ANTIDIABETIC ACTIVITY OF METHANOL EXTRACT OF BARK OF *Alstonia scholaris* FROM PAPUA

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ABSTRAK: Salah satu kekayaan hayati yang tumbuh di Papua adalah *Alstonia scholaris*, dikenal oleh suku Maybrat dengan nama tumbuhan Swe dan digunakan sebagai obat tradisional untuk penyakit diabetes. Pohon Swe mengandung banyak getah berwarna putih dan rasanya sangat pahit. Rasa pahit terdapat pula pada akar, kulit batang, dan daun sehingga diduga banyak mengandung senyawa bioaktif golongan alkaloid yang berpotensi sebagai antioksidan dan antidiabetes. Penelitan ini bertujuan untuk mengekstraksi dan menentukan aktivitas penghambatan enzim α-glukosidase, serta menentukan nilai IC_{50} ekstrak methanol kulit kayu *A. scholaris* asal Papua sebagai dasar bagi pengembangan obat antidiabetes berbasis sumber daya lokal. Metode yang digunakan adalah ektraksi dengan cara maserasi, skrining fitokimia, uji antidiabetes α-glukosidase dan analisis gugus fungsi mengunakan FTIR. Hasil skrining fitokimia menunjukkan bahwa ekstrak methanol kulit kayu Swe asal Papua positif kuat mengandung senyawa metabolit sekunder golongan alkaloid dan flavonoid. Analisis gugus fungsi dengan FTIR menunjukkan senyawa metabolit sekunder tersebut diduga mengandung gugus fungsi –OH, uluran C=C dan gugus C=O. Hasil uji penghambatan aktivitas enzim α-glukosidase, IC_{50} ekstrak methanol kulit kayu Swe adalah sebesar 0,97 µg/mL.

Kata kunci: *Alstonia scholaris*, antidiabetes, α- glukosidase, fitokimia

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease that is not contagious, generally caused by physical activity, an unhealthy diet, obesity, increased blood cholesterol and glucose (Singh & Kumar, 2015). According to the World Health Organization (WHO), diabetes mellitus is a chronic metabolic disease or disorder with multiple etiologies characterized by high blood sugar levels accompanied by impaired carbohydrate, lipid and protein metabolism as a result of insulin function insulfficiency, which can be caused by impaired insulin production by langerhans beta cells of pancreas gland or caused by the body's lack of responsiveness to insulin. Globally, an estimated 422 million adults lived with diabetes in 2014, compared with 1980 which only 108 million people or experienced an increase from 4.7% to 8.5% in the adult population. Indonesia ranks seventh in the world after China, India, the United States, Brazil, Russia, and Mexico with an estimated number of people with diabetes mellitus of 10 million.

The increasing prevalence of diabetes mellitus from year to year shows the need for serious attention in the treatment of these diseases. The tendency of people to do traditional treatments is increasing, this encourages the search for treatment to find alternative medicines with better efficacy so that diabetics have many treatment options to increase the chances of cure and minimum side effects and relatively lower costs (Mangallo *et al*., 2019).

The eastern part of Indonesia, namely Papua, is the easternmost region, which has an abundant diversity of flora and fauna while still having large areas of low tropical forest in Southeast Asia. One of the biological wealth that grows in Papua is blackboard tree (*A. scholaris*), this plant has the potential as a traditional medicine for the treatment of diabetes, especially by the Maybrat tribe, South Sorong district (Hara *et al*., 2012). The local name used by the Maybrat tribe for *A. scholaris* is Swe, which means a devil tree because the tree is tall and big. Devil's trees contain lots of sap. The sap is white and tastes very bitter. Bitter taste is also found in the roots, bark, and leaves so that it is thought to contain a lot of bioactive compounds of alkaloids. Alkaloid compounds have the potential as antioxidants and antidiabetic (Tiong *et al.*, 2013).

The content of active compounds from *A. scholaris* that grows in Papua and its potential as an antidiabetic has not been much studied. This is very important because *A. scholaris* has potential as a raw material for medicine in the world of pharmacology. The secondary metabolite compounds from A. scholaris include echitamin, echitamin chloride, scholarin, scholarisin, monoterpenoid, indole alkaloids, iridoids, coumarin, flavonoids, phenolics, steroids, saponins and tannins (Khyade & Vaikos, 2009). Research on the pharmacological properties of A. scholaris plants have been conducted, including as an antibacterial in chloroform and acetone extracts (Khyade *et al*., 2009; Khyade & Vaikos, 2009), methanol extract from leaves, stems and bark of the skin (Khan *et al*., 2003), anti-inflammatory ethanol extract from leaves (Shang *et al*., 2010), antioxidants, anticancer, and free radicals (Arulmozhi *et al*., 2008), and antimalarials in methanol extract of bark, ethyl acetate fraction and chloroform fraction (Maniagasi *et al*., 2013). However, research on the use of A. scholaris skin and its potential as an anti-diabetic is unknown.

This study aims to extract and determine the inhibitory activity of α-glucosidase, IC_{50} of methanol extract of *A. scholaris* bark from Papua as a basis for the development of antidiabetic drugs based on local resources.

MATERIALS AND METHODS

Material

The main materials used in this study is devil's tree bark. Other materials included as methanol (Merck), HCl (Merck), H2SO4 (Merck), FeCl3 (Merck), Dragendroff reagents, Mayer reagents, Wagner reagents, magnesium powders, ether, phosphate buffer, p-nitrophenyl-α-D-glucopyranoside, sodium carbonate (Na_2CO_3), and aquades.

Methods

Preparation and extraction process.

Devil's tree bark samples were washed thoroughly and then dried in an oven at 40^{o}C. Devil's tree bark samples were then smoothed using a hamermill.

A fine sample of 500 grams was macerated with 1000 ml methanol solvent for 3 x 24 hours, then filtered. The filtrate obtained was evaporated using a rotary evaporator until a thick

extract of methanol was obtained. The methanol extract obtained was then subjected to phytochemical screening and identification of functional groups using UV-VIS and FTIR spectroscopy.

Phytochemical screening test

Identification of the chemical content in the extract was carried out on compounds containing secondary metabolites: alkaloids, flavonoids, terpenoids or steroids, saponins and tannins (Harborne, 1987).

Alkaloid test

The 3 mL extract solution was added with 5 mL 2 N HCl and 6 ml distilled water, heated for 15 minutes. After that, it is cooled then filtered and the filtrate was collected. The filtrate was divided into 4 tubes, tube 1 added with Mayer reagent, tube 2 added with Dragendorff reagent, tube 3 added with Wagner reagent, and tube 4 as a blank. The presence of sedimentation indicated alkaloid positivity.

Flavonoid test

As much as 3 ml of the extract solution was added 5 mL of methanol, then heated for 10 minutes. After that, it is cooled and filtered to take the filtrate and divided into 2 tubes. Tube 1 as a blank, tube 2 added with 0.5 ml of concentrated HCl and zinc or magnesium powder. If there is a red to purple, green, yellow or blue discoloration, the test was considered to be flavanoid positive.

Steroid/Triterpenoid test

A total of 3 ml of the extract solution was evaporated to dryness, then the filtrate was added to an adequate amount of ether and Lieberman-Buchard reagent (3 drops of pthalic anhydride + 1 drop of H_2SO_4). If after a while a red or purple color occurs, the test was considered to be triterpenoid positive. If green was formed, then test positive for steroids.

Saponin test

Samples were taken as much as 3 ml and added 5 mL H_2O then heated 30 minutes then cooled and filtered to collect the filtrate, then shaken. If there is foam for about 15 minutes, the test was considered to be positive for saponin.

Tannin test

As much as 3 mL of the extract solution was added 5 mL of H_2O which was heated for 30 minutes, then cooled and filtered. The filtrate obtained was added with 5 drops of $FeCl_3$ 1%. Discoloration of green, greenish blue or blackish blue or the presence of sedimentation indicated positivity for tannin.

Inhibitory activity of α-glukosidase enzyme

The α-glucosidase enzyme inhibitory activity test was carried out to measure the anti-hyperglycemic activity of the extract. In this test, the enzyme α-glucosidase will hydrolyze p-nitrophenyl-α-D-glucopyranoside to p-nitrophenol which is yellow and glucose. Testing procedures include:

1. Reagent Preparation
   a. 1.3 mg of alpha-glucosidase enzyme is dissolved in 50 ml of phosphate buffer (pH 7.0)
   b. 5 mM of p-nitrophenyl α D-glucopyranoside substrate was prepared by dissolving 150 mg of substrate into 100 ml of phosphate buffer (pH 7.0)
   c. 200 mM of sodium carbonate is prepared by dissolving 2.12 grams of sodium carbonate in 100 ml of phosphate/water buffer
   d. Phosphate buffer pH 7
   e. DMSO

2. Processing/Mixing

Samples weighed 10 mg added with 100-1000 micro liters of DMSO, sonicated until dissolved, buffer added up to 10 ml to obtain 1000 ppm stock solution, then made a series of dilutions of 5 concentration series ie 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm by piping stock solutions as much as 50 µL, 100 µL, 150 µL, 200 µL and 250 µL from a stock solution of 1000 ppm and added the volume with phosphate buffer up to 1000 µL.

A sample of 60 µl was put into the well plate and then added with 20 µl p-nitrophenyl α-D-glucopyranoside 5 mM as a substrate. After mixing, incubated at 37ºC for 5 minutes, then added 20 µl of the enzyme solution and incubated at 37ºC for 5 minutes. The enzymatic reaction was stopped by the addition of 100 µl 200 mM sodium carbonate and the formed p-nitrophenol was measured at a wavelength of 405 nm.

For S0 or reduction of the color of the sample made by adding 60 µL samples from each concentration, adding 40 µL buffer and adding 100 µL Na_2CO_3, the absorption was measured using ELIZA spectrophotometer with a wavelength of 405 nm. The percentage of inhibition is measured using the equation:

$$\% \text{ Inhibition } = \frac{(C-S)}{C} x\ 100\% \ldots (1)$$

C = absorbance of negative control – absorbance of blanko.
S = absorbance of extract (difference of extract absorbance with enzyme and without enzyme).

IC_{50} values were calculated using linear regression equations, sample concentration as the x-axis and % inhibition as the y-axis.
The regression equation y = a + bx obtained is used to determine IC_{50} with the formula in equation 2.

$$IC_{50} = \frac{50-a}{b} \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Identification of the functional groups of compounds contained in the methanol extract of devil's tree (A. scholaris) bark using FTIR spectrophotometer.

RESULTS AND DISCUSSION

Devil's tree (A. scholaris) used in this study grew in Manokwari and is estimated to be 30 years old with a tree height of around ± 30 m. The sample used in this study is that the outer skin which was rough and grayish white. Sample preparation is carried out until the sample is obtained in the form of a brown powder (Figure 1), this aims to increase the surface area so as to enlarge the contact of the solvent with the component compounds present in the sample during the extraction process.

Figure 1. Preparation of Devil's tree Bark (*A. scholaris*)
(A) Devil's tree bark **(B)** Devil's tree bark powder

Extraction of active compound in devil's tree bark

In this study, the extraction process of devil's tree (A. scholaris) bark powder used maceration method. The maceration process was carried out by soaking 500 grams of devil's tree (A. scholaris) bark powder in 1000 mL methanol for 3x24 hours at room temperature. The macerated sample was then filtered and the filtrate obtained was concentrated using a rotary evaporator to sort out the thick extract (Figure 2).

Figure 2. Extraction Process of Devil's Tree Bark

The methanol extract obtained was dark brown with a yield of 2.57%. According to Harborne (1987), the yield is the ratio of the weight of the extract obtained to the initial weight of the sample used, the yield also shows the effectiveness of the solvent to an extracted material, so that the type of solvent used in the extraction process will affect the amount of yield produced. Methanol is a good solvent for dissolving secondary metabolites because it can extract almost all secondary metabolite compounds, both of which are semipolar to polar so that the extract obtained is high in yield.

Phytochemical screening

Phytochemical screening aims to determine qualitatively the content of secondary metabolites in devil's tree (*A. scholaris*) bark samples. Identification of the chemical content of devil's tree (*A. scholaris*) bark methanol extract was carried out on groups of alkaloid, flavonoid, steroid or terpenoid compounds, tannins and saponins. Phytochemical screening test results showed that the methanol extract of devil's tree (*A. scholaris*) bark was strongly positive containing secondary metabolites of alkaloids, flavonoids, steroids and tannins (Table 1).

Table 1. Phytochemical screening of methanol extract of devil's tree bark (*A. scholaris*)

Constituents	Methanol extract
Alkaloids	++++
Flavonoids	+++
Steroids	+++
Terpenoids	++
Saponins	+
Tannins	+++

Notes: + : Weak positive
++ : Medium positive
+++ : Strong positive
++++ : Very strong positive

FTIR analysis of Devil's tree bark extract

The results of the analysis with FTIR from devils' tree bark methanol extract showed an absorption in the region of wave numbers 3429 cm^{-1}, 2927 cm^{-1}, 2115 cm^{-1}, 1631 cm^{-1}, 1506 cm^{-1}, 1263 cm^{-1}, and 677 cm^{-1}. The FTIR spectra of devil's tree methanol extracts are presented in Figure 3.

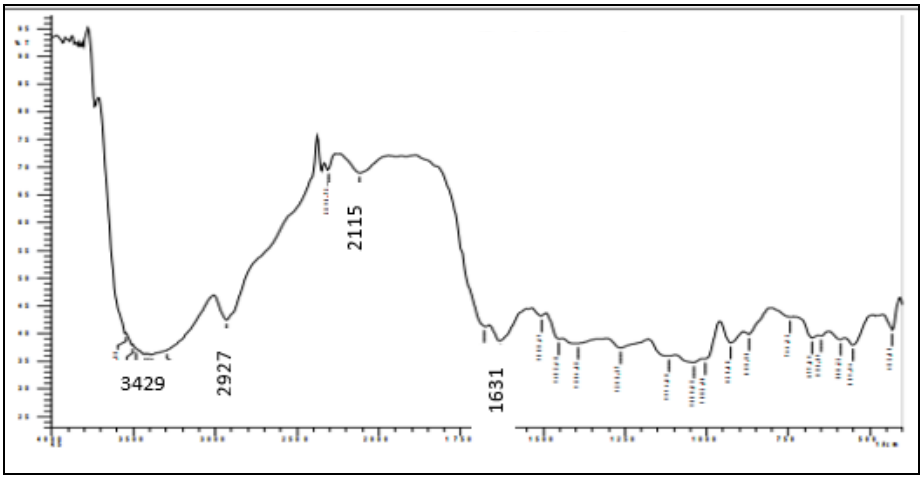

Figure 3. FTIR spectra of devil's tree bark methanol extract

The interpretation results for the obtained peaks showed that the devil's tree methanol extract contained groups of -OH, C-H, C=C, C=O, C-O, and C-H aromatic. The absorption peak of 3429 cm^{-1} indicates that the compound from the methanol extract contains a -OH functional group that can bind to hydrogen between molecules. The uptake at 2927 cm^{-1} showed a stretch of C-H while the bending of C-H was at 677 cm^{-1}. C=C stretches were seen at 2115 cm-1 uptake, uptake at 1631 cm^{-1} showed the presence of C=O groups (between aldehydes, ketones, esters or carboxyl), and C-O stretches were detected at peaks of 1263 cm^{-1} (Smith, 1999).

Inhibition activity of α-Glucosidase enzyme

The α-Glucosidase enzyme extract inhibition activity test for devil's tree bark is presented in Figure 4.

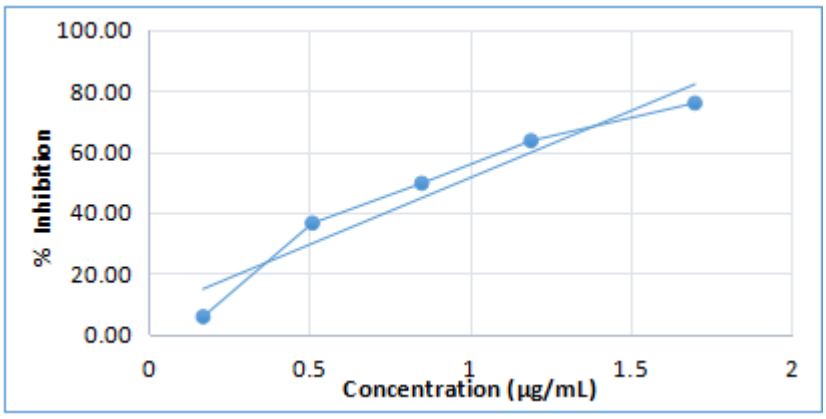

Figure 4. Effect of methanol extract inhibition for concentration variations

The IC_{50} (Inhibitory Concentration) value was determined based on the correlation curve between % inhibition and sample concentration and obtained a linear regression equation y = 43.817x + 7.5374. The calculation result of IC_{50} of devil's tree methanol extract was 0.97 µg/mL or lower when compared to the alpha-glucosidase inhibitory activity of ethanol extract of *A. scholaris* leaves with IC_{50} value of 0.43 µg/mL (Nagaraju *et al*., 2016).

CONCLUSION

Phytochemical screening results showed that devil's tree methanol extract was strongly positive containing secondary metabolites of alkaloids and flavonoids. Analysis of functional groups with FTIR showed that the secondary metabolite compound was thought to contain the -OH functional group, C=C stretch and C=O group. The results of the α-glucosidase enzyme inhibition test (IC_{50}) of devil's tree bark methanol extract was 0.97 µg/mL.

REFERENCES

Arulmozhi, S., Mazumder, P. M., Ashok, P., & Narayanan, L. S. (2008). In Vitro Antioxidant and Free Radical Scavenging Activity of *Alstonia scholaris* Linn. R.Br. *IJPT*, *6*, 6.

Hara, F. L. K., Nunaki, J. H., & Sadsoeitoeboen, M. J. (2012). Pemanfaatan Tumbuhan Sebagai Obat Tradisional Oleh Masyarakat Suku Maybrat di Kampung Renis Distrik Mare Kabupaten Sorong Selatan. *Jurnal Natural*, *8*(1). https://doi.org/10.30862/jn.v8i1.334

Harborne, 1987. Metode Fitokimia Edisi kedua. Patmawinata K. Soediro I. Penerjemah. Terjemahan dari: Phytochemical methods. Bandung: ITB.

Khan, M. R., Omoloso, A. D., & Kihara, M. (2003). Antibacterial activity of *Alstonia scholaris* and Leea tetramera. *Fitoterapia*, *74*(7–8), 736–740. https://doi.org/10.1016/S0367-326X(03)00192-8

Khyade, M. S., & Vaikos, N. P. (2009). Pharmacognostical studies on the Leaves of *Alstonia scholaris* R. Br. *Journal of Pharmacy Research*, *5*, 5.

Khyade, M, S., Vaikos, & N, P. (2009). Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br. *African Journal*

of Biotechnology, *8*(22), 6434–6436. https://doi.org/10.5897/AJB2009.000-9489

Mangallo, B., Putra, A. B. P., & Pulung, M. L. (2019). Uji Aktivitas Antidiabetes Dengan Metode Penghambatan Enzim α-Glukosidase dan Karakterisasi Kandungan Senyawa Aktif pada Fraksi Metanol dan Kloroform Daun Lavetar (Wedelia biflora (L).DC) Asal Biak. *Jurnal Natural*, *15*(1), 21–29. https://doi.org/10.30862/jn.v15i1.28

Nagaraju, B., Ramu, A., Vidyadhara, S., & Deepika, M. Y. (2016). Evaluation of in Vitro Anti-coagulant and in Vitro Anti Diabetic Activity of *Alstonia scholaris* Leaves. *International Research Journal of Pharmacy*, *7*(10), 23–26. https://doi.org/10.7897/2230-8407.0710117

Shang, J.-H., Cai, X.-H., Feng, T., Zhao, Y.-L., Wang, J.-K., Zhang, L.-Y., Yan, M., & Luo, X.-D. (2010). Pharmacological evaluation of *Alstonia scholaris*: Anti-inflammatory and analgesic effects. *Journal of Ethnopharmacology*, *129*(2), 174–181. https://doi.org/10.1016/j.jep.2010.02.011

Singh, S. P., & Kumar, Sugirtha. P. (2015). In vitro antidiabetic activity of compounds from Pithecellobium dulce fruit peel. *International Journal of Pharmaceutical Chemistry*.

Smith, B., 1999. Infrared Spectral Interpretation, a Systematic Approach, CRC Press, Boca Raton, FL.

Tiong, S., Looi, C., Hazni, H., Arya, A., Paydar, M., Wong, W., Cheah, S.-C., Mustafa, M., & Awang, K. (2013). Antidiabetic and Antioxidant Properties of Alkaloids from Catharanthus roseus (L.) G. Don. *Molecules*, *18*(8), 9770–9784. https://doi.org/10.3390/molecules18089770