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Antidiabetic, antioxidant activities and bioactive compound profile of extracts and fractions of tawar seribu root (*Bauhinia purpurea*)

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ABSTRACT: Diabetes mellitus is a global health challenge that requires innovative solutions. This study aimed to evaluate the antidiabetic and antioxidant potentials of the root extract and its fractions of Bauhinia purpurea (Tawar Seribu). Extraction was performed using 95% ethanol as a solvent through the maceration method, followed by stepwise fractionation with n-hexane, ethyl acetate, and water. The biological activities were evaluated using the α-glucosidase enzyme inhibition assay for antidiabetic activity and the DPPH assay for antioxidant activity. The results revealed that The aqueous fraction exhibited the strongest α-glucosidase inhibitory activity (IC_{50} = 26.61 ppm), whereas the ethyl acetate fraction exhibited the highest antioxidant activity (IC_{50} = 36.73 ppm). GC-MS analysis revealed the presence of bioactive compounds such as 24-norursa-3,12-diene, olean-12-en-3-ol acetate, and lupeol, which support these biological activities. This study concludes that tawar seribu root holds great potential as a natural alternative for managing diabetes and oxidative stress, paving the way for further development as an effective and safe herbal medicine.

KEYWORDS: Antioxidant; Bauhinia; diabetes mellitus; GC-MS; α-glucosidase.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease with a steadily increasing prevalence each year and is a significant cause of mortality in Indonesia. According to the International Diabetes Federation (IDF) data from 2021, Indonesia ranks fifth in the world for the highest number of diabetes cases (aged 20–79 years). The rising incidence of DM has become a serious health issue because of its impact on the socioeconomic conditions of society, productivity, and the national economy. The economic burden of diabetes management in Indonesia was recorded at US$ 323.8 per person in 2021 and is projected to increase to US$ 370.6 by 2030 and US$ 431.7 by 2045 . Diabetes also causes complications such as heart attacks, strokes, gangrene, kidney failure, and erectile dysfunction, further deteriorating the quality of life of patients.

Current DM treatment focuses on improving the patients' quality of life through lifestyle changes, oral diabetes medications, and insulin injections. However, these treatments have limitations, including side effects such as hypoglycemia and gastrointestinal disturbances, and high costs [1]. Therefore, alternative treatments that are more affordable, have minimal side effects, and are easily accessible are needed, one of which is the utilization of natural products.

The tawar seribu root (*Bauhinia purpurea*) is a plant with potential as an alternative treatment. This plant has been used by the Dayak Meratus tribe to treat diabetes and high cholesterol [2]. Previous studies have reported that secondary metabolites, such as flavonoids, tannins, phenolics, and alkaloids, exhibit antidiabetic activity through mechanisms involving glucose metabolism regulation, liver enzyme activity, and lipid profiles [3], [4]. Additionally, antioxidants are known to be effective in reducing diabetes complications by inhibiting intracellular free radical production and enhancing the capacity of the defense enzymes against oxidative stress [5].

Other studies have shown that *Bauhinia purpurea* extracts exhibit inhibitory activity against α-glucosidase, which plays a crucial role in reducing postprandial blood glucose levels. A study also reported that bioactive compounds, such as lupeol and oleanolic acid, found in this plant have antidiabetic effects by modulating

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insulin signaling pathways and reducing insulin resistance [6]. Furthermore, the strong antioxidant activity of this extract can help prevent oxidative damage to pancreatic cells, thereby supporting insulin secretion [5].

The novelty of this study lies in its specific investigation of the root of Bauhinia purpurea, which has been traditionally utilized by the Dayak Meratus community but remains scientifically underexplored. Employing a bioassay-guided fractionation approach, this study combined α-glucosidase inhibitory assays to assess specific antidiabetic activity, DPPH assays to determine antioxidant capacity related to oxidative stress reduction, and GC–MS analysis of the most active fractions to identify potential bioactive constituents.

This integrative methodology not only provides a scientific validation for the traditional use of Bauhinia purpurea root but also broadens the phytochemical and pharmacological understanding of the species. This approach is expected to support the advancement of safer and more effective diabetes treatments derived from natural products.

▪ MATERIALS AND METHODS

Materials

The tawar seribu roots obtained from the Banjarbaru area were identified at the Faculty of MIPA, Lambung Mangkurat University. The materials used include 95% ethanol, n-hexane (Smartlab), ethyl acetate (Smartlab), aquadest (Smartlab), 2,2-diphenyl-1-picrylhydrazyl, ascorbic acid, methanol p.a, aquadest (Onemed), 100% DMSO, 0.1 M Phosphate Buffer Saline (pH 6.9), glucosidase, 4-nitrophenyl α-D-glucopyranoside, sodium carbonate, aluminum foil, glucosidase enzyme, 4-nitrophenyl α-D-glucopyranoside (4-NPP) substrate, and acarbose.

Preparation of extraction and fractionation of tawar seribu roots

The tawar seribu roots were thoroughly washed, sliced thinly, and dried. The dried simplicia was ground into a fine powder using a blender and sieved using a 40-mesh sieve. Extraction was performed using the maceration method with 95% ethanol as the solvent for 72 h, maintaining a simplicia-to-solvent ratio of 1:20. The extract was then filtered, and the resulting filtrate was concentrated using a rotary evaporator to obtain a thick extract.

The thick extract was fractionated using a stepwise liquid-liquid extraction method with n-hexane, water, and ethyl acetate as solvents. The aqueous and organic phases were mixed in a 1:1 volume ratio. Each solvent extraction step was repeated three times to ensure thorough separation. Each fraction was concentrated using a rotary evaporator until thick fractions were obtained.

Alpha-glucosidase ınhibitory activity assay

In a 96-well plate, 50 µL of buffer was added to each well, followed by 10 µL of glucosidase enzyme (1 ppm) and 20 µL of sample or standard solution, with each concentration prepared in triplicates. The plate was incubated at 37 °C for 15 min under covered conditions using aluminum foil. Next, 20 µL of 4-NPP substrate was added to each well, and the mixture was incubated for an additional 20 min. The reaction was terminated by adding 50 µL of 0.1 M Na_2CO_3 solution to each well. Absorbance was measured at 405 nm using a microplate reader [7]. Acarbose was employed as the standard (positive control) in this assay, which is a clinically approved α-glucosidase inhibitor and generally used for the therapy of type 2 diabetes mellitus. One of the critical purposes of the acarbose serial was to create a standard inhibition curve and to assess our test sample for inhibitory effect.

DPPH antioxidant assays

A total of 80 µL of sample and standard solutions were pipetted into a 96-well plate at the desired concentrations, with each concentration tested in triplicate. For the control, a blank medium was prepared by mixing 80 µL of the solvent with 80 µL of 0.1 mM DPPH solution. To each well, 80 µL of 0.1 mM DPPH solution was added in the dark. The plate was incubated at 25°C for 30 min and covered with aluminum foil to maintain darkness. After incubation, the absorbance was measured at 492 nm using a microplate reader. [8]. Ascorbic acid (Vitamin C) was used as a positive control in this assay, as it is a well-established antioxidant compound that effectively scavenges DPPH radicals. Various concentrations of ascorbic acid solutions were prepared,

and the samples were examined under the same conditions for the bonus reference standard curve and the antioxidant activity was compared.

Gas Chromatography-Mass Spectrometry (GC-MS) assay

The analysis was conducted using a Gas Chromatography-Mass Spectrometry (GC-MS) system, consisting of a Gas Chromatograph (Perkin Elmer Clarus 500, USA) and a Mass Spectrometer (Perkin Elmer Clarus SQ 8S). The column used was a Perkin Elmer Elite-5ms capillary column with dimensions of 30 m × 0.25 mm I.D. × 0.25 µm. Helium was used as the carrier gas in this study. A 1 µL sample of tawar seribu root extract was injected using the split method at a ratio of 10:1. Helium was used as the carrier gas to transport the samples through the column. The separated components were then analyzed using a mass spectrometer detector, which processed the data to identify the components in the sample based on their molecular masses.

Analysis data

Statistical analysis using one-way ANOVA was conducted after confirming that the data were normally distributed and homogeneous, to determine significant differences among the extract, n-hexane, ethyl acetate, and aqueous fractions, as well as the standard, in antioxidant and α-glucosidase inhibitory activities, with a significance difference of $p < 0.05$.

▪ RESULTS AND DISCUSSION

This study demonstrated the antidiabetic and antioxidant potential of *Bauhinia purpurea* root extracts and fractions, supported by GC-MS profiling of bioactive compounds. The extraction process utilized the maceration method with 95% ethanol as the solvent, followed by sequential fractionation with n-hexane, ethyl acetate, and water to separate the compounds based on their polarity.

Antidiabetic activity

The α-glucosidase inhibitory activity showed that the aqueous fraction exhibited the most potent inhibition (IC_{50} 26.61 ppm), followed by the n-hexane fraction (IC_{50} 43.19 ppm), ethanol extract (IC_{50} 70.74 ppm), and ethyl acetate fraction (IC_{50} 91.67 ppm). The control drug, acarbose, had a significantly lower IC_{50} (0.01 ppm), indicating its superior potency. However, the strong activity of the aqueous fraction aligns with the existing literature that highlights the role of polar compounds, such as flavonoids, tannins, and phenolic acids, as effective α-glucosidase inhibitors through interactions with the enzyme's active site [9], [10]. These findings suggest that the aqueous fraction is a promising candidate for further development as a natural antidiabetic agent.

Table 1. Antidiabetic activity measurements.

Sample	IC_{50} (ppm)
Acarbose	0.01
Water fraction	26.61
n-Hexane fraction	43.19
95% ethanol extract	70.74
Ethyl acetate fraction	91.67

Studies on flavonoids and polyphenols have consistently demonstrated their ability to modulate glucose metabolism and inhibit carbohydrate-digesting enzymes, corroborating the current results. This mechanism is particularly relevant for managing postprandial hyperglycemia, which is a critical factor in diabetes control. The relatively moderate activity of the other fractions may reflect the lower concentrations of these polar bioactives.

Antioxidant activity

The DPPH radical scavenging assay was performed to evaluate the antioxidant capacity of the extracts. This method is based on the ability of antioxidant compounds to donate hydrogen atoms to the stable DPPH radical, resulting in a color change from purple to yellow, which reflects the radical scavenging activity [11]. The ethyl acetate fraction exhibited the strongest antioxidant activity, with an IC50 value of 36.73 ppm, which could be attributed to its semi-polar nature and the presence of bioactive compounds such as flavonoids and

triterpenoids, as revealed by GC-MS analysis. The ethyl acetate fraction often exhibits strong antioxidant activity because of its ability to extract flavonoids and triterpenoids [12]. The remarkable free radical-neutralizing capacity of this fraction is significant, considering the pivotal role of oxidative stress in the onset and progression of diabetes and its complications. The IC_{50} values of the other fractions indicated a declining antioxidant potential, corresponding to their solvent polarity and the composition of their compounds. The level of antioxidant properties is influenced by the type of chemical compound and its structural composition, as well as the concentration of phenolic or flavonoid content [13].

Table 2. Results of DPPH antioxidant activity assay.

Sample	IC_{50} (ppm)
Ascorbic acid	539
Ethyl acetate fraction	3673
n-Hexane fraction	99 48
95% Ethanol extract	15189
Water fraction	47445

The IC_{50} value of the ethyl acetate fraction was not significantly different from that of ascorbic acid ($p>0.05$). The administration of antioxidants aims to reduce intracellular free radical production or enhance the effectiveness of defence enzymes against free radicals, thereby preventing oxidative stress and vascular complications associated with diabetes [13]. The role of antioxidants in managing diabetes is well established, with flavonoids functioning as scavengers of reactive oxygen species (ROS) and boosters of the body's endogenous antioxidant defenses. The variation in antioxidant capacity among the fractions highlights the critical influence of solvent polarity on the extraction of bioactive compounds.

GC-MS analysis revealed the presence of 37 compounds in the 95% ethanol extract of tawar seribu root. The mass spectra of each peak were compared with those in the library database for tentative identification. Compound identification was not only performed using automated library matching but also confirmed by comparing the obtained retention times, molecular ion peaks, and fragmentation patterns. The similarity index (SI) with the identified compounds was >70%, and a good/high degree of confidence was provided for matching the spectra [14].

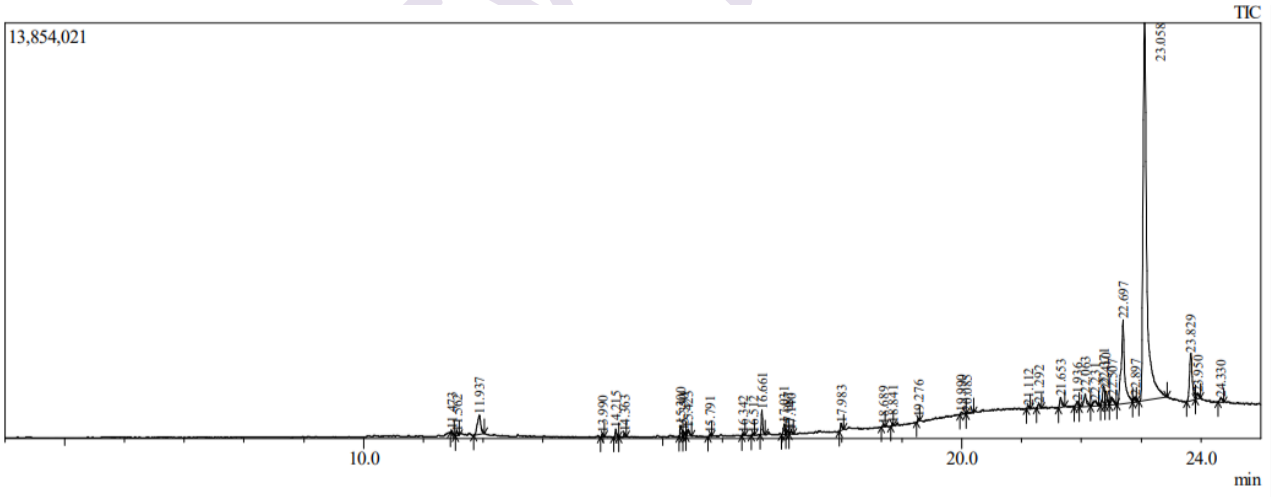

Figure 1. Chromatogram of *Bauhinia purpurea* root extract.

The major compounds identified through GC-MS analysis, including 24-Norursa-3,12-diene, Olean-12-en-3-ol acetate, and lupeol, are well recognized for their pharmacological activities. 24-Norursa-3,12-diene, a triterpenoid, dominated the composition and has been associated with anti-inflammatory and antidiabetic effects in previous studies. Similarly, Olean-12-en-3-ol acetate and Lupeol have documented roles as antioxidants and enzyme inhibitors relevant to glucose metabolism[15],16].

Tabel 3. Result of GCMS assay.

Peak number	Retention time	Area	Area (%)	Similarity	Base m/z	Compound name
34	23.058	55.992.055	61.58	88	218.10	24-Norursa-3,12-diene
32	22.697	11.665.698	12.83	94	218.10	Olean-12-en-3-ol, acetate, (3.beta.)-
35	23.829	5.314.765	5.85	84	189.05	(3S,6aR,6bR,8aS,12S,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl-icosahydro
3	11.937	2.530.890	2.78	82	87.05	3-O-Methyl-d-glucose
29	22.371	1.577.414	1.73	85	95.05	Lupeol
27	22.063	1.336.082	1.47	93	218.10	Olean-12-en-3-ol, acetate, (3.beta.)-
13	16.661	1.297.167	1.43	58	341.00	Naphthalen-1-yl(1-pentyl-1Hbenzo[d]imidazol-2-yl)methanone
30	22.410	1.216.164	1.34	72	218.10	Urs-12-en-3-ol, acetate, (3.beta.)-
25	21.653	850.098	0.93	83	280.90	β-Sitosterol
7	15.300	761.609	0.84	94	67.05	Linoleic acid ethyl ester

The combined effects of these metabolites may amplify the biological activities observed in this study, reinforcing the therapeutic potential of *Bauhinia purpurea* as a natural source of antidiabetic and antioxidant agents. This highlights the need for further in vivo studies to validate these effects and investigate the possible synergistic effects of these compounds.

▪ CONCLUSION

These findings establish a scientific basis for the traditional use of *Bauhinia purpurea* roots in diabetes management, particularly for its dual activity in glucose regulation and oxidative stress mitigation. The aqueous and ethyl acetate fractions, in particular, emerged as promising candidates for further investigation and development as herbal therapeutics.

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Conflict of interest statement: The authors declare no conflicts of interest.

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