.org/10.1186/s12906-020-02918-3

Lin, W.-Y., Y.J. Yu and T.R. Jinn. 2019. Evaluation of the virucidal effects of rosmarinic acid against enterovirus 71 infection via *in vitro* and *in vivo* study. Virol. J., 16(1): 94. https://doi.org/10.1186/s12985-019-1203-z

Maheswari, C., R. Maryammal and R. Venkatanarayanan. 2008. Hepatoprotective activity of "*Orthosiphon stamineus*" on liver damage caused by paracetamol in rats. Jordan J. Biol. Sci., pp. 1.

Malterud, K.F., I.M. Hanche-olsen and I. Smith-kielland. 1989. Flavonoids from *Orthosiphon spicatus* A new flavanone derivative from the leaves of T*suga diversfolia*. Planta Med., 55(6): 569-570. https://doi.org/10.1055/s-2006-962099

Matsubara, T., T. Bohgaki, M. Watarai, H. Suzuki, K. Ohashi and H. Shibuya. 1999. Antihypertensive actions of Methylripariochromene A from *Orthosiphon aristatus*. An Indonesian Traditional Medicinal Plant. Biol. Pharma. Bull., 22: 1083–1088. https://doi.org/10.1248/bpb.22.1083

Mohamed, E., A.J. Mohamed, M. Abdullah, A. Sadikun, O.S. Ebrika and M. Yam. 2011. Antihyperglycemic effect of *Orthosiphon stamineus* Benth leaves extract and its bioassay-guided fractions. Mol. (Basel, Switzerland). 16: 3787–3801. https://doi.org/10.3390/molecules16053787

Nandagopal, K., M. Halder, B. Dash, S. Nayak and S. Jha. 2017. Biotechnological approaches for production of anti-cancerous compounds resveratrol, podophyllotoxin and zerumbone. Curr. Med. Chem., pp. 24. https://doi.org/10.2174/0929867324666170404145656

Nuengchamnong, N., K. Krittasilp and K. Ingkaninan. 2011. Characterisation of phenolic antioxidants in aqueous extract of Orthosiphon grandiflorus tea by LC-ESI-MS/MS coupled to DPPH assay. Food Chem., 127(3): 1287–1293. https://doi.org/10.1016/j.foodchem.2011.01.085

Pauzi, N., K.S. Mohd, N. Hidayah and A. Halim. 2018. *Orthosiphon stamineus* extracts inhibits proliferation and induces apoptosis in uterine fibroid cells. Asian Pac. J. Cancer Prev., 19: 2737–2744.

Ripim, M., N. Fazil, K. Ibrahim, A. Bahtiar, C. Wai, N. Ibrahim and M. Nor. 2018. Antiviral properties of *Orthosiphon stamineus* aqueous extract in herpes simplex virus type 1 infected cells. Sains Malaysiana. 47(8): 1725–1730. https://doi.org/10.17576/jsm-2018-4708-11

Sanbongi, C., H. Takano, N. Osakabe, N. Sasa, M. Natsume, R. Yanagisawa, K. Inoue, K. Sadakane, T. Ichinose and T. Yoshikawa. 2004. Rosmarinic acid in perilla extract inhibits allergic inflammation induced by mite allergen, in a mouse model, clinical and experimental allergy. J. Br. Soc. Allerg. Clin. Immunol., 34: 971–977. https://doi.org/10.1111/j.1365-2222.2004.01979.x

Sarfaraj, M., S. Fareed, S. Ansari, M. Rahman, I. Ahmad and M. Saeed. 2012. Current approaches toward production of secondary

plant metabolites. J. Pharm. Bioall. Sci., 4: 10–20. https://doi.org/10.4103/0975-7406.92725

Sarkar, K., and R. Das. 2020. Preliminary identification of hamamelitannin and rosmarinic acid as covid-19 inhibitors based on molecular docking. Lett. Drug Design Dis., pp. 17. https://doi.org/10.2174/1570180817999200802032126

Sheena, E.V. and G.J. Jothi. 2015. *In vitro* propagation of *Orthosiphon stamineus* Benth (Lamiaceae) an important medicinal plant using nodal and leaf explants. Pharma Innov. J., 4(7): 06-10.

Takano, H., N. Osakabe, C. Sanbongi, R. Yanagisawa, K. Inoue, A. Yasuda, M. Natsume, S. Baba, E. Ichiishi and T. Yoshikawa. 2004. Extract of perilla *Frutescens enriched* for rosmarinic acid, a polyphenolic phytochemical, inhibits seasonal allergic rhinoconjunctivitis in humans. Exp. Biol. Med., 229: 247–254. https://doi.org/10.1177/153537020422900305

Takeda, Y., T. Matsumoto, H. Terao, T. Shingu, Y. Futatsuishi, T. Nohara and T. Kajimoto. 1993. Orthosiphol D and E, minor diterpenes from *Orthosiphon stamineus*. Phytochemistry, 33(2): 411–415. https://doi.org/10.1016/0031-9422(93)85530-5

Wai-leng, L. and C. Lai-keng. 2004. Establishment of *Orthosiphon stamineus* cell suspension culture for cell growth. Plant Cell, Tissue and Organ Culture. (January), pp. 101–106. https://doi.org/10.1023/B:TICU.0000022533.83592.37

Walker, T., H. Bais and J. Vivanco. 2002. Jasmonic acid-induced hypericin production in cell suspension cultures of *Hypericum perforatum* L. (St. John's wort). Phytochemistry, 60: 289–293. https://doi.org/10.1016/S0031-9422(02)00074-2

Wondmkun, Y.T. and O.A. Mohammed. 2020. Severe acute respiratory syndrome-coronavirus-2 (SARS-COV-2) Inhibition and other antiviral effects of ethiopian medicinal plants and their compounds traditional medicines for COVID-19 Treatment. iMedPub J., 19(2019): 1–7.

Woottisin, S., R.Z. Hossain, C. Yachantha, P. Sriboonlue, Y. Ogawa and S. Saito. 2011. Effects of *Orthosiphon grandiflorus*, *Hibiscus sabdariffa* and *Phyllanthus amarus* extracts on risk factors for urinary calcium oxalate stones in rats. J. Urol., 185(1): 323–328. https://doi.org/10.1016/j.juro.2010.09.003

Yam, M., R. Basir, M. Abdullah and Z. Ismail. 2007. Antioxidant and hepatoprotective effects of *Orthosiphon stamineus* Benth. Am. J. Chinese Med., 35: 115–126. https://doi.org/10.1142/S0192415X07004679

Youn, J., K. Lee, J. Won, S. Huh, H. Yun and W. Cho. 2003. Beneficial effects of rosmarinic acid on suppression of collagen induced arthritis. J. Rheumatol., 30(6): 1203-1207.

Yuniarto, A.R.I., E. Susilawati, I. Khairunnisa, D. Juanda and F. Setiawan. 2017. Antioxidant and gastric ulcer healing effect of *Orthosiphon stamineus* (Benth.) leaves extract in aspirin-induced rats. Asian J. Pharm. Clin. Res., 10(2): 2–4. https://doi.org/10.22159/ajpcr.2017.v10i2.15873