CYTOTOXICITY AND ANTIOXIDANT ACTIVITY OF STEM BARK EXTRACTS OF *Azanza garckeana* (kola of Tula)

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ABSTRACT

Plants have been used since ancient times as an important source of biologically active substances. The aim of the present study was to investigate the antioxidant potential and cytotoxicity against brine shrimp of the stem bark extract of Azanza garckeana (kola of Tula). The petroleum ether, ethyl acetate, acetone, methanol and water extracts, were obtain by serial extraction using soxhlet apparatus. The results showed that Acetone extract with LC_{50} of 3.98µg/ml, methanol extract LC_{50} of 47.66 µg/ml and ethyl acetate extracts LC_{50} of 100 µg/ml were active extracts while water extracts with LC_{50} of 138.04µg/ml is toxic where as petroleum ether extract with LC_{50} value of greater than 1000 µg/ml is in active. The result for the DPPH radical scavenging activity of the stem bark extracts of Azanza garckeana showed that the methanol extracts with IC_{50} value of less than 100 µg/ml and Acetone extracts with IC_{50} value of 160µg/ml had better activity than the standard Ascorbic acid with IC_{50} value of 220 µg/ml. The result has proved that the plant can be used as an antioxidant by the folk of total people.

Keywords: *Azanza garckeana, stem bark, cytotoxicity and antioxidant activity.*

INTRODUCTION

Different plant' parts have been used for treatment of various form of ailment. The investigation of medicinal properties of various plants attracted an increasing interest since last couple of decades because of their potent pharmacological activities. There is a growing interest in natural antioxidants, present in medicinal and dietary plants that might help attenuate oxidative damage (Silva *et al*., 2005, Shakeri *et al*., 2012). An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may therefore have health-promoting effects in the prevention of degenerative diseases. Epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Labibah, 2009).

Brine shrimp lethality test for cytotoxicity have also gained increasing interest among scientific community because it's has been considered as prescreening assay for, antimicrobial, antitumor, antimalarial, and insecticidal activities Umbreen *et al.,* (2015). Therefore it is suggested to be a convenient probe for the pharmacological activities of plant extracts (Yogesh *et al.,* 2012).

Though many plants have been screened for antimicrobial properties, cytotoxicity, and antioxidant capacity with some leading to the discovery of the derived drugs known today, a vast majority of them have not yet been adequately evaluated. In an effort to expand the spectrum of antibacterial, antitumor and antioxidants agents from natural resources, Azanza *garckeana* belonging to Malvaceae family has been selected for this work.

The plant A*zanza garckeana*, commonly known as Goron Tula, (kola of Tula) in Hausa, (Burkill, 1985), belong to the family Malvaceae, in the order Malvales, was reported to have some medicinal values. A decoction is made from the roots are taken orally for painful menstruation and to treat coughs and chest pains. An infusion made from the roots and leaves is dropped into the ear to treat earache or taken orally as an antiemetic according to Alfred, 2013. A decoction is made from the root for, treatment of venereal diseases and to treat coughs and chest pains. It is taken as a treatment drug for infertility and a drug that causes evacuation (purgative). A paste made from pounding its fruits is applied onto the cheek with abscess to draw it and onto boils in the mouth for relief. An infusion made from both its stem and leave is taken to treat liver problem (Ochukwu *et al.*, 2014).

MATERIALS AND METHODS
PREPARATION OF PLANT MATERIAL

The plant sample (root bark) of *Azanza garckeana* was collected in Tula Wange (Tantan) and Yiri (Bwane), Kaltungo local Government Area of Gombe State. The root bark obtained was air dried in the laboratory at room temperature and then pulverized using motorized miller. The extraction was carried out using the soxhlet extraction method with the following solvents: petroleum ether, ethyl acetate, acetone, methanol and water in order of increasing polarity.

Brine Shrimp Lethality Assay

Brine shrimp eggs were commercially available. For this experiment, brine shrimp egg without shells "Artemia Revolution" 120g were obtained from NT labs (Fry care) laboratories LTD UK, Serial No. 7//3380900038///3 Made in England. Eggs were stored in a refrigerator at 5^0C **(**NT labs, 2015).

Preparation of Artificial Sea water

Artificial sea water was prepared by dissolving 35g of sea salt in1litre of distilled water for hatching the brine shrimp eggs **(**NT labs, 2015).

Hatching of brine shrimp

Artificial seawater was prepared at full strength according to **(**NT labs, 2015). To obtain an optimum result a solution of specific gravity of 1.022 at 24^0C was prepared by dissolving 35g sea salt sodium chloride NaCl per liter of water. The seawater was added to the brine shrimp Hatcher in a heated aquarium aerate from bottom of the unit so that all eggs are kept in suspension and moving. The brine shrimp bottle was shaken before dispensing into the aquarium (each drop gives from 1500 to 2000 nauplii, three drops (5000 nauplii) and are hatched in approximately 250ml sea water **(**NT labs, 2015).

The hatcher is illuminated very well for a minimum of three hours preferably for 12hours. The hatching time depend on temperature at 24^0C (which is average tropical aquarium temperature) hatching take place between 24-48 hours (maximum hatch 44-48hours). The Nauplii is then used directly for the cytotoxicity test **(**NT labs, 2015).

Preparation of Test Sample

Samples were prepared by dissolving 20mg of the plant extracts in 10ml of suitable solvent (stock solution # 1). Solution of varying concentrations (1000, 500, 250,125,100 µg /ml) were obtained by the serial dilution technique.

Cytotoxicity Test (Bioassay)

Brine shrimp lethality bioassay was carried out using brine shrimp larvae (Artemia salina) to determine the Cytotoxicity of the plant extracts. To each sample vial corresponding to 1000,500,250,125, and 100µg/ml, 4ml of artificial seawater was added and 10 brine shrimps were introduced into the tubes using pipette, and the final volume in each vial was adjusted with artificial seawater to make a total volume of 5ml. The test tubes were left uncovered in the light, the nauplii were counted against a lighted background using magnifying hand lens and the number of the surviving shrimps were counted and recorded after 6, 12 and 24hours. Control test was also carried out using artificial seawater only. Nauplii were considered dead if they were lying immobile at the bottom of the vial.

Statistical Analysis

The percentage of deaths and (LC_{50}) were determined using statistical analysis. Percentage mortality (M %) was calculated by dividing the number of dead nauplii by the total number, and then multiply by 100%.

$$\text{Percentage of Death (\%M)} = \frac{Total\ number\ of\ nauplii - number of\ nauplii\ alive}{total\ number\ of\ nauplii} \text{X100}$$

Lethal concentration (LC_{50}) determination:

The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC50) was determined from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis ; the LC50 was derived from the best-fit line obtained. LC_{50} values were obtained from the best-fit line, plotted of concentration against Percentage mortality.

Antioxidant Activity of Plant Extracts

Antioxidant activity (DPPH free radical scavenging activity) of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical as described by Brand-Williams (Rajani et al 2013). The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard. 2ml of 0.002% of DPPH solution in methanol was mixed with 2ml of plant extract solution of varying concentrations (100, 125, 250, 500, and 1000 µg /ml). Corresponding blank sample were prepared and L-Ascorbic in (100-1000 µg /ml) was used as reference standard. Mixture of 2ml methanol and 2ml DPPH solution was used as control. These solutions were kept in dark for 30min, and optical density measured at 517nm using UV-Vis spectrometer, LT-290 Labtronics model**.** The reaction was carried out in duplicate and the decreased in absorbance was measured at 517nm. The DPPH radical scavenging activity (S %) or inhibition % was calculated using the equation.

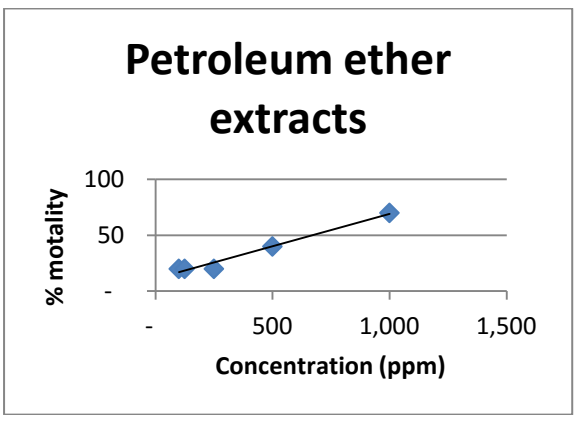
$$S\% = \frac{(A\ control\ - A\ sample)}{A\ control} \times 100$$

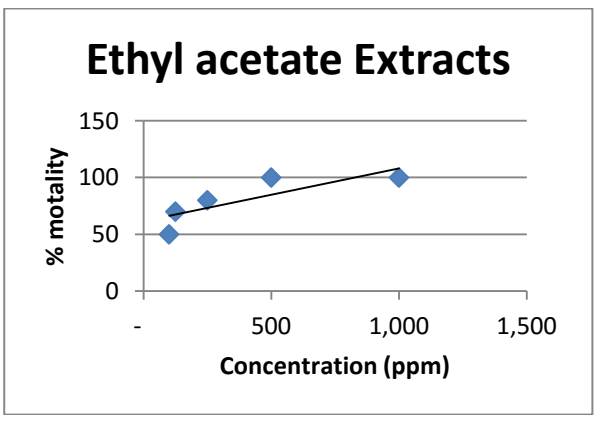
Where $A_{control}$ is the absorbance of the blank control (containing all reagent except the solution extract) and, A_{sample} is the absorbance of the sample.

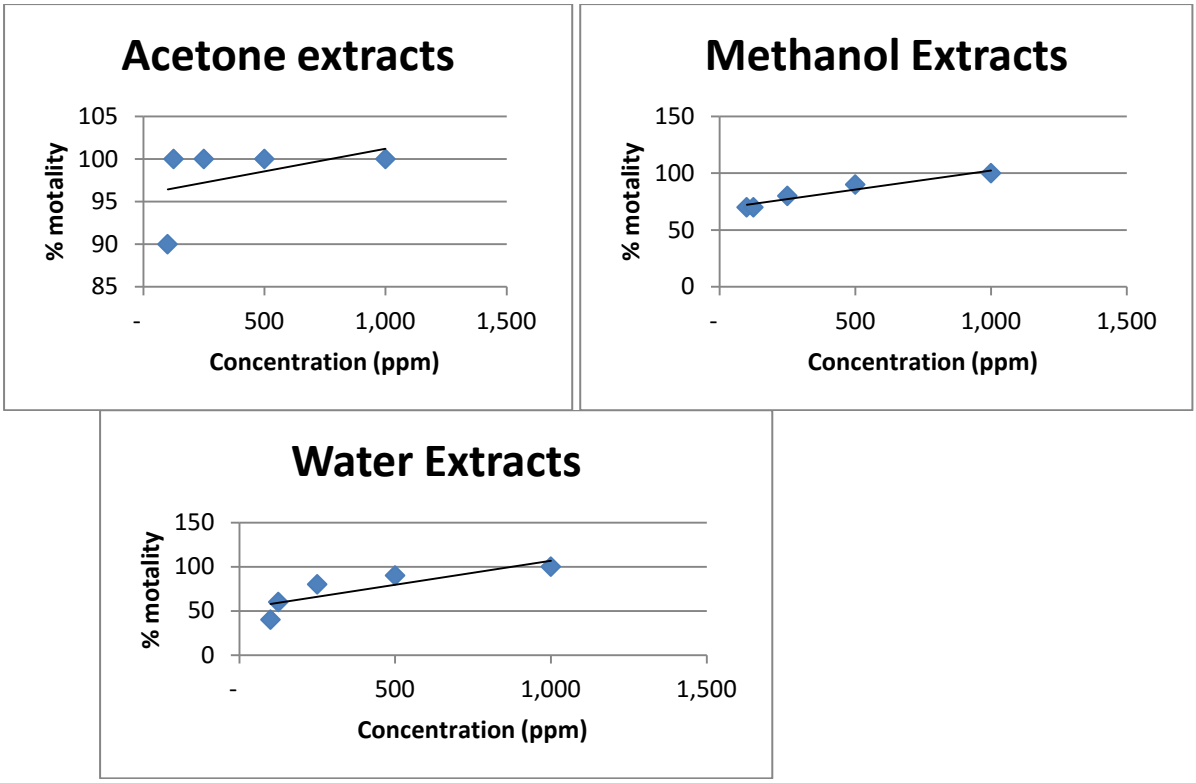
RESULTS AND DISCUSSION

Table 1: Brine shrimp lethality test of the stem bark of *Azanza garckeana* extracts

Root extracts	Conc. in µg/ml	Number of mortality nauplii after 24 hours					LC_{50} (µg/ml)
		1st	2nd	3rd	mean	%Mortality after 24hrs	
Water	1000	10	10	10	10	100	138.04
	500	9	8	7	9	90	
	250	8	9	7	8	80	
	125	4	5	9	6	60	
	100	4	4	4	4	40	
Methanol	1000	10	10	10	10	100	47.86
	500	10	10	8	9	90	
	250	8	8	8	8	80	
	125	7	7	6	7	70	
	100	7	7	7	7	70	
Acetone	1000	10	10	10	10	100	3.98
	500	10	10	10	10	100	
	250	10	10	10	10	100	
	125	10	10	10	10	100	
	100	9	10	8	9	90	
Ethyl acetate	1000	10	10	10	10	100	100
	500	10	10	10	10	100	
	250	8	8	7	8	80	
	125	7	7	7	7	70	
	100	5	5	5	5	50	
Petroleum ether	1000	7	6	8	7	70	>1000
	500	4	3	5	4	40	
	250	2	2	2	2	20	
	125	0	4	2	2	20	
	100	0	0	5	2	20	







Cytotoxicity effect of extracts of stem bark of Azanza garckeana on brine shrimp

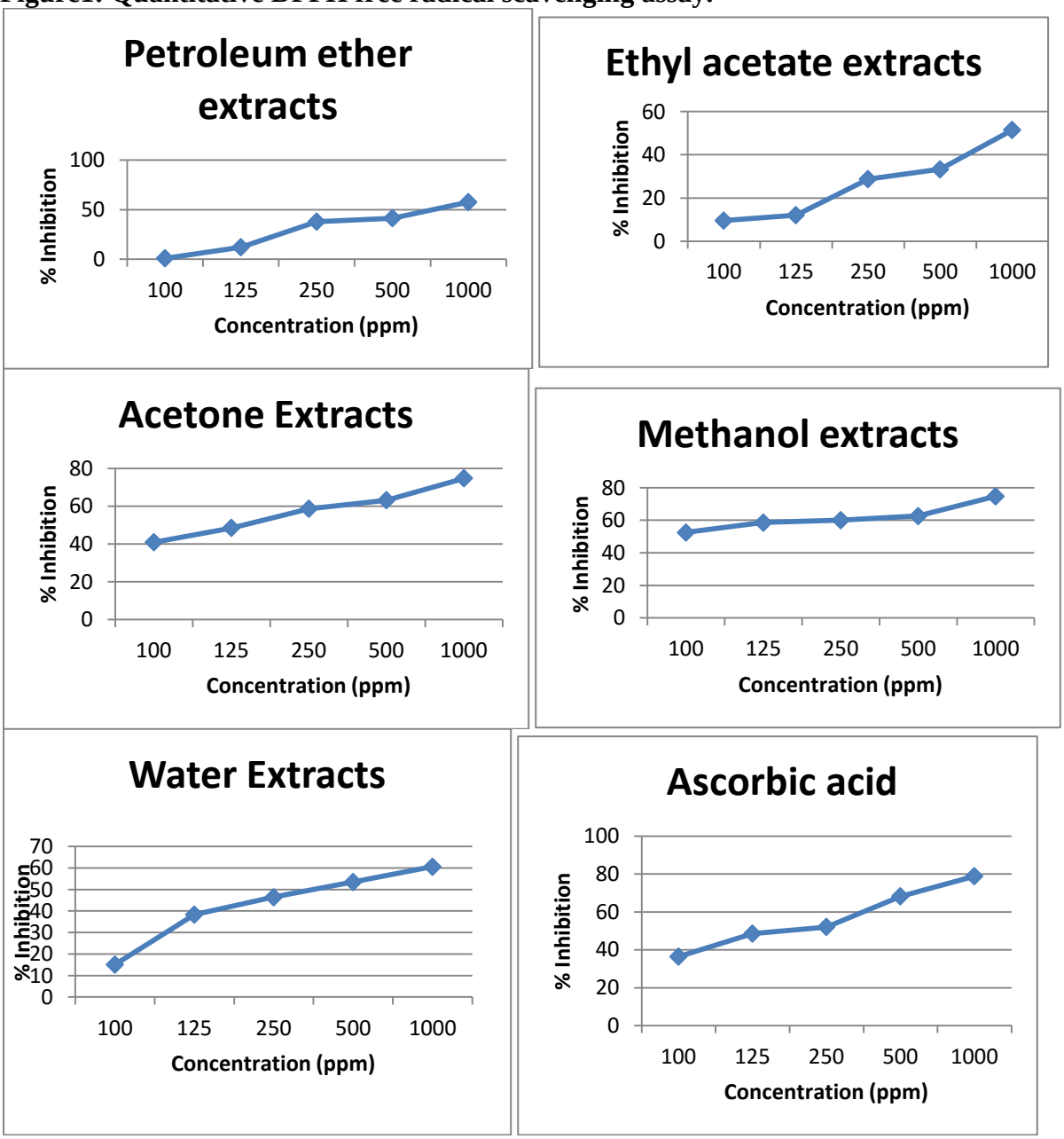
Table 3.0 DPPH Radical scavenging activity of ascorbic acid

Extracts	Concentration (ppm)	%inhibition	IC50
Water	100	15.15	580
	125	38.38	
	250	46.46	
	500	53.53	
	1000	60.60	
Methanol	100	52.52	< 100
	125	58.6	
	250	60.10	
	500	62.62	
	1000	74.74	
Acetone	100	40.90	160
	125	48.48	
	250	58.6	
	500	63.13	
	1000	74.74	
Ethyl Acetate	100	9.60	920
	125	12.12	
	250	28.78	
	500	33.3	
	1000	51.51	
Petroleum ether	100	1.01	

	125	12.12	960
	250	37.87	
	500	41.41	
	1000	57.57	
Ascorbic acid	100	36.36	220
	125	48.48	
	250	52.02	
	500	68.17	
	1000	78.78	

Results were recorded as a mean of tree replicates

Figure1: Quantitative DPPH free radical scavenging assay.



RESULTS AND DISCUSSIONS

Herbal medicines have received great interest as an alternative to clinical therapy, and the demand for these therapies has currently increased rapidly (Sahgal *et al.*, 2010). The use of Artemia sp. is essential in this study as a test species in toxicity, screening hepatotoxic cyanobacterial strains (Nunes, 2006), and natural products (Parra, 2001 and Alfred, 2013).
The brine shrimp test represents a rapid, inexpensive and simple bioassay for testing the plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. Most often, a desired biological response is not due to one component but rather due to a mixture of bioactive plant components. Therefore, crude extracts must be screened for biological activity. The brine shrimp lethality assay has been proved to be a convenient system for monitoring biological activities of natural products (Chanda and Bavalia, 2011).
The toxicity of herbal extracts expressed as LC_{50} values is commonly valorized either by comparison to Meyer's or to Clarkson's toxicity index. According to Meyer's toxicity index, extracts with LC_{50} < 1000 µg/ml are considered as toxic, while extracts with LC_{50} > 1000 µg/ml are considered as non-toxic (Meyer et al., 1982). Clarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts in the following order: extracts with LC_{50} above 1000 µg/ml are non-toxic, LC_{50} of 500 - 1000 µg/ml are low toxic, extracts with LC_{50} of 100 - 500 µg/ml are medium toxic, while extracts with LC_{50} of 0 - 100 µg/ml are highly toxic (Clarkson et al., 2004).

Brine shrimp LC_{50} values for the stem bark extracts evaluated is shown in table 1. The result showed that the acetone and methanol extracts are especially potent against the brine shrimp with an LC_{50} value of 3.9 µg /ml 8 and 47.86 µg /ml respectively. The ethyl acetate LC_{50} value of 100.00µg /ml and the Water extract with LC_{50} value of 138.04µg /ml indicated that the extracts are moderately cytotoxic to the brine shrimp larvacidal activity. The LC_{50} value of the petroleum ether extract of the stem barks is greater than 1000 µg /ml, hence it is inactive according to Cavallo *et al.*, (2002).

Based on the results, the brine shrimp lethality of extracts was found to be concentration-dependant that is the degree of lethality was directly proportional to the concentration of the extract. The cytotoxic property of plant extract may be due to the presence of antitumor compounds in Azanza garckeana.

Table 2 shows the DPPH radical scavenging activity of the extracts of Azanza garckeana stem bark as determined by the IC_{50} values. An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system (Rajani, 2013). The IC_{50} value is inversely related to the antioxidant activity of the extracts.

The inhibition effect of ascorbic acid (standard) expressed as IC_{50} value is 220µg/ml. The result of DPPH scavenging assey showed that, methanol extracts of stem bark was the most potent with an IC_{50} values less than 100µg/ml followed by the acetone extracts which showed the IC_{50} value of 160 µg/ml. The water extracts of stem bark with IC50 value of 580µg/ml while that of petroleum ether was 960µg/ml and ethyl acetate extract exhibited IC_{50} value 920µg/ml. The IC50 value of the methanol and acetone were more active than the standard ascorbic acid used in the test. The activity of the extracts may be due to the presence of active compounds present in the plant such as flavonoid, alkaloids, phenols, saponin, tannin,

cyanogenic glucoside and carotenoid (Adamu *et al.*, 2013: Michael *et al.*, 2015; Ochokwu *et al*., 2015; Reem *et al*., 2016)

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