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ASSESSMENT OF PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL OF THE STEM BARK OF *ALBIZIA CHEVALIERI*

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ABSTRACT

Background: Plants produce chemical compounds known as phytochemicals. These phytochemicals have many pharmacological properties which can be harness for the treatment of several diseases. **Study aim:** This study was carried out to evaluate the phytochemicals present in the methanolic stem bark extract of *Albizia chevalieri* with specific emphasis on investigating its antioxidant properties. **Methods:** The phytochemical screening was carried out on the extract using the spectrophotometric and the Gas Chromatography Mass Spectroscopy (GC-MS) methods. Antioxidant evaluation of the extract was investigated using Diphenyl-1-picrylhydrazyl (DPPH) and H_2O_2 free radical scavenging activities of the extract. **Results:** Spectrophotometric analysis showed the presence of Flavonoids, Steroids, Terpenoids, Saponins, Glycosides, Tannins, Alkaloids, Reducing sugars and Hydrogen cyanides. Quantitative screening showed Terpenoids as the most concentrated in the extract with average concentration of 65.03 ± 0.12µg/100g and hydrogen cyanide as the least concentrated with average concentration of 0.01 ± 0.00 µg/100g. The order of quantitative phytochemical compositions of the extract is: Terpenoids> Steroids> Saponins> Tannins> Reducing sugars >Flavonoids> Glycosides> Alkaloids> Hydrogen cyanides. GC-MS chromatogram showed 18 peaks of volatile bioactive compounds detected in the extract. The analysis of the volatiles yielded n-Hexadecanoic acid as the most available in the extract with percentage area of 20.75. The antioxidant analysis indicated a concentration dependent antioxidant activity of the extract. **Conclusion:** This study indicates a potent antioxidant activity of stem bark extract of *Albizia chevalieri* and suggest that it could be used as a natural antioxidant in the treatment of various ailments.

Keywords: Phytochemicals, Antioxidant**,** *Albizia chevalieri,* Diphenyl-1-picrylhydrazyl (DPPH), Chromatography Mass Spectroscopy (GC-MS)

INTRODUCTION

Plants provide man with different kinds of resources, which among them include several types of plant-based chemicals known widely as phytochemicals (Haung *et al*., 2016). Some of these substances called primary constituents are necessary for the survival of the plant. They are used by the plant for metabolism, and they include chlorophyll, proteins, sugar, and amino acids. The secondary constituents include terpenoids, alkaloids, flavonoids, phenolic compounds, and other metabolites which exhibit various important pharmacological activities such as antioxidant, anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities (Michael, 1990).

Oxidative stress related to the enhanced generation of Reactive Oxygen Species (ROS) has been implicated as the cause of over a hundred human diseases including diabetes, cancer, inflammation, metabolic disorders, stroke, malaria, and HIV/AIDS (Aliyu *et al*., 2009; Nawinda, 2016). Antioxidants are chemicals that can neutralize the harmful effects of the ROS through the endogenous enzymatic defense system such as the Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and Catalase (CAT) in human system. However, with the increase in the environmental pollution by factors such as cigarette and exhaust smoke, UV-rays, different damaging radiation and toxic chemicals, the endogenous defense system is weakened and this results to a phenomenal disturbance in the activity and balance of antioxidants reactions in living systems (Aliyu *et al*., 2009). This situation brings about damage to cell structures, including lipids, membranes, proteins, and DNA (Valko*et al*., 2006).

Antioxidants from plants are regarded as effective in controlling the effects of oxidative damage and therefore, have had an influence in what people eat and drink.(Pinder and Sandler, 2004; Aliyu *et al*., 2009). Thus, this stimulated the need to produce more of these plant derived antioxidants to combat increasing oxidative damage caused by the incredibly polluted environment. Antioxidant properties of plants have mostly been credited to phytochemicals such as terpenoids, flavonoids, alkaloids, and tannins (Aliyu *et al*., 2009). Antioxidant properties of these chemicals are due to their oxidation or reduction properties in which they can absorb or neutralize free radicals or quench singlet and triplet oxygen (Tsao and Akhtar, 2005).

Albizia chevalieri is a member of the mimosaceae and the sub-family mimosoidae. This family consists of more than 3200 species in 82 genera (Mathias, 2016). Legumes of the mimosoideae are primarily woody plants of the tropics, and the few species native to temperate parts of the world are mostly herbaceous(Mathias, 2016).*A. chevalieri* is being used in Nigeria as tropical medicine. *A. chevalieri* leaf has been used in Borno-North Eastern Nigeria as

medicine for dysentery, diarrhea, taenicide and also remedy for coughs(Aliyu *et al*., 2009). The leaf extract of *A. chevalieri* is used either as cold water decoction or ground sieved extract mixed with pap, for the management of diabetes mellitus by traditional medical practitioners in some parts of Niger Republic and Nigeria (Saidu *et al*., 2007). The stem bark has been also used as vermifuge and purgative for cough (Alhassane, 2013). Phytochemical screening of the methanolic stem bark extract of *A. chevalieri* revealed the presence of secondary metabolites such as saponins (Frothing test), triterpenes (Salkowski test), flavonoids (Shinoda test) and alkaloids (Mayer's and Draggendorff's test) (Aliyu *et al*., 2009). Screening of the roots and stem bark extracts of *Albizia anthelminthica* has also shown the presence of alkaloids, saponins and diterpenes, flavonoids, tannins, and phenolic compounds (Nawinda, 2016). Also, the presence of alkaloids, flavonoids, coumarin, saponin, tannins, terpenoids, phenolic compounds and cardiac glycosides in both bark and leaves extract of *Albizia saman* has been reported (Prema and Venkatachary, 2018). *Albizia lebbeck* has been found to possess antioxidant properties and hence, a very important medicinal plant used in Ayruvedic medicine (Kumar and Sharma, 2016).Similarly, the toxicity and oxidative stress potentials of the stem bark extract of *A. chevalieri* has been investigated by Ibiam *et al*. (2015). However, this study aimed to evaluate the plant-based phytochemicals and antioxidant properties of the stem bark of *A. chevalieri* with a view of unleashing the potentials of the plant as a natural antioxidant.

MATERIALS AND METHODS

Collection of plant sample

The stem bark sample of *Albizia chevalieri* was collected from the botanical garden, University of Nigeria Nsukka, Enugu State, Nigeria. It was taxonomically identified by a Professional Taxonomist at the Herbarium unit of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Preparation of plant sample for analysis

The plant sample was air-dried under shade (at 35 – 40 ºC) and thereafter pulverized. A measured quantity (200 g) was cold-macerated with methanol (Sigma-Aldrich analytical grade) for 48 hours. The liquid extract was concentrated using rotary evaporator (Buchi Rotavapor® R-215) under a reduced pressure to obtain the extract

Phytochemical Analysis

The qualitative and quantitative analysis of the extract was carried out using the spectrophotometric method of analysis and the GC-MS methods. Spectrophotometric methods investigated the secondary metabolites composition of the sample whereas the GC-MS methods evaluated the volatile compounds that can be found in the extract.

Spectrophotometric analysis of phytochemicals

Spectrophotometric screening of the extract was carried out to identify the secondary metabolites such as Tannins, Flavonoids, Steroids, Saponins, Alkaloids, Hydrogen Cyanides, Glycosides, Soluble carbohydrates and Reducing sugars,

according to the official methods of analysis of AOAC (Association of Official Analytical Chemists) international (AOAC, 2005).

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The analysis for volatiles contained in the extract was performed by the GC-MS instrument (Thermo Electron Corporation, USA) equipped with a Finnigan Trace DSQ and an electron impact (EI) ion source. The analytes were separated on a DB-5MS capillary column (30m × 0.25mm × 0.25 m; Agilent, USA) coated with phenyl arylene polymer. The oven temperature program was as follows: 50°C initially for 1 minute, increased to 145°C at 5°C /min, increased to 175°C at 7°C /min, increased to 195°C at 5°C /min, and then ramped to 250°C at 3°C /min; 250°C was maintained for 10 min. High pure helium (99.999%) was the carrier gas set at a constant flow rate of 1mL·min−1. The injection port, transfer line, and ion source temperatures were all set at 250°C. 70eV of EI was adopted, and the mass scanning range was set from 50 to 650 (amu) in full scan. The injection was performed by split mode with a split ratio of 10: 1. Solvent delay time was set for 3min for all samples generated by different methods. Xcalibur 2.0 workstation was used to process data. Most volatile components extracted from the extract were identified by comparing the Retention Indices (RIs) and obtained mass spectra of the analytes with those from the (GC/MS) authentic standards from the National Institute of Standards and Technology (NIST) and by comparing the RIs with the mass spectra published previously[25]. RIs were determined by analyzing a solution containing the homologous series of normal alkanes (C7–C22) and then calculated as described by Van Den Dool and Kratz (1963). Peak areas of all components were calculated by Xcalibur 2.0, and relative amounts (RAs) of volatile compounds were calculated on the basis of peak area ratios.

***In vitro* Antioxidant Analysis**

The antioxidant evaluation of the sample was ascertained using the diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H2O2) free radical scavenging assays and comparing their activity to that of ascorbic acid used as standard.

Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH scavenging activity was carried out following Mensor *et al*. (2001). Varying concentrations (800 - 25µg/ml) of the extract/fraction each was mixed (2:1 v/v) with 0.5 mM DPPH (in methanol) in a cuvette. After an incubation period of 30 minutes, the absorbance was read at 517 nm using a UV-Visble spectrophotometer, and the percentage antioxidant activity was calculated. Ascorbic acid served as a positive control.

Hydrogen peroxide (H2O2) scavenging assay

The method of Bokhari *et al*. (2013) was followed to investigate hydrogen peroxide scavenging capacity of samples. Hydrogen peroxide (2 mM) solution was prepared in phosphate buffer (50mM, pH 7.4). Samples (100µg) were pipette into flasks and their volume made up to 400 µ1 with 50 mM

phosphate buffer (pH 7.4). H2O2 solution (600 µ1) was added and absorbance at 230 nm was taken 10 min after vortexing the flasks. Percent scavenging activity was determined by following formula; H_2O_2% scavenging activity =

$$\left(1 - \frac{\text{absorbance of sample}}{\text{Absorbance of control}}\right) X\ 100$$

Ascorbic acid served as standard.

RESULTS

Phytochemical Screening Using the Spectrophotometric Method

Phytochemical screening of the methanolic extract of *Albizia chevalieri* stem bark revealed the presence of all the secondary metabolites tested, except for soluble proteins. The phytochemicals present were saponins, terpenoids, flavonoids, tannins, steroids, glycosides, hydrogen cyanides, and reducing sugars (Table 1). Further quantitative analysis showed that *A. chevalieri* stem bark extract contained high levels of terpenoids and steroids in the sample. Terpenoids concentration observed was 65.03 ± 0.12 µg/100g while steroid was 26.23 ± 0.05 µg/100g, respectively. On the other hand, hydrogen cyanide was the least concentration; it was found in the sample with an average concentration of 0.01 ± 0.00 µg/100g (Table 1). This was followed by the concentration of alkaloids. Generally, the phytochemicals observed in the stem bark of *A. chevalieri* were in the order of Terpenoids> Steroids> Saponins> Tannins> Reducing sugars >Flavonoids> Glycosides> Alkaloids> Hydrogen cyanides.

Table 1: Qualitative and quantitative phytochemical compositions of *A. chevalieri* stem bark extract

Phytochemical	Qualitative result	Quantitative result (µg/100g)
Saponins	+	22.97 ± 0.03
Tannins	+	12.22 ± 0.01
Alkaloids	+	0.72 ± 0.01
Terpenoids	+	65.03 ± 0.12
Steroids	+	26.23 ± 0.05
Glycosides	+	4.48 ± 0.20
Flavonoids	+	6.90 ± 0.02
Hydrogen cyanides	+	1.01 ± 0.0
Reducing sugars	+	7.37 ± 0.03
Protein	-	-

Key: - absent + present. All the values were performed in duplicates and the results were expressed as Mean ± SEM.

Phytochemical Screening Using the GC-MS Method of Analysis

The Gas Chromatography Mass Spectroscopy of the methanolic extract of *Albizia chevalieri* stem bark revealed eighteen peaks which represent different volatile compounds as shown in the total ion chromatogram. The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the Gas Chromatography-Mass Spectrometry library of NIST. The GC-MS chromatogram of the peaks and retention times (Rt) of the compounds detected is shown in Figure 1 below.

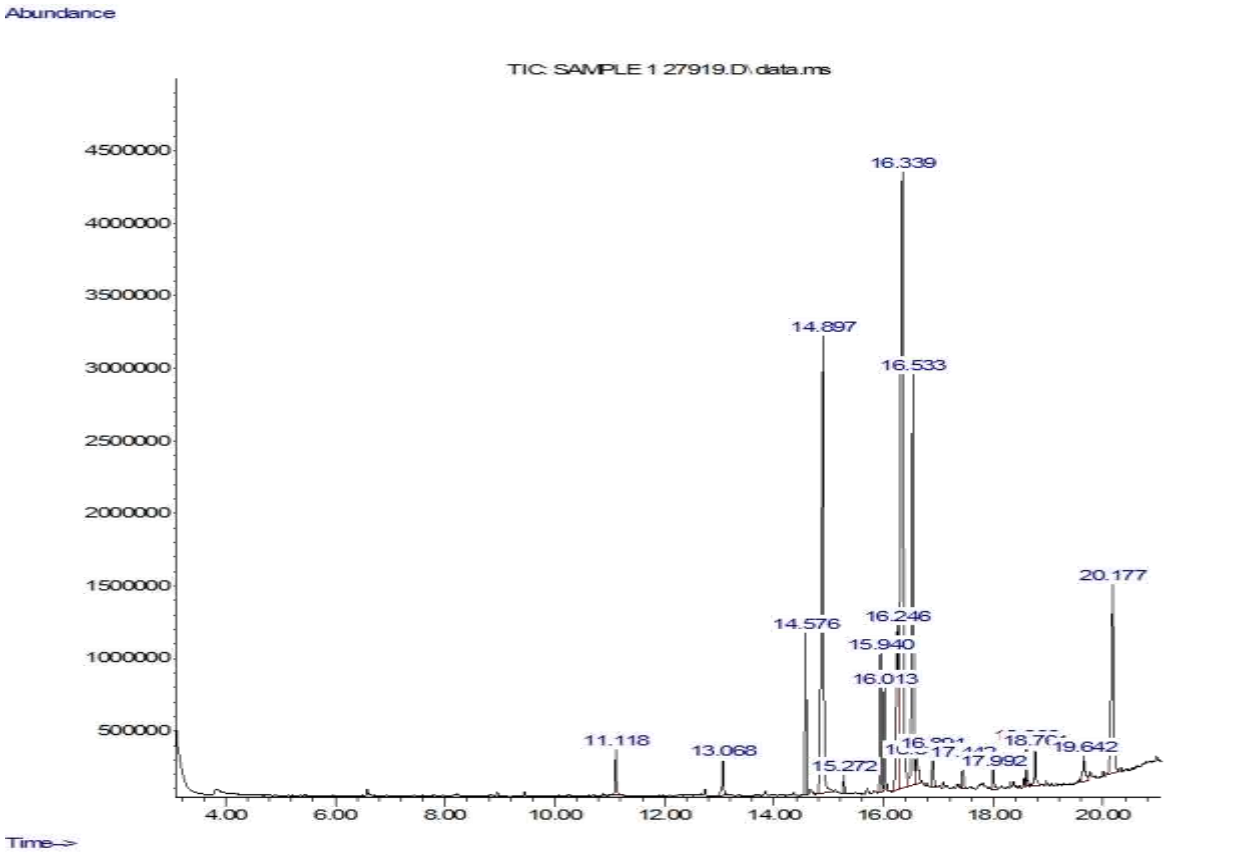
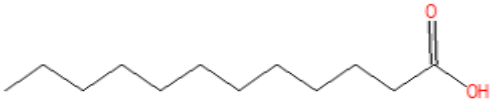
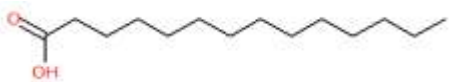
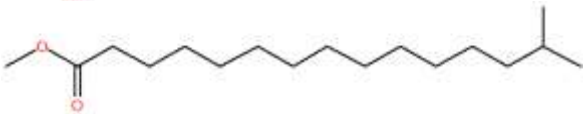
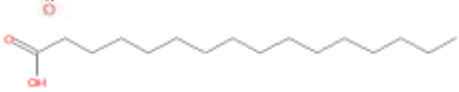
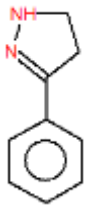
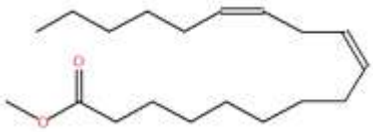
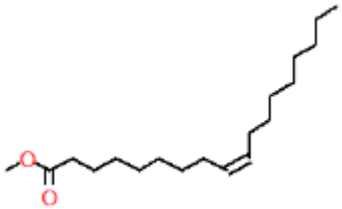
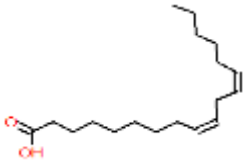
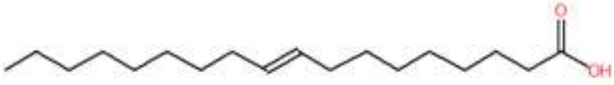


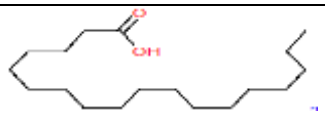
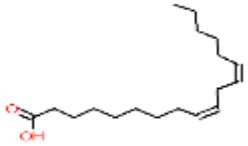
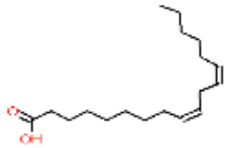
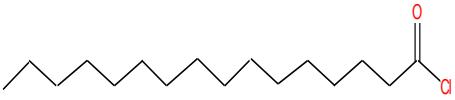
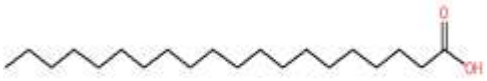
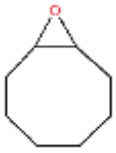
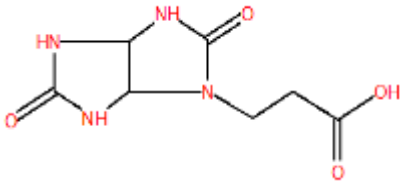
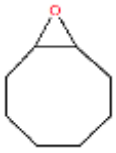
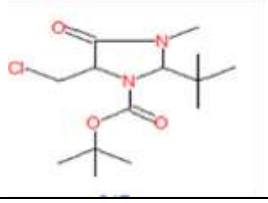
Figure 1: GC-MS chromatogram of methanolic extract of *A. chevalieri*

GC-MS analysis of the methanol extract of *A. chevalieri* showed the presence of eighteen major peaks and the compounds corresponding to the peaks according to the NIST library were determined as follows; Dodecanoic acid, Tetradecanoic acid, Pentadecanoic acid, 14-methyl-, methyl ester, n-Hexadecanoic acid, 1H-Pyrazole,4,5-dihydro-3- phenyl, 9,12-Octadecadienoic acid, 9-Octadecenoic acid (Z)-, methyl ester, 9,12- Octadecadienoic acid, 9-Octadecenoic acid, Octadecanoic acid, 9,12-Octadecadienoic acid, 9,12-Octadecadienoic acid, Palmitoyl chloride, Eicosanoic acid, 9-Oxabicyclononane, Propanoic acid, 3(perhydro-2,5-di-oxo-imidazo[4,5-d]imidazol-1yl, 9-Oxabicyclonane and 2-t-Butyl-5-

chloromethyl-3-methyl- 4-oxoimidazolidine-1-carboxylic acid, t-butyl ester, respectively. The detailed information on these compounds, their retention times and molecular structure of the major phytochemicals revealed by means of the GCMS methanolic extract analysis is shown in Table 2.

Table 2: Major phytochemical compounds identified in methanolic extract of *A. chevalieri* by GCMS analysis

Peaks	Retention time	Chemical Compound	Chemical Structure
1	11.118	Dodecanoic acid	
2	13.068	Tetradecanoic acid	
3	14.576	Pentadecanoic acid, 14-methyl-, methyl ester	
4	14.897	n-Hexadecanoic acid	
5	15.272	1H-Pyrazole,4,5-dihydro-3- phenyl-	
6	15.940	9,12-Octadecadienoic acid	
7	16.013	9-Octadecenoic acid (Z)-, methyl ester	
8	16.246	9,12-Octadecadienoic acid	
9	16.339	9-Octadecenoic acid	

1	**0**	16.533	Octadecanoic acid	
1	**1**	16.601	9,12-Octadecadienoic acid	
1	**2**	16.891	9,12-Octadecadienoic acid	
1	**3**	17.442	Palmitoyl chloride	
1	**4**	17.992	Eicosanoic acid	
1	**5**	18.603	9-Oxabicyclononane	
1	**6**	18.761	Propanoic acid, 3 (perhydro-2,5-di-oxo-imidazo[4,5-d]imidazol-1yl	
1	**7**	19.642	9-Oxabicyclononane	
1	**8**	20.177	2-t-Butyl-5-chloromethyl-3-methyl-4-oxoimidazolidine-1-carboxylic acid,t-butylester	

In vitro Antioxidant Activity of the Methanolic Extract of _A. chevalieri_ Stem Bark Using DPPH

The free radical scavenging activity of the 1, 1-diphenyl-2- picryl-hydrazyl (DPPH) assay showed percentage antioxidant activity (% AA) of _A. chevalieri_ stem bark extract, 67.11, 71.22 of various concentrations of 62.5, 125, 250, 500, and 1000 µg/ml of the methanolic extract of _A. chevalieri_ respectively (Figure

17). The greatest leap in antioxidant activity appeared when the concentration was raised from 62.5 to 125 µg/ml and the leap was from 48.13 to 61.41 i.e., 13.28 a significant increase in percentage antioxidant activity as compared to only 9.81 increases in percentage antioxidant activity observed when the concentrations were raised gradually to 1000 µg/ml, even though this had much higher increase in methanolic stem bark concentration (875 µg/ml).

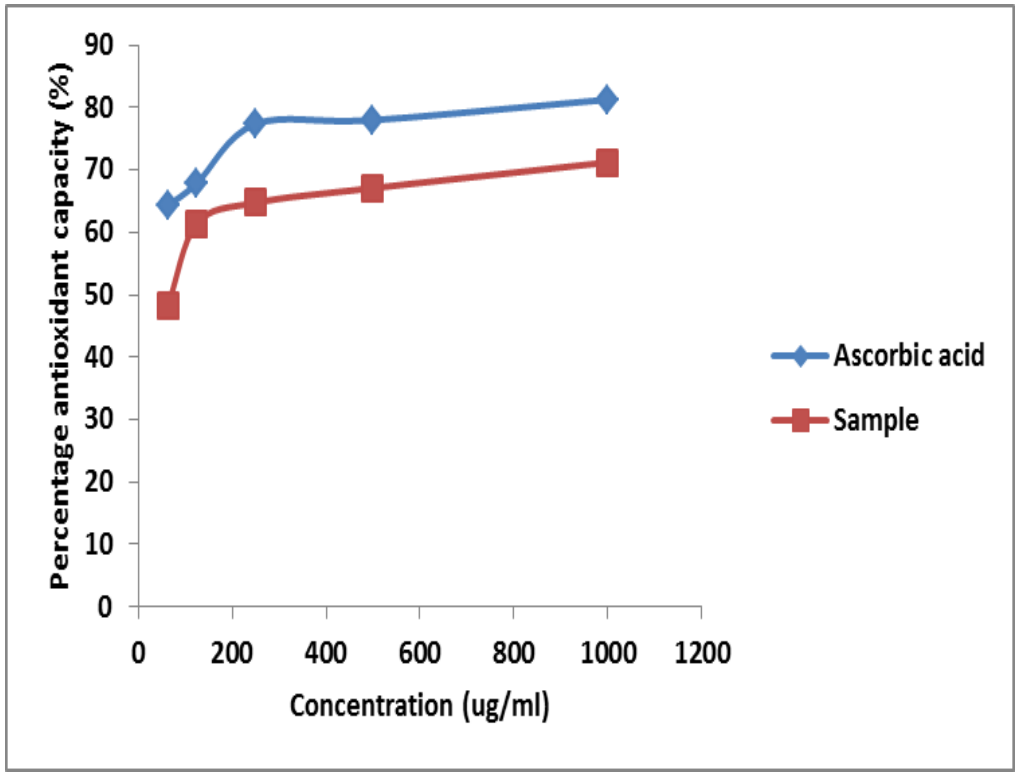


Figure 2: Percentage DPPH free radical scavenging activity of *A. chevalieri* bark extract

***In vitro* Antioxidant Activity of Methanolic Stem Bark Extract of *A. chevalieri* Using the H_2O_2 Free Radical Scavenging Activity**

Hydrogen peroxide free radical scavenging activity of the sample proved more insightful, especially at lower concentrations of the sample where the hydrogen peroxide free scavenging activity was higher than the ascorbic acid standard (Figure 18). The percentage antioxidant capacity of the sample was found to be 54.73 which is higher than the ascorbic acid standard of 52.45 percentage antioxidant activity at 62.5µg/ml concentration of the sample. At higher concentrations, the ascorbic acid standard exhibited marginally superior antioxidant capability, which can be seen when comparing the percentage antioxidant capacity of the sample (84.64) to that of the standard ascorbic acid (86.44) as shown in figure 18.

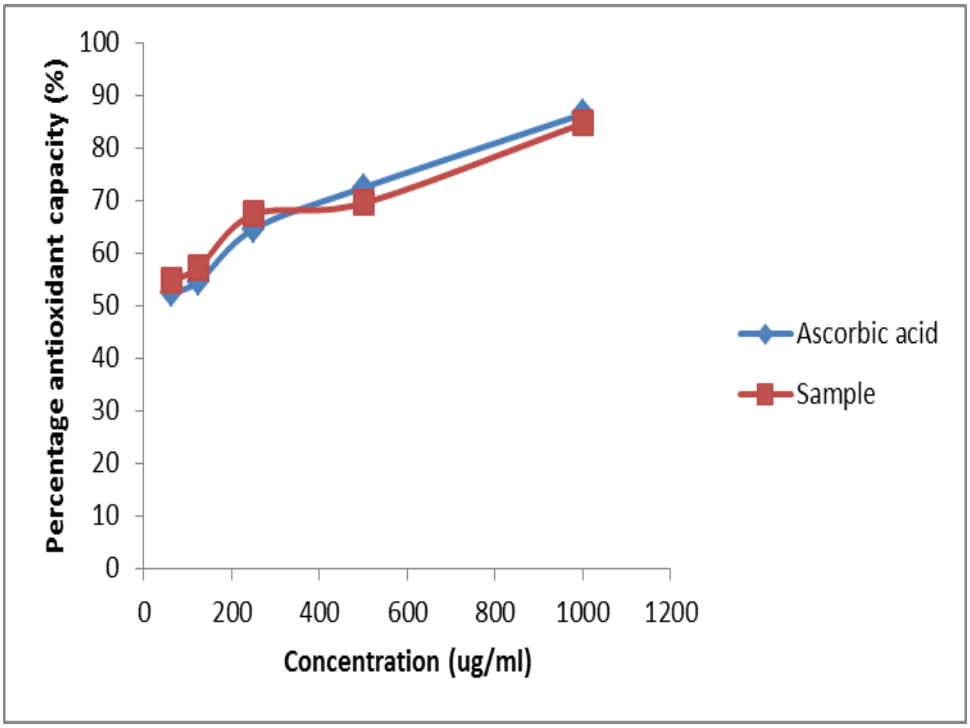


Figure 3: Percentage H_2O_2 free radical scavenging activity of *A. chevalieri* bark extract

DISCUSSION AND CONCLUSION

This phytochemical study of the methanolic extract of *A. chevalieri* stem bark reveals the presence of secondary metabolites and volatiles on evaluation with the spectrophotometric and GC-MS methods of analysis, respectively. Spectrophotometric analysis of the extract revealed the presence of Saponins, Tannins, Alkaloids, Terpenoids and Flavonoids in the methanolic stem bark extract of *A. chevalieri* which is in correlation with the results obtained by Bello *et al*. (2016) on aqueous stem bark extract of the plant. However, this present study reveals additional presence of steroids, glycosides, and hydrogen cyanides in the methanolic stem bark extract of *A. chevalieri*. This indicates that the stem bark extract of the *A. chevalieri* is a rich source of several phytochemicals. Meanwhile, qualitative phytochemical screening of the ethanoic stem bark extract of *A. lebbeck and A. saman* has shown the presence of hydrogen cyanide (Prema and Venkatachary, 2018). Quantitatively, our results had terpenoids as the most abundant with a concentration of 65.03 ± 0.12 µg/100g followed by saponins (22.97 ± 0.03 µg/100g) whereas hydrogen cyanide was the least with a concentration of 0.01 ± 0.00µg/100g. Nevertheless, results from Bello *et al*. (2016) on the aqueous stem bark extract of the plant showed flavonoids as the most abundant phytochemicals. The variations on the abundance of phytochemicals could be as a result of different in the solvent of extraction, time of collection, place of collection and the part of the plant being extracted.

GC-MS chromatogram of the methanolic extract of the *A. chevalieri* stem bark shows

18 peaks, representing 18 phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST 11 library, the eighteen phytocompounds were characterized and identified. Of the eighteen phytocompounds, the most prevailing compounds were n-Hexadecanoic acid with 20.750%, 2-t-Butyl-5-chloromethyl-3-methyl-4-oxoimidazolidine-1-carboxylicaciid,t-butyl ester with 20.177% and 9-Oxabicyclononane with19.644%. n-Hexadecanoic acid has been shown to possess antioxidant and anti-inflammatory properties, Tetradecanoic acid serves as lipid anchor in biomembranes, Octadecanoic acid as an antiseptic, Dodecanoic acid used as emulsifiers for cream, milky lotion, and hair conditioner and palmitoyl can be used for the preparation of lysophosphatidic acids which inhibit apoptosis (Yakubu and Onwuka, 2017). From our results, it could be inferred that *A. chevalieri* contains various phytocompounds that could be used for the treatments of quite numbers of health problems like cancer, inflammatory, diabetic, antioxidant, antibacterial, among others.

The antioxidant capacity of the plant was evaluated by the 1, 1-diphenyl-2- picryl-hydrazyl radical (DPPH) and Hydrogen peroxide (H_2O_2)Free radical scavenging activity using ascorbic acid as the standard. The DPPH assay has been largely used as a quick, reliable, and reproducible parameter to search for the *in vitro* general antioxidant activity of pure compounds as well as plant extracts (Koleva *et al*., 2002; Goncaves *et al*., 2005). Figure (2) compares the DPPH free radical scavenging activity of the methanolic stem bark extract of *A. chevalieri* to that of the standard, ascorbic acid. The result shows that their activities are concentration dependent as higher concentrations of the sample correlates with increased activity. It is interesting to know that there was rapid leap in antioxidant activity when the concentration was increased from 62.5 to 125 µg/ml and the leap was from 48.13 to 61.41 i.e. 13.28 increases in percentage antioxidant activity as compared to only 9.81 increases in percentage antioxidant activity observed when the concentrations were raised gradually to 1000µg/ml indicating that low concentrations like125 µg/ml could equally be effective for the use of the extract as antioxidants. The hydrogen peroxide (H_2O_2) free radical scavenging activity of the sample was also carried out using ascorbic acid as the standard (Figure 3). The result indicated higher reducing power of the sample at lower concentrations of the sample (i.e., below 70 µg/ml), but at increased concentrations, the reducing capacity of the sample was lower than the ascorbic acid standard. This indicates that lower concentrations of the sample could be very effective antioxidants. Although no correlation study was carried out, literature reports have shown that the reduction mechanism of DPPH is correlated with the presence of hydroxyl groups on the antioxidant molecules, which can be inferred that a good antioxidant activity of the polar extract of *A. chevalieri* is probably due to the presence of substances with an available hydroxyl group (Basnet *et al*., 1997; Cotelle *et al*., 1996). This structural requirement could be linked to the presence of flavonoids

or condensed tannins, which are known to occur in plant species belonging to the Leguminosae-Mimiosoideae family (Mensor *et al*., 2013). Our results show that *A. chevalieri* could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions such as cancer, hepatotoxicity, and cardiac arrest. The presence of high levels of antioxidant phytochemicals such as Flavonoids, Tannins, and Saponins in the methanolic stem bark extract of *A. chevalieri* could be attributed to the appreciable antioxidant activity of the extract. Thus, it could be inferred from this study that the stem bark extract of *A. chevalieri* could serve as a potential source for natural antioxidants.

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