*British Journal of Medicine & Medical Research*
*18(4): 1-7, 2016, Article no.BJMMR.28142*
*ISSN: 2231-0614, NLM ID: 101570965*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Histological and Aminotransferase Implications of Administration of Extracts of *Acalypha wilkesiana* Leaves in Normal Rabbits

**Omage Kingsley[1*], Azeke A. Marshall[2], Orhue N. E. Jerry[3] and Iseghohi O. Sylvia[3]**

*[1]Department of Biochemistry, College of Basic Medical Sciences, Igbinedion University, Okada, Edo State, Nigeria.*
*[2]Department of Biochemistry, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State. Nigeria.*
*[3]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Edo State, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BJMMR/2016/28142
Editor(s):
(1) Chan-Min Liu, School of Life Science, Xuzhou Normal University, Xuzhou City, China.
(2) Masahiro Hasegawa, Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu City, Mie, 514-8507, Japan.
Reviewers:
(1) Sameh Mohamed Farouk, Suez Canal University, Egypt.
(2) Walter Antonio Roman Junior, Regional Community University Chapecó, Brazil.
(3) Joao Batista de Sousa, Universitário Darcy Ribeiro, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/16575

***Original Research Article***

***Received 4th July 2016***
***Accepted 23rd August 2016***
***Published 15th October 2016***

## ABSTRACT

**Background and Aim:** Traditionally, *Acalypha wilkesiana* leaves are used in the management of hypertension, diarrhoea and dysentery. Thus, this study was carried out to evaluate the effects of oral administration of extracts of *Acalypha wilkesiana* leaves on serum aminotransferases activities and tissues of normal experimental rabbits.
**Methods and Design:** Eighteen adult rabbits of the New Zealand strain, were randomized into three groups (groups A, B and C) of six rabbits each and treated as follows; Group A (Treated with Aqueous Extract), Group B (Treated with Ethanol Extract), Group C (Non treated-Control), and used for the study.
**Statistical Analysis:** Data are represented as Mean ± S.E.M (n = 6). Significance of difference was tested by ANOVA at $P < 0.05$.

**Corresponding author: E-mail: omagekingsley@yahoo.com;*

**Results and Conclusion:** Administration of the extracts of *Acalypha wilkesiana* leaves at a dose of 300 mg/kg body weight to normal experimental rabbits resulted in significantly lower levels of serum ALT and AST activities. Histological studies revealed that administration of the aqueous extract of *Acalypha wilkesiana* leaves to normal rabbits resulted in moderate congestion of blood vessels in the heart, mild lymphocytosis of the glomerular of the kidney, and no effect on the liver. While administration of the ethanol extract resulted in moderate congestion in blood vessels of the heart, moderate congestion of the central portal vein of the liver, and marked lymphocytosis in the kidney.

*Keywords: Acalypha wilkesiana leaves; rabbits; ethanol extract; aqueous extract; histology; aminotransferases.*

## 1. INTRODUCTION

Traditional medicine is widely acclaimed to be important in preventing and curing various diseases. It is relevant in the health services of the low socio-economic class due to its ready availability and at a cost they can afford. Moreover, 30% of modern conventional drugs are derived from plant sources [1], and in West Africa, new drugs are often beyond the reach of the poor where up to 80% of the population use medicinal plants as remedy against infections and diseases [2,3].

*Acalypha wilkesiana* Muell Arg (copper leaf) is a medicinal plant from the family Euphorbiacaece. Traditionally, the leaves are used in managing the abnormal sodium and potassium metabolism that accompany hypertension [4]. The leaves are squeezed into water and the resulting solution drunk to treat diarrhoea and dysentery, while the fresh leaf juice is drunk for laryngitis. *Acalypha wilkesiana* leaves have been reported to be biologically active [5-7] due to its phytochemical, proximate and elemental composition [7-11].

In view of its many uses, especially in Nigeria, and the fact that traditional medicine practitioners prescribe and administer decoctions of the leaves to patients without regard to its possible adverse effects. Also, due to dearth of information on its histopathological implications in experimental animals, the present investigation was undertaken to assess the effects of *Acalypha wilkesiana* leaf extracts on the tissues of the heart, kidney and liver, as well as the activities of aminotransferases in normal experimental rabbits.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals and Design

Eighteen adult rabbits of the New Zealand strain, weighing between 1.0–1.4 kg, were used for this study. The experimental rabbits were maintained on a 12-hour light and dark cycle in clean disinfected cages, fed ad libitum with standard pelletized growers feed (from UAC- Vital Feed, Jos, Plateau State) and were allowed free access to water throughout the duration of the experiment. The animals were treated according to the International guidelines for the care and use of laboratory animals and allowed to acclimatize to the new environment for a period of three weeks. They were then randomized into three groups (groups A, B and C) of six rabbits each and treated as follows;

Group A: Treated with Aqueous Extract
Group B: Treated with Ethanol Extract
Group C: Non-treated (Control)

#### 2.1.1 Plant materials

Fresh *Acalypha wilkesiana* leaves were obtained from local gardens within Benin City and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The leaves were properly washed, air-dried and ground into fine powder.

#### 2.1.2 Preparation of ethanol extract

About 100 g of the powdered leaves was soaked in 400 ml of ethanol (95%) for 72 hours (3 days), with occasional stirring using a magnetic stirrer to ensure proper mixture of the vessel content. The content was then filtered using a sintered funnel, (which is equivalent to four folds of bandage or sheet of cheese cloth). The extract (filtrate) was then concentrated using rotary evaporator and weighed.

#### 2.1.3 Preparation of aqueous extract

About 100 g of the powdered leaves was soaked in 400 ml of distilled water for 72 hours (3 days), and treated as described above.

#### 2.1.4 Administration of extracts

Five grams of the dry mass (concentrated extracts) were suspended in distilled water for administration to the experimental animals. The extracts (aqueous or ethanol) were administered orally at a dose of 300 mg/kg body weight for a period of twenty-one (21) days.

#### 2.1.5 Collection of samples (Organs)

At the end of the treatment (day 22), the animals were sacrificed and the organs (Heart, Liver and Kidney) collected, rinsed in normal saline and stored in formalin solution. The tissues were then used for histological studies.

#### 2.1.6 Haematoxylin and eosin (H & E) staining method

The haematoxylin and eosin (H & E) staining technique was performed to demonstrate the general structure of the tissues. This was done on all tissues.

#### 2.1.7 Collection of blood

Prior to treatment (Basal/day 0) with the extract, blood samples were collected from the veins located on the dorsal side of the ear lobes of the experimental animals (rabbits), using sterilized hypodermic needles. At days 7, 14 and 21 after treatment with the extracts, blood samples were also collected. Samples were collected in plane (universal) bottles immersed in ice. Immediately after collection of blood, the tubes were centrifuged at 3,500 rpm for 10 minutes to obtain clear serum that were used for further analysis.

#### 2.1.8 Assay methods

Alanine aminotransferase (ALT) was by the method of Reitman and Frankel, [12], where ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4 – dinitrophenyl hydrazine at 546 nm. Aspartate aminotransferase (AST) was also by the method of Reitman and Frankel, [12] where AST was measured by monitoring the concentration of oxaloacetate formed with 2, 4- dinitrophenyl hydrazine at 546 nm.

### 2.2 Statistical Analysis

Data are represented as Mean ± S.E.M (n = 6). Significance of Difference was tested by Student t-Test, ANOVA and Turkey-Kramer test, using the GraphPad Instat Version 3 (GraphPad Software Inc. San Diego, California U.S.A.). Statistical Significance was set at $p < 0.05$.

## 3. RESULTS

The effects of oral administration of extracts (aqueous or ethanol) of *A. wilkesiana* leaves on serum ALT and AST, as well as the major organs (liver, heart and kidney) of normal experimental rabbits, are as shown below.

In Fig. 1, Group A (given aqueous extract) showed ALT levels significantly ($p < 0.05$) lower than that of Group C (control) at day 7.

In Fig. 2, at days 7 and 21 of treatment, the aqueous extract (group A) resulted in significantly ($P < 0.05$) lower AST levels as compared with the control.

Administration of the aqueous extract to normal rabbits resulted in moderate congestion of blood vessels in the heart, mild lymphocytosis of the glomerular of the kidney, and no effect on the liver. While administration of the ethanol extract resulted in moderate congestion in blood vessels of the heart, moderate congestion of the central portal vein of the liver, and marked lymphocytosis in the kidney.

## 4. DISCUSSION

ALT is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, pancreas and the blood. However, ALT activities in the blood can increase majorly due to liver damage or disease. In this study, administration of extracts of *Acalypha wilkesiana* leave (aqueous) resulted in significantly ($p < 0.05$) lower ALT levels as compared with the control. Significantly elevated levels of ALT often suggest the existence of diseases such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. While significantly lower levels suggest otherwise. Thus, the ALT activities as observed may suggest that no significant liver damage has occurred due to administration of the extracts.

Also, administration of the extracts at a dose of 300 mg/kg body weight to the normal experimental rabbits resulted in lower levels of serum AST of the test groups as compared with the control. Here, the effect of the aqueous extract is shown to be more significant. These

however, show the possible protective effect of the plant. AST is found in many tissues in the body, including the liver, heart, muscles, kidney, and brain. This means that AST is not as specific an indicator of liver damage as ALT. Striated muscle, myocardium, and liver tissues are the main sources of AST. A growing body of information suggests that determination of AST isoenzymes in human serum is useful in evaluating damage to some of these organs [13].

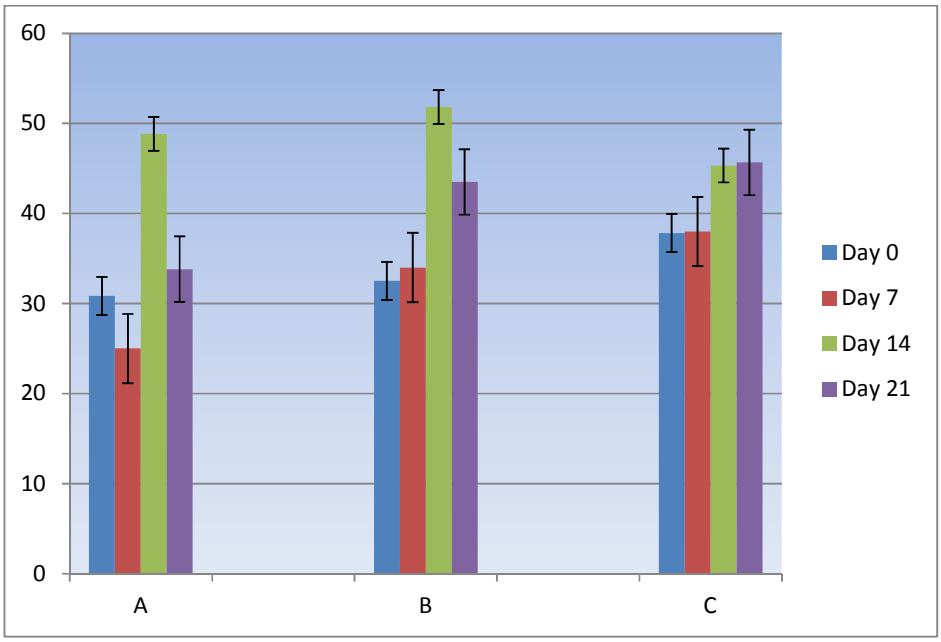


**Fig. 1. Serum ALT (U/L) of normal rabbits treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves with control (C)**

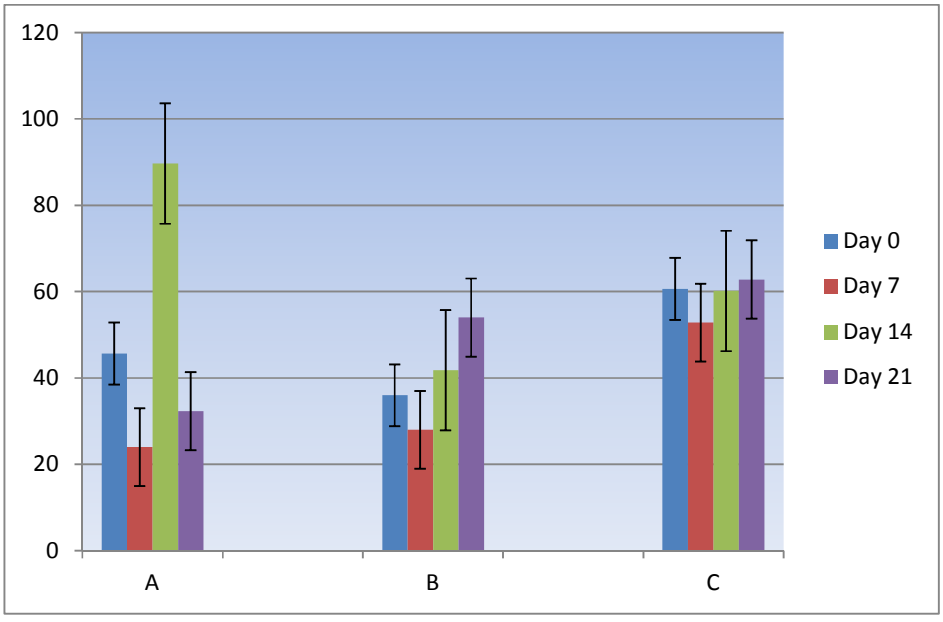


**Fig. 2. Serum AST (U/L) of normal rabbits treated with aqueous (A) and ethanol (B) extracts of *Acalypha wilkesiana* leaves with control (C)**

The heart is responsible for the pumping of blood in the body normally with the aid of the cardiac muscles. Administration of *Acalypha wilkesiana* (leaf extracts) as shown in this study resulted in moderate to severe congestion in the blood vessels of the cardiac muscles. This is dangerous as the heart will be unable to pump enough blood to meet the oxygen demand of the body and thus result in congestive heart failure (CHF). The portal vein appeared normal despite the administration of the extracts (aqueous or ethanol). However, treatment with the ethanol extract of the plant (leaves), resulted, possibly, in mild necrosis (which is a development of hepatocellular dysfunction without any prior liver disease). Administration of the aqueous extract of *Acalypha wilkesiana* leaves did not result in any definite effect on the hepatocytes, while administration of the ethanol extract showed a mild effect.

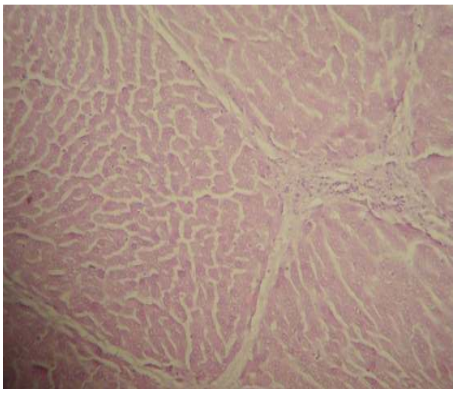

**Plate 1. Histological section of the liver. Group (A): given aqueous extract. The portal vein appears normal**

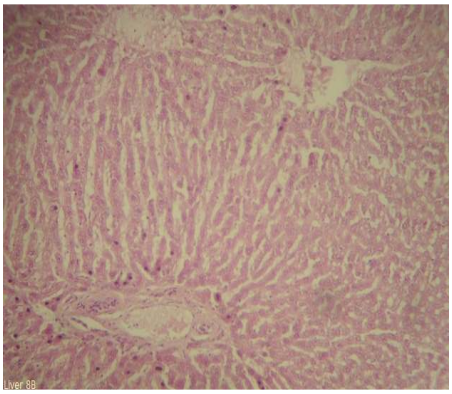

**Plate 2. Histological section of the liver. Group (B): given ethanolic extract. There is mild necrosis and central portal vein appears normal**

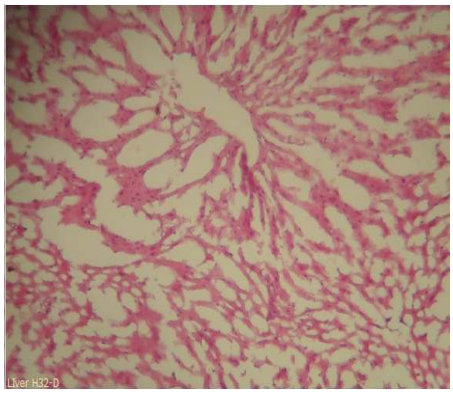

**Plate 3. Histological section of the liver. Control group (C): given distilled water The central portal vein appears normal**

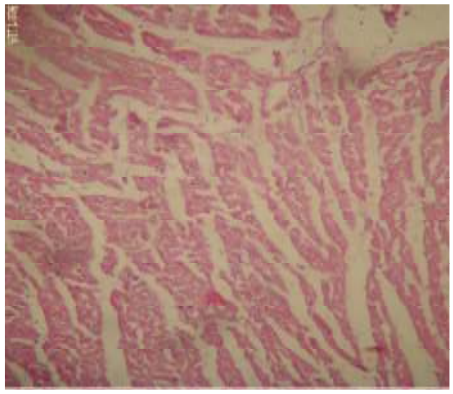

**Plate 4. Histological section of the heart. Group (A): given aqueous extract. The muscle appears to be moderate with severe congestion in blood vessels**

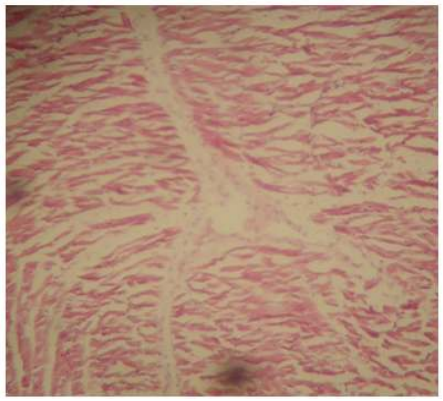

**Plate 5. Histological section of the heart. Group (B): given ethanolic extract. The muscles appear to be moderate with severe congestion in blood vessels**

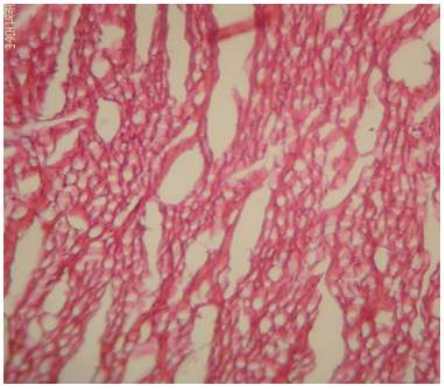

**Plate 6. Histological section of the heart Control group (C): given distilled water Shows normal cardiac muscle fibres**

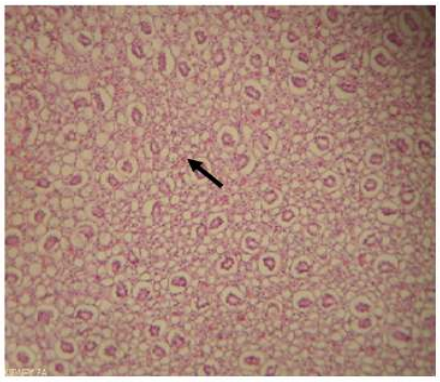

**Plate 7. Histological section of the kidney. Group (A): given aqueous extract. The arrow shows mild haemorrhage in glomerular of the kidney as well as mild lymphocytosis**

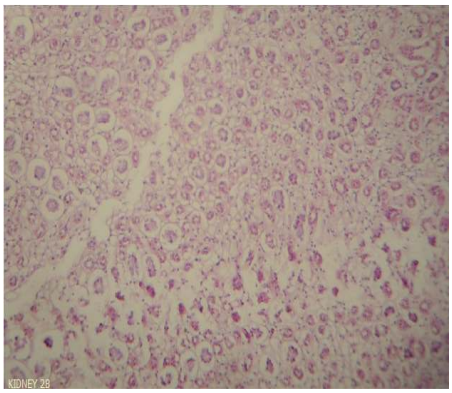

**Plate 8. Histological section of the kidney. Group (B): given ethanolic extract. The blood vessels appear normal. The glomeruli appear to have marked lymphocytosis with hyperchromatic nucleus**

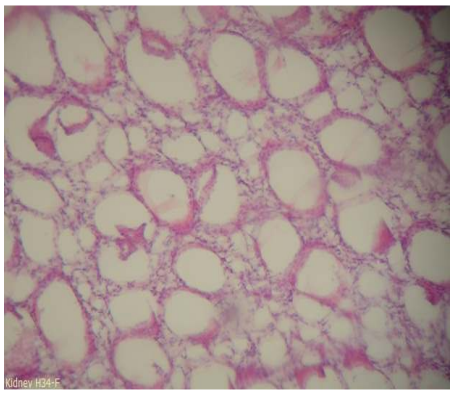

**Plate 9. Histological section of the kidney. Control group (C): given distilled water. Shows normal glomerular of the kidney**

The kidney is essential in the urinary system and also serves homeostatic functions such as regulation of electrolytes, maintenance of acid – base balance, and the regulation of blood pressure (via the maintenance of salt and water balance). Treatment with the aqueous extract (Group A) of *Acalypha wilkesiana* leaves resulted in mild haemorrhage (bleeding due to ruptured blood vessels) and also lymphocytosis of the glomerular (resulting from reduced level of glomerular filtration which could be due to a pre-existing disease or bleeding into the gastrointestinal tract). However, the ethanol extract showed no effect on the blood vessels, but there was marked level of lymphocytosis as well as hyperchromatic nucleus. The Hyperchromatic nucleus refers to abundant deoxyribonucleic acid – DNA (elevated chromatin) which suggests malignancy. Treatment with the aqueous extract of *Acalypha wilkesiana* leaves had no effect on the liver, a mild effect on the heart if controlled, and a less debilitating effect on the kidney. While treatment with the ethanol extract of *Acalypha wilkesiana* leaves had no effect on the blood vessels of the kidney but could cause malignancy and glomerular lymphocytosis. It had a mild effect on the heart.

## 5. CONCLUSION

Administration of the extracts of *Acalypha wilkesiana* leaf at a dose of 300 mg/kg body weight to normal experimental rabbits resulted in significantly lower levels of serum ALT and AST activities. Administration of the aqueous extract resulted in moderate congestion of blood vessels in the heart, no effect on the liver, mild lymphocytosis of the glomerular of the kidney;

while administration of the ethanol extract resulted in moderate congestion in blood vessels of the heart, moderate congestion of the central portal vein of the liver, and marked lymphocytosis in the kidney, in the normal test rabbits.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Murray MT. The Healing Power of Herbs, 2nd edition. Gramercy Books: New York, NY; 2004.
2. Kirby GC. Medicinal plants and the control of protozoal disease, with particular reference to malaria. Trans. R. Soc. Trop. Med. Hyg. 1996;90:605-609.
3. Hostettmann K, Marston A. Twenty years of research into medicinal plants: Results and perspectives. Phytochem. Rev. 2002; 1:275-285.
4. Ikewuchi JC, Ikewuchi CC. Effect of *Acalypha wilkesiana* muell Arg on the ATPase activities of salt-loaded rats. Pacific Journal of Science and Technology. 2009;10(2):823-828.
5. Seaforth CE, Adams CD, Sylvester Y. A guild to the medicinal plants of Trinidad and Tobago. The Commonwealth Secretariat, London; 1983.
6. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. J. Ethnopharmacol. 1993;39: 171-174.
7. Maharana DP, Sarengi SK, Singh RNB, Ali MH. Proceeding of the workshop on micronutrients. 22-23 Jan. 1992. Bhubaneswar, India. 1993;228-238.
8. Adesina SK, Idowu O, Ogundaini AO, Oladimeji HO, Olugbade TA, Onawunmi GO, Pais M. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytotherapy Res. 2000;14:371-374.
9. Ikewuchi CJ, Ikewuchi CC, Onyieke EN, Uwakwe AA. Nutritional potential of the leaves of *Acalypha wilkesiana* 'Godseffiana' Muell Arg. J. Appl. Sci. Environ. Manage. 2010;14(3):21–24.
10. Omage Kingsley, Azeke A. Marshall, Ihimire I. Inegbenose. Idagan A. Meg. Phytochemical, proximate and elemental analysis of *Acalypha wilkesiana* leaves. Scientific Journal of Pure and Applied Sciences. 2013;2(9):323–331.
11. Omage Kingsley, Azeke A. Marshall. Medicinal potential of *Acalypha wilkesiana* leaves. Advances in Research (AIR). 2014;2(11):655–665.
12. Panteghini M. Aspartate aminotransferase isoenzymes. Clin Biochem. 1990;23(4): 311-9.
13. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol. 1957;28:56-63.




*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://sciencedomain.org/review-history/16575*