HEPATOPROTective EFFECT OF AN AQUEOUS EXTRACT OF THE LEAVES OF ACALYPHA WILKESIANA ‘GODSEFFIANA’ MUell ARG (EUPHORBIACEAE) AGAINST CARBON TETRACHLORIDE INDUCED LIVER INJURY IN RATS

Jude C. Ikewuchi*, Augustine A. Uwakwe, Eugene N. Onyeike, Catherine C. Ikewuchi

Department of Biochemistry, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria

* corresponding author: E-mail: ecoli240733@yahoo.com; Tel.: +2348033715662

ABSTRACT

The potential of aqueous extract of the leaves of Acalypha wilkesiana, to protect against carbon tetrachloride induced liver damage was investigated in Wistar albino rats. The carbon tetrachloride was prepared 1:5 (v:v) in olive oil, and administered subcutaneously at 1 mL/kg body weight. The extract was administered to both normal and carbon tetrachloride treated rats at 100, 200 and 300 mg/kg. On fractionation and gas chromatographic analysis of the crude aqueous extract, thirty nine known alkaloids were detected, consisting mainly of akuamidine (69.027 %), voacangine (26.226 %), echitamine (1.974 %), echitamidine (0.599 %), lupanine (0.521 %) and augustamine (0.278 %). Compared to test control, the treatment dose dependently produced significantly lower ($P<0.05$) alkaline phosphatase, aspartate and alanine transaminase activities. Histopathological studies on the liver sections showed that pre-treatment with the extract protected against carbon tetrachloride induced fatty degeneration of hepatocytes, thus, confirming the results of the biochemical studies. The above results imply that treatment with the plant extract protects the liver against carbon tetrachloride induced hepatotoxicity, therefore, justifying the use of Acalypha wilkesiana in African traditional health care for the management of liver problems.

Keywords: Acalypha wilkesiana ‘Godseffiana’ Muell Arg (Euphorbiaceae), carbon tetrachloride, histopathology, hepatospecific markers

INTRODUCTION

Acalypha wilkesiana, a member of the spurge family (Euphorbiaceae), belongs to the genus Acalypha comprising about 570 species (Riley, 1963, as cited in Ogundaini, 2005). A large proportion of members of this genus are weeds while the others are ornamental plants. Acalypha wilkesiana is commonly called copperleaf, Joseph’s coat, fire dragon, beef steak plant and match-me-if-you-can (Christman, 2004). Although native to Fiji and nearby islands in the South Pacific, it has spread to most parts of the world, especially the tropics of Africa, America and Asia. It is a popular outdoor plant that provides colour throughout the year, although it is also grown indoors as a container plant. Many cultivars are available with different leaf forms and colours: A. wilkesiana ‘Godseffiana’ has narrow, drooping, green leaves with creamy-white margins; ‘Marginata’ has coppery-green leaves with pink or crimson margins; ‘Macrophylla’ has larger leaves, variegated with bronze, cream, yellow and red; while ‘Musaica’ has green leaves that are mottled with orange and red (Gilman, 1999; Christman, 2004). In Southern Nigeria, the expressed juice or boiled decoction of the leaves of A. wilkesiana cv. Godseffiana is
used in traditional health care practice, for
the management of gastrointestinal disor-
ders, fungal skin infections, hypertension
and diabetes mellitus. The leaf-poultice is
used in the treatment of headache, swell-
ings, colds and malaria (Akinyemi et al.,
2005). The seeds of *A. wilkesiana* are used
in compounding a complex plant mixture
used by traditional healers in South-West
Nigeria to treat breast tumours and inflam-
mation (Bussing et al., 1999; Taraphdar et
al., 2001). The antimicrobial (Akinyemi et
al., 2005; Ogundaini, 2005; Oladunmoye,
2006), hypolipidaemic (Ikewuchi, 2010;
Ikewuchi and Ikewuchi, 2010), diuretic
(Ikewuchi et al., 2009a), hypoglycaemic
(Ikewuchi et al., 2009a, 2011a) and anti-
hypertensive (Ikewuchi et al., 2009b,
2011b) properties of the leaves have been
reported.

Earlier analysis of the leaves of *Acaly-
pha wilkesiana* revealed the presence of
sesquiterpenes, monoterpenes, triterpe-
noids, polyphenols, gallic acid, corilagin,
eranin, quercetin 3-O-rutinoside,
kaempferol 3-O-rutinoside, saponins, tan-
nins, anthroquinone and glycosides (Ak-
ine, 1986; Adesina et al., 2000; Oladun-
 moye, 2006). They were also found to be
were rich in some potent hepatoprotective
agents like vitamin C (Ikewuchi and Ike-
wuchi, 2009), flavonoids and tannic acid
(Ikewuchi et al., 2010, 2011a). Therefore,
in this study, the ability of an aqueous ex-
tract of the leaves of *A. wilkesiana* ‘Godsef-
fiana’ Muell Arg, to protect against carbon
tetrachloride induced liver damage was in-
vestigated in Wistar albino rats.

**MATERIALS AND METHODS**

**Collection of plant samples and prepara-
tion of plant extract**

Samples of the fresh *Acalypha wilkesi-
ana* plants (Figure 1) were collected from
within the Choba and Abuja Campuses of
the University of Port Harcourt, Nigeria.
After due identification at the University of
Port Harcourt Herbarium, Port Harcourt,
they were cleaned, before removing their
leaves, which were then oven dried at 55 °C
and ground into powder. The resultant
powder was soaked in hot distilled water
for 12 h, after which the resultant mixture
was filtered and the filtrate (hereinafter re-
ferred to as the aqueous extract) was stored
in the refrigerator for subsequent use. A
known volume of this extract was evapo-
rated to dryness, and the weight of the resi-
due obtained, was used to determine the
concentration of the filtrate, which was in
turn used to determine the dose of adminis-
tration of the extract. The resultant crude
aqueous residue was subjected to phyto-
chemical analysis.

![Figure 1: Acalypha wilkesiana Muell Arg](image)

**Determination of the phytochemical
content of the crude aqueous leaf extract**

**Calibration, identification and quantifica-
tion**

Standard solutions were prepared in
methanol. The linearity of the dependence
of response on concentration was verified
by regression analysis. Identification was
based on comparison of retention times and
spectral data with standards. Quantification
was performed by establishing calibration
curves for each compound determined, us-
ing the standards.

**Determination of alkaloid composition**

The extraction of the alkaloids was car-
ried out according to the method of Tram et
al. (2002). The residue from the aqueous
extract above, was extracted with methanol
and subjected to gas chromatographic
analysis. Three grams of the crude aqueous
extract was extracted with 25 mL of metha-
ol for 6 h at room temperature, before fil-
tration. The filtrate was concentrated in a rotary evaporator, before “drying off” water using anhydrous sodium sulphate, prior to gas chromatography analysis. Gas chromatographic analyses was carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 [1206] software, to quantify and identify compounds. The column was a capillary DB-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 38 psi. The oven was programmed as follows: initial temperature at 60 °C for 5 min; first ramping at 10 °C/min for 20 min, followed by a second ramping at 15 °C/min for 4 min. The chromatogram of the extract is shown in Figure 2.

**Experimental design for the hepatoprotective study**

Wistar albino rats (180-200 g) were collected from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus. Studies were conducted in compliance with applicable laws and regulations for handling experimental animals. The rats were weighed and sorted into eight groups (Table 1) of five animals each, so that their average weights were approximately equal. The animals were housed in plastic cages at the animal house of the Department of Biochemistry, University of Port Harcourt. After a one-week acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), the treatment commenced. The extract was administered orally on daily basis for eight days. The dosage of administration of the extract was adapted, with modification, from Ikewuchi and Ikewuchi (2010) and Ikewuchi (2010). The carbon tetrachloride was prepared 1:5 (v:v) in olive oil, and administered subcutaneously at 1 mL/kg body weight of carbon tetrachloride, on days 4 and 8. The dosage and method of administration of carbon tetrachloride was adapted from Obi and Uneh (2003), with modification. Twenty four hours after the last administration of carbon tetrachloride, the rats were weighed and anaesthetized by exposure to chloroform. While under anesthesia, they were painlessly sacrificed and blood was collected from each rat into heparin sample bottles, after which the livers were collected and preserved in 10 % formalin, for histochemical analysis. The heparin anti-coagulated blood samples were centrifuged at 1000 g for 10 min, after which their plasma were collected and stored for subsequent analysis.

![Gas chromatogram of the alkaloid composition of an aqueous extract of Acalypha wilkesiana leaves](image-url)
Table 1: Experimental design for the hepatoprotective screening

| S/N | ID       | Treatment                                                                 |
|-----|----------|---------------------------------------------------------------------------|
| 1   | Normal   | Olive oil (1 mL/kg) and normal saline and Water                           |
| 2   | Test control | Carbon tetrachloride (1 mL/kg) and water                                 |
| 3   | Treatment control I (AWC1) | Olive oil (1 mL/kg) and extract (100 mg/kg)                              |
| 4   | Treatment control II (AWC2) | Olive oil (1 mL/kg) and extract (200 mg/kg)                              |
| 5   | Treatment control III (AWC3) | Olive oil (1 mL/kg) and extract (300 mg/kg)                              |
| 6   | Treatment I (AW1) | Carbon tetrachloride (1 mL/kg) and extract (100 mg/kg)                  |
| 7   | Treatment II (AW2) | Carbon tetrachloride (1 mL/kg) and extract (200 mg/kg)                  |
| 8   | Treatment III (AW3) | Carbon tetrachloride (1 mL/kg) and extract (300 mg/kg)                  |

Determination of plasma hepatospecific markers

The plasma activities of alanine and aspartate transaminases, and alkaline phosphatase were determined using Randox test kits (Randox Laboratories Ltd., Crumlin, England, UK). The activities of alanine and aspartate transaminases were respectively measured by monitoring at 546 nm the concentrations of pyruvate and oxaloacetate hydrazones formed with 2,4-dinitrophenylhydrazine. The activity of alkaline phosphatase was determined by monitoring the degradation of p-nitrophenylphosphate to p-nitrophenol, at 405 nm.

Plasma total bilirubin and protein concentrations were determined using Randox test kits (Randox Laboratories Ltd., Crumlin, England, UK). The wavelength for the determination of total bilirubin was 578 nm, while that of total protein was 560 nm.

Determination of percentage protection (% protection)

The percentage protection provided by the extract against carbon tetrachloride induced liver damage was calculated using the following formula adapted from Al-Qarawi et al. (2004).

\[
\% \text{ Protection} = \left( \frac{\text{Parameter}_{\text{Test control}} - \text{Parameter}_{\text{Control}}}{\text{Parameter}_{\text{Test control}}} \right) \times 100
\]

Histopathological study on the liver

The histopathology study on the liver samples was carried out by Professor S.O. Nwosu, from the Department of Anatomical Pathology, University of Port Harcourt Teaching Hospital. Small pieces of liver tissues were collected in 10 % formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 µm in thickness were cut, mounted on slide and stained with hematoxylin and eosin. The sections were then examined via light microscopy (Opticphot-2; Nikon, Tokyo, Japan) at x100 magnification.

Statistical analysis of data

All values are quoted as the mean ± s.e.m. (standard error in the mean). The values of the various parameters were analyzed for statistical significant differences between the groups, using the Student’s t-test, with the help of SPSS Statistics 17.0 package (SPSS Inc., Chicago Ill). \( P<0.05 \) was assumed to be significant. Graphs were drawn using Microsoft Office Excel, 2010 software.
RESULTS AND DISCUSSION

Table 2 shows the alkaloid composition of an aqueous extract of the leaves of *Acalypha wilkesiana*. Thirty nine known alkaloids were detected. The main constituents were 1.974 % echitamine, 26.226 % voacangine, 0.599 % echitamidine, 69.027 % akuamidine, 0.521 % lupanine, 0.278 % augustamine.

The effect of an aqueous extract of the leaves of *Acalypha wilkesiana* on the plasma hepatospecific markers of normal and carbon tetrachloride treated rats is given in Table 3. Compared to test control, the treatment produced significantly lower (*P*<0.05) plasma alkaline phosphatase, alanine transaminase and aspartate transaminase activities. The plasma total bilirubin and total bilirubin of the test groups were lower though not significantly, than that of the test control.

Table 2: Alkaloid composition of the aqueous extract of the leaves of *Acalypha wilkesiana*

| Compounds                        | Retention time (min) | Composition (x10^4 mg/kg) |
|----------------------------------|----------------------|---------------------------|
| Choline                          | 7.060                | 23.34                     |
| Trigonelline                     | 7.530                | 0.69                      |
| Angustifoline                    | 7.938                | 587.47                    |
| Sparteine                        | 8.927                | 44.78                     |
| Ellipicine                       | 9.745                | 95.73                     |
| Lupanine                         | 11.063               | 1422.77                   |
| 13-α-Hydroxyrhombifoline         | 11.358               | 77.31                     |
| 9-Octadecanamide                 | 12.828               | 38.75                     |
| Dihydro-oxo-demethoxyhaemanthamine | 14.156             | 179.31                    |
| Augustamine                      | 14.924               | 759.94                    |
| Oxoassoanine                     | 15.401               | 107.60                    |
| Cinchonidine                     | 16.251               | 195.75                    |
| Cinchonine                       | 16.374               | 117.95                    |
| Crinane-3α-ol                    | 16.459               | 245.13                    |
| Buphanidrine                     | 16.674               | 125.33                    |
| Indicine-N-oxide                 | 17.551               | 97.41                     |
| Powelline                        | 18.593               | 121.73                    |
| Undulatine                       | 18.843               | 90.20                     |
| Ambelline                        | 19.690               | 38.81                     |
| 6-Hydroxybuphanidrine            | 20.468               | 164.84                    |
| Acronycine                       | 21.102               | 113.47                    |
| Monocrotaline                    | 21.329               | 151.05                    |
| 6-Hydroxypowelline               | 21.822               | 210.00                    |
| Nitidine                         | 22.362               | 101.43                    |
| Crinamidine                      | 23.968               | 590.02                    |
| 1β,2β-Epoxambelline              | 24.726               | 56.04                     |
| 6-Hydroxyundulatine              | 24.792               | 69.00                     |
| Epoxy-3,7-dimethoxycrinane-11-one | 25.483             | 21.46                     |
| Akuamidine                       | 26.841               | 188641.00                 |
| Echitamidine                     | 26.953               | 1638.09                   |
| Voacangine                       | 27.065               | 71672.60                  |
| Mitraphylin                      | 27.650               | 0.05                      |
| Camptothecin                     | 28.194               | 44.95                     |
| Echitamine                       | 28.640               | 5393.51                   |
| Colchicine                       | 28.930               | 26.58                     |
| Emetine                          | 29.569               | 10.37                     |
| Tetrandrine                      | 29.677               | 5.36                      |
| Thalicarpin                      | 30.155               | 5.90                      |
| Paclitaxel                       | 32.108               | 1.46                      |
| **Total**                        | **-**                | **273287.00**             |
Table 3: Effect of an aqueous extract of the leaves of *Acalypha wilkesiana* on the plasma hepatospecific markers of normal and carbon tetrachloride treated rats

| Treatment group | Liver Function Indicator |
|-----------------|--------------------------|
|                 | Alkaline phosphatase activity (U/L) | Aspartate transaminase activity (U/L) | Alanine transaminase activity (U/L) | Total bilirubin content (µmol/L) | Total protein content (mg/dL) |
| Normal          | 327.52±89.49<sup>a,d</sup> | 17.85±0.36<sup>a,d</sup> | 23.17±1.97<sup>a</sup> | 7.52±0.47<sup>a</sup> | 58.31±3.06<sup>a,c</sup> |
| Test control    | 1217.16±118.33<sup>c</sup> | 49.88±1.67<sup>c</sup> | 122.72±1.02<sup>c</sup> | 9.10±1.25<sup>a</sup> | 61.91±3.57<sup>a,b,c</sup> |
| AWC1            | 260.13±43.22<sup>d,f</sup> | 20.85±3.32<sup>d</sup> | 20.89±3.38<sup>a</sup> | 2.41±0.17<sup>c</sup> | 55.32±1.74<sup>c,d</sup> |
| AWC2            | 540.04±90.35<sup>a,b,d,f</sup> | 20.74±2.50<sup>d</sup> | 20.57±1.58<sup>a</sup> | 3.24±0.19<sup>c</sup> | 59.55±2.91<sup>a,c</sup> |
| AWC3            | 523.85±137.31<sup>b,f</sup> | 18.66±1.05<sup>d</sup> | 22.53±4.83<sup>a</sup> | 2.54±0.19<sup>c</sup> | 48.90±1.93<sup>b,d</sup> |
| AW1             | 476.93±106.63<sup>a,b,d,f</sup> | 31.44±2.57<sup>b</sup> | 62.52±10.08<sup>b</sup> | 8.46±0.67<sup>a,b</sup> | 61.38±5.90<sup>a,b,c</sup> |
| AW2             | 394.68±82.26<sup>a,b,d,f</sup> | 43.58±2.18<sup>c</sup> | 119.51±1.09<sup>c</sup> | 8.18±0.54<sup>a,b</sup> | 60.67±2.11<sup>a</sup> |
| AW3             | 409.86±34.48<sup>a,b</sup> | 40.23±3.59<sup>b,c</sup> | 78.17±10.31<sup>b</sup> | 8.94±0.77<sup>a,b</sup> | 56.31±2.70<sup>a,b,c</sup> |

Values are mean ± s.e.m., n=5, per group  
<sup>a,b,c</sup> Values in the same column with different superscripts are significantly different at P<0.05.

The hepatoprotective activity of an aqueous extract of *Acalypha wilkesiana* leaves on carbon tetrachloride-induced hepatotoxicity in Wistar rats is shown in Figure 3. The protection against carbon tetrachloride damage seemed to be concentration dependent; with the 100 and 300 mg/kg doses being more effective. The treatment dose dependently significantly provided protection of about 83.21-92.45% in alkaline phosphatase, 19.65-57.56% in aspartate transaminase and 3.23-60.47% alanine transaminase activities. The frequency distribution of the effect of an aqueous extract of the leaves of *Acalypha wilkesiana* on the liver histology of normal and carbon tetrachloride treated rats is shown in Figure 4; while sections of the liver samples are shown in Figure 5.

![Figure 3](image-url)  
**Figure 3:** Hepatoprotective activity of an aqueous extract of the leaves of *Acalypha wilkesiana* on carbon tetrachloride-induced hepatotoxicity in Wistar rats.
Liver cirrhosis induced by carbon tetrachloride is perhaps the best-studied model of liver cirrhosis (Cornelius, 1993). The prevention of carbon tetrachloride-induced elevation of plasma aspartate and alanine transaminases and alkaline phosphatase activities, and plasma bilirubin level, in animals pretreated with the aqueous extract of the leaves of *Acalypha wilkesiana* shows its ability to protect normal functional status of the poisoned liver, in addition to protecting against subsequent carbon tetrachloride hepatotoxicity. The mechanism by which the extract produces its hepatoprotective activity is not certain. However, it is possible that β-sitosterol, a constituent of the extract of the leaves of *Acalypha wilkesiana* (Ikewuchi et al., 2011a), is at least partly
responsible for the protective activity against carbon tetrachloride hepatotoxicity. Earlier, Lin and Tome (1988) had reported that β-sitosterol was the anti-hepatotoxic principle in Sambucus formosana.

Carbon tetrachloride toxicity and its initiation of lipid peroxidation can be diminished by reducing the metabolic activation of carbon tetrachloride to trichloromethyl free radical by cytochrome P450 (Middleton et al., 2000). Therefore, any hepatoprotective agent should be able to inhibit the aromatase activity of cytochrome P450, and by so doing, favour liver regeneration. Flavonoids inhibit cytochrome P450 aromatase (Kowalska et al., 1990; Middleton et al., 2000), and may also inhibit lipid peroxidation by exerting a membrane-stabilizing action (Middleton et al., 2000). So, it can be suggested that flavonoids in A. wilkesiana leaves (Ikewuchi et al., 2010, 2011a), could be responsible for their hepatoprotective ability.

Earlier, Ikewuchi et al. (2010, 2011a) had reported that the leaves and aqueous extract of the leaves of A. wilkesiana were very rich in tannins and tannic acid. This may also be responsible for the hepatoprotective activity observed in this study. The hepatoprotective activity of tannic acid is well documented (Mittal et al., 2010; Pithayanukul et al., 2009).

Vitamin C, one of the major constituents of the leaves of A. wilkesiana (Ikewuchi and Ikewuchi, 2009) may also have contributed to, or be responsible for the hepatoprotection observed here. Studies have shown that hepatic microsomal drug metabolism is improved with vitamin C supplementation, probably due its augmentation of cytochrome P450 (Sato and Zannoni, 1976; Rikans et al., 1978; Burtis and Ashwood, 2001).

CONCLUSION

This study clearly demonstrates that extracts of the leaves of Acalypha wilkesiana are effective agents in the treatment and prevention of carbon tetrachloride-induced hepatic cytotoxicity. The data suggest that the daily oral consumption of an aqueous extract of the leaves of Acalypha wilkesiana was prophylactic to carbon tetrachloride poisoning.

ACKNOWLEDGEMENT

The authors wish to acknowledge the assistance of Mr. T. Mark-Balm, manager of the animal house of the Department of Biochemistry, University of Port Harcourt, for his assistance in taking care of the experimental animals.

REFERENCES

Adesina SK, Idowu O, Ogundaini AO, Oladimeji H, Olugbade TA, Onawunmi GO et al. Antimicrobial constituents of the leaves of Acalypha wilkesiana and Acalypha hispida. Phytother Res 2000;14:371-4.

Akinde BE. Phytochemical and microbiological evaluation of the oils from the Leaves of Acalypha wilkesiana. In: Sofowora A (ed.). The state of medicinal plant research in Nigeria. Nigeria: University of Ibadan Press, 1986 (pp. 362-3).

Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for antimethicillin resistant Staphylococcus aureus activity. BMC Complement Alternat Med 2005;5:6.

Al-Qarawi AA, Mousa HM, Ali BE-DH, Abdel-Rahman H, El-Mougy SA. Protective effect of extracts from Dates (Phoenix dactylifera L.) on carbon tetrachloride-induced hepatotoxicity in rats. Int J Appl Res Vet Med 2004;2:176–80.

Burtis CA, Ashwood ER. Tietz’s fundamentals of clinical chemistry. Vol. 2. Philadelphia, PA: WB Saunders, 2001.
Bussing A, Stein GM, Herterich-Akinpelu I, Pfüller U. Apoptosis-associated generation of reactive oxygen intermediates and release of pro-inflammatory cytokines in human lymphocytes and granulocytes by extracts from the seeds of *Acalypha wilke-siana*. J Ethnopharmacol 1999;66:301-9.

Christman S. *Acalypha wilkesiana*. Floridata.com LC, Florida, 2004. http://www.floridata.com/ref/A/acal_wil.cf

Cornelius CE. Animal models in liver research. San Diego, CA: Academic Press, 1993.

Gilman EF. *Acalypha wilkesiana*. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Fact Sheet FPS-6. October, 1999 (3pp.).

Ikewuchi JC. Changes in the weight, plasma lipid profile, and atherogenic indices of salt-loaded rats by aqueous extract of *Acalypha wilkesiana* Muell Arg: Potential for cardiovascular risk reduction. Pac J Sci Technol 2010;11:516-23.

Ikewuchi CC, Ikewuchi JC. Comparative study on the vitamin composition of some common Nigerian medicinal plants. Pac J Sci Technol 2009;10:367-71.

Ikewuchi JC, Ikewuchi CC. Hypocholesterolaemic effect of aqueous extract of *Acalypha wilkesiana* ‘Godseffiana’ Muell Arg on rats fed egg yolk supplemented diet: Implications for cardiovascular risk management. Res J Sci Technol 2010;2(4):78-81.

Ikewuchi JC, Ikewuchi CC, Onwuka FC. *Acalypha wilkesiana* Muell Arg induced diuresis in salt-loaded rats: Implication for the management of edema, obesity and hypertension. J Appl Sci Environ Manage 2009a;13(4):51-4.

Ikewuchi JC, Ikewuchi CC, Eriyamremu GE. Effect of *Acalypha wilkesiana* Muell Arg on the blood pressure and aorta contractility of salt-loaded rats. Pac J Sci Technol 2009b;10:829-34.

Ikewuchi JC, Ikewuchi CC, Onyeike EN, Uwakwe AA. Nutritional potential of the leaves of *Acalypha wilkesiana* ‘Godseffiana’ Muell Arg (Euphorbiaceae) on the hematology, plasma biochemistry and ocular indices of oxidative stress in alloxan induced diabetic rats. J Ethnopharmacol 2011a;137:1415–24. http://dx.doi.org/10.1016/j.jep.2011.08.015

Ikewuchi JC, Onyeike EN, Uwakwe AA, Ikewuchi CC. Effect of aqueous extract of the leaves of *Acalypha wilkesiana* ‘Godseffiana’ Muell Arg on blood pressure components and pulse rates of subchronic salt-loaded rats. Res J Sci Technol 2011b;3:264-9.

Kowalska MT, Brandt ME, Puett D. Inhibition of cytochrome P-450 aromatase activity by plant extracts. Planta Med 1990;56:675–7.

Lin C-N, Tome W-P. Antihypertensive components of *Sambucus formosana*. Planta Med 1988;54:223–4.

Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. Pharmacol Rev 2000;52:673-751.

Mittal DK, Joshi D, Shukla S. Protective effects of *Polygonum bistorta* (Linn.) and its active principle against acetaminophen-induced toxicity in rats. Asian J Exp Biol Sci 2010;1:951-8.
Obi FO, Uneh E. pH dependent prevention of carbon tetrachloride – induced lipoperoxidation in rats by ethanolic extract of *Hibiscus rosasinensis* petal. Biokemistri 2003;13:42–50.

Ogundaini AO. From greens into medicine: Taking a lead from nature. An inaugural lecture delivered at Oduduwa Hall, Obafemi Awolowo University, Ile-Ife, Nigeria. Ile-Ife, Nigeria: OAU Press, 2005 (Inaugural Lecture Series, No. 176). [http://www.oauife.edu.ng/faculties/pharmacy/aogund.pdf](http://www.oauife.edu.ng/faculties/pharmacy/aogund.pdf)

Oladunmoye MK. Comparative evaluation of antimicrobial activities and phytochemical screening of two varieties of *Acalypha wilkesiana*. Int J Trop Med 2006;1:134-6.

Pithayanukul P, Nithitanakool S, Bavovada R. Hepatoprotective potential of extracts from seeds of *Areca catechu* and nut galls of *Quercus infectoria*. Molecules 2009;14:4987-5000.

Rikans LE, Smith CR, Zannoni VG. Ascorbic acid and cytochrome P-450. J Pharmacol Exp Ther 1978;204:702–5.

Sato PH, Zannoni VG. Ascorbic acid and hepatic drug metabolism. J Pharmacol Exp Ther 1976;198:295–307.

Taraphdar AK, Roy M, Bhattacharya RK. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. Curr Sci 2001;80:1387-96.

Tram NTC, Mitova M, Bankova V, Handjieva N, Popov SS. GC-MS of *Crinum latifolium* L. alkaloids. Z Naturforsch 2002;57c: 239-42.