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Phytochemical analysis in conjunction with in vitro α-glucosidase inhibitory and antioxidant activities of three *Diospyros* species from East Kalimantan, Indonesia

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Abstract. *Ramadhan R, Rosyidah N, Firdaus YFH, Kurnia IT, Lestari DA, Phontree K, Phuwapraisirisan P, Riyadi L, Rahman A, Abdulgani N, Maretna SA, Fadhila KN. 2023. Phytochemical analysis in conjunction with in vitro α-glucosidase inhibitory and antioxidant activities of three* Diospyros *species from East Kalimantan, Indonesia. Biodiversitas 24: 4783-4790.* Locals who live in forested areas have long relied on Non-Timber Forest Products (NTFPs) for their traditional ceremonies, food, and medicine. As non-timber forest products, medicinal plants are of interest since they are used by locals in East Kalimantan regions, Indonesia, for local traditional medicine. In the search for new potential antidiabetics and antioxidants created from natural resources, the authors concentrated on medicinal plants traditionally used in East Kalimantan and ethnopharmacological data. The purpose of this study is to evaluate the dual roles of non-timber forest products, such as three *Diospyros* L. species, in α-glucosidase inhibition, antioxidant (free radical scavenging activity against DPPH and ABTS), and CUPRAC in-vitro activities. This study showed that the strongest α-glucosidase inhibitory activity was found in ethyl acetate and ethanol residue extracts of *Diospyros celebica* Bakh., with IC_{50} values of 4.5804±0.14 μg/mL and 3.8236±0.19 μg/mL respectively, which were more potent than quercetin as a positive standard. Additionally, the antioxidant test revealed that leaf extracts of three *Diospyros* species from East Kalimantan are excellent sources of natural antioxidants, as demonstrated by the SC_{50} values. The findings of this study support and validate the use of leaf extracts of three *Diospyros* species in traditional East Kalimantan medicine.

Keywords: Antidiabetic, antioxidant, biodiversity, diabetes, *Diospyros*, East Kalimantan

INTRODUCTION

Products from forests and their primary sources, such as medicinal plants, honey, mushrooms, resins, fruit and nuts, vegetables, barks, and natural fibers, are Non-Timber Forest Products (NTFPs). Local communities living near forests use NTFPs to support their way of life. As previously noted, medicinal plants are NTFPs utilized in traditional medicine since ancient times to cure various illnesses (Mipun et al. 2019; Mbopi et al. 2021). Humans have always depended on plants as their primary source of nutrition; the use of plants for medicinal purposes dates back to ancient civilizations (Paudel et al. 2014). Medicinal plants are well-known repositories of a variety of bioactive secondary metabolites, including phenolics, flavonoids, alkaloids, terpenes, and tannins, which have therapeutic effects, including antidiabetic, antioxidant, anti-inflammation, wound-healing, and antibacterial (Bibi et al. 2014; Odukoya et al. 2022). The tropical rainforests of East Kalimantan, Indonesia, are rich in medicinal plant species. It has various medicinal flora consisting of species that may provide therapeutic agents for treating various diseases (Falah and Hadiwibowo 2017). The majority of local people (Dayak ethnic group) in East Kalimantan rely on indigenous medicinal plants and use herbal remedies either alone or in combination with modern medications to treat several diseases. However, medicinal plants are only known by their specific characteristics or local names. Traditional medicinal plant species utilization has not yet been conducted with scientific methods. Traditional medicinal plant species are important for further research on phytochemical contents and their biological activities.

Hyperglycemia, often caused by an imbalance in the body's requirement for insulin, is a common symptom of diabetes mellitus. Hyperglycemia has a wide range of adverse effects, such as diabetic foot, heart diseases, renal failure, retinopathy, stroke, and problems during pregnancy (Ji et al. 2021). Therefore, to lower blood sugar levels in

the treatment of diabetes, several techniques have been devised, including an insulin secretagogue, an insulin sensitizer, an inhibitor of glucose recapture, and antihyperglycemia. Previous studies have shown that controlling postprandial blood glucose levels with antihyperglycemic drugs that block α-glucosidase can be a successful management method for managing the development of diabetes (Trinh et al. 2016). Patients with type 2 diabetes can employ well-known synthetic drugs, such as acarbose, miglitol, and 1-deoxynojirimycin, as digestive enzyme inhibitors. Although these drugs help reduce postprandial hyperglycemia and prevent impaired glucose intolerance, they cause gastrointestinal problems (Lee et al. 2020). Fortunately, by scavenging free radicals, a class of beneficial secondary metabolites known as phytochemicals found in medicinal plants may be able to treat hyperglycemia and its ramifications (Akyuz et al. 2022).

The physiologically active substances found in *Diospyros* L. species (Ebenaceae family) are abundant, and practically all portions of this plant genus have been employed as medicines (Cesari et al. 2013; Rashed et al. 2014; Zhang et al. 2018). According to reports, triterpene, coumarin, naphthoquinone, and other phenolic chemicals have all been found in *Diospyros* species. They are also recognized for their anti-inflammatory, antipyretic, antifungal, antioxidant, and antiplasmodial properties (Khan et al. 2016; Sulub-Tun et al. 2020; Tameye et al. 2020; du Preez-Bruwer et al. 2022; Tameye et al. 2022; Mujawah et al. 2023). Therefore, these methods inspired the authors to search and apply *Diospyros* sp. from East Kalimantan as it has dual functions, namely α-glucosidase inhibition and free radical scavenging activities, which would be more beneficial than common antidiabetic drugs with side effects. To the best of the authors' knowledge, this study is the first to report the evaluation of antidiabetic and antioxidant activities of *Diospyros* sp. from East Kalimantan tropical forests.

MATERIALS AND METHODS

Plant materials

A list of three species of *Diospyros* sp. (*Diospyros buxifolia* (Blume) Hiern; *Diospyros celebica* Bakh.; *Diospyros confertiflora* (Hiern) Bakh.) was obtained based on chemotaxonomy studies and ethnopharmacological information about plants that have been used widely for illness treatment and exhibited interesting pharmacological properties (Rathore et al. 2014; Peyrat et al. 2016). The three species of *Diospyros* sp. were collected in October 2020 from Balikpapan Botanical Garden, East Kalimantan, Indonesia.

Chemical reagents and instrument

The *1,1-diphenyl-2-picrylhydrazyl* (DPPH) and *2,2`-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid* (ABTS) were procured from Tokyo Chemical Industry Co. Ltd. (Japan). Potassium persulfate, ammonium acetate, neocuproine, and copper (II) chloride were purchased from Sigma Chemical Co (St. Louis, Missouri, USA). Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin, BHT, BHA, and ascorbic acid (Vitamin C) as positive standards of antioxidant were purchased from Sigma Chemical Co (St. Louis, Missouri, USA). Baker's yeast alpha-glucosidase (Sigma Aldrich) and all other solvents and chemicals were of the highest commercial grade. The absorbance was taken using a 96-well Thermo-Scientific Multiskan SkyHigh RE 6.1.1 microplate reader.

Extraction of medicinal plants

Plant leaves were washed with distilled water and dried at room temperature in a well-ventilated room. Next, the dried plant materials were ground to a fine powder using a grinder, separately extracted in ethanol (3 x 400 mL) and macerated at ambient temperature. Then, the mixture was filtered by filter papers, and the filtrate was dried under a vacuum in a rotary evaporator (Rotavapor R100, BUCHI) to obtain dry crude extract, which was then transferred into amber bottles. After that, the crude extract was stored at 4°C temperature for further bioassay analysis. Then, the ethanol crude extract was partitioned with n-hexane, and the residue was partitioned with dichloromethane and ethyl acetate. All solvents mentioned above were purchased from Merck (Darmstadt, Germany). Four obtained extracts were named *n*-hexane extract, dichloromethane extract, ethyl acetate extract, and ethanol residue extract. After that, all extracts were stored at 4°C temperature for further bioassay analysis. The scientific names, plant parts, and yields of dry extracts are shown in Table 1.

Phytochemicals analysis

Phytochemical analysis was conducted using a previous protocol applied by Surendra et al. (2016) and Singh et al. (2022) to determine the different chemical groups presence, such as alkaloid, flavonoid, saponin, phenolic, and terpenoid in the ethanol crude extracts. The analysis of each group's presence is described below.

Table 1. Extracts of *Diospyros* sp. from East Kalimantan, Indonesia and their yield based on weight

***Diospyros* Species**	**Part Used**	**Weight (g)**	**Yield (%)**
Diospyros buxifolia	Leaves		
Ethanol crude extract		8.95	9,07
n-Hexane extract		2.43	27,15
Dichloromethane extract		3.41	38,10
Ethyl acetate extract		0.92	10,28
Etanol residue extract		1.15	12,85
Diospyros celebica	Leaves		
Ethanol crude extract		18.28	16,86
n-Hexane extract		0.82	4.48
Dichloromethane extract		0.97	5.31
Ethyl acetate extract		12.57	68.76
Etanol residue extract		1.17	6.40
Diospyros confertiflora	Leaves		
Ethanol crude extract		16.55	18.56
n-Hexane extract		2.73	16.50
Dichloromethane extract		2.45	14.80
Ethyl acetate extract		3.75	22.66
Etanol residue extract		0.84	5.08

Alkaloid test

Mayer's test: 0.5 mL of Mayer's reagent was briefly added to 0.5 mL of extract and heated at 70°C for a few minutes. The yellow precipitate will form if their alkaloid is present in the extract.

Wagner's test: *Diospyros* sp. extract was added to the Wagner's reagent, and 0.5 mL of HCl was heated for a few minutes. The formation of a brown or reddish precipitate is evidence of alkaloids' presence.

Dragendroff's test: Dragendroff's reagent was added to 0.5 mL of *Diospyros* sp. extract. A positive presence of alkaloids will show a turbid orange color.

Flavonoid test

Alkaline reagent test: Briefly, 0.5 mL of extract was added to 1 mL of the sodium hydroxide solution (drop by drop) until it showed intense color. The yellow color should turn colorless after the addition of hydrochloride acid dilution.

Shinoda test: Approximately 5 mg of magnesium powder was added to 0.5 mL of extract, then dropwise at approximately 0.5 mL of concentrate hydrochloride acid. Crimson's red color appearance indicates the presence of flavonoids.

Saponin test

Foam test: About 0.5 mL of hot distilled water was added to Diospyros sp.'s ethanolic extract, cooled, and shaken vigorously for 10 seconds. Saponin is present if it forms 1-10 cm high foam that lasts for 10 minutes, and the foam does not disappear after 1 HCl 2 N is added.

Phenolic/Tannin test

Braymer's test: About 3 to 4 drops of ferric chloride solution were added to *Diospyros* sp.'s ethanolic extract and shaken vigorously for 10 seconds. The intense deep blue color of black indicates the presence of tannins/phenolics.

Steroid/Terpenes test

Salkowki's test: 0.5 mL of extract was briefly added to 0.5 mL of Salkowki's reagent. The red color on the upper layer and the yellow color on the lower layer indicate the presence of terpenes.

Liebermann Burchard's test: 0.5 mL of extract was treated with the addition of Liebermann Burchard's reagent (drop by drop) and observed for the formation of a dark pink or red color or reddish-brown ring, which indicates the presence of sterols.

In vitro antidiabetic activity

α-glucosidase inhibition assay

The α-glucosidase inhibitory activity of *Diospyros* sp. extract was examined using the chromogenic method described by Ramadhan et al. (2022) with slight modification. Briefly, various concentrations of extract (20 µL) were added with a phosphate buffer with pH 6.8, followed by 50 µL of α-glucosidase. After pre-incubation at 37°C for 10 minutes, 45 µL of *p*-nitrophenyl glucoside was added. After that, 90 µL of 0.1 M Na_2CO_3 solution was added, and after 30 minutes of reaction at 37°C, the absorbance was recorded at 405 nm. Acarbose and quercetin were standard references, and the results were expressed in half-maximal inhibitory concentration (IC_{50}) values.

Antioxidant properties

DPPH radical scavenging activity

The DPPH free radical was used to analyze Diospyros sp. extract's free radical scavenging potential. The method described by Khongkarat et al. (2020) was employed with minor modifications. Briefly, 20 µL of extract with final concentrations (3.13-50 µg/mL) were mixed with 0.1 mM of DPPH methanol solution. The reaction mixture was incubated in the dark, and after 30 minutes, absorbance was measured on a 96-well Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader at 517 nm. The samples' free radical scavenging activities were expressed in Percentage Inhibition (PI) using formula (1):

$$\%\ \text{Scavenging Activity} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

The analysis was conducted in triplicates, and the Inhibitory Concentration results of 50% (IC_{50}) were expressed as mean ± standard deviation in µg/mL.

ABTS radical scavenging activity

The antiradical activity was examined using the $ABTS^{\bullet+}$ free radical decolorization assay developed by Ramadhan et al. (2019). In brief, 20 µL of extract with final concentrations (3.13-50 µg/mL) was mixed with $ABTS^{\bullet+}$ working solution in a 96-well microplate. The absorption was measured by spectrophotometrically at 750 nm after 30 minutes. Next, the scavenging activity was calculated using the formula (1).

Cupric Reducing Antioxidant Capacity (CUPRAC)

The extract's antioxidant capacity was determined using the CUPRAC assay, performed with the method Pawłowicz et al. 2021 used with slight modification. 100 µL of extract with various concentrations was added with 50 µL of $CuCl_2$ solution, 50 µL of NH_4Ac buffer, and 50 µL of neocuproine. After 30 minutes of incubation, the reaction mixture was measured spectrophotometrically at 450 nm. The results were interpreted by calculating the mmol/g Trolox Equivalent Antioxidant Capacity (TEAC) compared to the standard antioxidant substance Trolox, which has a high reduction potential.

Data analysis

All data were presented as the mean±SD of three measurements and analyzed using nonlinear regression analysis. All measurement was carried out in triplicate. Quantitative data were presented in tables and exported into SigmaPlot 12.5 for analysis. Analysis of Variance (ANOVA) was used to analyze the means of different groups and whether there was any significant difference.

RESULTS AND DISCUSSION

Phytochemicals analysis

Phytochemical screening of ethanolic extracts of three of *Diospyros* sp. was carried out using various chemical assays to identify either the presence or absence of secondary metabolites, such as alkaloids, flavonoids, phenolics/tannins, saponins, and terpenoids/steroids. Table 2 shows the results of the phytochemical screening test.

As shown in Table 2, the phytochemical screening of ethanol extracts of *Diospyros* sp. leaves showed that the extracts contained phenolic/tannins, flavonoids, alkaloids, triterpenoids/steroids, and saponins. The ethanol extract of *D. buxifolia* and *D. celebica* revealed the presence of all secondary metabolites. Meanwhile, the ethanol extract of *D. confertiflora* showed the presence of all tested phytochemicals except saponins and triterpenoid/steroid. The presence of all secondary metabolites in *D. buxifolia* ethanolic leaf extract aligns with the work done by Rao et al. (2016). Chen et al. (2018) also reported the presence of several phytochemicals, including P-Cresol, 5-Hydroxymethylfurfural, Phenol, 2,6-dimethoxy, 2-Furancarboxaldehyde, 5-methyl and 6a,12a-Dihydro-6H-(1,3)-dioxolo(5,6)-benzofuro(3,2-c)-chromen-3-ol.

Additionally, Palakurthi et al. (2022) and Zreen et al. (2022) reported that the leaves of *Diospyros melanoxylon* Roxb. and *Diospyros malabarica* (Desr.) Kostel. contained several phytochemicals, such as phenolic/tannins, flavonoids, alkaloids, triterpenoids/steroids, and saponins. Several reports revealed that phytochemicals are responsible for protection from several chronic diseases, such as polygenic disorder, cancer, cardiovascular disease, and Alzheimer's disease (Rozirwan et al. 2022). Moreover, ingredients in leaves grown in different parts of the world are different, and the composition of individual plants may vary widely due to the different climates, geographical locations, and time of collection (Al-Owamri et al. 2023). Based on the authors' knowledge, this study is the first to report a phytochemical evaluation of ethanol extract from the leaves of three *Diospyros* species from East Kalimantan.

In vitro antidiabetic activity

The primary therapy strategy for reducing Postprandial Hyperglycemia (PPHG) is to inhibit glucose absorption by inhibiting α-glucosidase. An important factor in controlling postprandial hyperglycemia is α-glucosidase, which converts disaccharide α-1,4-glucosidic bonds into simpler sugars (Liu et al. 2016). In this study, the authors evaluated the effectiveness of 15 leaf extracts of *Diospyros* species as antidiabetic agents by inhibiting α-glucosidase, as shown in Table 3. The crude ethanol extract of three different *Diospyros* species was divided into four fractions by polarity, and the α-glucosidase inhibitory activities of these fractions were detected using *p*-NPG as the reaction substrate.

The crude ethanol extracts of three *Diospyros* species showed antidiabetic activities; the crude ethanol extract of *D. celebica* has the strongest α-glucosidase inhibitory activity, followed by *D. buxifolia* and *D. confertiflora*, with IC_{50} values of 5.79±0.32 µg/mL, 14.41±0.54 µg/mL, and 27.58±1.25 µg/mL respectively. Acarbose and quercetin as standard α-glucosidase also demonstrated inhibitory effects with IC_{50} values of 39.84±0.09 µg/mL and 8.36±1.47 µg/mL, respectively. Crude extracts can significantly inhibit if the IC_{50} value is below 50 µg/mL (Trinh et al. 2016). In this experiment, the dichloromethane and ethyl acetate extracts of *D. buxifolia* exhibited more potent α-glucosidase inhibitory effects than acarbose as the standard, and it was comparable to the quercetin as the positive control. The order of inhibitory activity of the four fractions by polarity of *D. buxifolia* against α-glucosidase from the lowest to the strongest was as follows: *n*-hexane extract < ethanol crude extract < ethyl acetate extract < ethanol residue extract < dichloromethane extract. This finding aligns with the phytochemical result above that revealed phenolic and flavonoids in the crude extract. Rao et al. (2016) stated that the methanolic crude extract of *D. buxifolia* leaves had antidiabetic activity by inhibiting α-amylase and amyloglucosidase. The results showed that crude extracts and four fractions of *D. celebica* and *D. confertiflora* also exhibited α-glucosidase inhibitory effects. Based on the findings in Table 3, the crude extracts and four fractions of *D. celebica* had stronger antidiabetic action by blocking α-glucosidase as they had the lowest IC_{50} values. The order of *D. celebica* extracts and fractions of antidiabetic action from the weakest to strongest is as follows: *n*-hexane extract (NI) < dichloromethane extract (24.71±1.31 µg/mL) < ethanol crude extract (5.79±0.32 µg/mL) < ethyl acetate extract (4.58±0.14 µg/mL) < ethanol residue extract (3.82±0.19 µg/mL). According to a study by Rathore et al. (2014), *D. melanoxylon* leaves showed antidiabetic effect by *in-vivo* reduction glucose level test. Demetillo et al. (2019) also reported that *Diospyros blancoi* A.DC. leaves had antidiabetic potency by evaluating alloxan-induced diabetes in mice. Based on recent studies, the phytochemical presence in the extract of three *Diospyros* sp. can be responsible for their biological activity against α-glucosidase. To the best of the authors' knowledge, there is no report on the α-glucosidase inhibitory activity of *D. buxifolia*, *D. celebica*, and *D. confertiflora* from East Kalimantan.

Antioxidant properties

Recent studies reported that chronic hyperglycemia produces an excessive generation of reactive radicals by autooxidation of glucose, such as Reactive Oxygen Species (ROS), contributing to complications by causing cell damage (Kanwugu et al. 2021). Thus, the antioxidant activities of selected medicinal plant extracts, namely DPPH and ABTS scavenging activities, were examined effectively using three approaches. It is well known that individual antioxidant assay alone cannot thoroughly analyze medicinal plant's antioxidant potentials. Hence, it is a prospective strategy to use α-glucosidase and ROS to screen potential medicinal plants with dual functions to combat multiple disorders of diabetes mellitus and its complications caused by free radicals.

The ability of *Diospyros* sp. from East Kalimantan to scavenge free radicals was examined using three distinct

assays, namely the DPPH, ABTS, and CUPRAC reducing power assays. It is widely known that no one antioxidant test can thoroughly assess the antioxidant capacity of plants. The comparatively low value of IC_{50} in DPPH and ABTS free radical scavenging tests is related to strong antioxidant potential. The ABTS and DPPH free radical scavenging activities of various plant extracts varied significantly. The ethanol crude extracts of all three *Diospyros* species had the highest antioxidant activities. The findings of the scavenging activities are represented in SC_{50} values, with a lower SC_{50} indicating a stronger scavenging ability. Table 4 displays the ethanol residue, *n*-hexane, dichloromethane, ethyl acetate, and ethanol leaf extracts' DPPH radical scavenging abilities.

These results showed that among three *Diospyros* species, the ethanol crude extract of *D. celebica* had a good inhibition against DPPH free radical with SC_{50} value of 13.74±1.77 µg/mL that is comparable with BHT as a positive standard with SC_{50} value of 15.94±2.33 µg/mL. Among fifteen fractions, *D. buxifolia* ethyl acetate extract had the highest DPPH antioxidant activity (SC_{50} 9.90±0.11 µg/mL), followed by its ethanol residue extract, ethanol crude extract, dichloromethane extract, and *n*-hexane extract. In addition, the best antioxidant activity against DPPH free radical was observed in ethyl acetate and ethanol residue extracts of *D. celebica* with SC_{50} equal to 8.80±0.21 µg/mL and 12.09±0.18 µg/mL, respectively. This finding was comparable to BHT, Trolox, and ascorbic acid as the positive standards. Thus, the ethyl acetate and ethanol residue extracts of *D. confertiflora* also showed the greatest antioxidant activity against DPPH free radicals with SC_{50} values lower than BHT as a positive standard. This study covers reports of free radical scavenger activities in various *Diospyros* species. These findings concur with Rehman et al. (2020) and Sembiring and Purba (2020), who described the antioxidant activity of methanol extracts of *D. malabarica* and *D. celebica* leaves against DPPH free radicals. Mujawah et al. (2023) also found that any part of other species, such as *Diospyros kaki* L.f., had antioxidant activity because of the presence of phytochemicals, including dinaphthodiospyrol S and 7'-dimethyl-[2,2-binaphthalene]-5,5',8,8'-tetraone.

Meanwhile, Arrisujaya et al. (2020) reported that methanol extracts of *D. blancoi* had the highest antioxidant activity against DPPH free radicals with an SC_{50} value of 2.72 µg/mL. The preceding results highlighted the antioxidant properties of several *Diospyros* species from East Kalimantan.

Well-known synthetic nitrogen-centered radical ABTS is also frequently used to assess antioxidant activity. The ABTS radical is generated by the oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants (Hussen and Endalew 2023). In ABTS scavenging activity, ethyl acetate and ethanol residue extracts of D. *celebica* demonstrated the strongest scavenging activity against $ABTS^{+\bullet}$ free radical, i.e., 13.74±0.42 µg/mL and 15.94±0.38 µg/mL, respectively, which are similar to the SC_{50} value of quercetin as a positive control (14.47±0.38 µg/mL). Meanwhile, ethanol residue extracts of *D. confertiflora* also exhibited the greatest antioxidant activity with an SC_{50} value of 16.67±0.28 µg/mL, as shown in Table 4. It is the first study on the antioxidant of three *Diospyros* species collected from East Kalimantan against $ABTS^{\bullet+}$ free radical.

Table 2. Phytochemicals of ethanolic crude extracts of *Diospyros* sp. from East Kalimantan, Indonesia

Phytochemical Test	*Diospyros buxifolia* (Blume) Hiern	*Diospyros celebica* Bakh.	*Diospyros confertiflora* (Hiern) Bakh
Alkaloid			
Mayer's test	+	+	+
Wagner's test	+	+	+
Dragendroff's test	+	+	+
Flavonoid			
Shinoda's test	+	+	+
Alkaline reagent test	+	+	+
Saponin			
Foam test	+	+	-
Phenolic/Tannins			
Braymer's test	+	+	+
Triterpenoid/Steroid			
Salkowski's test	+	+	-
Liebermann	+	+	-
Burchard's test			

Note: +: Presence; -: Absence

Table 3. α-Glucosidase inhibitory activity of *Diospyros* sp. extracts from East Kalimantan, Indonesia

Diospyros species	Part Used	IC_{50} (µg/mL) α-Glucosidase
Diospyros buxifolia	Leaf	
Ethanol crude extract		14.41±0.54
n-Hexane extract		NI
Dichloromethane extract		3.36±0.32
Ethyl acetate extract		11.54±0.47
Ethanol residue extract		$8.51±0.36^{A}$
Diospyros celebica	Leaf	
Ethanol crude extract		$5.79±0.32^{D}$
n-Hexane extract		NI
Dichloromethane extract		24.71±1.31
Ethyl acetate extract		$4.58±0.14^{D,E}$
Ethanol residue extract		$3.82±0.19^{E}$
Diospyros confertiflora	Leaf	
Ethanol crude extract		$27.58±1.25^{B}$
n-Hexane extract		NI
Dichloromethane extract		$8.66±0.29^{C}$
Ethyl acetate extract		13.20±0.76
Ethanol residue extract		$28.95±3.23^{B}$
Acarbose[a]		39.84±0.09
Quercetin[a]		$8.36±1.47^{A,C}$

Note: [a]: Positive control; [b] NI: No Inhibition, inhibitory effects were less than 30% at 40 µg/mL. Values with the same uppercase superscript letter represent that it is not significantly different (P>0.05, one-way ANOVA followed by Bonferroni test). Each value represents the mean ± SD (n=5)

Table 4. Antioxidant activity of *Diospyros* sp. extracts from East Kalimantan, Indonesia

Diospyros Species	Part Used	SC_{50} (µg/mL)		TEAC[b] (mmol/g)
		DPPH	ABTS	
Diospyros buxifolia (Blume) Hiern	Leaf			
Ethanol crude extract		36.26±1.32	NI[a]	0.22±0.01
n-Hexane extract		NI	NI	0.04±0.01
Dichloromethane extract		NI	NI	0.03±0.01
Ethyl acetate extract		9.90±0.11[A]	26.74±0.42	0.57±0.01
Ethanol residue extract		21.61±0.28	44.87±1.39	0.44±0.01
Diospyros celebica Bakh.	Leaf			
Ethanol crude extract		13.74±1.77[B]	27.29±1.54	0.92±0.14
n-Hexane extract		36.08±2.46	NI	0.05±0.01
Dichloromethane extract		24.18±3.97	NI	0.62±0.02[D]
Ethyl acetate extract		8.80±0.21[A]	13.74±0.42	0.72±0.02
Ethanol residue extract		12.09±0.18[B]	15.94±0.38[C]	0.60±0.02[D]
Diospyros confertiflora (Hiern) Bakh.	Leaf			
Ethanol crude extract		29.85±0.97	NI	0.34±0.01
n-Hexane extract		NI	NI	0.07±0.01[E]
Dichloromethane extract		NI	NI	0.08±0.01[E]
Ethyl acetate extract		9.53±0.11[A]	30.40±1.07	0.94±0.01
Ethanol residue extract		8.06±0.11[A]	16.67±0.28[C]	1.43±0.01
Positive standards				
Quercetin		3.12±0.11	14.47±0.38	
Ascorbic acid (Vitamin C)		6.97±1.01	6.60±0.18	
BHA (Butylated hydroxyanisole)		5.32±0.28	6.97±0.11	
BHT (Butylated hydroxytoluene)		15.94±2.33	8.25±0.21	
Trolox		6.42±0.11	10.63±0.21	

Note: [a]: No scavenging, scavenging effects were less than 40% at 50 µg/mL (final concentration); [b] TEAC: Trolox Equivalent Antioxidant Activity. Values with the same uppercase superscript letter are not significantly different (P>0.05, two-way ANOVA followed by Tukey's test). Each value represents the mean ± S.D (n=3)

Cupric Reducing Antioxidant Capacity (CUPRAC)

In assessing the activity of electron donation, an important mechanism of antioxidants, Cu^{2+} reduction, is frequently used. Therefore, the ability of three *Diospyros* species extracts to decrease Cu (II) was examined to gauge their electron-donating potential. Copper (II)-neocuproine reagent was used as the chromogenic oxidant in the CUPRAC test, which is based on the reduction of Cu (II) to Cu (I) by antioxidants contained in plant extracts. Table 4 shows the extraction's reduction activities; the results are represented as trolox equivalents for the linear equation of Trolox $y=0.009x+0.0548$, $R^2=0.9992$. The three *Diospyros* species in this study showed a larger range of CUPRAC values, ranging from 0.0346-2.5793 mmol/g TR-equivalent. The cupric-reducing ability of the three *Diospyros* species' leaf crude extracts tested was in the following order: *D. buxifolia* > *D. confertiflora* > *D. celebica*. In this study, spectrophotometry determined the mmol/g TEAC values of all fractions of the three *Diospyros* species. After copper (II) reduction capacities of standards were compared based on mmol/g TEAC values, it was found that ethyl acetate and ethanol residue extracts of *D. confertiflora* had the highest TEAC values. However, the lowest copper (II) reduction potential was measured in dichloromethane extract of *D. buxifolia*. The antioxidant mechanism for cupric reducing power of ethyl acetate and ethanol residue extracts might be caused by the high level of phenolic compounds that act as electron donors. Tameye et al. (2020) and Tameye et al. (2022) isolated napthalenone and norbergenin derivatives with good antioxidant activity. To the authors' knowledge, this is the first study on the Cupric-Reducing Antioxidant Capacity (CUPRAC) of three *Diospyros* species collected from East Kalimantan.

As a result of their ability to inhibit α-glucosidase as well as their capacity to neutralize free radicals, such as those that cause DPPH, ABTS, and CUPRAC, leaf extracts of three *Diospyros* species from East Kalimantan were selected for this study; however, ethnobotanical exploration of the subject region has never been observed before. This ethnobotanical study, influenced by the biological activities of three *Diospyros* species in East Kalimantan, can be considered the first. Additionally, this research offers crucial information for the bioassay guidance to isolate bioactive secondary metabolites from particular medicinal plants. Further research is therefore required to identify the specific components of some selected active extracts of three *Diospyros* species that may be responsible for preventing diabetes and associated consequences caused by free radicals.

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REFERENCES

Akyuz M, Yabo-Dambagi L, Kilic T, Cakir A. 2022. Antidiabetic, neuroprotective and antioxidant potentials of different parts of *Pistacia terebinthus* fruits. S Afr J Bot 147: 443-456. DOI: 10.1016/j.sajb.2022.01.040.

Al-Owamri FSN, Al Sibay LSA, Reddy SH, Hussain SA, Gangireddygari VSR. 2023. Phytochemical, antioxidant, hair growth and wound healing property of *Juniperus excelsa*, *Olea oleaster* and *Olea europaea*. J King Saud Univ Sci 35 (2): 102446. DOI: 10.1016/j.jksus.2022.102446.

Arrisujaya D, Susanty D, Hastuti LT. 2020. The effect of three variants of extracting solvents on the total phenolic content and antioxidant activity of *Diospyros blancoi* seeds. Intl J Fruit Sci 20 (S3): S1192-S1200. DOI: 10.1080/15538362.2020.1782803.

Bibi T, Ahmad M, Tareen RB, Tareen NM, Jabeen R, Rehman S-U, Sultana S, Zafar M, Yaseen G. 2014. Ethnobotany of medicinal plants in district Mastung of Balochistan Province - Pakistan. J Ethnopharmacol 157: 79-89. DOI: 10.1016/j.jep.2014.08.042.

Cesari I, Queiroz EF, Favre-Godal Q, Marcourt L, Caccialanza G, Moundipa PF, Brusotti G, Wolfender J-L. 2013. Extensive phytochemical investigation of the polar constituents of *Diospyros bipindensis* Gürke traditionally used by Baka pygmies. Phytochemistry 96: 279-287. DOI: 10.1016/j.phytochem.2013.09.005.

Chen J, Ni C, Lou J, Peng W. 2018. Molecules and functions of rosewood: *Diospyros celebica*. Arab J Chem 11 (6): 756-762. DOI: 10.1016/j.arabjc.2017.12.033.

Demetillo MT, Nuñeza OM, Uy MM, Senarath WTPSK. 2019. Phytochemical screening, antioxidant, and antidiabetic evaluation of leaf extracts from *Diospyros blancoi* A.DC. Intl J Pharm Sci Res 10 (8): 3951-3956. DOI: 10.13040/IJPSR.0975-8232.10(8).3951-56.

du Preez-Bruwer I, Mumbengegwi DR, Louw S. 2022. In vitro antimalarial properties and chemical composition of *Diospyros chamaethamnus* extracts. S Afr J Bot 149: 290-296. DOI: 10.1016/j.sajb.2022.06.006.

Falah F, Hadiwibowo N. 2017. Species identification of traditional medicine plants for women's health in East Kalimantan: Lesson learned from local wisdom. Indones J For Res 4 (1): 49-67. DOI: 10.20886/ijfr.2017.4.1.49-67.

Hussen EM, Endalew SA. 2023. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. BMC Complement Med Ther 23: 146. DOI: 10.1186/s12906-023-03923-y.

Ji Y, Liu D, Jin Y, Zhao J, Zhao J, Li H, Li L, Zhang H, Wang H. 2021. In vitro and in vivo inhibitory effect of anthocyanin-rich bilberry extract on α-glucosidase and α-amylase. LWT Food Sci Technol 145: 111484. DOI: 10.1016/j.lwt.2021.111484.

Kanwugu ON, Glukhareva TV, Danilova IG, Kovaleva EG. 2021. Natural antioxidant in diabetes treatment and management: Prospect of astaxanthin. Crit Rev Food Sci Nutr 65 (18): 5005-5028. DOI: 10.1080/10408398.2021.1881434.

Khan MA, Rahman MM, Sardar MN, Arman MSI, Islam MB, Khandakar MJA, Rashid M, Sadik G, Alam AHMK. 2016. Comparative investigation of the free radical scavenging potential and anticancer property of *Diospyros blancoi* (Ebenaceae). Asian Pac J Trop Biomed 6 (5): 410-417. DOI: 10.1016/j.apjtb.2016.03.004.

Khongkarat P, Ramadhan R, Phuwapraisirisan P, Chanchao C. 2020. Safflospermidines from the bee pollen of *Helianthus annuus* L. exhibit a higher in vitro antityrosinase activity than kojic acid. Heliyon 6 (3): e03638. DOI: 10.1016/j.heliyon.2020.e03638.

Lee A, Moulton D, Mckernan L, Russell A, Slaughter JC, Acra S, Walker L. 2020. (9000). Clinical hypnosis in Pediatric Crohn's Disease: A randomized controlled pilot study. J Pediatr Gastroenterol Nutrit 72 (3): e63-e70. 10.1097/mpg.0000000000002980.

Liu S, Yu Z, Zhu H, Zhang W, Chen Y. 2016. In vitro α-glucosidase inhibitory activity of isolated fractions from water extract of Qingzhuan dark tea. BMC Complement Altern Med 16 (1): 378. DOI: 10.1186/s12906-016-1361-0.

Mbopi PY, Fozeng HDS, Nguekeu YMM, Bitchagno GTM, Ngantchouko CBN, Awouafack MD, Opatz T, Ngouela SA, Morita H, Tene M. 2021. Chemical constituents, total phenolic content, antioxidant activity and bactericidal effect of *Dicliptera verticillate* (Acanthaceae). S Afr J Bot 142: 216-221. DOI: 10.1016/j.sajb.2021.07.001.

Mipun P, Bhat NA, Borah D, Kumar Y. 2019. Non-timber forest products and their contribution to healthcare and livelihood security among the Karbi Tribe in Northeast India. Ecol Process 8: 41. DOI: 10.1186/s13717-019-0194-4.

Mujawah A, Rauf A, Bawazeer S, Wadood A, Hemeg HA, Bawazeer S. 2023. In-vitro antioxidant, lipoxygenase inhibitory, and in-vivo muscle relaxant potential of the extract and constituent isolated from *Diospyros kaki* (Japanese Persimmon). Heliyon 9 (3): e13816. DOI: 10.1016/j.heliyon.2023.e13816.

Odukoya JO, Odukoya JO, Mmutlane EM, Ndinteh DT. 2022. Ethnopharmacological study of medicinal plants used for the treatment of cardiovascular diseases and their associated risk factors in Sub-Saharan Africa. Plants 11 (10): 1387. DOI: 10.3390/plants11101387.

Palakurthi VK, Aravind S, Sreepathi SB, Chippalapally SS, Beeravelli PR, Kedari S, Singisala NR. 2022. Phytochemical screening and antioxidant property of *Diospyros melanoxylon* used by Gothi Koya and Konda Reddi Tribes of Kinnerasani Wildlife Sanctury, Paloncha, Bhadradri Kothagudem District, Telangana. J Med Plants Stud 10 (6): 30-33. DOI: 10.22271/plants.2022.v10.i6a.1486.

Paudel B, Bhattarai HD, Kim IC, Lee H, Sofronov R, Ivanova L, Poryadina L, Yim JH. 2014. Estimation of antioxidant, antimicrobial activity and brine shrimp toxicity of plants collected from Oymyakon region of the Republic of Sakha (Yakutia), Russia. Biol Res 47 (1): 10. DOI: 10.1186/0717-6287-47-10.

Pawłowicz K, Ludowicz D, Karaźniewicz-Łada M, Wdowiak K, Cielecka-Piontek J. 2021. Analysis of the composition of lyophilisates obtained from *Aloe arborescens* gel of leaves of different ages from controlled crops. Molecules 26 (11): 3204. DOI: 10.3390/molecules26113204.

Peyrat L-A, Eparvier V, Eydoux C, Guillemot J-C, Stien D, Litaudon M. 2016. Chemical diversity and antiviral potential in the pantropical *Diospyros* genus. Fitoterapia 112: 9-15. DOI: 10.1016/j.fitote.2016.04.017.

Ramadhan R, Kristanti AN, Amirta R, Kusuma IW, Phuwapraisirisan P, Haqiqi MT, Saparwadi. 2019. Screening for potential antidiabetes and antioxidant activities of selected plants from East Kalimantan, Indonesia. Biodiversitas 20 (7): 1820-1826. DOI: 10.13057/biodiv/d200705.

Ramadhan R, Phuwapraisirisan P, Amirta R, Darmawan MFB, Ul-Haq K, Kusuma IW, Suwito H, Abdulgani N, Mukhdlor A, Saparwadi. 2022. The potency of selected ethnomedicinal plants from East Kalimantan, Indonesia as antidiabetic agents and free-radical scavengers. Biodiversitas 23 (4): 2225-2230. DOI: 10.13057/biodiv/d230458.

Rao PV, Krishinasamy E, Reddy IRM, Naidu MD, Gan SH. 2016. Phytochemical analysis and in vitro evaluation of antidiabetic activity of *Diospyros buxifolia*. Am J Biochem Mol Biol 6 (3): 95-101. DOI: 10.3923/ajbmb.2016.95.101.

Rashed K, Ćirić A, Glamoćlija J, Soković M. 2014. Antibacterial and antifungal activities of methanol extract and phenolic compounds from *Diospyros virginiana* L. Ind Crops Prod 59: 210-215. DOI: 10.1016/j.indcrop.2014.05.021.

Rathore K, Singh VK, Jain P, Rao SP, Ahmed Z, Singh VD. 2014. In-vitro and in-vivo antiadipogenic, hypolipidemic and antidiabetic activity of *Diospyros melanoxylon* (Roxb). J Ethnopharmacol 155 (2): 1171-1176. DOI: 10.1016/j.jep.2014.06.050.

Rehman R, Raza A, Mitu L. 2020. Evaluation of antioxidant prospective of *Diospyros malabarica* methanolic extract for improving oxidative stability of mustard oil. Rev Chim 71 (8): 183-194. DOI: 10.37358/RC.20.8.8292.

Rozirwan, Nugroho RY, Hendri M, Fauziyah, Putri WAE, Agussalim A. 2022. Phytochemical profile and toxicity of extracts from the leaf of *Avicennia marina* (Forssk.) Vierh. collected in mangrove areas affected by port activities. S Afr J Bot 150: 903-919. DOI: 10.1016/j.sajb.2022.08.037.

Sembiring HB, Purba YR. 2020. Isolation and characterization of an antioxidant compound from *Kayu Hitam* leaves (*Diospyros celebica* Bakh.F.). In: Wirjosentono B, Sembiring SB, Alfian Z, Mamat R, Nainggolan I, Gea S, Fischer M (eds). Proceedings of the 1st International Conference on Chemical Science and Technology Innovation (ICOCSTI 2019). Medan, 14-15 July 2019. DOI: 10.5220/0008919802340238.

Singh M, Rajput M, Yadav K, Singh N. 2022. Evaluation of antimicrobial activity and phytochemical qualitative analysis of *Ephedra foliata* Boiss. ex C.A. Mey. Herba Pol 68 (2): 70-75. DOI: 10.2478/hepo-2022-0007.

Sulub-Tun RA, Rodríguez-García CM, Peraza-Echeverría L, Torres-Tapia LW, Peraza-Sánchez SR, Pérez-Brito D, Vera-Ku BM. 2020. Antifungal activity of wild and nursery *Diospyros cuneata*, a native species of dune scrub. S Afr J Bot 131: 484-493. DOI: 10.1016/j.sajb.2020.03.029.

Surendra TV, Roopan SM, Arasu MV, Al-Dhabi NA, Sridharan M. 2016. Phenolic compounds in drumstick peel for the evaluation of antibacterial, hemolytic and photocatalytic activities. J Photochem Photobiol B Biol 161: 463-471. DOI: 10.1016/j.jphotobiol.2016.06.013.

Tameye NSJ, Akak CM, Happi GM, Frese M, Stammler H-G, Neumann B, Lenta BN, Sewald N, Nkengfack AE. 2020. Antioxidant norbergenin derivatives from the leaves of *Diospyros gilletii* DeWild (Ebenaceae). Phytochem Lett 36: 63-67. DOI: 10.1016/j.phytol.2020.01.012.

Tameye NSJ, Akak CM, Tabekoueng GB, Mkounga P, Bitchagno GTM, Lenta BN, Sewald N, Nkengfack AE. 2022. Chemical constituents from *Diospyros fragrans* Gürke (Ebenaceae). Biochem Syst Ecol 100: 104373. DOI: 10.1016/j.bse.2021.104373.

Trinh BTD, Staerk D, Jäger AK. 2016. Screening for potential α-glucosidase and α-amylase inhibitory constituents from selected Vietnamese plants used to treat type 2 diabetes. J Ethnopharmacol 186: 189-195. DOI: 10.1016/j.jep.2016.03.060.

Zhang Y, Zhao L, Huang S-W, Wang W, Song S-J. 2018. Triterpene saponins with neuroprotective effects from the leaves of *Diospyros kaki* Thunb. Fitoterapia 129: 138-144. DOI: 10.1016/j.fitote.2018.06.023.

Zreen Z, Hameed A, Kiran S, Farooq T, Zarooq MS. 2022. A Comparative study of *Diospyros malabarica* (Gaub) extracts in various polarity-dependent solvents for evaluation of phytoconstituents and biological activities. Biomed Res Intl 2022: 474623. DOI: 10.1155/2022/4746223.