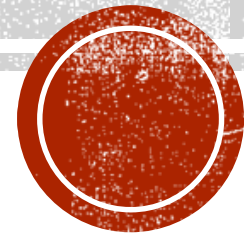




EVALUATION AND DETERMINANTS OF SECONDARY METABOLITES AND ITS ANTIOXIDANT ACTIVITIES OF VARIOUS FRACTIONS FROM *ALBIZIA MYRIOPHYLLA* BARK



Dr. Azmah Sa'at

MBBS (UM), MSc, PhD (UPM)



INTRODUCTION

- In Peninsular Malaysia, *Albizia myriophylla* (ABZ) aqueous bark extracts was used as a tonic drink which was mixed with other herbal supplementation.
- It was taken as an alternative medication for the treatment of diabetes.
- In 2012, *Albizia myriophylla* (ABZ) showed anti diabetic activity in streptozotocin and nicotinamide induced rats [1].
- Similar hypoglycaemic activity was seen when the ABZ aqueous extract with virgin coconut oil were administered to streptozotocin induced diabetic rats [2].
- The probable anti-oxidant and free radical scavenging activity in ABZ may be responsible in alleviating diabetes.



ANTIOXIDANTS

- Increasing inflammatory environment and enhanced oxidative stress conditions in the cells are the leading cause of chronic inflammatory related metabolic disease such as diabetes and obesity [3].
- When the generation and release of pro-inflammatory adipocytokines such as $\text{TNF}\alpha$ and Il-6 exceed the production of anti-inflammatory adipocytokines such as adiponectin and leptin, systemic inflammation and obesity associated metabolic disorders such as diabetes occurs [4].
- Over the last decade, there is renewed interest in antioxidants.
- Antioxidants are found to be essential in preventing and protecting against oxidative cell damage that can lead to chronic metabolic diseases.
- There is evidence that ABZ has a high content of polyphenol [5], which may have probable antioxidant properties with free radical scavenging activities.



OBJECTIVE OF THE RESEARCH

- Determining the phytochemical compound concentration and evaluating the antioxidant properties from the different solvents extracted from the ABZ bark.
- 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.





Figure 1. *A. myriophylla* tree with leaves and bark.



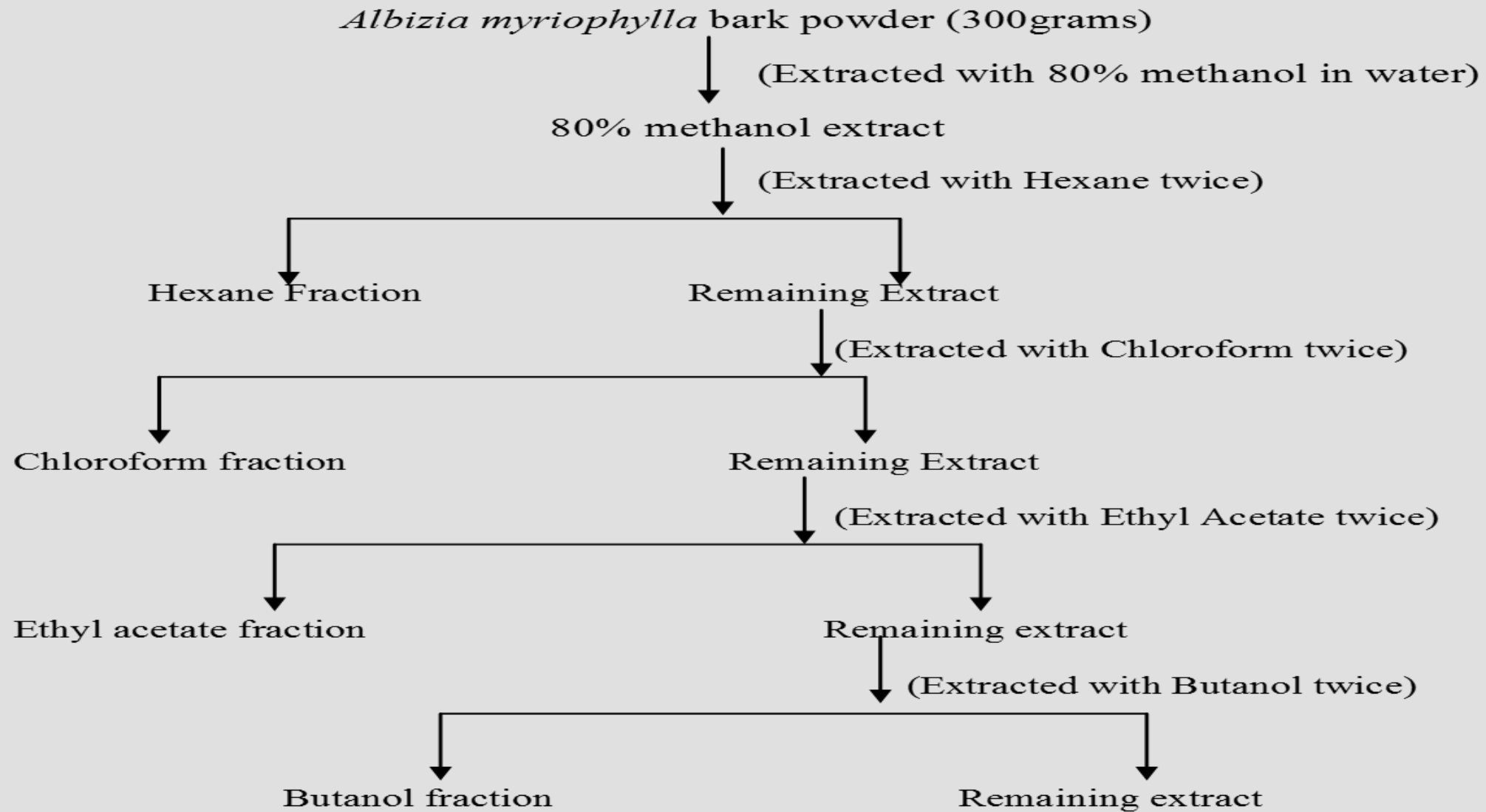
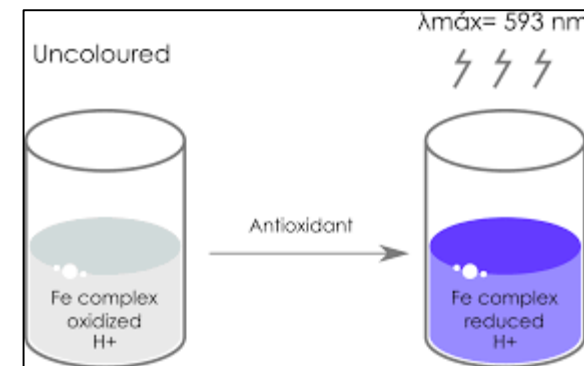
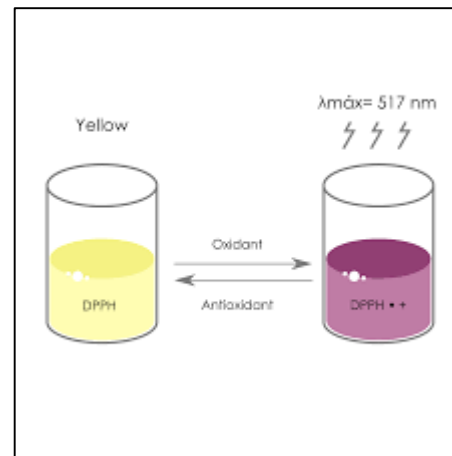
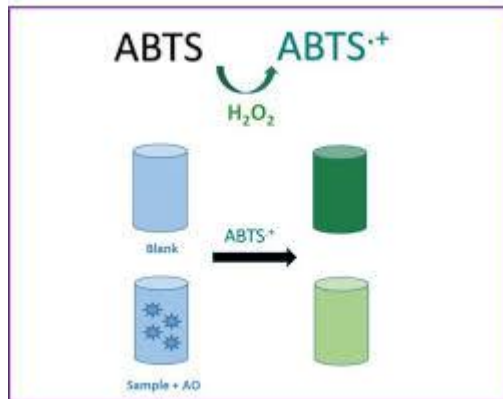


Figure 3.1. Extraction procedure of bark of *Albizia myriophylla*. Image adapted from Kaur *et al.*, (2008).



METHODS

- Following extraction methods, 250 μ g of aqueous, methanol, hexane, chloroform, ethyl acetate and butanol extract were tested for phenolics, flavonoid and saponin concentration.
- Antioxidant activity determinants which include ABTS, DPPH Scavenging Activity and FRAP assay were done following methods adapted from respective journals.



RESULTS

- *The Total Yield of Aqueous, Methanol, Hexane, Ethyl Acetate, Chloroform and Butanol Bark Extracts from 367 grams of ABZ bark*

Extracts	Quantity (grams)	Percentage (%)
Aqueous	18.35	5
Methanol	10.535	2.8
Hexane	0.281	0.07
Ethyl Acetate	1.226	0.33
Chloroform	0.402	0.11
Butanol	1.122	0.3



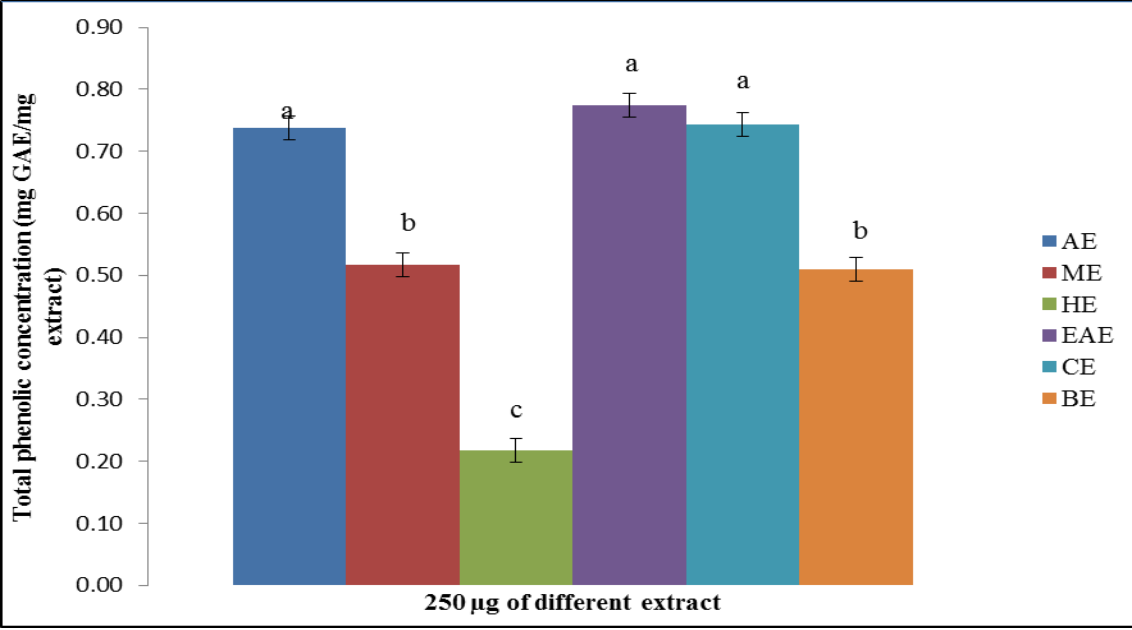


Fig. 2. Phenolic concentration of ABZ

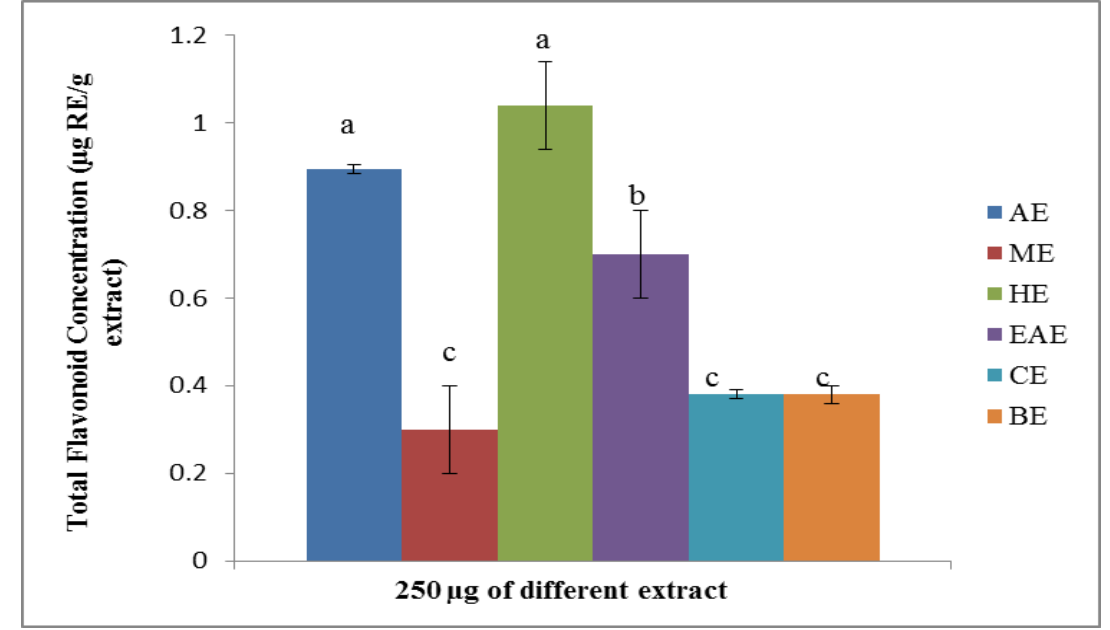


Fig. 3. Flavonoid concentration of ABZ

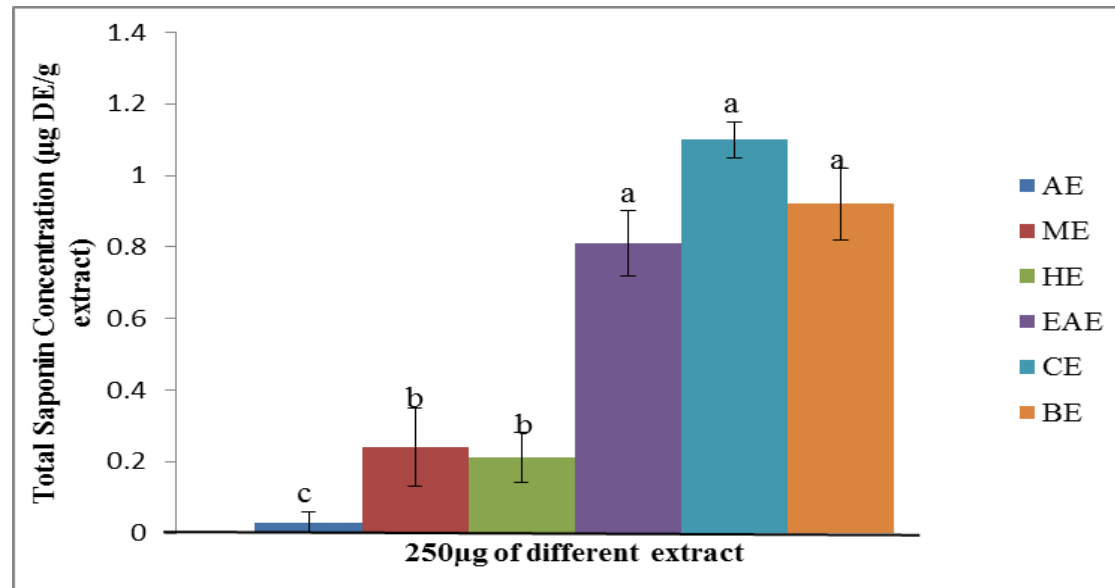


Fig. 4. Saponin concentration of ABZ



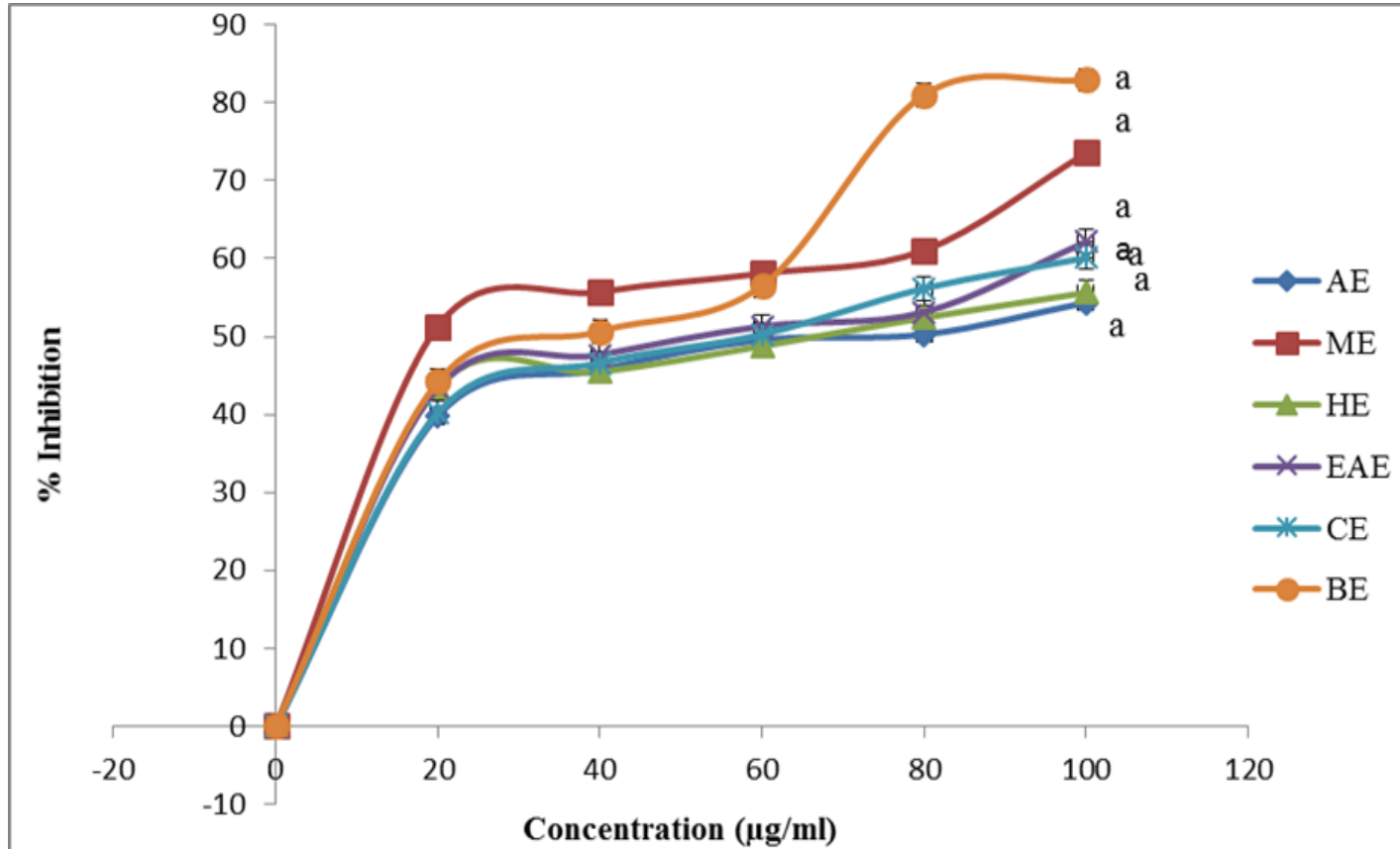


Figure 5. The percentage of inhibition in ABTS antioxidant test in 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of AE, ME, HE, EAE, BE and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups ($P < 0.05$). Results showed no significant difference between the extracts.



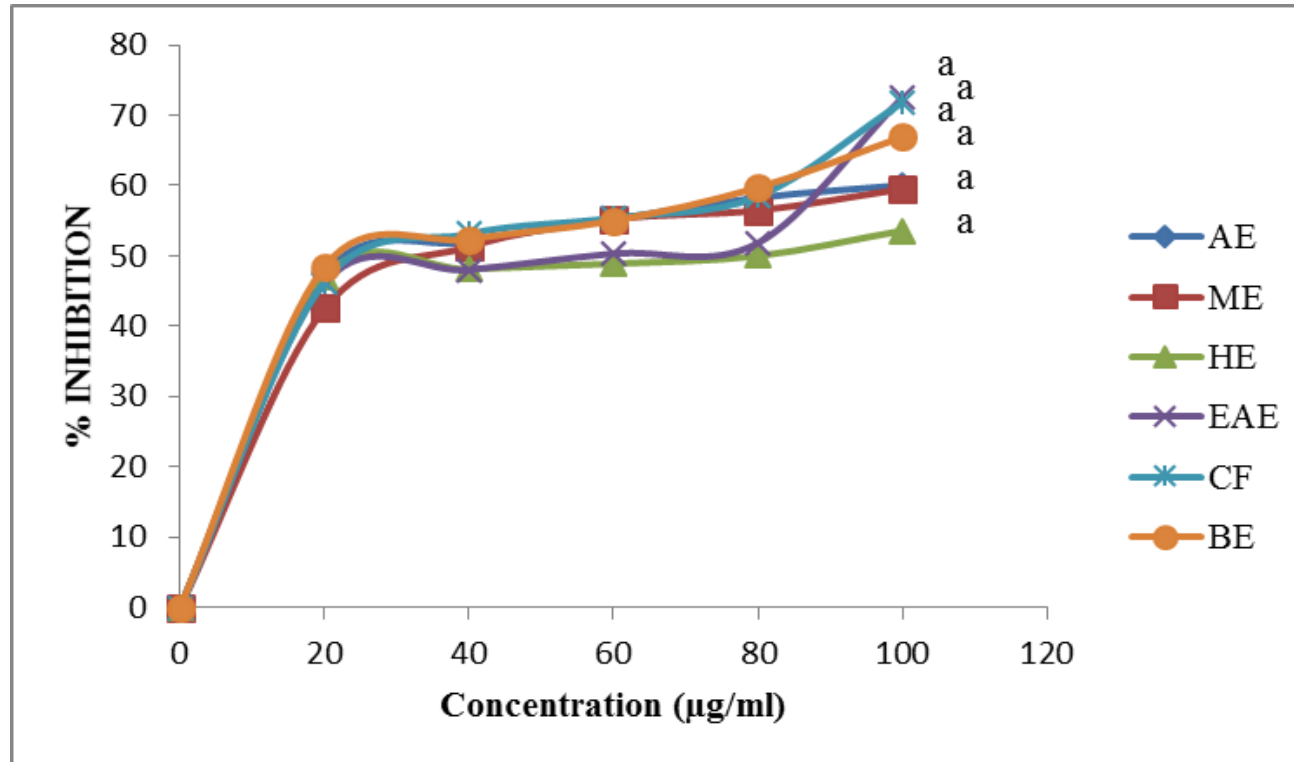


Figure 6. The percentage of inhibition in DPPH antioxidant test in 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of AE, ME, HE, EAE, BE and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups (P<0.05). Results showed no significant difference.



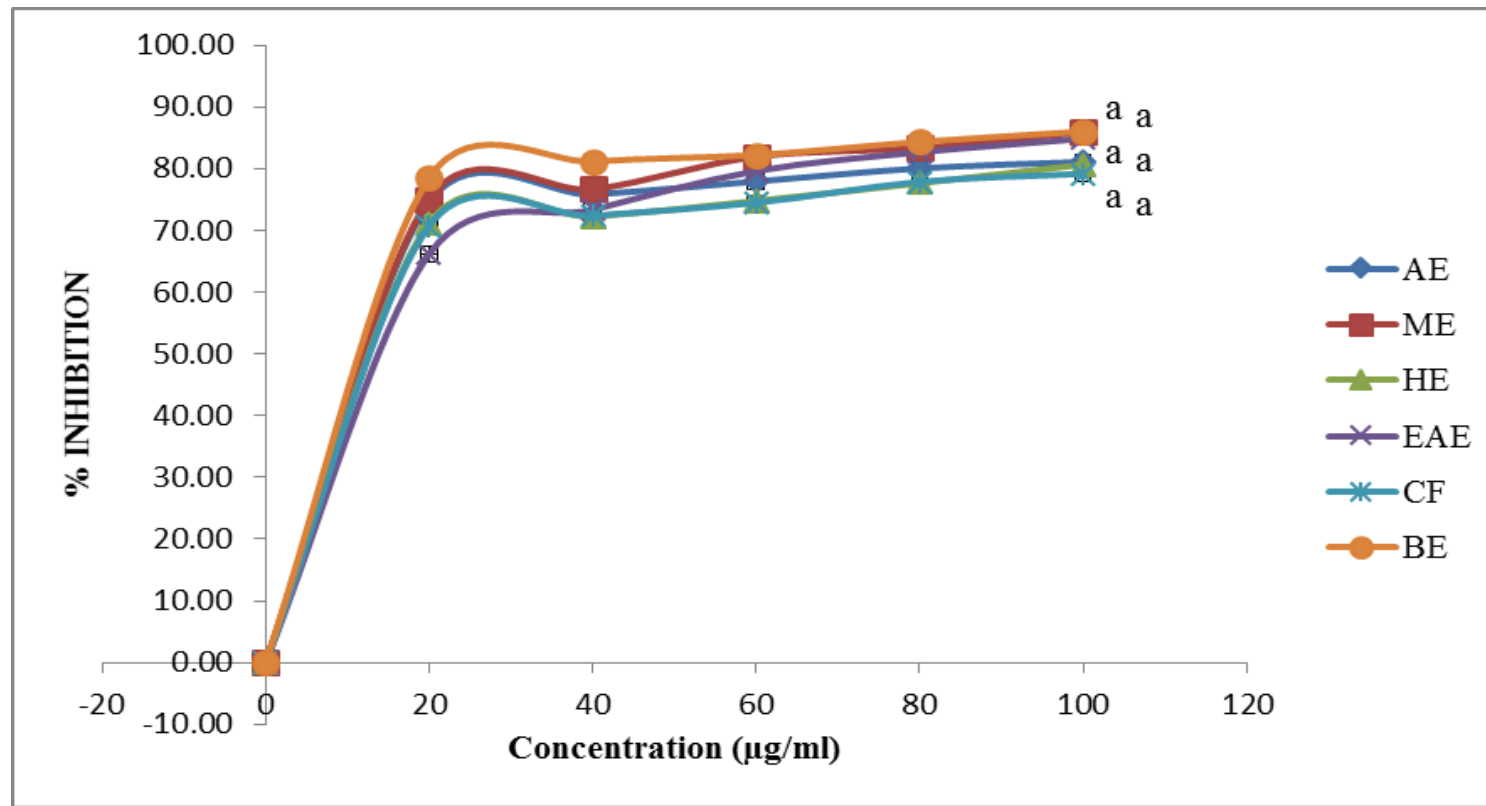


Figure 7. The percentage of inhibition in FRAP antioxidant test in 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of AE, ME, HE, EAE, BE and CE of ABZ bark extracts. Differing alphabets denotes significant difference between groups ($P < 0.05$). Results showed no significant difference.



DISCUSSION

Methanol Extract

- Methanol is a solvent commonly used for the extraction processes as it is generally known to be more effective in dissolving active mixtures [6]
- 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.
- From this study, the ME of ABZ bark had the most yields as compared to the other extracts.
- Therefore, based on the discussion above, methanol was used as a method of extraction of ABZ bark, with various fractions using different solvents.



PHENOL

- 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 [7].
- 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.



FLAVONOID

- Flavonoids have hydroxyl groups that are functional in promoting free radical scavenging activity and chelation of metal ions hence mediating the antioxidant activities [8,9].
- Numerous studies have shown the inhibitory effects of flavonoids against infections, obesity, cardiovascular diseases, and other metabolic disorders [9,10].
- 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

- Saponins could induce local immune stimulatory environment at the site of inoculation, characterized by the production of cytokines and chemokines [11].
- Information on pharmacological activities exhibited by some isolated saponins comprises hypocholesterolemic, anticarcinogenic, antioxidant, hypoglycemic, and antiprotozoans [12].
- 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.
- 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 [13].



ANTIOXIDANT ACTIVITY

- The antioxidant activities of different plants have different levels of antioxidant activity, with differing interactions within the antioxidant assay and extracts [14].
- However, the statistical analysis of DPPH, ABTS and FRAP scavenging activities amongst the various extract of ABZ showed that all the extracts were not significantly different from each other.
- From the DPPH, ABTS and FRAP results, all the extracts showed maximal 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



CONCLUSION

- 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 $\text{TNF}\alpha$ 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.



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