

IN VITRO ANTIBACTERIAL, ANTIOXIDANT, CYTOGENOTOXIC AND NUTRITIONAL VALUE OF *ALOE BARBADENSIS* MILL. (ASPHODELACEAE)

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ABSTRACT

This study evaluated the *in vitro* antibacterial, antioxidant, nutritional and cytogenotoxic potentials of *Aloe barbadensis* (AB) root extract. The antimicrobial activity of AB was determined using agar well diffusion and microdilution techniques while the nutritional analyses were done using standard procedures. The antioxidant activities were evaluated via the lipid peroxidation, 1, 1, diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing power (FRAP) assays. The cytogenotoxic effects were assessed using the *Allium cepa* and *Sorghum bicolor* assays. Five test bacterial isolates: *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* were found susceptible to the ethanolic extract of AB root. The extract at 50 mg/ml recorded the highest zone of inhibition (25.00±1.73 mm) against *S. saprophyticus*. The nutritional analysis gave a nitrogen free extract value of 24.66%, 30.05% crude fibre, 15.14 mg/kg of Na, 2.01 mg/kg of Mg, 37.42 mg/kg of Fe and 0.02 mg/kg of Zn. The fractions had antiradical activity in the lipid peroxidation, DPPH and FRAP assays. The ethanolic extract induced some chromosomal aberrations in the root tip cells of *Allium cepa*. The ethanolic extract had the highest (0.13±0.09 mm) antiproliferative activity at 20 mg/kg in the *Sorghum bicolor* assay. The relatively high nutrient content may be a possible mechanism for the aphrodisiac property of the plant. The results of the biological activities of AB root is an indication of its potential use in drug development for the management of infectious diseases and other related ailments.

Keywords: *Aloe barbadensis*, antibacterial, antioxidant, cytotoxicity, genotoxicity, microbial infections, phytochemicals

INTRODUCTION

Today medicinal plants are fast becoming an important key to unlock the ravaging effects of some diseases in the world. The World Health Organization (WHO) has supported traditional medicines, particularly in developing countries by promoting the incorporation of its useful elements into the national health care system (Akerle, 1987). This fortunately has led to the rising popularity of herbal medicine in developing countries (Obici *et al.*, 2008). This affirms the belief that medicinal plants are nature's gift to mankind.

Aloe barbadensis (AB) of the family- Asphodelaceae has been reported ethnomedicinally to have several folk uses with some validated scientific information. Its ethnomedical use as an aphrodisiac was earlier reported by Erhabor *et al.* (2013) and this was validated *in vivo* using an animal model (Erhabor and Idu, 2017). Other previously reported biological activities of this plant include the use of its gel in wound healing (Davis *et al.*, 1994; Maenthaisong *et al.*, 2007), inflammatory problems (Che *et al.*, 1991; Robbers and Tylers, 1999) as well as in moisturizing and anti-aging activities (West and Zhu, 2003; Sahu *et al.*, 2013). The leaves have been used for treating fungal infections associated with superficial mycoses (Shamim *et al.*, 2004). The antidiabetic property (Tanaka *et al.*, 2006; Yagi *et al.*, 2006) and its perceived reproductive functions in enhancing fertility (Oyewopo *et al.*, 2011; Iwu, 2014) have also been documented.

Some of the ethnomedical uses of *A. barbadensis* include its use in the treatment/management of intestinal ulcers (Ross, 1999), constipation, impotency, ringworm and eczema (Adodo, 2012). It is used as a purgative, appetite stimulant and emmenagogue, as well as in the treatment of colds, piles, asthma, cough and jaundice (Joseph and Raj, 2010). Interestingly, chemicals found in the plant have become known remedies for treating sexual disorders/reproductive diseases (Yakubu *et al.*, 2007; Joseph and Raj, 2010), microbial infections (Usman *et al.*, 2005; Idu *et al.*, 2011; Erhabor *et al.*, 2013; Mongalo *et al.*, 2017; Motihatlego *et al.*, 2018;), cancers (Venables *et al.*, 2016; Eboji *et al.*, 2017) and free radicals implicated diseases (Otang *et al.*, 2012; Sharma and Lall,

2014; Elisha *et al.*, 2015; Motihatlego *et al.*, 2018) as well as other relevant ailments/diseases. The medicinal value of plants lies in these chemical constituents or active ingredients and other nutrients for their actions (Edeoga, 2000; Okigbo *et al.*, 2008). These plant chemicals have been reported to offer a boost of nutritional value thereby improving sexual performance and libido (Yakubu *et al.*, 2007; Sumalatha *et al.*, 2010). This enhancement in the general health can thus lead to a burst of energy which translates into increased sexual appetite, besides increasing blood flow and intensity of ejaculation accompanied by anabolic and growth hormone stimulating properties (Smith *et al.*, 2000; Ahmad *et al.*, 2003; Yakubu *et al.*, 2007). These phytochemicals have also been reported to enhance erection and prolong ejaculatory latency in male albino rats (Yakubu *et al.*, 2005; Muanya and Odukoya, 2008; Sumalatha *et al.*, 2010). These bioactive substances from medicinal plants include saponins, alkaloids, flavonoids, tannins, sterols, protein, carbohydrates and several minerals (zinc, calcium, magnesium, potassium) (Olowokudejo *et al.*, 2008). *A. barbadensis* following previous studies undertaken mainly on the leaves and gel has many of these bioactive phytochemical substances (Ross, 1999; Joseph and Raj, 2010; Adodo, 2012; Sahu *et al.*, 2013; Idu *et al.*, 2014). Among, these phytochemicals are those which may scavenge reactive oxygen species that have been implicated in damaging biological molecules (DNA, protein, carbohydrate and lipids), leading to cancer, infertility and other ailments (Ashafa *et al.*, 2010; Raghuvver and Vyas, 2010). Despite the many merits of these phytochemicals, they are said to have a deleterious effect when not properly utilized or consumed. There is therefore a need to assess their safety levels either at the cell, tissue or organ levels.

In this study, our objective was to determine the antimicrobial, antioxidant, nutritional and phytochemical constituents of the root of *Aloe barbadensis* as a potential aphrodisiac. The cytotoxic and genotoxic effects of the plant were also investigated.

METHODS

Collection of plant sample

The roots of *A. barbadensis* were collected from Okene, Nigeria. It was authenticated by Mr. G. Ibhanebor of the Herbarium Unit of the Obafemi Awolowo University, Ile-Ife, Nigeria, with voucher number-IFE17004 where it was deposited.

Preparation of plant sample and extraction

AB roots were detached from the whole plant, rinsed in water, spread on laboratory tables and dried at room temperature. The roots were dried in an oven set at 40°C for 10 minutes before being reduced to a fine powder using a grinder. Two kilograms (2kg) of the powdered plant material was extracted with ethanol (5 L) using a Soxhlet extractor. The extract was concentrated to dryness using a water bath (HH-S Water Bath; Searchtech Instruments) set at an average temperature of 50 °C. The percentage yield of the ethanolic extract was determined using the formula (% yield= weight of extract/weight of powder material x 100/1).

Preparation of solvent fractions

Forty grams of the ethanolic extract was re-dissolved in 100 ml of 60 % methanol and partitioned exhaustively with chloroform (150 ml) in a separating funnel by decanting until a transparent layer was obtained. The lower layer-chloroform fraction (organic) was collected first and the upper layer- aqueous fraction (inorganic) was also collected. Water (70 ml) was added to the chloroform to remove compounds that entered the bottom layer due to agitation. The chloroform fraction was further concentrated to dryness using a water bath at a relatively low temperature. Ethyl acetate (300 ml) was added to the aqueous fraction and decanted appropriately. The ethyl acetate fraction, being less dense, was collected after decanting the aqueous fraction. Both fractions were then further concentrated using a water bath. The respective percentage yields of the fractions (chloroform, ethyl acetate and aqueous) were determined using the formula (% yield= weight of extracted fraction/weight of crude extract X 100/1).

Antimicrobial evaluation

Collection of semen samples

The semen samples were collected according to the method previously described by **Ekhaise and Richard (2008)**. The seminal fluid specimen was collected from five male patients attending a fertility clinic at two private hospitals in Benin City, Nigeria. The samples were collected from patients with secondary infertility using the masturbation method. The collected specimens were immediately transferred to the laboratory where they were further subjected to microbiological isolation and identification.

Isolation, Identification and Standardization of test organisms

The sperm specimens were streaked on both blood agar and nutrient agar. The plates were incubated at 37 °C for 24 hrs. After incubation, the bacterial species were identified via Gram staining and appropriate biochemical tests following standard procedures outlined by **Cheesbrough (2006)**. A loop full of stock culture of the organism was inoculated onto 5 ml of sterile Müller-Hinton agar and incubated for 24 hrs. Overnight culture of the organisms (0.2ml) was inoculated into 20 ml of sterile nutrient broth and incubated for 3-5 hrs. The turbidity of the culture was compared to that of a 0.5 Mac-Farland standard to standardize the culture to 10⁶ cfu/ml.

Susceptibility Testing

According to the method described by **Emeruwa (1982)**, 0.5ml of the standardized culture was spread onto a sterile plate to achieve confluent growth. Fifteen milliliters of Müller-Hinton agar at 45 °C was added to each plate and the plates rocked for even spread and proper mixing of bacteria and agar. The content of the plates was allowed to solidify and wells approximately 6 mm in diameter were bored on the surfaces of the agar medium using a sterile cork borer and the bottom of the holes seeded with molten agar. The reconstituted extract (0.2 ml) at the test concentrations was dropped into the holes while an aqueous solution of the standard antibiotic (chloramphenicol) at the same concentrations as the extract was used as positive control. Sterile distilled water (0.2 ml) was used as negative control. The plates were allowed to stand for 30 mins for pre-diffusion of the extract to occur and then incubated at 37 °C for 24hrs and zones of inhibition were measured to the nearest cm using a meter rule. The final measurements were converted to mm. The mean of duplicate results was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

Bacterial strains were cultured overnight at 37 °C on nutrient broth and were adjusted to a final density of 10⁶ cfu/ml. The standardized bacterial inocula were used to inoculate the 96-well microtitre plates containing appropriate dilutions of the extract (12.5 - 0.10 mg/ml) under sterile conditions. The plates were incubated under aerobic condition at 37 °C and examined after 24 hrs. As an indicator of bacterial growth, 40 µl of 0.2 mg/ml p-iodonitrotetrazolium violet was added to each well and incubated for 30 mins at 37 °C. The colourless tetrazolium salt was reduced to a red coloured product by the biological activity of the organisms. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of extract as indicated by a clear solution was required for it to be recorded as active (**Eloff, 1998**). Chloramphenicol was used as positive control in the experiment with sample free solutions of 10% DMSO as the negative control.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by removing a loopful of bacterial suspension from the MIC micro-titre plate that did not show any growth and sub-cultured into nutrient agar plates. The plates were incubated and the concentrations at which no visible growth was observed were recorded as MBC.

Antioxidant activity

Lipid peroxidation assay

This assay was carried out using a slightly modified method described by **Ohkawa et al. (1979)**. Liver homogenate was prepared from commercially available goat liver sourced from a local abattoir in Benin City, Nigeria. The liver was washed several times with ice-cold saline solution. Ten percent of the liver homogenate was prepared using ice-cold potassium chloride (KCl) (0.15M) in a blender. Lipid peroxidation was initiated in 1ml of tissue homogenate mixed with various concentrations of the ethanol extract (20, 40, 60, 80 and 100 µg/ml). This was followed by adding 0.1ml of ferric sulphate (2.5 mM), 0.1ml of ascorbate (100 mM) and 0.1ml of KH₃PO₄ (10 mM). The volume was made up to 3 ml with distilled water and incubated at 37 °C for one hour. One ml, each of 5 % trichloroacetic acid (TCA) and 0.6% thiobarbituric acid (TBA) was then added to the reaction mixture and the tubes boiled for 30 minutes in a water bath. The tubes were centrifuged at 3500 rpm for 10 minutes. The extent of inhibition of lipid peroxidation was evaluated by the estimation of thiobarbituric acid reactive substances (TBARS) level by measuring the absorbance at 532 nm. The lipid peroxidation inhibition percentage was calculated by using the formula below;

$$\text{Inhibition \%} = \frac{[A_{\text{control}} - A_{\text{test}}]}{A_{\text{control}}} \times 100$$

Where,

A_{control} = Absorbance of control

A_{test} = Absorbance in the presence of the extracts

The experiments were repeated in triplicates. α-Tocopherol was used as standard antioxidant.

DPPH (2, 2-diphenyl-1-picryl hydroxyl) radical scavenging assay

The radical scavenging activity of the crude ethanol extract and fractions of *A. barbadensis* root against the DPPH radical (Sigma-Aldrich) was determined by a slightly modified method using UV (**Chidambara Murthy et al., 2002; Leong and Shui, 2002**). Concentrations of 20, 40, 60, 80 and 100 µg/ml of the crude extract and Vitamin C were prepared in methanol (Analytical grade). One ml of the extract was placed in a test tube, followed by 2 ml of 0.1mM DPPH in methanol. A control solution was prepared containing the same amount of DPPH and methanol. This procedure was similarly conducted to determine the DPPH scavenging activity of the various fractions (chloroform, ethyl acetate and aqueous). The radical scavenging activity was calculated using the following formula;

$$\% \text{ inhibition} = [A_b - A_a] / A_b \times 100$$

Where,

A_b = the absorbance of the blank sample

A_a = the absorbance of the extract and fractions

Ferric reducing power assay

The previously outlined method by **Kumar et al. (2005)** was used in assessing the ferric reducing power of the samples. This was determined by mixing various concentrations of the crude extract, fractions and standard ascorbic acid solutions (20, 40, 60, 80 and 100 µg/ml) in 1ml of methanol with phosphate buffer (2.5 ml, 0.2 M at pH 6.6) and potassium ferricyanide K₃[Fe(CN)₆] (2.5 ml, 1%). The

mixture was incubated at 50 °C for 20 minutes. Then 2.5ml of 10 % trichloroacetic acid (TCA) was added to the mixture which was centrifuged at 3000 rpm for 10 minutes at room temperature. An aliquot of 2.5ml of the supernatant was mixed with 2.5 ml distilled water and ferric chloride (FeCl₃) (0.5ml, 0.1%) and the absorbance of the reaction mixture was measured at 700 nm to detect increased reducing power. All the tests were performed in triplicate.

Phytochemical and nutritional analysis

The qualitative phytochemical screening of the crude ethanol extract and fractions was carried out to determine the different metabolites (glycosides, cardiac glycosides, saponins, flavonoids, phenolic, tannins, phlobatannins, terpenoids, alkaloids, steroids, polysaccharides and reducing sugars) using the methods described by Sofowora (1993), Trease and Evans (1996). The total phenolic and flavonoid content was determined using the method of Lin (2007) while the saponin content was done using the method of Obadoni and Ochuko (2002); Ejikeme et al. (2014). The alkaloidal content was determined using the method of Harborne et al. (1973). The proximate analysis (gross chemical composition) was determined by the recommended method of Horwitz and Latimer (2000). The determined six food categories were moisture content, ash content, crude oil, crude fibre, crude protein and nitrogen free extract. For the mineral element's composition, a weighed aliquot of 5 g of the powdered root was ashed at 550°C in the Muffle furnace for 5 hours and the residue dissolved in 100 ml deionized water. Standard solutions of the minerals were prepared and used to calibrate the atomic absorption spectrophotometer (AAS) (model 969AA, Unicam Series) using acetylene-air flame at specific wavelengths. Aliquots of the ash solutions were injected into the AAS and from the standard curve the various concentrations were obtained.

Cytotoxic and genotoxic Study

Allium cepa assay

The relatively same sized purple variety of onion bulbs were obtained commercially from a local market in Benin City, Edo State, Nigeria. The bulbs were stored under dry and well aerated condition for three weeks before use to prevent rot and enhance viability. The outer scaly leaves of the onion bulbs were carefully removed with hand while the dried roots were shaved off with a sharp razor blade to expose the fresh meristematic tissues. The bulbs were placed into freshly obtained distilled water to protect the primordial cell from drying off. Mouldy bulbs and those with shooting green leaves were discarded. The macroscopic evaluation (root growth inhibition) and microscopic analysis (induction of chromosomal aberrations) were subsequently done. Photomicrographs of the root tip cells were taken using an Olympus model microscope with a 5 mega pixel digital microscopic eyepiece camera. The standard procedures earlier described by Rank and Nielsen (1994); Olorunfemi et al. (2011) was adopted for this bioassay with the under listed parameters determined as displayed in the formulas below.

$$\text{Percentage(\%)} \text{ root growth of control} = \frac{\text{overall mean root length of test solution}}{\text{overall mean root length of control}} \times 100$$

Mitotic index was computed by determining the mitotic cell frequency at the tip as

$$\frac{\text{Number of dividing cells}}{\text{Total number of cells counted}} \times 100$$

The mitotic inhibition was determined using the following formula:

$$\frac{\text{Mitotic index in control} - \text{Mitotic index in treatment}}{\text{Mitotic index in control}} \times 100$$

Frequency of Chromosomal aberration:

$$\frac{\text{Number of Aberrant cells}}{\text{Total number of cells counted}} \times 100$$

Sorghum bicolor assay

The growth inhibitory effects of the ethanol extract of *A. barbadensis* was carried out following the method described by Fajana (2013). Seeds of *Sorghum bicolor* (Guinea corn) were purchased locally in Edo State, Nigeria. A simple viability test was carried out by placing a handful of the seeds in distilled water. The viability of the seeds was determined by their ability to sink in water. Those that remained submerged in water were removed and air dried for use while those

which floated were discarded. The viable seeds were sterilized with 96 % ethanol for 1 minute and then rinsed with distilled water. The, 10 ml different concentrations (5, 10, 15, 20, 25 and 30 mg/ml) of the ethanol extract containing 3.3% tween 80 in water were poured into 9 cm full Petri dishes lined with cotton wool and filter paper (Whatman No 1). Twenty (20) viable seeds were spread on each and incubated in the dark. The lengths (mm) of the radicles emerging from the seeds were taken at 24, 48, 72, and 96 hours. The control seeds were only treated with 3.3% Tween 80 in distilled water containing no extracts. The experiments were carried out in triplicates.

Statistical analysis

Data are shown as mean ± SEM and mean ± SD of the corresponding replicates. One Way ANOVA was done where applicable as well as a Duncan's multiple range test to analyse differences among different means and the interaction between the variables using SPSS 15.0 computer software package. Differences at *P*<0.05 or *P*<0.01 were considered statistically significant.

RESULTS

Percentage yield of extract and fractions

Two kilograms of the powdered root plant material yielded 124.65g (6.23%) of the ethanol extract. The fractionation of 40 g of the ethanol extract gave various yields of 10.96g (27.4%), 2.69g (6.73%) and 22.89g (57.23%) corresponding to the chloroform, ethyl acetate and aqueous fractions respectively (Tab1).

Table 1 Percentage extract/fractions yield of *A. barbadensis* root

Extract/ Fractions	Yield in (%) and (g)
Ethanolic extract	6.23% (124.65g)
Aqueous fraction	57.23% (22.89g)
Chloroform fraction	27.4% (10.96g)
Ethyl acetate fraction	6.73% (2.69g)

Antimicrobial effect of *A. barbadensis* root extract on test organisms

The susceptibility of the test organisms to the ethanolic extract of *A. barbadensis* root is shown in Tables 2 – 3. The tested concentrations of the extract of AB had a certain degree of inhibitory activity against the tested organisms with the highest zone of inhibition of 25.00±1.73 mm recorded against *Staphylococcus saprophyticus* at a concentration of 50 mg/ml (Tab 2). It was observed that the extract had a MIC of 6.25 mg/ml against *E. coli* and *S. saprophyticus* while the MIC was 12.5 mg/ml against *P. mirabilis*, *P. aeruginosa* and *K. pneumoniae*. The extract had the same MBC value of 12.5 mg/ml against the tested organisms (Tab 3). The positive control, chloramphenicol was active against all test organisms but significantly at the highest concentration of 50 mg/ml. The commercial antibiotic (chloramphenicol) was most active against *Staphylococcus saprophyticus* with an inhibitory diameter of 24 mm and least active against *E. coli* and *P. aeruginosa* with no measurable zone of inhibition (Tab 4).

Table 2 Effect of various concentrations of the ethanolic root extract of *Aloe barbadensis* on test organisms

Test organisms	Concentrations of Extract (mg/ml)		
	12.5	25	50
<i>Escherichia coli</i>	8.00±0.58	12.67±0.88	16.00±1.00
<i>Klebsiella pneumoniae</i>	4.67±0.88	6.67±0.88	15.00±2.00
<i>Proteus mirabilis</i>	3.00±0.58	4.33±0.88	12.00±1.00
<i>Pseudomonas aeruginosa</i>	4.33±0.67	8.00±0.58	14.67±1.45
<i>Staphylococcus saprophyticus</i>	9.70±0.88	13.33±1.20	25.00±1.73

Values are mean±SEM (zone of inhibition in mm); n = 3

Table 3 Antibacterial activity (MIC and MBC) of the ethanol extract of *A. barbadensis* root

Isolates	MIC (mg/ml)	MBC (mg/ml)
<i>Escherichia coli</i>	6.25	12.5
<i>Klebsiella pneumoniae</i>	12.5	12.5
<i>Pseudomonas aeruginosa</i>	12.5	12.5
<i>Proteus mirabilis</i>	12.5	12.5
<i>Staphylococcus saprophyticus</i>	6.25	12.5

Table 4 Antibiotic sensitivity of the test organisms with growth inhibitory zones

Organisms	Chloramphenicol		
	12.5 mg/ml	25 mg/ml	50 mg/ml
<i>Escherichia coli</i>	NMZI	NMZI	10.06±0.05
<i>Klebsiella pneumoniae</i>	4.00±0.04	10.02±0.07	18.00±0.03
<i>Proteus mirabilis</i>	NMZI	10.09±0.03	16.00±0.09
<i>Pseudomonas aeruginosa</i>	NMZI	NMZI	12.00±0.02
<i>Staphylococcus saprophyticus</i>	10.05±0.06	15.00±0.03	24.00±0.02

Legend: NMZI= No measurable zone of inhibition; n = 2; Values are mean±SEM (zone of inhibition in mm)

Antioxidant activities of *A. barbadensis* root extract

Lipid peroxide scavenging activity of *A. barbadensis* root extracts

The result of the lipid peroxide scavenging activity of the extract and fractions are presented in Figure 1. It was noticed that the ethanolic extract had a concentration-dependent activity. The chloroform fraction had a maximum inhibition of 48.28 ± 0.34%, followed by the crude ethanolic extract with 46.57 ± 0.69%, ethyl acetate had an inhibition zone of 30.58 ± 1.04%. The aqueous fraction had the least inhibition of 30.07 ± 1.89%. This scavenging activity happened at the same concentration of 100 µg/ml. The standard positive control, α-tocopherol had inhibition of 97.33 ± 0.54% at 100 µg/ml (Figure 1).

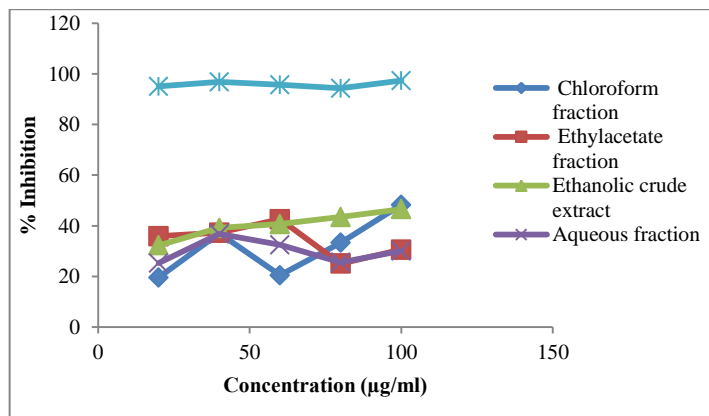


Figure 1 Lipid peroxide free radical scavenging activity of the crude ethanolic extract and fractions of *A. barbadensis* root

DPPH scavenging activity of *A. barbadensis* root extracts

The ethyl acetate fraction had the highest free radical scavenging ability with a percentage inhibition of 82.2 ± 0.44 % at 100 µg/ml while the chloroform fraction had the lowest inhibition of 27.52 ± 2.32 % at the same concentration of 100 µg/ml. The standard positive control (Vitamin C) had an inhibition percentage range of 93.39 % to 94.72 % (Figure 2).

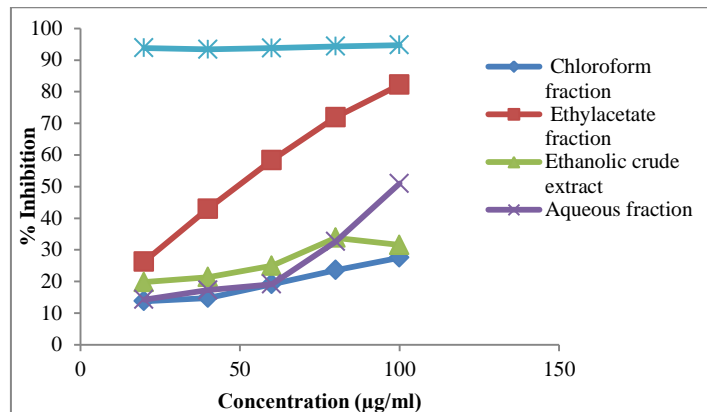


Figure 2 DPPH scavenging activities of crude ethanolic extract and fractions of *A. barbadensis* root with Vitamin C.

Ferric reducing power of *A. barbadensis* root extracts

The ferric reducing power potential of the ethanolic extract and fractions are shown in Figure 3. The highest reducing power activity was observed in the ethyl acetate fraction, with an average absorbance range of 0.145 ± 0.01 to 0.36 ± 0.00 nm. (Figure 3). The positive control (Vitamin C) had a concentration-dependent ferric reducing power within an absorbance range of 0.962 to 1.513 nm.

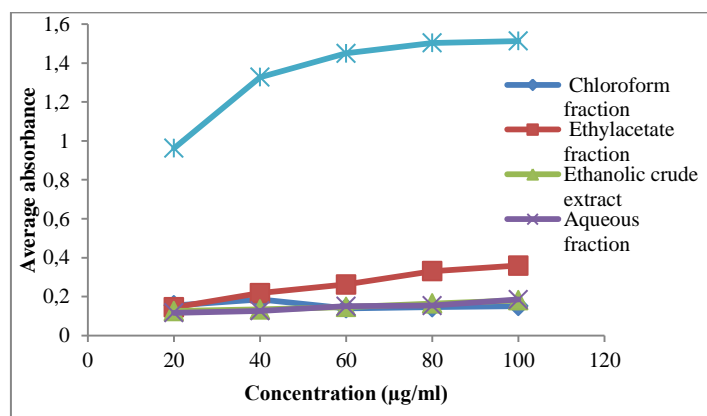


Figure 3 Ferric reducing powers of the crude ethanolic extract and fractions of *A. barbadensis* root

Preliminary phytochemistry and nutritional attributes of *A. barbadensis* root extract

Qualitative phytochemistry of ethanol root extract of *A. barbadensis* and its fractions

The prefatory phytochemical screening of the crude ethanolic root extract and fractions (chloroform, ethyl acetate and aqueous) of *A. barbadensis* revealed the presence of condensed tannins, phenolic compounds, flavonoids, glycosides and reducing sugars in all the tested extracts. Saponins were absent only in the chloroform extract while terpenoids were present in the chloroform and ethyl acetate fractions only. Alkaloids were present in the ethanol extract and ethyl acetate fraction only. Steroids were only present in the ethyl acetate fraction (Tab 5).

Table 5 Qualitative phytochemical composition of the ethanol extract and fractions of *A. barbadensis* root

Phytochemicals	Ethanol extract	Fractions		
		Chloroform	Ethyl acetate	Aqueous
Glycoside	+++	++	+	++
Cardiac glycoside	+	-	-	-
Saponin	++	-	+	++
Flavonoid	++	+++	+	+
Phenolic compound	++	++	++	+
Hydrolysable tannin	-	-	-	-
Condensed tannin	++	++	++	+
Phlobatannins	+	-	-	-
Terpenoid	-	++	++	-
Alkaloid	+	-	+	-
Polysaccharide/Starch	-	-	-	-
Reducing sugar	+++	++	++	++
Steroids	-	-	+	-

Legends: +++; appreciable amount; ++: moderate amount; +: minute amount; -: not detected

Quantitative analysis of *A. barbadensis* root.

The percentage total phenolic content was found to be highest (2.12 ± 0.003) quantitatively of the secondary metabolites determined, closely followed by saponins (1.75 ± 0.001). Alkaloids had the lowest concentration of $0.121 \pm 0.001\%$ (Tab 6).

Table 6 Quantitative Composition of the ethanol extract of *Aloe barbadensis* root

Parameters	Composition (%)
Alkaloids	0.12 ± 0.001
Flavonoids	1.21 ± 0.002
Saponins	1.75 ± 0.001
Phenolics	2.12 ± 0.003

Values represent Mean \pm SEM of three (3) determinations.

Proximate composition of *A. barbadensis* root

The percentage composition on a dry weight basis presented in Table 7 indicated that the root of *A. barbadensis* contained a moderate amount of crude fibre ($30.05 \pm 2.33\%$) and $24.66 \pm 1.89\%$ nitrogen free extract.

Table 7 Proximate Composition of the root of *Aloe barbadensis*

Parameters	Composition (%)
Moisture Content	10.23 ± 0.04
Ash Content	18.48 ± 0.32
Crude Oil	9.15 ± 1.34
Crude Protein	7.44 ± 0.62
Crude Fibre	30.05 ± 2.33
Nitrogen Free Extract (NFE)	24.66 ± 1.89

Values represent Mean \pm Standard deviation of two (2) determinations on dry Weight basis.

Mineral element composition of the root of *A. barbadensis*.

The root extract was found to have a considerable concentration of iron (37.42 ± 0.001 mg/kg), sodium (21.01 ± 0.002 mg/kg) and potassium (15.14 ± 0.001 mg/kg) (Tab 8). The amount of zinc quantified was low (0.02 ± 0.003), while cadmium and lead were not detected.

Table 8 Minerals Composition of the root of *Aloe barbadensis*

Minerals	Concentration (mg/kg)
Potassium	15.14 ± 0.001
Calcium	3.12 ± 0.003
Sodium	21.01 ± 0.002
Magnesium	2.01 ± 0.002
Iron	37.42 ± 0.001
Zinc	0.02 ± 0.003
Manganese	0.10 ± 0.001
Lead	ND
Cadmium	ND

Legend: ND- Not Detected; Values represent Mean \pm SEM of three (3) determinations on a dry weight basis.

Cytotoxic and genotoxic evaluation of AB root extract

Effect of AB root extract on root length and meristematic cells of *Allium cepa*

The highest growth rate (root length) was observed at 0.25 mg/ml with a root length of 1.74 ± 0.05 cm, while the lowest growth was observed at 1 mg/ml with a corresponding root length of 0.76 ± 0.03 cm (Tab 9). The mitotic indices, types and numbers of aberrations of the different concentrations of the crude ethanol extract of *Aloe barbadensis* root were also recorded in this study. It was observed that the mitotic index decreased with an increase in concentration of the extract (Table 10) while several chromosomal aberrations were induced in the *Allium cepa* root cells when exposed to the ethanol extract of *A. barbadensis* (Figure 4).

Table 9 Mean root length of *Allium cepa* exposed to crude ethanolic extract of *Aloe barbadensis* root.

Concentrations of extract (mg/ml)	Number of Roots	Mean root length (cm)	% Root Growth of control
Control	100	3.11 ± 0.07	100.00
0.25	100	1.74 ± 0.05	55.95
0.5	90	1.15 ± 0.05	36.98
0.75	100	1.14 ± 0.05	36.66
1	93	0.76 ± 0.03	42.44

Values are Mean \pm SEM of five replicates; Control – distilled water

Table 10 Cytological effects on *Allium cepa* root tip cells grown in various concentrations of ethanolic extract of *Aloe barbadensis* root

Concentrations of extract (mg/ml)	No. of dividing Cells	Total No. of cells counted	Mitotic Index \pm S.E (cm)	Mitotic Inhibition	Laggard	Polar deviation	Bridge	C – mitosis	Disturbed cell	Vagrant	Multipolar	Stickiness	% Aberrant Cell \pm S.E (cm)
Control	174	2113	8.63 ± 0.38	0.00	1		2			1		1	0.24 ± 0.25
0.25	155	2043	7.40 ± 0.42	13.79	4	7	5	5	3	5		5	1.66 ± 0.46
0.50	137	2145	6.29 ± 0.38	27.11	6	6	9	4	4	8		7	2.05 ± 0.71
0.75	128	2118	6.04 ± 0.43	30.01	6	8	9	6	4	8	1	5	2.27 ± 0.97
1	116	2045	7.36 ± 0.34	34.88	9	4	11	7	4	10	1	12	2.84 ± 1.39

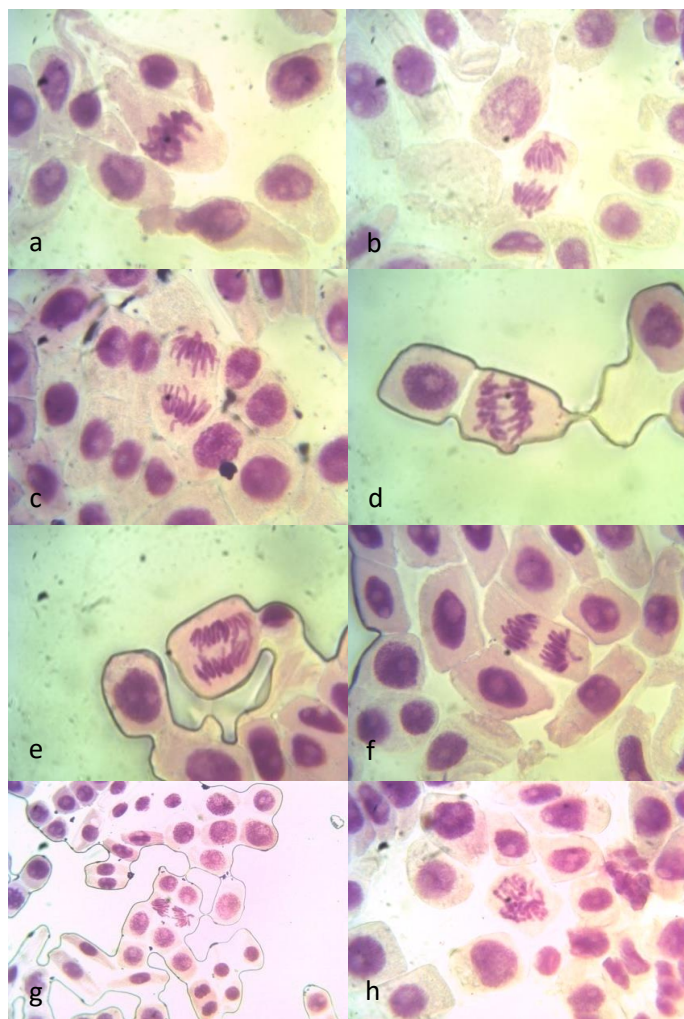


Figure 4 Chromosomal aberrations induced in *Allium cepa* root exposed to ethanol extract of *A. barbadensis* root (a) Vagrant + Polar deviation, (b) Sticky metaphase, (c) Vagrant cell on anaphase, (d) Multiple bridges, (e) Bridge, (f) Vagrant, (g) Polar deviation, (h) C- mitosis

Effect of AB root extract on root length of *Sorghum bicolor* (guinea corn)

A significant reduction ($P < 0.01$) in the radicle length of *Sorghum bicolor* when exposed to the ethanolic extract of *A. barbadensis* during the entire period of the study was noticed (Tab 11). After 24 hr, a significant reduction in the average radicle length (0.13 ± 0.09 mm) of the seeds of guinea corn was recorded at the 20 mg/ml concentration of the extract compared to an average length of 4.17 ± 0.42 mm observed in the control. The effect of the extract on the radicle length of *S.bicolor* at all tested concentrations after 24 and 48 hrs (except 5 mg/ml) were not significantly different from each other but differed significantly ($P < 0.01$) from the control.

DISCUSSION

Antimicrobial potential

Infectious diseases, according to Okigbo and Ajalie (2005), are responsible for over one percent of deaths occurring in tropical countries. Infertility is one ailment that has been linked to these infections and accounts for two-thirds of the

recorded infertility cases in Nigeria (Okonofua *et al.*, 1995). Similarly, polymicrobial infections have been linked to many cases of erectile dysfunction (Momoh *et al.*, 2011). In this study, ethanolic root extract of *A. barbadensis* was active against the Gram-positive and Gram-negative bacterial species isolated from the semen samples with a zone of inhibition of ≥ 10 mm (Andrews, 2005; Usman *et al.*, 2005). The inhibitory activity of the extract was found to be concentration-dependent (Tab 2). The isolated bacterial species from this study were similar to species isolated from earlier work (Ekhaise and Richard, 2008; Ibadin and Ibeh, 2008; Komolafe and Awoniyi, 2013). This probably reflects the prevalence of these bacterial species associated with male infertility due to their ability to cause polymicrobial infections. The ethanolic extract of *A. barbadensis* at 50 mg/ml had the highest inhibitory activity against the isolated organisms. It is noteworthy that the extract suppressed the growth of the isolated Gram-negative bacterial species. This indicated a very good antimicrobial property of the extract which agrees with the report of Ashafa *et al.* (2008) on the ability of the stem extracts of *Felicia muricata* to inhibit all Gram-negative bacterial strains tested. Of the isolated bacterial species, *E. coli*, *P. aeruginosa* and *S. saprophyticus* had lower tolerance to the extract when compared to the control drug, at 50 mg/ml (Tab 2). The MIC and MBC values of the extract (Tab 3) suggested that the tested organisms had low resistance. According to the MIC result, the extract was most active against *E. coli* and *S. saprophyticus* with the same MIC value of 6.25 mg/ml compared to a MIC of 12.5 mg/ml obtained for the other organisms. The extract had bactericidal activity against all the tested bacteria with an MBC value of 12.5 mg/ml for all the isolated species. It can, therefore, be inferred that the bactericidal activity of the extract against the isolated organism may prevent the inhibition of spermatogenesis and sperm function impairment in infertile males (Ibadin and Ibeh, 2008).

Antioxidant study

Antioxidants are chemical substances used to prevent human diseases and disorders, including male infertility. They scavenge or inhibit the production of free radicals (reactive oxygen species), which are very toxic to different forms of biological molecules (DNA, lipids, protein and carbohydrates) (Ashafa *et al.*, 2010; Raghuvver and Vyas, 2010). In the antioxidant assays, the ethanol extract and fractions of *A. barbadensis* exhibited strong scavenging activity against the free radicals. Prasad and Ramakrishnan (2012) reported that the exposure of unsaturated lipids in cell membranes and liver tissues to reactive oxygen species (ROS) make them very susceptible to peroxidation. The crude extract and fractions from the results obtained from this assay displayed a significant antiperoxidant effect (Figure 1). The chloroform fraction had the highest scavenging/ inhibitory activity of 48.28 ± 0.34 %, followed by the crude ethanolic extract (46.57 ± 0.69 %). The antiperoxidant effect of the ethanolic extract which was observed to be concentration dependent was similar to the findings of a previous study elsewhere (Prasad and Ramakrishnan, 2012) where the lipid peroxide radical scavenging potential of *Rumex vesicarius* was investigated. The inhibitory lipid peroxidation potential of the extract and fractions reflects their ability to probably reduce the risk factors associated with the induction and development of atherosclerosis (Godwin and Prabhu, 2006); slow down the aging process and boost immune responses (Prasad and Ramakrishnan, 2012). The lipid peroxidation inhibitory capacity of the extract and fractions is also very remarkable as it can be linked to the ability of the plant to prevent peroxidative damage to the plasma membrane of the sperm and loss of DNA structural integrity. This also ensures prevention of cell mortality and enhanced fertility (Lombardo *et al.*, 2011). The potent lipid peroxidation inhibitory effect of the extract and fractions can also be an indication that the plant may prevent loss of sperm membrane fluidity, which in turn improves sperm motility and sperm oocyte fusion (Raghuvver and Vyas, 2010). The lipid peroxidation inhibition capacity in this study supports the use of the plant in treating reproductive disorders/ailments, as previously reported by Erhabor and Idu (2017). For the DPPH assay (Figure 2), the extract and fractions had a high scavenging activity against the reactive oxygen species produced.

Table 11 Growth inhibitory effects of the ethanol extract of *A. barbadensis* on the radicle length (mm) of guinea corn.

Groups	24hrs	48hrs	72hrs	96hrs	P-Value
Control	4.17 ^{a#} ±0.42	16.63 ^{a###} ±2.8	22.83 ^{a####} ±0.08	30.47 ^{a#####} ±0.02	**P<0.01
5mg/ml	0.30 ^{b#} ±0.06	7.57 ^{c###} ±0.88	2.63 ^{c####} ±0.01	3.33 ^{c#####} ±0.03	*P<0.05
10mg/ml	0.20 ^{b#} ±0.58	0.65 ^{b#} ±0.40	1.03 ^{c###} ±0.02	2.03 ^{c####} ±0.01	**P<0.01
15mg/ml	0.33 ^{b#} ±0.09	1.23 ^{b###} ±0.27	3.03 ^{b####} ±0.03	2.03 ^{c#####} ±0.01	**P<0.01
20mg/ml	0.13 ^{b#} ±0.09	0.27 ^{b#} ±0.03	1.41 ^{d###} ±0.01	2.50 ^{d####} ±0.02	**P<0.01
25mg/ml	0.80 ^{b#} ±0.26	1.63 ^{b#} ±0.9	3.11 ^{b###} ±0.01	3.78 ^{b####} ±0.01	**P<0.01
P-Value	**P<0.01	**P<0.01	**P<0.01	**P<0.01	

Values are mean±SEM; n = 20, Note: **P<0.01-Highly Significant, *P<0.05-Significant, P>0.05-Not Significant
Different superscript letters (in columns) shows that the mean is significant from others.
Different[#](in rows) shows there is a significant difference across the sampled means across the hours.

The ethyl acetate fraction had the highest activity compared to the crude extract and other fractions. The concentration-dependent scavenging activity of the ethanol extract until the penultimate concentration (80 µg/ml) was observed. It was also noticed that the fractions had a concentration-dependent scavenging activity. The ability of the extract to inhibit the oxidation of DPPH implies that it can donate protons and scavenge other stable radicals. This agrees with the findings of Falodun and Irabor (2008) where a concentration-dependent scavenging ability of the methanol extract of *Calliandra surinamensis* was observed against the DPPH radical. Within the concentrations used in the ferric reducing power assay, the ethanolic extract, fractions (ethyl acetate and aqueous) and Vitamin C (positive control) exhibited a reducing power that increased as the concentration increased (Figure 3). This reducing power may be attributed to the ability of the extract and fractions to donate hydrogen that can react with reactive oxygen species to stabilize and end radical chain reactions (Sudha et al., 2011). It may also be due to hydrophilic polyphenolic compounds in the extract and fractions (Okolie et al., 2011; Yen et al., 1993), which have been implicated as being effective hydrogen donors (good oxidants). These findings are in sync with previous reports (Al-Fartosy, 2011; Okolie et al., 2011; Sudha et al., 2011) where the reductive capacities (antioxidant potential) of the ethyl acetate extract of pepino fruit; ethanol root extract of *Rauwolfia vomitoria* and methanol extract of *Inula graveolus* increased proportionally to their respective concentrations.

Phytochemical and nutritional composition of AB root

Investigations into the phytochemical constituents of the ethanol extract and its fractions revealed that glycosides, flavonoids, phenols, tannins and reducing sugars were present in the extract and fractions (Tab 5). The saponin content of the crude extract may have been responsible for the earlier reported aphrodisiac property of *A. barbadensis* root (Erhabor and Idu, 2017). This is because saponin has been previously postulated as a possible constituent responsible for the aphrodisiac property of *Tribulus terrestris*. It has also been found to increase testosterone in the body by raising the concentrations of luteinizing hormone which helps to sustain testosterone concentrations (Gauthaman, et al., 2002). Also, the aphrodisiac potential of clove (*Syzygium aromaticum*) has been attributed to the presence of sterols or phenolic compounds from an earlier study (Ahmad et al., 2004). The crude extract from the phytochemical results contained phenols but lacked sterols which were present only in the ethyl acetate fraction. Alkaloids have been reported to increase the dilation of blood vessels in the male sex organ (Zamblé et al., 2008) and are said to be one of the mechanisms of action through which aphrodisiac substances act. The ethanol extract of *A. barbadensis* was also found to contain an alkaloid, which can be linked to the increased blood flow noticed in the histological studies (Erhabor, 2015). Therefore, phenols, alkaloids and saponins may synergistically or individually be responsible for the aphrodisiac activity of the ethanolic extract of *A. barbadensis*. This agrees with the assertion that phytoconstituents potentiates erection and prolong ejaculatory latency in male rats (Muanya and Odukoya, 2008; Sumalatha et al., 2010; Yakubu et al., 2005). The occurrence of phenol in the extract may be responsible for its probable cytotoxic and antiproliferative properties. This can be inferred from earlier studies adding the cytotoxic and antiproliferative activity of phenols against melanocytes cell lines (Ayinde and Aghakwuru, 2010). Hour et al. (1980) also reported that saponins and flavonoids had antitumor and anticancer properties and as a result, their presence in *A. barbadensis* could be linked to its relatively toxic effect on cells. Again, phenols and flavonoids have been suggested to be responsible for the radical scavenging activities of most drugs (Falodun et al., 2011). Therefore, it is possible that these secondary metabolites can be responsible for the antioxidant property of the ethanolic extract and fractions of *A. barbadensis*. However, the tannins in the root extract of *A. barbadensis* may cause its antimicrobial activity as earlier reported by Sodipo (1991). Flavonoids which have also been reported to have antimicrobial properties by Yebpella et al. (2011) are one of the chemical constituents in the extract of *A. barbadensis*. It is pertinent to note that the percentage composition of alkaloids, flavonoids and phenols (0.12%; 1.21% and 2.12% were lower than those recorded for the leaves of *Cymbopogon citratus* which had concentrations of 1.38%, 4.76% and 3.58% respectively. The saponin content (1.75%) was, however greater than that of *C. citratus* leaves (1.25%) (Uraku et al., 2016).

The proximate constituents of a plant indicate nutrients available in the plant. The nitrogen free extract (NFE) in *A. barbadensis* root (Tab 7) may have provided the required energy for cellular activities such as arousal of the penis when metabolized in the body as reported by Odesanmi et al. (2012) on the nutritional prospect of *Microdesmis keayana*. The NFE of *A. barbadensis* was found to be lower than that of *Acorous calamus* (75.03%) as reported by Barua et al. (2015). The root of *A. barbadensis* also has protein which may form a pool for amino acids such as arginine which have been suggested to potentiate the aphrodisiac properties of medicinal plants (Sumalatha et al., 2010). The protein value from this present study was found to be lower than that reported by Kibar and Temel (2016); Kibar and Kibar (2017) for some wild edible plants. The moisture and ash contents are two values that can show the stability, quality and purity of a powdered crude drug. The moisture and ash contents of *A. barbadensis* root (Table 7) were relatively high when compared to that of *Microdesmis keayana*

root (Odesanmi et al., 2012). It was also observed that the ash content of *A. barbadensis* root (18.48%) was higher than that of *C. citratus* leaves (7.15%) as reported by Uraku et al. (2016). Interestingly, *A. barbadensis* root collected from the wild was also found to have higher ash content than the values reported by Kibar and Temel (2016); Kibar and Kibar (2017) for some wild edible plant species. The high values can be linked to its ability to store water for a long time, being a drought-resistant plant.

Micronutrients play vital roles in metabolism and enzymatic reactions when they function as co-factors and co-enzymes. The magnesium concentration in the root sample of *A. barbadensis* (Tab 8) can be described as low (2.01±0.002 mg/kg) when compared to that of *Microdesmis keayana* root (458.8±5.44 ppm). However, magnesium is useful in the biosynthesis of sex hormones such as androgen, oestrogens and neurotransmitters (dopamine and norepinephrine which aid in sex modulation) (Odesanmi et al., 2012). Potassium which function as a major cation of intercellular fluid, regulate the electrode potential and concentration of aldosterone and cell membrane permeability as reported by Robert et al. (2003) and was detected in moderate amount (15.14±0.001mg/kg) in the root of *A. barbadensis*. The concentrations of iron (37.24±0.001 mg/kg) and sodium (21.01±0.002 mg/kg) were higher than those of *Microdesmis keayana* which recorded values of 5.62±0.48 ppm and 5.68±0.25 ppm. The quantification of calcium in the root of *A. barbadensis* was very important as it helps in protecting the structural integrity of the skeleton and provides energy for contraction via a complex reaction involving vital enzymes regulating metabolism at the intermediary level (Breslau, 1991). Another mineral element, zinc, functions in boosting the immune system and is necessary for the effective synthesis of testosterone and as such can prevent or be used in managing male infertility (Odesanmi et al., 2012). The presence of zinc maybe responsible for the ability of the plant to boost the immune system and increased testosterone which supports the aphrodisiac use of the plant.

Cytotoxic and genotoxic effects of *A. barbadensis* root

Earlier report by Grant (1994) reported the ability of the meristematic cells of *Allium cepa* root tip to interact with mutagenic substances and as a result, these are used to detect the cytotoxic and genotoxic effects of harmful chemicals. The macroscopic evaluation of the *A. cepa* root showed that the extract at all concentrations decreased the root growth of the tested onion bulbs when compared to the control (distilled water) (Tab 9). This finding implied that the extract may be relatively toxic and can inhibit tumour-producing cells. From an earlier study, inhibition of the root growth of *A. cepa* suggests a reduction in the number of dividing cells (Olorunfemi et al., 2011), which reflects the mitodepressive effect of the extract on the ability of *A. cepa* cells to divide. Thus, the extract may have the ability to inhibit the synthesis of DNA and nucleus – proteins, a process that had been reported with extract of other plants (Stephen, 1980; Timothy et al., 2014). These results support report in this study where the effect of the extract inhibited the radicle growth of *Sorghum bicolor* seeds.

Following the previous report by Smaka-Kincl et al. (1996), a decrease in the rate of mitotic index is one acceptable measure for determining the cytotoxic effect of harmful substances on all living organisms. The ethanol extract of *A. barbadensis* showed a concentration dependent decrease in mitotic index (MI) which implies a cytotoxic effect (Tab 10). This is consistent with an earlier report by Timothy et al. (2014) where the extract of *Icacina trichantha* decreased the MI of *A. cepa* root cells. Also, Olorunfemi et al. (2014) stated that chromosomal aberration and abnormalities are useful parameters for assessing genotoxic activity of a substance. These aberrations include sticky chromosomes, vagrant chromosomes, bridges, polar deviations and spindle disturbance. It was observed that the extract induced certain levels of chromosomal aberrations at all the tested concentrations (Figure 4). This concentration-dependent activity of the extract on the chromosomes of the *A. cepa* root cells implies a cyto-genotoxic effect of the extract. Therefore, it can be inferred that the extract has the functional therapeutic ability to destroy cancerous cells (Dahof, 1983). This finding also agrees with previous studies by Timothy et al., (2014) where the cyto-genotoxic activity of the leaf extract of *Icacina trichantha* on *A. cepa* root cells was tested.

It has been reported that meristematic tissues of seeds can rapidly multiply when exposed to favourable conditions. The level of proliferation is reflected in an increase in the radicle length of the seeds during a defined period (96 hrs) (Ikpefan et al., 2013). Also, germination inhibition and subsequent growth of the *Sorghum* spp radicle have been suggested as a measure to determine the herbicidal potential of a plant extract or its ability to induce dormancy and retard the growth of tumor-producing cells (Sogbaike et al., 2002). Administration of the ethanol extract of *A. barbadensis* at all concentrations and within the tested period significantly ($p<0.01$) inhibited the growth of *Sorghum bicolor* radicle (Tab 11). The extract displayed a concentration-dependent reduction in the radicle length of *S. bicolor* seeds when compared to the control. The extract of *A. barbadensis* root exerted the greatest significant ($p<0.01$) inhibitory effect against the growth of the *S. bicolor* radicle at 20 mg/ml. The result implies that the extract possessed an anti-proliferative activity which suggests that it can be used to retard the growth of cells that produce tumours. This finding agrees with earlier reports by Fajana (2013), Ikpefan et al. (2013) on the growth inhibitory activity of the methanol extract of *Tridax procumbens* and *Cnidioscolus*

acontifolius on the radicle length of guinea corn seed. This *in vitro* cytotoxic result does not, however, agree with previous findings by Erhabor and Idu (2017) on the *in vivo* acute toxicological effect of the *A. barbadensis* root extract within the utilized dose range (100 to 400 mg/kg).

CONCLUSION

This study revealed the potential of *A. barbadensis* root extract as a strong scavenger of free radicals with relatively good antimicrobial activities though at moderately high concentrations. Lead and cadmium were not detected in the plant which probably showed lack of contamination of the plant material. It should be noted, however, that the fractions of AB root were not assessed for antimicrobial, quantitative phytochemical, cytotoxic and genotoxic activities due to insufficient samples. The moderately high nutrient content of the extract may be a possible mechanism for the aphrodisiac property of the plant. The results of the biological activities of *A. barbadensis* root is an indication of its potential use in drug development for managing infectious diseases and other related ailments. Although the results suggest that the extract may be cytotoxic, there is a need to assess the plant in other *in vitro* and *in vivo* assays using relevant cell lines, and in long term animal model studies.

List of abbreviations: **AB:** *Aloe barbadensis*, **DPPH:** 1, 1, diphenyl-2-picrylhydrazyl, **FRAP:** ferric reducing power, **MIC:** minimum inhibitory concentration, **MBC:** minimum bactericidal concentration.

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