

**TOXICOLOGICAL EVALUATION OF THE METHANOL EXTRACT AND BUTANOL  
FRACTION OF *CHRYSOPHYLLUM ALBIDUM* COTYLEDONIN RATS**

**SHOBO Akinmayowa Adedoyin**

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**AUTHOR:** Shobo, Akinmayowa Adedoyin

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-----  
**Date**

## CERTIFICATION

We certify that this work was carried out in the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria by Shobo, Akinmayowa Adedoyin under our supervision.

-----  
**Dr. O.M. Daniyan**

**(Supervisor)**

**Department of Pharmacology,**

**Faculty of Pharmacy, OAU**

**Ile-Ife, Nigeria.**

-----  
**Date**

-----  
**Dr. G. Olayiwola**

**(Co-Supervisor)**

**Department of Clinical Pharmacy and Pharmacy Administration,**

**Faculty of Pharmacy, OAU**

-----  
**Date**

**Ile-Ife, Nigeria.**

-----  
**Prof. M.A. Akanmu**  
**(Head of Department)**  
**Department of Pharmacology,**  
**Faculty of Pharmacy, OAU**  
**Ile-Ife, Nigeria.**

-----  
**Date**

### **DEDICATION**

This work is dedicated to my phenomenal parents, Mr. and Mrs. E.A. Shobo and to my siblings, Femi and Bimpe.

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**LIST OF ABBREVIATIONS**

<b>CA</b>	<i>Chrysophyllum albidum</i>
<b>ME</b>	Methanol extract
<b>BF</b>	Butanol fraction
<b>TM</b>	Traditional medicine
<b>LD<sub>50</sub></b>	Median lethal dose
<b>LC<sub>50</sub></b>	Median lethal concentration
<b>EMEA</b>	European Medicinal Evaluation Agency
<b>FDA</b>	Food and Drug Administration
<b>SOT</b>	Society of Toxicology
<b>TG</b>	Test guideline
<b>OECD</b>	Organization for Economic Development and Cooperation

<b>P.O.</b>	<i>Per oral</i>
<b>NOEL</b>	No Observed Effect Level
<b>EDTA</b>	Ethylene diamine tetraacetic acid
<b>ALT</b>	Alanine aminotransferase
<b>AST</b>	Aspartate aminotransferase
<b>HCT</b>	Hemocrit
<b>PCV</b>	Packed cell volume
<b>MCV</b>	Mean corpuscular volume
<b>MCH</b>	Mean corpuscular hemoglobin
<b>MCHC</b>	Mean corpuscular hemoglobin concentration
<b>IBIL</b>	Indirect bilirubin
<b>DBIL</b>	Direct bilirubin
<b>TBIL</b>	Total bilirubin
<b>CCAC</b>	Canadian Council on Animal Care
<b>BMD</b>	Benchmark Dose
<b>SWG</b>	Scientific working group
<b>DPX</b>	Distrene Plasticizer and Xylene

### ABSTRACT

The seed extracts of *Chrysophyllum albidum*(CA) have been reported to possess anti-microbial, anti-diabetic, hypolipidemic, antihyperglycemic properties among others. As with other phytomedicines, there is the risk of adverse effects due to its indiscriminate use which could be attributed but not limited to the perceived safety of herbal formulations. The study therefore investigated the toxicological profile of the methanol extract (ME) and butanol fraction (BF) of the CA cotyledon.

Dried cotyledons were subjected to extraction and fractionation processes using methanol and butanol respectively. Single dose (150, 300, 600 mg/kg ME and 40, 80, 160 mg/kg BF; n = 5) and repeated dose (100, 300 mg/kg ME and 50, 150 mg/kg BF; n = 10) toxicity tests were conducted via acute and sub-acute oral exposure of nulliparous female Wistar rats to the ME and BF of CA cotyledon by evaluation of various endpoints including functional observational battery (FOB); haematological; biochemical and histopathological parameters, in accordance with the guidelines of OECD – 420 and – 407. A 21 – day non – dosing recovery study was subsequently conducted to ascertain the reversibility (or persistence) potential for toxicity.

Results revealed that ME and BF were relatively toxic, having LD<sub>50</sub> of 760 and 200 mg/kg (p.o.) respectively. The test substances were found to have depressant activity following the FOB. The ME and BF did not cause adverse effect with respect to their body and organ weights following acute and sub-acute oral exposure. Following acute doses of the ME and BF, there were no significant alterations in the hematological parameters. Repeated administration of ME however, caused significant reduction in the RBC count (t = 4.350, P = 0.002) and HCT at 300 mg/kg while sub-acute doses of the BF resulted on significantly reduced WBC count (t = 3.32, P = 0.01), HCT (t = 2.854, P = 0.02), MCV (t = 4.306, P = 0.003) and MCH (t = 3.59, P = 0.01) at 150 mg/kg. Acute doses of the ME also caused significant elevation in ALT activity (t = 2.951, P = 0.02) at 300 mg/kg while at 600 mg/kg, significant increase in creatinine levels (t = 3.909, P = 0.01) was noted. Single dose administration of BF also caused significant increase (t = 4.76, P = 0.00) in ALT activity at 80 mg/kg while at 160 mg/kg, significant decrease in indirect bilirubin (t = 2.452, P = 0.04) was noted. Repeated administration of the ME resulted in significant increase in ALT activity (t = 3.066, P = 0.02), AST activity (t = 4.000, P = 0.00), creatinine levels (t = 4.444, P = 0.00) at 100 mg/kg while at 300 mg/kg, decreases in indirect (t =

2.620,  $P = 0.03$ ) and total bilirubin ( $t = 2.56$ ,  $P = 0.03$ ) was noted. The AST activity ( $t = 2.419$ ,  $P = 0.04$ ) was significantly decreased in animals repeatedly administered the BF at 50 mg/kg. Histological examination of liver, kidney and brain did not reveal significant changes in the treatment groups compared to the control.

The study concluded that the test materials had potential to cause moderate but reversible forms of toxicity; therefore there is need for caution in the consumption of *Chrysophyllum albidum* cotyledon.

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## CHAPTER ONE

### 1.0 INTRODUCTION AND LITERATURE REVIEW

#### 1.1 TRADITIONAL MEDICINE

Traditional medicine (TM) also known as ‘Complementary and Alternative’ medicine is an indigenous form of medicine (WHO, 2005a; Alves and Rosa, 2007). TM is an essential aspect of the rich cultural heritage that has survived through many generations and includes the indigenous way of preventing, diagnosing and managing diseases. Among the widely known and globally practiced forms of TM are: Ayurveda, a form of traditional medicine in India (Morgan, 2002); Unani, an indigenous form of medicine originating from Greece (Sofowora, 2012); Tibetan medicine, a derivative of methods from both Ayurvedic medicine and the Chinese traditional medicine (Li, 2000); Neo - Western Herbalism, encompassing the European and American herbal medicine (Elvin-Lewis, 2001; WHO, 2002); Traditional Chinese Medicine, which accounts for around 40% of all health care delivery in China (Wu, 2005); and African Traditional Medicine, accounting for up to 80% of the African health care needs (Gurib-Fakim, 2006). These underscore the global relevance and increasing recognition of traditional medicine as a veritable tool in addressing Man’s health needs.

In a bid to accommodate the worldwide diversity of cultures and their indigenous mode of traditional medicine practices, the World Health Organization (WHO) defines traditional medicine as: “the sum total of all the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures whether explicable or not, used in maintenance of health as well as in the prevention, diagnosis, improvement or treatment of

physical and mental illness” (WHO, 2005b). In addition, Medicinal plant are defined as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products (WHO, 2001).

Man’s knowledge of medicinal plants and traditional system of medicine dates back to 1500 BC from the Eberus Papyrus in Egypt. Such knowledge may have been acquired through instinct, experiences or careful observations of the effects of effects of such plants on domestic animals, and subsequently passed from generation to generation through tutelage or other anecdotal forms of communication (Sofowora, 2012).

Traditional medicine in Africa has grown considerably, having approximately 60,000 of the world’s higher plant species (Dzoyem *et al.*, 2013). Its ease of accessibility and affordability has made it “the most economical and available system of health care and highly favoured by a large number of the African population in rural and semi-urban areas” (Kasilo *et al.*, 2010; Kamsu-Foguem and Foguem, 2014). Despite its popularity, information bordering on African traditional medicine is still largely insufficient when compared to its contemporaries around the world (Ndhlala *et al.*, 2009; Egharevba *et al.*, 2015a,b) due to several challenges. One of such is the quality control of herbal medicine, an issue of global importancethat is indispensable for the advancement of the herbal medicine system (Sen *et al.*, 2011). Zhang *et al.* (2012) pointed out that issues on quality control of herbal medicines involve internal factors arising from the drug and external factors in clinical use. Another important challenge is the issue of adverse effects caused by herbal medicines. Kamsu-foguem and Foguem (2014) noted thatfrom the huge patronage of herbal medicine in Africa countries, it is most likely that many adverse drugs reactions will go unnoticed and unrecorded, either as a result of patients failing to report cases of

adverse effect to health services, or non-availability of pharmacovigilance analysis. In spite of this, a few African countries notably South Africa, Nigeria, and Cameroon have subsequently introduced herbal/traditional medicine as part of their pharmacovigilance systems (Fokunang *et al.*, 2011).

### 1.1.1 Traditional Medicine in Nigeria

Nigeria abounds in its huge biodiversity of flora especially medicinal plants used in the treatment of many tropical diseases. This curative property has been attributed to the presence of certain phytochemicals present in these tropical medicinal plants (Okwu and Okwu, 2004; Onwuliri, 2004).

In Nigeria, the use of herbal medicine singly or in combination with orthodox medicine for management of various ailments is a frequent practice (Ezuruike and Prieto, 2014). Several studies have reported effective use of herbal medicine in Nigeria for the management of diseases including those of adults with various forms of chronic illness (Amira and Okubadejo, 2007; Ogbera *et al.*, 2010); on pregnant women (Fakeye *et al.*, 2009); children with chronic illness (Oshikoya *et al.*, 2008). Also, increasing patronage of herbal preparations has been reported for, among other purposes - the treatment of malaria and hypertension (Oreagba *et al.*, 2011). Such increasing patronage may be attributed to but not limited to the perceived safety of herbal formulation when compared to orthodox medicine (Amira and Okubadejo, 2007; Fakeye *et al.*, 2009), mostly due to their natural origin, efficacy and perceived lack of adverse effect (Oreagba *et al.*, 2011). Typical example of some Nigerian Medicinal Plants with their folkloric uses are: *Rauwolfia vomitoria* (used in hypertension, stroke, insomnia and convulsion) (Amole *et al.*, 2009); *Citrus parasidi* seed (treatment of urinary tract infections) (Oyelami *et al.*, 2005); *Carica*

*papaya* L. (treatment of intestinal parasitosis) (Okeniyi *et al.*, 2007); *Garcinia kola* (treatment of osteoarthritis) (Adegbehingbe *et al.*, 2008); *Pygeum africanum* (prostatitis, aphrodisiac, Laxative) (Kim *et al.*, 2012); *Securidac longepedunculata* (epilepsy,

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