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Determination of Antidiabetic Activity of Ethanolic Extract of Karas Tulang Leaves (*Chloranthus Erectus*) In Alloxan-Induced Wistar Rats

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ABSTRACT

Diabetes mellitus is a chronic condition that affects the metabolism of carbohydrates (glucose) in the body. According to data from the 2018 Basic Health Research (Riskesdas), there was a 2.1% increase in the prevalence of diabetes mellitus in Indonesia. The objectives of this study are: 1) To determine the antidiabetic activity of ethanol extract from *Karas Tulang* leaves in Wistar strain rats modeled for diabetes mellitus induced by alloxan, and 2) To perform phytochemical screening of the ethanol extract of *Karas Tulang* leaves (*Chloranthus erectus*).The research method utilized an experimental design with four groups of Wistar strain white rats. The phytochemical screening conducted included tests for alkaloids, saponins, flavonoids, and tannins using the thin-layer chromatography (TLC) method. Based on the results of the phytochemical screening tests, it was found that *Karas Tulang* leaves contain alkaloids, saponins, flavonoids, and tannins. The effectiveness of the antidiabetic activity was analyzed using SPSS through statistical tests, including normality, homogeneity, Kruskal-Wallis, and Mann-Whitney tests. Based on the statistical test results, it was concluded that the ethanol extract of *Karas Tulang* leaves at doses of 50 and 100 mg/kgBW is effective in reducing blood glucose levels.

KEYWORDS: Type 2 Diabetes Mellitus, Karas Tulang Leaves, Phytochemical Screening, Thin-Layer Chromatography, Wistar Strain White Rats

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I. INTRODUCTION

Diabetes mellitus is a chronic condition that affects the metabolism of carbohydrates (glucose) in the body. Poor glucose control leads to an increase in blood glucose levels, a condition known as hyperglycemia (Hananti, R., S., Hidayat, S., Yanti, 2018). A person is considered to have diabetes mellitus if their fasting blood glucose level exceeds 126 mg/dL and their random blood glucose level reaches or exceeds 200 mg/dL (Haryoto & Nur'aini, 2018). According to the 8th edition of the International Diabetes Federation (IDF), as documented in the 2017 Diabetes Atlas, approximately 425 million people worldwide suffer from diabetes. This number is projected to increase by 48% by 2045, reaching around 629 million people with diabetes (Djahi, S., N., S., Lidia, K., Pakan, P., D., Amat, A., L., 2021). According to data from the 2018 Basic Health Research (Riskesdas), there has been an increase in the prevalence of diabetes mellitus in Indonesia. In 2013, the prevalence of diabetes mellitus was 2.4%, whereas in 2018, this figure rose to 4.5%. This indicates a significant increase over the past five years, with a rise of 2.1% (Imanda, Y., L., Indriani, M., Fatoni, A., Rahajeng, V., 2023).

Currently, the treatment of diabetes mellitus is still limited to the use of Oral Hypoglycemic Agents (OHA) such as biguanides, sulfonylureas, glinides, thiazolidinediones, and acarbose, as well as insulin injections. The use of these medications may cause side effects such as diarrhea, dizziness, headaches, nausea, vomiting, and weight gain (Puspitasari, V., Choerunisa, 2021). Therefore, the use of traditional medicine is needed as an alternative for treating and preventing complications of diabetes mellitus. One commonly used traditional treatment is herbal plants. One herbal plant that has the potential for diabetes mellitus treatment is *Karas Tulang* leaves (*Chloranthus erectus*) (Firdaus, M., M., Subandrate., Sinulingga, 2023).

The genus *Chloranthus* belongs to the family Chloranthaceae and includes 14 species worldwide (Liu, Y., Y., Li, Y., Huang, S., Zhang, H., Deng, C., Song, X., Zhang, D., D., Wang, 2022). Several studies have reported hypoglycemic effects in this genus. It was found that

shizukaol D, isolated from *C. japonicus*, can activate AMP-activated protein kinase and regulate glucose metabolism, indicating that this genus has potential for diabetes mellitus treatment. Additionally, chlorabietols A-C, isolated from the roots of *C. oldhamii*, showed some inhibitory effects on complexinase (Hu et al., 2017). In another study, a hybrid abietane phloroglucinol compound was isolated from *Chloranthus oldhamii*. This hybrid compound demonstrated Protein Tyrosine Phosphatase 1B (PTP1B) activity, with IC50 values of 5.35 and 4.92 μM, respectively (Hussain et al., 2019).

Based on the background above, this study aims to determine the antidiabetic activity of the ethanolic extract of *Karas Tulang* leaves (*Chloranthus erectus*) in Wistar rats with alloxan-induced diabetes mellitus, and to identify the compounds responsible for the antidiabetic effects in the ethanolic extract of *Chloranthus erectus* leaves.

II. METHOD

A. Types Of Research

This research uses an experimental design with a Completely Randomized Design (CRD). The study involves 30 healthy male white rats (*Rattus norvegicus*) aged 3 months, divided into 4 treatment groups: Group 1 (Alloxan + *Karas Tulang* leaf extract at a dose of 50 mg/Kg body weight); Group 2 (Alloxan + *Karas Tulang* leaf extract at a dose of 50 mg/Kg body weight); Group 3 (Alloxan + 30% glucose as a negative control); and Group 4 (Alloxan + glibenclamide at a dose of 10 mg/Kg body weight as a positive control).

B. Time and Place of Research

This research was conducted at the Pharmacology and Toxicology Laboratory, Faculty of Pharmacy, Muhammadiyah University of Surakarta. The study was carried out over a period of 4 months.

C. Extract Making

The preparation of the ethanolic extract of *Karas Tulang* leaves was carried out at the Pharmacology and Clinical Pharmacy Laboratory, Muhammadiyah University of Surakarta. The dried *Karas Tulang* leaves were purchased from the Bandung area, weighing 1 kilogram. The leaves were then blended into a fine dry powder. Approximately 100 grams of the powder was soaked in 70% ethanol with a 1:10 ratio for 3 days. Afterward, the mixture was filtered using filter paper to obtain the liquid extract. The filtered liquid was then evaporated using a rotary vacuum evaporator to obtain the *Karas Tulang* leaf extract. The extract was further evaporated with a water bath to yield a concentrated extract.

D. Alkaloid Test

The alkaloid test was conducted by spotting the extract on a Thin Layer Chromatography (TLC) plate, followed by elution using a mobile phase of n-hexane and ethyl acetate in a 7:3 ratio. The spots were then identified using Dragendorff's reagent. A positive result is indicated by a color change to orange or red (Jawa La et al., 2020).

E. Flavonoid Test

The flavonoid test was conducted by spotting the extract on a Thin Layer Chromatography (TLC) plate, followed by elution using a mobile phase of n-hexane and ethyl acetate in a 7:3 ratio. For visualizing the spots, a 1% AlCl3 reagent was used. A positive reaction is indicated by the formation of yellow-brown spots (Fajriaty et al., 2018).

F. Tannin Test

The tannin test was conducted by spotting the extract on a Thin Layer Chromatography (TLC) plate, followed by elution using a mobile phase of n-hexane and ethyl acetate in a 7:3 ratio. The spots were identified by spraying with FeCl3. A positive result is indicated by a color change to black (Dewi et al., 2021).

G. Saponin Test

The saponin test was conducted by spotting the extract on a Thin Layer Chromatography (TLC) plate, followed by elution using a mobile phase of n-hexane and ethyl acetate in a 7:3 ratio. The spots were identified by applying the Lieberman-Burchard reagent. A positive reaction is indicated by the formation of green-blue spots (Suhaenah & Nuryanti, 2017).

H. Preparation of Test Solution

The preparation of a 30% sugar solution is done by weighing 30 grams of sugar and dissolving it in 100 mL of distilled water (aquades), ensuring it is fully dissolved. The alloxan test solution is prepared at a dose of 170 mg/Kg body weight. The dose conversion is calculated using the formula: (200/1000 × 170 = 34 mg/200g body weight), and the volume for administration is calculated using the formula: (body weight/200 × 1 mL). A 0.5% CMC-Na solution is prepared by weighing 500 mg of CMC-Na powder and adding it to 100 mL of distilled water. The glibenclamide test solution is prepared at a dose of 10 mg/Kg body weight, which is converted for mice by using the formula: (200/1000 × 10 mg = 2 mg/200g body weight). A stock solution is made by weighing 200 mg of glibenclamide powder and adding it to 100 mL of CMC-Na solution.

The preparation of *Karas Tulang* leaf extract at a dose of 100 mg/Kg body weight involves dose conversion using the formula: (200/1000 × 100 = 20 mg/200g body weight). Then, a 100 mL stock solution is made by calculating the concentration as follows: (20 mg/2 mL = 10 mg/mL; 10 mg/mL × 100 mL = 1000 mg). Therefore, 1 gram of the extract is weighed and dissolved in 100 mL of 0.5% CMC-Na solution. For the *Karas Tulang* leaf extract at a dose of 50 mg/Kg body weight, the dose conversion is done using the formula: (200/1000 × 50 = 10 mg/200g body weight). A 100 mL stock solution is prepared by calculating the concentration as follows: (10 mg/2 mL = 5 mg/mL; 5 mg/mL

× 100 mL = 500 mg). Therefore, 0.5 grams of the extract is weighed and dissolved in 100 mL of 0.5% CMC-Na solution.

III. RESULT AND TABLE

Table 1. Rf Value of Each Compound

Description	Tannin	Alkaloid	Saponin	Flavonoid
Rf Value	0,04	0,22	0,509	0,75

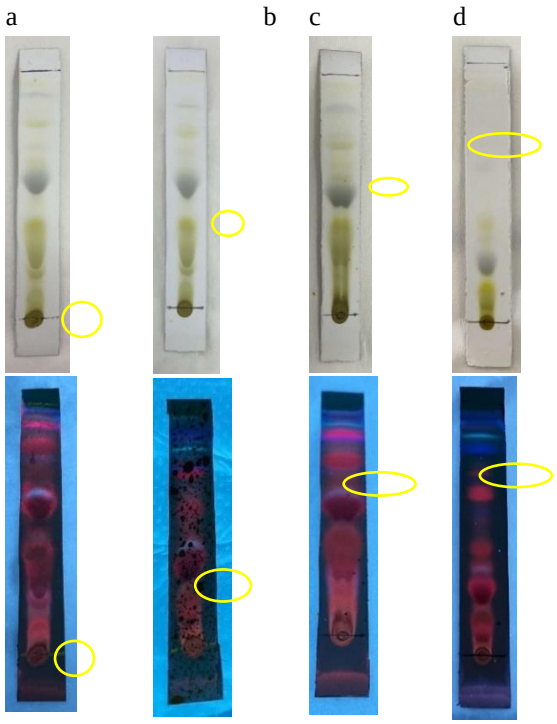

Figure 1. Phytochemical Screening of Compounds of the Groups (a) Tannin, (b) Alkaloid, (c) Saponin, and (d) Flavonoid.

IV. DISCUSSION

The principle of separation in the Thin Layer Chromatography (TLC) method is based on adsorption and partition processes. TLC has several advantages, including simple analysis, the use of color reagents to identify separated components, flexible elution that can be performed upward, downward, or in two dimensions, and better accuracy in concentration determination because the compounds measured form stationary spots that do not move (Sundhani et al., 2020).

The purpose of the Thin Layer Chromatography (TLC) method is for qualitative analysis, specifically to detect the presence of secondary metabolite compounds in the leaf extract of Karas Tulang (Hasan et al., 2024). In the phytochemical screening results, it was found that the sample tested positive for tannins, alkaloids, saponins, and flavonoids. The alkaloid test was conducted using Dragendorff's reagent. The positive result from the Dragendorff test is indicated by a color change to orange-red, which occurs due to the reaction between nitrogen used to form a coordinate covalent bond with the K+ ion, a metal ion. The tannin test was performed by spraying with FeCl3, which gave a positive result. The phenolic group in tannins can be identified through a color change to green or dark blue, which happens because the FeCl3 reacts with Fe^{3+} ions, forming a complex compound (Ergina, Nuryanti. S., 2014).

Similarly, from the analysis of the Rf values, the leaf extract of Karas Tulang showed four spots with Rf values of 0.04, 0.22, 0.509, and 0.75. These results indicate that the ethanol extract of Karas Tulang leaves positively contains secondary metabolite compounds such as tannins, alkaloids, saponins, and flavonoids. This is in line with previous research, where the total flavonoid content (TFC) of *Chloranthus erectus* was found to be 15.46 g RE/g in ethanol extract, 13.54 g RE/g in methanol extract, and 5.00 g RE/g in water extract (Jeeno et al., 2023). Additionally, the IC50 value of *Chloranthus erectus* for DPPH radical scavenging activity is 6.98 ± 0.93 mg/mL in the methanol extract and 4.00 ± 1.78 mg/mL in another study. The highest antioxidant activity was observed in the methanol extract, both from the leaves (88.36 ± 0.24%) and from the twigs (91.25 ± 0.10%) (Zemry et al., 2023).

For 3 weeks, diabetes-induced rats were orally administered ethanol extract of Karas Tulang leaves. Alloxan enters through the glucose transporter 2 (GLUT2) and tends to damage certain types of insulin produced by pancreatic cells, leading to reduced glucose absorption by peripheral organs and ultimately triggering diabetes. This is due to the ability of alloxan to stimulate the production of free radicals through redox processes, which cause tissue damage and cell degeneration (Muhtadi et al., 2015).

The statistical data analysis began with testing the antidiabetic activity to examine the normality of the distribution using the Shapiro-Wilk test. The results showed that the data from each group in the blood glucose level (BGL) check on Days 4-20 followed a normal distribution ($p > 0.05$), except for the positive control group on Day 20 ($p < 0.05$). Next, a Homogeneity test was conducted, and the results indicated that the data variation from each test on Days 4-20 was not homogeneous ($p < 0.05$). Statistical analysis continued with the Kruskal-Wallis test, which showed significant values for BGL checks after treatment administration on Days 8, 12, 16, and 20, with values of 0.005, 0.035, 0.013, and 0.010 ($p < 0.05$). This indicates a significant effect of the treatment on blood glucose levels among the four test groups.

The analysis was then continued with the non-parametric Mann-Whitney test. There were four tests conducted. The first was between the 50 mg/kg body weight Karas Tulang leaf extract treatment group and the negative control group, showing values on Days 8, 12, 16, and 20 of 0.009, 0.465, 0.009, and 0.009, respectively. These results indicate a significant difference between the two treatment groups ($p < 0.05$). The second test was between the 50 mg/kg body weight Karas Tulang leaf extract treatment group and the positive

control group, with values on Days 8, 12, 16, and 20 of 0.117, 0.251, 0.917, and 0.347, respectively. These results indicate no significant difference between the two treatment groups ($p > 0.05$). The third test was between the 100 mg/kg body weight Karas Tulang leaf extract treatment group and the negative control group, with values on Days 8, 12, 16, and 20 of 0.009, 0.016, 0.009, and 0.009, respectively. These results indicate a significant difference between the two treatment groups ($p < 0.05$). The final test was between the 100 mg/kg body weight Karas Tulang leaf extract treatment group and the positive control group, with values on Days 8, 12, 16, and 20 of 0.465, 0.465, 0.754, and 0.917, respectively. These results indicate no significant difference between the two treatment groups ($p > 0.05$).

The results from the Mann-Whitney test of the ethanol extract of Karas Tulang leaves at doses of 50 and 100 mg/kg body weight, compared to the positive control (Glibenclamide at a dose of 10 mg/kg body weight), showed no significant difference. This indicates that the Karas Tulang leaf extract is effective in reducing blood glucose levels. The ability of the ethanol extract of Karas Tulang leaves to lower blood glucose levels is suspected to be related to its flavonoid content. The mechanism of action of flavonoids is thought to stimulate regeneration and the release of insulin by pancreatic β cells (Dheer & Bhatnagar, 2010).

This is consistent with research on *C. henryi*, which demonstrated moderate hepatoprotective activity with IC50 values of 0.19, 0.66, 0.09, and 0.18 mM, respectively (Wang et al., 2015). In addition, several studies have reported on the hypoglycemic activity of the *Chloranthus* genus. It was found that shizukaol D, isolated from *C. japonicus*, can activate AMP-activated protein kinase (AMPK) and regulate glucose metabolism. This suggests that this genus has potential for the treatment of diabetes mellitus (DM) (Hu et al., 2017). In another study, a hybrid abietane phloroglucinol compound was isolated from *Chloranthus oldhami*. This hybrid compound exhibited Protein Tyrosine Phosphatase 1B (PTP1B) activity with IC50 values of 5.35 and 4.92 μM, respectively (Hussain et al., 2019). PTP1B is an intracellular protein tyrosine phosphatase involved in the negative regulation of insulin and leptin signaling systems. PTP1B has emerged as a validated therapeutic target for the treatment of type 2 diabetes (Tamrakar et al., 2014).

CONCLUSIONS

Based on the results of the research and discussion, it can be concluded that the ethanol extract of *Chloranthus erectus* leaves at doses of 50 and 100 mg/kg body weight has antidiabetic efficacy in reducing blood glucose levels. The group of compounds responsible for the antidiabetic effect in *Chloranthus erectus* leaves is flavonoid compounds.

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REFERENCES

I. Dewi, I. S., Saptawati, T., & Rachma, F. A. (2021). Skrining Fitokimia Ekstrak Etanol Kulit dan Biji Terong Belanda (Solanum betaceum Cav.) Phytochemical Screening of Tamarillo Peel and Seeds Ethanol Extracts (Solanum Betaceum Cav.). *Prosiding Seminar Nasional UNIMUS*, 1210–1218.

II. Dheer, R., & Bhatnagar, P. (2010). A study of the antidiabetic activity of Barleria prionitis Linn. *Indian Journal of Pharmacology*, *42*(2), 70–73. https://doi.org/10.4103/0253-7613.64493

III. Djahi, S., N., S., Lidia, K., Pakan, P., D., Amat, A., L., S. (2021). Uji Efek Antidiabetes Ekstrak Etanol Daun Sereh (Cymbopogon Citratus) Terhadap Penurunan Glukosa Darah Tikus Putih Sprague Dawley Diinduksi Aloksan. *Cendana Medical Journal (CMJ)*, *9*(2). https://doi.org/10.35508/cmj.v9i2.5981

IV. Ergina, Nuryanti. S., P. I. D. (2014). UJI KUALITATIF SENYAWA METABOLIT SEKUNDER PADA DAUN PALADO (Agave angustifolia) YANG DIEKSTRAKSI DENGAN PELARUT AIR DAN ETANOL. *J. Akad. Kim*, *3*(3), 165–172.

V. Fajriaty, I., I H, H., Andres, & Setyaningrum, R. (2018). Skrining Fitokimia Lapis Titpis Dari Ekstrak Etanol Daun Bintangur (Calophyllum soulattri Burm . F .). *Jurnal Pendidikan Informatika Dan Sains*, *7*(1), 54–67.

VI. Firdaus, M., M., Subandrate., Sinulingga, S. (2023). Efek Antihiperglikemik Ekstrak n-Heksana Daun Benalu Kersen pada Tikus Putih Jantan Model Diabetes Melitus. *Jurnal Kedokteran Dan Kesehatan*, *19*(2), 105–115.

VII. Hananti, R., S., Hidayat, S., Yanti, L. (2018). UJI AKTIVITAS ANTIDIABETES EKSTRAK ETANOL KULIT KAYU MANIS (Cinnamomum burmanii Nees ex.Bl.) DIBANDINGKAN DENGAN GLIBENKLAMID PADA MENCIT JANTAN GALUR Swiss Webster DENGAN METODE TOLERANSI GLUKOSA. *Jurnal Sains Dan Teknologi Farmasi Indonesia*, *1*(1), 13–21. https://doi.org/10.58327/jstfi.v1i1.10

VIII. Haryoto, & Nur'aini, A. R. (2018). Antidiabetes Ethanol Mellitus Extract Of Yellow Potato Sticks And Leaves (Ipomoea Batatas Linn.) On Blood

Glucose Levels Of Male Rats. *Journal of Science and Practical Pharmacy*, *IV*(2), 1–8.

IX. Hasan, H., Djuwarno, E. N., Hiola, F., & Ramadhani, F. N. (2024). Penapisan Fitokimia Dan Uji Efek Antidiabetes Ekstrak Metanol Daun Brotowali (Tinospora crispa L .) Pada Mencit (Mus musculus). *Journal of Pharmacology and Natural Products (JPNP)*, *1*, 20–32.

X. Hu, R., Yan, H., Fei, X., Liu, H., & Wu, J. (2017). Modulation of glucose metabolism by a natural compound from Chloranthus japonicus via activation of AMP-activated protein kinase. *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/s41598-017-00925-y

XI. Hussain, H., Green, I. R., Abbas, G., Adekenov, S. M., Hussain, W., & Ali, I. (2019). Protein tyrosine phosphatase 1B (PTP1B) inhibitors as potential anti-diabetes agents: patent review (2015-2018). *Expert Opinion on Therapeutic Patents*, *29*(9), 689–702. https://doi.org/10.1080/13543776.2019.1655542

XII. Imanda, Y., L., Indriani, M., Fatoni, A., Rahajeng, V., N. (2023). UJI EFEK ANTIDIABETES EKSTRAK ETANOL UMBI TALAS (Colocasia esculenta L.) PADA TIKUS PUTIH JANTAN YANG DIINDUKSI ALOKSAN. *Jurnal Ilmiah Bakti Farmasi*, *8*(2), 71–79. https://doi.org/10.61685/jibf.v8i2.107

XIII. Jawa La, E. O., Sawiji, R. T., & Yuliawati, A. N. (2020). Skrining Fitokimia Dan Analisis Kromatografi Lapis Tipis Ekstrak Etanol Kulit Buah Naga Merah (Hylocereus polyrhizus). *Indonesian Journal of Pharmacy and Natural Product*, *3*(1), 45–58. https://doi.org/10.35473/ijpnp.v3i1.503

XIV. Jeeno, P., Yadoung, S., Yana, P., & Hongsibsong, S. (2023). Phytochemical Profiling and Antioxidant Capacity of Traditional Plants, Northern Thailand. *Plants*, *12*(23), 1–15. https://doi.org/10.3390/plants12233956

XV. Liu, Y., Y., Li, Y., Huang, S., Zhang, H., Deng, C., Song, X., Zhang, D., D., Wang, W. (2022). Genus Chloranthus: A comprehensive review of its phytochemistry, pharmacology, and uses. *Arabian Journal of Chemistry*, *15*(11). https://doi.org/10.1016/j.arabjc.2022.104260

XVI. Muhtadi, Primarianti, A. U., & Sujono, T. A. (2015). Antidiabetic Activity of Durian (Durio Zibethinus Murr.) and Rambutan (Nephelium Lappaceum L.) Fruit Peels in Alloxan Diabetic Rats. *Procedia Food Science*, *3*, 255–261. https://doi.org/10.1016/j.profoo.2015.01.028

XVII. Puspitasari, V., Choerunisa, N. (2021). Kajian Sistematik : Efek Anti Diabetes Buah Pare (Momordica charantia Linn.) Terhadap Kadar Glukosa Darah pada Tikus yang Diinduksi Aloksan. *Generics: Journal of Research in Pharmacy*, *1*(2), 18–27. https://doi.org/10.14710/genres.v1i2.11052

XVIII. Suhaenah, A., & Nuryanti, S. (2017). SKRINING FITOKIMIA EKSTRAK JAMUR KANCING (Agaricus bisporus). *Jurnal Fitofarmaka Indonesia*, *4*(1), 199–204. https://doi.org/10.33096/jffi.v4i1.228

XIX. Sundhani, E., Diniatik, Hakim, Z. R., Nurzijah, I., Prakoso, A., Fajrina, N., Rifki, M., & Dasa, Z. A. M. C. (2020). Studi Interaksi Obat Antidiabetes Metformin dan Glibenklamid dengan Jamu pada Tikus Diabetes yang Diinduksi Aloksan. *JFIOnline | Print ISSN 1412-1107 | e-ISSN 2355-696X*, *12*(1), 29–37.

XX. Tamrakar, A. K., Maurya, C. K., & Rai, A. K. (2014). PTP1B inhibitors for type 2 diabetes treatment: A patent review (2011-2014). *Expert Opinion on Therapeutic Patents*, *24*(10), 1101–1115. https://doi.org/10.1517/13543776.2014.947268

XXI. Wang, A. R., Song, H. C., An, H. M., Huang, Q., Luo, X., & Dong, J. Y. (2015). Secondary metabolites of plants from the genus Chloranthus: Chemistry and biological activities. *Chemistry and Biodiversity*, *12*(4), 451–473. https://doi.org/10.1002/cbdv.201300376

XXII. Zemry, I. H., Hasan, N., Hasbullah, N. I., Nawahwi, M. Z., Azzeme, A. M., Ahmad, S. N. D., & Ariffin, S. (2023). Antioxidant Potential of Chloranthus erectus (Chloranthaceae) from various solvents extract. *Journal of Experimental Biology and Agricultural Sciences*, *11*(1), 75–80. https://doi.org/10.18006/2023.11(1).75.80