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GC-MS analysis of three *Diospyros* species and evaluation of its in vitro rat intestinal α-glucosidase and FRAP activity

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Abstract. *Hariyanto NA, Ramadhan R, Raharjo Y, Firdaus YFH, Kurnia IT, Lestari DA, Suwandari S, Mahmuda NF, Nathanael J, Phontree K, Phuwapraisirisan P, Riyadi L, Rahman A, Abdulgani N. 2024. GC-MS analysis of three* Diospyros species *and evaluation of its* in vitro *rat intestinal α-glucosidase and FRAP activity. Biodiversitas 25: 2442-2453.* The vast diversity of natural products has long been recognized as a potentially valuable source of bioactive compounds derived from nature. The *Diospyros* species, belonging to the Ebenaceae family, thrive in tropical forest regions and have been traditionally used for treating various disorders such as ischemic stroke, hypertension, and infectious diseases. This research aims to assess the phytochemical constituents, rat intestinal α-glucosidase, and Ferric-Reducing Antioxidant Power (FRAP) of non-polar (*n*-hexane) and semi-polar (dichloromethane) extracts from three *Diospyros* species, i.e., *Diospyros buxifolia* (Blume) Hiern; *Diospyros celebica* Bakh.; *Diospyros confertiflora* (Hiern) Bakh). Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified numerous bioactive secondary metabolites in the extracts. The study revealed that the semi-polar extracts of *D. buxifolia* exhibited potent rat intestinal α-glucosidase inhibitory activities, with an IC_{50} value of 33.31±3.23 µg/mL. The IC_{50} of acarbose as a positive control was 6.31±0.31 µg/mL. The FRAP test demonstrated that the non-polar and semi-polar leaf extracts of the three *Diospyros* species from East Kalimantan are potential sources of natural antioxidants, with the IC50 in the range of 6.48±0.38 µmol Fe/g - 11.35±0.16 µmol Fe/g. The results of this study confirm the therapeutic properties of leaf extracts derived from 3 *Diospyros* species, which align with their traditional medicinal use.

Keywords: Antidiabetic, *Diospyros* species, FRAP, GC-MS, rat intestinal α-glucosidase

INTRODUCTION

Non-Timber Forest Products (NTFPs) are products other than timber produced in forests, such as medicinal plants, honey, mushrooms, resins, fruit and nuts, vegetables, bark, and natural fibers. NTFPs have an essential role in supporting the livelihood of local communities around forest areas (Moukette et al. 2015). Medicinal plants, one of the NTFPs, have been used to cure several diseases since ancient times for therapeutic purposes (Silva et al. 2020). East Kalimantan has vast tropical forest biodiversity, which includes medicinal plants that Dayak people traditionally used for generations. In the search for the most potent medicinal plants, scientific approaches should be conducted to develop new, effective, inexpensive, and less toxic traditional medications (Hutauruk et al. 2018). The *Diospyros* genus encompasses approximately 350 species, some known for their medicinal properties. *Diospyros* plants are found in various warm regions globally, including China, Korea, Japan, Brazil, Turkey, Italy, India, Pakistan, the Middle East, parts of Africa, and the United States. While predominantly pantropical, certain *Diospyros* species can also thrive in temperate climates. The *Diospyros* species are adaptable to diverse habitats such as lowland dry forests, mixed forests, dry deciduous forests, rainforests, and lowland forests (Hossain et al. 2018).

Diabetes is a carbohydrate, fat, and protein metabolism disorder attributed to diminished insulin production or increased resistance to its action. Obesity is also an increasing problem worldwide and induces many diseases like diabetes, atherosclerosis, and other metabolic syndromes (Mazumder et al. 2021; Akyuz et al. 2022). Inhibition of two digestive enzymes, α-glucosidase and α-amylase, can significantly reduce hyperglycemia after consuming carbohydrates. Therefore, important strategies are needed in treating type 2 diabetic patients (Ji et al. 2021). One of the most effective therapeutic strategies for managing postprandial blood glucose levels involves the inhibition of

intestinal α-glucosidase. Various inhibitors targeting postprandial hyperglycemia have been developed to address this enzyme (Kim et al. 2022). The glycosidases, a diverse group of enzymes responsible for processing complex carbohydrates, have been identified as a significant therapeutic target. By partially inhibiting the enzymatic breakdown of complex carbohydrates, amylase, and α-glucosidase inhibitors can potentially mitigate postprandial hyperglycemia and delay glucose absorption (Wang et al. 2022). Commonly used inhibitors such as acarbose, voglibose, and miglitol are often prescribed alone or in conjunction with insulin secretagogues for individuals with type II diabetes. However, these inhibitors have been associated with adverse effects, including liver complications, flatulence, and abdominal discomfort. Numerous studies have explored natural agents, including active natural compounds and crude extracts, to overcome these effects as potential α-glucosidase inhibitors with reduced or minimal side effects (Wei et al. 2021). Recent research has focused on investigating *Diospyros* species, medicinal plants traditionally utilized for managing several diseases (Adu et al. 2022; Hossain et al. 2023), as a promising avenue for further exploration.

Due to its use in folk medicine, the above taxon has attracted the attention of many scientists who investigated the chemical constitution of many species and reported the presence of various classes of compounds, including hydrocarbons, terpenes, naphthoquinones, and coumarins (Chen et al. 2018; Adu et al. 2022; du Preez-Bruwer et al. 2022). The *Diospyros* genus belongs to the Ebenaceae family, consisting of approximately 500 species of trees and shrubs distributed in tropical and subtropical regions of the world (Bawazeer and Rauf 2021; Yunusa et al. 2023). Plants of this genus have various ethnomedicinal applications, including the treatment of dysentery, whooping cough, hemorrhages, leprosy, fungal infections, incontinence, rheumatoid arthritis, cardiovascular disorders, diabetes, and various types of cancers (Rathore et al. 2014; Khan et al. 2016; Adu et al. 2022; Hossain et al. 2023). There has been a lack of comprehensive research on *Diospyros* species regarding comparative phytochemical and biological investigations. Therefore, a recent study examined the α-glucosidase inhibitory activities of *Diospyros* species from East Kalimantan against rat intestinal α-glucosidase sources in vitro for the first time. Additionally, the study aimed to analyze the phytochemical composition of these species using Gas Chromatography-Mass Spectrometry (GC-MS) and assess their antioxidant capacity through the Ferric Reducing Antioxidant Power (FRAP) assay.

MATERIALS AND METHODS

Plant materials

Three species of *Diospyros* spp., i.e., *Diospyros buxifolia* (Blume) Hiern; *Diospyros celebica* Bakh.; *Diospyros confertiflora* (Hiern) Bakh) was collected in October 2020 from Balikpapan Botanical Garden and identified by Mr. Trisno as a botanist at Balikpapan Botanical Garden. Voucher specimens of these plants were deposited at Balikpapan Botanical Garden. The leaves were extracted in successive steps, with non-polar (*n*-hexane) and semi-polar (dichloromethane).

Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) of *n*-hexane and dichloromethane extracts was determined according to the Folin-Ciocalteu method following the protocol outlined by a previous study (Rojas-Ocampo et al. 2021). 0.5 mL of the plant extract, at a concentration of 1.0 mg/mL, was mixed with 0.5 mL of Folin-Ciocalteau reagent (0.5 N) and incubated at room temperature for 5 minutes. Subsequently, 2.0 mL of saturated sodium carbonate was added to the mixture and then incubated at room temperature for 30 minutes. The absorbance of the resulting solution was measured at 765 nm using a spectrophotometer. Gallic acid was used as the standard solution. TPC was estimated from a standard curve of gallic acid ($y=ax+b$, $R^2 = 0.9937$), and results were expressed as mg gallic acid equivalents (GAE)/100 g fresh weight (mg GAE/g).

Total Flavonoid Content (TFC)

The total flavonoid content was determined using the aluminum chloride method with slight modification (Koley et al. 2019), with quercetin as the reference standard ($y=ax+b$, $R^2 = 0.9926$). 1.0 mL of the sample was mixed with 4.0 mL of distilled water. Then, 0.3 mL of 5% $NaNO_2$ solution was added and thoroughly mixed. After a 5-minute incubation, 0.3 mL of 10% $AlCl_3$ solution was added and thoroughly mixed. The mixture was allowed to settle at room temperature (25°C) for 6 minutes. To achieve a final volume of 10 mL, 2 mL of 1 M NaOH solution was added, followed by double-distilled water. After allowing the mixture to sit for 15 minutes, the absorbance was measured at 510 nm using a spectrophotometer. The result was reported as milligrams of quercetin equivalent per gram of sample extract.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The extracts from the leaves of three *Diospyros* species were analyzed by Gas Chromatography (GC) Agilent 7890 GC series equipped with an HP-5 silica capillary column (5% diphenyl-and 95% dimethyl-polysiloxane, 30 m x 0.32 mm ID, 250 μm film thickness) coupled to Mass Spectrometry (MS) 5977B MS selective detector with slight modification (Eswaraiah et al. 2020). Initially, the oven temperature was programmed at 40°C, then increased by 2°C/min to 230°C. The detection was carried out using full-scan mode between 30 and 1000 m/z, with the ion source and the quadrupoles temperatures set at 230°C and 280°C. The chemical components of the extract were identified by matching their mass spectra using the NIST 2017 MS library.

Antidiabetic activity

Rat intestinal α-glucosidase

The rat intestinal α-glucosidase inhibition assay was performed according to the method previously employed by Worawalai et al. (2015). The sources of maltase and sucrose were obtained from the crude enzyme solution

prepared from rat intestinal acetone powder. Rat intestinal acetone powder (1 g) was homogenized in 30 mL of 0.9% NaCl solution. After centrifugation (12,000 rpm × 30 min), the aliquot was used for antidiabetic assay. 10 µL of buffer (pH 6.9, 30 µL), 20 µL of the substrate solution (maltose and sucrose) in 0.1 M phosphate buffer, glucose kit, and the crude enzyme solution were mixed. The reaction mixture was then incubated at 37°C for 40 minutes. The concentration of glucose released from the reaction mixture was detected by the glucose oxidase method using a glu-kit (Human, Germany). Enzymatic activity was quantified by measuring absorbance at 503 nm. The percentage of inhibition was calculated by [(A0-A1)/A0] × 100, where A0 is the absorbance without the sample, and A1 is the absorbance with the sample. The IC_{50} value was determined from a plot of percentage inhibition versus sample concentration. Acarbose® and quercetin were used as standard control, and the experiment was performed in triplicate.

Antioxidant capacity

Ferric Reducing Antioxidant Power (FRAP)

The efficacy of *Diospyros* sp. leaf extracts in reducing iron was assessed using a method developed by Wairata et al. (2022) with a slight modification. This assay was conducted to reduce a colorless iron complex of Fe^{3+}-tripyridyltriazine to a blue complex of Fe^{2+}-tripyridyltriazine. The FRAP reagent was combined with a prepared 300 mM acetate buffer, 40 mM HCl, 10 mM TPTZ, and 20 mM $FeCl_3.6H_2O$. Standard curves were created using various concentrations of $FeSO_4.7H_2O$, and all solutions were prepared in fresh conditions. 20 µL of the sample solution at various concentrations, 80 µL of distilled water, and the FRAP reagent were mixed and incubated at 37°C for 30 minutes. The absorbance was then measured at 593 nm. The difference between the samples' absorbance and the blank's absorbance was calculated and used to obtain the extracts' FRAP value and reduction capacity. The FRAP value was expressed as µmol/g, and the experiment was performed in triplicate.

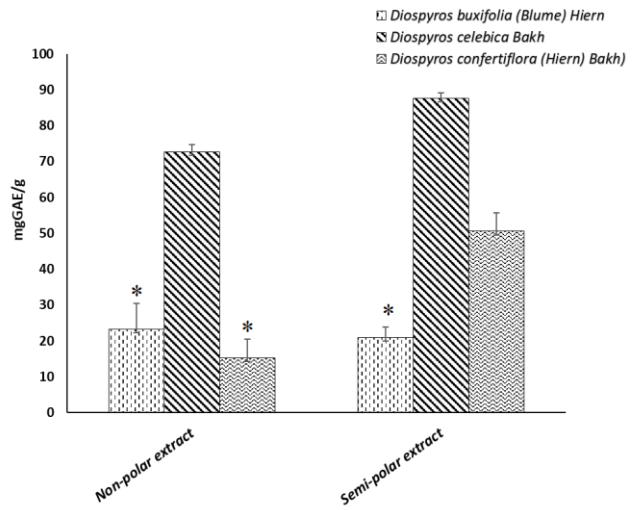

Figure 1. The total phenolic content (TPC) of non-polar and semi-polar extracts of *Diospyros* spp. Values with the same symbols represent insignificant differences (P>0.05, one-way ANOVA followed by Bonferroni test

Data analysis

The obtained quantitative data was analyzed descriptively. All measurements were conducted in triplicate. The quantitative data was tabulated and exported into SigmaPlot 12.5 for further analysis. The obtained quantitative data were analyzed descriptively.

RESULTS AND DISCUSSION

Quantitative phytochemical analysis

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC and TFC of hexane and dichloromethane extracts were presented in Figures 1 and 2.

The Total Phenolic Content (TPC) values were determined using a calibration curve (y=0.0006x+0.0135) with an R^2 value of 0.9937, where x represents absorbance and y represents the concentration of a gallic acid solution expressed as mg GAE/g dry powder extract. The results indicated that the non-polar extract of *D. celebica* Bakh exhibited significantly higher total phenolic content than *D. buxifolia* and *D. confertiflora* (Hiern) Bakh. Similarly, the semi-polar extract of *D. celebica* Bakh also demonstrated higher total phenolic content than the other two species. Previous studies by Vijayan et al. (2020) and Adu et al. (2022) reported significant total phenolic contents in the leaf of *Diospyros ebenum* J. Koenig Ex Retz and *Diospyros villosa* (L.) De Winter. Grygorieva et al. (2020) found that leaf extracts of *Diospyros lotus* and *Diospyros virginiana* contain total phenolic content within the 10 - 15 mgGAE/g range. Diouf et al. (2022) also reported a total phenolic content of 272.65 mgGAE/mg in leaf ethanol extract of *Diospyros mespiliformis* Hochst Ex. DC. These studies collectively suggest that phenolic content in *Diospyros* spp. extracts may contribute to their biological activities. Notably, there is currently no available data on the total phenolic content of the leaf extracts of three specific species of *Diospyros* spp. (*Diospyros buxifolia*; *Diospyros celebica*; *Diospyros confertiflora.*

The flavonoid content in both non-polar extracts of *D. buxifolia* and *D. celebica* Bakh and semi-polar extracts exhibited similar trends to total phenol content, with no significant differences observed. The Total Flavonoid Content (TFC) determined by the aluminum chloride colorimetric method ranged from 162.61 mgQE/g to 1257.33 mgQE/g.

Figure 2 illustrates the flavonoid content of the three *Diospyros* species' non-polar extracts in the order: *D. confertiflora<D. buxifolia<D. celebica* Similarly, the flavonoid content of the three Diospyros species' semi-polar extracts followed the order: *D. buxifolia<D. celebica<D. confertiflora*, as depicted in Figure 2. These findings align with previous studies on the total flavonoids of various *Diospyros* species, such as *Diospyros mespiliformis* Hochst Ex. DC (Diouf et al. 2022), *Diospyros kaki* L., *Diospyros lotus* L., *Diospyros virginia* L. (Hossain et al. 2018; Grygorieva et al. 2020), and *Diospyros malabarica* (Gaub) (Zreen et al. 2022). Notably, this study represents the first investigation into the total

flavonoid content of three *Diospyros* species collected from East Kalimantan.

Phytochemicals by GC-MS analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of non-polar and semi-polar extracts of three *Diospyros* species revealed the presence of numerous bioactive compounds. These compounds were identified by comparing their peak retention time, peak area percentage, and similarity index with known compounds in the National Institute of Standard and Technology (NIST) library. Figures 3-8 display the GC-MS chromatograms of the non-polar and semi-polar extracts obtained from *D. buxifolia*, *D. celebica*, and *D. confertiflora* (Hiern) Bakh.

The GC-MS analysis of non-polar extracts of three *Diospyros* species revealed the phytoconstituents of the extract. The identified major bioactive compounds from non-polar *D. buxifolia* leaf extract were phytol (SI: 98; 12.95%), β-amyrin (SI: 99; 10.26%), α-amyrin (SI: 99; 4.85%), 1-naphthalenol, 1,2,3,4-tetrahydro - acetate (SI: 43; 2.54%), hexadecane (SI: 98; 2.24%), as shown in Table 1. Meanwhile, the identified major bioactive compounds from semi-polar *D. buxifolia* leaf extract were 1H-indole, 2-methyl- (SI: 43; 6.52%), 9,12-octadecadienoic acid (Z, Z) (SI: 91; 5.92%), α-amyrin (SI: 99; 4.12%), 2(1H) naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl) (SI: 38; 3.86%), hexadecane (SI: 98; 3.70%), tetradecane (SI: 98; 2.87%), octadecane (SI: 98; 2.75%), 2,5-dihydroxybenzoic acid (SI: 53; 2.69%), and eicosane (SI: 99; 2.01%), as shown in Table 2.

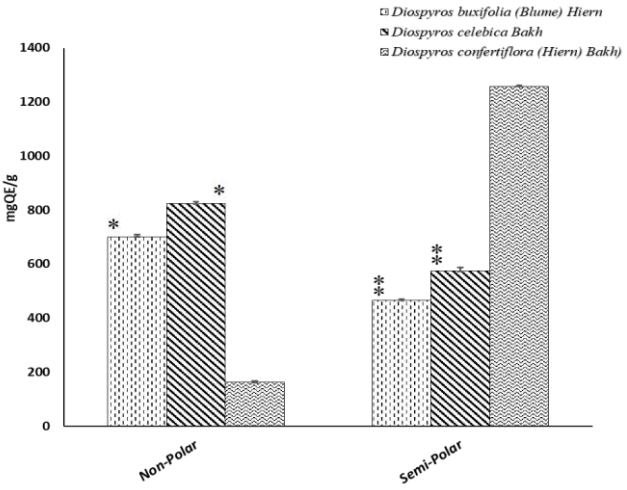

Figure 2. The Total Flavonoid Content (TFC) of non-polar and semi-polar extracts of *Diospyros* spp. Values with the same symbols represent insignificant differences (P>0.05, one-way ANOVA followed by Bonferroni test)

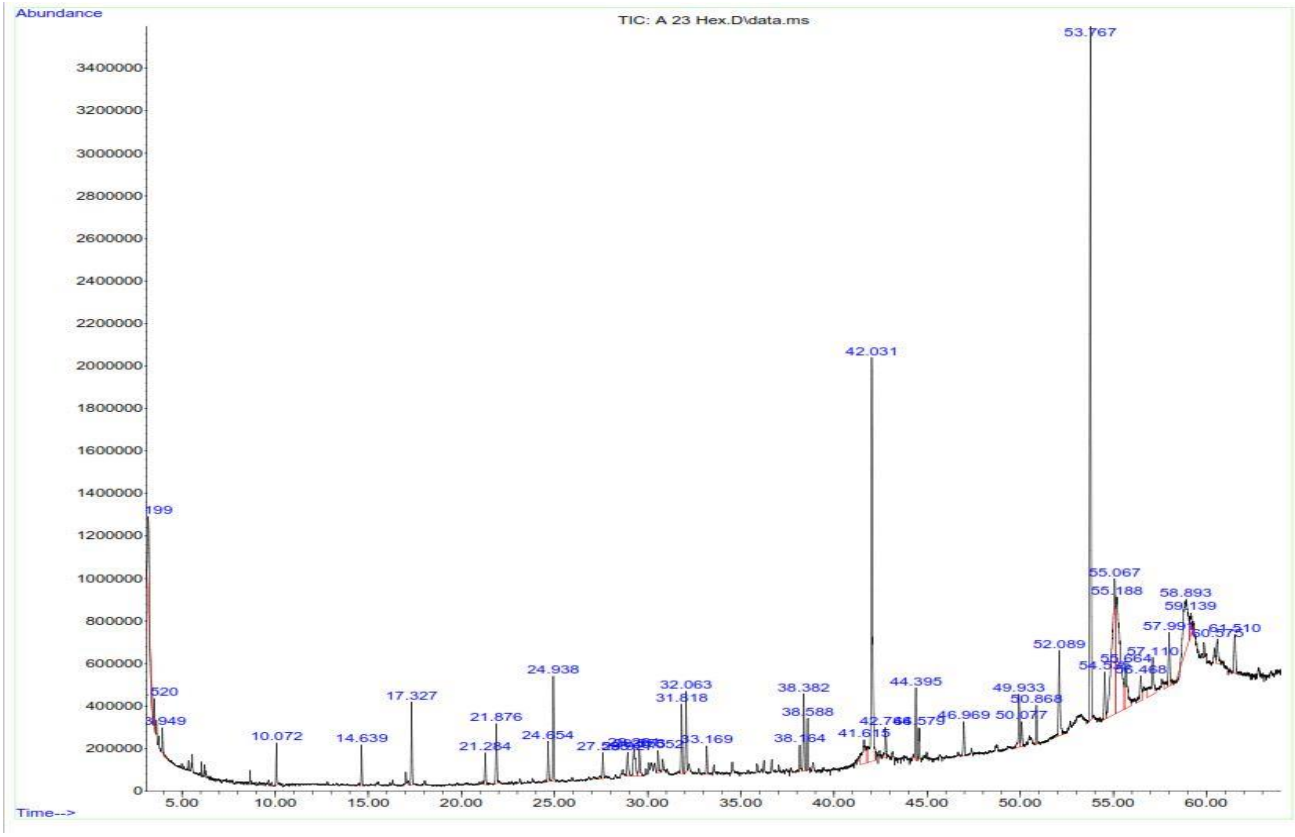

Figure 3. The GC-MS chromatogram of the *n*-hexane extract of *Diospyros buxifolia*

Table 1. Phytochemicals of the *n*-hexane extract of *Diospyros buxifolia*

Retention time (min)	Phytochemicals	SI (Similarity Index)	Peak area (%)
3.199	1-Heptadecene	96	1.79
17.327	Tetradecane	98	1.58
21.876	2,4-Di-tert-butylphenol	95	1.38
24.654	3-Hexadecene, (Z)-	94	0.95
24.938	Hexadecane	98	2.24
31.818	1-Octadecene	96	1.55
32.063	Octadecane	98	1.72
38.382	Heptadecyl trifluoroacetate	94	1.75
38.588	Eicosane	99	1.11
41.615	Oleic Acid	93	1.79
42.031	Phytol	98	12.95
44.395	1-Hexacosanol	94	1.54
49.933	Cyclotetracosane	99	1.38
54.535	2,5-Dihydroxy-benzoic acid	91	1.25
55.067	β-Amyrin	99	10.26
58.893	α-Amyrin	99	4.85
60.575	Squalene	94	0.93

Table 2. Phytochemicals of the dichloromethane extract of *Diospyros buxifolia*

Retention time (min)	Phytochemicals	SI (Similarity Index)	Peak area (%)
3.211	9,12-Octadecadienoic acid (Z,Z)-	91	5.92
6.194	4,4'-Bi-1,3,2-dioxaborolane, 2,2'- diethyl-, (R*,S*)	83	1.01
10.066	Dodecane	97	1.50
14.635	Cyclohexasiloxane, dodecamethyl-	91	1.34
17.318	Tetradecane	98	2.87
21.275	Cycloheptasiloxane, tetradecamethyl	93	1.19
21.853	2,4-Di-tert-butylphenol	95	2.43
24.926	Hexadecane	98	3.70
27.587	Cyclooctasiloxane, hexadecamethyl-	94	1.77
32.047	Octadecane	98	2.75
33.159	Cyclononasiloxane, octadecamethyl-	87	1.03
38.153	Cyclodecasiloxane, eicosamethyl-	91	1.23
38.572	Eicosane	99	2.01
42.209	Phytol	91	1.28
44.550	Docosane	96	1.21
55.166	β-Amyrin	95	1.33
58.997	α-Amyrin	99	4.12

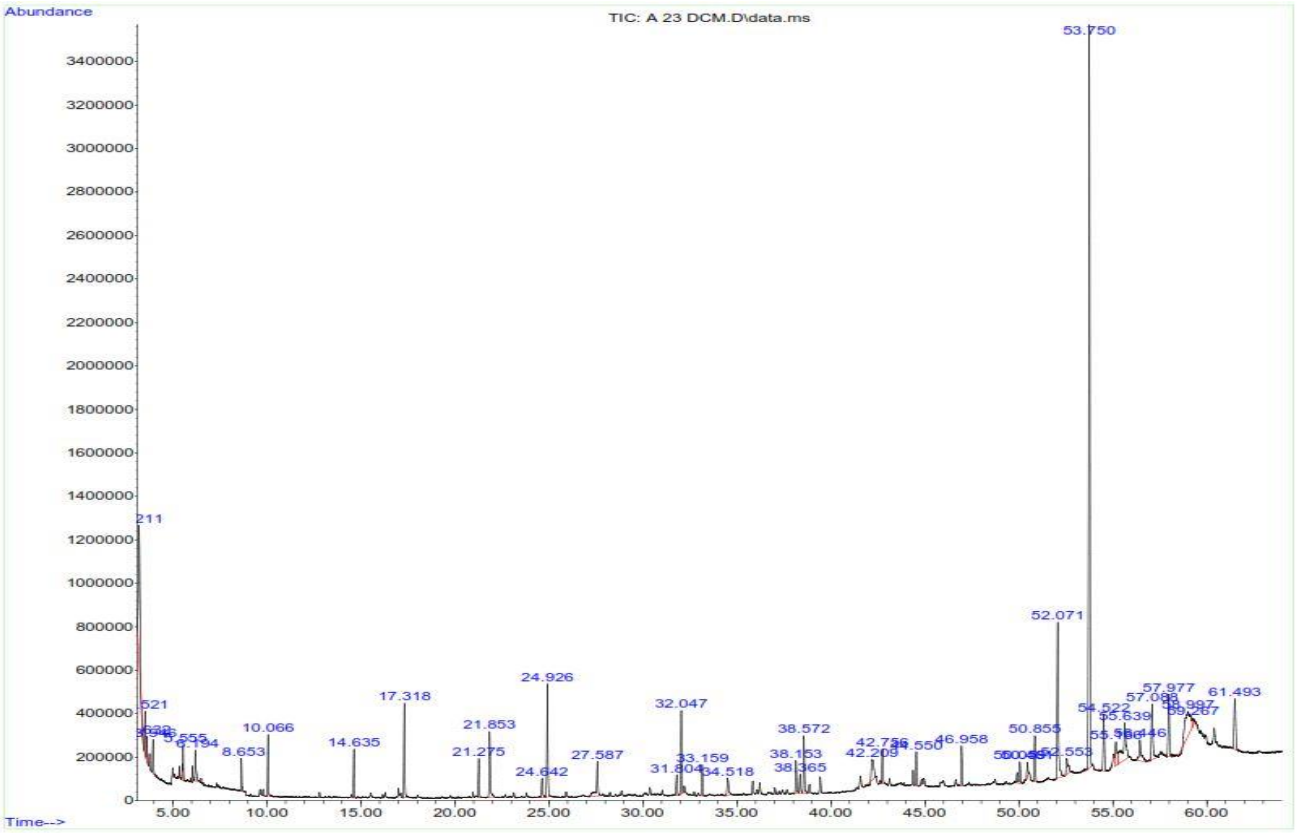

Figure 4. The GC-MS chromatogram of the dichloromethane extract of *Diospyros buxifolia*

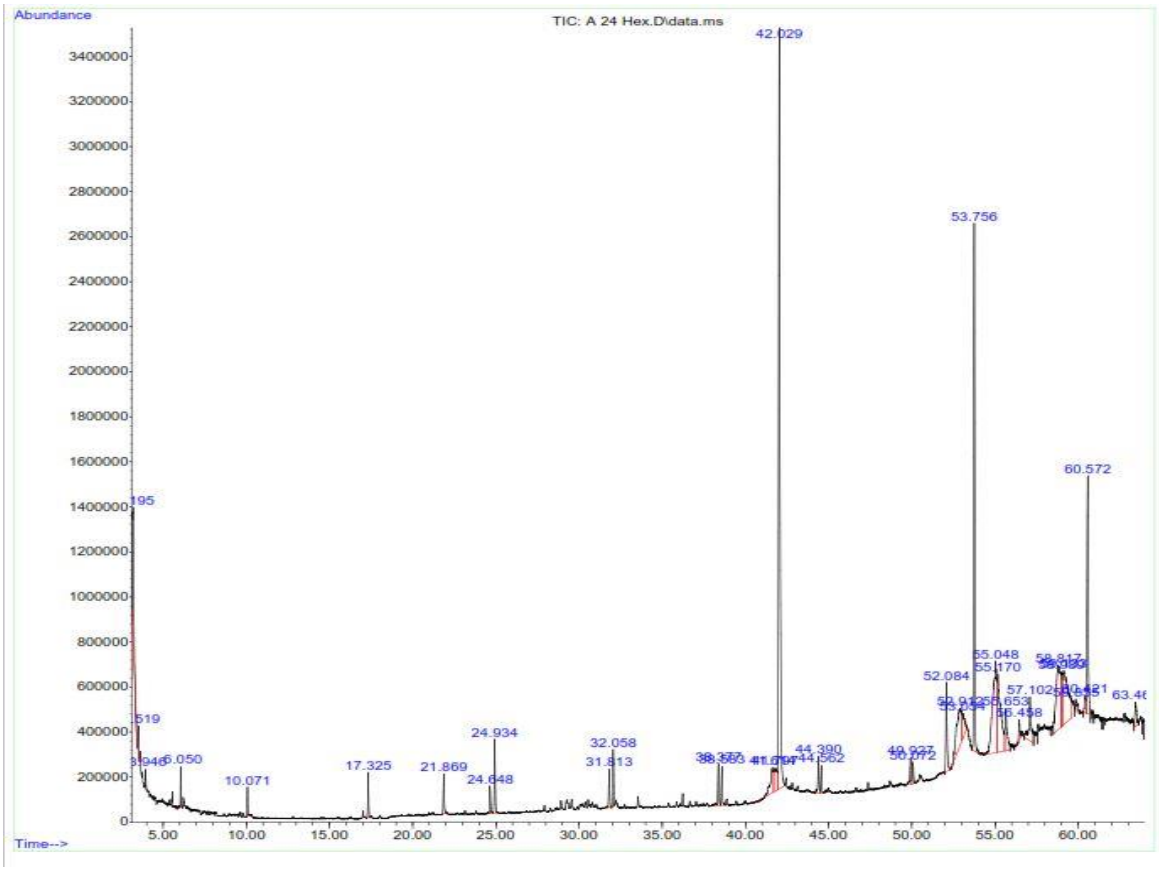

Figure 5. The GC-MS chromatogram of the n-hexane extract of *Diospyros celebica*

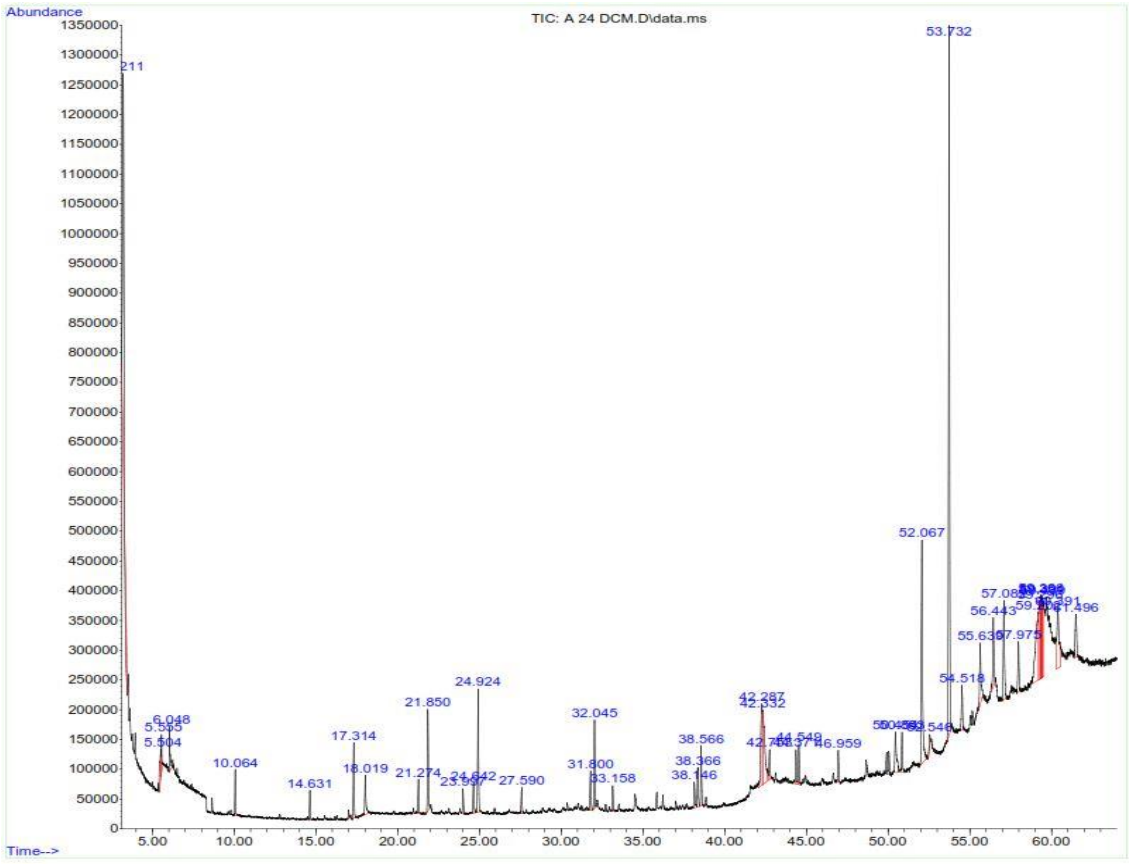

Figure 6. The GC-MS chromatogram of the dichloromethane extract of *Diospyros celebica*

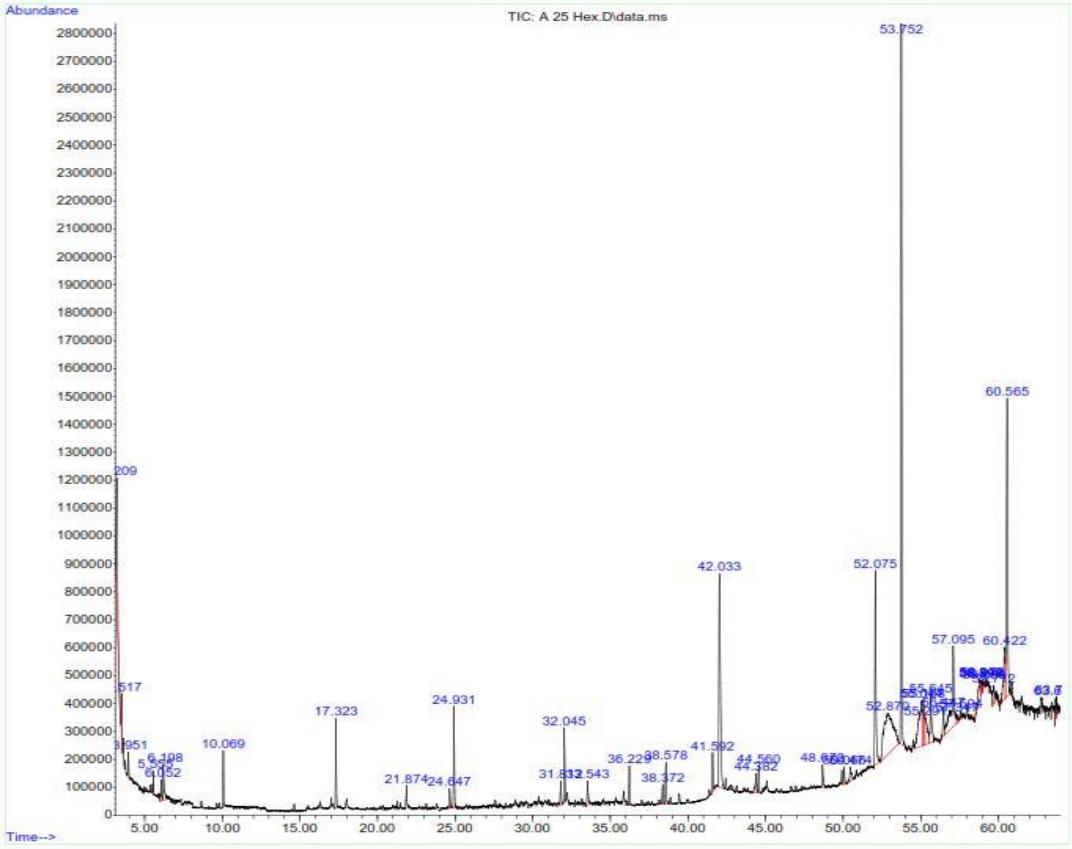

Figure 7. The GC-MS chromatogram of the *n*-hexane extract of *Diospyros confertiflora*

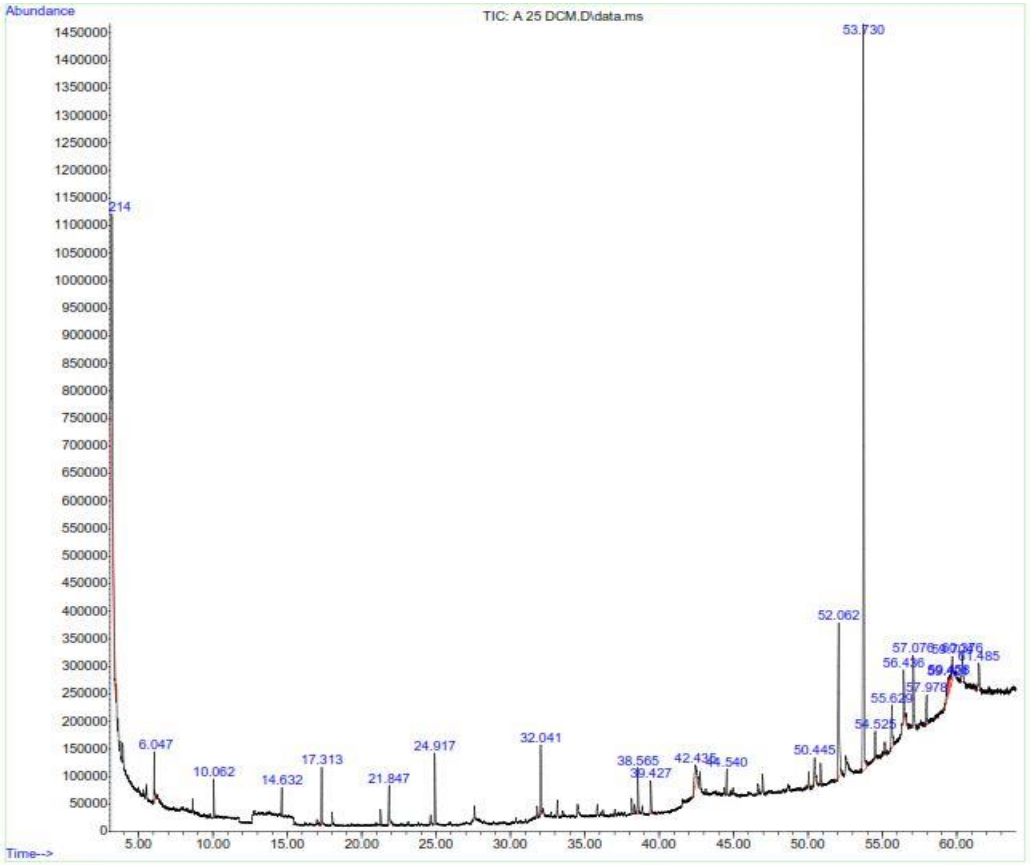

Figure 8. The GC-MS chromatogram of the dichloromethane extract of *Diospyros confertiflora*

Table 4. Phytochemicals of the dichloromethane extract of *Diospyros celebica*

Retention time (min)	Phytochemicals	SI (Similarity Index)	Peak area (%)
3.211	Cyclopentadecanone, 2-hydroxy-	91	11.21
5.504	Oleic Acid	76	0.99
17.314	Tetradecane	98	1.64
18.019	2-Hydroxy-4-hydroxylaminopyrimidine	72	1.20
21.850	2,4-Di-tert-butylphenol	95	2.48
24.924	Hexadecane	98	2.94
31.800	1-Octadecene	98	0.99
32.045	Octadecane	99	2.12
38.366	Cycloeicosane	96	1.00
38.566	Eicosane	99	1.41
42.287	Phytol	90	3.28
50.454	Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl	98	1.62
59.202	α-Amyrin	99	5.11

Table 5. Phytochemicals of the *n*-hexane extract of *Diospyros confertiflora*

Retention time (min)	Phytochemicals	SI (Similarity Index)	Peak area (%)
3.209	Cyclopentadecanone, 2-hydroxy-	96	3.65
6.198	2-Pyrrolidinone, 1-methyl-	80	1.07
10.069	Dodecane	94	1.05
17.323	Tetradecane	98	1.96
24.931	Hexadecane	98	2.45
32.045	Octadecane	99	1.49
38.578	Eicosane	99	1.03
41.529	cis-13-Octadecenoic acid	98	1.09
42.033	Phytol	96	8.62
55.044	β-Amyrin	97	4.21
58.849	α-Amyrin	97	1.05
60.565	Squalene	99	10.40

Table 6. Phytochemicals of the dichloromethane extract of *Diospyros confertiflora*

Retention time (min)	Phytochemicals	SI (Similarity Index)	Peak area (%)
3.214	Cyclopentadecanone, 2-hydroxy-	98	16.42
6.047	2-Ethyl-1-hexanol	87	1.31
10.062	Dodecane	97	1.21
17.313	Tetradecane	97	2.03
21.847	2,4-Di-tert-butylphenol	93	1.76
24.917	Hexadecane	99	3.00
32.041	Octadecane	98	2.84
38.565	Eicosane	99	2.00
44.540	Pentadecane, 8-heptyl-	91	1.10
50.445	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	98	1.03
59.421	β-Amyrin	90	1.64
59.704	Oleic Acid	86	1.92

Table 3. Phytochemicals of the *n*-hexane extract of *Diospyros celebica*

Retention time (min)	Phytochemicals	SI (Similarity Index)	Peak area (%)
21.689	2,4-Di-tert-butylphenol	94	0.81
24.934	Hexadecane	98	1.53
32.058	Octadecane	97	1.12
38.377	1-Hexacosanol	94	1.03
41.614	9-Octadecenoic acid, (E)-	96	1.60
42.029	Phytol	99	21.45
55.048	β-Amyrin	99	8.01
58.817	α-Amyrin	99	6.13
60.572	Squalene	99	6.54

The phytoconstituents in the non-polar leaf extract of *D. celebica* Bakh were found to be phytol (SI: 99; 21.45%), β-amyrin (SI: 99; 8.01%), squalene (SI: 99; 6.54%), α-amyrin (SI: 99; 6.13%), 1,4-dimethyl-8-isopropylidene tricyclo [5.3.0.0(4,10)] decane (SI: 50; 4.22%), 1H-indole, 2-methyl (SI: 43; 2.33%), and 1,4-cyclohexanediol, (Z) (SI: 49; 2.28%), which are presented in Table 3. Meanwhile, the major phytochemicals in the semi-polar leaf extract of *D. celebica* Bakh include cyclopentadecanone, 2-hydroxy (SI: 91; 11.21%), α-amyrin (SI: 99; 5.11%), phytol (SI: 90; 3.28%), 1,2,3,4-tetrahydro-1-naphthylamine (SI: 46; 3.05%), hexadecane (SI: 98; 2.94%), 2,4-di-tert-butylphenol (SI: 95; 2.48%), octadecane (SI: 99; 2.12%), Z)-2-((8R,8aS)-8,8a-Dimethyl-3,4, 6,7,8,8a-hexahydronaphthalene-2(1H) -ylidene) propanal (SI: 38; 2.05%), cinnamyl cinnamate (SI: 38; 2.04%), tetradecane (SI: 98; 1.64%), phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl (SI: 98; 1.62%), and 2-hydroxy-4-hydroxylaminopyrimidine (SI: 72; 1.20%), as shown in Table 4.

In addition, the GC-MS analysis of a non-polar extract of *D. confertiflora* (Hiern) leaf revealed the presence of sixteen chemical compounds, namely squalene (SI: 99; 10.40%), 8-amino-2-hydroxymethyl-6-methoxyquinoline (SI: 44; 10.24%), phytol (SI: 96; 8.62%), naphthalene, 1,2,3,4-tetrahydro-1-methoxy (SI: 40; 5.78%), β-amyrin (SI: 97; 4.21%), cyclopentadecanone, 2-hydroxy- (SI: 96; 3.65%), D-ribo-hexonic acid, 3-deoxy-2,5,6 -tris-O-(trimethylsilyl)-, lactone (SI: 14; 2.56%), hexadecane (SI: 98; 2.45%), tetradecane (SI: 98; 1.96%), 3-methyl-2-thioxo-1,2-dihydro-3H-1,3,4-benzotriazepine (SI: 25; 1.95%), and octadecane (SI: 99; 1.49%), as shown in Table 5. The chromatogram results on the semi-polar extract of *D. confertiflora* (Hiern) leaf were found to be cyclopentadecanone, 2-hydroxy- (SI: 98; 16.42%), naphthalene, 1,2,3,4-tetrahydro-1-ethoxy (SI: 50; 8.72%), hexadecane (SI: 99; 3.0%), octadecane (SI: 98; 2.84%), tetradecane (SI: 97; 2.03%), eicosane (SI: 99; 2.0%), oleic acid (SI: 86; 1.92%), pentyl dotriacontyl ether (SI: 43; 1.97%), β-amyrin (SI: 90; 1.64%), and 2,5-dihydroxybenzoic acid (SI: 27; 1.43%), as presented in Table 6.

Table 7. Rat intestinal α-glucosidase inhibitory activity of *Diospyros* spp. extracts

Diospyros species	Rat intestinal α-glucosidase IC_{50} (μg/mL)	
	Maltase	**Sucrase**
Diospyros buxifolia		
n-Hexane extract	NI	NI
Dichloromethane extract	33.31 ± 3.23	NI
Diospyros celebica		
n-Hexane extract	50.17 ± 1.76	NI
Dichloromethane extract	53.24 ± 3.46	NI
Diospyros confertiflora		
n-Hexane extract	97.04 ± 6.62	NI
Dichloromethane extract	48.83 ± 0.91	NI
Acarbose[a]	6.31 ± 0.31	15.31 ± 1.10
Quercetin[a]	6.07 ± 0.60	21.97 ± 0.49

Note: [a] Positive controls; [b] NI, No inhibition, inhibitory effects less than 40% at 100 μg/mL. Each value represents the mean ± S.D (n=3)

This investigation was conducted to analyze the phytochemical composition of non-polar and semi-polar extracts from three *Diospyros* species using GC-MS analysis, with a focus on the pharmaceutical significance of this genus. The GC-MS results revealed that the n-hexane fractions of the three *Diospyros* species contain various secondary metabolites, including terpenes and fatty acids, which possess biological activities. Notably, phytol, a diterpene present in all *n*-hexane fractions of the three *Diospyros* species, has been previously reported to exhibit anti-inflammatory (Silva et al. 2014), antioxidant (Pejin et al. 2015), anticancer (Sakthivel et al. 2018), and antidiabetic properties (Wang et al. 2017). In previous studies, α and β-amyrin, also present in the n-hexane extracts of the three *Diospyros* species, have been associated with pharmaceutical properties. For instance, α and β-amyrin isolated from *Myrcianthes pungens* leaves demonstrated antioxidant activities (Karen Cardoso et al. 2020), while those isolated from *Celastrus hindsii* leaves exhibited antioxidant, anti-tyrosinase, and anti-xanthine oxidase properties (Viet et al. 2021). Furthermore, α and β-amyrin have been reported to display antidiabetic potency through insulin-mimetic action in C2C12 myoblasts (Giacoman-Martínez et al. 2021). Fatty acid components present in the non-polar extracts have been reported to possess anticancer (Jóźwiak et al. 2020), antioxidant and antimicrobial (Gidik 2021), and antidiabetic properties (Hetta et al. 2017). Squalene, another secondary metabolite found in the non-polar extracts of the *Diospyros* species, has been associated with anti-inflammatory (Cárdeno et al. 2021), antioxidant (Zhang et al. 2023), antibacterial (Nazemi et al. 2022), and antidiabetic effects in previous studies (Widyawati et al. 2021). This study has demonstrated that *n*-hexane extract yielded bioactive compounds positively correlated with biological activities, particularly in developing these species for pharmaceutical purposes. Furthermore, the GC-MS analysis of the dichloromethane extract from three *Diospyros* species revealed the presence of various secondary metabolites. The extracts contained alkanes such as dodecane, tetradecane, hexadecane, octadecane, eicosane, pentadecane, and 8-heptyl. Previous research has indicated that the presence of 2,4-di-tert-butylphenol in these species may exhibit antioxidant (Choi et al. 2013), antibacterial (Aissaoui et al. 2019), and anticancer properties (Seenivasan et al. 2022). Additionally, the 9,12-Octadecadienoic acid (Z, Z)- has been reported to play a significant role in prostaglandin biosynthesis, with various biological functions including anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective effects (Mensah-agyei et al. 2020). It has also been suggested that the risk of heart disease be lowered by contributing to maintaining normal heart rhythm and pumping action (Naghshi et al. 2021). Furthermore, the extracts contain phenolic compounds, esters, aldehydes, alkenes, and ketones, which have demonstrated antiulcer, anti-inflammatory, anti-arthritic, antidiabetic, hypolipidemic, and cytotoxic activities (Olivia et al. 2021). This study uses GC-MS to represent the first reported phytochemical evaluation of extracts from the leaves of three *Diospyros* species from East Kalimantan.

In vitro antidiabetic activity

The primary therapeutic approach for Reducing Postprandial Hyperglycemia (PPHG) involves the inhibition of α-glucosidase to prevent glucose absorption. α-Glucosidase is crucial in regulating postprandial hyperglycemia by breaking down α-1,4-glucosidic bonds in disaccharides into simpler sugars (Liu et al. 2016). This study assessed the potential anti-diabetic properties of non-polar and semi-polar leaf extracts from various *Diospyros* species by evaluating their inhibitory effects on rat intestinal α-glucosidase, as detailed in Table 7. The inhibitory activities of the non-polar and semi-polar extracts from three different *Diospyros* species were tested using maltase and sucrase as the reaction substrates.

In this investigation, the inhibitory effects of α-glucosidase were examined in non-polar and semi-polar extracts of three *Diospyros* species using maltase and sucrase as substrates for the reaction. This study represents the first attempt to assess the antidiabetic potential of three *Diospyros* species from East Kalimantan in inhibiting rat intestinal α-glucosidase (maltase and sucrase). The results presented in Table 7 indicate that the dichloromethane extract of *D. buxifolia* exhibited inhibitory effects against maltase, with an IC_{50} value of 33.31±3.23 μg/mL. In this investigation, the dichloromethane extracts of *D. buxifolia* and *D. confertiflora* demonstrated more potent inhibitory effects on intestinal α-glucosidase than other extracts tested. The order of inhibitory activities of the semi-polar extracts of the three *Diospyros* species against intestinal α-glucosidase, from the weakest to the strongest, was as follows: dichloromethane extract of *D. buxifolia* < dichloromethane extract of *D. confertiflora* < dichloromethane extract of *D. celebica* Acarbose and quercetin were employed as positive controls for the rat intestinal α-glucosidase (maltase) assay, and they exhibited inhibitory activities with IC_{50} values of 6.31±0.31 μg/mL and 6.07±0.60 μg/mL, respectively. This finding is

consistent with the study conducted by Rao et al. (2016), which reported that the methanolic crude extract of *D. buxifolia* leaf displayed antidiabetic activities by inhibiting α-amylase and amyloglucosidase. Additionally, Rathore et al. (2014) demonstrated the antidiabetic effect of *Diospyros melanoxylon* Roxb. leaves through in-vivo reduction of glucose levels. Demetillo et al. (2019) also reported the antidiabetic potency of *D. blancoi* leaves by evaluating alloxan-induced diabetes in mice. Furthermore, Ramadhan et al. (2023) documented the screening of antidiabetic agents from three *Diospyros* species via baker's yeast α-glucosidase inhibition activities.

Based on recent studies, phytochemicals in the extracts of the three *Diospyros* species may account for their biological activities against rat intestinal α-glucosidase. To the best of the authors' knowledge, there are no reports on the rat intestinal α-glucosidase inhibitory activity of *D. buxifolia*, *D. celebica*, and *D. confertiflora* (Hiern) Bakh from East Kalimantan.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP analysis measures the reduction capability of antioxidants using a complex of ferric ions and tripyridyltriazine (TPTZ) as reagents. In this assay, antioxidants reduce the ferric ion (Fe(III))-TPTZ complex to ferrous ion (Fe(II))-TPTZ complex, resulting in a concentrated blue color that can be measured at 593 nm (Chohra et al. 2020). The results of the antioxidant activity of *Diospyros* spp. are shown in Table 8.

Table 8. Antioxidant activity (FRAP) of extracts of three *Diospyros* species

***Diospyros* species**	**FRAP (µmol Fe/g)**				
	31.25 µg/mL	**62.5 µg/mL**	**125 µg/mL**	**250 µg/mL**	**500 µg/mL**
Diospyros buxifolia					
n-Hexane extract	5.12 ± 0.32	5.58± 0.43	6.39± 0.78	7.14± 0.30	8.01± 0.02
Dichloromethane extract	4.23 ± 0.24	4.66 ± 0.16	5.27 ± 0.06	5.71 ± 0.14	6.48 ± 0.38
Diospyros celebica					
n-Hexane extract	5.73 ± 0.06	6.50 ± 0.22	7.05 ± 0.17	7.37 ± 0.09	8.35 ± 0.06
Dichloromethane extract	6.03 ± 0.20	6.27 ± 0.11	7.25 ± 0.05	8.89 ± 0.23	11.35 ± 0.16
Diospyros confertiflora					
n-Hexane extract	6.00 ± 0.23	6.12 ± 0.15	6.28 ± 0.18	6.71 ± 0.06	7.12 ± 0.35
Dichloromethane extract	6.26 ± 0.06	6.68 ± 0.19	6.78 ± 0.04	7.16 ± 0.14	7.95 ± 0.24
Trolox	12.08 ± 0.35	16.54 ± 0.59	29.27 ± 0.50	37.84 ± 0.37	40.64 ± 0.66
Ascorbic acid	17.14 ± 0.18	27.96 ± 0.68	44.08 ± 0.29	45.15 ± 0.92	48.88 ± 0.50

Table 8 displays the result of the Ferric Reducing Antioxidant Power (FRAP) test, indicating the levels of electron-donating antioxidants and the conversion of ferric iron (Fe^{3+}) to ferrous ion (Fe^{2+}). The linear equation for the ferrous ion (Fe^{2+}) is y=0.0004x+0.1232, with an R^2 value of 0.9971. The three *Diospyros* species examined in this study exhibited a wide range of FRAP values, spanning from 4.23 to 11.35 µmol Fe/g equivalent. The non-polar and semi-polar extracts demonstrated significant reducing power based on their antioxidant capabilities. Notably, the non-polar and semi-polar extracts from *D. celebica* Bakh exhibited the strongest antioxidant properties, with FRAP values of 8.35±0.06 and 11.35±0.16 µmol Fe/g respectively. This antioxidant activity indicates the presence of reductive compounds, such as hydrogen and electron donors (Spiegel et al. 2020). Previous studies have suggested that the reducing properties of polyphenols and their ability to form stable complexes with transition metals, particularly iron and copper, can influence various biological processes involving the redox state of metal ions. The interaction of copper and iron ions with polyphenols, particularly flavonoids, is often proposed as one mechanism of action for the antioxidant properties of these natural products. These findings align with those of Murthy et al. (2022) and Adu et al. (2022), who reported on the FRAP antioxidant activity of extracts from *Diospyros chloroxylon* Roxb and *Diospyros villosa* (L.) De Winter, respectively. Additionally, Huang et al. (2016) found that isolariciresinol, a Diospyros kaki Thunb metabolite, exhibited more potent activity in the FRAP assay. To the authors' knowledge, this study represents the first investigation on the Ferric-Reducing Antioxidant Power (FRAP) of non-polar and semi-polar extracts from three *Diospyros* species collected from East Kalimantan.

In summary, the findings of this study suggest that extracts from three *Diospyros* species, i.e., *D. buxifolia*; *D. celebica*; *D. confertiflora*. from East Kalimantan, demonstrate potential antidiabetic and antioxidant agents as presented by their ability to inhibit rat intestinal α-glucosidase activity and their Ferric-Reducing Antioxidant Power (FRAP). It is due to bioactive secondary metabolites, as revealed by GC-MS profiling. Further investigation is necessary to identify specific components within the active extracts of these *Diospyros* species that may contribute to their potential to prevent diabetes and mitigate the associated consequences of free radicals.

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