Vietnam Journal of Agricultural Sciences

ISSN 2588-1299

VJAS 2022; 5(1): 1375-1388
https://doi.org/10.31817/vjas.2022.5.1.06

Phytochemical Analysis and Antioxidant and Alpha-glucosidase Inhibitory Activities of the Stem Bark of *Dialium cochinchinense* Pierre

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Abstract

The purpose of this study was to determine the phytochemical compositions and to evaluate the biological activity of the stem bark of *Dialium cochinchinense*. The phytochemical analysis showed the presence of steroids, terpenoids, anthraquinones, saponins, and phenolics in the stem bark of *D. cochinchinense*. The optimal extraction conditions were 70% ethanol at 30°C for 40min with a material/solvent ratio of 1/20. Under the optimal conditions, the corresponding predicted response value for the total phenolic content was 100.80 ± 0.40 mg GAE/g DW. The evaluation of antioxidant activity indicated that the polyphenol-rich extract was a good source of antioxidants as measured by the DPPH assay with the IC_{50} of 3.81 ± 0.58 μg mL^{-1}. The extract also demonstrated a strong α-glucosidase inhibitory activity with the IC_{50} value of 2.14 ± 0.05 μg/mL. Therefore, *D. cochinchinense* could be useful as a potential preventive intervention for free radicals in mediated diseases as well as an antioxidant drug and a potential source in treating diabetes mellitus in the pharmaceutical industry.

Keywords

Antioxidant, Caesalpinioideae, *Dialium cochinchinense*, Phytochemical, α-glucosidase

Introduction

Diabetes mellitus is a metabolic disorder characterized by a congenital (type I insulin-dependent diabetes mellitus/IDDM) or acquired (type II noninsulin-dependent diabetes mellitus/NIDDM) inability to transport glucose from the bloodstream into cells. Type I is usually diagnosed in childhood, and the body makes little or no

Received: June 18, 2021
Accepted: February 16, 2022

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insulin. Type II diabetes is an insulin resistance condition that occurs in adulthood, and as it afflicts approximately 90% of all diabetics, it is the major focus of diabetes research. Sustained high blood glucose levels (such as in the case of diabetes) are damaging to many organ systems. Thus, type II diabetes is associated with severe long-term complications which contribute to high levels of morbidity and mortality. For example, vascular and nerve damages caused by chronic hyperglycemia hasten the progression of cardiovascular disease and can lead to vision loss and renal failure (Reinehr, 2013). Diabetes-induced retinopathy is the primary cause of preventable blindness, while diabetic nephropathy is a major contributor to kidney disease (Roglic, 2016). The global prevalence of diabetes has reached epidemic proportions, and with worldwide incidence projected to increase from 424 million in 2017 to 629 million by 2045 (Idf, 2017), the global economic burden of the disease can be expected to skyrocket. Therefore, the appropriate management of hyperglycemia is a key goal of diabetes treatments and aims to minimize the development of chronic complications.

An important therapeutic approach for treating type II diabetes is to decrease post-prandial hyperglycemia by retarding the absorption of glucose through the inhibition of α-glucosidase in the digestive tract. The drugs used to treat diabetes are often expensive and have side effects such as obesity, jaundice, and hypoglycemia, etc., causing many difficulties while treating and taking care of patients (Lee *et al.*, 2014a). The scientific investigation of plants for their medicinal value is possibly used as an alternate and effective strategy for the discovery of novel therapeutic agents. Today, herbal medicines have been gaining increased popularity for treating the above-mentioned diseases because of their safety and lack of side effects. Herbal medicines have been used widely for treating various diseases since ancient times in India, China, and many other countries. In India, more than 800 traditional plants have been used for the treatment of obesity and diabetes (Saxena & Vikram, 2004). Such examples have demonstrated that the scientific investigation into evidence-based medicinal plants for discovering bioactive natural products with therapeutic applications is a promising avenue to establish novel therapies for the diseases.

Polyphenols are the most abundant antioxidants in the human diet and are the common and most widespread constituents in plants. Recently, they have emerged as critical phytochemicals in type II diabetes prevention and treatment. Their hypoglycemic action results from their antioxidative effects involved in restoring insulin-secreting machinery in pancreatic cells, or in their abilities to inhibit the activity of carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) (Xiao *et al.*, 2013). In addition, some flavonoids and polyphenols as well as sugar derivatives (acarbose) are inhibitors for α-glucosidase (Lee *et al.*, 2014b; Anand Priya *et al.*, 2019). Therefore, the discovery of α-glucosidase inhibitors from traditional plants is very helpful to develop new antidiabetic drugs.

Dialium is a genus of legume in the Caesalpinioideae family. The genus consists of about 30 species, of which most species occur in Africa (Junior *et al.*, 2016) and some species in Asia (India, Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, Singapore, Brunei, and Indonesia) (Schmidt & Nguyen, 2005). Previous phytochemical studies of plants in the genus *Dialium* have revealed the presence of steroids, terpenoids, polyphenols, and oleanolic acid glycosides (Awantu *et al.*, 2011; Adeleye *et al.*, 2014). Some bioactivities including antioxidant, antimicrobial, antibacterial, and antifungal, among others, have already been reported (Adeleye *et al.*, 2014). For example, the fruits of *D. guineese* are rich in minerals, sugars, and tartaric, citric, malic, and ascorbic acids (Ayessou *et al.*, 2014; Niyi, 2015). In spite of the use of *D. cochinchinense* in traditional medicine, there have been no reports on its phytochemical composition or biological activity.

In this study, we focused on the analysis of phytochemical compositions, antioxidant and alpha-glucosidase inhibitory activities of the stem bark of *D. cochinchinense*. This work may

help to guide researchers in the phytochemistry and pharmaceutical sciences in the future.

Meterials and Methods

Plant materials

The stem barks of *D. cochinchinense* Pierre were collected in Kon Chu Rang (Gia Lai province, Vietnam) in 2005. The plant was identified by Dr. Nguyen Quoc Binh (Vietnam Academy of Science and Technology). A voucher specimen (VN-1478) was deposited at the Herbarium of the Institute of Ecology and Biological Resources of the Vietnam Academy of Science and Technology, Hanoi, Vietnam.

Chemicals

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, ascorbic acid, α-glucosidase enzyme (CAS No 9001-42-7), and other chemicals such as *p*-nitrophenyl-α-D-glucopyranoside, 4-nitrophenol, and dimethyl sulfoxide were purchased from Sigma-Aldrich (USA). The sodium carbonate and other chemicals and reagents (AR) were purchased from MΛCKLIN (China). All solvents used in the extraction were redistilled.

Phytochemical screening of the stem bark extracts

The phytochemical tests were carried out following the methods described by Ijoma and Ajiwe with some modifications (Ijoma & Ajiwe, 2017). The stem barks of *D. cochinchinense* were taken for the phytochemical analyses to identify the presence of the bioactive constituents.

Screening for steroids and terpenoids

Fifty mL of 96% ethanol was added to 2.0g of dry powder of the stem bark in a round bottom flask. This was refluxed for a few minutes and warmly filtered using a Whatman filter paper grade 1. The filtrate was concentrated to 2.5mL on a boiling water bath followed by the addition of 5.0mL of distilled water. The mixture was allowed to stand for 1 hour. The presence of waxy matter indicated the presence of steroids.

Then, the mixture was filtered. Two mL of the filtrate was heated to dryness on a water bath. Three mL of glacial acetic acid was then added to the mixture and heated to almost dryness, followed by 1mL of sulphuric acid and subsequent heating. A grey coloration indicated the presence of terpenoids.

Screening for anthraquinones

To screen for anthraquinone, 0.5g of dry powder of the stem bark was extracted with 30.0 mL of 90% ethanol for 30 minutes x three times at 30°C. Twenty mL of the extract was added to 10.0mL of sulphuric acid and filtered. The filtrate was shaken with chloroform. The chloroform layer was pipetted into another test tube and a 10% ammonia solution was added. An aqueous ammonia layer showing yellow color indicated the presence of anthraquinone.

Screening for glycosides

The dry powder of the stem bark (0.5g) was extracted with 50.0mL of 90% ethanol for 30 minutes x three times at 30°C. A mixture of 2.0mL of acetic acid and 2.0mL of chloroform was added to 2.0mL of the filtrate. The solution was cooled and concentrated H_2SO_4 was added. A resulting green color of the solution showed the presence of glycosides.

Screening for saponins

Fifty mL of distilled water at 40°C was added to 0.5g of dry powder of the stem bark in a 200mL conical flask. The mixture was soaked in a thermostatic bath at 40°C for 20 minutes. The mixture was filtered into a 100mL beaker while warm and allowed to cool to room temperature. Then, the filtrate was used for the following tests:

Frothing test: One mL of filtrate was diluted with 4.0mL of distilled water and vigorously stirred in a test tube. Persistent (foaming) frothing showed the presence of saponin.

Fehling test: One mL of 1 M $CuSO_4$ solution was mixed with 1.0mL of 2 M NaOH solution and 1.0mL of 10% sodium-potassium tartrate solution. This solution was added to 5.0mL of filtrate and heated. Reddish precipitate confirmed the presence of saponin. This was further heated with two drops of sulphuric acid

and a brick red precipitate confirmed the presence of saponin.

Screening for phenolic

The dry powder of the stem bark (0.5g) was extracted with 50.0mL of 90% ethanol for 30 minutes at 30°C and filtered. One mL of the filtrate and 1.0mL of iron (III) chloride were mixed. Phenolic compounds made the solution turn green.

Selection of the extraction procedure factors and determination of total phenolic content

Selection of the extraction procedure factors

A study of the influence of the polyphenol extraction process of the stem bark of *D. cochinchinense* on the total phenolic content was conducted using the one-factor-at-a-time approach. The experiments were conducted independently from each other. Phenolic contents were affected by the extraction solvents (ethanol, acetone, methanol, and water), solvent concentration, material/solvent ratio, extraction temperature, and extraction time.

Extraction solvent

Ethanol, acetone, methanol, and water were used to test their ability to extract the total phenolics from the stem bark of *D. cochinchinense*. The dry powder of stem bark (0.5g) was extracted with 10.0mL of each solvent in 70% concentration *(v/v)* and ultrasound was used for 30min at 30°C. The mixtures were centrifuged at 6000rpm for 10min at room temperature and the supernatants were collected to determine the total phenolic content.

Solvent concentration

The dry powder of the stem bark of *D. cochinchinense* (0.5g) was extracted with 10.0mL ethanol and ultrasound was used for 30min at 30°C. The ethanol concentrations evaluated were 60, 70, 80, 90, and 99.7% in water (*v/v*). The other extraction conditions were kept constant. The mixtures were centrifuged at 6000 rpm for 10 min at room temperature and the supernatants were collected to determine the total phenolic content.

Material/solvent ratio

The dry powder of the stem bark of *D. cochinchinense* was mixed with the ethanol solution at the concentration chosen from the experiment described above in order to obtain material-solvent ratios of 1/5-1/25 (*w/v*) and ultrasound was used for 30min at 30°C. The mixture was centrifuged at 6000rpm for 10min at room temperature and the supernatants were collected to determine the total phenolic content.

Extraction temperature

The dry powder of the stem bark of *D. cochinchinense* (0.5g) was extracted with 10.0mL of the ethanol solution selected from the concentration experiment described above and ultrasound was used for 30min at different temperatures (30, 40, 50, and 80°C). The mixtures were centrifuged at 6000rpm for 10min at room temperature and the supernatants were collected to determine the total phenolic content.

Extraction time

The dry powder of the stem bark of *D. cochinchinense* (0.5g) was extracted with 10.0mL of the ethanol solution selected from the concentration experiment described above and ultrasound was used for various times ranging from 20 to 90min at 30°C. The mixtures were centrifuged at 6000rpm for 10min at room temperature and the supernatants were collected to determine the total phenolic content.

Optimal conditions

The dry powder of the stem bark of *D. cochinchinense* (3.0g) was mixed with the ethanol solution with the conditions of ethanol concentration, extraction temperature, and extraction time chosen from the experiments described above. The mixtures were centrifuged at 6000rpm for 10min at room temperature and the supernatants were collected to determine the total phenolic content. Then, after the removal of the solvent under reduced pressure, the polyphenol-rich extract was estimated for antioxidant and α-glucosidase inhibitory activities.

Total phenolic content

The extract of *D. cochinchinense* was used to determine the total phenolic contents by the Folin-Ciocalteu method (Singleton & Rossi, 1965). Specifically, 2.5mL of 10% Folin-Ciocalteu reagent was added to 0.5mL of the plant extract and shaken for 5min. Then, 2.0mL of 7.5% sodium carbonate solution was added and the mixture was kept in the dark for 2 hours. Absorbance was monitored at 760nm using a Cary 60 UV-VIS spectrophotometer. Results were expressed as mg of gallic acid equivalents per gram of dry weight of the sample (mg GAE/g DW).

DPPH scavenging assay

The scavenging activity of DPPH free radicals of the extract was determined by the method of Tabart *et al*. (2009). Three mL of extract at the concentrations of 2, 4, 6, 8, and 10.0 µg mL^{-1}, respectively, were prepared in methanol and added to 1.0mL of DPPH (0.1mM). The solutions were set aside in dark conditions for 30min, and the absorbance of each solution was measured at 517nm. Ascorbic acid was used as a reference. The inhibition percentages of DPPH were calculated using the equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_x}{A_0} \cdot 100\ (\%)$$

where A_0: the absorbance of the control (3.0mL methanol and 1.0mL of DPPH) and A_x: the absorbance of the sample.

Assay for *α*-glucosidase inhibition

The *α*-glucosidase inhibition assay was performed according to the previously described methods of Moradi-Afrapoli *et al.* (2012). The sample solution (2.0mL sample extract at the concentrations of 1, 4, 16, 64, and 256 µg mL^{-1}, respectively, dissolved in dimethyl sulfoxide; DMSO) and 40.0mL *α*-glucosidase 0.5 U mL^{-1} (40mL) were mixed in 120.0mL of 0.1 M phosphate buffer (pH 7.0). After a 5min pre-incubation period, 40.0mL of 5mM *p*-nitrophenyl-*α*-D-glucopyranoside solution was added, and the solution was incubated at 37°C for 30min. The absorbance of the released 4-nitrophenol was measured at 405nm by using a microplate reader (Molecular Devices, Sunnyvale, CA). Acarbose was used as the positive control.

Statistical Analysis

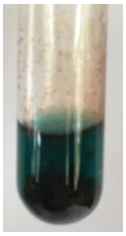
Experiments were carried out in three replicates. The value of IC_{50} was calculated using GraphPad Prism 8.0. Data were presented as means ± standard deviation. One-way analysis of variance (ANOVA) was performed using Minitab ver. 16.0 to analyze statistical significances. Significance levels were tested at $P < 0.05$.

Results and Discussion

Phytochemical constituents

Phytochemical screening is an important step in the effort to uncover the potential of medicinal plant resources such as antibiotics, antioxidants, and anticancer agents. This screening was carried out to provide an overview of the classes of compounds contained in the stem bark of *D. cochinchinense*. These results were then used for further analyses. Phytochemical analysis of the stem bark of *D. cochinchinense* showed the presence of a variety of secondary compounds, namely steroids, terpenoids, anthraquinones, saponins, and phenolics, but not glycoside compounds. The results are shown in **Table 1**. Several works have reported on the phytochemical constituents of the pulp, seeds, leaves, and stem bark of the *Dialium* genus including David *et al.* (2011), Oluwole-Banjo (2019), Ijoma & Ajiwe (2017), and Bui *et al.* (2019). These reports are in agreement with our results. The presence of various phytochemical compounds in the sample extracts may adduce evidence for the reported ethnomedicinal uses of *D. cochinchinense* plants in Vietnam because the potency of medicinal plants is attributed to the action of the phytochemical constituents (Balandrin *et al.*, 1985).

Table 1. Phytochemical analysis of *D. cochinchinense*

Chemical groups					
Steroids and terpenoids test	Anthraquinones test	Glycosides test	Saponins Frothing test	Saponins Fehling test	Phenolic test
+	+	-	+	+	+
					

Selection of the extraction procedure factors

Effect of extraction solvent

In order to find the best conditions for phenolic extraction from the stem bark of D. cochinchinense, different extraction solvents were tried out. The total phenolic (TP) content varied in response to the solvent used ($P < 0.05$) (Figure 1). The average efficiencies of the studied solvents in the extraction of TP from the stem bark of *D. cochinchinense* were placed in order as follows: ethanol > acetone = methanol > water. Ethanol was the most efficient solvent for the extraction of phenolic compounds with the TP content of 80.05 ± 0.30 mg GAE/g DW, and water as an extraction solvent occupied the lowest place in phenolic extraction yields with the TP content of 42.80 ± 0.70 mg GAE/g DW. These results are in accordance with a previous study reporting that solvents with very high polarity, such as water, do not give good extraction results (Liu *et al.*, 2000). Moreover, the use of water as the only solvent yielded an extract with a high content of impurities (e.g., organic acids, sugars, and soluble proteins), which could interfere in phenolic identification and quantification. In accordance with previously reported studies, ethanol was employed for the extraction of TP compounds from grapes (Spigno *et al.*, 2007) and *Orthosiphon stamineus* (Chew *et al.*, 2011). As the extraction with ethanol gave the highest TP values from the stem bark of *D. cochinchinense* and as this solvent is the most frequently used in phenolic extractions, it was chosen as the solvent for the following steps.

Effect of solvent concentration

The recovery of phenolic compounds as a function of the solvent concentration was evaluated. The use of water in combination with other organic solvents contributes to the creation of a moderately polar medium that ensures the extraction of polyphenols (Lapornik *et al.*, 2005; Liyana-Pathirana & Shahidi, 2005). Water-ethanol mixtures were used as the extraction solvent in this study. The selection of ethanol as the extraction solvent was justified by the fact that ethanol is a food-grade solvent, and is less toxic and more abundant as compared to acetone, methanol, and other organic solvents. It has also been widely used to extract phenolic compounds in previous studies (Stoica *et al.*, 2013; Nedyalkov *et al.*, 2015; Waszkowiak & Gliszczyńska-Świgło, 2016; Maulana *et al.*, 2019). The ethanol concentration in the extraction medium had a significant effect on the recovery of TP content (**Figure 2**) ($P < 0.05$). The TP content increased when the ethanol concentration increased, reaching a maximum TP content at 70% (*v/v*) with the TP content of 97.04 ± 3.04 mg GAE/g DW. However, this value was not significantly different from the ethanol concentrations of 60 and 80% (*v/v*). The results also showed that the TP content decreased when the ethanol concentration increased to 100%. A general principle in solvent extractions is "like dissolves like", which means that a solvent only extracts those phytochemicals which have similar polarity with the solvent (Zhang *et al.*, 2007). Our results showed that the polyphenols in the stem bark had a polarity

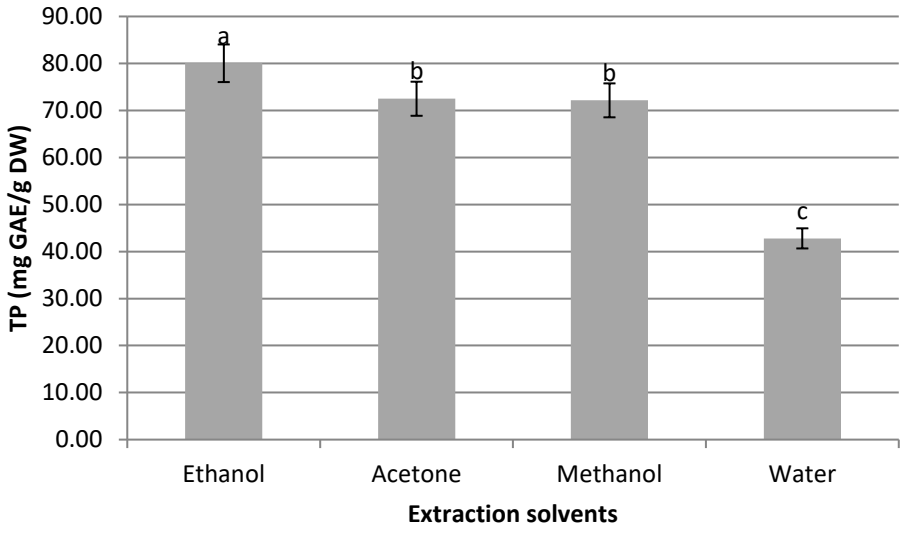


Figure 1. Effect of extraction solvents on the TP content of the stem bark of *D. cochinchinense*
Note: Columns with different letters (a, b, or c) are significantly different (P <0.05)

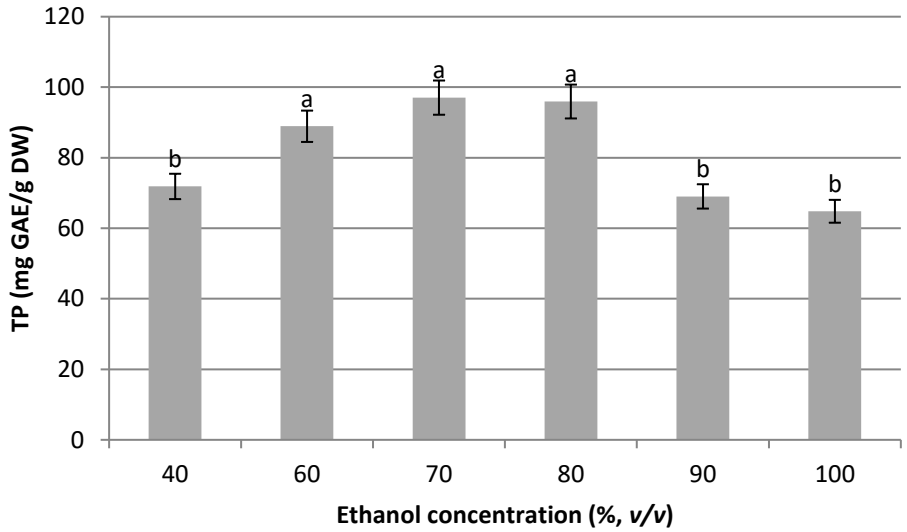


Figure 2. Effect of the solvent/water ratio on the TP content of the stem bark of *D. cochinchinense*
Note: Columns with different letters (a, b, or c) are significantly different (P <0.05)

corresponding to 70% ethanol. Due to the results obtained so far, it was decided to continue working with 70% ethanol for the evaluation of the other parameters.

Effect of the material/solvent ratio

The impact of the material/solvent ratio on the extraction of phenolic compounds from the stem bark of *D. cochinchinense* was determined with five ratios (1/5, 1/10, 1/15, 1/20, and 1/25 g mL^{-1}) for a 30min extraction period, with a 70% ethanol solution, at 30°C. The amounts of TP extracted are presented in **Figure 3**. The results of the one-way analysis of variance showed that there were significant differences among the material/solvent ratios studied (P <0.05). The TP content increased when the ratio increased from 1/5 to 1/10, remained fairly constant at 1/20, and then reached a maximum value at the 1/25 ratio. The high solubility of polyphenols in a hydroalcoholic solution, especially when they are in a glycoside form (Silva *et al.*, 2007), may

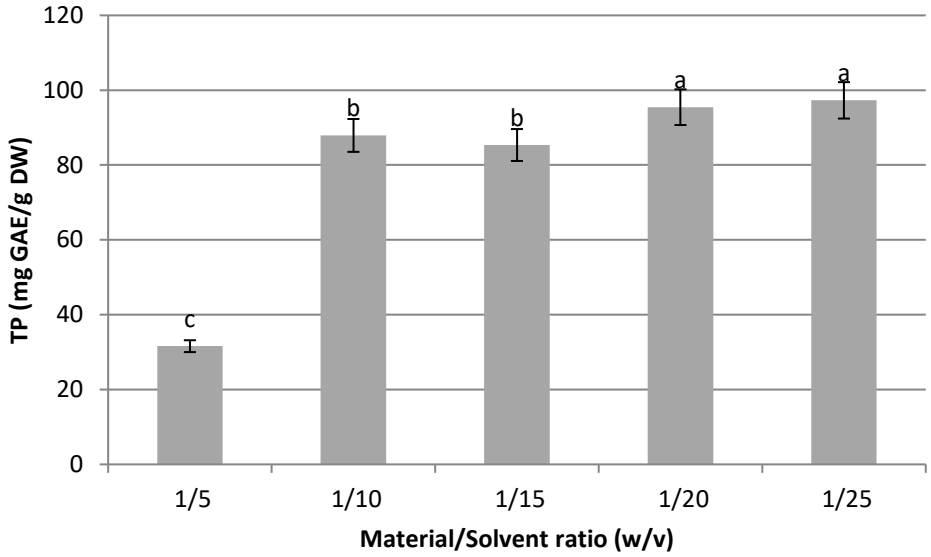


Figure 3. Effect of the material/solvent ratio on the TP content of the stem bark of *D. cochinchinense*
Note: Columns with different letters (a, b, or c) are significantly different (P <0.05)

explain the absence of variability for the higher ratios. According to our results, for any ratio chosen above 1/20, the quantity of phenolic compounds extracted would remain the same.

This allows for the choosing of any value above this limit, but one should avoid the use of an excessive quantity of solvent in the design of a process. Therefore, the material/solvent ratio of 1/20 was chosen for the evaluation of the other parameters.

Effect of extraction temperature

Temperature has a strong influence on the extraction of secondary compounds in general and polyphenol compounds in particular. As the temperature increases, the diffusion rate increases, and, therefore, the amount of polyphenols extracted from the material is increased. However, if the temperature rises too high, this may lead to the degradation of some phenolic compounds during extraction coupled with a relatively longer extraction time (Chirinos *et al.*, 2007). In this experiment, the polyphenol compounds were extracted with 70% ethanol and the material/solvent ratio of 1/20 for 30 minutes at different temperatures from 30 to 80°C. **Figure 4** shows the effects of temperature on the extraction of polyphenols from the stem bark of *D. cochinchinense*. The statistical analysis of TP content showed that there were no statistically significant differences among the extraction temperatures ranging from 30 to 80°C (P <0.05). This result may be explained by the small particles of the material which facilitate the extraction of polyphenol compounds (Pinelo *et al.*, 2007; Kossah *et al.*, 2010). An extraction temperature of 30°C was chosen as the optimal temperature for the extraction of polyphenols from the stem bark of *D. cochinchinense*.

Effect of extraction time

The effect of time on the extraction of polyphenols from the stem bark of *D. cochinchinense* is shown in **Figure 5**. The results showed that the extraction of polyphenols increased as time increased from 20min to 30min. Longer extraction times decreased the content of polyphenols, possibly because of some loss of phenolic compounds via oxidation, and these products might have been polymerized into insoluble compounds (Shi *et al.*, 2003). However, no significant differences (P <0.05) were observed among the amounts of polyphenols with the extraction times of 30 to 90min. Therefore, 40min was considered as the optimal time for the extraction of polyphenols

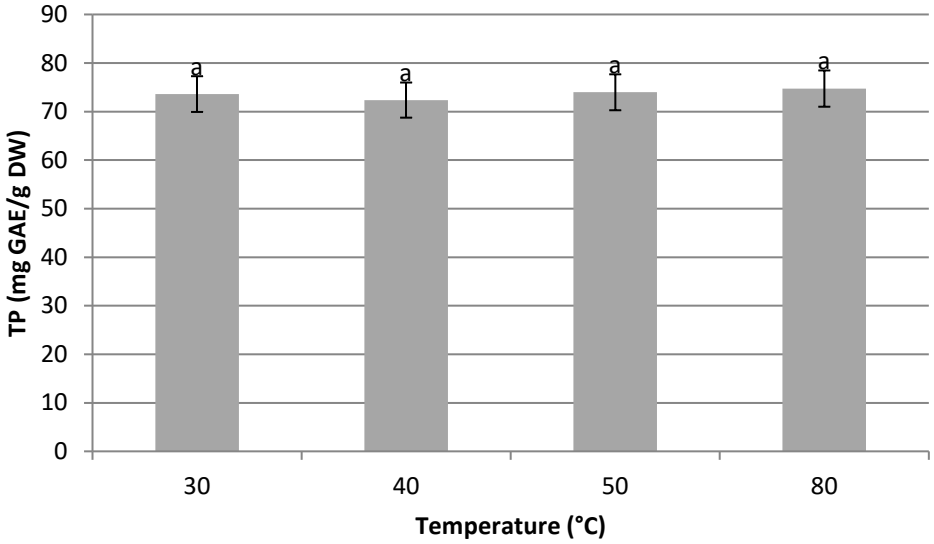


Figure 4. Effect of extraction temperature on the TP content of the stem bark of *D. cochinchinense*
Note: Columns with different letters (a, b, or c) are significantly different (P <0.05)

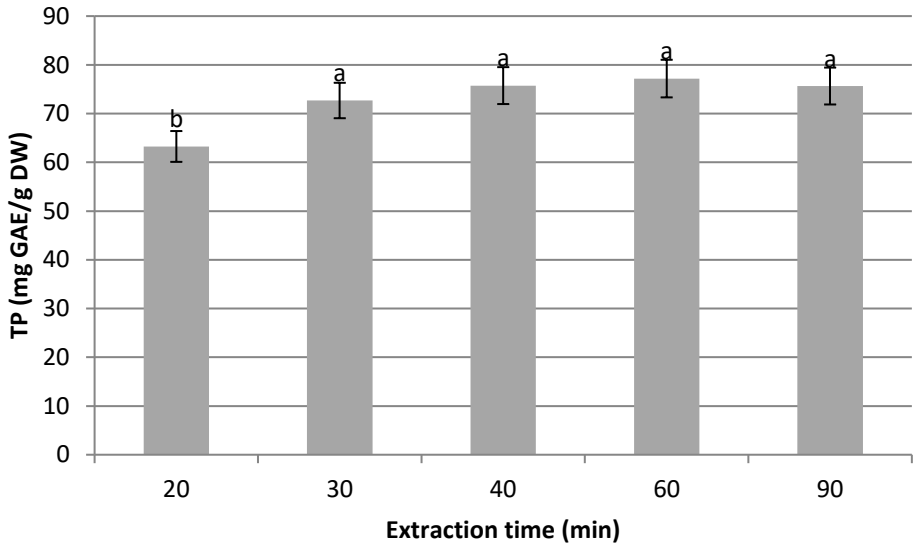


Figure 5. Effect of extraction time on the TP content of the stem bark of *D. cochinchinense*
Note: Columns with different letters (a, b, or c) are significantly different (P <0.05)

from the stem bark of *D. cochinchinense*.

Optimal conditions

Extraction studies were done using the one-factor-at-a-time approach. The ideal extraction conditions for carrying out the determination of TP of the stem bark of *D. cochinchinense* were 70% ethanol at 30°C for 40min with a material/solvent ratio of 1/20. Under the ideal extraction conditions, the TP content was found to be 100.80 ± 0.40 mg GAE/g DW. This value is smaller than the TP content in seeds of this species (133.5 ± 2.87 mg/g DW) (Bui *et al.*, 2019), but more than five times the TP content in the seeds of *Dialium guineense* (Oluwole-Banjo, 2019). These differences are due to the effects of plant parts and species on the total phenolic content.

In comparison to the bark of traditional Chinese medicinal plants associated with anticancer treatments, including *Paeonia*

suffruticosa Andr (2.9 mg GAE/g DW), *Phellodendron amurense* Rupr. (16.8 mg GAE/g DW) (Cai *et al.*, 2004), and 56 other selected Chinese medicinal plants of which the total phenolic content ranged from 0.18 ± 0.01 to 59.43 ± 1.03 mg GAE/g DW (Song et *al.*, 2010), *D. cochinchinense* had a much higher total phenolic content. The total phenolic content of *D. cochinchinense* bark was four times higher than that of *Lannea discolor* bark (27.64 ± 0.09 mg GAE/g DW), which some traditional herbal practitioners in Malawi claim is effective in the management of cancer (Mwamatope et *al.*, 2020). The bark of *D. cochinchinense* was much richer in phenolic compounds than three wild medicinal plants from Bahrain, namely *Aizoon canariense*, *Asphodelus tenuifolius*, and *Emex spinosus* (135.84 - 442.44 mg GAE/100g DW) (Al-Laith *et al.*, 2015). However, *D. cochinchinense* bark contained fewer phenolic compounds than *Prunus africana* bark (148.55 ± 4.05 mg GAE/g DW) and *Bridelia micrantha* bark (128.79 ± 1.54 mg GAE/g DW), which have wide distributions in the mountainous regions of Africa and were selected due to several studies on their medicinal potency, including having antimicrobial, anti-inflammatory, antidiabetic, antimalarial, fever-reducing, and anti-cancer properties (Siangu *et al.*, 2019). In general, the bark of *D. cochinchinense* could be considered a polyphenol source in Asia and could be the material of future studies for medicinal purposes.

Antioxidant activity

The polyphenol-rich extract of the stem bark of *D. cochinchinense* was studied for its antioxidant activity using DPPH. The results of the free radical scavenging activity of the DPPH assay showed the percentages of antioxidant activities of 31.67, 54.39, 68.16, 75.29, and 78.77% for the 1.5, 3.0, 4.5, 6.0, and 7.5 µg mL^{-1} extracts, respectively (**Figure 6**). There was no significant difference (P <0.05) in the antioxidant activity of the polyphenol-rich extract at 3.0 µg mL^{-1} when compared with the standard ascorbic acid. DPPH is a relatively stable free radical scavenger that converts the unpaired electrons to paired ones by hydrogen proton donation. The scavenging of DPPH radicals in this study indicates the potency of the plant extracts in donating hydrogen protons to the lone pair of electrons of the radicals. The methods have proven the effectiveness of the extracts in a concentration-dependent manner. The strong activity observed in the DPPH radical scavenging assay (with an IC_{50} value of 3.81 ± 0.58 µg mL^{-1}) may be a result of the high level of the TP content in the plant extracts. The antioxidant activity of the stem bark was greater

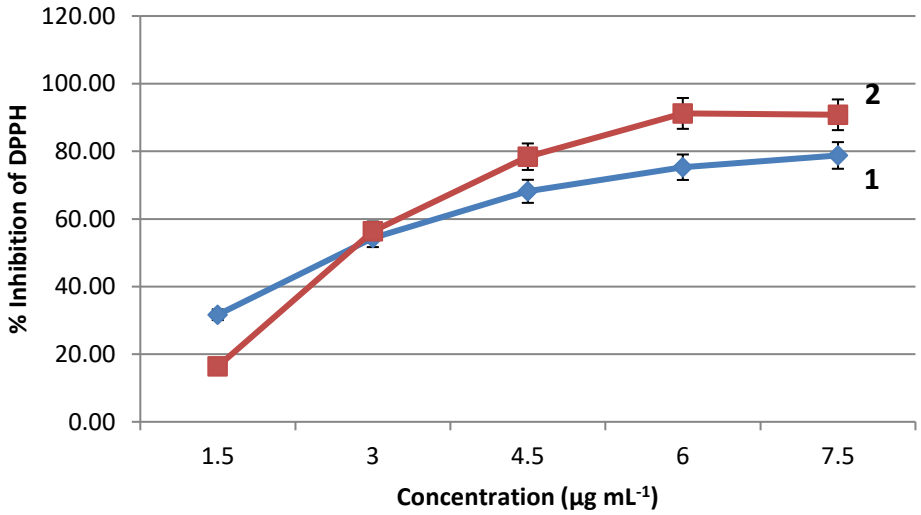


Figure 6. Inhibition of DPPH radicals by the polyphenol-rich extract of the stem bark of *D. cochinchinense* (1) and control (Ascobic acid) (2)

than that of the seeds (with IC_{50} of 33.6 ± 1.66 µg mL^{-1}) (Bui *et al.*, 2019). Thus, it can be affirmed that polyphenolic compounds are highly determinant in the antioxidant activity of this plant.

In comparison with 90 Vietnamese medicinal plant extracts having DPPH IC_{50} values ranging from 4.9 to more than 100.0 µg $µL^{-1}$ (Phan Thi Anh Dao *et al.*, 2012), *D. cochinchinense* had a high antioxidant capacity. This plant bark had an antioxidant capacity at least ten times higher than the eight plant species (*Selaginella* sp., *Ficus bhotanica*, *Lygodium microphyllum*, *Ipomoea cymosa*, *Melastoma malabathricum*, *Naravellia zeylanica*, *Glochidion arborescens*, and *Rubus ellipticus*) used in Indian folk medicines which had DPPH IC_{50} values ranging from 37.26 ± 0.05 to 1212.51 ± 37.25 µg mL^{-1} (Handique & Gogoi, 2016). Interestingly, although *D. cochinchinense* bark contained fewer polyphenols than the bark of two selected Kenyan medicinal plants (*Prunus africana* and *Bridelia micrantha*) with DPPH IC_{50} values of 0.033 and 0.038 mg mL^{-1}, respectively (Siangu *et al.*, 2019), its DPPH scavenging capacity was higher. This could be explained by the difference in the phenolic profiles of the three plants.

α-Glucosidase inhibitory activity

The polyphenol-rich extract of the stem bark of *D. cochinchinense* was evaluated for its α-glucosidase inhibitory activity. This extract exhibited 99.0, 99.0, 98.0, 98.0, and 20.5% inhibitory activities at 256, 64, 16, 4, and 1 µg mL^{-1}, respectively. The IC_{50} value of this polyphenol-rich extract was found to be 2.14 ± 0.05 µg mL^{-1}, while the IC_{50} value for acarbose was calculated to be 197.33 ± 2.51 µg mL^{-1} (**Figure 7**). From these results, it was shown that the polyphenol-rich extract effectively inhibited the action of the α-glucosidase enzyme by a dose-dependent manner. In particular, there were no significant differences (P <.05) in the α-glucosidase inhibitory activities of the extract at the concentrations of 4 to 256 µg mL^{-1}. Various studies have suggested the role of plant extracts as α-glucosidase inhibitors, clearly indicating the potential of these extracts to manage hyperglycemia (Abesundara *et al.*, 2004; Iwai *et al.*, 2006; Qaisar *et al.*, 2014; Liu *et al.*, 2016; El-Manawaty & Gohar, 2018; Joycharat *et al.*, 2018). The activity of these plant extracts can be attributed to the phytoconstituents that are present in them, such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, and

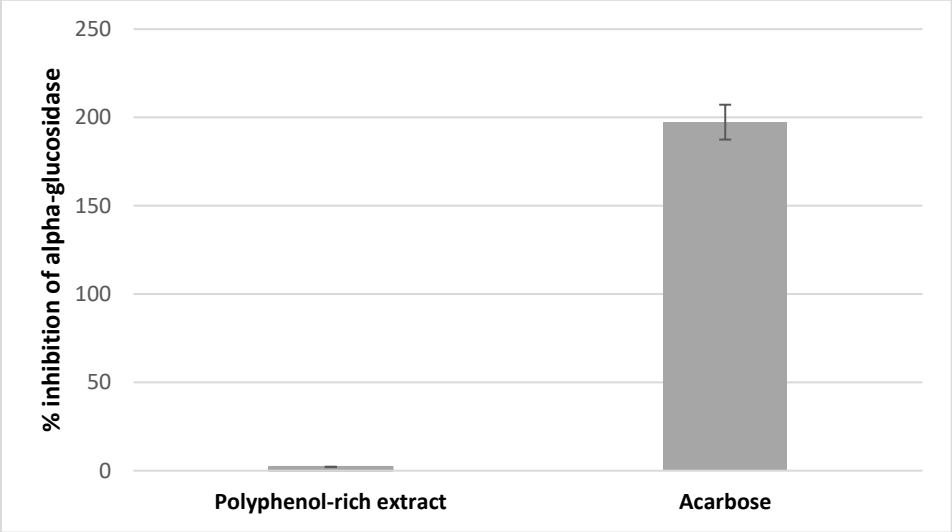


Figure 7. Inhibition of α-glucosidase by the polyphenol-rich extract of the stem bark of *D. cochinchinense* with control (Acarbose)

phenolic compounds. In the present study, the high TP content may be explained by the strong ability of the extract to inhibit the α-glucosidase enzyme (Xiao *et al.*, 2013). Although phenolic compounds may be involved and play an important role in the α-glucosidase inhibitory activity of the plant, there may be other compounds in *D. cochinchinense* that could contribute to the α-glucosidase inhibitory activity in addition to the phenolics.

Conclusions

In this study, the phytochemical constituents of the stem bark of *D. cochinchinense* collected in Vietnam were investigated. The results showed the presence of steroids, terpenoids, anthraquinones, saponins, and phenolics. The optimal conditions for the extraction of the total phenolic content were also surveyed and were as follows: 70% ethanol (*v/v*) with a material/solvent ratio of 1/20 at 30°C for 40min. The study demonstrated the antioxidant and α-glucosidase inhibitory activities of the polyphenol-rich extract of the stem bark of *D. cochinchinense*. The considerable content of phenolic compounds of the extract could be linked to their antioxidant and α-glucosidase inhibitory activities. Further chemical investigations and evaluations of the antioxidant activity of the isolated compounds should be carried out to provide further evidence for a new source of antioxidants and α-glucosidase inhibitory activities from plants in Vietnam.

Acknowledgments

This work was financially supported by an institutional project sponsored by the Vietnam – Belgium Project at Vietnam National University of Agriculture, Vietnam, through a grant to Ms. Vu Thi Huyen. We would like to express our thanks to Dr. Nguyen Quoc Binh for his valuable help in plant collection and botanical determination.

References

Abesundara K. J., Matsui T. & Matsumoto K. (2004). α-Glucosidase inhibitory activity of some Sri Lanka plant extracts, one of which, *Cassia auriculata*, exerts a strong antihyperglycemic effect in rats comparable to the therapeutic drug acarbose. Journal of Agricultural and Food Chemistry. 52(9): 2541-2545.

Adegboye M., Akinpelu D. & Okoh A. (2008). The bioactive and phytochemical properties of *Garcinia kola* (Heckel) seed extract on some pathogens. African Journal of Biotechnology. 7(21): 3934-3938.

Adeleye A. O., Ajiboye T. O., Iliasu G. A., Abdussalam F. A., Balogun A., Ojewuyi O. B. & Yakubu M. T. (2014). Phenolic extract of *Dialium guineense* pulp enhances reactive oxygen species detoxification in aflatoxin B1 hepatocarcinogenesis. Journal of Medicinal Food. 17(8): 875-885.

Al-Laith A. A., Alkhuzai J. & Freije A. (2019). Assessment of antioxidant activities of three wild medicinal plants from Bahrain. Arabian Journal of Chemistry Volume 12(8): 2365-2371.

Anand Priya V. V. M., Kranthi K., Punnagai K. & David D. C. (2019). Evaluation of Alpha-Glucosidase Inhibitory Activity of *Vinca Rosea*. Biomedical and Pharmacology Journal. 12(2): 783-786.

Awantu A. F., Lenta B. N., Bogner T., Fongang Y. F., Ngouela S., Wansi J. D., Tsamo E. & Sewald N. (2011). Dialiumoside, an Olean-18-ene Triterpenoid from *Dialium excelsum*. Zeitschrift für Naturforschung B. 66(6): 624-628.

Ayessou N. C., Ndiaye C., Cisse M., Gueye M., Sakho M. & Dornier M. (2014). Nutrient composition and nutritional potential of wild fruit *Dialium guineense*. Journal of Food Composition and Analysis. 34(2): 186-191.

Balandrin M. F., Klocke J. A., Wurtele E. S. & Bollinger W. H. (1985). Natural plant chemicals: sources of industrial and medicinal materials. Science. 228(4704): 1154-1160.

Bui L. T. K., Nguyen Q. T., Dao L. M., Nguyen H. L., Lam M. V. & Hoang C. T. (2019). Evaluation of antimicrobial, antioxidant and cytotoxic activities of *Dialium cochinchinensis* seed extract. Indian Journal of Pharmaceutical Sciences. September-October: 975-980.

Cai Y., Luo Q., Sun M. & Corke H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences 74: 2157-2184.

Chew K., Khoo M., Ng S., Thoo Y., Aida W. W. & Ho C. (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Orthosiphon stamineus* extracts. International Food Research Journal. 18(4): 1427.

Chirinos R., Rogez H., Campos D., Pedreschi R. & Larondelle Y. (2007). Optimization of extraction

conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers. Separation and Purification Technology. 55(2): 217-225.

David A.A., Olaniyi A. T., Mayowa A. O., Olayinka A. A & Anthony O. I (2011). Anti-Vibrio and preliminary phytochemical characteristics of crude methanolic extract of the leaves of *Dialium guineense* (Wild). Journal of Medicinal Plant Reasearch. 5(11): 2398-2404.

El-manawaty M. & Gohar L. (2018). In vitro alpha-glucosidase inhibitory activity of Egyptian plant extracts as an indication for their antidiabetic activity. IN VITRO. 11(7): 360-367.

Handique J. G., Gogoi D. (2016). Antioxidant Activities of the Medicinal Plants Used for Preparation of Fermentation Cakes of "Haanj", the Rice Based Alcoholic Beverage of Ahom Community People of Assam, India. International Journal of Pharmacognosy and Phytochemical Research. 8(2): 217-222.

IDF (2017). IDF Diabetes Atlas, 8th edition. International Diabetes Federation. Pages 150 pages.

Ijoma K. & Ajiwe V. (2017). Phytochemical Screening of *Dialium Indum* Leaf extract (Velvet Tarmarind). International Journal of Phytopharmacy. 7(1): 06-13.

Iwai K., Kim M.-Y., Onodera A. & Matsue H. (2006). *α*-Glucosidase Inhibitory and Antihyperglycemic Effects of Polyphenols in the Fruit of *Viburnum dilatatum* Thunb. Journal of agricultural and food chemistry. 54(13): 4588-4592.

Joycharat N., Issarachote P., Sontimuang C. & Voravuthikunchai S. P. (2018). Alpha-glucosidase inhibitory activity of ethanol extract, fractions and purified compounds from the wood of *Albizia myriophylla*. Natural Product Research. 32(11): 1291-1294.

Junior M. J. d. A. F., Pinto R. B. & Mansano V. D. F. (2016). A Taxonomic Revision of the genus *Dialium* (Leguminosae: Dialiinae) in the Netotropics. Phytotaxa. 283(2): 123-142.

Kossah R., Nsabimana C., Zhang H. & Chen W. (2010). Optimization of extraction of polyphenols from Syrian sumac (*Rhus coriaria* L.) and Chinese sumac (*Rhus typhina* L.) fruits. Research Journal of Phytochemistry. 4(3): 146-153.

Lapornik B., Prošek M. & Wondra A. G. (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering. 71(2): 214-222.

Lee M. Y., Choi D. S., Lee M. K., Lee H. W., Park T. S., Kim D. M., Chung C. H., Kim D. K., Kim I. J. & Jang H. C. (2014a). Comparison of acarbose and voglibose in diabetes patients who are inadequately controlled with basal insulin treatment: randomized, parallel, open-label, active-controlled study. Journal of Korean Medical Science. 29(1): 90-97.

Lee S., Mediani A., Nur Ashikin A., Azliana A. & Abas F. (2014b). Antioxidant and *α*-glucosidase inhibitory activities of the leaf and stem of selected traditional medicinal plants. International Food Research Journal. 21(1): 165-172.

Li J.-K., Liu X.-D., Shen L., Zeng W.-M. & Qiu G.-Z. (2016). Natural plant polyphenols for alleviating oxidative damage in man: Current status and future perspectives. Tropical Journal of Pharmaceutical Research. 15(5): 1089-1098.

Liu F. F., Ang C. Y. & Springer D. (2000). Optimization of extraction conditions for active components in *Hypericum perforatum* using response surface methodology. Journal of Agricultural and Food Chemistry. 48(8): 3364-3371.

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 Complementary and Alternative Medicine. 16(1): 378 (8 pages).

Liyana-Pathirana C. & Shahidi F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry. 93(1): 47-56.

Malik E. M. & Müller C. E. (2016). Anthraquinones as pharmacological tools and drugs. Medicinal Research Reviews. 36(4): 705-748.

Maulana T., Falah S. & Andrianto D. (2019). Total phenolic content, total flavonoid content, and antioxidant activity of water and ethanol extract from Surian (*Toona sinensis*) leaves. IOP Conference Series: Earth and Environmental Science. IOP Publishing. 012021.

Moradi-Afrapoli F., Asghari B., Saeidnia S., Ajani Y., Mirjani M., Malmir M., Bazaz R. D., Hadjiakhoondi A., Salehi P. & Hamburger M. (2012). *In vitro* *α*-glucosidase inhibitory activity of phenolic constituents from aerial parts of *Polygonum hyrcanicum*. DARU Journal of Pharmaceutical Sciences. 20(1): 37 (6 pages).

Mwamatope B., Tembo D., Chikowe I., Kampira E., Nyirenda C. (2020). Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. Scientific African. 9(2020): e00481.

Nedyalkov P., Kaneva M., Mihaylova D., Kostov G. & Kemilev S. (2015). Influence of the ethanol concentration on the antioxidant capacity and polyphenol content of *Alchemilla mollis* extracts. Comptes rendus de l'Académie bulgare des Sciences. 68(12): 1491-502.

Niyi O. H. (2015). Analytical and Nutritional Evaluation of Velvet Tamarind (*Dialium guineense*) Pulps. American Chemical Science Journal. 6(2): 69-76.

Ogu G. I., Ezeadila J. & Ehiobu J. M. (2013). Antioxidant and antimicrobial activities of *Dialium guineense* (Willd) leaf extract. Pharmacy and Pharmacology

Research. 1(1): 1-7.

Oluwole-Banjo A. K. (2019). Phytochemical and antioxidant properties of methanolic extract of pulp, seed, leaf án stem bark of velvet tamarind (*Dialium guineense*) plant. Journal of Underutilized Legumes. 1(1):159-168.

Orji J., Alo M., Anyim C. & Okonkwo E. (2012). Antibacterial activities of crude leaf and bark extracts of "icheku" *Dialium guineense* on bacterial isolates from bronchitis patients. IOSR Journal of Pharmacy and Biological Science 1: 21-25.

Patocka J. (2003). Biologically active pentacyclic triterpenes and their current medicine signification. Journal of Applied Biomedicine. 1(1): 7-12.

Phan T. A. D., Nguyen X. H., Nguyen T. N., Tran L. Q., & Nguyen T. T. M. (2012). Study on DPPH Free Radical Scavenging and Lipid Peroxidation Inhibitory Activities of Vietnamese Medicinal Plants. Natural Product Sciences 18(1):1-7.

Pinelo M., Tress A. G., Pedersen M., Arnous A. & Meyer A. S. (2007). Effect of cellulases, solvent type and particle size distribution on the extraction of chlorogenic acid and other phenols from spent coffee grounds. American Journal Food Technology 2(7): 641-651.

Qaisar M. N., Chaudhary B. A., Sajid M. U. & Hussain N. (2014). Evaluation of *α*-glucosidase inhibitory activity of dichloromethane and methanol extracts of *Croton bonplandianum* Baill. Tropical Journal of Pharmaceutical Research. 13(11): 1833-1836.

Reinehr T. (2013). Type 2 diabetes mellitus in children and adolescents. World Journal of Diabetes. 4(6): 270.

Roglic G. (2016). WHO Global report on diabetes: A summary. International Journal of Noncommunicable Diseases. 1(1): 3.

Saxena A. & Vikram N. K. (2004). Role of selected Indian plants in management of type 2 diabetes: a review. The Journal of Alternative and Complementary Medicine. 10(2): 369-378.

Schmidt L. H. & Nguyen V. A. (2005). *Dialium cochichinense* Pierre. Seed Leaflet. 91: 1-2.

Shi J., Yu J., Pohorly J., Young J. C., Bryan M. & Wu Y. (2003). Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. Journal of Food, Agriculture and Environment. 1(2): 42-47.

Siangu B. N., Sauda S., John M. K. & Njue W. M. (2019). Antioxidant activity, total phenolic and flavonoid content of selected Kenyan medicinal plants, sea algae and medicinal wild mushrooms. African Journal of Pure and Applied Chemistry. 13(3): 43-48.

Silva E., Rogez H. & Larondelle Y. (2007). Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology. Separation and Purification Technology. 55(3): 381-387.

Singleton V. L. & Rossi J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 16(3): 144-158.

Song F-L., Gan R-Y., Zhang Y., Xiao Q., Kuang L. & Li H-B. (2010). Total Phenolic Contents and Antioxidant Capacities of Selected Chinese Medicinal Plants. International Journal Molecular Sciences. 11:2362-2372; doi:10.3390/ijms11062362.

Spigno G., Tramelli L. & De Faveri D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering. 81(1): 200-208.

Stoica R., Velea S., Ilie L., Calugareanu M., Ghimis S. B. & Ion R.-M. (2013). The influence of ethanol concentration on the total phenolics and antioxidant activity of *Scenedesmus opoliensis* algal biomass extracts. Revista de Chimie. 64(3): 304-306.

Tabart J., Kevers C., Pincemail J., Defraigne J.-O. & Dommes J. (2009). Comparative antioxidant capacities of phenolic compounds measured by various tests. Food Chemistry. 113(4): 1226-1233.

Waszkowiak K. & Gliszczyńska-Świgło A. (2016). Binary ethanol–water solvents affect phenolic profile and antioxidant capacity of flaxseed extracts. European Food Research and Technology. 242(5): 777-786.

Xiao J., Kai G., Yamamoto K. & Chen X. (2013). Advance in dietary polyphenols as α-glucosidases inhibitors: a review on structure-activity relationship aspect. Critical Reviews in Food Science and Nutrition. 53(8): 818-836.

Zhang Z.-S., Li D., Wang L.-J., Ozkan N., Chen X. D., Mao Z.-H. & Yang H.-Z. (2007). Optimization of ethanol–water extraction of lignans from flaxseed. Separation and Purification Technology. 57(1): 17-24.

Zwenger S. & Basu C. (2008). Plant terpenoids: applications and future potentials. Biotechnology and Molecular Biology Reviews. 3(1): 1-7.