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Phytochemical Investigation of methanolic fractions of *Acalypha ornata* (Hochst) leaves for antioxidant and toxicity activities

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ABSTRACT

Secondary metabolites of medicinal importance phenolics, resins, flavonoids, steroids, tannins and carbohydrates were obtained in the crude methanolic extract of Acalypha ornata (Hoscht). The methanolic extract was separated into 262 fractions by using a mixture of hexane, ethylacetate and methanol and silica gel 70-230 mesh as adsorbent in a gradient elution - column chromatographic technique. The bulked fractions were subjected to antioxidant screening and brine shrimp lethality assay. The activity of the fractions was compared with three antioxidant agents; ascorbic acid, butylatedhydroxyanisole (BHA) and α-tocopherol. Samples W_4, W_5, W_6, W_7 and W_{10} possessed high percentage inhibition than vitamin C at 1.0 to 0.0625 mg/ml. BHA showed a generally high percentage inhibition than all test samples at 1.0 and 0.5 mg/ml while fractions W_4, W_5 and W_6 possessed weak free radical scavenging activity. The better scavenging activity of A. ornata could be linked to the presence of secondary plant products like flavonoids and phenols which have ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals. Brine shrimp lethality test showed that fractions W_{10}, W_{16} and W_{17} were not toxic as the LC_{50} (μg/ml) values were greater than 1000 μg/ml at 95% confidence level.

Key words: *Acalypha ornata*, antioxidant, toxicity, 2, 2-Diphenyl-1-picrylhyrazyl radical, ascorbic acid, butylatedhydroxyanisole, α-tocopherol.

INTRODUCTION

Plants bio-synthesize a large variety of chemical substance which have been used in the treatment and cure of human and other animal ailments. These substances include flavonoids, terpenes, steroids, alkaloids, glycosides and host of other chemical substances which are of no apparent importance to the plants' existence [1-4]. Many studies have shown that phytochemicals with antioxidant activity may reduce the risk of cancer and heart disease. Antioxidants are chemicals that offer up their own electrons to free radicals, thus preventing

cellular damage. Continuous intake of antioxidants is therefore necessary and important to reduce cellular damage and ageing process [5-7].

Acalypha ornata is a horticultural shrub that is open to forest zone of Nigeria and widespread across tropical Africa. It is used in traditional medicine to treat a wide array of disease conditions [8-9]. Much phytochemical work has been carried on many *Acalypha species* and compounds of medicinal importance have been isolated and characterized [10-19] but little is known about the biology and chemistry of *A. ornata*.

In this paper, we report the phytochemical constituents of *A. ornata*, toxicity of fractions obtained from chromatographic separation using Brine shrimp lethality assay and the antioxidant activity determined by photometric assay of the effect on 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and scavenging effect on hydrogen peroxide. DPPH radical gives strong absorption at 517nm (deep violet colour) in visible spectroscopy. The absorption vanishes or is decolourized as the electron becomes paired off in the presence of a free radical scavenger. The ability of the fractions to scavenge the dangerous hydroxyl radical generated by hydrogen peroxide was determined by scavenging effect on hydrogen peroxide carried out at 285 nm. These two tests revealed the ability of the fractions as a proton donor or as a hydroxyl radical scavenger respectively [20-23].

EXPERIMENTAL SECTION

Materials and Methods

Reagents and chemicals: Hexane, ethyl acetate, methanol, butanol, and chloroform, hydrochloric acid, ammonia solution, naphthol, bismuth nitrate, potassium iodide, sodium hydroxide, sodium chloride, copper sulphate pentahydrate, sodium potassium tartarate, 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 and silica gel 70 - 230 microns (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).

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 (Unico1200 & Perkin Elmer lambda 25 models), Glass Column chromatographic materials and fraction collectors.

Plant Material

Fresh leaves of *A. ornata* were collected in October 2009 at the Botanical Garden, University of Ibadan and were identified by Mr. Esimenkhuai Donatus of the Department of Botany and Microbiology, University of Ibadan. The leaves were air-dried under mild sunshine for 2 weeks and milled at Wood Extraction Unit of the Department of Chemistry, University of Ibadan and then subjected to solvent extraction.

Extraction and Fractionation Procedure

The powdered leaves (2 kg) of *A. ornata* were extracted with methanol by soxhlet extraction. The filterate was evaporated to dryness in a rotary evaporator at 37°C and stored in desiccators prior to further 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 by using DPPH as a spray reagent. Yellow coloration on the spots on the TLC plates indicates that the methanolic extract of *A. ornata* has antioxidant activity. The crude methanolic extract (12.2 g) was pre-adsorbed in 5 g of silica gel, allowed to dry and added to the column in the dry state. Silica gel (100 g) was packed into the column and the adsorbed hexane mixture was added on top. The mobile phase was introduced into the adsorbed sample in the glass column. The proportion of the more polar solvent was increased gradually in the non-polar one. This produced the stock solutions used with the gradient mixer. 20ml each of the eluent were collected in the fraction collectors and analysed by TLC. Solvent system used was hexane, EtOAc and methanol and silica gel $60F_{254}$, 70 - 230 microns was used as adsorbent. Different solvent systems employed for TLC included 100% (EtOAc), EtOAc: MeOH (9:1), EtOAc: MeOH (7:3), EtOAc: MeOH (5:5), Hexane: EtOAc (6:4), Hexane: EtOAc (1:1), Hexane: EtOAc (2:3), Hexane: EtOAc (2:8) and adsorbent for TLC was silica gel 60 F_{254} precoated aluminium plates. The retention factor R_f obtained from TLC analysis of the 262 fractions obtained was used as the basis for bulking the fractions into 17 fractions (W_1- W_{17}). Thereafter, toxicity test using Brine shrimp lethality assay and free radical scavenging activity test were carried out on the bulked fractions using the following spectrophotometric experiments; scavenging effect on DPPH and hydroxyl radical generated by hydrogen peroxide. Fractions W_1- W_3 were not analyzed because they were mainly solvent used for fractionation and did not show activity at preliminary qualitative screening.

Phytochemical Screening of the Crude Extract

The extract was screened for different classes of secondary metabolites. The screening tests carried out include test for phenols, resin, flavonoids, sterol, tannins, alkaloids, carbohydrates, cardiac glycosides and saponins [24].

Scavenging Effect on DPPH

A solution of 2, 2-diphenyl-1-picryhydrazyl radical (DPPH), a stable radical was prepared by dissolving 3.94 mg in 100ml methanol 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 bulked fractions with doses ranging from 1.0 to 0.0625 mg/ml [25-27]. The actual decrease in absorption at 517 nm of DPPH in UV-Visible spectrophotometer was measured 10 minutes later against that of the control and the percentage inhibition was also calculated. The same experiment was carried out on ascorbic acid, butylatedhydroxyanisole (BHA) and α-tocopherol which are known antioxidants. All test and analysis were run in triplicates and the results obtained were averaged.

The Percentage Inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_2 - A_1}{A_2} \times \frac{100\%}{1}$$

Where A_1 is average absorbance run in triplicates and A_2 is average absorbance of control reaction.

Scavenging Effect on Hydrogen Peroxide

Hydroxyl radical scavenging activity of methanolic fractions of *A .ornata* was determined spectrophotometrically at 285 nm. A solution of 2 mM hydrogen peroxide was prepared in phosphate buffered-saline (PBS) pH 7.4. The fractions at 1.0 to 0.0625 mg/ml were added to the H_2O_2 solution. Decrease in absorbance of H_2O_2 at 285 nm was determined 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 [28-29].

Toxicity Analysis

Brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the toxicity of the fractions [30-32]. 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 (10000, 1000 and 100 ppm) 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 that killed fifty percent of the larvae (LC_{50}) was determined using the Finney computer programme [33-34].

RESULTS AND DISCUSSION

The extraction yielded 21.58% crude methanolic extract. *A. ornata* leaves extract contained phenols, resins, sterols, flavonoids, tannins and carbohydrates while alkaloids, cardiac glycosides and saponins were absent (Table 1). These metabolites are known to exhibit medicinal activity [9, 35].

Table 1: Phytochemical Screening of *A. ornata* methanolic extract

Test	Inference*
Phenols	+
Resins	+
Flavonoids	+
Sterols	+
Tannins	+
Alkaloids	-
Carbohydrates	+
Cardiac glycosides	-
Saponins	-

***** *+ = present, - = absent*

The presence of these secondary metabolites in methanolic extract of *A. ornata* leaves agrees with the literatures and other studies on *Acalypha* spp. Some of the species *A. racemosa*, *A. maginata*, *A. fruticosa* have also been shown to contain flavonoids and saponin [19, 36]. Also a recent study confirmed the presence of phlobatannin and hydroxylanthraquinone in methanolic leaf extract of *A. hispida* and absence of glycosides and alkaloids in *A. marginata* and *A. racemosa*. The methanolic extract was separated by column chromatography because it gave instant colour reaction within 0-5 minutes with DPPH spray reagent. A total of 262 fractions were obtained and pooled according to their R_f values into 17 fractions.

Free radical scavenging activities

Scavenging activities of the bulked samples of methanolic fractions of *A. ornata* on 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and hydrogen peroxide (H_2O_2) are as shown in Tables 2, 3, 4 and 5. Their free radical scavenging activities were compared with the activities of known antioxidants ascorbic acid, butylatedhydroxyanisole (BHA) and α-tocopherol. The stable radical 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and hydrogen peroxide (H_2O_2) which are known to generate free radicals were used as the radical sources.

Scavenging effects on DPPH

A stable free radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) accepts an electron or hydrogen radical to become a stable diamagnetic molecule and absorbs at 517 nm in the visible region of a UV-Visible Spectrophotometer [29, 37]. The reduction in absorbance of DPPH at 517 nm caused by the samples was measured in triplicate after 10min. Table 2 shows that the absorbance increases or decreases with increase in concentrations for some of the fractions and the standards (ascorbic acid, α-tocopherol and BHA). Fractions W_4 and W_5 showed weak scavenging activity towards DPPH radical at 517 nm but all the other fractions scavenged very effectively than ascorbic acid and α-tocopherol.

However, BHA scavenged more effectively than the fractions. Table 3 shows the percentage inhibition of the samples and standards on DPPH radical at 517 nm. All the tested fractions with the exception of W_4, W_5, W_6, W_7 and W_{10} possessed a fairly higher percentage inhibition than ascorbic acid at all concentrations (0.0625 to 1.0 mg/ml). BHA showed a generally higher percentage inhibition than all the fractions. The antioxidant activity of α-tocopherol towards DPPH radical is comparatively weak. The better scavenging activity of *A. ornata* could be linked to the presence of secondary plant products like flavonoids and phenolics [35].

Table 2: Scavenging effects of methanolic fractions of *A. ornata* leaves (W_4- W_{17}) on 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)*

	Concentrations (mg/ml) **				
	1.0	0.5	0.25	0.125	0.0625
W_4	0.770 ± 0.029	0.843 ± 0.007	0.866 ± 0.003	0.886 ± 0.002	0.895 ± 0.004
W_5	0.731 ± 0.019	0.888 ± 0.085	0.875 ± 0.009	0.787 ± 0.016	0.792 ± 0.018
W_6	0.166 ± 0.001	0.283 ± 0.003	0.566 ± 0.004	0.678 ± 0.012	0.792 ± 0.003
W_7	0.190 ± 0.003	0.072 ± 0.001	0.060 ± 0.002	0.058 ± 0.002	0.046 ± 0.003
W_8	0.056 ± 0.000	0.115 ± 0.001	0.059 ± 0.001	0.332 ± 0.003	0.623 ± 0.006
W_9	0.068 ± 0.001	0.060 ± 0.001	0.056 ± 0.006	0.147 ± 0.002	0.066 ± 0.007
W_{10}	0.101 ± 0.003	0.080 ± 0.001	0.069 ± 0.002	0.165 ± 0.001	0.097 ± 0.098
W_{11}	0.082 ± 0.003	0.074 ± 0.008	0.057 ± 0.003	0.056 ± 0.008	0.068± 0.007
W_{12}	0.072 ± 0.003	0.067 ± 0.002	0.080 ± 0.030	0.062 ± 0.003	0.072± 0.006
W_{13}	0.051 ± 0.005	0.040 ± 0.004	0.026 ± 0.006	0.039 ± 0.004	0.041 ± 0.008
W_{14}	0.056 ± 0.001	0.101 ± 0.069	0.064 ± 0.009	0.079 ± 0.004	0.093 ± 0.002
W_{15}	0.062 ± 0.002	0.071 ± 0.002	0.069 ± 0.006	0.082 ± 0.014	0.198 ± 0.037
W_{16}	0.061 ± 0.001	0.062 ± 0.001	0.066 ± 0.001	0.135 ± 0.037	0.317 ± 0.006
W_{17}	0.079 ± 0.006	0.073 ± 0.003	0.069 ± 0.001	0.263 ± 0.060	0.453 ± 0.005
BHA	0.037 ± 0.006	0.048 ± 0.002	0.049 ± 0.004	0.065 ± 0.003	0.160 ± 0.091
ASC	0.085 ± 0.010	0.289 ± 0.028	0.298 ± 0.024	0.320 ± 0.082	0.515 ± 0.015
TOC	0.680 ± 0.029	0.704 ± 0.004	0.705 ± 0.007	0.707 ± 0.007	0.721 ± 0.017

** The scavenging activity of methanolic fractions of A. ornata, ASC (Ascorbic acid), BHA (Butylatedhydroxylanisole) and TOC (α-Tocopherol) on DPPH was measured at 517 nm*

*** Each value represents the mean ± standard deviation of triplicate analysis. Absorbance of DPPH at 517 nm = 0.9330*

Phenolic compounds especially flavonoids have been known to act as either antioxidant or pro - oxidant entities due to its ability to undergo autoxidation, reduce $Cu^{2+}$and $Fe^{3+}$and become involved in redox reaction and have been shown to lower the risk of heart disease [6, 38].

Scavenging effects on Hydrogen peroxide (H_2O_2)

The scavenging activities of fractions collected from chromatographic separation of *A. ornata* and known antioxidants ascorbic acid, butylatedhydroxyanisole (BHA) and α-tocopherol on H_2O_2 is shown in Table 4 and 5. Scavenging effects on H_2O_2 was measured in triplicates after 10 min of incubation at 285 nm. It has been shown that H_2O_2 has only a weak activity to initiate

lipid peroxidation, but its activity as an active - oxygen specie comes from its potential to produce the highly reactive hydroxyl radical through the Fenton reaction [39-40]. Interestingly, one of the known antioxidants showed an exceptionally high antioxidant activity in scavenging OH radical when compared to its activity in DPPH.

Table 3: Percentage Inhibition of Samples and Standards on DPPH radicals*

Sample	Concentration (mg/ml)*				
	1.0	0.5	0.25	0.125	0.0625
W_4	17.43	9.65	7.22	5.07	4.07
W_5	21.61	4.79	6.18	15.68	15.11
W_6	82.17	69.70	39.37	27.33	15.15
W_7	79.67	92.32	93.60	93.75	95.11
W_8	93.99	87.67	93.64	64.42	33.19
W_9	92.68	93.60	93.99	84.28	92.95
W_{10}	89.17	91.46	92.64	82.32	89.57
W_{11}	91.21	92.10	93.85	93.96	92.75
W_{12}	92.25	92.78	91.46	93.39	92.25
W_{13}	94.57	95.68	97.18	95.78	95.61
W_{14}	93.96	89.17	93.10	91.50	89.99
W_{15}	93.35	92.43	92.57	91.17	78.74
W_{16}	93.50	93.32	92.89	85.57	65.99
W_{17}	91.57	92.14	92.64	71.81	51.48
BHA	95.40	93.99	93.91	91.92	80.10
ASC	90.80	68.67	67.78	65.37	44.30
A-Toc	15.42	12.44	12.35	12.06	10.36

**Absorbance of DPPH at 517 nm = 0.9330± 0.001*

Table 4: Scavenging effects of methanolic fractions of *A. ornata* leaves (W_4- W_{17}) on hydrogen peroxide radicals*

	Concentration (mg/ml) **				
	1.0	0.5	0.25	0.125	0.0625
W_4	0.0885 ± 0.0057	0.0598 ± 0.0027	0.0233 ± 0.0050	0.0137 ± 0.0070	0.0538 ± 0.0005
W_5	0.0818 ± 0.0003	0.0521 ± 0.0009	0.0489 ± 0.0017	0.0566 ± 0.0004	0.0318 ± 0.0003
W_6	0.0507 ± 0.0008	0.0712 ± 0.0026	0.0246 ± 0.0020	0.0216 ± 0.0009	0.0298 ± 0.0011
W_7	0.2238 ± 0.0325	0.1770 ± 0.0017	0.0995 ± 0.0021	0.0276 ± 0.0068	0.2340 ± 0.0077
W_8	1.1208 ± 0.0743	0.1477 ± 0.0241	0.0735 ± 0.0066	0.0546 ± 0.0104	0.1035 ± 0.0006
W_9	0.7134 ± 0.0013	0.3226 ± 0.0084	0.1728 ± 0.0007	0.0675 ± 0.0007	0.2109 ± 0.0009
W_{10}	0.1084 ± 0.0062	0.1352 ± 0.0083	0.0653 ± 0.0083	0.0129 ± 0.0051	0.2266 ± 0.0155
W_{11}	1.2900 ± 0.0308	0.1875 ± 0.0131	0.0650 ± 0.0196	0.0571 ± 0.0240	0.3916 ± 0.5044
W_{12}	0.1266 ± 0.0039	0.2646 ± 0.0047	0.1196 ± 0.0050	0.0505 ± 0.0079	0.2200 ± 0.0067
W_{13}	1.5318 ± 0.0373	0.2871 ± 0.0469	0.1345 ± 0.0286	0.0492 ± 0.0007	0.1762 ± 0.0216
W_{14}	0.2424 ± 0.0017	0.2122 ± 0.0010	0.1316 ± 0.0007	0.0658 ± 0.0046	0.1947 ± 0.0049
W_{15}	0.1786 ± 0.0047	0.0594 ± 0.0105	0.2702 ± 0.1999	0.0222 ± 0.0066	0.2574 ± 0.0437
W_{16}	0.0177 ± 0.0076	0.0481 ± 0.0044	0.0473 ± 0.0072	0.0116 ± 0.0018	0.2549 ± 0.0017
W_{17}	0.1649 ± 0.0146	0.2550 ± 0.0115	0.0266 ± 0.0001	0.3046 ± 0.0053	0.0174 ± 0.0221
BHA	0.0413 ± 0.0016	0.0617 ± 0.019	0.0740 ± 0.0015	0.0947 ± 0.0003	0.0174 ± 0.0014
ASC	0.1952 ± 0.000	0.2078 ± 0.012	1.2645 ± 0.0012	2.7586 ± 0.0049	2.9236 ± 0.021
TOC	0.0321 ± 0.045	0.0633 ± 0.032	0.1552 ± 0.061	0.1807 ± 0.015	0.5150 ± 0.049

** The scavenging activity of methanolic fraction of A. ornate, ASC (Ascorbic acid), BHA (Butylatedhydroxyanisole) and TOC (α-Tocopherol) on H_2O_2 was measured at 285 nm*

*** Each value represents the mean ± standard deviation of triplicate analysis.*

Absorbance of hydrogen peroxide at 285nm = 3.7692 ± 0.0536

Table 4 shows that the fractions can scavenge hydroxyl radical very effectively when compared with the standards. With the exception of W_8, W_9, W_{11} and W_{13}, all the fractions scavenged more effectively than ascorbic acid at all concentrations. Fractions W_8, W_9, W_{11} and W_{13} possessed low percentage inhibitions than α-tocopherol at 1.0 mg/ml. At 0.125 and 0.0625 mg/ml, all test samples possessed higher percentage inhibitions than α-tocopherol (Table 5).

Antioxidant principles containing plants prevent formation of free radicals in biological system when eaten as food or used as medicine. A growing number of diseases are reported to be caused or influenced by free radicals. Free radicals are the toxic agents behind ageing and cellular damage. Reactive oxygen species capable of damaging DNA, proteins, carbohydrates and lipids are generated in aerobic organisms. A system of enzymic and non-enzymic antioxidants checks and controls the effects of the reactive oxygen species. The antioxidants eliminate pro-oxidant and scavenge free radicals from causing cellular damage [41]. Oxygen-radical, superoxide radical for example when formed could lead to other radical formation. The toxicity of oxygen radical in living organism is due to its conversion into OH radical and into reactive radical-metal complexes. The test samples showed high activities towards scavenging hydrogen peroxide and eventually contributes to the inhibition of peroxidation of lipid. Elimination of OH radical is one of the most important effective defence of a living body against diseases [21, 41-42].

Table 5: Percentage Inhibition of Samples and Standards on hydrogen peroxide radicals*

Sample	Concentration (mg/ml)*				
	1.0	0.5	0.25	0.125	0.0625
W_4	97.73	98.41	99.38	99.64	98.57
W_5	97.83	98.62	98.70	98.50	99.15
W_6	98.65	98.11	99.34	99.43	99.21
W_7	94.06	95.28	97.36	99.28	93.80
W_8	70.27	96.08	98.05	98.55	97.25
W_9	81.07	91.44	95.42	98.21	94.41
W_{10}	97.12	96.41	98.27	99.66	93.99
W_{11}	65.76	95.02	98.28	98.48	89.61
W_{12}	96.64	92.98	96.83	98.66	94.61
W_{13}	59.36	92.38	96.43	98.69	95.32
W_{14}	93.57	94.37	96.51	98.26	94.83
W_{15}	95.26	98.42	92.83	99.41	93.17
W_{16}	99.53	98.72	98.74	99.69	93.24
W_{17}	95.62	93.24	99.29	91.92	99.54
BHA	98.90	98.36	98.04	97.49	97.02
ASC	94.82	94.49	66.45	26.81	22.43
α-Toc	99.15	98.32	95.88	95.21	86.34

**Absorbance of H_2O_2 at 285 nm 3.7690 ± 0.004*

Consequently, this investigation shows that methanolic fractions of *A. ornata* possessed antioxidant activities which could impart health benefits by scavenging free radicals causing cellular damage and ageing. From literature, other *Acalypha spp* such as *A. wilkesiana, A. communis* demonstrated a significant antioxidant activity. *A. fruticosa* and *A. semiflabellata* were also effective free radical scavengers in a DPPH – assay [43-44]. Thus, the results of this study showed that the methanolic fractions of *A. ornata* possess antioxidant activity, which could exert a beneficial action against pathological alterations caused by the presence of free and hydroxyl radicals.

Toxicity analysis

The toxicity of the fractions was determined by brine shrimp lethality test (BST). The concentration that killed 50% of larvae (LC_{50}) showed that fractions W_{10}, W_{16} and W_{17} were not toxic as the LC_{50} (μg/ml) values were greater than 1000 μg/ml at 95% confidence level. These fractions being non-toxic showed significant antioxidant activities at scavenging free radicals (Table 6).

Table 6: Result of Brine shrimp lethality test

	Survival						
Sample	100ppm	1000ppm	10000 ppm	Degree of freedom	Upper limit	Lower limit	LC_{50}
W_4	30	28	0	0.28	166.95	5.00	61.12
W_5	24	14	3	0.31	234.22	14.45	301.31
W_6	20	12	2	0.43	322.23	23.42	231.11
W_7	12	9	6	0.39	970.96	26.95	307.65
W_8	30	10	2	0.10	561.32	132.34	319.10
W_9	30	0	0	0.13	529.08	194.63	324.81
W_{10}	19	14	6	0.19	1730.70	360.93	763.45
W_{11}	23	12	6	0.20	911.65	160.59	403.69
W_{12}	21	12	3	1.41	148.05	6.55	9.62
W_{13}	27	24	21	3.76	35.14	3713.52	0.03
W_{14}	22	15	8	2.98	32.49	3036.81	429.86
W_{15}	27	15	0	0.26	1.59	1.70	6.33
W_{16}	26	15	0	0.23	1367.89	516.77	848.65
W_{17}	30	18	9	0.29	1395.48	573.49	865.28

** LC_{50} < 1000 μg/ml is toxic while LC_{50} > 1000 μg/ml is not toxic*

The ability of the fractions to be used as primary antioxidant is significant to its being non-harmful to organism's cells. The remaining fractions have varied degree of toxicity and also showed significant antioxidant activity but their use at high dose should be properly controlled.

CONCLUSION

In vitro assessment of the antioxidant activity of methanolic fractions of *A. ornata* to scavenge 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and highly reactive hydroxyl radicals showed that the semi - pure compounds present in the fractions are useful potential source of antioxidants and can be used in the therapy of diseases like cancer, coronary heart disease, ageing and any other disease related to oxidative stress. The presence of secondary plant metabolites like the flavonoids and phenolics may have been responsible. The activity was concentration dependent and the results obtained are comparative to the standards, ascorbic acid, butylatedhydroxyanisole (BHA) and α-tocopherol used. The toxicity test carried out to determine the least concentration that killed 50% of larvae (LC_{50}) showed that some of the fractions were non-toxic at 95% confidence level. These fractions being non-toxic showed significant antioxidant activity at scavenging free radicals. They also significantly scavenge hydroxyl radical which is known to cause cellular damage. Other fractions though toxic also showed significant antioxidant activity but their use at high dose should be properly monitored. More work therefore needs to be carried out on the fractions in other to isolate, purify and characterize the active chemical compounds responsible for antioxidant activity. The pure compounds will also be subjected to further toxicological analysis.

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