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Biological and Analytical Investigations of Alpha-Glucosidase Inhibitory and Anti-Oxidant Activities on Selected Malaysian Medicinal Plants

(Penyelidikan Biologi dan Analisis Perencatan Alfa-Glukosidase serta Aktiviti Anti-Oksidan dalam Tumbuhan Ubat-ubatan Terpilih di Malaysia)

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ABSTRACT

This study was performed to establish anti-diabetic and anti-oxidant properties, and to carry out phytochemical analysis of selected local plants which are traditionally used as medicinal plants. Local plants involved in this study were Lawsonia inermis, Punica granatum, Dryobalanops aromatica, Ziziphus mauritiana, *and* Ocimum basilicum. *Solvent extraction was performed using maceration method with solvents of increasing polarity. Alpha-glucosidase inhibition assay was performed on all extracts to ascertain their anti-diabetic potentials. The extracts were screened for antioxidant activity using anti-oxidant assays (FRAP, DPPH, TAOC, ABTS, and BCB) and quantitative phytochemical analyses (TPC and TFC). Chemical profiling using LCMS and GCMS was performed on extracts with high biological activities. Methanol extracts of* D. aromatica *bark and leaves showed the most potent inhibition of alpha-glucosidase with* IC_{50} *values of 0.63 ± 0.03 µg/mL and 0.98 ± 0.02 µg/mL, respectively. Both extracts exhibited similar anti-oxidant activity on all five assays and possessed high phenolic contents with values of 266.79 and 261.69 GAE, respectively. The results obtained suggested that amongst the selected plants studied,* D. aromatica *showed high anti-oxidant activity and anti-diabetic activity (*via *inhibition of alpha-glucosidase). This is the first report that highlights the anti-diabetic potential of* D. aromatica.

Keywords: Alpha-glucosidase; anti-diabetic; anti-oxidant; Dryobalanops aromatica; *medicinal plants*

ABSTRAK

Kajian ini dijalankan bagi mengenal pasti ciri-ciri anti-diabetik dan anti-oksidan, serta analisis fitokimia terhadap tumbuhan tempatan terpilih yang digunakan secara tradisi sebagai tumbuhan ubatan. Tumbuhan tempatan yang terlibat dalam kajian ini adalah Lawsonia inermis, Punica granatum, Dryobalanops aromatica, Ziziphus mauritiana *dan* Ocimum basilicum. *Pengekstrakan pelarut telah dijalankan melalui kaedah maserasi menggunakan pelarut-pelarut dengan peningkatan kepolaran. Asai perencatan alfa-glukosidase telah dijalankan ke atas semua ekstrak bagi mengenal pasti keupayaan anti-diabetik. Kesemua ekstrak juga telah disaring untuk aktiviti anti-oksidan menggunakan asai-asai anti-oksidan (FRAP, DPPH, TAOC, ABTS dan BCB) dan analisis fitokimia kuantitatif (TPC dan TFC). Pemprofilan kimia telah dijalankan ke atas ekstrak dan fraksi yang mempunyai aktiviti biologi yang tinggi dengan menggunakan LCMS dan GCMS. Ekstrak metanol bagi kulit dan daun* D. aromatica *menunjukkan perencatan alfa-glukosidase yang paling tinggi dengan nilai* IC_{50} *masing-masing adalah 0.63 ± 0.03 µg/mL dan 0.98 ± 0.02 µg/mL. Kedua-dua ekstrak menunjukkan aktiviti anti-oksidan yang tinggi ke atas kelima-lima ujian anti-oksidan, serta mempunyai kandungan fenol yang tinggi dengan nilai masing-masing 266.79 dan 261.69 GAE. Hasil uji kaji yang diperoleh menunjukkan bahawa* D. aromatica *mempunyai aktiviti anti-oksidan dan aktiviti anti-diabetik yang tinggi (melalui perencatan alfa-glukosidase). Laporan ini adalah yang pertama kalinya merekodkan keupayaan anti-diabetik bagi* D. aromatica.

Kata kunci: Alfa-glukosidase; anti-diabetik; anti-oksidan; Dryobalanops aromatica; *tumbuhan ubatan*

Introduction

The American Diabetes Association (ADA) classifies diabetes mellitus as a complex metabolic disease (ADA 2006). It is a chronic endocrine disorder that disturbs the metabolism process of proteins, carbohydrates, fat, water, and electrolytes (Nair et al. 2013). It is characterized by glucose intolerance and hyperglycemia which occur because of insulin deficiency, defect in the insulin's action or a combination of both (Deutschländer et al. 2009). There are different types of diabetes mellitus where type 1 and 2 are the most common. Less than 10% of all diabetic cases are due to the autoimmune destruction of beta cells resulting in type 1 diabetes, whereas about 90% are affected by type 2 diabetes due to the impaired effectiveness of insulin secretion and/or action (Szkudelski & Szkudelska 2011). Type 2 diabetes mellitus is a progressive and complex disorder which in the long term is difficult to manage effectively and considered to be a non-curable but controllable disease (Kim et al. 2009).

Studies showed that absorption inhibitors play a role in preventing diabetes mellitus in high risk populations (Cheng & Josse 2004). Hence, a method to treat diabetes is to decrease postprandial hyperglycemia by inhibiting the carbohydrate hydrolysing enzymes such as alpha-amylase and alpha-glucosidase. These enzymes play a crucial role in the digestion of carbohydrates (Nair et al. 2013). Alpha-amylase is an important enzyme that is present in the pancreatic juice and saliva. It breaks down long carbohydrate chains such as large insoluble starch molecules into molecules that can be absorbed (Kazeem et al. 2013). Alpha-glucosidase on the other hand is a glycosylphosphatidyl-anchored enzyme in the mucosal brush border of the small intestine that catalyses the final step in carbohydrate digestion by hydrolysing the carbohydrate on the 1,4-alpha linkages, releasing alpha-D-glucose (Sivasothy et al. 2016). Blocking the process of carbohydrate breakdown, thereby diminishing the postprandial blood glucose excursion, can be achieved by inhibiting the alpha-amylase and alpha-glucosidase enzymes (Kwon et al. 2007). New inhibitors of alpha-amylase and alpha-glucosidase enzymes have been shown as potential leads in developing new drugs for the treatment of diabetes (Nair et al. 2013).

At present, anti-diabetic drugs such as acarbose, which is a commercial inhibitor, is used as a treatment for diabetes patients to control postprandial hyperglycemia (Tabussum et al. 2013). Even though current drugs are able to reduce hyperglycemia in diabetic patients, prolonged usage is associated with side effects such as liver toxicity, abdominal distention, flatulence, meteorism, and increased occurrence of diarrhoea (Hollander 1992; Sivasothy et al. 2016). Therefore, compounds from natural resources as new inhibitors with potentially less side effects may offer better treatment outcomes (Rege & Chowdhary 2014). Inhibitors from plants had been reported to display high inhibition against alpha-glucosidase activity and low inhibition against alpha-amylase activity, which is preferable because excessive inhibition of the pancreatic alpha-amylase will result in increased bacterial fermentation of undigested carbohydrates in the colon, leading to an increased incidence of diarrhoea (Kim et al. 2009). It was noted by the World Health Organization (WHO) that traditional plant remedies for diabetes may offer better treatment options because of their effectiveness, fewer side effects and, are preferable for oral therapy (Shokeen et al. 2008).

Malaysia is known for its abundant tropical biodiversity rich with many medicinal plants that were inspired from different practices, religions, and traditional beliefs. In this study, among the locally grown plants chosen to be tested for anti-diabetic and anti-oxidant activities were *Lawsonia inermis* (henna), *Punica granatum* (pomegranate), *Dryobalanops aromatica* (Borneo camphor tree), *Ziziphus mauritiana* (jujube) and *Ocimum basilicum* (basil). These plants were also mentioned in Al-Quran and Hadith, hence, are used amongst muslim communities. Additionally, the plants were also chosen because of their wide use in Malaysia not only as food but also used as traditional medicines to treat different ailments (Ahmad et al. 2015; Ganesh & Amit 2013; Le et al. 2016; Salleh et al. 2019; Syamsudin & Winarno 2008; Zhang et al. 2010).

Dryobalanops aromatica belongs to the family Depterocarpaceae and is grown in Malaysia. This plant has been a source of camphor (obtained from the bark) as early as in the ninth century. *D. aromatica* is more commonly known as camphor tree, Borneo camphor, Malay camphor or Sumatran camphor. Locally it is called '*pokok kapur barus*' (Ashton 1983; Donkin 1999). The branches and leaves of this plant are employed orally for gum swelling, cholera, and breast pain (Ali 2014). It has been reported that this plant contains terpenoids such as D-borneol, terpinen-4-ol, alpha-terpineol, alpha-pinene, and caryophyllene, which is known for its anti-microbial, cytotoxic, and anti-inflammatory activities (Le et al. 2016).

Punica granatum, from the family Punicaceae, is commonly known as pomegranate and locally called '*delima'* (Radhika et al. 2011). The ash of the young leaves of pomegranate is used traditionally by mixing with water and consumed to treat gastrointestinal ailments. The juice and/or peel has been used to treat vaginal white discharges, leucorrhea, treating intestinal worms and for slimming purposes (Ong & Norzalina 1999; Ong et al. 2011). *P. granatum* had been reported to exhibit anti-proliferative, anti-bacterial and anti-diarrheal activities (Radhika et al. 2011; Zhang et al. 2010). It contains anthocyanins, phenolics, polyphenols, tannins, flavonoids, proanthocyanidin, ellagitannins, and minerals such as nitrogen, calcium, magnesium, sodium, phosphorus and potassium (Heftmann et al. 1966; Moneam et al. 1988; Poyrazoğlu et al. 2002; Viuda-Martos et al. 2010).

Lawsonia inermis, commonly known as henna and locally called *inai*, belongs to the family Lythraceae. Henna has been used traditionally in Malaysia as colouring and used traditionally for ringworm, dandruff, inflammatory conditions, calculus affliction, jaundice, stomach disorder, sore throat, skin diseases, diabetes, and rheumatism (Alia et al. 1995; Badoni Semwal et al. 2014; Othman et al. 2020; Sultana et al. 2009). *L. inermis* has various biological activities such as anti-inflammation, hypoglycemic, analgesic, immunostimulant, anti-microbial and anti-viral (Agarwal et al. 2014; Muhammad & Muhammad 2005). Phytochemical studies of *L. inermis* reported the presence of lawsone, gallic acid, tannic acid, alpha-D-glucose, esculetin, fraxetin, isoplumbagin, scopoletin, betulin, betulinic acid, hennadiol, lupeol, lacoumarin, and laxanthone (Nik et al. 2012; Sultana et al. 2009).

Ziziphus mauritiana, locally known as '*pokok bidara'* and commonly known by many names such as jujube, Indian jujube, and chinese apple, is a tropical fruit tree from the Rhamnacease family (Ganesh & Amit 2013). The fruit is well known as it is used to treat ailments such as cuts and ulcers as well as for fevers and pulmonary ailments. Traditionally, the plant part is used as a tonic, anti-cancer, sedative, and for treating fever (Ganesh & Amit 2013). Furthermore, it has been used in treating insomnia, hemorrhage, diarrhea, and anxiety problems (Salleh et al. 2019; Sun et al. 2011). Studies on *Z. mauritiana* had shown biological activities such as anti-microbial, anti-cancer, anthelmintic, hepatoprotective, anti-obesity, and wound healing (Ganesh & Amit 2013). The plant is reported to contain flavonoids, alkaloids, saponins, resins, polyphenols, mucilage and vitamins (Jain et al. 2012; Jarald et al. 2009; Rahman 2012).

Ocimum basilicum, commonly called basil, common basil or sweet basil, and locally known as '*selasih*', is part of the Lamiaceae family, and is widely cultivated for its medicinal and religious purposes (Ahmad et al. 2015). The plant is used as a tonic, and the infusion made from the leaves is used in treating nausea, flatulence, and dysentery. It is also used in alleviation of spasm, rhinitis, mental fatigue, cold, and for wasp stings and snake bites (Ahmad et al. 2015). *O. basilicum* extract showed potential biological activities such as analgesic, anti-inflammation, hypoglycemic and hepatoprotective, anti-microbial, anti-cancer, and anti-ulcer (Ahmad et al. 2015). The plant is reported to contain chemical constituents such as monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, flavonoids, oxygenated monoterpenes, triterpenes, and aromatic compounds (Marwat et al. 2011).

One of the major hypotheses proposed that the imbalance between the reactive oxygen species capacity and the anti-oxidant defense capacity results in the onset of diabetes (Dehghan et al. 2016). It has been reported that oxidative stress could have a role in the pathogenesis of secondary diabetic complications (Ceriello 2000), and anti-oxidants have been considered as treatments (Cunningham 1998). Plants are known to contain a substantial amount of anti-oxidants such as flavonoids (Larson 1988). Hence, it can be postulated that using anti-oxidants can be useful in scavenging various reactive oxygen species and the prevention of diabetes mellitus.

Phenolic compounds are known to have anti-oxidant properties. These compounds usually exhibit oxidative activities *via* several mechanisms of action which include singlet oxygen quencher, hydrogen donating anti-oxidant, free radical scavenger, and metal ions chelator (Ruhomally et al. 2015). The biological activities of extracts might be due to the phenolic compounds contained in the extracts (Saeed et al. 2012). Consequently, this study will also explore the anti-oxidant activity of all the plant extracts as well as quantitative phytochemical analysis on each crude extract. Chemical profiling on LCMS and GCMS was done for extracts that exhibited high biological activities.

Materials and Methods

Plant materials

All plant materials were collected locally in Malaysia, identified and confirmed by a botanist from the Faculty of Science, Universiti Malaya, Malaysia. The herbarium specimens of *L. inermis* (voucher no: KL5824), *P. granatum* (KL5826), *D. aromatica* (KL5829), *Z. mauritiana* (KL5828), and *O. basilicum* (KL5825) were prepared and stored in the herbarium of the Department of Chemistry, Faculty of Science, Universiti Malaya, Malaysia.

Preparation of plant extracts

Parts of the plant, barks, leaves and seeds were rinsed with water, sliced and air dried at room temperature. The dried plant materials were put through the extraction process by maceration method. The dried plant samples were soaked in hexane with periodical stirring for a period of three days. On the third day, the hexane extracts were filtered and concentrated using the rotary evaporator (BUCHI R-114). This process of extraction was repeated two more times. Following this, the maceration extraction process was repeated using solvents with increasing polarities, which were ethyl acetate, followed by methanol (Handa et al. 2008). The plant materials were then soaked in distilled water and boiled three times up to 30 min each time to yield the water extracts. Once the solutions had cooled down, the extracts were filtered and freeze dried to obtain the dried water extract (Chen et al. 2009).

Biological evaluations of plant extracts and quantitative phytochemical analysis

Alpha-glucosidase inhibitory activity

Two-fold serial dilution was carried out from a stock solution of 100 µg/mL of the extracts, and 40 µL of various concentrations of the extracts were pre-incubated with 80 µL of 2 unit/mL alpha-glucosidase enzyme (dissolved in 67 mM potassium phosphate buffer, pH 6.8) for 10 min. A total of 40 µL of 5 mM p-nitrophenyl-alpha-D-glucopyranoside solution (p-NPG) was added and incubated for 10 min. To stop the reaction, 60 µL of 100 mM of sodium carbonate was added and the absorbance taken at a wavelength of 415 nm (Infinite M200PRO). The standard inhibitor used in the experiment was acarbose, and the determination of the half maximal inhibitory concentration (IC_{50}) was carried out using GraphPad Prism 6 statistical package (GraphPad Software, USA). The data was expressed as mean ± standard deviation of triplicate determinations (Sivasothy et al. 2016).

Anti-oxidant assay

All extracts were screened for their anti-oxidant activities using five assays, namely DPPH (2, 2-diphenyl-1-picrylhydrazyl), Beta-Carotene Bleaching (BCB), Ferric Reducing Power (FRAP), Total Anti-oxidant Capacity (TAOC), and ABTS 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). DPPH radical-scavenging activities of all extracts were determined using the method described by Nordin et al. (2014). Gallic acid was used as a standard in this experiment and absorbance read at 517 nm using microplate absorbance reader (Infinite M200PRO) after 30 min incubation at room temperature.

The BCB assay was conducted according to the protocol by Zamakshshari et al. (2019). Gallic acid was used as a positive control. The absorbance was measured at 470 nm at 0 and 2 h. The percentage of BCB between all extracts (1 mg/mL) and positive control (100 µg/mL) were compared. The reducing power of the extracts and gallic acid were determined according to Nordin et al. (2014). A standard curve of gallic acid was constructed and a standard equation was determined. This equation would result in finding the reducing power (FRAP) for these extracts. The reducing power results were expressed relative to gallic acid equivalent (GAE, µg of gallic acid/mg of extract). Each extract was screened at 1 mg/mL.

TAOC assay is a non-enzymatic assay that is capable to measure the anti-oxidant capacity. This assay was performed following the protocol reported by Sun et al. (2011). All extracts were screened at a concentration of 1 mg/mL and analysed at 695 nm. The total anti-oxidant activity was expressed as equivalent to ascorbic acid.

Free radical scavenging assay was performed by using the ABTS method as described by Dudonne et al. (2009), with slight modification. The method involved 10 µL of 1 mg/mL sample (diluted in DMSO) pipetted into a 96-well microplate, followed by 300 µL of ABTS+● solution. DMSO was used as a blank. The plate was kept for 10 min at 30 ºC. The absorbance was then recorded at 743 nm. The ABTS was decolorized as a result of the scavenging activity of the anti-oxidant.

TOTAL PHENOLIC CONTENT (TPC) AND TOTAL FLAVONOID CONTENT (TFC)

The total phenolic content (TPC) of each extract was determined using the Folin-Ciocalteu assay, a method by Kahkonen et al. (1999). The TPC assay was carried out in a 96-well flat bottom plate. Gallic acid was used as a standard. The TPC of each crude extract was expressed as gallic acid equivalent, GAE (µg of gallic acid/mg extract). Meanwhile, the total flavonoid contents (TFC) of the extracts were determined according to the method by Ceriello (2000). Quercetin was used as a standard in this experiment. The TFC was expressed as quercetin equivalent, QE (µg of QU/mg of crude extract).

CHEMICAL PROFILING USING LCMS

A solvent system in LCMS consisting of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) was used with the following gradient: starting with 100% B and installing a gradient to obtain 50% B at 18 min, and finally to 5% B from 18 at 20 min. The system controller was stopped at the end of 20 min. The solvent flow rate was 0.8 mL/min. Samples (10 µL) were injected onto a C18 reversed-phase column (150 mm × 4.6 mm i.d, 3.0 µm particle size). Mass spectrometric detection was performed with a QTOF-MS operated in the positive mode. Information dependent acquisition was done using a QTOF-MS survey scan 100 - 1100 Da (100 ms) and up to 10 dependent QTOF-MS scans 100 - 1100 Da (100 ms) using Collision Energy (CE) of 45 V with Collision Energy Spread (CES) of ± 30 V. The identification of the peaks was conducted against the Metlin and Chemspider databases.

CHEMICAL PROFILING USING GCMS

The GCMS column used was RTX-5MS fused-silica capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) with helium as the carrier gas and was run at a constant pressure of 100.0 kPa. Injection was conducted using the splitless mode at an injector temperature of 300 °C. The oven temperature was ramped from 40 to 160 °C (5 min hold) at a rate of 4 °C/min, and 160-280 °C (15 min hold) at 5 °C/min (rate). The total run time for each sample was approximately 74 min. The GCMS interface temperature was set to 280 °C. MS mode was used during analytical scanning from 45-500 atomic mass units (amu). The ion source temperature was set to 280 °C. The identification of the peaks was conducted against the National Institute of Standard and Technology Mass Spectral Library (NIST08 and 08s).

STATISTICAL ANALYSIS

The alpha-glucosidase inhibitory activity, anti-oxidant test, and phytochemical analysis data were represented as mean ± standard deviation, and were carried out in triplicate independent analyses. The graphs were generated using Microsoft Excel Software (Version 2010). Data were analyzed using one-way ANOVA via Tukey's post hoc test (SPSS 14.0) to determine the significant differences between sample activities. Meanwhile, the independent sample t-test (SPSS 14.0) was used to determine any significant difference in activity between samples and standard drugs. Pearson correlation (SPSS 14.0) was used to determine the correlation of alpha-glucosidse activity with TPC and TFC. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

The inhibitory activities of forty-four plant extracts of *Dryobalanops aromatica*, *Ziziphus mauritiana*, *Lawsonia inermis*, *Ocimum basilicum,* and *Punica granatum* against alpha-glucosidase were screened and the results obtained are shown in Figure 1. Furthermore, alpha-glucosidase assay was conducted as plants were noted to have higher inhibition against alpha-glucosidase and lower inhibition against alpha-amylase leading to reduced side effects. Further investigation of the antidiabetic activity of potential extracts will be conducted after preliminary screening (Kim et al. 2009).

The enzyme used to investigate the alpha-glucosidase inhibitory activity was obtained from yeast (*Saccharomyces cerevisiae*). This assay was evaluated based on the development of yellow colour of p-nitrophenol by employing p-NPG as the substrate (Rege & Chowdhary 2014). Out of the forty-four plant extracts, thirty-four of them displayed inhibitory activity against alpha-glucosidase enzyme with IC_{50} ranging from 0.63 to 94.71 µg/mL, which were better inhibition compared to the standard acarbose (IC_{50} = 1067.67 µg/mL). Twenty-eight of the plant extracts showed IC_{50} less than 50.00 µg/mL, while only six of the plant extracts showed IC_{50} in the range of 50.00 - 100.00 µg/mL. From the twenty-eight extracts with IC_{50} below 50.00 µg/mL, fourteen of them showed high

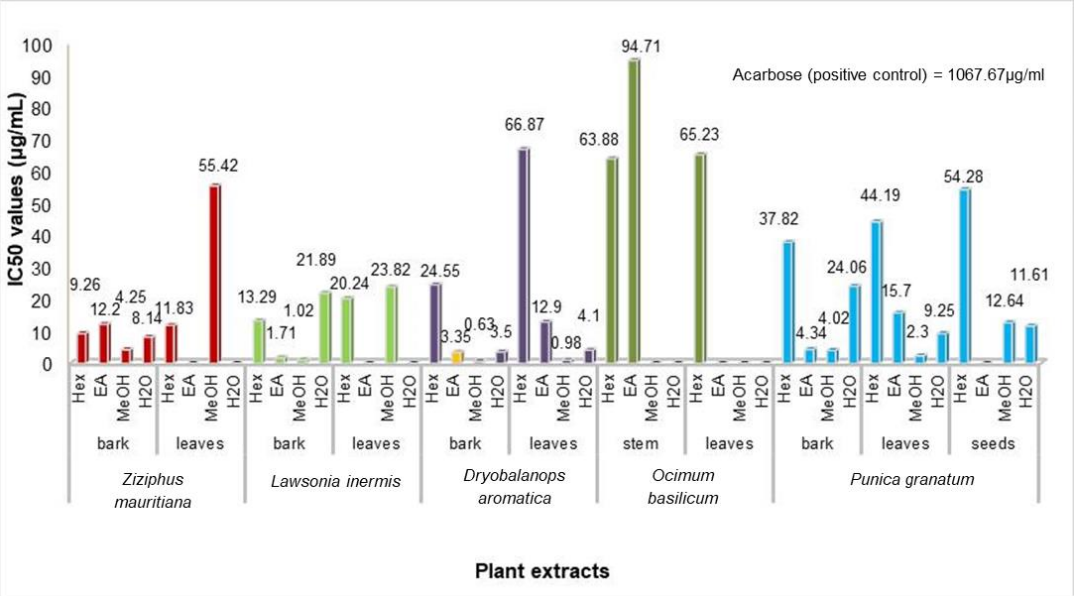


FIGURE 1. The IC_{50} values of different plants extracts against *alpha*-glucosidase
(*Hex = Hexane, EA = Ethyl acetate, MeOH = Methanol, H2O = Water)

inhibitory activity with IC_{50} less than 10.00 µg/mL. The methanol extract of *D. aromatica* bark showed the highest inhibitory activity at IC_{50} of 0.63 µg/mL, followed by the methanol extract of *D. aromatica* leaves with IC_{50} of 0.98 µg/mL. Except for hexane extract, all the extracts of *D. aromatica* showed the highest potential for anti-diabetic activity with IC_{50} of less than 13.00 µg/mL. Conversely, *O. basilicum* plant extracts exhibited the least inhibitory activities with only three of the extracts showed activities with IC_{50} of more than 60.00 µg/mL. The methanol extracts of the barks of *D. aromatica*, *Z. mauritiana,* and *L. inermis*, and of the seeds of *P. granatum* showed the highest inhibitory activities amongst all of the various extracts within each respective plant. It could be deduced that there was a general trend of increasing inhibition activity with increasing polarity of the extraction solvent. However, while the water is a more polar solvent than methanol, the methanol extracts of the mentioned plants exhibited more potent inhibitory activities compared to their respective water extracts. This could be attributed to the different chemical properties of the phytochemical constituents of the respective extracts.

Except for *O. basilicum* which has stem instead of bark extracts, in general, it could be observed that the inhibitory activities of the bark extracts were stronger than the leaves extract for each solvent type. This suggested that the bark extracts have higher potential for anti-diabetic applications compared to the leaves extracts. From this study, it can be inferred that *D. aromatica*, *P. granatum*, *Z. mauritiana,* and *L. inermis* are promising plant candidates for further investigations to be developed into anti-diabetic agents. Contrary to the results obtained for the alpha-glucosidase inhibitory activity, inhibition assay against alpha-amylase showed only the methanol extract of *D. aromatica* bark was active with an IC_{50} value of 95.51 µg/mL, which was less active than acarbose (IC_{50} = 70.31 µg/mL) (SD1). This observation corroborated with the report by Kim et al. (2009) stating that, generally, natural inhibitors from plant extracts have displayed higher inhibition against alpha-glucosidase enzyme and lower inhibition against alpha-amylase enzyme with minimal side effects.

Phytochemical analysis was carried out on the extracts which included the phenolic content assay and total flavonoid assay. These two assays will quantitively determine the amount of phenolic and flavonoid compounds, respectively, in each extract. The principle of the total phenolic content assay is based on the redox

reaction properties of anti-oxidant compounds that react with Folin-Ciocalteu reagent to enhance the measurement of the phenolic concentrations (Norshazila et al. 2010). The most polar extract showed high phenolic content compared to the semi-polar and less polar extracts, as shown in Figure 2. However, the total flavonoid content was seen highest in semi-polar extracts (Figure 3). Furthermore, correlation studies were conducted on TPC and TFC values of the extracts to determine whether they played a role in inhibiting alpha-glucosidase. The correlation coefficient of TPC with alpha-glucosidase assay is r = -0.441, p = 0.004, and TFC with alpha-glucosidase assay is r = 0.183, p = 0.259. According to Zamakshshari et al. (2019), correlation coefficients that range from 0.1 to 0.4 indicate a weak correlation, followed by 0.5 to 0.7 correlation coefficients showing moderate correlation and 0.8 to 0.9 correlation coefficients indicate strong correlations. Therefore, coefficient correlations for both TPC and TFC indicate that the alpha-glucosidase inhibitory activities of the extracts are not directly related to the phenolic and flavonoid compounds.

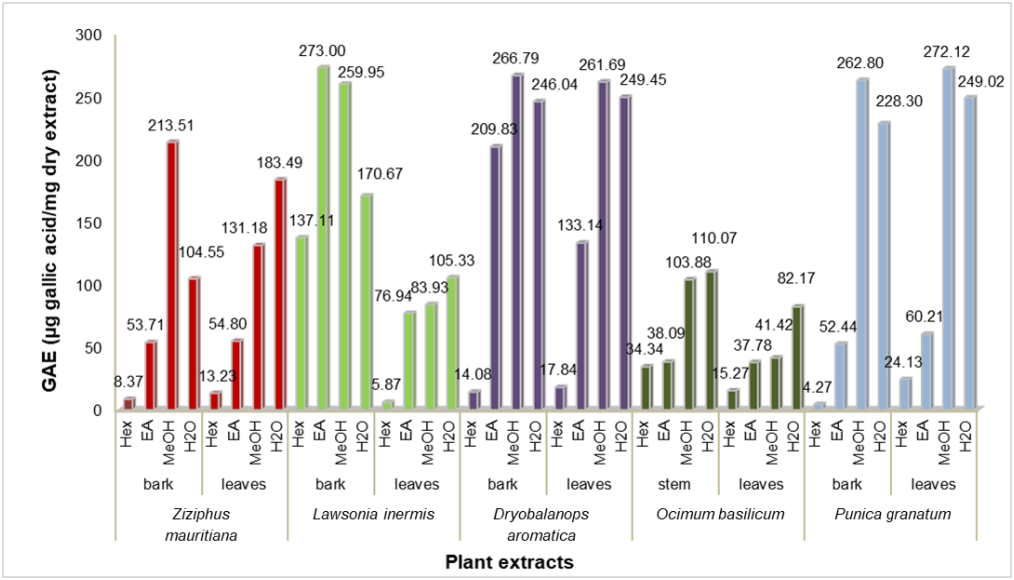


FIGURE 2. Total phenolic content of different plant extracts in GAE
(*Hex = Hexane, EA = Ethyl acetate, MeOH = Methanol, H_2O = Water)

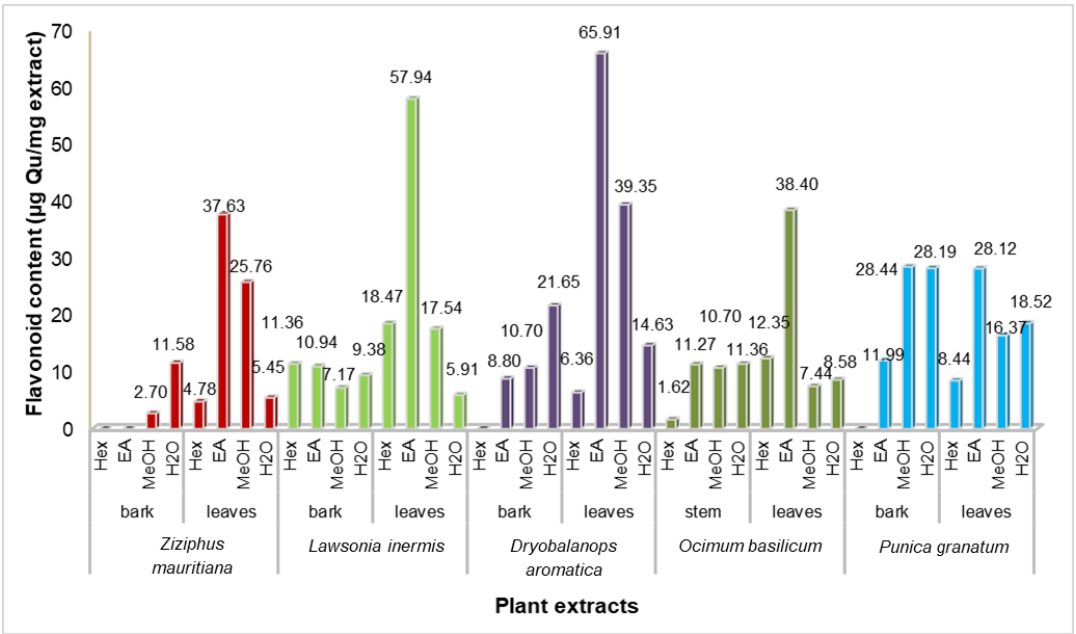


FIGURE 3. Total flavonoid content of different plants extracts
(*Hex = Hexane, EA = Ethyl acetate, MeOH = Methanol, H_2O = Water)

Five anti-oxidant tests were performed and out of 40 plant extracts, the methanol extract from leaves and bark of *D. aromatica* showed the highest anti-oxidant activity in several anti-oxidant assays compared to others. Table 1 shows methanol leaves extract of *D. aromatica* having the highest ability in reducing Fe^{3+} to Fe^{2+} at 1084.13 ± 20.03 GAE followed by methanol bark extract of *D. aromatica* with 972.71 GAE.

TABLE 1. Anti-oxidant activity of the crude extracts of the Malaysian ethnomedicinal plants used in this study

Plants	Extract	Plant part	FRAP	DPPH	TAOC	ABTS	BCB
			GAE (µg gallic acid/ mg dry extract)	EC_{50} (µg/mL extract)	AAE (µg Ascorbic Acid/ mg of dry extract)	% of scavenging of extract at 1 mg/mL	% of β-carotene bleaching of 1 mg extract
Dryobalanops aromatica	Hex	Bark	NA	>	$48.74 \pm 8.90^{a,b,c}$	2.62 ± 0.46*	62.27 ± 1.14*
	Hex	Leaf	NA	>	$48.62 \pm 0.77^{a,b,c}$	7.70 ± 1.02	34.35 ± 2.63*
	EA	Bark	609.97 ± 8.97^{j}	56.65 ± 0.53*	$132.43 \pm 0.50^{k,l}$	92.27 ± 0.40*	88.18 ± 1.48
	EA	Leaf	$306.31 \pm 28.86^{f,g}$	112.12 ± 2.05*	$116.46 \pm 21.62^{e,f,g,h,i,j}$	77.53 ± 3.62	86.84 ± 6.80
	MeoH	Bark	972.71 ± 46.99^{m}	24.86 ± 0.76*	225.22 ± 10.81^{o}	93.77 ± 0.48	91.26 ± 1.13
	MeoH	Leaf	1084.13 ± 20.03^{n}	19.32 ± 1.24*	$203.05 \pm 16.74^{n,o}$	93.86 ± 0.49	91.40 ± 1.68
	H_2O	Bark	714.93 ± 32.79^{k}	45.74 ± 0.58*	$142.11 \pm 7.26^{j,k,l}$	93.20 ± 0.55*	86.44 ± 1.35
	H_2O	Leaf	807.69 ± 39.72^{l}	39.73 ± 0.59*	$119.66 \pm 15.47^{h,i,j}$	93.17 ± 0.54	86.29 ± 1.44*
Ziziphus mauritiana	Hex	Bark	NA	106.32 ± 2.80	34.76 ± 2.18^{a}	4.91 ± 0.14*	34.90 ± 3.06*
	Hex	Leaf	NA	>	$46.17 \pm 4.73^{a,b}$	7.06 ± 0.75*	53.50 ± 2.79*
	EA	Bark	$131.96 \pm 2.27^{b,c}$	>	$84.16 \pm 11.85^{b,c,d,e,f,g,h}$	48.98 ± 2.38	69.06 ± 3.45*
	EA	Leaf	52.60 ± 0.47^{a}	>	$89.45 \pm 5.51^{d,e,f,g,h,i}$	22.85 ± 1.93	68.75 ± 1.01*
	MeoH	Bark	574.86 ± 14.05^{j}	>	224.10 ± 12.63^{o}	90.61 ± 1.14	85.80 ± 1.61
	MeoH	Leaf	$298.80 \pm 13.11^{f,g}$	139.27 ± 1.09*	$136.90 \pm 10.66^{j,k}$	59.94 ± 0.91*	83.73 ± 1.49
	H_2O	Bark	$188.63 \pm 15.23^{c,d}$	333.31 ± 6.22*	$91.54 \pm 7.84^{d,e,f,g,h,i}$	43.40 ± 3.28	78.02 ± 2.23*
	H_2O	Leaf	475.64 ± 22.20^{i}	102.71 ± 6.55	$168.99 \pm 12.29^{k,l,m,n}$	85.32 ± 1.80	84.32 ± 2.25*
Lawsonia inermis	Hex	Bark	$261.76 \pm 20.37^{e,f}$	324.49 ± 27.07	$86.18 \pm 13.54^{c,d,e,f,g,h}$	68.96 ± 1.29	77.38 ± 4.56
	Hex	Leaf	NA	>	$68.71 \pm 15.40^{a,b,c,d}$	11.52 ± 1.64*	67.81 ± 4.27*
	EA	Bark	804.59 ± 28.61^{l}	73.04 ± 0.80*	$176.71 \pm 14.04^{l,m,n}$	93.30 ± 0.19	87.42 ± 2.45
	EA	Leaf	$70.91 \pm 14.50^{a,b}$	698.04 ± 13.02	$127.38 \pm 5.65^{i,j}$	27.53 ± 0.92*	59.77 ± 2.31*
	MeoH	Bark	965.09 ± 18.15^{m}	34.84 ± 0.93*	$189.74 \pm 14.88^{m,n,o}$	93.47 ± 0.27	88.80 ± 0.70
	MeoH	Leaf	$168.89 \pm 7.18^{c,d}$	194.21 ± 2.99*	$85.88 \pm 28.42^{c,d,e,f,g,h}$	55.59 ± 3.68*	86.07 ± 7.76
	H_2O	Bark	386.43 ± 14.88^{h}	103.63 ± 3.82*	$92.47 \pm 10.66^{d,e,f,g,h,i}$	77.79 ± 0.26*	87.39 ± 2.31
	H_2O	Leaf	$348.45 \pm 25.15^{g,h}$	154.15 ± 40.88	$79.00 \pm 27.34^{b,c,d,e,f,g}$	77.86 ± 2.91	86.10 ± 5.74

Ocimum basilicum	Hex	Bark	NA	>	$55.92 \pm 2.76^{a,b,c,d}$	10.27 ± 0.41*	62.27 ± 1.14*
	Hex	Leaf	NA	>	$155.53 \pm 4.75^{j,k,l,m}$	8.97 ± 1.32	51.68 ± 4.00*
	EA	Bark	12.89 ± 5.78^{a}	>	$78.62 \pm 4.77^{b,c,d,e,f}$	18.35 ± 3.13	73.33 ± 1.60*
	EA	Leaf	NA	>	$118.71 \pm 13.28^{e,f,g,h,i,j}$	16.10 ± 1.38	71.91 ± 2.71*
	MeoH	Bark	$223.48 \pm 18.43^{d,e}$	>	$91.60 \pm 17.52^{d,e,f,g,h,i}$	44.88 ± 1.56*	86.05 ± 2.90
	Meoh	Leaf	57.06 ± 1.48^{a}	>	$71.85 \pm 7.41^{a,b,c,d}$	16.11 ± 0.32*	69.24 ± 1.34*
	H_2O	Bark	$220.80 \pm 17.44^{d,e}$	>	$70.51 \pm 15.26^{a,b,c,d}$	38.05 ± 1.55*	85.75 ± 5.76
	H_2O	Leaf	$170.43 \pm 3.7^{c,d}$	>	$74.08 \pm 9.01^{b,c,d}$	37.27 ± 4.71*	80.98 ± 1.56*
Punica granatum	Hex	Seed	NA	>	$53.29 \pm 8.15^{a,b,c,d}$	4.24 ± 0.62	53.11 ± 4.96*
	Hex	Leaf	NA	>	$117.79 \pm 5.37^{f,g,h,i,j}$	9.26 ± 0.98*	62.33 ± 4.16*
	EA	Seed	45.88 ± 9.88^{a}	567.37 ± 8.44*	$74.72 \pm 7.40^{b,c,d}$	27.92 ± 1.70	76.51 ± 3.41*
	EA	Leaf	$61.40 \pm 4.81^{a,b}$	633.59 ± 31.18*	$77.47 \pm 11.65^{b,c,d,e}$	32.50 ± 2.99*	76.54 ± 3.04*
	MeoH	Seed	979.28 ± 48.38^{m}	36.58 ± 1.02*	$132.58 \pm 11.19^{j,k}$	94.01 ± 0.28	90.19 ± 0.09
	MeoH	Leaf	1006.86 ± 40.45^{n}	37.53 ± 1.71*	$139.02 \pm 4.51^{j,k,l}$	93.93 ± 0.27	89.41 ± 1.51
	H_2O	Seed	630.25 ± 54.62^{j}	55.94 ± 3.62*	$88.72 \pm 4.49^{d,e,f,g,h,i}$	92.95 ± 0.09*	87.32 ± 0.78
	H_2O	Leaf	968.18 ± 75.28^{m}	41.46 ± 0.70*	$118.15 \pm 2.99^{g,h,i,j}$	94.01 ± 0.21	86.82 ± 1.90
Standard compounds		Gallic acid	ND	5.56 ± 0.70	ND		
		Gallic acid (100µg/mL)				94.05 ± 0.25	88.58 ± 1.42

Hex = Hexane, EA = Ethyl acetate, MeOH = Methanol, H_2O = Water
NA = No activity, ND=not detected, > = E_C50 more than 1 mg/mL
The (*a-m*) symbol denotes a significant difference between sample ($p<0.05$)
The (*) symbol denote a significant difference between sample and standard drug ($p<0.05$)

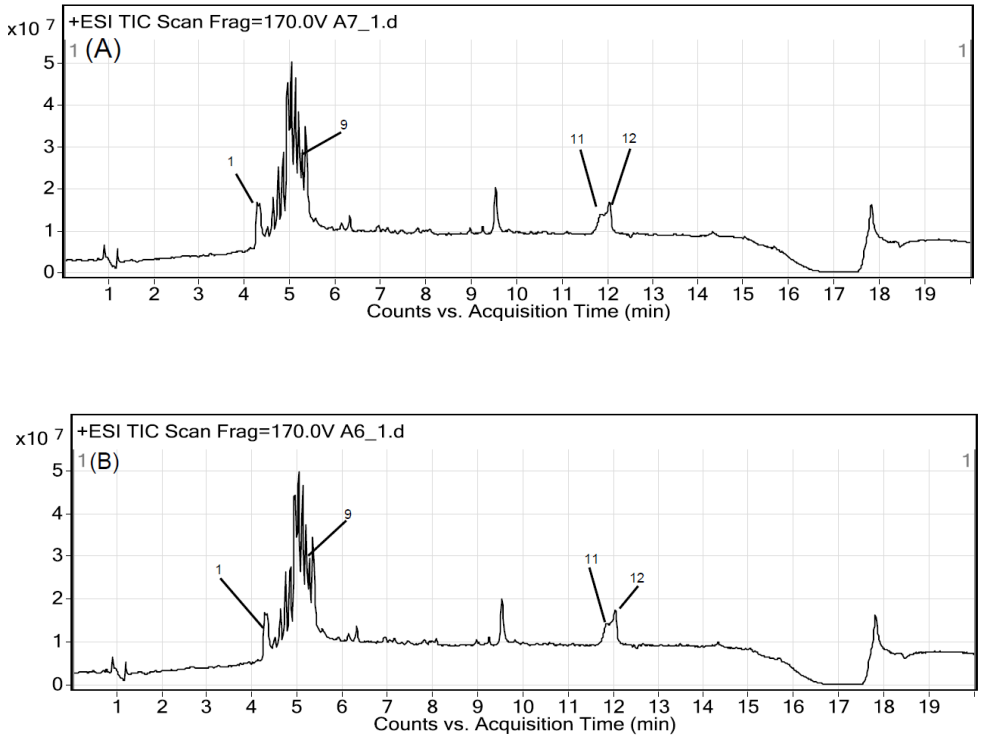
In DPPH assay, the methanol extracts for the leaves and bark of *D. aromatica* together with gallic acid gave scavenging activities EC_{50} at 19.32, 24.86, and 5.56 µg/mL, respectively. Both extracts exhibited good radical scavenging activity, which was close to the standard compound as seen in Table 1. Another anti-oxidant assay used to measure the radical scavenging activities is the ABTS assay. The green-blue stable radical cationic chromophore, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) is produced by oxidation. In the presence of anti-oxidant compounds, this cationic radical can receive an electron or hydrogen and becomes a stable diamagnetic molecule (Roberta et al. 1999). Table 1 shows the free radical scavenging ABTS assay of all extracts from the five plants. Almost all polar extracts, such as the methanol extracts of the leaves and bark of *D. aromatica*, gave a similar percentage of ABTS scavenging activities compared to standard compound gallic acid, except for *O. basilicum*. This could be due to the low phenolic and flavonoid contents observed in *O. basilicum* extracts. The TAOC assay is a non-enzymatic assay that is capable in measuring the anti-oxidant capacity directly. It quantifies the presence of anti-oxidants by formation of green phosphate molybdenum V from reducing anti-oxidant activity (Prieto et al. 1999).

The TAOC values for all extracts are displayed in Table 1. The highest TAOC value was exhibited by the methanol extract of the bark of *Z. mauritiana* with a value of 224.10 AAE, followed by the methanol extracts of the bark and leaves of *D. aromatica* with values of

225.22 and 203.05 AAE, respectively. Meanwhile, for the BCB assay, the principle is to measure the ability of an anti-oxidant to inhibit lipid peroxidation. The model system of this assay is to make the beta-carotene and linoleic acid undergo a rapid discoloration in the absence of an anti-oxidant (Othman et al. 2014). The percentage inhibitions by each extract together with the standard compound are shown in Table 1. In the BCB assay, most methanol extracts for each plant showed good beta-carotene bleaching and comparable to BHT (an anti-oxidant compound). Each anti-oxidant assay mentioned measures the anti-oxidant activity of the extracts based on different reaction mechanisms, leading to different results. It is important to determine which extract has good activities in all the anti-oxidant assays. From this research, it is shown that the methanol extract of the leaves from *D. aromatica* showed good anti-oxidant activity on all the anti-oxidant tests, which can be due to the high phenolic content in the extracts.

The alpha-glucosidase inhibitory and the antioxidant results obtained may be attributed to *D. aromatica* genus which had been reported to be a source of resveratrol oligomers such as trans-3,4′,5-trihydroxystilbene and bergenin. These oligomers were reported to have several bioactivities such as anti-diabetic and anti-oxidant activities (Wibowo et al. 2012, 2011). Other than that, *P. granatum* had been reported to exhibit anti-oxidant and anti-diabetic activities (Radhika et al. 2011; Zhang et al. 2010). *L. inermis* leaf extract was reported to have exhibited anti-diabetic activity in diabetic mice (Syamsudin & Winarno 2008) and also have anti-oxidant biological activity (Agarwal et al. 2014). Studies on *Z. mauritiana* had shown anti-diabetic (Ganesh & Amit 2013) and anti-oxidant properties (Salleh et al. 2019). Lastly, *O. basilicum* had been reported to have anti-oxidant activity (Ahmad et al. 2015) and the aqueous extract showed potential anti-diabetic activities when tested for hypoglycemic activity (El-Beshbishy & Bahashwan 2012). According to Radhika et al. (2011), plants with anti-oxidant properties have the potential to treat diabetes due to their therapeutic benefits.

Since methanol extracts of the leaves and bark of *D. aromatica* showed the most potentials for anti-oxidant and anti-diabetic activities, the chemical contents of both extracts were further profiled using GCMS (for volatile compounds) and LCMS (for non-volatile compounds. Figure 4(a) and 4(b) shows LCMS chromatograms of methanol extracts of *D. aromatica* leaves and bark, respectively. It can be seen that both spectra showed a similar pattern. Based on the library (Metlin and Chemspider database), 12 phytochemical compounds were identified in both extracts (Tables 2 and 3). The compounds are spirilloxanthin, spinoside A, 8E,11Z-hexadecadienal, nostocyclopeptide A3, scytophycin C, α-viniferin, ε-viniferin, kurilensoside I, methyl gallate, clerodin, N-linoleoyl taurine, and guazatine. Previous report indicated that *D. aromatica* was rich with oligostilbenoid that gave rise to biological activities. Compounds such as ε-viniferin in extracts of *D. aromativa* yielded cytotoxic activities against two cancer cell lines, MCF7 and A549 (Wibowo et al. 2014). Meanwhile, α-viniferin was found to give good anti-proliferative activity against HL-60 cell line (Wibowo et al. 2011).



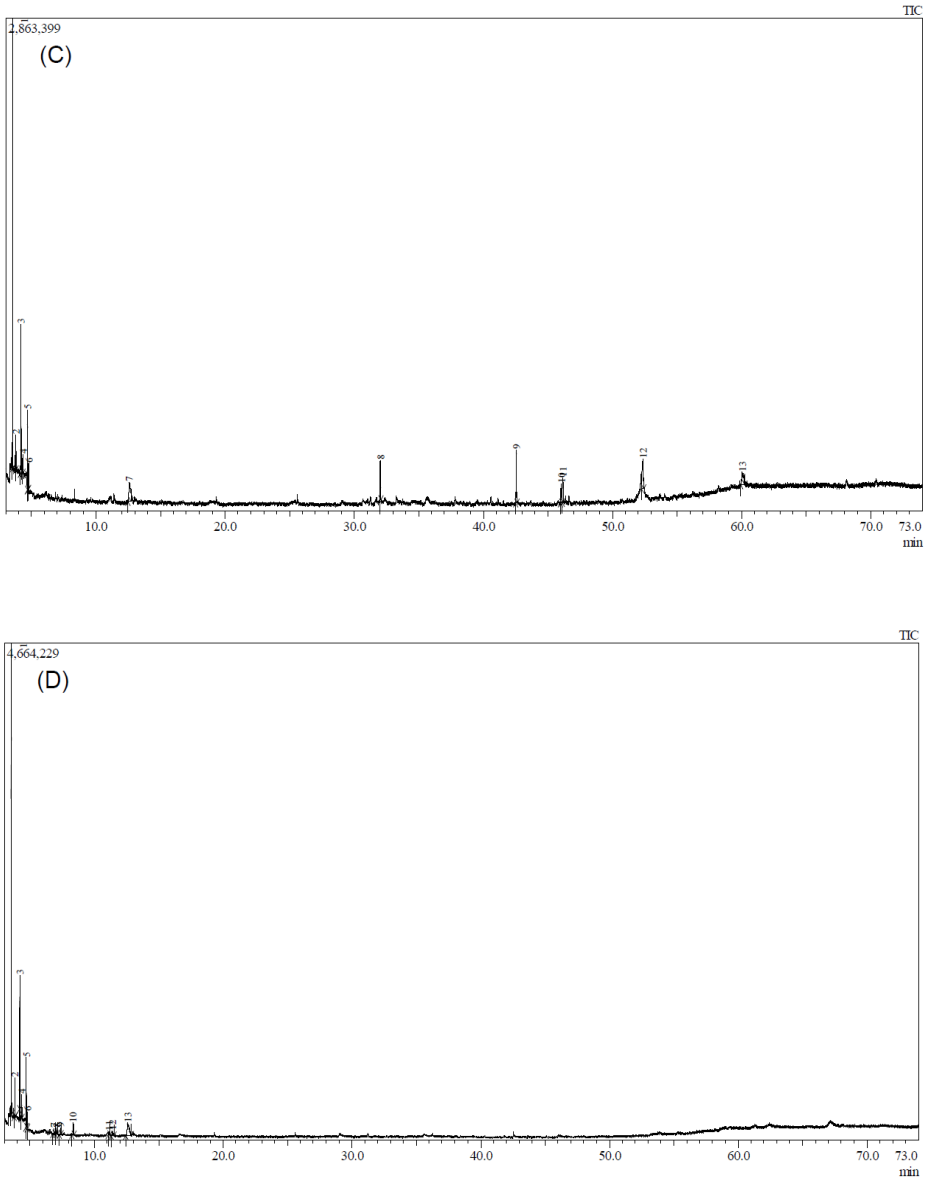


FIGURE 4. Typical total ion chromatograms for phytochemical analysis, (A) LCMS analysis of methanol extracts of *D. aromatica* leaves, (B) LCMS analysis of methanol extracts of *D. aromatica* barks, (C) GCMS analysis of methanol extracts of *D. aromatica* leaves, and (D) GCMS analysis of methanol extracts of *D. aromatica* barks

Meanwhile, phytochemical analysis of the volatile components was performed using GCMS for both of the active extracts (Figure 4(c) and 4(d)). The profiling of two *D. aromatica* extracts with GCMS showed that there are 13 metabolites appeared in each extract. Of the 13 metabolites in *D. aromatica* methanol leaves extract, 11 compounds were assigned with a compound name while two compounds were labeled as 'unknown' since the SI of these compounds did not achieve 80% when the mass spectrum was compared with the NIST library

(Table 4). Meanwhile, 8 compounds were assigned out of 13 metabolites from *D. aromatica* methanol bark extract based on the NIST library (Table 5). Five similar compounds were found in both extracts which were performic acid, acetic acid, decane, glycerine, and propanoic acid. It is thus suggested that these compounds which were present in both extracts contributed to the anti-oxidant and anti-diabetic activities of *D. aromatica*. Performic acid appeared abundantly in both extracts and is known as a disinfectant agent used in the food industry as it has antibacterial properties (Helvi & Harri 2010).

TABLE 2. Information on the metabolites of *Dryobalanops aromatica* leaves methanol extract analyzed using LCMS

No	RT (min)	Ion	Molecular formula	Measured mass (m/Z)	Tentative identification
1	4.108	$[M+H]^+$	$C_{42}H_{60}O_2$	597.4696	Spirilloxanthin
2	4.612	$[M+H]^+$	$C_{39}H_{56}O_{12}$	717.3855	Spinoside A
3	4.792	$[M+H]^+$	$C_{16}H_{28}O$	237.2204	8E,11Z-hexadecadienal
4	4.829	$[M+H]^+$	$C_{41}H_{56}N_8O_9$	805.4277	Nostocyclopeptide A3
5	4.926	$[M+H]^+$	$C_{45}H_{75}NO_{11}$	828.5213	Scytophycin C
6	5.015	$[M+H]^+$	$C_{43}H_{72}O_{19}$	428.3240	Kurilensoside I
7	5.016	$[M+H]^+$	$C_{28}H_{22}O_6$	455.8115	ε-viniferin
8	5.057	$[M+H]^+$	$C_8H_8O_5$	185.1606	Methyl gallate
9	5.198	$[M+H]^+$	$C_{24}H_{34}O_7$	435.2377	Clerodin
10	7.791	$[M+H]^+$	$C_{20}H_{37}NO_4S$	388.2534	N-linoleoyl taurine
11	11.877	[M+H] +	$C_{42}H_{30}O_9$	669.4432	α-viniferin
12	12.049	$[M+H]^+$	$C_{18}H_{41}N_7$	356.3486	Guazatine

TABLE 3. Information on the metabolites of *Dryobalanops aromatica* bark methanol extract analyzed using LCMS

No	RT (min)	Ion	Molecular formula	Measured mass (m/Z)	Tentative identification
1	4.107	$[M+H]^+$	$C_{24}H_{60}O_2$	597.4695	Spirilloxanthin
2	4.612	$[M+H]^+$	$C_{39}H_{56}O_{12}$	717.3855	Spinoside A
3	4.610	$[M+H]^+$	$C_{16}H_{28}O$	237.2204	8E,11Z-hexadecadienal
4	4.824	$[M+H]^+$	$C_{41}H_{56}N_8O_9$	805.4277	Nostocyclopeptide A3
5	4.922	$[M+H]^+$	$C_{45}H_{75}NO_{11}$	828.5213	Scytophycin C
6	5.011	$[M+H]^+$	$C_{43}H_{72}O_{19}$	428.3240	Kurilensoside I
7	5.012	$[M+H]^+$	$C_{28}H_{22}O_6$	455.8115	ε-viniferin
8	5.052	$[M+H]^+$	$C_8H_8O_5$	185.1607	Methyl Gallate
9	5.195	$[M+H]^+$	$C_{24}H_{34}O_7$	435.2377	Clerodin
10	7.789	$[M+H]^+$	$C_{20}H_{37}NO_4S$	388.2534	N-linoleoyl Taurine
11	11.875	[M+H] +	$C_{42}H_{30}O_9$	669.4438	α-viniferin
12	12.048	$[M+H]^+$	$C_{18}H_{41}N_7$	356.3486	Guazatine

TABLE 4. Information on metabolites of *Dryobalanops aromatica* leaves methanol extract analyzed using GCMS

Peak	R. Time (min)	Area (%)	SI	Compound name
1	3.52	24.86	86	Performic acid
2	3.78	2.21	92	Acetic acid
3	4.17	10.73	91	Glycerin
4	4.33	1.24	90	Propanoic acid
5	4.69	6.20	94	Glycerin
6	4.77	2.35	77	Unknown
7	12.56	10.97	93	Decane
8	32.0	7.07	94	Dodecanoic acid
9	42.55	8.54	95	Hexadecenoic acid
10	46.03	2.57	90	9,12-octadecadienoic acid
11	46.15	4.86	84	Cyclopropanebutanoic acid
12	52.35	11.28	81	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene- pentadic-4-enyl) cyclohexane
13	60.05	7.11	63	Unknown

TABLE 5. Information on metabolites of *Dryobalanops aromatica* bark methanol extract analyzed using GCMS

Peak	R. Time (min)	Area (%)	SI	Compound name
1	3.54	41.43	86	Performic acid
2	3.80	4.23	94	Acetic acid
3	4.19	14.12	92	Glycerin
4	4.35	3.26	96	Propanoic acid
5	4.72	11.07	95	Glycerin
6	4.79	2.38	76	Unknown
7	6.85	1.47	81	p-Dioxane-2,3-diol
8	7.05	1.11	66	Unknown
9	7.35	1.37	79	Unknown
10	8.34	2.63	72	Unknown
11	11.137	1.01	79	Unknown
12	11.40	1.35	84	Octane
13	12.56	13.58	95	Decane

Conclusion

It can be concluded from the current study, that out of all the plant extracts investigated, the methanol extracts of *D. aromatica* showed potential in inhibiting alpha-glucosidase, as well as containing high anti-oxidant properties. The results obtained corroborate with the claim that plants with anti-oxidant properties could have correlations with anti-diabetic properties. Thus, the current study could suggest that the anti-oxidant activities of *D. aromatica* extracts might have some association with the anti-diabetic effects of these extracts. This is the first report that highlights the antidiabetic potential of *D. aromatica*. Therefore, further investigation is warranted on *D. aromatica*, where bioassay-guided isolation of the potent extracts and phytochemical characterization of isolated bioactive constituents can be performed. Further work on this plant will open up more options in the management and treatment of diabetes.

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