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Original Research Article

Effects of Solvent Variation on The Antioxidant, Anti-Inflammatory, and Alpha-Glucosidase Inhibitory Activity of *Andrographis paniculata* (Burm.f.)Wall Leaves Extract

Candra Irawan[1] , Riri Enriyani[2]*, Ismail[2], Maman Sukiman[3], Imalia Dwi Putri[1], Andita Utami[2], Lintannisa Rahmatia[2], Anisa Lisandi[2]

[1]*Department of Food Nanotechnology Politeknik AKA Bogor, Bogor, Indonesia 16158*
[2]*Department of Analytical Chemistry Politeknik AKA Bogor, Bogor, Indonesia 16158*
[3]*Department of Industrial Waste Treatment, Politeknik AKA Bogor, 16158, Indonesia*

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ABSTRACT

Andrographis paniculata (Burm.f.) Wall, Acanthaceae, is a plant that grows in the lowlands at 700 meters above sea level. This study aimed to determine the antioxidant activity, alpha-glucosidase enzyme inhibition, and anti-inflammatory activity of *Andrographis paniculata* (Burm.f.) Wall leaf extract using ethanol and butanol as extraction solvents. The extraction process was accomplished using the Ultrasonic Assist Extraction (UAE) method. The result showed that ethanol and butanol extract yields were 8.88% and 9.02%, respectively. The ethanol and butanol extracts exhibited potent antioxidant activity with IC_{50} values of (2 – 45) mg/L from the FRAP assay. The extracts also demonstrated potent anti-inflammatory activity with an IC_{50} range of 0.5-15 mg/L. Also, the ethanol extract showed good alpha-glucosidase inhibitory activity with IC_{50} = 9.49 ± 0.04 mg/L. The butanol extract showed inhibitory activity against alpha-glucosidase enzyme with an IC_{50} value of 29.12 ± 0.019 mg/L. The results also showed that the antioxidant, anti-inflammatory, and alpha-glucosidase inhibitory activity of the ethanol leaf extract of *Andrographis paniculata* (Burm.f.) Wall was slightly higher but not significantly different from butanol; as such, butanol could be used as an alternative solvent to ethanol.

***Keywords*:** Ultrasonic Assist Extraction, antioxidant, anti-inflammatory, alpha-glucosidase, *Andrographis paniculata* (Burm.f.) Wall.

Introduction

Diabetes mellitus (DM), also referred to as hyperglycemia, is characterized by elevated blood glucose levels. DM can be treated with oral hypoglycemic drug therapy (OHO), specifically designed for individuals with type II diabetes. One method of oral antidiabetic therapy with OHO is by using alpha-glucosidase inhibitor (AGI) agent, which increases the absorption of glucose by catalysing the hydrolytic cleavage of oligosaccharides into monosaccharides in the small intestine. This process results in an increase in blood glucose levels after a meal. AGI is required to limit or delay the intestinal concentration of glucose, thereby preventing an increase in post-prandial blood glucose levels.[1,2]

AGIs are antidiabetic agents that inhibit alpha-glucosidase enzymes. Meanwhile, reducing intestinal absorption of dietary carbohydrates is a treatment for post-prandial hyperglycemia.[3] Before entering the blood circulation through epithelial absorption, complex polysaccharides are hydrolyzed by amylase enzyme and alpha-glucosidase to form dextrins and glucose, respectively. Amylase and acarbose are synthetic AGIs commonly used to treat type II DM, but these medications have been reported to induce a variety of adverse effects.[4]

*Corresponding author. E mail: enriyani.riri@gmail.com
Tel: +6281267213539

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Long-term use of acarbose can cause several adverse effects, including disturbances in the digestive tract system, such as nausea, vomiting, abdominal pain, and bloating. Consequently, considering natural medicines as an alternative therapy is vital because of the potential and minimal side effects. As a result, efforts have been made to advance AGI from natural sources to treat diabetes. Previous studies showed that combining AGI and antioxidants was more effective in type II DM prophylaxis.[5,6]

Andrographis paniculata (Burm.f.) Wall grow in the lowlands at 700 meters above sea level. The plant thrives at temperatures of 25-32°C with moderate humidity and light intensity and at a pH range of 5.5 - 6.5.[7] The leaves contain diterpene lactones and glycosides, such as anndrographolide, deoxyandrographolide, 11, 12-didehydro-14 eoxyandrographolide, neoandrographolide, and flavonoids. Andrographolide content in the leaves has been shown to stimulate insulin release and inhibit glucose absorption by restraining alpha-glucosidase enzymes and alpha-amylase.[8] This study investigated the antioxidant, alpha-glucosidase inhibitory (AGI), and anti-inflammatory activity of *Andrographis paniculata* (Burm.f.) Wall leaf extract using two different organic solvents (ethanol and butanol) for extraction.

Materials and Method

The leaves of *Andrographis paniculate* (Burm.f.) Wall were collected in the at Politeknik AKA Bogor (February, 2023). The plant sample was identified by a Taxonomist (*Andrographis paniculata* (Burm.f.) Wall) at National Research and Innovation Agency, Indonesia, a herbarium specimen with a voucher number: B-104/II.6.2/IR.01.02/2/2023 was deposited at Politeknik AKA Bogor

Preparation and Extraction of plant material
The fresh leaves of *Andrographis paniculata* (Burm.f.) Wall were washed with water, dried under shade for a week, and ground to powder. The powdered leaf samples (5 kg) weighed into separate beakers were extracted with 40 L each of ethanol and butanol by ultra-sonification for 30 minutes at ambient temperature with an amplitude of 0.6 m.

Total of Phenolic Content Determination
The total phenolic content of the extracts was determined by the Folin-Ciocalteu reagent method. Briefly, 400 µL of the crude extract (1 mg/mL) was diluted to 6 mL with distilled water and thoroughly mixed with 1 mL Folin-Ciocalteu reagent for 3 minutes. This was followed by adding 2.5 mL of 10% (w/v) sodium carbonate and 10 mL of distilled water and then homogenised. The mixture was allowed to stand in the dark for 60 minutes before the absorbance at 650 nm was measured. Gallic acid, at concentrations of 0, 2, 4, 6, and 8 mg/L, was used to generate the calibration curve to determine the total phenolic content of the extracts. The results were expressed as milligrams of gallic acid equivalent per gram of extract weight.[9]

Antioxidant Activity by Ferric Reducing Antioxidant Power (FRAP)
The extract (5 mg) was dissolved in 5 mL ethanol to give a sample concentration of 1 mg/L. The solution was pipetted at 40, 80, 160, 320, and 640 µL separately into 5 mL measuring flasks. Furthermore, 0.4 mL of 0.001 M citric acid, 0.2 mL of 0.002 M Fe^{3+} solution, and 0.4 mL of 0.2% o-phenanthroline solution were added to the extracts to make extract contractions of 8, 16, 32, 64, and 128 mg/L, respectively. The solutions were then filtered and homogenised. After incubating the solution at 37°C for 35 minutes, the absorption at 510 nm was measured using a visible light spectrophotometer. This procedure had two iterations, namely the use of a gallic acid comparator with concentrations of 0.5, 1.0, and 1.5 mg/L, and an identical procedure was also carried out.[10]

Evaluation of Anti-Inflammatory Activity
The extract solutions (500 µL) and diclofenac sodium with 0.2% BSA were diluted to a final volume of 5 mL. The dilutions yielded 12.5, 25, 50, 100, and 200 parts per million (ppm). The mixtures were incubated at 37°C for 15 minutes. After 5 minutes of warming at 70°C, the mixture was allowed to settle to room temperature. The solution was agitated vigorously, the agar in the tube was shaken to prevent clustering, and the absorbance measurements were taken at the wavelength of 660 nm.[11]

Alpha-Glucosidase Inhibitory Activity
The standard reference (acarbose) and the sample were weighed and dissolved in phosphate buffer pH 6.8. Initially, intractable samples in phosphate buffer were dissolved in DMSO at a concentration of 10%. Standard solutions and specimens were diluted to multiple concentrations. After adding 30 µL of standard solution and 17 µL p-Nitrophenyl-α-D-glucopyranoside (PNPG) substrate to each sample, the solution was incubated for 5 minutes at 37°C and 17 µL alpha-glucosidase solution was added. The solution was re-incubated at 37°C for 15 minutes, and 100 µL of 200 mM sodium carbonate was added. The absorbance of the solution was measured using a microplate reader at 405 nm. After the initial incubation, 100 µL of 200 mM sodium carbonate and 17 µL alpha-glucosidase solution were added, respectively, for the control test. The absorbance of the solution was measured using a microplate reader at 405 nm, as shown in Table 1.
The percentage of alpha-glucosidase inhibition was calculated from the equation below: equation:

% Inhibition=((A_0-A_1))/A_0 x100%

A0 control = absorbance of the blank corrected by the control blank
A1 = Sample absorbance corrected by the control sample

IC50 value was calculated using the regression equation y = bx + a, where the x and y-axis represent sample concentration and the percentage of inhibition, respectively.[12]

Statistical analysis
All data were presented as mean ± standard deviation (SD) of at least three replicates.

Table 1: *In-vitro* alpha-glucosidase test procedure

Reagent	Volume (µL)			
	Blank Control (KB)	**Blank (B)**	**Sample Control (KS)**	**Sample (S)**
Standard reference/Sample	-	-	30	30
Dapar Fosfat pH 6.8	66	66	36	36
PNPG (0,25; 0,5; 1; 2; 3; dan 4 mM)	17	17	17	17
Incubation at 37ºC, 5 minutes				
Alpha-glucosidase (Optimum Concentration)	-	17	-	17
Sodium Carbonate 200 mM	100	-	100	100
Incubation at 37ºC, 15 minutes				
Alpha-glucosidase (Optimum Concentration)	17	-	17	-
Sodium Carbonate 200 mM	-	100	-	100

Result and Discussion

The solvent of extraction plays a significant role in plant extracts' phytochemical composition and pharmacological activity. This study explores the role of solvent variation in the antioxidant, anti-inflammatory, and alpha-glucosidase inhibitory activity of *Andrographis paniculata* (Burm.f.) Wall leaf extract. Ultrasonic-assisted extraction (UAE) was used to extract the phytochemicals in the plant leaf using ethanol and butanol as extracting solvents. The extract yields are reported in Table 2. The result showed that butanol extract of *Andrographis paniculata* (Burm.f.) Wall leaves had a higher yield than ethanol. This is closely related to the polarity of the phytoconstituents contained in the extract and the solvent used. Polar compounds dissolve only in polar solvents, whereas nonpolar compounds dissolve only in nonpolar solvents.[13]
Total phenolic content was determined using the Folin-Ciocalteu method. The colour changes from yellow to blue when the Folin-Ciocalteu reagent interacts with a phenolic compound. The observed blue colour is proportional to the concentration of phenolic ions formed. A greater concentration of phenolic compounds results in a higher formation of ions, resulting in a more intense blue colour.[14] Only alkaline solutions contain phenolics, but the Folin-Ciocalteu reagent and its derivatives are unstable in alkaline conditions. The addition of Na_2CO_3 to the phenolic test was intended to establish an alkaline

condition, facilitating the Folin-Ciocalteu reduction reaction of the hydroxyl groups in the phenolic compounds of the sample.[15] Figure 1 shows the gallic acid standard curve using the linear regression formula y = 0.0884x + 0.0122 and the R2 value of 0.9979. From the gallic acid linear regression equation, the total phenolic content in the ethanol extract was (1105.43 ± 0.5) mg/L, and the butanol extract was (70.51 ± 0.4) mg/L, as shown in Table 3. This difference in the two extracts' total phenolic content (TPC) was significant. The TPC of the ethanol extract was greater than that of butanol due to its higher polarity. Therefore, ethanol has the potential to attract hydroxyl groups in aromatic compounds in plant samples, which is directly proportional to the antioxidant content.[16]

Similarly, FRAP assay was used to assess the antioxidant activity of the plant extracts. The result showed that electrons were transferred from *Andrographis paniculata* (Burm.f.) Wall leaf extract to the complex compound Fe^{3+} -TPTZ. Fe^{3+} -TPTZ represents an oxidizing compound that may be present in the body and can damage cells. Under these conditions, Fe^{3+} ions are reduced to Fe^{2+} ions, as shown by the formation of blue colour in the solution[17]. From the study results, the standard linear regression equation for BHT, ethanol, and butanol extracts was obtained, as shown in Table 4.

IC50 value of each extract and BHT standard was determined using the linear regression equation. Specifically, a compound has a "very strong" antioxidant when the IC_{50} value is less than 50 ppm, "strong" when the value is between 50 and 100 ppm, and "weak" for 150 - 200 ppm[18]. Standard BHT, ethanol, and butanol extracts of *Andrographis paniculata* (Burm.f.) Wall leaves exhibited highly potent antioxidant activity with IC50 value < 50 ppm, namely (0.76 ± 0.41) mg/L, (2.63 ± 0.05) mg/L, and (40.89 ± 0.01) mg/L, respectively. However, ethanol extract had a lower IC_{50} value than butanol. This value is directly proportional to the total phenolic value of each extract, with IC50 for antioxidant activity decreasing as the total phenolic value increases[19]. The extract was also evaluated for anti-inflammatory and antidiabetic potential.

The anti-inflammatory study results are shown in Table 5. The anti-inflammatory activity of the two extracts was compared to that of Diclofenac sodium, the positive control agent. The extracts exhibited potent anti-inflammatory action, with ethanol extract showing better activity (7.76 ± 0.11) mg/L than butanol extract (14.68 ± 0.025) mg/L. Both extracts were, however, less potent than the positive control agent, diclofenac sodium. IC_{50} value of diclofenac sodium is determined to quantify the anti-inflammatory activity of medicinal agents.[20] Table 5 shows IC_{50} values of the positive control, ethanol, and butanol extracts through the linear equation. The IC_{50} values of the extracts range from 8 to 15 mg/L, implying that the plant possesses good anti-inflammatory phytochemicals and can be further explored for anti-inflammatory leads for drug development. Literature references abound of natural products for the treatment of inflammation. Plants have been a major source of new chemical entities with different biological activity, including anti-inflammations. Medicinal plants have been investigated in in vitro and in vivo studies for their anti-inflammatory activities, and these have been shown to exhibit potent anti-inflammatory activity via different mechanisms, reduction of inflammatory mediators (cytokines, PGs), inhibition of COX and NF-kB activities.[21,22]

Inhibitors of alpha-glucosidase enzymes, such as acarbose, operate by delaying glucose absorption in the intestine to prevent a rise in post-prandial blood sugar levels. Therefore, alpha-glucosidase enzyme is one of the target enzymes for type II DM treatment[23]. This study evaluated ethanol and butanol extracts of *Andrographis paniculata* (Burm.f.) Wall leaves as potential AGI against alpha-glucosidase enzymes using acarbose as a standard. The activity of the extracts indicated by their inhibitory potential against alpha-glucosidase enzyme (IC_{50}) was computed from their linear regression equations shown in Table 6. The IC50 values of the extract were compared to the standard acarbose, as shown in Figure 2. Based on the IC_{50} value, antidiabetics can be divided into three categories, namely "very active," "active," and "inactive" at IC50 of < 11 mg/L, 11-100 mg/L, > 100 mg/L, respectively[24,25]. The result showed that the standard acarbose and butanol extract exhibited inhibitory activity against alpha-glucosidase enzymes and fell within the 'active' category with IC_{50} values of (36.58 ± 0.061) mg/L and (29.12 ± 0.019) mg/L, respectively. In contrast, the ethanol extract exhibited potent antidiabetic activity with an IC_{50} value of (9.49 ± 0.04) mg/L, which fell into the very active category.

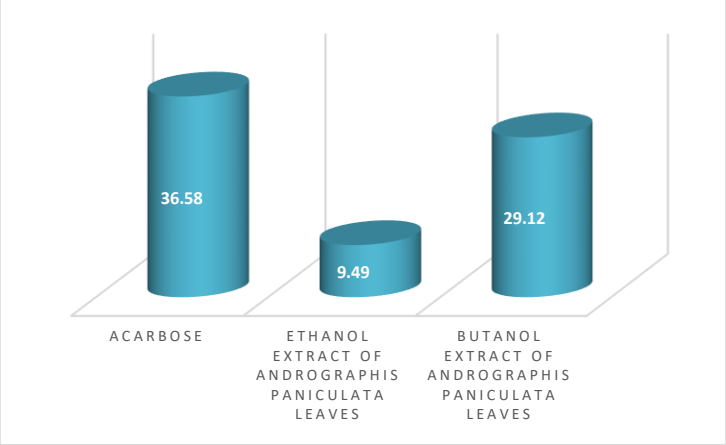

Figure 2: IC_{50} values of acarbose, ethanol, and butanol extracts of *Andrographis paniculate* (Burm.f.) Wall leaves against alpha-glucosidase

Table 2: Yield of ethanol and butanol extracts from *Andrographis paniculate* (Burm.f.) Wall leaves

Sample	Solvent	Mass of sample (g)	Mass of extract (g)	Yield (%)
Andrographis paniculate (Burm.f.) Wall leaves	Ethanol	21.5213	1.9120	8,88
	Butanol	46.2605	4.1727	9.02

Table 3: Total phenolic content of ethanol and butanol extracts of *Andrographis paniculate* (Burm.f.) Wall leaves

Sample	Solvent	Total phenolic content (mg GAE/g extract)
Andrographis paniculate (Burm.f.) Wall leaves	Ethanol	1105.43 ± 0.5
	Butanol	70.51 ± 0.4

Table 4: Antioxidant activity of *Andrographis paniculate* (Burm.f.) Wall leaves using FRAP method

Sample	Pelarut	Linear regression	R^2	IC_{50} (mg/L)
BHT		y = 82.108x - 12.782	0.9941	0.76 ± 0.41
Andrographis paniculate (Burm.f.) Wall leaves	Ethanol	y = 15.105x + 10.1499	0.9889	2.63 ± 0.05
	Butanol	y = 0.6782x + 22.268	0.9801	40.89 ± 0.01

Table 5: Anti-inflammatory activity of ethanol and butanol extracts from *Andrographis paniculate* (Burm.f.) Wall leaves

Sample	Solvent	Linear regression	R^2	IC_{50} (mg/L)
Positive Control		y = 32.896x - 6.3119	0.9979	1.71 ± 0.027
Andrographis paniculate (Burm.f.) Wall leaves	Ethanol	y = 5.0417x + 10.864	0.9944	7.76 ± 0.11
	Butanol	y = 1.4926x + 28.188	0.9999	14.68±0.025

Table 6: Alpha-glucosidase inhibitory (AGI) activity of ethanol and butanol extracts from *Andrographis paniculate* (Burm.f.) Wall leaves

Sample	Solvent	IC_{50} ± SD	Linear regression	R^2
Acarbose		36.58 ± 0.061	y = 1.0093x + 13.079	0.9882
Andrographis paniculate (Burm.f.) Wall leaves	Ethanol	9.49 ± 0.04	y = 2.69x + 24.453	0.9865
	Butanol	29.12 ± 0.019	y = 0.6865x + 30.009	0.9809

Conclusion

In conclusion, this study revealed that the solvent used in extracting plant material plays a significant role in the biological activity of the extracts. It shows that the polarity of the solvent influences the class of compounds extracted and its pharmacological activity. The extracts showed good antioxidant, anti-inflammatory, and alpha-glucosidase inhibitory activities. In all the investigations, the ethanol extract exhibited better activity than butanol. In particular, the ethanol extract significantly inhibited the *α*-glucosidase enzyme with an IC_{50} value of 9.49 ± 0.04 mg/L. Results showed that the leaf extract of *Andrographis paniculata* (Burm.f.) Wall contained phytoconstituents with potential antidiabetic properties and could be further explored for developing drugs for managing diabetes and oxidative-related diseases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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