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LC-MS/MS analysis and antioxidant activity of ethanol fraction of *Aglaonema modestum* leaves

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Abstract. *Silaban S, Nainggolan B, Ikhwan J, Harliananda N, Simorangkir M. 2024. LC-MS/MS analysis and antioxidant activity of ethanol fraction of* Aglaonema modestum *leaves. Biodiversitas 25: 4756-4762.* Free radicals are one of the triggers of various diseases. Excessive free radicals have the potential to cause oxidative stress, such as diabetes, heart disease, Alzheimer's, and Parkinson's disease. Antioxidants can be used to prevent free radicals. Synthetic antioxidants have unnecessary side effects, and natural antioxidants can be a safer alternative. Natural antioxidants can come from plants, which contain phenolic compounds in the form of flavonoids. *Aglaonema modestum*, with the local name simargaolgaol, contains this compound. This plant is often found in Barus, North Sumatra, Indonesia, and is usually used by the community as traditional medicine for internal and external wounds. This study aims to identify the potential antioxidant activity of the leaves ethanol fractions of *A. modestum*. Purification was done using the standard column chromatography method, antioxidant activity analysis using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method with vitamin C as a control, and fraction compound analysis using LC-MS/MS. Antioxidant activity value (IC_{50}) of ethanol fraction of *A. modestum* consecutively at 30.953 ppm (very strong), 109.809 ppm (moderate), and vitamin C with an IC_{50} value of 2.304 ppm (very strong). The results of LC-MS/MS analysis of the ethanol fraction identified three compounds, namely Cadsurenin K (RT 5.91 minutes), Methyl ophiopogonone B (RT 6.37 minutes), and Schizandrin C (RT 7.30 minutes). The ethanol fraction of *A. modestum* leaves has alternative potential as a natural antioxidant.

Keywords: *A. modestum*, antioxidant, chromatography, DPPH, LC-MS/MS

INTRODUCTION

Oxidative stress occurs due to high levels of free radicals in the body. Free radicals are molecular fragments with unpaired electrons in their outer orbit. They result from a process called homolytic cleavage, which is the breaking of a chemical bond in a way that each fragment retains one of the shared electrons, leading to the formation of two free radicals (Karundeng and Aloanis 2018). Damage due to free radical oxidation in living cells causes chronic diseases such as Parkinson's disease (Khan et al. 2018), Alzheimer's disease (Peña-Bautista et al. 2019), diabetes mellitus (Sharifi-Rad et al. 2018; D'Oria et al. 2020), cancer (Liskova et al. 2020), aging (Chaudhary et al. 2023; Jomova et al. 2023), heart attacks (Caiati et al. 2023), cardiovascular disease (Alfei et al. 2024), cataracts, chronic obstructive pulmonary disease (Suryadinata 2018), inflammation, and several other diseases (Nurcholis et al. 2021; Maigoda et al. 2022). Free radicals are molecular fragments that contain one or more unpaired electrons in their molecular orbitals, so they are highly reactive and can oxidize the surrounding molecules (Martemucci et al. 2022). Free radicals, both the reactive nitrogen species (RNS) and reactive oxygen species (ROS), are the most widely known types of free radicals (Gurning and Haryadi 2022). ROS are very reactive because they are unstable (have unpaired electrons) (Tian et al. 2017; Menezes et al. 2024). Free radicals can interfere with the production of DNA and affect blood vessels, prostaglandins, and other proteins such as enzymes in the body (Martemucci et al. 2022). Free radicals that take electrons from DNA can cause changes in the structure of DNA so that mutant cells arise. If this mutation occurs for a long time, it can cause cancer.

Substances to reduce the negative impact of free radicals include the use of antioxidants. One way to reduce the harmful effects of free radicals is to use antioxidants. Antioxidants are compounds that can bind free radicals and highly reactive molecules, so they can inhibit the oxidation of molecules that produce free radicals and inhibit the occurrence of free radical formation reactions (Noviarni et al. 2020). Antioxidants are needed as a free radical antidote and to prevent oxidative stress. Antioxidants delay or inhibit molecules' oxidation process (Vona et al. 2021; Sinaga et al. 2022). Antioxidants are easily oxidized, so free radicals will oxidize antioxidants, thus protecting other molecules in the cell from damage due to oxidation by reactive oxygen or free radicals. Antioxidants, based on their source, are grouped into endogenous antioxidants, namely enzymes, and exogenous antioxidants, namely antioxidants obtained from outside the body/food (Ayoka et al. 2022). Naturally, the human body can produce antioxidants to balance the number of incoming oxidants in the body, but because the amounts of incoming oxidants exceed the limit

abilities that can be accepted by natural antioxidants in the body, which are necessary for other antioxidants that come from outside. The human body can neutralize free radicals when their amounts are not excessive, with endogenous antioxidant defense mechanisms. When endogenous antioxidants are insufficient, the body needs antioxidants from the outside (Simorangkir et al. 2019a; Simorangkir et al. 2019b).

Antioxidants from outside the body can be obtained in synthetic form or from natural ingredients. Widely used synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butyl hydroquinone (TBHQ) are believed to be effective in inhibiting oxidation. However, the use of synthetic antioxidants is limited by government regulations because use that exceeds the limit can cause toxins in the body and is carcinogenic, so alternative antioxidants that are safe to use are needed. One potential source of natural antioxidants is plants (Ji et al. 2023; Saragih et al. 2023). Using natural antioxidants has become an alternative with fewer negative effects than synthetic antioxidants. Natural antioxidants from plants are generally phenolic compounds such as flavonoids (Shen et al. 2022).

One of the plants that can act as an antioxidant is *Aglaonema modestum* (Figure 1). *A. modestum* leaf extract is reported to contain secondary metabolites, including alkaloids, flavonoids, saponins, steroids, and tannins (Zega et al. 2021; Silaban et al. 2022). Other species under Aglaonema have been reported (Arnob 2014). This study aims to extract and purify ethanol extract of *A. modestum* leaves using column chromatography, antioxidant activity analysis with the DPPH method, and analysis of the ethanol fraction using LC-MS/MS.

MATERIALS AND METHODS

Materials

The tools used are a vacuum rotary evaporator (Heidolph), micropipettes, a set of chromatography column tools, UV lamps, capillary pipes, UV-VIS spectrophotometers (Spectroquant Pharo 300 Merck), and LC-MS/MS, Xevo G2-XS QTOF (Waters Corporation, Milford, USA). Next, 2,2-Diphenyl-1-picrylhydrazyl (Sigma-Aldrich), 96% ethanol (Merck), *n*-hexane (Merck), technical ethyl acetate (Merck), aquades, vitamin C (Sigma-Aldrich), 99.8% methanol (Merck), silica gel (Merck), silica gel $G_{60}F_{254}$ (Merck).

Preparation of DPPH 0.1 mM stock solution

The 0.1 mM DPPH stock is made by weighing 3,9 mg DPPH (molecular weight 394.32 g/mol) and dissolved in a 100 mL measuring flask using methanol to the limit mark. The flask is then covered with aluminum foil. The preparation of a 0.1 mM DPPH stock solution is carried out in a dark place.

Vitamin C standard stock solution

A standard 1,000 ppm solution of vitamin C is prepared by weighing 10 mg of vitamin C powder and dissolved in a 10 mL measuring flask using methanol to the limit. The solution is pipetted at 20, 40, 80, 160, and 320 ppm to obtain concentrations of 2, 4, 8, 16, and 32 ppm.

Preparation and extraction

Following previous research with minor modifications, we made the stock solution of *A. modestum* leaf ethanol extract by weighing 10 mg of extract, dissolving it in a 10 mL measuring flask, and then adding methanol to the boundary mark (Silaban et al. 2022). Stock solutions are picked up by 125, 250, 500, 1,000, and 2,000 µL. Each concentration is put into a 10 mL measuring flask, and methanol is added to the limit mark. This method is also carried out in the manufacture of fractionated stock solutions.

Antioxidant activity test of *A. modestum* leaves ethanol extract

The antioxidant activity of *A. modestum* leaves ethanol extract was tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Soltanzadeh et al. 2018; Situmorang et al. 2022). The concentrations of each test solution are 12.5, 25, 50, 100, and 200 ppm. Each concentration was pipetted as much as 2.5 mL into the test tube and then added 2.5 mL of 0.1 mM DPPH solution. The same way is carried out in the solution of fractionation results. Blank solutions are made without a solution of ethanol extract of *A. modestum* leaves. Analysis was carried out in a dark place and incubated for 30 minutes. Furthermore, the absorption process uses a UV-Vis spectrophotometer at a wavelength of 517 nm (Lu and Foo 2000; Juwitaningsih et al. 2022).

Fractionation of *A. modestum* leaves ethanol extract

Preliminary analysis of spot stains with thin-layer chromatography was performed by weighing 0.1 g of leaf extract and then dissolving it with 1 mL of ethanol solvent. Furthermore, the sample was placed on the silica gel plate $G_{60}F_{254}$, and placed in a chamber containing 5 mL of eluent which had been saturated with the ratio of eluents, namely *n*-hexane and ethyl acetate with volume variations of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9, ethyl acetate and ethanol with volume variations of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 respectively. The $G_{60}F_{254}$ silica gel plate is then removed from the chamber, dredged, irradiated, and irradiated under an ultraviolet lamp at a wavelength of 254 nm.

Figure 1. Simargaolgaol plant (*Aglaonema modestum*)

The purification process was carried out using silica gel 60 with column chromatography with a length of 20 cm and a diameter of 2 cm (Gurning et al. 2021a). 30 g of silica gel was activated in the oven at 100℃ for 4 hours, then cooled in a vacuum desiccator. Then, the silica gel is made into a slurry by dissolving it with *n*-hexane and putting it into the column gradually. The silica slurry that has been inserted into the column is mandated first to ensure that no air bubbles are scattered in the silica gel in the column. A sample of 1 g was dissolved with 4 mL of the mobile phase and then inserted into the column, then flowed *n*-hexane eluent: ethyl acetate in a ratio of 4:6. The rate of motion phase is set at 3 mL/2 min past the column accommodated on the vial bottle with a volume of every 5 mL. The eluate obtained was monitored using the pattern of stains produced on thin-layer chromatography plates. It was then combined based on the similarity of the stain pattern, and the solvent was evaporated using a rotary evaporator. The separation results were tested for purity with a thin layer chromatography plate with various motion phase mixtures, namely *n*-hexane:ethyl acetate and ethanol:*n*-hexane:ethyl acetate with variations in ratio of 6:4, 7:3, 2:8, 5:5, ethyl acetate, and ethanol with variations in the ratio of 2:8, 8:2, *n*-hexane, and ethyl acetate and ethanol in a ratio of 2:4:2.

Analysis of bioactive compounds using the LC-MS/MS instrument

The structure of the ethanol fraction was determined using LC-MS/MS analysis equipped with an electrospray ionization source coupled with Ultra Performance analysis performed using Waters Acquity. An inverted-phase HSS T3 C-18 column (2.1 × 100 mm, particle size 1.8 µm) was used and maintained at 40ºC. The motion phase consisted of A (0.1% formic acid in water) and B (acetonitrile in 0.1% formic acid). Gradient elution was performed at a flow rate of 0.3 mL/min with an injection volume of 1 µL. Gradients are as follows: 5% B (0-8 min), 40% B (8-11 min), and 100% B (11-16 min). Data range from 50-1,200 m/z. All LC-MS/MS data is processed, topped, and analyzed using UNIFI's informatics platform. The intensity of each ion produces a matrix consisting of m/z values, retention time (TR), and peak area. Variables of interest are then identified using UNIFI software (Vreeke et al. 2022).

RESULTS AND DISCUSSION

Antioxidant activity analysis

The results obtained showed a decrease in the absorbance value of DPPH, which was directly proportional to the increase in concentration. Antioxidant activity test (IC_{50}) with a variation in concentration range (12.5-200 ppm) of *A. modestum* leaves ethanol extract of 30.953 ppm (Table 1) are particularly noteworthy. The antioxidant activity of Vitamin C is determined by making concentration variations in the range of 2-32 ppm. The results showed that there was a reduction in the absorbance value of DPPH, which was directly proportional to the increase in the concentration of the solution, which showed a stronger free radical damping ability. The antioxidant activity value (IC_{50}) of vitamin C was 2.304 ppm (Table 1). The fractionation results obtained were tested for antioxidant activity using the same method. Measurement of antioxidant activity of ethanol fraction from *A. modestum* leaves with a concentration interval (12.5-200 ppm) obtained an antioxidant activity value (IC_{50}) of 109.809 ppm (Table 1). The key finding here is that the antioxidant activity of *A. modetum* leaves in the ethanol fraction showed lower activity when compared to vitamin C.

Table 1. Results of antioxidant activity test with the DPPH method

Types of solutions	Concentration (ppm)	Absorbance	%Inhibition	IC_{50} (ppm)	Antioxidant categories
Ethanol extract	12.5	0.495	33.956	30.953	Very strong
	25	0.421	43.747		
	50	0.314	58.077		
	100	0.196	73.787		
	200	0.082	89.008		
Ethanol fraction	12.5	0.714	4.673	109.809	Moderate
	25	0.609	18.736		
	50	0.491	34.490		
	100	0.373	50.245		
	200	0.295	60.614		
Vitamin C	2	0.454	39.430	2.304	Very strong
	4	0.252	66.400		
	8	0.125	83.311		
	16	0.055	92.701		
	32	0.005	99.288		

Based on the results of the IC_{50} value obtained, it is known that the ethanol extract of the leaves of *A. modestum* shows a very strong activity as an antioxidant. Ethanol fraction results of *A. modestum* leaves showed the ability of antioxidant activity in a moderate category, and vitamin C showed antioxidant activity in a very strong category. The ability of antioxidant activity of *A. modestum* leaves from ethanol fraction showed lower activity when compared to vitamin C. Nevertheless, the antioxidant activity of leaves ethanol extract *A. modestum* has a very active antioxidant power, so it can be used as a source of natural antioxidants. The ability of very strong antioxidant activity (IC_{50} < 50 ppm) of the ethanol extract of the leaves of *A. modestum* is due to the abundant content of secondary metabolites. Based on the antioxidant activity value (IC_{50}) produced, the strength of the activity is divided into several categories, including IC_{50} < 50 ug/mL in the very strong category, 50 < IC_{50} < 100 in the strong category, 100 < IC_{50} < 250 in the moderate category, 250 < IC_{50} < 500 in the weak category, and IC_{50} > 500 in the inactive category as an antioxidant (Herawati et al. 2021; Sinaga et al. 2023).

The antioxidant activity test of the ethanol fraction from the leaves of *A. modestum* and vitamin C as a control was carried out using the DPPH method, which was measured at a maximum wavelength of 517 nm using UV-vis spectrometry (Gurning et al. 2021b). This method is the most common and has been used for a long time to determine antioxidant activity because it has a high sensitivity for samples with small concentrations and a relatively fast measurement time (Novriyanti et al. 2022). In addition to vitamin C, another compound often used to compare the antioxidant activity of natural products is quercetin and its derivatives (Hassanpour and Doroudi 2023). However, the availability of vitamin C and the relatively lower price compared to quercetin made vitamin C a positive control standard solution in this study.

The potent antioxidant activity of the crude ethanol extract of *A. modestum* leaves is influenced by the content of active compound groups that abound in the extract. The content of the class of bioactive compounds shows synergistic activity in providing antioxidant activity so the potential antioxidant activity in the crude ethanol extract of *A. modestum* leaves shows better activity than the ethanol fraction. If the various content contained in the crude ethanol extract of *A. modestum* leaves is antagonistic, it will weaken its potential as an antioxidant (Prieto et al. 2014; Simorangkir et al. 2022).

Defense mechanisms against oxidants are divided into 3 types, namely primary, secondary, and tertiary. The primary defense mechanism works through the principle of neutralizing free radicals, namely by giving one electron to reactive molecules. Examples of these antioxidants are tocopherol, ascorbic acid, and flavonoids. The secondary defense mechanism works by binding metals and removing transition metals that can trigger free radicals. Examples of these antioxidants are albumin and transferrin. The tertiary defense mechanism works by preventing the buildup of biomolecules from causing further damage. Examples include the repair of damaged DNA by the enzyme methionine reductase and oxidized proteins by proteolytic enzymes (Wulansari 2018).

Fractionation of ethanol extract

The results of determining the eluent with thin-layer chromatography obtained the best eluent used as the phase of motion, namely the ratio between *n*-hexane solvents: ethyl acetate (4:6). Eluent with this scaling produces five stain spots (Figure 2). Furthermore, separation using gravity column chromatography with silica gel $G_{60}F_{254}$ yielded 73 bottles of eluate. Each eluate contained in a vial bottle is monitored for a given stain spot so that a fraction with an isolate weight of 0.1 g is obtained. The purity of the fractions was monitored using thin-layer chromatography with various eluent mixtures as mobile phases, namely *n*-hexane, ethyl acetate, and ethanol. The eluent ratio of *n*-hexane: ethyl acetate with variations of 6:4, 7:3, 2:8, 5:5; ethyl acetate: ethanol with variations of 2:8, 8:2; and *n*-hexane: ethyl acetate: ethanol with a ratio of 2:4:2 were applied.

The results obtained from monitoring on thin-layer chromatography plates with various types and ratios of eluents showed there is one spot in the stain (Figure 3). The spot of the stain obtained is still elongated, indicating that qualitatively, the active compound obtained is not yet in its pure state. This occurs due to less effective separation in the column. In the stationary phase, as much as 25 g will provide better separation in columns 1 cm in diameter than in columns with a diameter of 2 cm.

Meanwhile, the ethanol fraction of *A. modestum* leaves, a key focus of this antioxidant research, demonstrated a moderate IC_{50} in the results of column chromatography purification. This moderate antioxidant ability is likely due to the limited content of active compounds resulting from purification. However, it's important to note that antagonistic interactions may occur. The interaction of metabolite compounds present in natural materials can be synergistic or antagonistic in providing pharmacological activity. The interaction between chemical components is synergistic and enhances antioxidant activity but weakens after separation.

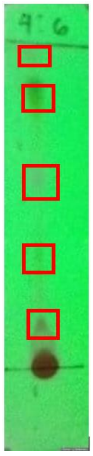

Figure 2. Propyl separation of ethanol extract of *Aglaonema modestum* leaves using *n*-hexane:ethyl acetate (4:6) solvents

Analysis of bioactive compounds using the LC-MS/MS instrument

Thin layer chromatography aims to determine which eluent is used as the mobile phase in eluting and to provide good separation between the components of the compound from the mixture (Dongare et al. 2023; Radoičić et al. 2024). Besides, the size of the column diameter and the amount of silica gel stationary phase used in column chromatography significantly affect the separation effectiveness in the column. It's been found that the smaller the diameter and the higher the stationary phase, the better the separation (Kondeti et al. 2014).

The results of the ethanol fraction analysis using LC-MS/MS yielded several chromatogram peaks with varying retention times (RT). LC-MS/MS results obtained three peaks, namely (1) retention time (RT) 5.91 minutes having a molecular weight of 343.153 g/mol (89.57%) with the molecular formula $C_{20}H_{22}O_5$ (Cadsurenin K compound) which is a derivative of neolignane group (Figure 4); (2) retention time (RT) 6.37 minutes has a molecular weight of 327.12 g/mol (2.49%) with the molecular formula $C_{19}H_{18}O_5$ (Methyl ophiopogonone B compound) which is a derivative of the homoisoflavanone compound group (Figure 5); and (3) retention time (RT) 7.30 minutes has a molecular weight of 384.16 g/mol (7.94%) with the formula $C_{22}H_{24}O_6$ g/mol (Schizandrin C compound) which is a derivative of tannin group compounds (Figure 6).

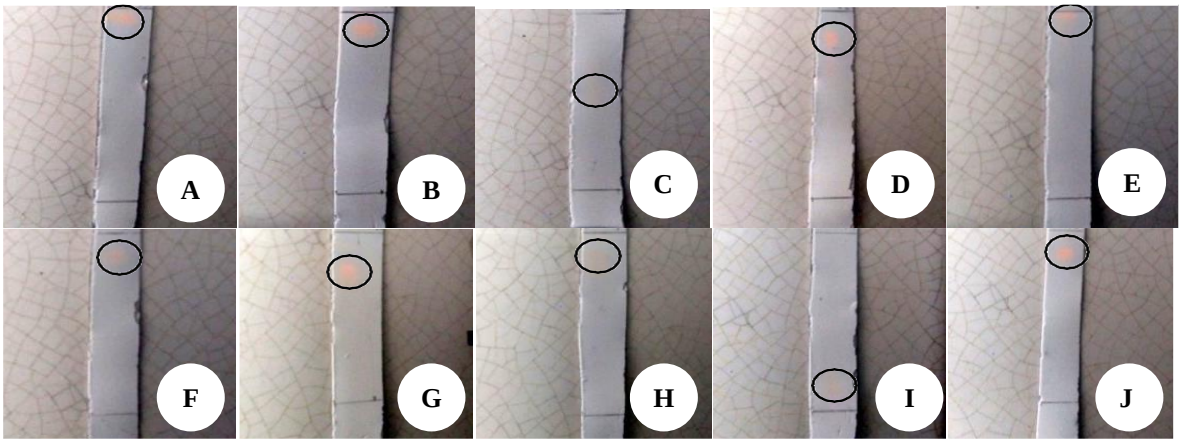

Figure 3. Results of thin layer chromatography purification with various types and ratios of eluents. A. *n*-Hexane; B. Ethyl acetate; C. Ethanol; D. *n*-Hexane:Ethyl acetate (6:4); E. *n*-Hexane:Ethyl acetate (7:3); F. *n*-Hexane:Ethyl acetate (2:8); G. *n*-Hexane:Ethyl acetate (5:5); H. Ethyl acetate:Ethanol (2:8); I. Ethyl acetate:Ethanol (8 :2); J. *n*-Hexane:Ethyl acetate:Ethanol (2:4:2)

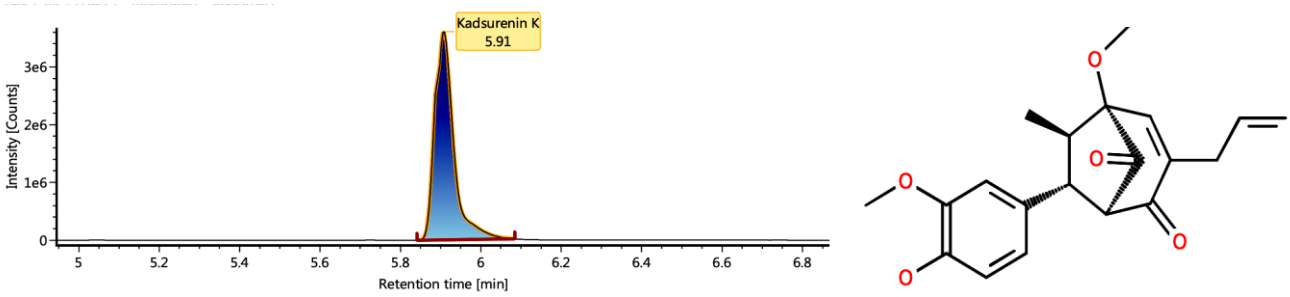

Figure 4. Results of LC-MS analysis of Cadsurenine K compounds (RT 5.91 min)

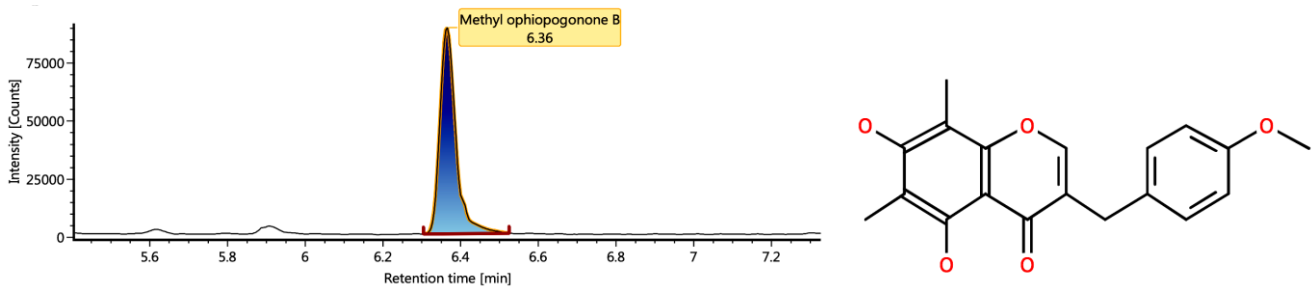

Figure 5. Results of LC-MS analysis of Methyl ophiopogonone B compound (RT 6.36 min)

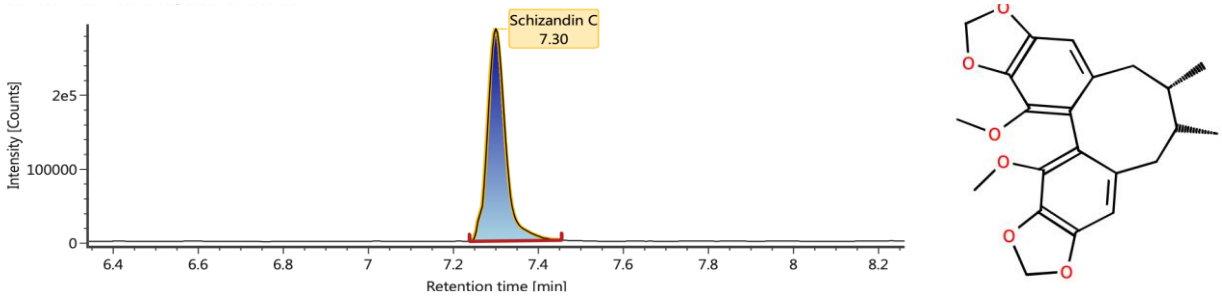

Figure 6. Results of LC-MS analysis of Schizandrin C compounds (RT 7.30 min)

In conclusion, this study's findings suggest ethanol fraction from *A. modetum* leaves from North Sumatra demonstrates potential antioxidant agents as presented by their ability to reduce the activity of free radicals with an IC_{50} value of 109.809. Monitoring of the extract showed more potent antioxidant activity with an IC_{50} value of 30.953. This is caused by the synergy between chemical components in the extract, which provides activity but weakens after separation. Further investigation is necessary to identify specific components within the active fraction of *A. modestum* that may contribute to their potential free radicals.

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REFERENCES

Alfei S, Schito GC, Schito AM, Zuccari G. 2024. Reactive oxygen species (ROS)-mediated antibacterial oxidative therapies: Available methods to generate ROS and a novel option proposal. Intl J Mol Sci 25 (13): 7182. DOI: 10.20944/preprints202405.1628.v1.

Arnob SY. 2014. Cytotoxic and Antioxidant Evaluation of Methanolic Extract of *Aglaonema hookerianum*. [Dissertation]. Department of Pharmacy East West University, Dhaka, Bangladesh.

Ayoka TO, Ezema BO, Eze CN, Nnadi CO. 2022. Antioxidants for the prevention and treatment of non-communicable diseases. J Explor Res Pharmacol 7 (3): 179-189. DOI: 10.14218/JERP.2022.00028.

Caiati C, Stanca A, Lepera ME. 2023. Free radicals and obesity-related chronic inflammation contrasted by antioxidants: A new perspective in coronary artery disease. Metabolites 13 (6): 712. DOI: 10.3390/metabo13060712

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D, Sharifi-Rad J. 2023. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Front Chem 11: 1158198. DOI: 10.3389/fchem.2023.1158198.

D'Oria R, Schipani R, Leonardini A, Natalicchio A, Perrini S, Cignarelli A, Laviola L, Giorgino F. 2020. The role of oxidative stress in cardiac disease: From physiological response to injury factor. Oxid Med Cell Longev 2020 (1): 5732956. DOI: 10.1155/2020/5732956.

Dongare MVS, Kohale NB, Rathod SB. 2023. A review of chromatograph: Principal, classification, application. Intl J Humanit Soc Sci 3 (2): 367-373.

Gurning K, Haryadi W, Sastrohamidjojo H. 2021a. Isolation and charactericzation of antioxidant compounds of *bangun-bangun* (*Coleus amboinicus* L.) leaves from Nort Sumatra, Indonesia. Rasayan J Chem 14 (1): 248-253. DOI: 10.31788/rjc.2021.1416077.

Gurning K, Haryadi W. 2022. Potential antioxidants of secondary metabolite isolates ethyl acetate fraction *Coleus amboinicus* Lour. leaves. ScienceRise: Pharm Sci 39 (5): 100-105. DOI: 10.15587/2519-4852.2022.266401.

Gurning K, Lumbangaol S, Situmorang RF, Silaban S. 2021b. Determination of phenolic contents and antioxidant activity test of ethanol extract of red betel (*Piper crocatum* Ruiz & Pav.) leaves using the DPPH method. Jurnal Pendidikan Kimia 13 (2): 137-142. DOI: 10.24114/jpkim.v13i2.26984.

Hassanpour SH, Doroudi A. 2023. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna J Phytomed 13 (5): 354. DOI: 10.22038/AJP.2023.21774.

Herawati E, Ramadhan R, Ariyani F, Marjenah, Kusuma IW, Suwinarti W, Mardji D, Amirta R, Arung ET. 2021. Phytochemical screening and antioxidant activity of wild mushrooms growing in tropical regions. Biodiversitas 22: 4716-21. DOI: 10.13057/biodiv/d221102.

Ji X, Liang J, Wang Y, Liu X, Li Y, Liu Q, Liu R. 2023. Synthetic antioxidants as contaminants of emerging concern in indoor environments: Knowns and unknowns. Environ Sci Technol 57 (51): 21550-21557. DOI: 10.1021/acs.est.3c06487.

Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. 2023. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Arch Toxicol 97 (10): 2499-2574. DOI: 10.1007/s00204-023-03562-9.

Juwitaningsih T, Roza D, Silaban S, Hermawati E, Windayani N. 2022. Phytochemical screening, antibacterial, antioxidant, and anticancer activity of coffee parasite acetone extract (*Loranthus ferrugineus* Roxb). Pharmacia 69: 1041-1046. DOI: 10.3897/pharmacia.69.e91427.

Karundeng M, Aloanis AA. 2018. Analisis pemerangkapan radikal bebas ekstrak etanol buah beringin (*Ficus benjamina* Linn.). Fullerene J Chem 3 (2): 37-39. DOI: 10.37033/fjc.v3i2.36. [Indonesian]

Khan F, Kumar Garg V, Kumar Singh A, Tinku T. 2018. Role of free radicals and certain antioxidants in the management of huntington's disease: A review. J Anal Pharm Res 7: 386-392. DOI: 10.15406/japlr.2018.07.00256

Kondeti RR, Mulpuri KS, Meruga B. 2014. Advancements in column chromatography: A review. World J Pharm Sci 2: 1375-1383.

Liskova A, Stefanicka P, Samec M, Smejkal K, Zubor P, Bielik T, Biskupska-Bodova K, Kwon TK, Danko J, Büsselberg D, Adamek M, Rodrigo L, Kruzliak P, Shleikin A, Kubatka P. 2020. Dietary phytochemicals as the potential protectors against carcinogenesis and their role in cancer chemoprevention. Clin Exp Med 20: 173-190. DOI: 10.1007/s10238-020-00611-w.

Lu Y, Foo LY. 2000. Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chem 68 (1): 81-85. DOI: 10.1016/S0308-8146(99)00167-3.

Maigoda T, Judiono J, Purkon DB, Haerussana ANEM, Mulyo GPE. 2022. Evaluation of *Peronema canescens* leaves extract: Fourier transform infrared analysis, total phenolic and flavonoid content,

antioxidant capacity, and radical scavenger activity. Open Access Maced J Med Sci 10 (A): 117-124. DOI: 10.3889/oamjms.2022.8221.

Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. 2022. Free radical properties, source and targets, antioxidant consumption and health. Oxygen 2 (2): 48-78. DOI: 10.3390/oxygen2020006.

Menezes FA, Oliveira JG, Guimarães AO. 2024. Electron paramagnetic resonance applied to free radicals and reactive oxygen species detection in plant systems. Appl Magn Reson 55 (4): 335-355. DOI: 10.1007/s00723-023-01625-9.

Noviarni I, Batubara I, Putri SP. 2020. Antiglycation and antioxidant activity from methanol extract and fraction of *Xylocarpus granatum* stem. Jurnal Kimia Sains dan Aplikasi 23 (1): 21-27. DOI: 10.14710/jksa.23.1.21-27.

Novriyanti R, Putri NEK, Rijai L. 2022. Phytochemical screening and antioxidant activity testing ethanol extract of lime skin (*Citrus aurantifolia*) using DPPH method. Proceeding Mulawarman Pharm Conf 15: 165-170. DOI: 10.25026/mpc.v15i1.637.

Nurcholis W, Putri DNSB, Husnawati H, Aisyah SI, Priosoeryanto BP. 2021. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits. Ann Agric Sci 66 (1): 58-62. DOI: 10.1016/j.aoas.2021.04.001.

Peña-Bautista C, Baquero M, Vento M, Cháfer-Pericás C. 2019. Free radicals in Alzheimer's disease: Lipid peroxidation biomarkers. Clin Chim Acta 491: 85-90. DOI: 10.1016/j.cca.2019.01.021.

Prieto MA, Murado MA, Vázquez JA. 2014. Quantification, characterization and description of synergy and antagonism in the antioxidant response. Food Res Intl 60: 218-229. DOI: 10.1016/j.foodres.2013.09.033.

Radoičić A, Šegan S, Milojković-Opsenica D. 2024. Exploring separation mechanisms and lipophilicity in hydrophilic interaction chromatography conditions by thin-layer chromatography of anesthetics and adjuvant drugs as polar model compounds. J Sep Sci 47 (12): 2400099. DOI: 10.1002/jssc.202400099.

Saragih MH, Silaban S, Eddiyanto E. 2023. The impact of temperature and antioxidants on oxidation and the formation of trans fatty acids in several palm oil derivatives. al Kimiya: Jurnal Ilmu Kimia dan Terapan 10 (2): 74-86. DOI: 10.15575/ak.v10i2.25256.

Sharifi-Rad J, Sharifi-Rad M, Salehi B, Iriti M, Roointan A, Mnayer D, Soltani-Nejad A, Afshari A. 2018. In vitro and in vivo assessment of free radical scavenging and antioxidant activities of *Veronica persica* Poir. Cell Mol Bio 64 (8): 57-64. DOI: 10.14715/cmb/2018.64.8.9.

Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. 2022. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chem 383: 132531. DOI: 10.1016/j.foodchem.2019.04.105.

Silaban S, Nainggolan B, Simorangkir M, Zega TS, Pakpahan PM, Gurning K. 2022. Antibacterial activities test and brine shrimp lethality test of simargaolgaol (*Aglaonema modestum* Schott ex Engl.) leaves from North Sumatra, Indonesia. Rasayan J Chem 15 (2): 745-750. DOI: 10.31788/RJC.2022.1526911.

Simorangkir M, Hutabarat W, Nainggolan B, Silaban S. 2019b. Antioxidant and antibacterial activities of nonpolar to polar solvent extracts of sarang banua (*Clerodenrum fragrans* Vent Wild) leaves. Rasayan J Chem 12 (2): 959-965. DOI: 10.31788/rjc.2019.1225095.

Simorangkir M, Nainggolan B, Silaban S. 2019a. Antioxidant activity of vacuum column chromatography fractions of ethanol extract of sarang banua (*Clerodenrum fragrans* vent willd) leaves. J Phys: Conf Ser 1374 (1): 012016. DOI: 10.1088/1742-6596/1374/1/012016.

Simorangkir M, Silaban S, Roza D. 2022. Anticholesterol activity of ethanol extract of *ranti hitam* (*Solanum blumei* Nees ex Blume) leaves: in vivo and in silico study. Pharmacia 69 (02): 485-492. DOI: 10.3897/pharmacia.69.e84913.

Sinaga SP, Lumbangaol DA, Iksen I, Situmorang RFR Gurning K. 2022. Determination of phenoloc, flavonoid content, antioxidant and atibacterial activities of seri (*Muntingia calabura* L.) leaves ethanol extract form North Sumatera, Indonesia. Rasayan J Chem 15 (02): 1534-1538. DOI: 10.31788/RJC.2022.1526730.

Sinaga SP, Situmorang RFR, Singarimbun N, Lestari W, Gurning K. 2023. Determination of total phytochemical compounds from ethanol extract nangka (*Artocarpus heterophyllus* Lam.) leaves and antioxidant activity from North Sumatera, Indonesia. Rasayan J Chem 16 (1): 19-23. DOI: 10.31788/RJC.2023.1618113.

Situmorang RFR, Gurning K, Kaban VE, Butar-Butar MJ, Perangin-Angin SAB. 2022. Determination of total phenolic content, analysis of bioactive compound components, and antioxidant activity ethyl acetate seri (*Muntingia calabura* L.) leaves from North Sumatera, Province, Indonesia. Open Access Maced J Med Sci 10 (A): 240-244. DOI: 10.3889/oamjms.2022.8362.

Soltanzadeh H, Acik L, Türk M, Houshmand M, Shahsavari G. 2018. Antimicrobial, antioxidant, cytotoxic and apoptotic activities of *Satureja khuzestanica*. Gazi Med J 29 (3): 264-270. DOI: 10.12996/gmj.2018.75.

Suryadinata RV. 2018. Effect of free radicals on inflammatory process in chronic obstructive pulmonary disease (COPD). Amerta Nutr 2 (4): 317-324. DOI: 10.20473/amnt.v2i4.2018.317-324.

Tian T, Wang Z, Zhang J. 2017. Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. Oxid Med Cell Longev 2017 (1): 4535194. DOI: 10.1155/2017/4535194.

Vona R, Pallotta L, Cappelletti M, Severi C, Matarrese P. 2021. The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. Antioxidants 10 (2): 201. DOI: 10.3390/antiox10020201.

Vreeke GJ, Lubbers W, Vincken JP, Wierenga PA. 2022. A method to identify and quantify the complete peptide composition in protein hydrolysates. Anal Chim Acta 1201: 339616. DOI: 10.1016/j.aca.2022.339616

Wulansari AN. 2018. Alternatif *cantigi ungu* (*Vaccinium varingiaefolium*) sebagai antioksidan alami: Review. Farmaka 16 (2): 419-429. [Indonesian]

Zega TS, Pakpahan PM, Siregar R, Sitompul G, Silaban S. 2021. Antibacterial activity test of simargaolgaol (*Aglaonema modestum* Schott ex Engl) leaves extract against *Escherichia coli* and *Salmonella typhi* bacteria. Jurnal Pendidikan Kimia 13 (2): 151-158. DOI: 10.24114/jpkim.v13i2.26989.