

Article

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 and Suryati Mohd. Thani ³, Azmah Saat ^{3,*}

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

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

³ Department of Human Anatomy, Faculty of Medicine and Health Sciences, 43400 Universiti Putra Malaysia 3; razifabas@upm.edu.my (R.A.); rahman60@hormail.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

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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 mechanism for the observed beneficial effects. Hence, this study examined the phenolic, flavonoid, 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'-azino-bis (3-ethyl-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 elucidate BE as having the highest percentage of inhibition which is 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 Asians population 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-hyperglycaemic agents [6] and subsequently, 12 phenolic acids were isolated qualitatively [3]. 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 hypoglycaemic properties in streptozotocin-induced diabetic rats [4]. These studies show that ABZ has potential hypoglycaemic properties [7] with probable antioxidant activity.

In recent years, antioxidants are fast 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 is 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 [3], 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 disease 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 been 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 is 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 following 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).

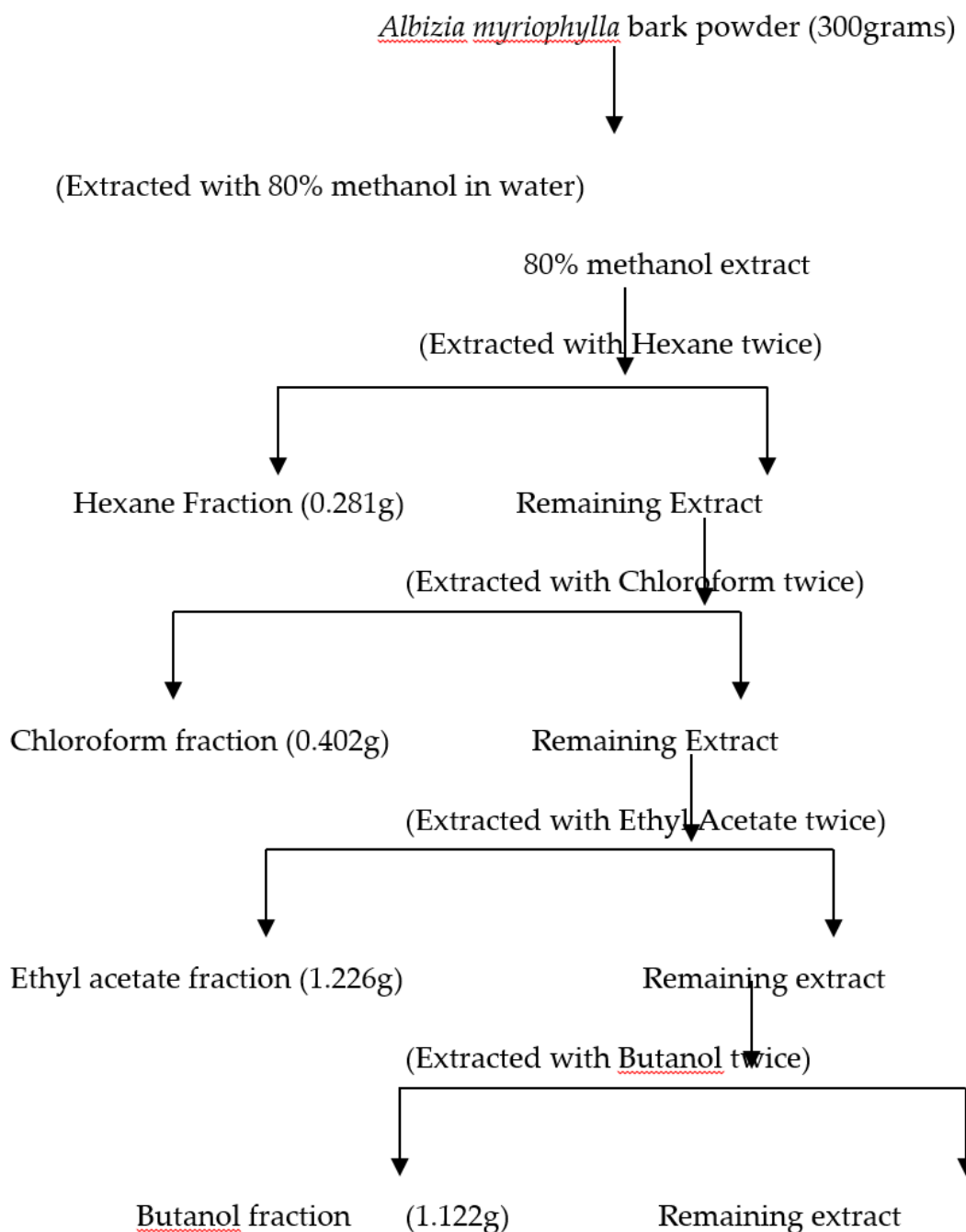


Figure 1. Extraction procedure of bark of *Albizia myriophylla*. Methods adapted from Kaur et al.

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]. 300 mg 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 is 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. 1 mg of ABZ extracts were individually dissolved in dimethyl sulfoxide (DMSO). 150 μ L of each extract was mixed with 150 μ L of 2 % Aluminium Chloride. The mixture absorbent 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 (μ g 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]. 500 mg/mL of ABZ extracts 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 is infused slowly on the inner side of the wall. The solution is 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 (μ g 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 60C 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 (μ g DE/g extract).

2.8. Antioxidants Activity Determinations

2.8.1. Total Antioxidants Assay Using ABTS method

Preparation of ABTS (2-2'-azinobis 3-ethyl-benzothiazoline -6-sulfonic acid) method were adapted from Fidrianny et al. with minor modification [13]. Two solutions were used to prepare the ABTS solution which was 7.4 mM of ABTS and 2.6 mM of potassium persulfate. Both of this solution 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. 0.1 mL of each extract at 20, 40, 60, 80, 100 μ g, respectively, 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:

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

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 adapted 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. 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 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 includes 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 is 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 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 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 its phenolic, flavonoid, 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. Where else, 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 quantity 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 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 is

significantly reduced compared to AE. This is 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, both had a significantly reduced amount of phenolics as compared to the aqueous extract.

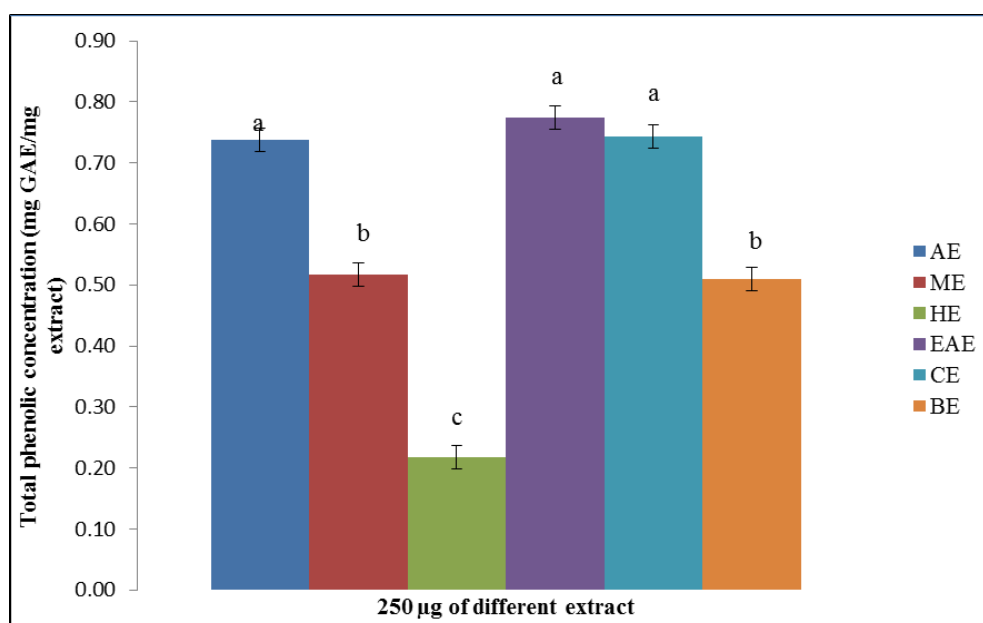


Figure 2. The phenolic contents in 250 µg of AE, ME, HE, EAE, CE and BE of ABZ bark extract. Values were expressed as mean ± SEM. Differing alphabets denote significant difference between groups ($p < 0.05$).

3.3. The Flavonoid Concentrations of ABZ Bark Extracts

Figure 3 showed definite and positive findings of flavonoids in all extracts with the highest flavonoid detected in the HE at 1.04 µgRE/g and AE at 0.895 µgRE/g, respectively. The least 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.

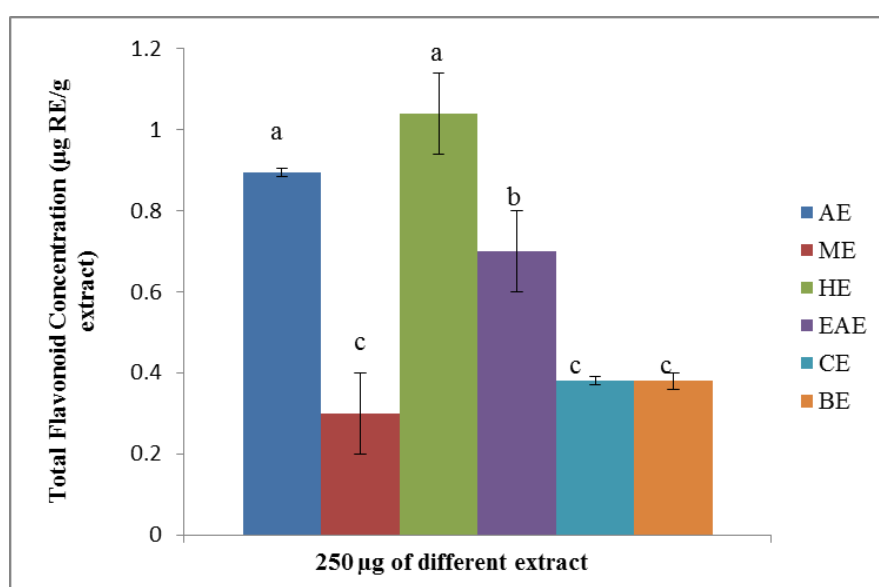


Figure 3. The flavonoid levels in 250 µg of AE, ME, HE, EAE, CE and BE of ABZ bark extract. Values were expressed as mean ± SEM. Differing alphabets denotes significant difference between groups ($p < 0.05$).

3.4. The Saponin Concentrations of ABZ Bark Extracts

The results depicted in Figure 4 showed that qualitatively, saponins can be found in *Albizia myriophylla*, with the highest amount detected in CE at 1.1 $\mu\text{gDE/g}$, followed closely by BE and EAE at 0.92 $\mu\text{gDE/g}$, 0.81 $\mu\text{gDE/g}$ respectively. A moderate amount of saponins were detected in the HE and ME at 0.21 $\mu\text{gDE/g}$, 0.24 $\mu\text{gDE/g}$, respectively. However, the AE had the least amount of saponin as compared to the other extract at 0.03 $\mu\text{gDE/g}$.

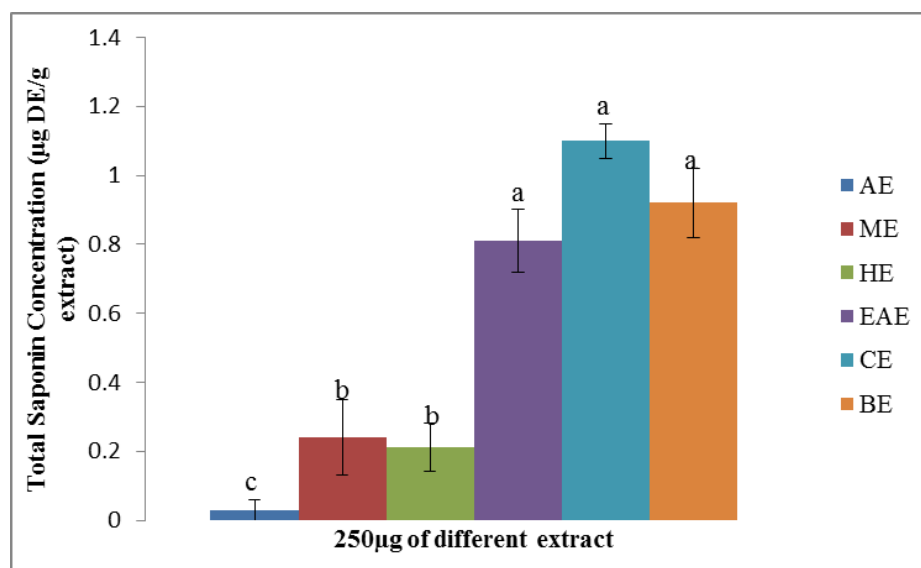


Figure 4. The saponin levels in 250 μg of AE, ME, HE, EAE, CE and BE of ABZ bark extract. Values were expressed as mean \pm SEM. Differing alphabets denotes significant difference between groups ($p < 0.05$).

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 showed that BE had the highest percentage of inhibition at 100 $\mu\text{g/mL}$ with an inhibition of 82.91%. This is 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.

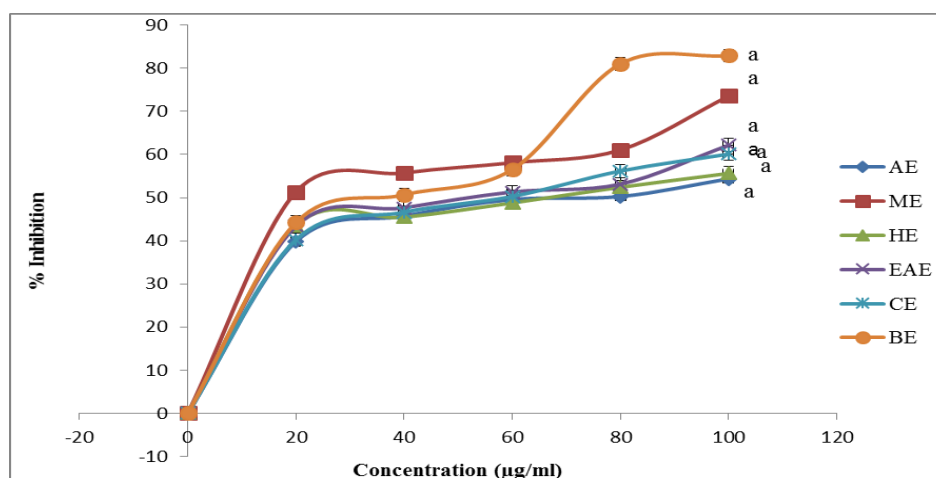


Figure 5. The percentage of inhibition in ABTS antioxidant test in 20 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 60 $\mu\text{g/mL}$, 80 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ of AE, ME, HE, EAE, BE and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups ($p < 0.05$). 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 reaches a plateau till 80 $\mu\text{g/mL}$. From 80 $\mu\text{g/mL}$, it peaks 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 is closely followed by CE which showed a 71.89 % of inhibition at 100 $\mu\text{g/mL}$. AE and ME both have 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.

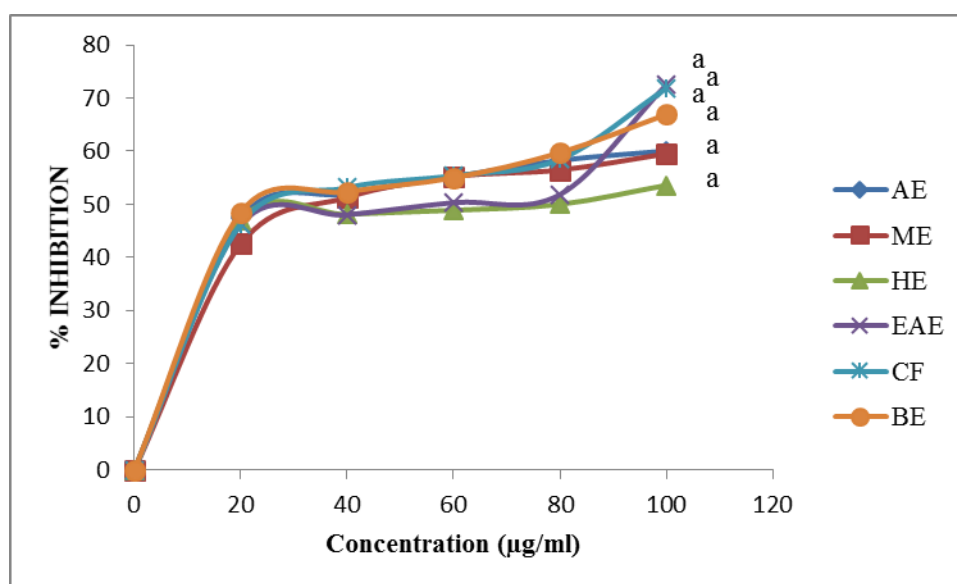


Figure 6. The percentage of inhibition in DPPH antioxidant test in 20 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 60 $\mu\text{g/mL}$, 80 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ of AE, ME, HE, EAE, BE and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups ($p < 0.05$). 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 reaches a plateau to 100 $\mu\text{g/mL}$. At 100 $\mu\text{g/mL}$, the BE reaches the highest percentage of inhibition which is 86.04%. ME comes to a close second at 85.90% of inhibition. This is 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 depicts that with increasing amount of extracts leads to an increase in the reducing power. Thus, the present results showed that highest reducing power was evident in BE of ABZ which is statistically insignificant.

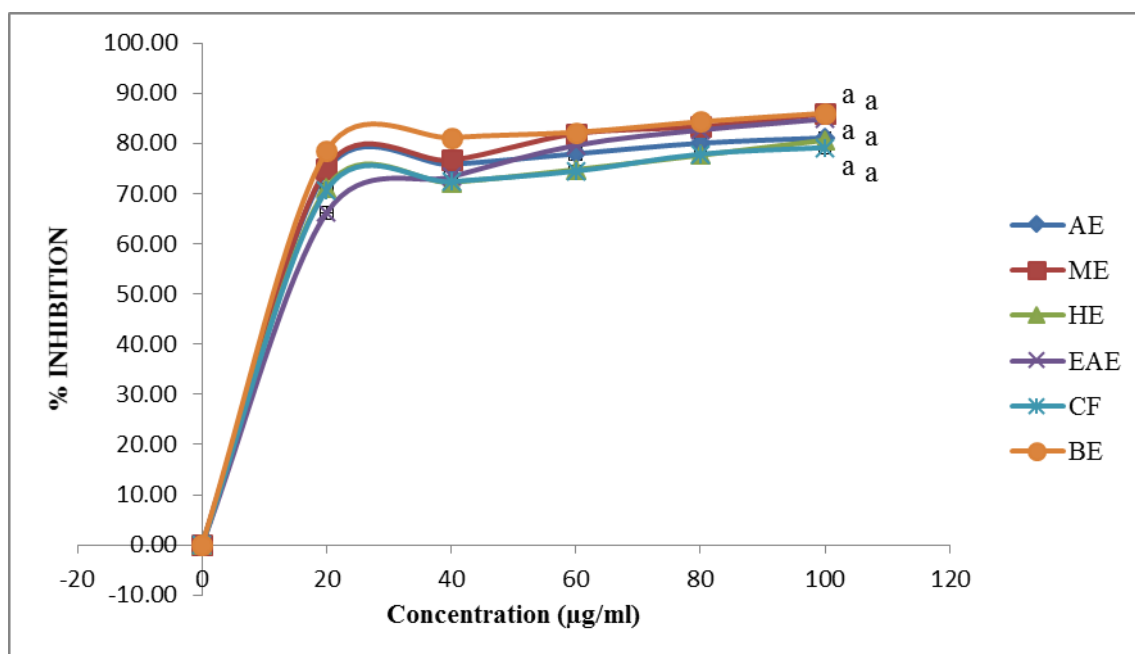


Figure 7. The percentage of inhibition in FRAP antioxidant test in 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL of AE, ME, HE, EAE, BE and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups ($p < 0.05$). Results showed no significant difference.

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 [3] 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 act 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 will be 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. had shown ME of *p. 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 the 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 was 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 result of our research had 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 humans or animals consumption, it would be wiser to use the AE or ME of ABZ, as chloroform is a toxic solvent that is able to cause tumor 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 & 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 shown 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 degree 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 also 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 becomes a lighter shade [47]. As 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, was 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 lebeck* 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.

The previous study postulated that the reducing properties of antioxidant activity 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 initiates 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 includes AE, ME, EAE, CE, HE, 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, BE of ABZ bark showed various concentrations of phenolics, flavonoids and saponin with varying degree of antioxidant activities in the ABTS, DPPH and FRAP test. 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 is related in 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 the 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.

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