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# IN-VITRO EVALUATION OF ANTI-OXIDANT AND ANTI-DIABETIC ACTIVITIES OF *MELIA AZEDARACH* (MELIACEAE) LEAF, STEM-BARK AND ROOT EXTRACTS

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## ABSTRACT

**Introduction**: Diabetes mellitus is a major health burden in developing countries. Formations of free radicals due to oxidative stress can cause beta-cell death, tissue insulin resistance with increased diabetic complications. Extracts from *Melia azedarach* is used in traditional medicine to treat various diseases which includes diabetes, but this claimed activity has not been evaluated. This study was therefore designed to evaluate the antioxidant and anti-diabetic activities of *M. azedarach* extracts. **Materials and methods:** The dried powdered leaves, stem-bark and root of *M. azedarach* were studied for phytochemical analysis. The antioxidant activity of the extracts and standards (ascorbic acid and gallic) were determined at 517nm with spectrophotometer using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) model. Anti-diabetic activity was assessed using in-vitro alpha-amylase inhibitory assay with Metformin tablet 500mg as the positive control. The absorbance was measured at 540nm. Graph-pad prism was used for statistical analysis. **Results:** Phytochemical investigation revealed presence of secondary metabolites like tannins and alkaloids. The root extract exhibited the highest radical-scavenging activity as well as the highest inhibitory effect on alpha-amylase enzymes. **Conclusion:** All the extracts of *M. azedarach*, possess anti-diabetic activities which seems to be more in the root extract. The anti-diabetic effect of the extract could be linked to their antioxidant and alpha-amylase enzymes inhibitory effects. Therefore, the folkloric use of this plant extracts in the management of diabetes is justified. **Recommendation**: In vivo anti-diabetic study of these extracts using animal model is recommended.

**KEYWORDS:** *Melia azedarach*, Anti-oxidant, Type 2 diabetes, Root extract.

## 1. INTRODUCTION

**1.1 Background:** Medicinal plants have continued to attract attention in the global search of plants for the treatment of various diseases affecting humans (Afrisham *et al.*, 2015). Many important drugs used in medicine today are directly or indirectly derived from plants due to their bioactive constituents such as alkaloids, steroids, saponins and tannins Afrisham *et al.*, 2015).

Diabetes mellitus is a metabolic disease characterized by a high blood sugar level over a prolonged period of time. There are three main types of diabetes mellitus: Type 1, Type 2 and Gestational diabetes. Complications which include diabetic ketoacidosis, stroke, chronic kidney disease, damage to the eyes are associated with type 2 diabetes (WHO, 2013). In 2019, diabetes was said to be the cause of approximately 4.2 million deaths (Diabetes Atlas ninth edition, 2019). It is the 7$^{th}$ leading cause of death globally (Centers for Disease Control and Prevention, 2020).

**1.2. Antioxidants:** There is an increased focus in the development of natural antioxidants from plant extracts for the treatment of diseases such as cancer, cardiovascular diseases and lung diseases. These diseases are associated with oxidative stress. Antioxidants are used to counter the effects of oxidative stress because they promote optimal cellular and systemic health. Previous studies have demonstrated some medicinal plants possess both antioxidant and hypoglycemic effects

(González et al., 2011). This study was therefore aimed at evaluating the antioxidant and anti-diabetic activities of the aqueous methanol extract of the leaf, stem bark and root of *M. azedarach* and to establish the relationship between their antioxidant and anti-diabetic activities.

Antioxidants mop up free radicals which are molecules with one or more single pair of the electron that can quickly react with constituents such as proteins, nucleic acid and lipids. The reactive molecule comprises the reactive oxygen species (ROS) and reactive nitrogen species (RNS) derived from oxygen and nitrogen respectively (Fang *et al.,* 2002). These reactive particles are generated in cell membrane, mitochondria, nucleus, lysosome, peroxisome and endoplasmic reticulum. These atoms, called free radicals, scavenge the body to seek out other electrons so they can become a pair (Fang *et al.,* 2002). Nevertheless, free radicals play a very important role in the activation of different signaling pathways inside the cell, such as the mitogen activated protein kinase (MAPK) path way and extracellular-signal regulated kinase (ERK) pathway which alter gene expression (Fang *et al.,* 2002). For instance, RNS produced by neurons act as neurotransmitters and those generated by macrophages act as mediators of immunity. Similarly, ROS is involved in gene transcription, single transduction and regulation of other activities in cell (Fang *et al.,* 2002). In coordination with superoxide dismutase, free radical can also initiate cell death (Cho *et al.,* 2003). The pairing of these electrons causes damage to cells, proteins and DNA (Cho *et al.,* 2003). Hence, free radicals are associated with human diseases (Gabriele *et al.,* 2017). Although free radicals are produced naturally in the body, lifestyle factors, such as air pollution, cigarette smoke, alcohol intake and stress can accelerate their production (Smilin *et al.,* 2013).

**1.3: Oxidative stress:** Oxidative stress plays an important role in the development of vascular complications in type 2 diabetes (Pham-Huy and He 2008). Oxidative stress manifests as a result of an imbalance between the production of free radicals and the ability of the body to counteract their harmful effects through neutralization by antioxidants (Lipinski, 2001). Several antioxidants such as Vitamin E, Vitamin C, Flavonoids and Polyphenol have been exploited for their beneficial effect against oxidative stress (Lipinski, 2001). The variation in the levels of antioxidants (catalase, superoxide dismutase and glutathione) makes the tissue susceptible to oxidative stress which can lead to the development of chronic diseases like diabetes (Lipinski, 2001).

**1.4. Diabetes**: Free radicals play a major role in the onset and progression of diabetes due to their ability to damage lipids, proteins and DNA (Ayepola *et al.,* 2014) Elevated levels of blood-glucose (hyperglycemia) in diabetes could be caused by insufficiency in insulin production or resistance to the action of insulin produced by the beta cells of the pancreas in the body (Maritim *et al*., 2003). Long term elevation of blood glucose is associated with macro- and micro-vascular complications which could lead to heart diseases, stroke, blindness and kidney disease (Loghmani, 2005). Apart from hyperglycemia, there are other factors that play some role in pathogenesis of diabetes such as hyperlipidemia (Kangralkar *et al.,* 2010). Pharmacological management of diabetes includes the use of drugs like Metformin, Thiazolidinediones and Sulfonylureas while the non-pharmacological management involves dietary control, regular exercises and lifestyle modifications.

Some plants such as *Allium sativa* (galic), *Psidium guajava* (guava) *and Aloe vera* (aloe) have demonstrated both anti-diabetic and antioxidant activities (Deepa *et al.,* 2018). The antioxidants protect the beta-cell which produces insulin against oxidative stress induced-apoptosis thereby preserving the function of beta-cells (Pi *et al*., 2014). *Melia azedarach* Linn. (Meliaceae**)** is commonly known as white cedar, umbrella tree, bead tree, ichenkurdi (Hausa), and eke-oyinbo (Yoruba) (Figure 1). In folklore medicine, extracts from different parts of *M. azedarach* plant are used as athelmintic, astringent, anti-malaria recipe, and in the treatment of leprosy (Sabira *et al*., 2014).

**Figure 1: *Melia azedarach* fruits, leaves and flowers.**

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Plant materials

***Sample collection and identification***

The leaves, stem barks and roots of *M. azedarach* were obtained from the botanical garden of University of Ibadan, Osun state Nigeria. This was identified and authenticated at Forest Research Institute in Ibadan with voucher number FHI108966.

#### 2.1.2. Instruments

Analytical weighing balance (Bioevopeak, U.SA), test tubes, conical flasks, separating funnel (Glassco Lab. USA), Water-bath (Bio Techno Lab Mumbai India), measuring cylinder, Aluminum foil, Cotton wool, UV Spectrophotometer (Spectrolab SPM-752 pro India),

#### 2.1.3. Drugs

Alpha-amylase solution (EC 3.2.1.1 type VI, Sigma), Metformin tablet 500 mg (Nigerian-German Chemical, Nigeria), Gallic acid and Ascorbic acid.

#### 2.1.4 Chemicals and reagents

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical (Cayman Chemical, USA), Potato starch solution (SiccaDania Denmark), sodium chloride and methanol (Sigma, India).

### 2.2 Methods

#### 2.2.1 Extraction of leaves, stem barks and roots of *M. azedarach*

The leaves stem barks and roots of *M. azedarach* were shade-dried for 5 days and ground into fine powder with grinding machine. Each powdered plant parts were weighed (500g) and soaked in 400 ml aqueous methanol (70% v/v) for 48 hours and then filtered with muslin cloth. The marc was soaked again until it was completely extracted and the filtrates were combined and concentrated at $40^{oC}$ using a rotary evaporator. The weights of the dried extracts were obtained using the weighing balance, the percentage yields were calculated.

#### 2.2.2 Qualitative phytochemical screening of the extracts

Preliminary phytochemical screening for secondary metabolites of the extracts to identify the presence of alkaloids, flavonoid, anthraquinone, glycoside, steroid, tannin and triterpenes was carried out according to the procedures outlined by Habourne (1998).

#### 2.2.3 Antioxidant assay using DPPH free radical scavenging activity

The ability of the plant extracts to scavenge DPPH free radical was assessed using the method described by Baba and Malik, (2015) with a little modification. The stock solutions of the various plant extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Serial dilutions were made with methanol to obtain concentrations of 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml,15.63µg/ml and 7.8125µg/ml. Two positive controls were used; Gallic and Ascorbic acid (100µg/ml-3.125µg/ml) while negative control contained only the solvent which was 70 % v/v methanol (the blank). The assay mixture contains 1000µL (of leaf, stem bark, root extract or standard) plus 1000µL of Methanol plus 1000 µL of freshly prepared DPPH solution (0.04 mg/mL in 50 mL of methanol). The mixture was incubated at room temperature in the dark for 30 minutes. The change in color from deep violet to light yellow was measured spectrophotometrically at 517nm using UV spectrophotometer (Spectrolab SPM-752 pro). The ability of the extract to scavenge DPPH radical was calculated using the equation below:

$$\% \text{ DPPH Scavenging activity} = \frac{Absblank - Abssample}{Absblank} \times 100$$

The experiment was conducted in duplicate and $IC_{50}$ values were determined as the concentration of the extract that caused 50% DPPH radical inhibition. The lower the $IC_{50}$ value the higher the antioxidant activity of the extract.

#### 2.2.4 Anti-diabetic assay using Alpha-amylase inhibition assay

The alpha amylase inhibition assay was performed using modified chromogenic method (Hasenah *et al.,* 2006). A 0.5 g of porcine alpha-amylase (EC 3.2.1.1 type VI, Sigma) was dissolved in 100ml of ice-cold distilled water to give a 0.5% w/v solution and potato starch (0.5% w/v) in 20 mM phosphate buffer (pH 6.9) containing 6.7mM sodium chloride was used as a substrate solution. The aqueous methanol extracts were dissolved in 1mL distilled water (1000 µg/ml to 31.25 µg/ml), followed by 1 ml of alpha-amylase solution and allowed to incubate for 5mins at $25^{O}$C. A 1ml of potato starch solution was added and the mixture was further incubated for 5mins at $25^{O}$C. The reaction was stopped by adding 0.5 ml of DNS reagent and boiled at $90^{O}$C in a water bath for 15mins, cooled and diluted with 2 ml of distilled water. The absorbance of the test (alpha-amylase + extract + patatoe), blank (without alpha-amylase) and control (without extract) were measured at 540nm using the UV spectrophotometer. The ∝-amylase inhibitory activity was expressed as percentage inhibition and calculated using the equation below.

$$\% \propto\text{-amylase inhibition} = \frac{Absblank - Abssample}{Absblank} \times 100$$

The concentration of extract exhibiting 50% inhibition of enzyme activity ($IC_{50}$) was determined.

#### 2.2.5 Statistical analysis

The data was analyzed by Computer Software, Graphpad prism using one-way analysis of variance (ANOVA) to check for the level of significance and determination of $IC_{50}$.

## 3. RESULTS

### 3.1. Yields of extracts

The yield of extract from the powdered leaf, stem bark and root were 32.41, 17.56 and 6.08 % w/w respectively. The leaves had the highest yield of extract while the root had the smallest yield (Table 1).

### 3.2. The Phytochemical screening of *M. azedarach* crude extracts

The phytochemical analysis of the crude extracts of *M. azedarach* revealed the abundance of
alkaloids, glycosides, triterpenoids, tannin, saponins, carbohydrates, flavonoids and steroids in the leaves, stem bark and root extracts. Though there was abundance of anthracene glycoside in stem bark and root, its absence was observed in the leaves (Table 2).

### 3.3. Antioxidant study

The concentration of the extracts and the controls that caused 50% DPPH radical inhibition ($IC_{50}$) was observed to be 4.742 µg/ml (leaf), 4.390 µg/ml (stem bark), 4.105 µg/ml (root), 4.184 (Ascorbic acid) and 14.71 µg/ml (Gallic acid ) (Table 3a and 3b). The lower the $IC_{50}$ value the higher the antioxidant activity of the extract. From this result the sample with the highest anti-oxidant property was the root extract followed by the stem bark extract then, the leaf extract. The $IC_{50}$ value of the root extract (4.105 µg/ml) was also observed to be lower than that of the two positive controls, Ascorbic acid (4.184 µg/ml) and Garlic acid (14.71 µg/ml) ( Table 3a and 3b).

From this result the root extract of *M. azedarach* probably posses higher anti-oxidant property than Ascorbic acid and Garlic. The leaf, stem-bark and root aqueous extracts of M. *azedarach*, significantly ($P<0.05$) inhibition DPPH free radical when compared to the control, Ascorbic acid (Tables 3a and 3b).

**Table 1: Percentage Yields of *M. azedarach* extracts.**

| Plant parts | Weight of powder (grams) | Volume of solvent(ml) | Weight of bottle (grams) | Weight of bottle + extract(grams) | Weight of extract (grams) | Percentage yield (%) |
|---|---|---|---|---|---|---|
| **Leaf** | 80 | 1500 | 61.012 | 86.938 | 25.93 | 32.41 |
| **Stem bark** | 105 | 1500 | 69.40 | 87.84 | 18.44 | 17.56 |
| **Root** | 100 | 1500 | 67.52 | 73.69 | 6.08 | 6.08 |

**Table 2: Phytochemical screening of *M. azedarach* crude extracts.**

| Phytochemical test | Leaf | Stem bark | Root |
|---|---|---|---|
| **Alkaloids** | +++ | +++ | +++ |
| **Glycosides** | +++ | +++ | +++ |
| **Anthracene glycoside** | - - - | +++ | ++ |
| **Tannins** | +++ | +++ | +++ |
| **Saponins** | ++ | +++ | ++ |
| **Carbohydrates** | +++ | +++ | +++ |
| **Flavonoids** | +++ | +++ | +++ |
| **Triterpenes** | +++ | +++ | +++ |
| **Steroid** | +++ | +++ | +++ |

**Key: +++ =abundance, ++ =moderately present, - - - = Absent**

**Table 3a: Antioxidant assay using DPPH free radical scavenging activity.**

| | (Mean absorbance) | | | % Inhibition | | |
|---|---|---|---|---|---|---|
| **Conc (µg/ml)** | **leaf** | **S/bark** | **Root** | **leaf** | **s/bark** | **Root** |
| 500 | 0.386±0.54 | 0.325±1.69 | 0.356±0.65 | 79.03* | 82.35* | 80.66* |
| 250 | 0.368±0.98 | 0.350±0.48 | 0.360±1.22 | 80.01* | 80.99* | 80.45* |
| 125 | 0.370±0.82 | 0.379±0.57 | 0.344±0.52 | 79.90* | 79.41* | 81.32* |
| 62.5 | 0.466±1.15 | 0.401±0.72 | 0.323±1.88 | 74.69* | 78.27* | 82.46* |
| 31.25 | 0.727±0.34 | 0.508±1.36 | 0.431±1.39 | 60.51 | 72.41* | 76.59* |
| 15.625 | 0.593±0.30 | 0.626±0.50 | 0.560±0.99 | 67.79 | 66.00 | 69.58 |
| 7.8125 | 0.522±0.66 | 0.561±1.47 | 0.543±0.69 | 71.65* | 69.53 | 70.51* |
| $IC_{50}$ values | **4.742** | **4.390** | **4.105** | | | |

Data analyzed by ONE WAY ANOVA. Values are presented as mean ± Standard error of mean of experiment in duplicate. *$P<0.05$ is statistically significant

**Table 3b: Antioxidant assay using DPPH free radical scavenging activity.**

| Conc (µg/ml) | Mean absorbance | | % Inhibition | |
|---|---|---|---|---|
| | Ascorbic acid | Garlic acid | Ascorbic acid | Garlic acid |
| 100 | 0.366±0.63 | 0.351±1.32 | 80.12* | 80.93* |
| 50 | 0.362±0.51 | 0.358±0.89 | 80.34* | 80.55* |
| 25 | 0.970±0.65 | 0.362±1.44 | 47.31* | 80.34* |
| 12.5 | 0.334±2.47 | 0.367±0.26 | 81.86* | 80.07* |
| 6.25 | 0.458±0.68 | 0.382±0.57 | 75.12* | 79.25* |
| 3.125 | 0.615±1.42 | 0.373±1.43 | 66.59 | 79.74* |
| 1.563 | 0.573±0.77 | 0.375±1.19 | 68.88 | 79.63* |
| **$IC_{50}$ values** | **4.184** | **14.71** | | |

Data analyzed by ONE WAY ANOVA. Values are presented as mean ± Standard error of mean of experiment in duplicate. *P<0.05 is statistically significant

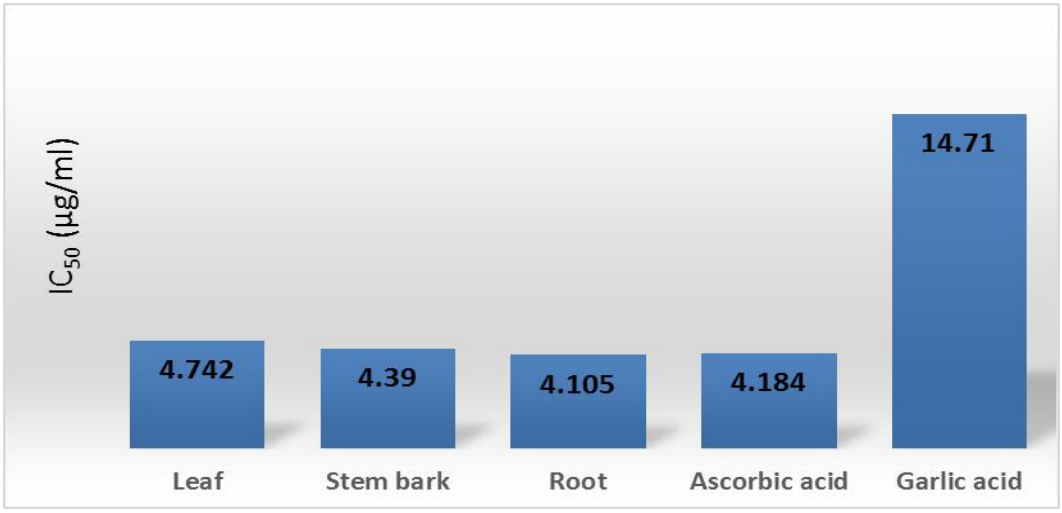


**Figure 2: IC50 of various extracts and standards.**

### 3.4. The Inhibitory effect of the aqueous extracts of *M. azedarach* on alpha-amylase

The invitro anti-diabetic study was carried out in order to determine the ability of this plant extracts to inhibit alpha-amylase enzymes. Inhibition of these enzymes is one of the mechanisms through which some anti-diabetic agents exert their hypoglycemic effect. The $IC_{50,}$ which was the concentration of the extract required to cause 50 % inhibition on the enzymes was measured. The lower the $IC_{50}$ value the higher the inhibitory activity. The $IC_{50}$ for the various extracts and the reference standard were found to be as follows; the leaf (63.88 µg/ml,), stem bark (35.37 µg/ml), root (12.39 µg/ml) and Metformin (3.236 µg/ml) (Table 4). Though the reference standard drug Metformin exhibited the lowest $IC_{50,}$ all the extract were able to cause significant (P<0.05) inhibition on the alpha-amylase enzymes when compared to control even at the lowest concentration of 31.25 µg/ml.

It was also observed, that at the lowest concentration of 31.25ug/ml treatment, the percentage inhibition exhibited on alpha-amylase by the root extract (44.36%) and Metformin (45.13%) were comparable (Table 4).

**Table 4: Anti-diabetic assay using Alpha-amylase inhibition assay.**

| | (Mean absorbance) | | | | % Inhibition | | | |
|---|---|---|---|---|---|---|---|---|
| Conc (µg/ml) | leaf | S/bark | Root | Metformin | leaf | s/bark | Root | Metformin |
| 1000 | 2.27±0.33 | 2.26±0.49 | 1.844±0.38 | 1.501±0.68 | 3.81 | 4.24 | 21.86 | 36.40 |
| 500 | 2.22±1.67 | 2.14±1.51 | 1.838±0.67 | 1.049±0.36 | 5.93 | 9.32* | 22.12 | 55.55* |
| 250 | 1.80±0.99 | 2.17±0.68 | 1.525±0.46 | 1.302±1.66 | 23.73* | 8.05* | 35.38* | 44.83* |
| 125 | 2.15±1.44 | 2.32±0.79 | 1.526±0.29 | 1.386±2.04 | 8.90* | 1.70 | 35.34* | 41.27* |
| 62.5 | 2.02±0.61 | 2.26±0.37 | 1.191±1.22 | 1.059±0.44 | 14.41* | 4.24 | 49.53* | 55.13* |
| 31.25 | 2.12±0.84 | 2.13±1.53 | 1.313±0.26 | 1.295±0.79 | 10.17* | 9.75* | 44.36* | 45.13* |
| **$IC_{50}$=** | **63.88** | **35.37** | **12.39** | **3.236** | | | | |

**Data analyzed by ONE WAY ANOVA. Values are presented as mean ± Standard error of mean of experiment in duplicate. *P<0.05 is statistically significant**

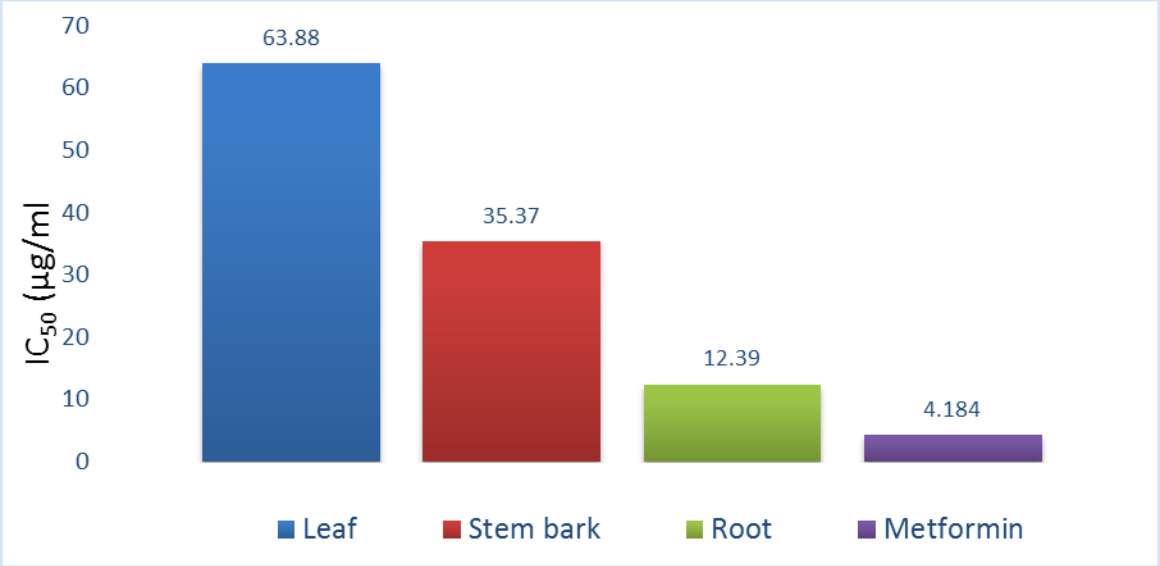


**Figure 3: Alpha-Amylase inhibitory activities of various extract and Metformin.**

### 4.1 DISCUSSION

Diabetes mellitus is a group of metabolic disorders that is caused by insulin deficiency or resistance to insulin (Ahren and Pacini 2005). It is assumed that formation of free radicals due to oxidative stress contributes to diseases such as cancer, cardiovascular disease and diabetes (Taniyama and Griendling 2003). Though free radicals have cell signaling biological role in human system (Halliwell 2007), at higher level it is capable of manifesting negative effects such as beta-cell death and tissue damage (Halliwell, 2007). Beta-cells are the only cells that produces insulin hormone (Boland *et al.*, 2018). Hence, the use of antioxidants such as Vitamin C and E is usually recommended in the management of diabetes mellitus (Deepa *et al.*, 2018). Many medicinal plants have been found to exhibit anti-diabetic effects and such plants include Ch*amaecostus cuspidatus* (Bhat *et al.*, 2010) and *Momordica charantia* (Ranabir *et al.*, 2019) These plants have been found to contain natural antioxidants like flavonoids, tannins and terpenoids (Bhat *et al.*, 2010, Ranabir *et al.*, 2019).

Phytochemical screening of *M. azedarach* extracts (leaf, stem-bark and root) had revealed the presence of bioactive compounds with antioxidant activities such as alkaloids, flavonoids, terpenoids, tannins and flavonoids (Mohammed *et al.*, 2012). Alkaloids, flavonoids, terpenoids, and tannins are free radical scavengers as well as powerful antioxidants (Galleano *et al.*, 2010). Flavonoids can activate antioxidant enzymes (Cos *et al.*, 1998) and can inhibit oxidases (Lima *et al.*, 2014). These properties are beneficial in management of diabetes. Tannins are also noted for their antioxidant effects (Kaisarun *et al.*, 2016). Hence, the presence of these bioactive compounds may have contributed to the observed anti-diabetic activity of these extracts.

The *in-vitro* antioxidant study of the crude extracts of *M. azedarach* was carried out using DPPH free radical scavenging model which is a method based on electron transfer that produces a violet solution in ethanol. DPPH free radicals are reduced in the presence of an antioxidant molecules giving rise to light yellow color. The percentage scavenging is concentration dependent, with the highest concentration of antioxidant molecules mopping up most of the reactive oxygen species leading to decreased absorbance and high percentage scavenging. In this study, the scavenging activity of leaf, stem-bark, and root extracts of *M. azedarach* and standards on DPPH free radicals were revealed. The $IC_{50}$ values, for Garllic and Ascorbic acid were 14.71 and 4.184 µg/ml, respectively. For the leaf, stem-bark and root extracts, the $IC_{50}$ values were found to be 4.742, 4.390 and 4.105 µg/ml respectively. $IC_{50}$ represents the concentration of a substance that is required for 50% inhibition *in-vitro* and is a measure of the effectiveness of antioxidant property of a substance (Ahmed *et al.*, 2012, Munir 2012). All the extracts of *M. azedarach* exhibited antioxidant activity which was higher than that of Garllic acid but comparable to that of ascorbic acid. The ability of flavonoids to donate the hydrogen atoms in their hydroxyl groups Lugasi *et al.*, (2003) may have played a role in the observed DPPH free radical scavenging activities of the extracts in this study. Tannins also have the ability to scavenge free radicals by inhibiting lipid peroxidation (IIhami *et al.*, 2010). The antioxidant activity of these extracts could therefore be a combination of the roles played by the different phytochemicals present in the extracts.

Metformin tablet is a synthetic analog of the natural product guanidine which is known to enhance insulin sensitivity of muscles and fats (Wiernsperger *et al.*, 1999). Metformin decreases hepatic glucose production, and intestinal absorption of glucose (Wiernsperger *et al.*, 1999). Alpha-amylase is an enzyme in the body that is involved in the digestion of carbohydrates. The inhibitors of these enzymes have an important role in reducing postprandial hyperglycemia (Cheng and Josse 2004),

thereby preventing increase in blood glucose level. Using the alpha-amylase inhibitory assay, Metformin, (standard control) had an $IC_{50}$ of 3.236 µg/ml. For the leaf, stem-bark and root extracts, an $IC_{50}$ of 63.88, 35.37 and 12.39 µg/ml were observed respectively. Again, the root extract exhibited the lowest $IC_{50}$ value, suggesting that out of the three extracts, it has the highest ability to inhibit this enzyme and probably has the highest ability to cause reduction in blood glucose level. Flavonoids are a family of polyphenolic compounds which are wide spread in nature (González *et al.*, 2011). Polyphenols are capable of reducing oxidative stress as well as inhibit carbohydrate hydrolyzing enzymes (Keerthana *et al.*, 2013). Therefore, the ability of these extracts to have significantly ($P<0.05$) inhibited alpha-amylase enzymes could be attributed to their antioxidant properties. Furthermore, since the inhibition of these enzymes lead to reduction in hyperglycemia (Cheng & Josse, 2004), the extracts of M. *azedarach* could play important role in the treatment and management of diabetes. The fact that drugs from plant extracts are attributed with little or no side effect could be an added advantage if this plant extract is eventually developed into a novel anti-diabetic agent. Currently, some alpha-amylase inhibitors in use include Acarbose and Miglitol; they have the ability to reduce postprandial hyperglycemia.

### 4.2 CONCLUSION

The antioxidant-property of these extracts could be linked to their anti-diabetic effect since the root extract which showed the highest antioxidant effect also exhibited the highest alpha-amylase-inhibitory effect. Alpha-amylase inhibitory effect is a measure of the anti-diabetic property.

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