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BIOLOGICAL ACTIVITIES OF EXTRACTS AND SECONDARY METABOLITE OF *Macaranga mappa* BARK AS AN ANTIDIABETIC BY α-GLUCOSIDASE INHIBITOR, ANTIOXIDANT, AND CYTOTOXICITY

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ABSTRACT

The biological activities of extracts and isolated compounds of *Macaranga mappa* stem bark for antidiabetic herbal medicine, antioxidant, and cytotoxicity activities have been determined. The antidiabetic, antioxidant, and cytotoxicity activities of the sample were carried out by inhibition value of α-glucosidase, DPPH method, and brine shrimp larvae method, respectively. The initial methanol extract and its fractions have good activities against antidiabetics and antioxidants, except for the hexane fraction. The fractions with the highest activity for the antidiabetic and antioxidant tests were the water fraction IC_{50} 6.654 ppm and the butanol fraction IC_{50} 32.34 ppm, respectively. The toxicity test results with the BSLT method showed that all fractions were non-toxic LC_{50}>1000 ppm. The isolated compound from the ethyl acetate fraction was a coumarin derivative, namely scopoletin, with antidiabetic and antioxidant activities.

Keywords: Biological Activity, Antidiabetic, α-glucosidase Inhibitor, Antioxidant, *Macaranga mappa*, Secondary Metabolite.

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INTRODUCTION

Diabetes mellitus (DM) is a disease in which carbohydrate metabolism disorders occur due to relative or absolute insulin deficiency, which is characterized by blood sugar levels exceeding normal values (hyperglycemia).[1,2] Diabetes mellitus can be classified into type-1 diabetes, type-2 diabetes, other types of diabetes, and gestational diabetes. Among the existing types, type 2 diabetes is the most common type, which is more than 90%.[3] Type-2 diabetes mellitus is hyperglycemia caused by the insensitivity of cells to insulin. The resulting insulin levels are slightly decreased or within normal levels. Type 2 diabetes is considered non-insulin-dependent Diabetes Mellitus (NIDDM) because pancreatic beta cells can still produce insulin.[4,5] One of the healing therapies for type 2 diabetes is the administration of oral drugs as a hypoglycemic agent.[6] One of the oral antidiabetic drugs used is a class of α-glucosidase inhibitors such as acarbose and miglitol.[7] This class of drugs works to inhibit the action of the α-glucosidase enzyme so that it can reduce the breakdown of complex carbohydrates and digestion, thereby reducing the increase in glucose levels in diabetics after eating.[8] However, standardized antidiabetic drugs that have been circulating in the market have limitations and side effects, so it is necessary to look for alternative medicines from natural products.[9] One of the plants that can be used as antidiabetic medicine and a source of natural antioxidants is the Euphorbiaceae plant group. The results showed that 148 types of plants had potential as traditional medicines Euphobiaceae.[10] The potential of chemical compounds isolated from the Euphorbiacea tribe, namely *M. tanarius*, had good inhibitory activity against the α-glucosidase enzyme. The genus Macaranga also has anticancer, antimicrobial, antioxidant, and antidiabetic bioactivity.[11] In this study, we investigated the potential of crude extracts and chemical compounds isolated from the *Macaranga mappa* species and evaluated the bioactivities as an antidiabetic by α-glucosidase inhibitor, antioxidant, and

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cytotoxicity activities.

EXPERIMENTAL

Plant Material

The plant used in this study was the bark of the *Macaranga mappa* species obtained from the Mekongga forest area, Kolaka Regency, Southeast Sulawesi Province, Indonesia. The sample plants were identified by the staff of Herbarium Bogoriensis, Center for Biological Research, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia.

Extraction and Isolation

A total of 1.9 kg of air-dried *M. mappa* bark powder was macerated with methanol over three days. The macerated extract was concentrated with a rotary evaporator at a temperature of 40-50°C and dried in an oven at a temperature of 40-50°C to obtain crude methanol extract. A total of 100 grams of methanol extract was fractionated successively with n-hexane, ethyl acetate, and butanol. The extract obtained from each solvent was concentrated with a rotary evaporator until all the solvent evaporated. The fractions of each solvent were analyzed by phytochemical screening. The isolation of the active compounds was carried out by a separation process using the column chromatography method. A total of 3.5 grams of the ethyl acetate fraction was eluted with n-hexane, n-hexane: ethyl acetate, ethyl acetate: methanol, and methanol in a gradient manner. The resulting fractions were monitored by TLC which was then purified by the recrystallization process. Structure elucidation of the pure isolate was analyzed using Fourier transform Infrared Spectroscopy (FTIR), Mass spectrometry (MS), and Nuclear Magnetic Resonance (NMR).

Bioactivities Tests

Isolated compound and all extracts from each solvent were assayed in vitro antidiabetic activity through α-glucosidase inhibition referred to the way previous research,[12,13] antioxidant using DPPH (2,2-diphenyl-1-picryl-hydrazine-hydrate) assay,[14–16] and cytotoxicity using brine shrimp larvae (*Artemia salina*).[17–19]

RESULTS AND DISCUSSION

A preliminary test in the form of phytochemical screening for the content of secondary metabolites was carried out on the bark extract of *M. mappa*. This test is qualitative with visual observations in the form of color changes and the formation of a precipitate with the addition of reagents in each test.[20,21] Phytochemical tests identified that the bark extract of *M. mappa* contained alkaloids, flavonoids, saponins, steroids/terpenoids, and tannins, as shown in Table-1.

Table-1: Profile of Secondary Metabolites in the Bark Extract of *M. mappa*.

No	Secondary metabolite	Methanol extract	Fraction			
			n-Hexane	Ethyl acetate	Butanol	Water
1	Alkaloids	+	-	-	-	+
2	Flavonoids	+	-	+	+	+
3	Saponins	+	+	+	+	+
4	Terpenoids/steroids	+	+	+	-	-
5	Tannins	+	+	-	-	-

The molecular structures of isolated compounds were identified by functional groups, molecular weights, and molecular structures using FTIR, LC-ESI-MS, and ^{1}HNMR and ^{13}CNMR, respectively. The FTIR spectrum (Fig.-1a) showed the stretching vibration of the -OH functional group at 3.220 cm^{-1}, -C=O (carbonyl ketone) group at 1.711 cm^{-1}, -C=C-aromatic group at 1.657 cm^{-1} and 1.610 cm^{-1}, C-H sp^2 at 2924 cm^{-1}, and C-H sp^3 at 2856 cm^{-1}.[22] The results of the analysis using LC-ESI-MS (Fig.-1b) showed that the isolated compound had a molecular weight (*m/z*) of 193 $[M+H]^+$. Thus, the isolated compound has a molecular weight of (m/z) 192, with the molecular formula $C_{10}H_8O_4$. ^{1}H-NMR spectrum analysis (Fig.-1c) showed that the isolated compound had two aromatic protons with singlet peaks at chemical shift values of δ_H 7.12 ppm and δ_H 6.77 ppm. The formation of a singlet peak indicates that the two protons are localized and are not close to each other. Then, two protons doublet in the chemical shift region δ_H 6.21 ppm (d, J = 9.75 Hz) and δ_H 7.86 ppm (d, J = 9.75 Hz). At the δ_H 3.91 ppm, there is a single peak with a high intensity with an integration value of three H atoms which indicates the presence of a methoxy group (-OCH_3). The

results of the ^{13}CNMR spectrum analysis showed that the isolated compound had 10 carbon atoms. The chemical shift of δ_C 56.91 ppm is an indication of the methoxy carbon atom and δ_C 164.24 ppm is the chemical shift of the carbonyl carbon atom. The chemical shift of the carbon atoms of the double bond is found at δ_C 112.70 ppm and δ_C 146.31 ppm. The aromatic carbon atoms are present in the chemical shift δ_C 110.019 ppm; 112.62 ppm;147.21 ppm; 151.51 ppm; 104.05 ppm, and 153.02 ppm. From this analysis, the arrangement of H atoms and C atoms is shown in Table-2.

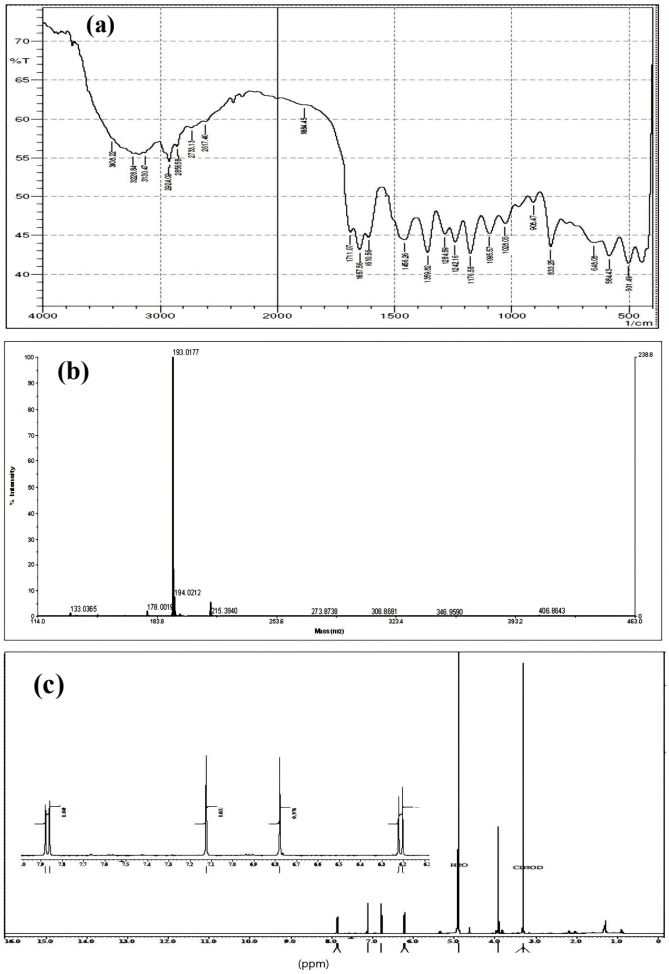

Fig.-1: Spectra of (a) FTIR, (b) Mass, and (c) HNMR of the Isolated Compound

Table-2: ^{1}HNMR and ^{13}CNMR Data of Isolated Compounds

No	δ_H (ppm), (mult., ΣH, *J* Hz)	δ_C (ppm)
1	-	164.24
2	6.21 (d; 1H; 9.75)	112.70
3	7.86 (d; 1H; 9.75)	146.31
4	-	112.66
5	7.12 (s; 1H)	110.011
6	-	147.21
7	-	153.02
8	6.77 (s; 1H)	104.05
9	-	151.51
10	3.91 (s; 3H)	56.91

Based on functional group vibration data from FTIR, molecular weight data (*m/z*) 192 from MS analysis and strengthened by ^{1}HNMR and ^{13}CNMR data, it is estimated that the synthesized compound was a coumarin derivative compound which has a methoxy substituent known as scopoletin with the structural formula as shown in Fig.-2.

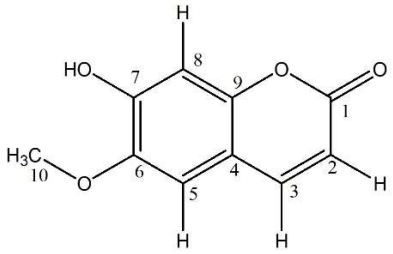

Fig.-2: Molecular Structure of Isolated Compound (Scopoletin)

Bioactivity for all extracts and fractions of *M. mappa* bark was carried out on antidiabetic, antioxidant, and toxicity tests. Enzyme activity of antidiabetic by α-glucosidase inhibition was observed using a spectrophotometer at 404 nm.[23] As a comparison, the compound quercetin was chosen as a positive standard which is known to have the ability to treat type-2 diabetes mellitus. The percentage of inhibitory activity of each *M. mappa* bark extract and quercetin can be seen in Fig.-3.

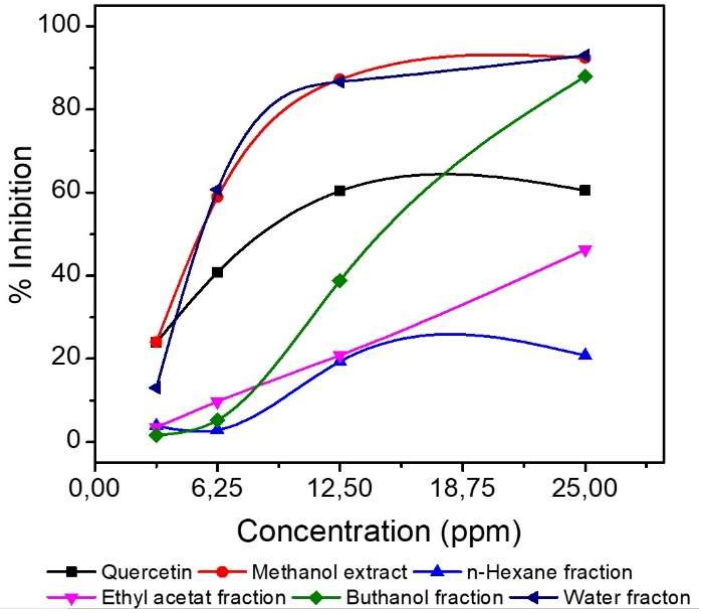

Fig.-3: The Percentage of α-glucosidase Inhibition Activity of each *M. mappa* Bark Extract and Quercetin at Various Concentrations

Based on the data in Fig.-3, it can be seen that the higher the concentration of the extract, the better the enzyme inhibitory activity. Furthermore, the inhibition (%) data of each test sample was used in determining the IC_{50} value. Based on the calculations, the IC_{50} value of the positive standard (quercetin) was 11,075 ppm with an inhibitory activity of 60.4491% and was declared active as an antidiabetic. Compared to the inhibition of the initial methanol extract and the aqueous fraction, both had better inhibitory properties than standard quercetin. The IC_{50} value of the methanol extract was 7.204 ppm with an inhibitory activity of 92.3379%, and the water fraction at 6.654 ppm with an inhibitory activity of 92.9707%. Then, the n-hexane fraction gave the weakest inhibition with an inhibitory power of 20.763% with an IC_{50} value of 335.357 ppm, followed by the ethyl acetate and butanol fractions which had medium inhibition ability with an inhibitory power of 46.326% and 87.975%, with an IC_{50} value of 26,897 ppm and 15,817 ppm, respectively. This indicates that the compounds contained in the polar fraction have better antidiabetic activity than the non-polar fraction. The percentage of α-glucosidase inhibition by each extract, fraction, and positive standard is shown in Fig.-4.

The measurement of antioxidant activity on the methanol extract and fraction of *M. mappa* bark using the free radical scavenging method (DPPH assay). The antioxidant ability of an extract or compound is assessed based on the ability to scavenge free radicals through hydrogen atoms or electron donors.[24] Antioxidant

activity is expressed in IC_{50} value, where the smaller the IC_{50} value, the more potential the sample is as an antioxidant.[25] The percentage of the free radical scavenging ability of each extract and fraction of *M. mappa* bark is shown in Fig.-5.

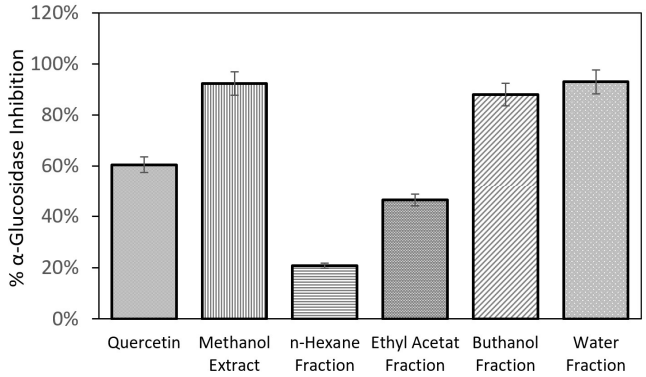

Fig.-4: The Percentage of α-glucosidase Inhibition by Each Extract, Fraction, and Positive Standard

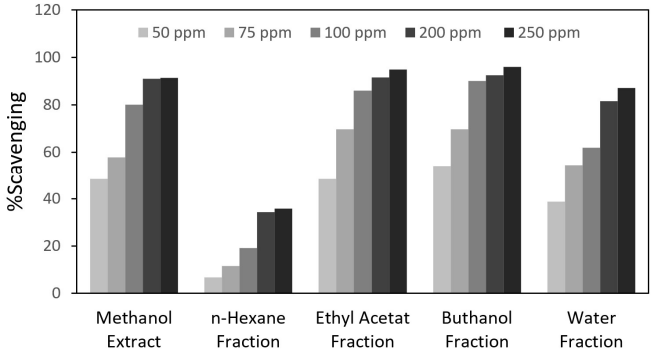

Fig.-5: The Percentage of the Free Radical Scavenging Ability of Each *M. mappa* Bark Extract and Fraction

The antioxidant ability of the initial methanol extract and the bark fraction of *M. mappa* had quite an active antioxidant activity except for the n-hexane fraction. The IC_{50} values for free radical scavenging by methanol extract, n-hexane fraction, ethyl acetate fraction, butanol fraction, and water fraction were 48.47 ppm, 326.06 ppm, 32.70 ppm, 32.34 ppm, and 77.16 ppm, respectively. Thus, the ethyl acetate and butanol fractions are the fractions that have the best antioxidant activity because they have the smallest IC_{50} value. In contrast to the toxicity test, the initial methanol extract, n-hexane fraction, ethyl acetate fraction, butanol fraction, and water fraction of *M. mappa* bark did not show any mortality in test animals (*Artemia salina*). In other words, it has a 0% mortality value for all test samples at all concentration levels. The bioactivity tests on isolated compounds (scopoletin) were also carried out on antidiabetic, antioxidant, and toxicity tests. The IC_{50} value for the ability as an antidiabetic is 15.33 ppm which is lower than the positive standard of quercetin. The ability as an antioxidant in scavenging free radicals with an IC_{50} value of 83.75 ppm, which is much lower than the standard vitamin C measured under the same conditions, namely 16.250 ppm. Like other extracts and fractions of *M. mappa* bark, scopoletin also did not have the ability as antitoxic.

CONCLUSION

In summary, the secondary metabolite isolated from the ethyl acetate fraction of *M. mappa* bark has been successfully carried out with instrument data showing scopoletin compounds. The scopoletin compound along with the methanol extract and the fraction of *M. mappa* bark showed good activity in antidiabetic tests through α-glucosidase inhibition and antioxidants through the DPPH assay but were not active in the cytotoxic test.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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