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Phytochemical, Cytotoxicity and Free radical scavenging activities of *Acalypha torta* leaf extracts (Euphorbiaceae)

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ABSTRACT

Phytochemical screening of the leaf methanolic extract of Acalypha torta revealed the presence of alkaloids, flavonoids, tannins, resins, glycosides, saponins and carbohydrates. The crude methanolic extract was partitioned successively in n-hexane, ethyl acetate and butanol to give different fractions. Brine shrimp lethality test (cytotoxicity) carried out on the methanol extract and each of the fractions obtained, gave lethal doses: LC_{50} of 6.9030 µg/ml (hexane fraction), 45.0958 µg/ml (ethyl acetate fraction), 0.7210 µg/ml (butanol fraction) and 0.0002 µg/ml (methanol), indicating their toxicity. $LC_{50} \geq 1000$ µg/ml is considered to be non-toxic. The free radical scavenging activity of A. torta was determined by three methods not yet reported in literature for this plant namely: scavenging effect on 2,2-diphenyl-1-picryhydrazyl radical (DPPH), hydroxyl radical and peroxide oxidation by ferric thiocyanate method. Comparison of the results obtained with the three antioxidant standards used in the assay revealed that the fractions possessed antioxidant activity. Butylated hydroxyl anisole (BHA), ascorbic acid and α-tocopherol were used as reference standards. The results obtained support the ethno medicinal applications of Acalypha torta.

Keywords: Cytotoxicity, free radicals, *Acalypha torta*, 2, 2-diphenyl-1-picrylhydrazyl radical, hydroxyl radical, ferric thiocyanate.

INTRODUCTION

The medicinal properties of plants have not been sufficiently harnessed. The difficulty encountered with alternative medicine has been that of reliable documentation of known traditional herbal medicine since uses vary from tribe to tribe. Indigenous medical practices have been the subject of much attention in the literature of various disciplines to date but the specificity, mode of action and clinical efficacy of most traditional plants have not been established in a manner consistent with standards of modern pharmacognosy and pharmacology. Alternative medicine however, cannot be discredited since a large number of modern day medicines have their origin from plants. For instance, the drugs used for treatment of malaria, a

prevailing ailment in tropical Africa: chloroquine and more recently artemisinin have their origin from plant sources - from *Cinchona* bark and *Artemisia annua*, respectively [1-5].

The plant *Acalypha torta* belongs to the family Euphorbiaceae which is a family of dicotyledonous plants that includes shrubs and trees. They are primarily found in the tropical region of Africa. Euphorbiaceae plants have been intensively investigated and contain alkaloids, saponins, flavonoids, tannins, resins and carbohydrate amongst others. Various pharmacological activities have been reported for some *Acalypha* species. *A. wilkesiana* have been reported to have *in vitro* antimicrobial activities. Antioxidant activities have also been reported in *A. guatemalensis* and *A. hispida* [6,7]. *Acalypha torta* is used medicinally for the treatment of some fungal skin diseases. The plant is also useful traditionally in the treatment of neonatal jaundice [8-11]. Oke and Hamburger [12] reported the *in vitro* antioxidant activity of *A. torta* using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) only; the experiment was only qualitative and was not conclusive. The aim of this research work however is to determine the toxicity of the plant using Brine shrimp larvae eggs and to subject the fractions to free radical scavenging activity. The quantitative antioxidant property was determined by three methods not yet reported in literature for this plant namely: scavenging effect on 2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH), hydroxyl radical generated by hydrogen peroxide and peroxide oxidation by ferric thiocyanate method. Butylated hydroxylanisole (BHA), ascorbic acid and α-tocopherol were used as reference standards. The mechanism of action of the antioxidant effect of *A. torta* was also determined in this assay [13-15].

MATERIALS AND METHODS

Chemicals and Reagents

Hexane, ethyl acetate, methanol, butanol, chloroform, hydrochloric acid, ammonia solution, naphthol, bismuth nitrate, potassium iodide, sodium hydroxide, copper acetate, NaOH, sodium chloride, copper sulphate pentahydrate, ferric chloride, conc. tetraoxosulphate (VI) acid, Conc. HCl, ammonia solution, sodium potassium tartarate, linoleic acid, ammonium thiocynate, ethanol, ferrous chloride, hydrochloric acid, potassium chloride, glacial acetic acid, disodium hydrogen phosphate, and dihydrogen potassium phosphate were all BDH general purpose chemicals and distilled prior to use. Dimethylsulphoxide (M&B, England), hydrogen peroxide (Merck, Germany) and 2, 2 - diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, butylatedhydroxylanisole (BHA) and α-tocopherol were obtained from Sigma Chemical Co (St Louis, MO). Brine shrimp larvae eggs were obtained from Ocean Star International, Inc. Company, USA.

Equipment and Apparatus

Soxhlet apparatus, Mettler analytical balance H80 (UK), Water Bath (Gallenkamp), Rotavapor RII0 (Buchi, England), silica gel GF_{254} (precoated aluminium sheets - Merck Germany), pH meter (Jenway model), UV-Visible spectrophotometer (UV-Visible spectrophotometer (UVD-2960 model equipped with a UVWIN software version LABOMED INC, USA).

Plant collection and identification

Fresh leaves of *A. torta* was identified by Mr Donatus of Department of Botany and Microbiology of the Faculty of Science, University of Ibadan and were collected at Botanical Gardens, University of Ibadan, Oyo State in June, 2010 and confirmed at the Federal Research Institute, Ibadan (FRIN) where a voucher specimen is deposited (FHI 107324). .

Reference Standards
Ascorbic acid, Butylated hydroxyanisole (BHA) and α-Tocopherol for antioxidant activity. Dimethylsulphoxide (DMSO) for toxicity studies.

Sample preparation
Fresh leaves of *A. torta* was collected, weighed and air-dried for 3 weeks until the weight was constant and then pulverized using mill machine. The pulverized samples were weighed and kept for further analysis.

Extraction/ partitioning procedure
The dried plant material (2 kg) was extracted with 5l of methanol using soxhlet apparatus. The extracts were collected and concentrated with the aid of a Bucchi rotavapor and stored in a desiccator prior to analysis. Thin Layer Chromatography (TLC) was employed using silica gel 60 F_{254} precoated plates and solvent system: Ethyl acetate/methanol (8:2) to detect antioxidant activity with 2, 2-diphenyl-1-picrylhydrazylradical (DPPH) as a spray reagent. Yellow coloration on the spots on the TLC plates indicated that the methanolic extract of *A. torta* had antioxidant activity. The crude methanolic extract was then partitioned successively in hexane, ethyl acetate and butanol. Thereafter, toxicity test using Brine shrimp lethality assay and free radical scavenging activity test were carried out on the fractions using the following spectrophotometric experiments: scavenging effect on DPPH, scavenging effect on hydroxyl radical generated by hydrogen peroxide and peroxide oxidation by ferric thiocyanate method.

Phytochemical screening
For the purpose of this study, phytochemical screening was carried out on the crude methanol extract obtained to confirm the presence or absence of the following plant secondary metabolites: alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides, reducing sugars, anthraquinones, carbohydrates, resin and cardiac glycosides [16].

Cytotoxicity analysis
Brine shrimp lethality test
The brine shrimp lethality test (BST) was used to determine the toxicity of the fractions [17]. The shrimp's eggs were hatched in sea water for 48 h at room temperature. The nauplii (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the extracts were made in DMSO, at varying concentrations (1000, 100, and 10 µg/ml) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24 h the vials were examined against a lighted background and the average number of larvae that survived in each vial was determined. The concentration at fifty percent mortality of the larvae (LC_{50}) was determined using the Finney computer programme [18,19].

Antioxidant activities of *Acalypha torta* extracts
Scavenging Effect on DPPH
The antioxidant activity or the capacity to scavenge the "stable" free radical DPPH was determined using the DPPH free – radical scavenging method. A 3.94 mg of 2, 2-diphenyl-1-picryhydrazyl radical (DPPH), a stable radical was dissolved in methanol (100ml) to give a 100 µm solution. To 3.0 ml of the methanolic solutions of DPPH was added 0.5 ml of each of the fractions with doses ranging from 1.0 mg/ml to 0.0625 mg/ml [19-21]. The decrease in absorption at 517 nm of DPPH was measured 10 minutes later. The actual decrease in absorption was measured against that of the control and the percentage inhibition was also calculated. The same experiment was carried out on butylated hydroxylanisole (BHA), α-tocopherol and

ascorbic acid which are known antioxidants. All test and analysis were run in triplicates and the results obtained were averaged. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below:

% inhibition = $\{(A_{DPPH} - A_S)/A_{DPPH}\}$ x 100

Where A_S is the absorbance of the solution and A_{DPPH} is the absorbance of the DPPH solution [22]

Scavenging Effect on Hydrogen Peroxide

Spectrophotometric determination of the extracts of *A. torta* was carried out at 285 nm. A solution of 2 mM hydrogen peroxide was prepared in phosphate buffered-saline (PBS) pH 7.4. The fractions at the following concentrations; 0.1- 0.00625 mg/ml was added to the H_2O_2 solution. Decrease in absorbance of H_2O_2 at 285nm was determined spectrophotometrically 10 minutes later against a blank solution containing the test extract in PBS without H_2O_2. All tests were run in triplicates and averaged [23,24]. The same experiment was carried out on Butylatedhydroxyanisole (BHA), ascorbic acid and α-tocopherol which are known antioxidant standards.

Antioxidant activity by ferric thiocyanate method

The antioxidant activities of hexane, ethyl acetate and butanol fractions of the plant material were determined by ferric thiocyanate method [25]. 10 mg of each extract was dissolved separately in 99.5% of ethanol and various concentrations (50, 100, 250, 500 µg/ml) were prepared. A mixture of a 2 ml of sample in 99.5% ethanol, 2.0 ml of 2.51% linoleic acid in 99.5% ethanol, 4 ml of 0.05 M phosphate buffer (pH 7.0) and 2 ml of water was placed in a vial with a screw cap and placed in an oven at 60oC in the dark. To 0.1 ml of this sample solution, 10 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate was added. After the addition of 0.1 ml of 2 x 10-2 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red colour developed was measured in 3 min at 500 nm. The control and standards were subjected to the same procedures as the sample, except that for the control, only solvent was added, and for the standard, sample was replaced with the same amount of Butylatedhydroxyanisole (BHA), ascorbic acid and α-tocopherol (reference compounds) [26]. All test and analysis were run in triplicates and the results obtained were averaged. The inhibition of lipid peroxidation in percentage was calculated using this equation:

% Inhibition = 1 - (A1/A2) X 100

Where A1 is the absorbance of the test sample and A2 is the absorbance of control reaction.

RESULTS AND DISCUSSION

The methanol extract of *A. torta* was found to contain alkaloids, flavonoids, tannins, carbohydrate, glycosides, saponins and resins. These secondary metabolites exhibit various biological activities such as anti-microbial and anti-oxidation activities which have been associated with their intrinsic reducing capability as pro-oxidants. The presence of these secondary metabolites especially alkaloids and flavonoids justifies the use of *Acalypha torta* in ethno medicine.

Brine shrimp lethality test

The extracts of *A. torta* (methanol, hexane, ethylacetate and butanol) were toxic to brine shrimp larvae having very low LC_{50} (Lethal concentration) values which were indicative of high level of toxicity. $LC_{50} \geq 1000$ µg/ml is considered to be non-toxic (Table 1). Toxicity level as determined by Finney computer programme gave the following lethal concentration: methanol (LC_{50} of 0.0002 µg/ml), hexane extract (LC_{50} of 6.9030 µg/ml), ethyl acetate extract (LC_{50} of 45.0958 µg/ml) and butanol extract (LC_{50} of 0.7210 µg/ml). The result indicated that the methanol extract of the leaves of *A. torta* was the most toxic (0.0002µg/ml) while the ethyl acetate fraction of the leaves was the least toxic. The result corroborated the presence in the plant of medicinally active compounds. Toxic chemical compounds are beneficial in the therapy of some ailments involving cell or tumour growth. It had also been observed by previous workers that medicinally active natural products are most times toxic to *Artemia silina* nauplii [27,28].

Table 1: Results of Brine shrimp lethality test of *Acalypha torta* leaf extracts*

Conc.	10000ppm		1000ppm		100ppm		Control		
Extracts	S	D	S	D	S	D	S	D	LC_{50} (µg/ml)
Methanol	1	29	6	24	3	27	10	0	0.0002
Hexane	0	30	2	28	6	24	10	0	6.9030
Ethylacetate	0	30	3	27	11	19	10	0	45.0958
Butanol	5	25	9	21	9	21	10	0	0.7210

* LC_{50} < 1000 µg/ml =Toxic, LC_{50} > 1000 µg/ml = Not Toxic. S- Survivor, D-Death

Antioxidant Activity

The antioxidant activities of the methanol, hexane, ethyl acetate and butanol extracts of *A. torta*, were determined by three methods: scavenging effect on 2, 2-diphenyl-1-picryhydrazyl radical (DPPH), hydroxyl radical generated by hydrogen peroxide and ferric thiocyanate (FTC) method. The results are presented in Tables 2-4 and Figures 1-3.

Scavenging effects on DPPH

2,2-diphenyl-1-picryhydrazyl radical (DPPH) is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [24]. At 517 nm, the absorbance of the DPPH solution (2,2-diphenyl-1-picryhydrazyl radical solution) was 0.933 nm. The reduction in absorbance of DPPH at 517nm caused by the samples was measured in triplicate after 10min. The tested samples showed very good activity when compared to the standards used (Table 2). There was decrease in absorption at 517 nm indicating that the fractions have hydrogen donating ability or can scavenge free radicals. From our analysis, a larger percentage of the samples showed the ability to scavenge the free radical used in a concentration dependent manner. The observation was further corroborated by the calculated percentage inhibition. Fractions from *A. torta* have good activities as free radical scavengers when compared with controls: ascorbic acid, butylatedhydroxylanisole (BHA) and α –Tocopherol (Table 2). The fractions gave %inhibition of 70 - 93% at 1.0 – 0.0625 mg/ml. The activity is however lower than that of BHA but better than that of ascorbic acid and α –tocopherol. The hexane fraction showed better inhibition than the entire tested fractions at 0.125-1.0 mg/ml (Figure 1). The presence of flavonoids may have been responsible for the observed activity [29,30]. The biological functions of flavonoids include protection against allergies, inflammation, free radicals scavenging, platelets aggregation, microbes, ulcers, hepatoxins, viruses and tumours.

Table 2: Absorbance values from scavenging effect of extracts from *Acalypha torta* on DPPH at 517 (nm)*

Conc. (mg/ml)	C.E	H. F	E. F	B. F	Vit. C	BHA	α-TCP
1.0	0.066±0.002	0.061±0.004	0.007±0.002	0.146±0.003	0.217±0.001	0.053±0.001	0.205±0.001
0.5	0.083±0.003	0.006±0.004	0.149±0.001	0.201±0.001	0.246±0.001	0.025±0.001	0.298±0.001
0.25	0.087±0.007	0.068±0.002	0.149±0.001	0.203±0.004	0.386±0.005	0.094±0.001	0.930±0.000
0.125	0.111±0.001	0.068±0.002	0.161±0.003	0.243±0.002	0.091±0.002	0.018±0.001	0.840±0.000
0.0625	0.169±0.001	0.125±0.005	0.175±0.004	0.293±0.020	0.068±0.001	0.034±0.002	0.734±0.001

***DPPH scavenging activity of extracts and standards at 517 nm. Conc. = concentration, C.E = Crude extract (methanol), H.F = n-hexane fraction, E.F = Ethyl acetate fraction, B.F = Butanol fraction, Vit. C = Ascorbic acid, BHA = Butylated hydroxyl anisole, α-TCP = α-Tocopherol*

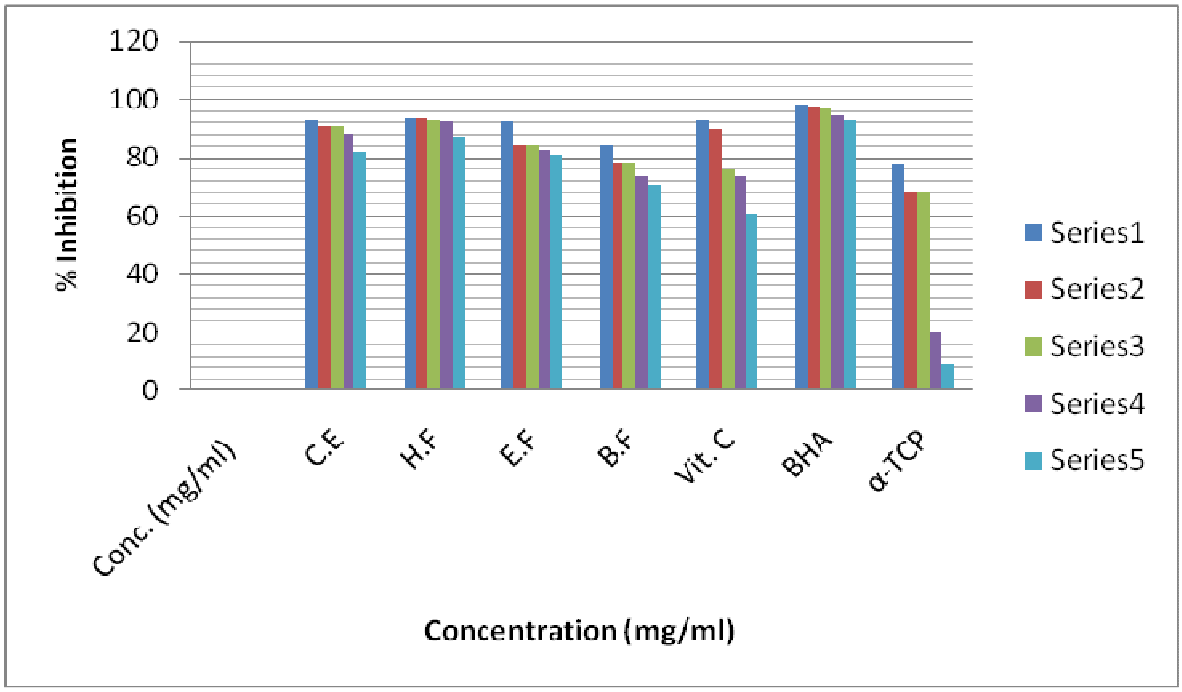

Figure 1: DPPH Free radical scavenging activity of extract from the leaves of *Acalypha torta*

C.E = Crude extract (methanol), H.F = n-hexane fraction, E.F = Ethyl acetate fraction, B.F = Butanol fraction, Vit. C = Ascorbic acid, BHA = Butylated hydroxyl anisole, α-TCP = α-Tocopherol. Series 1 = 1.0 mg/ml, series 2 =0.5 mg/ml, series 3 = 0.25 mg/ml, series 4 = 0.125 mg/ml, series 5 = 0.0625 mg/ml.

Scavenging effects on Hydrogen peroxide (H_2O_2)

The scavenging activities of fractions and antioxidants standards, ascorbic acid, Butylated hydroxyanisole (BHA) and α-tocopherol on H_2O_2 is shown in Table 3. Scavenging effects on H_2O_2 was measured in triplicates after 10min of incubation at 285nm.

Table 3: Scavenging Effect of hydroxyl radical generated by H_2O_2 of the Leaf Extract of *Acalypha torta* at 285nm*

CONC (mg/ml)	A_1	A_2	A_3	A_4	ASCORBIC ACID	BHA	ALPHA TOCOPHEROL
0.1	0.298±0.001	0.509±0.007	0.292±0.001	0.252±0.000	0.1952±0.001	0.0413±0.016	0.0321±0.045
0.05	0.165±0.001	0.258±0.001	0.146±0.018	0.137±0.053	0.2078±0.012	0.0617±0.019	0.0633±0.032
0.025	0.294±0.018	0.169±0.001	0.075±0.001	0.084±0.013	1.2645±0.119	0.0740±0.015	0.1552±0.061
0.0125	0.247±0.019	0.116±0.000	0.059±0.002	0.052±0.001	2.7586±0.049	0.0947±0.003	0.1807±0.015
0.00625	0.164±0.046	0.131±0.000	0.060±0.001	0.034±0.000	2.9236±0.211	0.1126±0.014	0.4940±0.017

**Absorbance measurement of A1 (butanol fraction), A2 (ethyl acetate fraction), A3 (n-hexane fraction), A4 (Crude methanol extract), Ascorbic Acid, BHA and α- Tocopherol at 285nm measured in triplicate.*

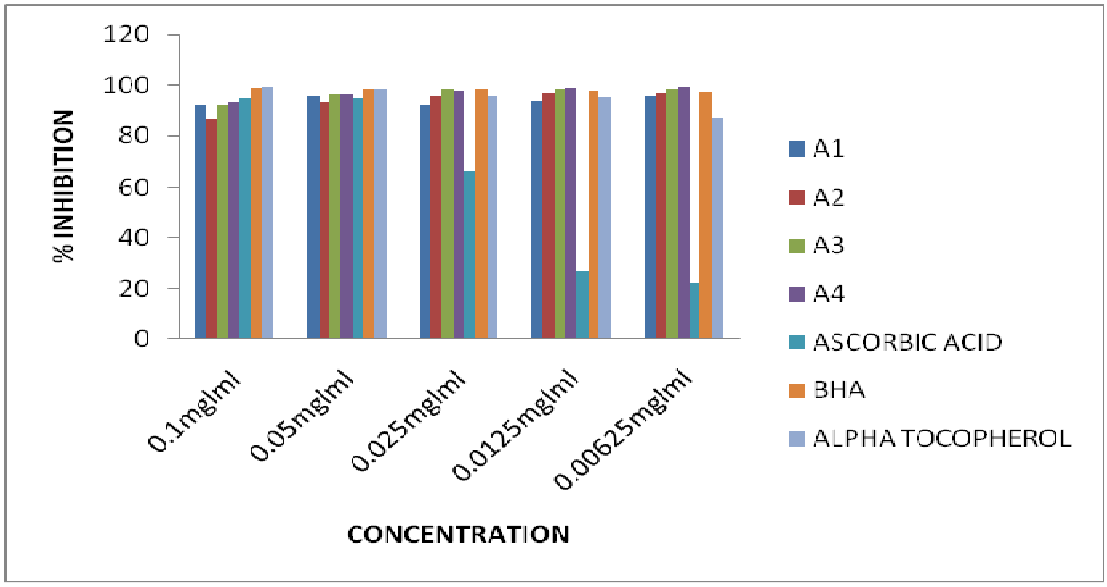

Figure 2: H_2O_2 Free radical scavenging activity of the extracts from the leaves of *Acalypha torta* and standards at 285 nm. A1 (Butanol fraction), A2 (Ethyl acetate fraction), A3 (n-Hexane fraction), A4 (Crude methanol extract)

Scavenging of hydroxyl radical generated from hydrogen peroxide by fractions from *A. torta* indicated that at concentration of 0.1 - 0.0065 mg/ml, the fractions had high scavenging activities when compared to standards. The % inhibition was between 86-98% at all the concentrations used (Fig 1). Activity was found to be better especially with the n-hexane fraction. *A. torta* therefore is a source of antioxidant compounds especially in scavenging the highly reactive hydroxyl radicals. It has been observed that H_2O_2 through the Fenton reaction is an active - oxygen specie and has potential to produce the highly reactive hydroxyls radical which are often involved in free radical chain reactions known to cause damage to biological macromolecules. Thus plants with ability to scavenge these radicals will alleviate the oxidative diseases [31,32].

Table 4: Peroxide oxidation of fractions from *Acalypha torta* Extract at 500 nm using the Ferric thiocyanate method*

CONC (mg/ml)	A_1	A_2	A_3	A_4	ASCORBIC ACID	BHA	ALPHA TOCOPHEROL
0.8	0.029±0.000	0.120±0.006	0.274±0.009	0.350±0.121	0.173±0.008	0.326±0.006	0.133±0.004
0.4	0.031±0.002	0.085±0.030	0.430±0.201	0.447±0.063	0.173±0.008	0.375±0.008	0.164±0.006
0.2	0.032±0.001	0.063±0.005	0.489±0.021	0.471±0.016	0.245±0.008	0.431±0.008	0.184±0.009
0.1	0.034±0.001	0.062±0.001	0.515±0.253	0.535±0.010	0.275±0.006	0.616±0.005	0.195±0.023
0.05	0.036±0.002	0.056±0.003	0.528±0.130	0.575±0.047	0.287±0.050	0.647±0.004	0.294±0.004
0.025	0.040±0.002	0.053±0.015	0.619±0.214	0.582±0.080	0.367±0.004	0.653±0.008	0.340±0.069
0.0125	0.059±0.005	0.050±0.015	0.640±0.262	0.681±0.089	0.516±0.008	0.747±0.003	0.360±0.005
0.00625	0.060±0.003	0.028±0.002	0.694±0.098	0.690±0.089	0.668±0.002	0.750±0.001	0.377±0.008

**Absorbance measurement of A1 (butanol fraction), A2 (ethyl acetate fraction), A3 (n-hexane fraction), A4 (Crude methanol extract), Ascorbic Acid, BHA and α- Tocopherol at 500 nm measured in triplicate.*

Antioxidant activity by Ferric thiocyanate method (FTC)

The Ferric thiocyanate method was used to determine the amount of peroxide which oxidized ferrous chloride ($FeCl_2$) to a reddish ferric chloride ($FeCl_3$) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases. Crude methanol extract, hexane, ethyl acetate and butanol fractions at various concentration (0.00625 – 0.8 mg/ml), showed antioxidant activities in a concentration dependent manner (Table 4). However, butanol fraction at all the concentration showed an antioxidant activity (91-96%) better than the activities of all the reference compounds, ascorbic acid, BHA and α- tocopherol. It has been observed that the more polar extracts exhibited stronger activity (with reference to organic solvents used for

extraction), indicating that highly polar organic compounds may play important roles in the activities [33]. This result has given an indication that the most polar butanol fraction of *A. torta* is able to scavenge the highly reactive hydroxyl radical through peroxide oxidation unlike in the hydrogen peroxide assay where the non polar n-hexane fraction inhibits or scavenge hydroxyl radical better than the entire fractions. This analysis has shown to us that different mechanism exist in the free radical scavenging activities of the plant extracts and that polarity (and as a result, the solvent used for extraction) also play a very important role.

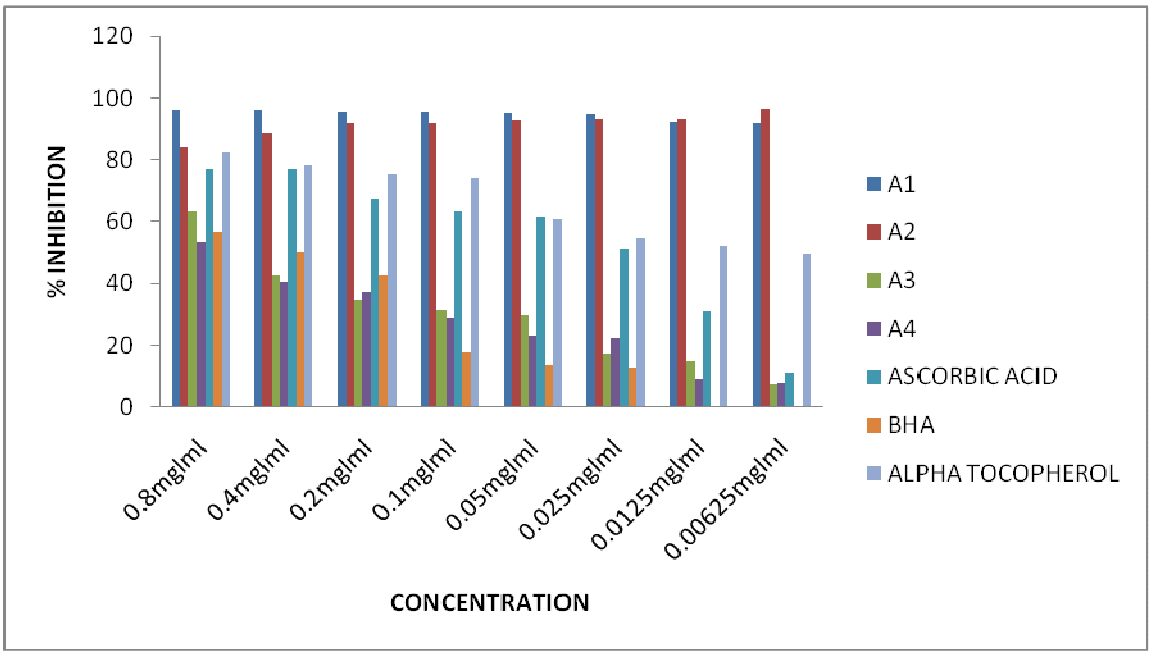

Figure 3: Peroxide oxidation of the extracts from the whole plant of *Acalypha torta* and standards at 500 nm. A1 (Butanol fraction), A2 (Ethyl acetate fraction), A3 (n-Hexane fraction), A4 (Crude methanol extract).

CONCLUSION

Brine shrimp lethality test showed that the extracts from the plant *Acalypha torta* were toxic, all having LC_{50} values much less than 1000 µg/ml. Alkaloids, flavonoids, tannins, resins, glycosides, saponins and carbohydrates are the major secondary plant metabolites found in *A. torta*. The high antioxidant activity of the plant at low concentration shows that the plant could be very useful for the treatment of ailments resulting from oxidative stress such as Parkinson's disease, Alzheimer's disease, cancer, cardiovascular disorders, bacterial and viral infections and inflammation, coronary heart disease and stroke. The presence of secondary plant metabolites like alkaloids and flavonoids and the toxicity results corroborated the ethno medicinal importance of *A. torta*. Further studies therefore need to be carried out to isolate the active compounds responsible for the observed activities as this could be a potential source of useful drugs.

REFERENCES

[1] T Swain; Plants in the Development of Modern Medicine. Harvard University press; Cambridge, **1988,** 940- 955.
[2] G Abdul; The State of Medicinal Plants Research in Nigeria. Sofowora, A. (ed). Unife Press; Ile-Ife, **1989**, 56-78.

[3] M Hamburger and K Hostettmann; *Phytochemicals*, **1991,** 3864-3874.
[4] JS Glasby; Dictionary of plants containing Secondary Metabolites. Burgess Science press; Great Britain, **1991**, 172.
[5] P Andrew; The constituent of medicinal plants Kerry Bone publication; UK Third Edition, **2009,** 56 – 86.
[6] PA Onocha; GK Oloyede and OA Bamkole; *European Journal of Scientific Research* **2011**, 49(3): 322-321.
[7] PA Onocha; GK Oloyede and QO Afolabi; Experimental and Clinical sciences (EXCLI) Journal **2011**, 10:1-8.
[8] HM Burkill; Useful plants of West Tropical Africa Second edition, vol. 1, Royal Botanical Gardens; Kew, **1987**,88-91.
[9] PI Akube; Nigerian medicinal plants Pharmacology and toxicology; the state of medicinal plant research in Nigeria. **1990**,53-54.
[10] GM Cragg; DJ Newmann and KM Snader; *Journal of Natural Products* **1997**,60: 52-60.
[11] SK Adesina; O Idowu; AO Ogundaini; H Oladimeji; TA Olugbade and GO Onawunmi; *Phytother Res*. **2000,** 14: 371-4.
[12] JM Oke and MO Hamburger; *African journal of Biomedical Research* **2002**, 5: 77-79.
[13] W Bors and M Saran; *Horticultural science*. **1991,** 26: 66-68.
[14] H Sies; Oxidative stress: Introductory remarks. In: Sies H, editor. Oxidative stress, FL academic press; Orlando **1997**, 3-18.
[15] II Koleva; TA Van- Beck and A Evstaliva; *Phytochem.Anal*. **2002**, 13: 8-17.
[16] JB Harborne; "Phytochemical Methods" Harborne JB ed. Chapman & Hall; London **1998**.
[17] BN Meyer; RN Ferrign; JE Putnam; LB Jacobson; DE Nicholas and JL McLaughlin; *Planta Medica* **1982**,45: 31-34.
[18] GK Oloyede; MJ Oke; Y Raji and AT Olugbade; *World Journal of Chemistry* **2010**, 5(1): 26- 31.
[19] MO Falope; H Ibrahim and Y Takeda; *International Journal of pharmacognosy* **1993**,37 (4): 230-254.
[20] IM Gulcin; OK Oktay and A Aslan; *Ach. Journal of Ethnopharmacology* **2002**, 79: 325-329.
[21] AF Mutee; SM Salhimi; MF Yam; CP Lim; GZ Abdullah; OZ Ameer; MF Abdulkareem and MZ Asmawi;. *International Journal of Pharmacology* **2010**, 6: 686-690.
[22] T Hatano; H Kagawa; T Yasuhora and T Okuta; *Chemical and Pharmaceutical Bullettin* **1988**, 36: 200-209.
[23] GK Oloyede and OE Farombi; *World Journal of Chemistry* **2010**, 1(5): 32-36.
[24] JR Soares; TC Dinis; AP Cohn and LM Almeida; *Free Radical Research* **1997**,26: 469-478.
[25] R Mackie and C McCartney; In Practical Medicinal Microbiology 3rd edtion, Churchill Livingstone (Publishers); London, UK. **1989,** 2: 100- 491.
[26] GK Oloyede; PA Onocha; J Soyinka; O Oguntokun and E Thonda; *Annals of Biological Research* **2010**,1 (2): 114-120.
[27] P Dvorack; E Sucman; D Cervenakona; K Korinnek and R Blechova; Utilization of bioassay with *Artemia Silina* in Pharmacology. In: Collection of abstracts from 9th Conference on toxicity biodegradability of wastes and chemicals significant in aquatic environment. Solan, Czech Republic: **1999**,9-15.
[28] EO Ajaiyeoba; PA Onocha; SO Nwozo and W Sama; Fitoterapia **2003**, 72 (7-8): 706-709
[29] Y Gow-chin and D Pin-Der; *Journal of Agriculture and Food Chemistry* **1994**,42: 629 - 632.
[30] L Alan and ND Miller; *Alternative Medicine* **1996**,1: 103-111.
[31] M Namiki; *Crititical Review of Food Science and Nutrition.* **1990**,29 (4): 273 - 300.

[32] A Lugasi; P Honvahorich (deceased) and A Dworschark; *Phytotherapy Research* **1999**, 13: 160- 162.
[33] G Wolf; *J of Nutr*. **2005**, 135 (3): 363–6.