CYTOTOXICITY AND ANTIOXIDANT ACTIVITY OF LEAVES AND STEM BARK EXRTACT OF TERMINALIA SCHIMPERIANA HOSTCH.

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Abstract.

The aim of this study was to investigate the antioxidant potential and cytotoxicity of the leaves and stem bark of Teminalia schimperianaplant against brine shrimp. Ethyl acetate, ethanol, acetone hexane and water extracts of leaves and stem bark of the plant were obtained by serial extraction using Soxhlet apparatus. Logit and Probit analysis was used to determined the LC_{50} values for the cytotoxicity of the various extracts. The results shows that all the extracts are less toxic against the brine shrimp as only extracts giving LC_{50} values lower than 30 µg/ml were considered to be cytotoxic. Aqueous extract (stem bark) have low toxicity among the various extracts with LC_{50}values of 843.3 and 828.9 µg/ml for both the logit and probit function while ethanol extract (both leaves and stem bark) showed little toxicity with LC_{50} ranging between 68.1 and 32.2 µg/ml for the leaves and stem bark respectively. The difference between Logistic and Probit analysis lie in the distribution of error. Antioxidant activity (DPPH) free radical scavenging activity) result shows that both leaves and bark extract shows better antioxidant activity when compared with reference standard ascorbic acid. They exhibit strong antioxidant DPPH radical scavenging activity with IC_{50} ranging from 25 to 33.0µg/ml for the leaves and stem bark respectively when compared with reference standard ascorbic acid which is 18.5 µg/ml, regarding the fact that the extract is a mixture of a great number of compounds as opposed to pure ascorbic acid used as standard reference. Aqueous extract (stem bark) with IC_{50} value of 25µg/ml has better antioxidant activity among the extracts. It can be deduced that T. Schimperiana is less toxic and has better antioxidant activity when compared with standard ascorbic acid.It is therefore recommend that the drug should be used for medicinal purpose because of its therapeutics effect. Further investigations are needed for chemical characterization of the active compounds and more comprehensive biological assays.

key words*: Terminalia Schimperiana, stem bark, leaves, cytotoxicity and antioxidant activity.*

INTRODUCTION.

Terminalia Schimperiana Hostch is a family of *combrataecia* plant. It is a family of *Terminalia* native to tropical Africa from Guinea to Sierra Leon east to Uganda and Ethiopia,(Abonier,S.2004). It is a broad leave small tree that can reach up 7 to 14 meters height, variably deciduous in the dry season to semi- ever green, depending on the climate. The plant is used for the treatment of wounds, bronchitis and dysenteryrespectively (Eucaria, & Eunice 2013). In part of west Africa it is used as medicinal plant,(Sofonara, 1982). The bark is applied to wounds**,** and the twigs may be chewed to promote oral hygiene, **(**Abornier, 2004). In laboratory experiment, extract of the plant were found to have in-vitro antibiotics properties against staphylococcus, (Akande& Hayashi,1998). Bark decoction are administeredas purgative and to treat malaria, diarrhea and pinworm infection. Leaf decoctions are applied to treat burns and headache, and are taken against malaria, stomach ache, hepatitis, amenorrhea, cough, asthma, diabetes, obesity and elephantiasis. Fruit serve as vermifuge,(Schimelzer & Fakin;2013). In some part of western Nigeria, fresh matured root (hand full) are cooked along with one- half tea spoon of potash in water and the concentrated decoction is taken orally for treatment of diarrhea.(Bhat, et al, 1990). Many *Terminalia* Species have uses in African traditional medicine and contain a large number of compounds.(Kaur et al, 2002), identify polyphenols which includes flavones, flavanols, phenyl-propanoid and tannis from extracts of *Terminalia* plants. These compounds are claimed to treat different ailments, including fractures, ulcers, blood diseases, anemia and asthma, (Kaur et al 2002). Evidence exist that *Terminalia* species poses both anti fungal and anti bacterial activities,(Silver et al,2002; Fyhrquist et al,2004; Mosoko et al, 2005).

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, (Silver et al, 2005, Shekari 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 shown that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular diseases and cancer,(Labibah, 2009). Cytotoxicity studies are useful initial step in

determining the potential toxicity of a test substance, including plants extracts or biological active compounds isolated from plants. Minimal or no toxicity is essential for the successful development of a pharmacological or cosmetic preparation and in this regard, cellular toxicity studies play a crucial role. (Lyndy & Jocobos ,2014).

Brine shrine lethality test for cytotoxicity have also gained increasing interest among scientific community because it has been considered as prescreening assay for antimicrobial, antitumor anti malarial and insecticidal activities,(Umbreen et al,2015). It was suggested to be a convenient probe for pharmacological activities of plant extracts,(yogesh et al, 2012).

Though many plants have been screened for antimicrobial properties, cytotoxicity and antioxidant activity with some, leading to the discovery of some derived drugs today, a vast majority of the plants have not yet been adequately evaluated. The basic aim of this research is to determined the antioxidant activity and cytotoxicity of the *Terminalia Schimperiana Hostch* plant in vitro, in an effort to expand the spectrum of antibacterial, antitumor and antioxidant agents from natural resources.

MATERIAL AND METHOD.

PREPARATION OF PLANT MATERIAL. The plant sample (leaves and stem bark) of *Terminalia Schimperiana Hostch* was collected in Kaltungo local government area, Gombe state Nigeria in the month of June 2019. The leaves and stem bark obtained was air dried in the laboratory at room temperature and then pulverized using motorized miller. the extraction was carried out using soxhlet extraction method with the following solvent; N-hexane, ethyl acetate, acetone, ethanol and water.

Cytotoxicity of the plant extract.

Brine Shrimp Lethality Assay. For this experiment brine shrimp eggs without shells "Artemia Revolution" 120g were obtained from NT labs (Fry care) laboratories LTD UK, Serial No. 7//3380900038///3 made in England. **Preparation of Artificial Sea Water.** Artificial sea water was prepared by dissolving 35g of sea salt in a one liter of distilled water for hatching the brine shrimp eggs, (NT labs, 2015).

Hatching Of Brine Shrimp. Artificial seawater was prepared atfull strength according to NT labs, 2015. The sea salt water was added to the brine shrimp Hatcher in an aquarium aerated from the bottom of the unit so that all the 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, 3 drops 5000 nauplii) and are hatched in approximately 250ml sea water, (NT labs, 2015). The Hatcher was illuminated very well for 3hours and allowed to hatched for 24hours at 24^0C, (which is average topical aquarium temperature), (NT labs 2015)

.**Preparation of Test Sample.** samples were prepared by dissolving 20mg of the plant extracts in 10ml of solvent (stock solution 1) Solution of various concentrations (1000, 500, 200,100 and 10µg/ml was prepared by serial dilution technique.

Cytotoxicity Test.(Bioassay)Brine shrimps eggs were hatched in the laboratory as explain above. 5ml sea salt water and 2ml of the extract in concentration of 10, 100, 200,500 and 1000µg each were added to a vial. 2ml of brine shrimps stock solution (contain about 15 to 20 shrimps) was added in to the tube using a pipette. Control test was also carried out using artificial sea water only. The test tube were maintain under illumination. Survivors were counted with the aid of 3X magnifying glass after 24 hours and the percentage death at each dose and the control were also determined. Nauplii were considered dead if they were lying immobile at the bottom of the vial.

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

(M%) = (total nauplii - alive nauplii)/ total nauplii × 100. Lethal Concentration (LC_{50}) resulting in 50% mortality of the brine shrimp was determined from the 24 hour count by using Logit and Probit analysis. The difference between the logistic and probit analysis lie in the distribution of error.

Antioxidant Activity of Plant extracts

Antioxidant activity (DPPH free radical scavenging activity) of the extracts was carried out. The antioxidant activity of the plant extracts and the standard was assessed based on the basis of the radical scavenging effect of the stable 1, 1-diphenyle-2-picrylhydrazyl (DPPH). Free radical activity was describe by (Marwa et al, 2007). The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard. 10-50µg/ml solution of the extracts was used. 0.002% of DPPH was prepared in methanol and 1ml of this solution was mixed with 1ml of sample solution (10-50µg/ml) and standard solution separately. These solution mixtures were kept in dark for 30 minutes and optical density was measured at 517 nm using UV-Vis-Spectrophotometer model –LT-290 (Labtronics). Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was use as blank. The optical density was recorded and % inhibition was calculated using the formula below:

Percent (%) inhibition of DPPH activity = $(\frac{A-B}{A})$ X 100

Where A = optical density of the blank and B = optical density of the sample.

IC_{50} value was determined by plotting a graph of percentage inhibition against concentration.

Statistical analysis

All the experimental results were as mean ± SD of three parallel measurements. The data was entered into a Microsoft Excel© database and analyzed using Logit and Probit anlysis for the LC_{50} values and IC_{50} values were obtained by the linear regression analysis. Extracts giving LC_{50} values lower than 30 µg/ml were considered to be toxic. The extracts with IC_{50} values lower than 50 µg/ml showed antioxidant activity.

Results:

Table 1. Brine shrimp cytotoxicity test for the leaves and stem bark of *Terminalia Schimperiana Hostch* with different extract.

Plant parts	Leaves. (conc. µg/ml)		Stem bark. (conc. µg/ml)	
Extracts	LC50 (LF)	LC50 (PF)	LC50 (LF)	LC50 (PF)
Water	285.8	290.6	843.3	828.9
Ethanol	68.1	68.4	32.3	32.2
Ethyl acetate	640.2	640.3	45.9	45.6
Acetone	46.0	45.6	843.3	828.9
Hexane	482.4	486.8	80.8	80.8

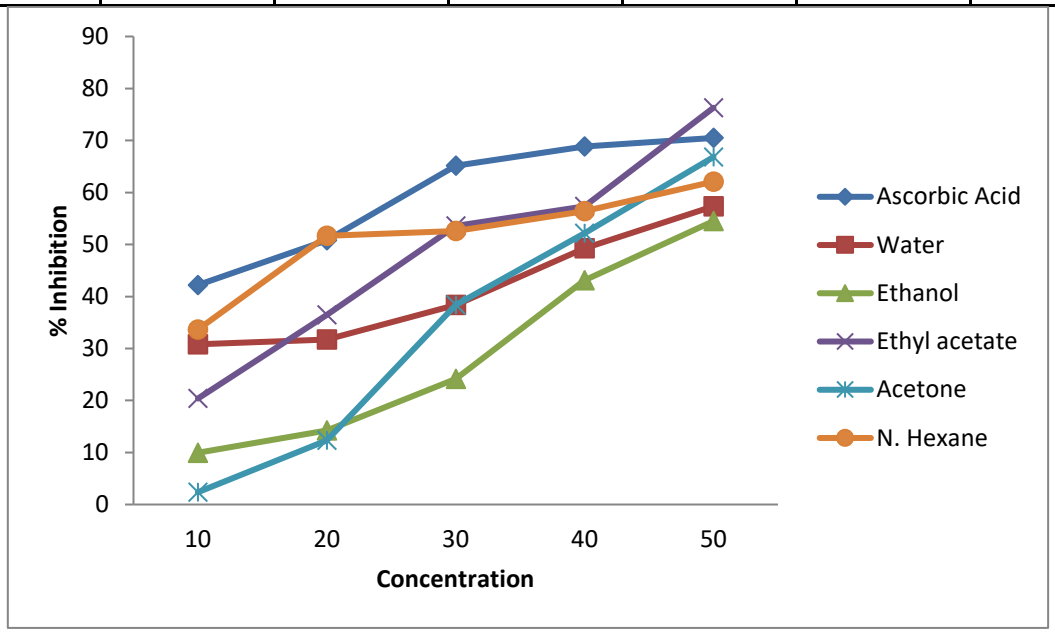
Key:PF = Probit function. LF = Logit function. LC_{50} = 50% Lethal concentration.

Table 2. **Percentage inhibition in various concentrations of leaves extracts of schimperiana by UV visible spectrophotometerat 517nm. (DPPH scavenging assay method).**

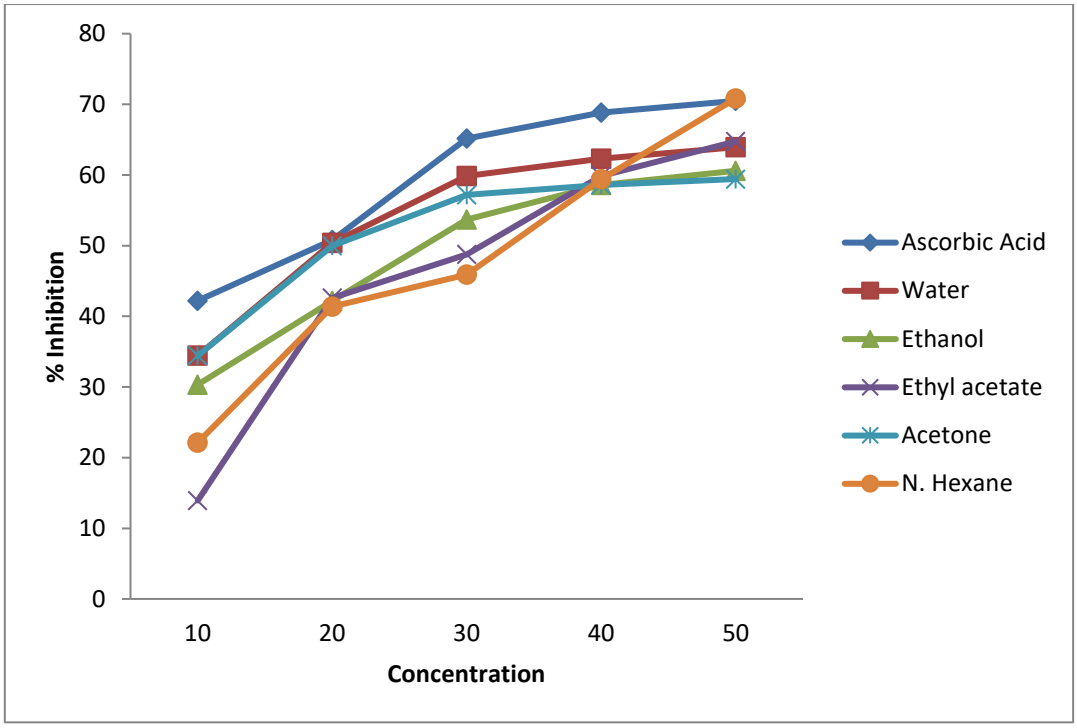
Conc. in µg/ml	Ascorbic Acid	Water	Ethanol	Ethyl acetate	Acetone	N. Hexane
10	42.21	34.43	30.33	13.93	34.43	22.13
20	50.82	50.41	42.21	42.62	50	51.39
30	65.16	59.84	53.69	48.77	57.17	45.9
40	68.85	62.3	58.61	59.84	58.61	59.43
50	70.49	63.93	60.6	64.75	59.43	70.82

Table 3. **Percentage inhibition in various concentrations of stem bark extracts of schimperiana by UV visible spectrophotometerat 517nm. (DPPH scavenging assay method).**

Conc. in µg/ml	Ascorbic Acid	Water	Ethanol	Ethyl acetate	Acetone	N. Hexane
10	42.21	30.81	9.95	20.38	2.37	33.65
20	50.82	31.75	14.22	36.49	12.32	51.66
30	65.16	38.39	24.17	53.55	38.39	52.61
40	68.85	49.29	43.13	57.35	52.13	56.4
50	70.49	57.35	54.5	76.3	66.82	62.09



Percentage inhibition in various concentrations of leaves extracts of schimperiana by UV visible spectrophotometerat 517nm. (DPPH scavenging assay method).



Percentage inhibition in various concentrations of stem bark extracts of schimperiana by UV visible spectrophotometerat 517nm. (DPPH scavenging assay method).

Table 4. IC_{50}: Inhibition concentration at 50 percent for the leaves extracts

Extracts	Ascorbic Acid	Water	Ethanol	Ethyl acetate	Acetone	N. Hexane
IC_{50}	18.5	41.29	46.88	30.87	39.09	28.27

Table 5. IC_{50}: Inhibition concentration at 50 percent for the stem bark extracts

Extracts	Ascorbic Acid	Water	Ethanol	Ethyl acetate	Acetone	N. Hexane
IC_{50}	18.5	25.04	31.08	33.00	27.42	31.74

Discussion

Cytotoxicity of plant extracts

Results of the toxicity of the extracts against brine shrimp (LC_{50} values) are shown in Table 1. A total of 20 different extracts for both the leaves and the stem bark were tested for their toxicity against the brine shrimp using the brine shrimp lethality assay. The results shows that all the extracts are less toxic against the brine shrimp as only extracts giving LC_{50} values lower than 30 µg/mL were considered to be toxic.(Wanyoike et al,2004). Only the extract of ethanol (stem bark) showed little toxicity against brine shrimp (LC_{50} 32.3 and 32.2µg/mL) for both logit and Probit function.

Antioxidant activity of the plant extract.

Antioxidant activity (DPPH) free radical scavenging activity) result shows that both leaves and bark extract shows antioxidant activity when compared with reference standard ascorbic acid. They exhibit strong antioxidant DPPH radical scavenging activity with IC_{50} ranging from 28.27 to 46.88µg/ml for the leaves and 25.04 to 33.00 for stem bark extract respectively. Although less potent when compared with ascorbic acid which is 18.5 µg/ml, this is due to the fact that the extract is a mixture of a great number of compounds as opposed to pure ascorbic acid used as standard reference. Water extract (stem bark) with IC_{50} value of 25.04 µg/ml has better antioxidant activity among the extracts. The activity of the extracts may be due to the presence of some active compounds in the plant such as flavanoid, alkaloids, saponin, phenols tannin cyanogenic gucoside and carotenoid.(Mshelia, *et al*, 2016).

Recommendations.

It is recommends that further investigations be carried out for chemical characterization of the active compounds and more comprehensive biological assays for the purpose of producing more potent antibacterial drugs from the plant. Analysis on the plant roots should also be considered for the same purpose.

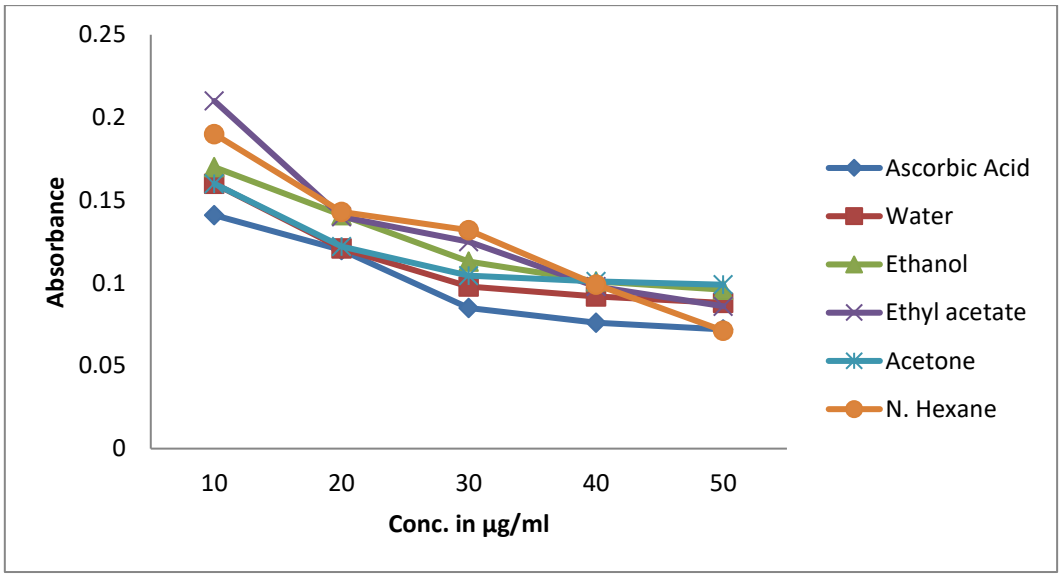


Chart 1. Absorbance against concentrations of leaves extracts of T. schimperiana by UV visible spectrophotometerat 517nm. (DPPH scavenging assay method).

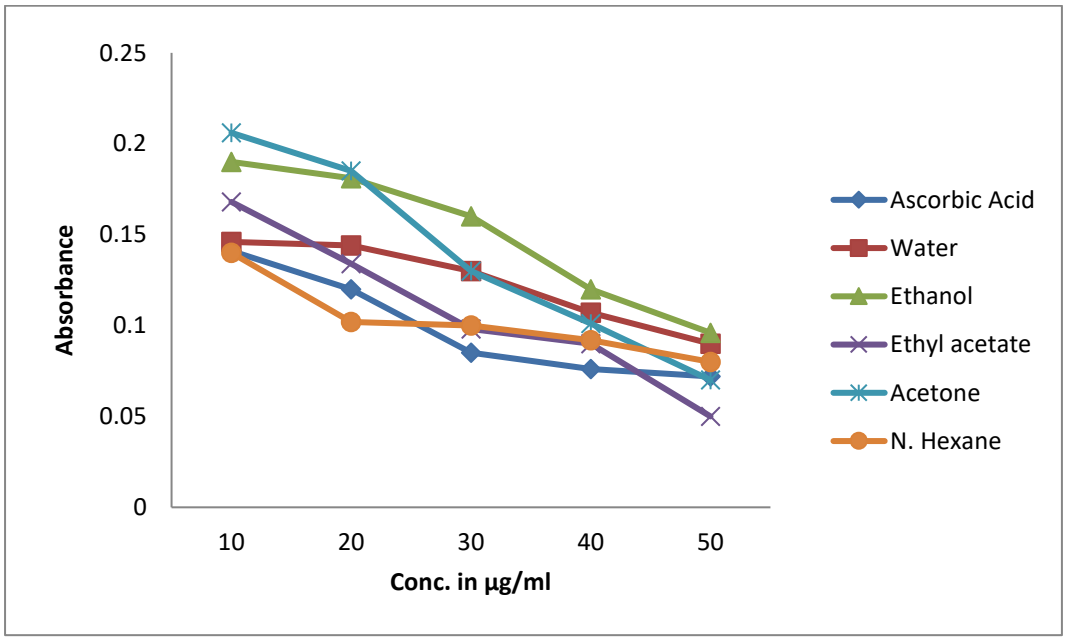


Chart 2. Absorbance against concentrations of stem bark extract of T. schimperiana by UV visible spectrophotometerat 517nm. (DPPH scavenging assay method).

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