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The potential of five wild growing aromatic plants from Hemaq Beniung Customary Forest, West Kutai District, Indonesia on antidiabetic activity

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Abstract. *Kuspradini H, Putri AS, Kiswanto, Sa'adah H, Fajriansyah, Rizqullah MA, Larasati AG, Zulfa NA, Egra S, Yamauchi K, Mitsunaga T. 2023. The potential of five wild growing aromatic plants from Hemaq Beniung Customary Forest, West Kutai District, Indonesia on antidiabetic activity. Biodiversitas 24: 2156-2162.* Several aromatic plants have been used for millennia across the globe for their hyperglycemic properties. The in vitro inhibitory activity of extracts from *Blumea balsamifera* (L.) DC., *Cinnamomum parthenoxylon* (Jack) Meisn., *Clausena excavata* Burm.fil., *Neouvaria acuminatissima* (Miq.) AiryShaw, and *Piper porphyrophyllum* N.E.Br. were tested. The aim of this study was to determine the phytochemical constituent, total phenolic and flavonoid content of five aromatic plant extracts obtained from West Kutai, East Kalimantan, Indonesia as well as the antioxidant and antidiabetic activity via in vitro assays. Assays for total phenol and flavonoid content were based on Follin-Ciocalteu and aluminum chloride reagents, respectively. In this study, the antidiabetic activity was determined by measuring the inhibitory activity of α-glucosidase enzyme, while the in vitro antioxidant activity was measured using the DPPH scavenger method. The order of inhibition of DPPH free radical was *P. porphyrophyllum* ethanol extract > *N. acuminatissima* ethanol extract > *B. balsamifera* ethyl acetate extract, which was found to be directly proportional to their total phenolic content. On the other hand, the order of inhibition of α-glucosidase enzyme was found to be *N. acuminatissima* ethanol extract > *C. parthenoxylon* ethanol extract > *N. acuminatissima* ethyl acetate extract. Out of the 10 extracts studied, only 3 showed inhibition activity on α-glucosidase. These findings indicate the aromatic plant extract's potential as a natural therapy for preventing and treating carbohydrate metabolic problems. As a result, the *N. acuminatissima* ethanol extract, which exhibits both antidiabetic and antioxidant activity, can be used in the pharmaceutical industry for the production of functional foods and herbal medicines. This is our first report on *N. acuminatissima* as an antioxidant and antidiabetic agent.

Keywords: *Blumea balsamifera*, *Cinnamomum parthenoxylon*, *Clausena excavata*, *Neouvaria acuminatissima*, *Piper porphyrophyllum*

INTRODUCTION

Medicinal and aromatic plants have been used since ancient times. Because of their therapeutic and inherent pharmacological qualities, aromatic herbs are frequently employed as natural remedies. Plants' low-volume production of secondary metabolites, in particular, is the basis for their therapeutic qualities. Medicines, flavors, colors, and scents are all examples of secondary metabolites' many uses (Abdulhafiz 2022). Phytochemicals, also known as secondary metabolites, are substances found in plants. These compounds have a well-defined chemical structure, which allows for further categorization. Glycosides, alkaloids, flavonoids, phenolics, terpenoids, and saponins make up the largest group of secondary metabolites (Roopan and Madhumitha 2018). Plant-based medicines have shown promising results in treating various illnesses, but many plant and the active compounds they yield remain poorly characterized. It is well known that aromatic plants possess antioxidant activity. Growing circumstances, processing/extraction methods, and, most significantly, antioxidant contents all have a role in determining whether or not aromatic plants have an anti-inflammatory or antioxidant effect (Dolghi et al. 2022).

The increased number of diabetic patients is mainly (>95%) type 2 diabetes that develops due to insulin resistance and pancreatic β-cell dysfunction, leading to hyperglycemia (Lacroix et al. 2014). The mechanisms of action of natural compounds that inhibited enzyme activity were presented in numerous pathways, such as the inhibition of alpha-glucosidase. Anti α glucosidase activity is a typical model for studying anti-hyperglycemic activity (Abbas et al. 2017). The postprandial glucose surge results from the digestion of dietary starch by carbohydrate hydrolyzing enzymes like α-glucosidase, which breaks down the oligosaccharides into glucose. Type 2 diabetes

patients can manage hyperglycemia to some extent by decreasing glucosidase production, and the medication acarbose is the leading alpha-glucosidase inhibitor available. Researchers have recently investigated natural sources like plants for alpha-glucosidase inhibitors and discovered many secondary metabolites, such as alkaloids, flavonoids, phenols, and terpenoids, which show promise as potential candidates. Therefore, developing inhibitors from natural products is an alternative strategy for controlling hyperglycemia.

Moraceae, Cucurbitaceae, Liliaceae, Anacardiaceae, Myrtaceae, Fabaceae, Solanaceae, Asteraceae and many other families reported having antidiabetic properties (Qais et al. 2018). Natural compounds that were investigated from natural resources were significantly reported to have anti-alpha glucosidase activity and exhibited high potential effects, such as terpenes, alkaloids, quinones, phenolics (flavonoids, phenols, phenylpropanoids), steroids, and other compounds (Yin et al. 2014).

West Kutai, located in East Kalimantan, is known for its many aromatic plants, some of which have traditionally been used for medicinal purposes (Kusuma et al. 2014; Matius et al. 2018; Lestari and Syafah 2019; Paramita et al. 2022; Sundari et al. 2022). However, only a few of these plants have been scientifically validated for their medicinal properties, such as those with antidiabetic effects. In this study, we used a medicinal and aromatic plants approach to test 5 aromatic plant species in West Kutai against the α-glucosidase enzyme and DPPH free radical.

The study focused on five aromatic plants: *Blumea balsamifera* (L.) DC., *Cinnamomum parthenoxylon* (Jack) Meisn., *Clausena excavata* Burm.fil., *Neouvaria acuminatissima* (Miq.) AiryShaw, and *Piper porphyrophyllum* N.E.Br. Specifically, some of these plants were chosen because they are part of plant genera that have been found to exhibit antidiabetic activity. For example, *B. balsamifera* (Asteraceae) leaves are traditionally used for treating diabetes in India (Roy et al. 2013), while in West Java and North Sumatra, Indonesia, decoctions and juice preparations from *B. balsamifera* leaves have been used to treat diabetes (Emilda and Heriyati 2017; Raja et al. 2018). Clausena, which belongs to the family Rutaceaea, is a rich source of secondary plant substances, including alkaloids, coumarins, limonoids, and essential oils (Arbab et al. 2012). *C. excavata* root has been reported to have potential antidiabetic effects (Thant et al. 2019). Several plant species from the *Piperaceae* and *Cinnamommum* genera have been traditionally used to treat various diseases, including diabetes. Cinnamon, a ubiquitous medicinal herb, is consumed daily by people all over the world. In addition to its antimicrobial properties, cinnamon has been shown to reduce the incidence and severity of diabetes and its complications (Kuspradini et al. 2016a; Al-Samydai et al. 2018; Kuspradini et al. 2022).

However, there is currently no specific information available on the antidiabetic properties of *P. porphyrophyllum*, *C. parthenoxylon* and *N. acuminatissima* species. The genus Neouvaria belongs to the family Annonaceae. Although extracts from *N. acuminatissima* have been found to have antiplasmodial activity, information on the phytochemical constituents and other biological activities of the genus *Neouvaria* or its plant species is still lacking.

Our bioprospecting research aims to discover antioxidant and antidiabetic agents from medicinal and aromatic plants located in East Kalimantan. As part of this research, we seek to determine the inhibitory potential of 10 extracts derived from 5 aromatic plants that are found in West Kutai, East Kalimantan, against alpha-glucosidase, which is a crucial enzyme involved in the breakdown of carbohydrates. Additionally, we intend to identify the antioxidant properties and phytochemical constituents of these plants. The free radical DPPH method will be utilized to assess the capacity of antioxidants to neutralize radicals.

MATERIALS AND METHODS

Time and location

This research was conducted from February to June 2022 in Pepas Asa Village, West Kutai District, East Kalimantan, Indonesia (-0.165978°N, 115.681712°E) (Figure 1). The laboratory experiments were conducted at the Forest Product Chemistry and Renewable Energy Laboratory, University of Mulawarman, Indonesia.

Sampling and extraction

Five aromatic plants (*Piper porphyrophyllum* N.E.Br.; *Blumea balsamifera* (L.) DC.; *Neouvaria acuminatissima* (Miq.) AiryShaw; *Cinnamomum parthenoxylon* (Jack) Meisn.; *Clausena excavata* Burm.fil.) were collected from Customary Forest of Hemaq Beniung. All plant species were identified by a taxonomist from the Forestry Faculty, Universitas Mulawarman, Samarinda, Indonesia.

After being air-dried, the samples were ground into powder in a mechanical grinder. In that order, maceration with n-hexane, ethyl acetate, and ethanol solvents was used to extract the powder (10 g). After 24 hours of n-hexane extraction at a 1:1 (w/v) ratio, the resulting solution was filtered. Additional extraction was performed using ethyl acetate by leaving the residue in the solvent at a ratio of 1:1 (w/v) for 24 hours. Extract ethyl acetate was made from the filtrate. Ethanol was used to extract the residue at a ratio of 1:1 (w/v) for 24 hours, and the resulting filtrate was collected as ethanol extract. Orbital shakers were used for all of the extraction. Extract powder was dry and used to calculate the yield percentage (Sulmartiwi et al. 2018). Extract (g)/dried sample (g) 100 was used to determine the yield (D'Auria et al. 2021). In addition, the phytochemical, antioxidant, and alpha-glucosidase inhibitory activities were studied using an ethyl acetate and ethanol extract.

Phytochemical analysis

Qualitative analysis

This plant underwent a qualitative phytochemical test to determine whether or not it contained any active chemicals. The Kokate technique evaluated alkaloid, flavonoid, and tannin availability. The carotenoid and coumarin levels were determined using the Senthilmurugan method, whereas the saponin, carbohydrate, triterpenoid, and steroid levels were determined using the Harborne and Kokate method (Harborne 2013; Kokate et al. 2017). The visual color change reaction is used as a primary indicator of the presence of a given phytochemical compound in all these qualitative determinations.

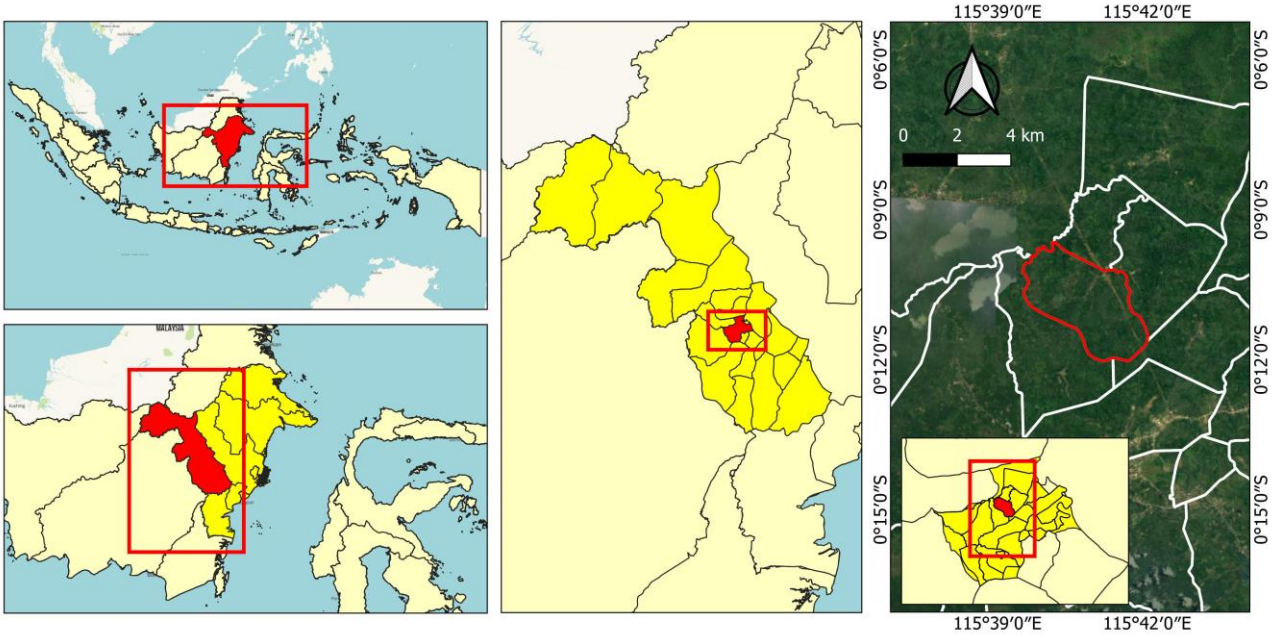

Figure 1. Customary Forest of Hemaq Beniung, Pepas Asa Village, Kutai Barat, East Kalimantan, Indonesia

Quantitative analysis

The microplate Total Phenolic Content (TPC) method was based on the 96-well microplate Folin-Ciocalteu method given by Bobo-García et al. (2015) with some modifications. A total of 20 µL of the diluted extract were mixed with 100 µL of Folin-Ciocalteu reagent in a flat-bottom 96-well microplate. The mixture was left for 5 minutes and then 100 µL of sodium carbonate solution (7.5%) was added and the mixture was shaken at medium-continuous speed for 1 min. After 30 minutes at room temperature, the absorbance was measured at 730 nm using the microplate reader. The absorbance of the same reaction with methanol (blank) instead of the extract or standard was subtracted from the absorbance of the reaction with the sample. Gallic acid dilutions (0, 25, 50, and 75ppm) were used as standards for calibration. TPC was expressed as mg of gallic acid equivalents per gram of dry weight (mg QE/g DW), through a calibration curve with gallic acid.

Total Flavonoids Content (TFC) was analyzed with aluminum chloride reagent using the colorimetric method adapted from Koley et al. (2019) with a slight change. Aliquots (10 µL) of each extract's / blank / standard accession were placed in 96-well plate containing 10 µL of 10% aluminum chloride, 6 µL of methanol, and 10 µL of 1 M CH3COOK. The mixtures were incubated for 30 min at room temperature in the dark. The absorbance at 415 nm was measured. Gallic acid dilutions (0, 25, 50, 75, dan 100 ppm) were used as standards for calibration. TFC was expressed as mg of quercetin equivalents per gram of dry weight (mg QE/g DW), through a calibration curve with quercetin. All samples were performed in three replicates.

DPPH free radical scavenging assay

To determine antioxidant activity using the DPPH free radical scavenging method, adopted from a method of Kuspradini et al. (2016b). An aliquot 33 µL of the diluted extract was mixed with 467 µL and 500 µL of 2.7% DPPH. The final concentration of samples 200, 100, 50, 25, 12.5 ppm. The solution was incubated for 20 minutes and absorbance was measured at 517 nm. Ascorbic acid was used as comparative control.

α-glucosidase inhibitory assay

Inhibition of α-glucosidase activity was determined using yeast α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (pNPG) as described before with slightly modification (Poovitha et al. 2016). Fifty microliters of a 0.1 M phosphate buffer (pH 7.0), twenty-five microliters of 0.1 mM 4-nitrophenyl-D-glucopyranoside (in 0.1 M phosphate buffer, pH 7.0), ten microliters of a test sample (at a concentration of 500 g mL-1), and twenty-five microliters of α-glucosidase solution (0.04 unit mL-1) made up. The resulting reaction mixture was then kept in an incubator at 37 degrees Celsius for 30 minutes. The reaction was halted after adding 100 µL of 0.2 M sodium carbonate solution. To follow the enzymatic hydrolysis of the substrate, we measured the amount of p-nitrophenol released in the reaction mixture at 410 nm. All experiments were done in triplicate. All experiments were done in triplicate.

RESULTS AND DISCUSSION

Sample collection and yield

Five aromatic plants were collected from Customary Forest (*Hutan Adat*) Hemaq Beniung, Pepas Asa Village, Kutai Barat, East Kalimantan, and identified. The five plants are shown in Figure 2.

The extraction efficiency of ethanol and ethyl acetate solvents was analyzed. The extraction yield did not change noticeably between solvent types, except for *C. excavata* extracts (Table 1).

In addition to the extraction method, the solvent is crucial in determining the extract's yield and biological activity. Extraction through a series of macerations with progressively more polar solvents is the second most common method. Which solvent is best for extraction relies on the compounds of interest and the plant materials used (D'Auria et al. 2021).

Phytochemicals

The results obtained from the phytochemical screening conducted on the ethyl acetate- and ethanol extracts are presented in Table 1. All the samples contained alkaloids, flavonoids and carbohydrates. Carotenoids, tannins, and saponins were absent in all extracts in this study. Steroid, coumarin and triterpenoid were only detected in *P. porphyrophyllum* (ethyl acetate extract), *P. porphyrophyllum* (ethanol extract), and *C. excavata* (ethyl acetate extract), respectively. There were several reports that alkaloids, triterpenes, and flavonoids could inhibit a-glucosidase (Zafar et al. 2016; Proença et al. 2017; Ur Rehman et al. 2018).

We found the phytochemical results studied here (Table 2) were different from the previous study. According to the previous study, chloroform extract of *P. porphyrophyllum* obtained by the soxhlet extraction method shows the presence of alkaloids and flavonoids (Yudi and Nugroho 2017). However, our study found additional compounds such as steroids in the ethyl acetate extract and coumarin in the ethanol extract, in addition to alkaloids and flavonoids. Several studies have shown that B. balsamifera contains over a hundred phytochemical compounds, some volatile and others not. Compounds belonging to the polyphenolic, fatty acid, terpenoid, and flavonoid classes were found on them (Widhiantara and Jawi 2021). Wei et al. (2017) identified two benzenoids, one chromene dimer, two steroids, and three flavonoids in the leaves of *C. parthenoxylon*. However, in our study, we did not observe the presence of steroid compounds. Different extraction and purification techniques have been used to isolate a wide variety of secondary metabolites from *C. excavata*. These metabolites primarily include alkaloids, coumarins, and a small number of limonoids and essential oils, which have a pleasant odor (Guo et al. 2018). Phytochemical content in plants is highly sensitive to extraction techniques and solvents. Besides that, the environmental factors (biotic or abiotic) on plant growth dependent on altitude might affect the production of specialized metabolites (Volf et al. 2022).

Table 3 represents the analytical data for total phenolics and flavonoid content of the ethyl acetate and ethanol extracts of five aromatic plants. Data clearly show the amount of total phenolic and flavonoid content. Most ethanol extracts were higher than the ethyl acetate extract in the levels of TPCs in this study. This might be caused by the possible complex formation of some phenolic compounds in the extract that is soluble in ethanol solvent. Babbar et al. (2014) stated that the total phenolic content in the extract depends on the polarity of the solvent used for extraction. The high solubility of phenolic compounds in polar solvents results in highly concentrated extracts.

Table 1. The yield of five aromatic plant extract

Sample	Family	Solvent	Yield (%)
Piper porphyrophyllum	Piperaceae	EtOAc	13.55
		EtOH	12.54
Blumea balsamifera	Asteraceae	EtOAc	8.21
		EtOH	4.34
Neouvaria acuminatissima	Annonaceae	EtOAc	4.00
		EtOH	3.08
Cinnamomum parthenoxylon	Lauraceae	EtOAc	5.44
		EtOH	4.88
Clausena excavata	Rutaceae	EtOAc	6.30
		EtOH	49.73

Note: EtOAc: Ethyl acetate extract, EtOH: Ethanol extract

Figure 2. Five aromatic plants from Pepas Asa village, West Kutai East Kalimantan, Indonesia

Table 2. Qualitative phytochemical analysis on five aromatic plant extracts

Sample	Extract	1	2	3	4	5	6	7	8	9
Piper porphyrophyllum	EtOAc	+	+	-	-	+	++	-	-	-
	EtOH	+	+	-	-	-	++	-	+	-
Blumea balsamifera	EtOAc	+	+	-	-	-	++	-	-	-
	EtOH	+	+	-	-	-	++	-	-	-
Neovaria acuminatissima	EtOAc	+	+	-	-	-	+++	-	-	-
	EtOH	+	+	-	-	-	++	-	-	-
Cinnamomum parthenoxylon	EtOAc	+	+	-	-	-	++	-	-	-
	EtOH	+	+	-	-	-	++	-	-	-
Clausena excavata	EtOAc	+	+	-	+	-	++	-	-	-
	EtOH	+	+	-	-	-	++	-	-	-

Note: EtOAc: Ethyl acetate extract, EtOH: Ethanol extract, 1: Alkaloid, 2: Flavonoid, 3: Tannin, 4: Triterpenoid, 5: Steroid, 6: Carbohydrate, 7: Carotenoid, 8: Coumarin, 9: Saponin

Table 3. Total phenolic and flavonoid content

Sample	Extract	TFC (mg QE/g extract)	TPC (mg GA/g extract)
Piper porphyrophyllum	EtOAc	40.8	9.93
	EtOH	24.4	20.41
Blumea balsamifera	EtOAc	32.8	18.88
	EtOH	44.8	13.92
Neovaria acuminatissima	EtOAc	35.2	6.36
	EtOH	14.4	19.77
Cinnamomum parthenoxylon	EtOAc	34.8	11.95
	EtOH	12.4	14.33
Clausena excavata	EtOAc	24.4	6.84
	EtOH	30	8.27

Note: TFC: Total Flavonoid Content, TPC: Total Phenolic Content

Table 4. DPPH and α-glucosidase inhibitory activity

Sample	Extract	IC50 (mg/mL)	
		DPPH	α-glucosidase
Piper porphyrophyllum	EtOAc	0.13 Weak	nd
	EtOH	0.01 **Strong**	nd
Blumea balsamifera	EtOAc	0.03 **Strong**	nd
	EtOH	0.04 **Strong**	nd
Neovaria acuminatissima	EtOAc	0.13 Weak	2.43
	EtOH	**0.02 Strong**	**0.73**
Cinnamomum parthenoxylon	EtOAc	0.18 Weak	nd
	EtOH	0.10 Intermediate	1.39
Clausena excavata	EtOAc	0.18 Weak	nd
	EtOH	0.18 Weak	nd

Note: EtOAc: Ethyl acetate extract, EtOH: Ethanol extract, nd: not detected

Antioxidant activity

The scavenging actions against DPPH radicals were dose-dependent, with the IC50 value showing the concentration required to scavenge 50% of the free radicals. All plant extracts showed anti-DPPH free radical activity, albeit varying degrees depending on the concentration tested. The extracts with IC50 values between 0.01 and 0.05 mg/mL and 0.5 to 0.1 mg/mL are considered to have strong and intermediate antioxidant activity, respectively. Antioxidant activity is considered low in extracts with an IC50 value of 0.1 mg/mL or higher (Farshi et al. 2022). As shown in Table 4, showed that the extract of *P. porphyrophyllum* (EtOH), *B. balsamifera* (EtOAc and EtOH), and *N. acuminatissima* (EtOH) have strong antioxidant activity according to the categories above.

The plant's medicinal properties have been attributed to the presence of compounds from each class. The lack of flavonoid groups, which are water-soluble antioxidants with free radical scavenging properties, may account for the low levels of antioxidant activity. The antioxidant activity depended on the solvent used to extract the compound. Maximum antioxidant extraction and DPPH free radical inhibition were observed with methanol (Ghasemzadeh et al. 2011).

Medicinal plants have played a crucial role in diabetic treatment for a long time. Only 3 out of 10 extracts in this study have inhibition activity on α glucosidase. Among the different plants, *N. acuminatissima* (ethanol extract) exhibited 50 percent inhibitory (IC50) activity at 0.73 mg/ml. The other ethanolic extracts were less effective against the α-glucosidase enzyme. Their ability to inhibit the alfa-glucoside enzyme, plant alkaloids, and flavonoids showed promise as potential antidiabetic agents (Dirir et al. 2022).

Traditional uses for people with diabetes aside, the other three medicinal plant species tested failed to demonstrate any inhibitory impact on α-glucosidase. This study's lack of bioactivity towards glucosidase suggests that this enzyme may not have mediated the antidiabetic effect. The metabolites in these plants may lower blood sugar through some other method, such as by mimicking the effects of insulin (De Block et al. 2022). The conversion of inert molecules into active chemicals is another potential mechanism through which the extracts demonstrated bioactivity in vivo (Singh et al. 2022).

Although over 400 plant species are known to exhibit hyperglycemic activity, the search for new antidiabetic medications from natural plants remains appealing since these compounds show novel and safe effects on diabetes mellitus. Plants typically include compounds with antidiabetic effects, such as alkaloids, glycosides, terpenoids, carotenoids, flavonoids, etc. (Gandhi et al. 2022). Diabetic-induced zebrafish were demonstrate the antidiabetic effect of *Psychotria malayana* Jack leaf extract (Nipun et al. 2020). There are perhaps several alkaloids in this plant, but so far, none have been shown to have antidiabetic effects. The antidiabetic effect of medicinal plants is due to compounds that exhibit a reduction in blood glucose levels, such as flavonoids, polyphenols, coumarins, terpenoids, and others.

In conclusion, this research explained the effect of wild aromatic plant growth in the customary forest of Hemaq Beniung. All the extracts (*P. porphyrophyllum; B. balsamifera; N. acuminatissima; C. parthenoxylon; C. excavata*) in this study show an ability to inhibit DPPH free radicals. Compared to other extracts, the ethanolic extract

of *P. porphyrophyllum* showed the highest levels of antioxidant activity followed by *N. acuminatissima* ethanol extract, and *B. balsamifera* ethyl acetate and ethanol extract. The ethanolic extract of *P. porphyrophyllum* also gives the highest TPC in this study, followed by *N. acuminatissima* ethanol extract, and *B. balsamifera* ethyl acetate. Interestingly, in this study, we found the ethanol extract of *N. acuminatissima* was potent in inhibiting α-glucosidase, with an IC50 value of 0.73 mg/ml. This is the first report on *N. acuminatissima* plant species as an antioxidant and antidiabetic agent. Based on these findings, ethanol appears to be the most effective solvent for extracting bioactive chemicals from *N. acuminatissima* for inhibiting the DPPH free radical and α-glucosidase enzyme. To determine the effectiveness of a compound extracted from a source, it is necessary to isolate and identify it as a pure compound in future research. This process allows for the study of the individual effects of each compound and can help identify potential therapeutic benefits. The antidiabetic ability to inhibit α-glucosidase should be further explored using experimental in vivo models to confirm the results of this study.

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