

Evaluation and Determinants of Secondary Metabolites and its Antioxidant Activities of Various Fractions from *Albizia myriophylla* Bark [†]

Goh Yong Meng ^{1,2}, Razif Abas ³, Nurmawati Syakroni ³, Nur Izah Abdul Razak ³,
Nurul Huda Mohd. Nor ³, Siti Fadziyah Mohamad Asri ³, Safuraa Salihan ³,
Nurul Hayati Mohamad Zainal ³, Suryati Mohd. Thani ³ and Azmah Saat ^{3,*}

¹ Department of Veterinary Preclinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; ymgoh@upm.edu.my

² Deputy Director Office, Research Grant Division, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia

³ Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; razifabas@upm.edu.my (R.A.); rahman60@hotmail.com (N.S.); nurizah@upm.edu.my (N.I.A.R.); hudamohdnor@upm.edu.my (N.H.M.N.); fadziyah@upm.edu.my (S.F.M.A.); safuraa@upm.edu.my (S.S.); mz_nurul@upm.edu.my (N.H.M.Z.); th_suryati@upm.edu.my (S.M.T.)

* Correspondence: azmahsaat@upm.edu.my; Tel.: +60-192755432

[†] Presented at the 1st International Electronic Conference on Nutrients—Nutritional and Microbiota Effects on Chronic Disease, 2–15 November 2020; Available online: <https://iecn2020.sciforum.net/>.

Published: 30 October 2020

Abstract: *Albizia myriophylla* (ABZ) is a plant used in Thailand and Peninsular Malaysia for the treatment of diabetes mellitus type II. The antioxidant activities of ABZ have been suggested as one of the mechanisms for the observed beneficial effects. Hence, this study examined the phenolic, flavonoid, and saponin contents and antioxidant activity from methanol extract (ME) and its derived fractions hexane (HE), chloroform (CE), ethyl acetate (EAE), butanol (BE), and aqueous fraction of the bark of ABZ (AE). Amongst the extracts, EAE showed the highest total phenolic content of about 0.77 mg of gallic acid equivalent/g of extract (mg GAE/mg). However, the highest flavonoid content was detected in HE at 1.04 µg retinol equivalent ((RE)/g extract), while the saponin content was highest in CE at 1.1 µg diosgenin equivalent ((DE)/g extract). In 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test, EAE at 100 µg/mL had the highest percentage of inhibition of about 72.55%. 2-2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS radical scavenging test) showed BE had the highest percentage of inhibition at 100 µg/mL at 82.91%. Ferric reducing antioxidant power elucidated BE as having the highest percentage of inhibition which was 86.04% followed closely by ME at 85.90%. Thus, the different extracts of ABZ displayed various antioxidant capacity with probable free radical scavenging activity, which may be useful for the treatment of chronic inflammatory related metabolic diseases, such as diabetes and obesity.

Keywords: antioxidant activity; phenolic; flavonoid; saponin; *Albizia myriophylla*

1. Introduction

Albizia myriophylla (ABZ) otherwise known as tebu gajah in Malaysia belongs to the subfamily Mimosoideae and genus *Albizia*. This genus consists of about 145 species, distributed in tropical and subtropical regions in the world such as Thailand, Malaysia, Vietnam, and other countries [1]. It is used in traditional medicine as mouth washes in Thailand [2] and for rice beer production [3]. A

substantial proportion of South-East Asian populations uses traditional medicine to treat diabetes, and this includes the use of ABZ as an anti-diabetic agent [1,4]. In addition, an in vitro study has shown that it has a α -glucosidase inhibitory activity [5]. Phenolics have been found to be effective anti-hyperglycemic agents [6] and subsequently, 12 phenolic acids were isolated qualitatively [6]. Saat et al. showed ABZ had anti-diabetic activity in streptozotocin- and nicotinamide-induced rats. This is followed by another study where ABZ in combination with virgin coconut oil displayed hypoglycemic properties in streptozotocin-induced diabetic rats [3]. These studies show that ABZ has potential hypoglycemic properties [7] with probable antioxidant activity.

In recent years, antioxidants have fast been becoming recognized as a crucial area which is being extensively investigated amongst scientists around the world. The antioxidants are reputedly known as having valuable effects especially on the human health aspect. It has become an essential element for maintaining health in humans. The beneficial effects of antioxidant are mainly due to its capability of providing protection against oxidative cellular damage, thus preventing diseases such as cancer, chronic heart diseases, and diseases associated with chronic inflammation and degeneration [8].

In view of the above statement, coupled with the fact that ABZ has a relatively high constituent of phenolic compounds [6], the current investigation was conducted to investigate antioxidant activities in various extracts of ABZ. In this study, different solvents were used for the extraction processes, in the hope of evaluating their antioxidant properties, respectively. These possible antioxidant activities may help to alleviate and reduce the inflammatory precursors that causes chronic inflammatory related metabolic diseases such as diabetes and obesity.

2. Materials and Methods

2.1. Collection of Plant Material

The ABZ barks were collected in January 2015 from a growing tree in Pasir Mas, Kelantan, Malaysia. The barks were kept in a dry sack and was brought to Universiti Putra Malaysia in a lorry. The plant was sent to Dr. Samsul Kamis, a botanist at the Institute of Bioscience (IBS), Universiti Putra Malaysia, for authentication. A voucher specimen (ACPO 122) was approved by UPM Agriculture Conservatory Park, IBS, Universiti Putra Malaysia.

2.2. Preparation of Dried Barks of *Albizia myriophylla*

The freshly collected barks of ABZ were then chopped into coarse chips using a wood cutting machine. These coarse chips of ABZ were then air dried for 1 week, under direct sunlight. These chips were then oven-dried for 24 h at 40 °C.

2.3. Preparation of Methanolic Extract of *Albizia myriophylla* Bark

The powdered bark was extracted using 80% methanol (by adding 20% of distilled water) by employing maceration methods. The powdered barks were left macerated in methanol and shaken for 24 h using a rotary shaker. The methanol solution was then separated from the powdered bark of ABZ and concentrated at 40 °C. The concentrated methanol extract was then freeze-dried into powdered form. The 80% methanol extract was later fractionated with different solvents via butanol, chloroform, ethyl acetate, and hexane.

2.4. Preparation of Hexane, Chloroform, Ethyl Acetate, and Butanol *Albizia myriophylla* Bark Extract

From the total amount of freeze-dried methanol ABZ extract, about 75% was used to obtain the hexane fraction. The methanol ABZ extract was dissolved first in 500 mL of distilled water, and then followed by a mixture of 500 mL hexane. This mixture was left shaken for 24 h. The hexane extract was later separated from the mixture by using a funnel separator. The hexane extract was then concentrated by using a rotary vacuum evaporator (Laborota 4000 efficiency: Heidolph, Germany) at 40 °C. The hexane ABZ extract was then freeze-dried to dry powdered form. The steps stated above

were repeated during the extraction of chloroform, ethyl acetate, and butanol fractions by using the 500 mL of the remaining aqueous extract, separated from every fraction [9] (as explained in Figure 1).

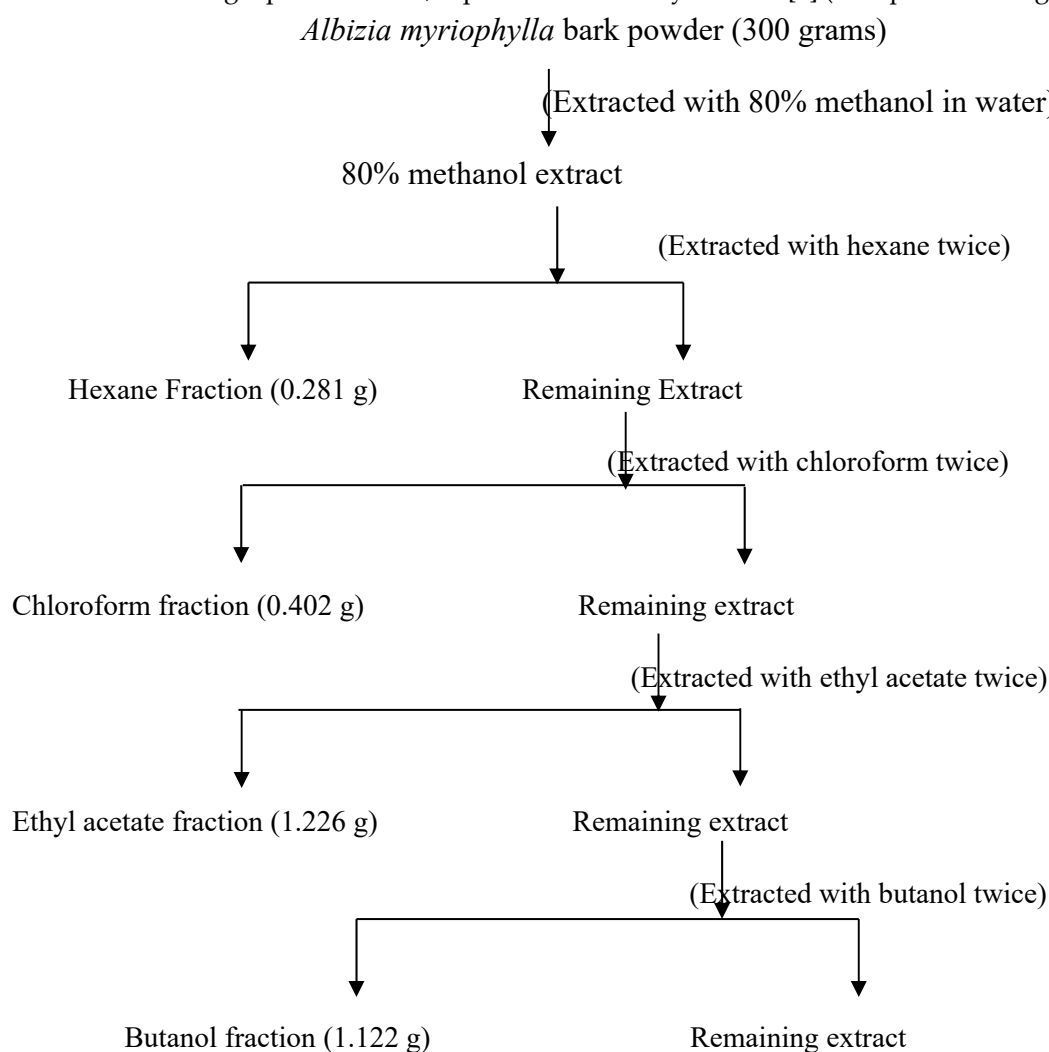


Figure 1. Extraction procedure of bark of *Albizia myriophylla*. Methods adapted from Kaur et al.

2.5. Determination of Total Phenolic Contents of *Albizia myriophylla* Bark Extracts

Total phenol contents of ABZ extracts were determined using Folin-Ciocalteu assay [10]. Three hundred milligrams of each extract was mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent and 2.0 mL of 7.5% of sodium carbonate. Three replicates were made for each test sample. Following this, the mixture was incubated at 40 °C for 30 min. The absorbance of the reaction mixtures was measured at 760 nm by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). Gallic acid was used as a standard and the total phenol content of the ABZ extracts was expressed in milligram gallic acid equivalents.

2.6. Determination of Total Flavonoid Contents of *Albizia myriophylla* Bark Extracts

The total flavonoid content was calculated by the aluminium calorimetric method [11] using rutin as a reference. ABZ extracts of 1 mg were individually dissolved in dimethyl sulfoxide (DMSO). A measurement of 150 µL of each extract was mixed with 150 µL of 2% aluminium chloride. The mixture absorbance was measured in 435 nm using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan) following a 10 min incubation at ambient temperature. The total flavonoid content in micrograms was expressed by extract (µg RE/g) as rutin equivalents. For each test sample, three replicates were made.

2.7. Determination of Total Saponin Contents of *Albizia myriophylla* Bark Extracts

Determination of total saponin was carried out according to the method of Pasaribu et al. with some modifications, using diosgenin as a standard solution [12]. ABZ extracts of 500 mg/mL were used. The extract was mixed with 0.25 mL of the vanillin reagent, followed later with 2.5 mL of 72% sulfuric acid, which was infused slowly on the inner side of the wall. The solution was mixed well and transferred to a water bath at 60 °C for 10 min. After 10 min, the tubes were cooled in ice-cold water for 3 to 4 min, and absorbance was measured at a wavelength of 544 nm. The test was performed in triplicate. The total saponin content was expressed as diosgenin equivalents in microgram per gram extract (µg DE/g extract).

Total saponin content was determined according to Pasaribu et al. with some modifications by using diosgenin as a standard solution. In this study, 500 mg/mL of ABZ extract were used. It was mixed with 0.25 mL of vanillin reagent and 2.5 mL of 72% sulfuric acid by slow infusion on the inner side of the wall. It was later transferred to a water bath at 60 °C for 10 min before being cooled in ice-cold water. The absorbance was measured at 544 nm wavelength. This test was performed in triplicate. The total saponin content was expressed as diosgenin equivalents in microgram per gram extract (µg DE/g extract).

2.8. Antioxidants Activity Determinations

2.8.1. Total Antioxidants Assay Using ABTS Method

Preparation of ABTS (2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) method was adopted from Fidrianny et al. with minor modifications [13]. Two solutions were used to prepare the ABTS solution, which were 7.4 mM of ABTS and 2.6 mM of potassium persulfate. Both solutions were mixed in equal quantities and were allowed to react in the dark at room temperature for 12 to 18 h. The ABTS solution was diluted with 95% methanol to obtain an absorbance of 1.1 ± 0.2 units at wavelength 734 nm using a spectrophotometer. Then, 0.1 mL of each extract at 20, 40, 60, 80, and 100 µg was pipetted into 0.4 mL of diluted ABTS and incubated for 5 min at room temperature to initiate the reaction. The absorbance was read at wavelength 734 nm. Methanol 95% was used as a blank and ABTS solution was used as a standard. The test was performed in triplicate for standard and each extract. All measurement procedures were done in the dark. The test was done in triplicate. The ABTS radical scavenging activity (%) was calculated by using the following formula:

$$\text{ABTS radical scavenging effect (\%)} = 1 - \frac{A_{734}(\text{sample})}{A_{734}(\text{blank})} \times 100$$

$$A_{734}(\text{blank})$$

This test was performed according to method described by Fidrianny et al. (2013) with minor modification.

2.8.2. DPPH Scavenging Activity

Preparation of 2,2-diphenyl-1 picrylhydrazyl (DPPH) method was adopted from Thaipong et al. with some modifications [14]. The stock solution was prepared by dissolving 24 mg of DPPH with 100 mL methanol and then stored at −20 °C until needed. The working solution was prepped by

mixing 10 mL of stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at wavelength of 515 nm, detected using the spectrophotometer. Next, 100 μ L of the ABZ extracts at 20, 40, 60, 80, and 100 μ g reacted with 400 μ L of diluted DPPH and were incubated in the dark, at room temperature for 60 min. Triplicates were conducted for each test. Then, the reading of the absorbance was performed at a wavelength of 515 nm. Methanol 95 % was used as a blank solution. The DPPH radical scavenging activity (%) was calculated by using the following formula:

$$\text{DPPH radical scavenging effect (\%)} = 1 - \frac{A_{515}(\text{sample})}{A_{515}(\text{blank})} \times 100$$

2.8.3. FRAP Assay

Preparation of ferric reducing antioxidant power (FRAP) method was also adapted from Thaipong et al. The stock solution needed to prepare the FRAP reagent included 300 mmol/L of acetate buffer at pH 3.6, 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mmol/L hydrochloric acid, and 20 mmol/L ferric chloride solution. The working solution was prepared by combining all the stock solution in the ratio of 10:1:1, accordingly. Then, 100 μ L of the ABZ extracts at 20, 40, 60, 80, and 100 μ g reacted with 400 μ L of the prepared FRAP reagent in a dark condition, at room temperature for 30 min. Using the spectrophotometer, the absorbance reading was conducted at a wavelength of 593 nm. Trolox was used as a standard from 0 to 200 μ g/mL. Results are expressed in unit of μ M TE/g fresh mass. Triplicates were performed for each extract individually. The FRAP radical scavenging activity (%) was calculated by using the following formula:

$$\text{FRAP radical scavenging effect (\%)} = 1 - \frac{A_{593}(\text{sample})}{A_{593}(\text{blank})} \times 100$$

2.9. Statistical Analysis

Data were analyzed by one-way ANOVA, using the mixed procedure of the SAS software package, version 9.1 (SAS Inst. Inc., Cary, NC, USA). The data were cross-checked/screened for normality using the univariate procedure of SAS software with a post hoc Tukey test. Differences of the data analyses in a p value of < 0.05 were significant.

3. Results

Various fractions of ABZ, which includes methanol, hexane, ethyl acetate, chloroform, butanol, and aqueous, were collected during different processes of macerations and were quantitatively analyzed for their phenolic, flavonoid, and saponin content and antioxidant activities.

3.1. The Total Quantity of Aqueous, Methanol, Hexane, Ethyl Acetate, Chloroform, and Butanol Bark Extracts of ABZ

From the processed 367 g of bark powder of ABZ, only 5 % of the aqueous bark extract (AE) was obtained through this method whereas the total quantity of methanol extract (ME) from the ABZ bark powder was 10.535 g, which was 2.8%. From the ME, via maceration process, the total quantities of its fraction which includes hexane extract (HE), ethyl acetate extract (EAE), chloroform extract (CE), and butanol extract (BE) were 0.281 g (0.07%), 1.226 g (0.33%), 0.402 g (0.11%), and 1.122 g (0.3%), respectively.

3.2. The Total Phenolic Content of ABZ Bark Extracts

From Figure 2, the highest amount of phenolic acid content was found in the EAE at 0.77 mg gallic acid equivalent (GAE)/mg extract. A similar amount of 0.74 mg GAE/mg extract of phenolic acid was obtained in the AE and CE. The methanol extract of ABZ had 0.52 mg GAE/mg extract of phenolic acid

content which was significantly reduced compared to AE. This was followed closely by BE with 0.51 mg GAE/mg extract. The least amount of phenolic acid content was obtained from the HE at 0.22 mg GAE/mg extract. BE, HE, and ME all had a significantly reduced amount of phenolics as compared to the aqueous extract.

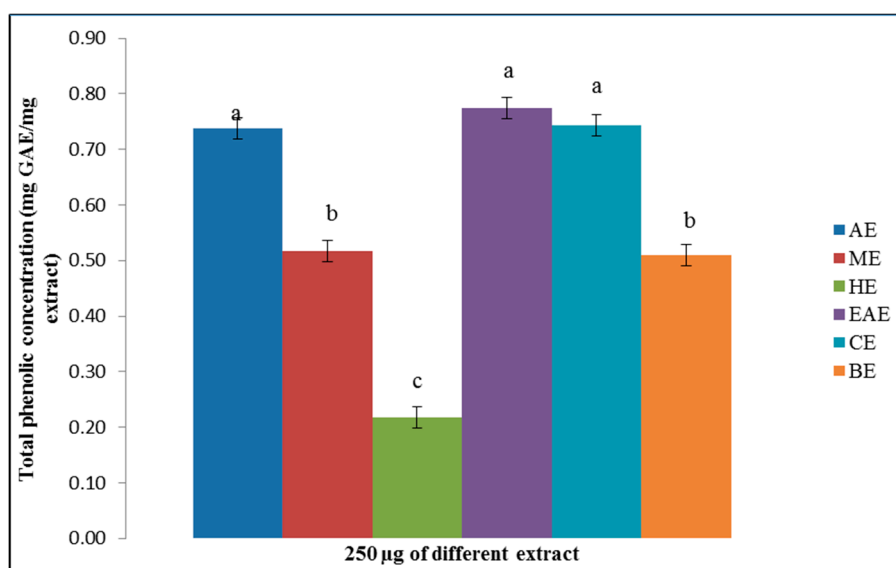


Figure 2. The phenolic contents in 250 µg of aqueous fraction (AE), methanol extract (ME), hexane (HE), ethyl acetate extract (EAE), chloroform extract (CE), and butanol extract (BE) of *Albizia myriophylla* (ABZ) bark extract. GAE, gallic acid equivalent. Values were expressed as mean ± SEM. Differing alphabets denote significant difference between groups ($p < 0.05$).

3.3. The Flavonoid Concentrations of ABZ Bark Extracts

Figure 3 shows definite and positive findings of flavonoids in all extracts with the highest flavonoid detected in the HE at 1.04 µg retinol equivalent (RE)/g and AE at 0.895 µgRE/g. The smallest amount of flavonoids was extracted from the CE, BE, and ME at 0.38 µgRE/g, 0.38 µgRE/g, and 0.3 µgRE/g, respectively.

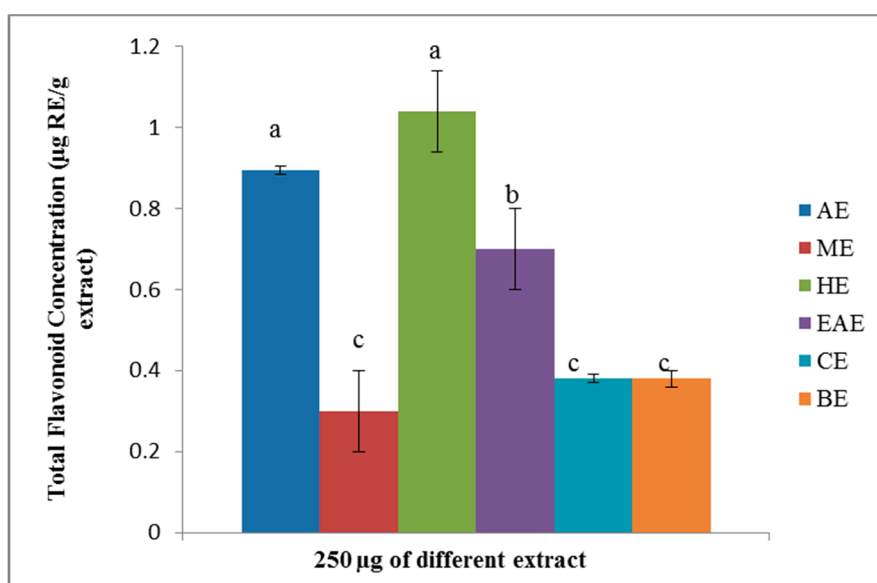


Figure 3. The flavonoid levels in 250 µg of AE, ME, HE, EAE, CE, and BE of ABZ bark extract. RE, retinol equivalent. Values were expressed as mean ± SEM. Differing alphabets denotes significant difference between groups ($p < 0.05$).

3.4. The Saponin Concentrations of ABZ Bark Extracts

The results depicted in Figure 4 show that qualitatively, saponins can be found in *Albizia myriophylla*, with the highest amount detected in CE at 1.1 μg diosgenin equivalent (DE)/g, followed closely by BE and EAE at 0.92 μg DE/g and 0.81 μg DE/g, respectively. A moderate amount of saponins were detected in the HE and ME at 0.21 μg DE/g and 0.24 μg DE/g, respectively. However, the AE had the least amount of saponin as compared to the other extracts at 0.03 μg DE/g.

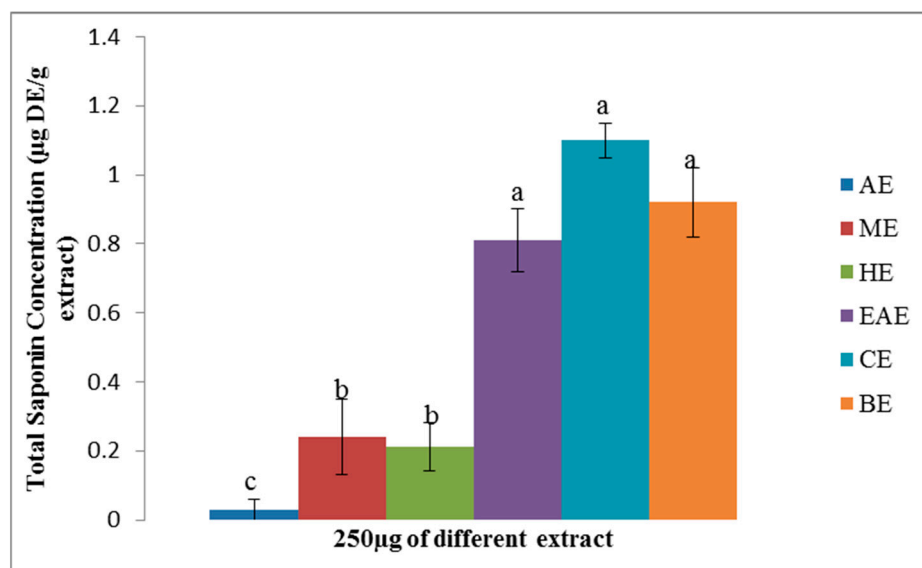


Figure 4. The saponin levels in 250 μg of AE, ME, HE, EAE, CE, and BE of ABZ bark extract. DE, diosgenin equivalent. Values were expressed as mean \pm SEM. Differing alphabets denotes significant difference between groups ($p < 0.05$).

3.5. ABTS Assay of ABZ Bark Extracts

All the extracts showed a steady increase in the percentage of inhibition ranging from 20 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$. However, the results obtained from Figure 5 show that BE had the highest percentage of inhibition at 100 $\mu\text{g/mL}$ with an inhibition of 82.91%. This was followed by ME with 73.55 % of inhibition. All the other extracts which include EAE, CE, HE, and AE had almost similar readings with an inhibition of 62.16%, 60.11%, 55.62%, and 54.37% at 100 $\mu\text{g/mL}$, respectively.

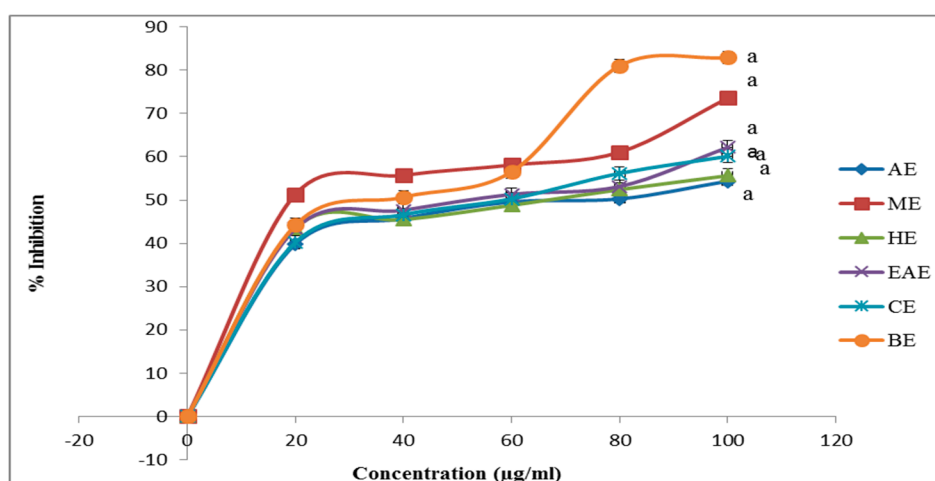


Figure 5. The percentage of inhibition in 2,2'-azinobis 3-ethyl-benzothiazoline -6-sulfonic acid (ABTS) antioxidant test in 20 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 60 $\mu\text{g/mL}$, 80 $\mu\text{g/mL}$, and 100 $\mu\text{g/mL}$ of AE, ME, HE, EAE, BE, and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups ($p < 0.05$). Results showed no significant difference between the extracts.

3.6. DPPH Assay of ABZ Bark Extracts

In Figure 6, all the extracts showed a marked increase at 20 $\mu\text{g/mL}$ but following that reached a plateau till 80 $\mu\text{g/mL}$. From 80 $\mu\text{g/mL}$, it peaked to its highest point at 100 $\mu\text{g/mL}$. EAE, at 100 $\mu\text{g/mL}$, had the highest percentage of inhibition of about 72.55%. This was closely followed by CE which showed a 71.89 % of inhibition at 100 $\mu\text{g/mL}$. AE and ME had almost similar readings at 60.07% and 59.53% of inhibition at 100 $\mu\text{g/mL}$. HE had the lowest percentage of inhibition at 100 $\mu\text{g/mL}$ which was 53.59%. However, these results did not denote any statistical significance within the groups.

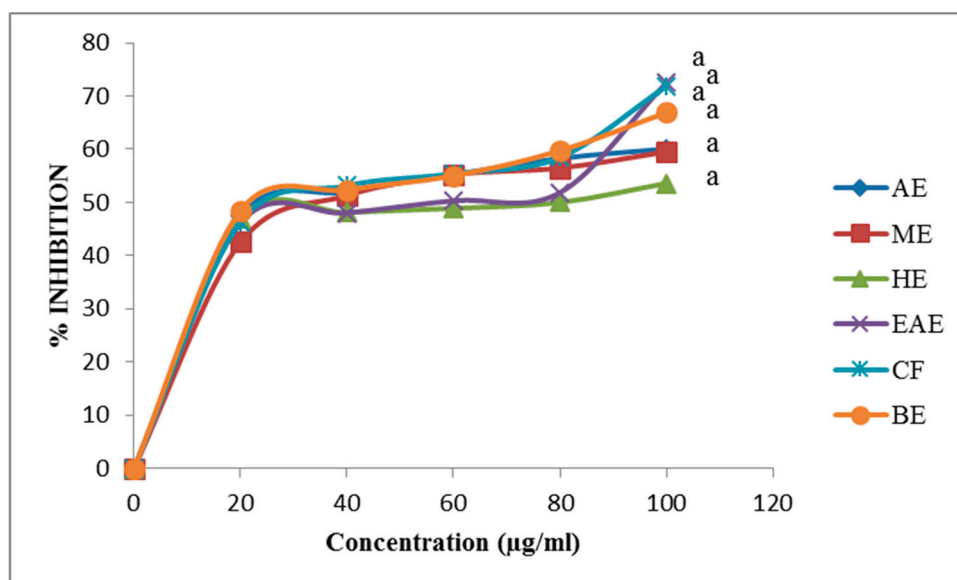


Figure 6. The percentage of inhibition in 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant test in 20 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 60 $\mu\text{g/mL}$, 80 $\mu\text{g/mL}$, and 100 $\mu\text{g/mL}$ of AE, ME, HE, EAE, BE, and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups ($p < 0.05$). Results showed no significant difference.

3.7. FRAP Assay of ABZ Bark Extracts

As depicted in Figure 7, all the extracts showed a similarly huge increase from 0 $\mu\text{g/mL}$ to 20 $\mu\text{g/mL}$, which later reached a plateau to 100 $\mu\text{g/mL}$. At 100 $\mu\text{g/mL}$, the BE reached the highest percentage of inhibition which is 86.04%. ME came to a close second at 85.90% of inhibition. This was closely followed by EAE at 84.87% of inhibition. AE at 100 $\mu\text{g/mL}$ had a percentage of inhibition of 81.09%, while HE had 80.66% of inhibition. The lowest percentage of inhibition was from the CE at 79.18% of inhibition. The results depict that with increasing amount of extracts, there is an increase in the reducing power. Thus, the present results show that highest reducing power was evident in BE of ABZ, which is statistically insignificant.

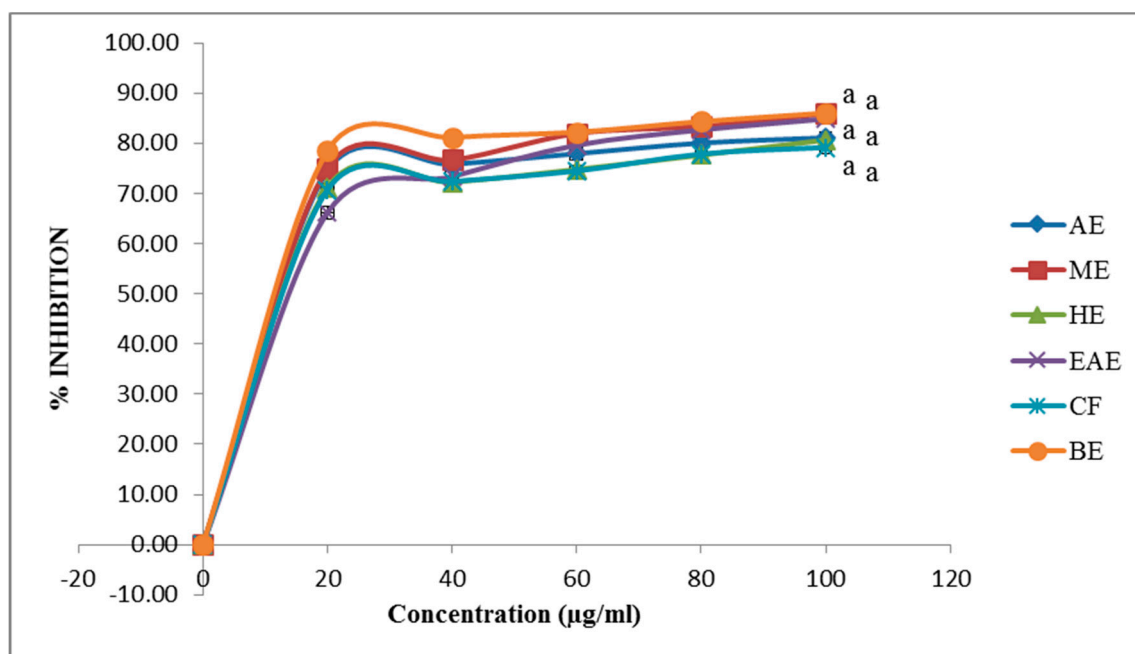


Figure 7. The percentage of inhibition in the ferric reducing antioxidant power (FRAP) antioxidant test in 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL of AE, ME, HE, EAE, BE, and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups ($p < 0.05$). Results showed no significant difference.

4. Discussion

4.1. The Phenolic Acid, Flavonoids, and Saponin in Various Extracts of ABZ.

There are various potential antioxidant mechanisms of polyphenols. The polyphenols act as a free radical scavenging agent, inhibiting the development of free radicals by binding the metallic ions and disrupting the progression sequences of the free radical chain. The association between polyphenol constituents with additional biological antioxidants provides another potential antioxidant pathway [15]. The ABZ bark extracts have probable antioxidant activities as it contains high levels of phenolic acids [6] in the various extracts, as seen in Figure 2.

Free radicals are usually associated with the development of various chronic metabolic disorders like obesity, coronary artery diseases, and obesity [16]. Antioxidants are known to have free radical scavenging activity, which acts against free radicals and acts as prevention against a variety of diseases.

In a study by Sivakrishnan et al., ethanol was used for the extraction of the *Albizia procera* bark [17]. However, the fractionation process of the ethanol extracts with various solvents at different polarity was not done. In our study, methanol was used to replace ethanol in the extraction process of the ABZ bark. Methanol is a solvent commonly used for the extraction processes as it is generally known to be more effective in dissolving active admixtures [18]. Therefore, the penetration of a cellular membrane is made easier, especially in extracting the intracellular constituents from herbal properties [18,19]. Tiwari et al. identified that various bioactive compounds such as saponins, flavonoids, and polyphenols are easier to obtain when methanol is used as a solvent during the extraction process [20]. Boeing et al. also described that the antioxidants potentials in extracts found are totally dependent on the types of solvent used. This is because the bioactive compounds have differing polarity displaying different rates of antioxidant activity. Thus, the various results obtained from this study would imply that the methanol solvent used in extraction would be the preferred choice in extracting polyphenols. From this study, the ME of ABZ bark had the most yields as compared to the other extracts. Furthermore, a study by Iloki-Assanga et al. showed that the ME of *Phoradendron californicum* oak had the highest yield of phenols and flavonoids [21].

Polyphenol compounds are generally linked with the antioxidant components found in fruits and vegetables [22]. Most of these compounds are categorized as antioxidants with hydrophilic activity [23]. This may be an added reason why the phenolic compounds in fruits and vegetables are able to dissolve better in methanol. This happens as a result of the interactions between the solvent's polar sites and antioxidant molecules with the hydrogen bond [18]. Even though the polarity of ethanol and methanol are almost similar, ethanol was found to be less effective in extracting the antioxidants compounds compared to methanol [24]. This is probably due to the low solvability of antioxidant molecules from ethanol extraction, as a result of the existence of the ethyl radical which has higher solvability than the methyl radical found in methanol [24]. Therefore, based on the discussion above, methanol was used as a method of extraction of ABZ bark, with various fractions using different solvents.

Phenolic compounds' ingredients of the plant are considered crucial because of their characteristics consisting of numerous hydroxyl groups, which are responsible for their scavenging capability [25]. Phenolics found in leaves and stem barks of various plants have received worldwide recognition because of their antioxidant activities [26]. Herbal resources rich in phenolics are highly valued and are used increasingly in the food industry, as they are famously regarded having antioxidant activities, thus improving the nutritional value and quality of the nutrient [27]. In our study, the EAE showed the highest total phenolic content, followed by AE, CE, ME, BE, and the least found in the HE. These phenolic contents in ABZ may be responsible for the radical scavenging and reducing capacity, as exhibited by a study by Khatoon et al. which showed similar results with *Albizia procera* [28]. Hence, the beneficial properties of ABZ stem bark may be possibly derived from the phenolic compounds found in its constituents. The other phytochemical test was done in this study also indicated the presence of flavonoids in these extracts.

Aside from phenolics, flavonoids, which are characteristically comprised of a polyphenolic compound with a benzo- γ -pyrone structure, are also commonly extracted in plants. Studies have shown that secondary metabolites produced from phenolic compounds may be responsible for various pharmacological actions [29,30]. Current interests in these elements have been propagated by the potential antioxidant activities found in these polyphenolic compounds, which may be beneficial for health. Flavonoids have hydroxyl groups that are functional in promoting free radical scavenging activity and chelation of metal ions, hence mediating the antioxidant activities [25,31]. The metal ions chelated could be essential in suppressing the free radicals' production, thus reducing further damage biomolecules components [32]. With these self-proclaimed antioxidant activities, flavonoids are assumed to have health beneficial properties [33]. Numerous studies have shown the inhibitory effects of flavonoids against infections, obesity, cardiovascular diseases, and other metabolic disorders [33–36]. In the present study, among the six different extracts of ABZ, the HE contained the highest number of flavonoids, followed by AE, EAE, CE, BE, and ME.

Saponin is a bioactive constituent which is able to produce foam in water even in small concentrations, as it is capable of reducing the surface tension of water [37]. The foam-producing capacity of saponins is a desired effect and often used in chemical and food manufacturers, especially for producing shampoos and liquid detergents [38]. The foamability of saponins is produced because of the interaction between the non-polar section of the saponins and the water-soluble side chain [39]. The basic principle in obtaining a high yield of saponins depends on the surface area of the plant used. Grinding the plant materials into the smallest size will increase the surface area, hence increasing the rate of extraction when dissolved in various solvents (Tiwari et al., 2011). The raw material should be prepared to the smallest size, if possible, in powder form, as this will ensure easier extraction of the bioactive compounds and increasing the yield of the extract obtained [12]. This investigation was in agreement with Zubairi et al. which demonstrated that larger particle size ranging from 2 to 5 mm produced a lesser yield of rotenone compared to that of smaller particle size ranging from 0.5 to 2 mm [40]. Results which were in parallel with this study were also illustrated by Zhang et al. in *Zingiber officinale* root [41]. In our research, amongst six extracts of ABZ, the CE demonstrated the highest amount of saponin content, followed by BE, EAE, ME, HE, and the lowest amount of saponin was detected in the AE. The extraction yield of saponin content is totally

dependent on the polarity of the solvent. The results of our research demonstrated that chloroform fraction produces the highest saponin content compared to the other extracts of ABZ. This suggests that ABZ has bioactive saponins which probably have the same polarity as the chloroform solution. Similar to this study, the highest quantity of saponins was also acquired from the chloroform extract of Tibetan herbal medicine *Dracocephalum tanguticum* [42]. However, for the safety of human or animal consumption, it would be wiser to use the AE or ME of ABZ, as chloroform is a toxic solvent that is able to cause tumors in animals [43].

Saponins are also known to have antioxidant and anti-bacterial activities, as demonstrated by Akinpelu et al. which used the saponin fraction of *Erythrophyleum suaveolens* stem bark extract through an antioxidant in vitro study [44]. Hence, saponin can be used against free radicals and reduce infections caused by pathogenic bacteria [44]. This was further substantiated by a study from Smith and Adanlawo, which illustrated the in vitro and in vivo antioxidant activity of saponin extracted from the root of *Garcinia kola* on alloxan induced diabetic rats [45]. Thus, the saponins found in *A. myriophylla*, may also play a contributory role in the antioxidant activity.

Hence, from these results, we can propose that the phenolic compounds, saponin, and flavonoids found in ABZ are the important contributors responsible for the antioxidant action.

4.2. The Antioxidant Activity of Various Extracts of ABZ Bark.

In this study, ME and its derived fractions at various concentrations showed a significant presence of antioxidant activity using DPPH radical scavenging assay, ABTS, and reducing power capacity method. The DPPH test is a tool which is often used to investigate the free radical scavenging activity of various plant extracts [37]. The basis of this method depends on the DPPH methanol solvent being reduced by the presence of antioxidants found in the various extracts. As a result of this reaction, the non-radical DPPH-H was produced. The reduction of the stable DPPH by the antioxidants in these extracts will cause a change in the color from purple to yellow, in various degrees of color. This outcome will depend on the quantity of the antioxidant found in these extracts. The varying degree of discoloration will reflect on the free radical scavenging activity of the extract [46]. Results obtained from this study showed that the EAE of ABZ bark at 100 µg/mL had the highest capacity to neutralize the radicals found in DPPH. The other extracts showed moderate activity in the following order, CE, AE, ME, with the least activity seen in the HE.

Like DPPH, ABTS are stable free radicals. These stable free radicals can dissolve in either methanol or ethanol. In ABTS, when there is antioxidant activity elicited by the interaction of the solvents and extracts, the colors of ABTS assay solution become a lighter shade [47]. As with DPPH, ABTS have been widely used as a tool to evaluate the free radical scavenging activity of extracts [13]. In our result, the ABTS antioxidant test revealed the antioxidant activities of various extracts of ABZ at 100 µg/mL were within the range of 54.37% and 82.91%. The BE had the highest ABTS scavenging activity (82.91%), while the lowest activity (54.37%) was produced by the AE. In contrast, a study by Siahpoosh and Mehrpeyma demonstrated that the ME of *Albizia lebbek* showed a higher antioxidant activity in the ABTS antioxidant assay [48]. The differences in these results may be due to the differing methods of extraction, with different plants used. The antioxidant activities of different plants have different levels of antioxidant activity, with differing interactions within the antioxidant assay and extracts [32]. However, the statistical analysis of DPPH and ABTS scavenging activities amongst the various extract of ABZ showed that all the extracts were not significantly different from each other.

Khatoun et al. postulated that the reducing properties of antioxidant activity were illustrated by donation of one hydrogen atom, in order to break the free radical chain [28]. The antioxidants present in the ME of ABZ bark and its other fractions initiate the reduction process of Fe³⁺-ferricyanide complex to the ferrous form, which eventually determines the reducing power. The ferric reducing power activity of the ME of ABZ bark and its fractions might be due to the existence of polyphenol compounds. The reducing capacity of herbal extract may assist as a significant gauge of its potential antioxidant capacity. Khennouf et al. demonstrated that the herbal extract having reducing power may be able to avert liver injury by suppressing the production of lipid peroxides [49]. Like the DPPH and ABTS radical scavenging activity, the reducing power of the AE, ME, BE, CE, EAE, and HE of

ABZ bark increased with increasing concentrations. However, similar to DPPH and ABTS assay, there was no significant difference noted between groups.

From the DPPH, ABTS, and FRAP results, all the extracts showed inhibition at 100 µg/mL of concentration denoting the antioxidants activity. These antioxidant properties from all the different extracts, which included AE, ME, EAE, CE, HE, and BE of ABZ bark, may propagate the free radical scavenging activities, thus enabling to control and reduce the inflammatory precursors which contributes to chronic inflammatory related metabolic disease, such as diabetes and obesity.

5. Conclusions

The AE, ME, EAE, CE, HE, and BE of ABZ bark showed various concentrations of phenolics, flavonoids, and saponin with varying degree of antioxidant activities in the ABTS, DPPH, and FRAP tests. The free radical scavenging activity of these antioxidants from phenolics, flavonoids, and saponins compound may play a vital role in reducing the pre-inflammatory precursors, such as tumor necrosis factor alpha and cytokines, that are related to the development of various chronic inflammatory related metabolic diseases. Thus, ABZ bark of various extract may have potential in controlling the chronic metabolic diseases influenced by oxidating factors such as diabetes mellitus and obesity.

Author Contributions: A.S., G.Y.M., and N.S. were the group leaders; S.F.M.A., N.H.M.Z., S.M.T., S.S., and N.I.A.R. performed the conceptualization; R.A. and N.H.M.N. conducted the formal analysis. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding: This research received no external funding.

Acknowledgments: The authors are grateful to Faculty of Veterinary Medicine, Universiti Putra Malaysia, for providing the necessary facilities.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Saat, A.; Syakroni, N.; Rosli, R. Potential hypoglycemic property of *Albizia myriophylla* in streptozotocin-nicotinamide induced diabetic rats. *Int. J. Pharm. Pharm. Sci.* **2012**, *4*, 427–431.
2. Amornchat, C.; Kraivaphan, P.; Dhanabumi, C.; Tandhachoon, K.; Trirattana, T.; Choonharcongdej, S. Effect of cha-em Thai mouthwash on salivary levels of mutans streptococci and total Ig A. *Southeast. Asian J. Trop. Med. Public Health* **2006**, *37*, 528–531. <https://pubmed.ncbi.nlm.nih.gov/17120974>.
3. Panmei, C.; Singh, P.K.; Gautam, S.; Variyar, P.S.; Shantibala Devi, G.A.; Sharma, A. Phenolic acids in Albizia bark used as a starter for rice fermentation in Zou preparation. *JFAE* **2007**, *5*, 147–150. Available online: <https://www.researchgate.net/publication/261675944> (accessed on 22 April 2007).
4. Saat, A.; Syakroni, N.; Rosli, R. Potential hypoglycemic activity of *Albizia myriophylla* and virgin coconut oil in streptozotocin induced diabetic rats. *Int. J. Pharm. Pharm. Sci.* **2013**, *5*, 199–202. Available online: <https://innovareacademics.in/journal/ijpps/Vol4Issue3/4144.pdf> (accessed on 20 August 2013).
5. Tunsaringkarn, T.; Rungsiyothin, A.; Ruangrunsi, N. α-Glucosidase inhibitory activity of Thai Mimosaceous plant extracts. *Int. J. Health Res.* **2008**, *22*, 29–33. Available online: <https://www.researchgate.net/publication/242753490> (accessed on 15 November 2007).
6. Manickam, M.; Ramanathan, M.; Farboodinay Jahromi, M.A.; Chansouria, J.P.N.; Ray, A.B. Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*. *J. Nat. Prod.* **1997**, *60*, 609–610. doi:10.1021/np9607013.
7. Pumthong, G.; Nathason, A.; Tuseewan, M.; Pinthong, P.; Klangprapun, S.; Thepsuriyanon, D.; Kotta, P. Complementary and alternative medicines for diabetes mellitus management in ASEAN countries. *Complement. Ther. Med.* **2015**, 1–9. doi:10.1016/j.ctim.2015.01.016.
8. Forni, C.; Facchiano, F.; Bartoli, M.; Pieretti, S.; Facchiano, A.; D’Arcangelo, D.; Norelli, S.; Valle, G.; Nisini, R.; Beninati, S.; et al. Beneficial Role of Phytochemicals on Oxidative Stress and Age-Related Diseases, *BioMed Res. Int.* **2019**, 8748253. doi:10.1155/2019/8748253.
9. Kaur, R.; Arora, S.; Singh, B. Antioxidant activity of the phenol rich fractions of leaves of *Chukrasia tabularis* A. Juss. *Bioresour. Technol.* **2008**, *99*, 7692–7698. doi:10.1016/j.biortech.2008.01.070.

10. Meda, A.; Lamien, C.E.; Romito, M.; Millogo, J.; Nacoulma, O.G. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem.* **2005**, *91*, 571–577. doi:10.1016/j.foodchem.2004.10.006.
11. Quettier-Deleu, C.; Gressier, B.; Vasseur, J.; Dine, T.; Brunet, C.; Luyckx, M.; Cazin, M.; Cazin, J.C.; Bailleul, F.; Trotin, F. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J. Ethnopharmacol.* **2000**, *72*, 35–42. doi:10.1016/S0378-8741(00)00196-3.
12. Pasaribu, T.; Astuti, D.A.; Wina, E.S.; Setiyono, A. Saponin content of *Sapindus rarak* pericarp affected by particle size and type of solvent, its biological activity on *Eimeria tenella* oocysts. *Int. J. Poult. Sci.* **2014**, *13*, 347–352. Available online: <https://www.researchgate.net/publication/275584738> (accessed on 25 January 2014).
13. Fidrianny, I.; Permatasari, L.; Wirasutisna, K.R. Antioxidant activities from various bulbs extracts of three kinds allium using DPPH, ABTS assays and correlation with total phenolic, flavonoid, carotenoid content. *Int. J. Pharm. Sci. Res.* **2013**, *4*, 438–444. Available online: <https://pharmascope.org/index.php/ijrps/article/view/682> (accessed on 15 July 2013).
14. Thaipong, K.; Boonprakob, U.; Crosby, K.; Zevallos, L.C.; Byrne, D.H. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* **2006**, *19*, 669–675. doi:10.1016/j.jfca.2006.01.003.
15. Li, A.; Li, S.; Zhang, Y.; Xu, X.; Chen, Y.; Li, H. Resources and biological activities of natural polyphenols. *Nutrients* **2014**, *6*, 6020–6047. doi:10.3390/nu6126020.
16. Steven, S.; Frenis, K.; Oelze, M.; Kalinovic, S.; Kuntic, M.; Jimenez, M.T.B.; Vujacic-Mirski, K.; Helmstädter, J.; Kröller-Schön, S.; Münzel, T.; et al. Vascular inflammation and oxidative stress: Major triggers for cardiovascular disease. *Oxidative Med. Cell. Longev.* **2019**, 7092151, doi:10.1155/2019/7092151.
17. Sivakrishnan, S.; Kavitha, J.; Muthu, A.K. Antioxidant potential, total phenolic and flavonoids content of aerial parts of ethanolic extract of *Albizia procera* (Family: Mimosoideae). *Asian J. Pharm. Clin. Res.* **2012**, *6*, 108–110. Available online: <https://www.researchgate.net/publication/331802940> (accessed on 23 March 2019).
18. Boeing, J.S.; Barizão, E.O.; Silva, B.C.; Montanher, P.F.; Almeida, V.C.; Visentainer, J.V. Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: Application of principal component analysis. *Chem. Cent. J.* **2014**, *8*, 1–9. doi:10.1186/s13065-014-0048-1.
19. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot, D.A. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants* **2017**, *6*, 42. doi:10.3390/plants6040042.
20. Tiwari, P.; Kumar, B.; Kaur, M.; Kaur, G.; Kaur, H. Phytochemical screening and extraction: A review. *Inter. Pharm. Sci.* **2011**, *1*, 98–106. Available online: <http://docshare01.docshare.tips/files/9403/94036813.pdf> (accessed on 22 February 2011).
21. Iloki-Assanga, S.B.; Lewis-Luján, L.M.; Lara-Espinoza, C.L.; Gil-Salido, A.A.; Fernandez-Angulo, D.; Rubio-Pino, J.L.; Haines, D.D. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC Res. Notes* **2015**, *8*, 1–14. Available online: <https://www.researchgate.net/publication/281376622> (accessed on 28 August 2015).
22. Oliveira, L.L.; Carvalho, M.V.; Melo, L. Health promoting and sensory properties of phenolic compounds in food. *Rev. Ceres* **2014**, *61*, 764–779. doi:10.1590/0034-737x201461000002.
23. Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J.A.; Prior, R.L. Highthroughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric. Food Chem.* **2002**, *50*, 4437–4444. doi:10.1021/jf0201529.
24. Truong, D.; Nguyen, D.H.; Ta, N.T.A.; Bui, A.V.; Do, T.H.; Nguyen, H.C. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. *J. Food Qual.* **2019**, 8178294. doi:10.1155/2019/8178294.
25. Tungmunthum, D.; Thongboonyou, A.; Pholboon, A.; Yangsabai, A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines* **2018**, *5*, 93. doi:10.3390/medicines5030093.

26. Tanase, C.; Coşarcă, S.; Muntean, D. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. *Molecules* **2019**, *24*, 1182. doi:10.3390/molecules24061182.
27. Lourenço, S.C.; Moldão-Martins, M.; Alves, V.D. Antioxidants of natural plant origins: From sources to food industry applications. *Molecules* **2019**, *24*, 4132. doi:10.3390/molecules24224132.
28. Khatoon, M.; Islam, E.; Islam, R.; Rahman, A.A.; Alam, A.H.M.K.; Khondkar, P.; Rashid, M.; Parvin, S. Estimation of total phenol and in vitro antioxidant activity of *Albizia procera* leaves. *BMC Res. Notes* **2013**, *6*, 1–7. doi:10.1186/1756-0500-6-121.
29. Dzialo, M.; Mierziak, J.; Korzun, U.; Preisner, M.; Szopa, J.; Kulma, A. The Potential of plant phenolics in prevention and therapy of skin disorders. *Int. J. Mol. Sci.* **2016**, *17*, 160. doi:10.3390/ijms17020160.
30. Lin, D.; Xiao, M.; Zhao, J.; Li, Z.; Xing, B.; Li, X.; Kong, M.; Li, L.; Zhang, Q.; Liu, Y.; et al. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules* **2016**, *21*, 1374. doi:10.3390/molecules21101374.
31. Wang, T.; Li, Q.; Bi, K. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian J. Pharm. Sci.* **2018**, *13*, 12–23. doi:10.1016/j.ajps.2017.08.004.
32. Kumar, R.S.; Raj Kapoor, B.; Perumal, P. Antioxidant activities of *Indigofera cassioides* Rottl. Ex. DC. Using various in vitro assay models. *Asian Pac. J. Trop. Biomed.* **2012**, *2*, 256–261. doi:10.1016/S2221-1691(12)60019-7.
33. Kumar, S.; Gupta, A.; Pandey, A.K. *Calotropis procera* root extract has capability to combat free radical mediated damage. *Pharmacology* **2013**, *2013*, 1–8. doi:10.1155/2013/691372.
34. Evans, C.A.R.; Miller, N.J.; Bolwell, P.G.; Broamley, P.M.; Pridham, J.B. The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Radic. Res.* **1995**, *22*, 375–383. doi:10.3109/10715769509145649.
35. Cook, N.C.; Samman, S. Review: Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. *J. Nutr. Biochem.* **1996**, *7*, 66–76. doi:10.1016/S0955-2863(95)00168-9.
36. Pandey, A.K. Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Parthenium hysterophorus*: An in vitro study. *Natl. Acad. Sci. Lett.* **2007**, *30*, 383–386.
37. Sparg, S.G.; Light, M.E.; van Staden, J. Biological activities and distribution of plant saponins. *J. Ethnopharmacol.* **2004**, *94*, 219–243.
38. Chan, R.S.M.; Woo, J. Prevention of overweight and obesity: How effective is the current public health approach. *Int. J. Environ. Res. Public Health* **2010**, *7*, 765–783.
39. Musman, M. Toxicity of *Barringtonia racemosa* (L.) kernel extract on *Pomacea canaliculata* (Ampullariidae). *Trop. Life Sci. Res.* **2010**, *21*, 41–50.
40. Zubairi, S.I.; Sarmidi, M.R.; Aziz, R.A. The effects of raw material particles size, types of solvents and solvent-to-solid ratio on the yield of rotenone extracted from *Derris elliptica* roots. *Sains Malays.* **2014**, *43*, 707–71.
41. Zhang, G.F.; Yang, Z.B.; Wang, Y.; Yang, W.R.; Jiang, S.Z.; Gai, G.S. Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status and serum metabolites of broiler chickens. *Poult. Sci.* **2009**, *88*, 2159–2166.
42. Wang, Y.C.; McPherson, K.; Matsh, T.; Gortmaker, S.L.; Brown, M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet* **2011**, *378*, 815–825.
43. Hara, A.; Radin, N.S. Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* **1978**, *90*, 420–426.
44. Akinpelu, B.A.; Igbeneghu, O.A.; Awotunde, A.I.; Iwalewa, E.O.; Oyedapo, O.O. Antioxidant and antibacterial activities of saponin fractions of *Erythrophyleum suaveolens* (Guill and Perri.) stem bark extract. *Sci. Res. Essays* **2014**, *9*, 826–833.
45. Smith, A.Y.R.; Adanlawo, I.G. In vitro and in vivo antioxidant activity of saponin extractd from the root of *Garcinia kola* (bitter kola) on alloxan-induced diabetic rats. *World J. Pharm. Pharm. Sci.* **2014**, *3*, 8–26.
46. Kedare, S.B.; Singh, R.P. Genesis and development of DPPH method of antioxidant assay. *Food Sci. Technol.* **2011**, *48*, 412–422. Available online: <https://www.cabi.org/ISC/abstract/20083133473> (accessed on 18 January 2007).
47. Li, X.C.; Wang, X.Z.; Chen, D.F. Antioxidant activity and mechanism of protochatechuic acid in vitro. *J. Func. Food Health Dis.* **2011**, *1*, 232–244. doi:10.31989/ffhd.v1i7.127.
48. Siahpoosh, A.; Mehrpeyma, M. Antioxidant effects of *Albizia lebbek* and *Prosopis juliflora* barks. *Inter. J. Biosci.* **2014**, *5*, 273–284. Available online: <https://www.cabdirect.org/cabdirect/FullTextPDF/2015/20153063562.pdf> (accessed on 16 January 2014).

49. Khennouf, S.; Amira, S.; Arrar, L.; and Baghiani, A. Effect of some phenolic compounds and quercus tannins on lipid peroxidation. *World Applied Sci.* **2010**, *8*, 1144–1149. Available online: <https://www.researchgate.net/publication/268045543> (accessed on 20 November 2009).

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).