Toxicity Test on *Anogeissus leiocarpus* Plant with Potential for use as Tea Substitute  
Authors  
Aisha Umar Auwal¹, Rabiu Sani Shawai², Abdulrazak Ado³, Dahiru Naziru⁴, Jamilu Yusuf Muhammad⁵, Sale Ali Ibrahim⁶, Bala Sidi Aliyu⁷.  
¹,²,³,⁴,⁵,⁶&⁷ Student of M.Tech, Dept. of Biotechnology School of Engineering & Technology Sharda University, Plot 32-34 Knowledge Park III, Greater Noida U.P.- 201306.  
⁷ Professor Dept. of Plant Science, Faculty of Science, Bayero University Kano, Nigeria.  

ABSTRACT  
Powdered leaves of *Anogeissus leiocarpus* were extracted with ethanol and fractionated with Pet-ether, N-hexane, Ethyl acetate, Chloroform, and Ethanol using partition technique. The extracts and fractions were tested for toxicity on brine shrimp larvae and phytochemically screened for the presence of secondary metabolites. Brine shrimp test result showed that the fraction of ethyl acetate was found to be more toxic with LC₅₀ of 0.0017mg/ml. The fraction obtained from chloroform was found to be moderately toxic with LC₅₀ of 18.58 mg/ml to brine shrimps. Concentrations of 10, 100, 1000 in mg/ml of extracts were administered. The lethal concentrations (LC₅₀) at lower concentrations (10mg/ml) were less toxic and are highly toxic at higher concentrations. The Phytochemical screening of the fractions revealed the presence of Alkaloids, Flavonoids, Steroids, Reducing sugars, and Tannins. Thus it is appropriate to use the plant as a tea substitute.  

Key words: *Anogeissus leiocarpus*, brine shrimp, Toxicity, Alkaloids, Flavonoids, Steroids, Reducing sugars, Tannins, Ethanol, Pet-ether, N-hexane, Ethyl acetate, Chloroform, Methanol.  

1. INTRODUCTION  
Toxicity is the degree to which something is poisonous or state of being poison to the body. A poison is any substance that produces disease conditions, tissue injury or otherwise interrupts natural life processes when in contact with or absorbed into the body. Most poisons taken in sufficient quantity are lethal. A poisonous substance may originate as a mineral vegetable or an animal and it may assume the form of solid, liquid or gas. A poison depending on the type may attack the surface of the body or most seriously internal organs or the central nervous system. Poisonous plants are plants containing substances that, when taken into the body of humans or animals in small or moderate amounts, provoke a harmful reaction resulting in illness or death. A plant may vary in toxicity as it grows, generally becoming more toxic with maturity, certain plants however, can be highly toxic when young and harmless later (John, 2007). It considered unethical to use human test subjects for acute (chronic) toxicity research. However, some information can be gained from investigating accidental human exposures. Otherwise, most acute toxicity data comes from animal testing or recently, invitro testing methods and inference from data on similar substances (MSD, 2006).  
Toxicology is the science of poison embracing the physical and chemical study of all the known poisonous substances, as well as the methods of testing them and their action on living body (Encarta, 2007). However, it can be considered as a branch of pharmacology since the later discipline also deals with adverse effect of drugs (Andres, 1976). Literally toxicology can be defined as the scientific study of poisons, their effects and...
their antidotes. It is clear that plants play a vital role in our lives, with the primary use being a source of food and medicine. Plants have been reported to contain comparatively high amounts of valuable nutrients such as vitamins A and C, and other antioxidant micronutrients (Szeto et al, 2002, Jimoh et al, 2008). In area of medicinal plants for instance, it has now become possible to isolate and structurally elucidate microgram quantities of potent bioactive natural products (Day, 1998).

Tea is a name given to a lot of brews but purists consider only: green tea, white tea, oolong tea and pu-erh tea, the real thing is that they are all derived from the Camellia sinensis plant, a shrub native to China and India, and contain unique antioxidant called flavonoid. All these teas also have caffeine and thiamine, which affect the brain and seem to heighten mental alertness. The more processed the tea leaves, usually the less polyphenol content, which contain flavonoids. Oolong and black teas are oxidized or fermented, so they have lower concentrations of polyphenols than green tea, but their antioxidanting powder is still high (Anonymous, 2005). Darkening of tea is due to enzymatic oxidation, among the health benefits of tea include: normalizing blood pressure, improving beneficial intestinal microflora, providing immunity against intestinal disorder, protecting cell membrane from oxidative damage, also for boosting energy and warming the body (Wikipedia, 2010).

1.1 Anogeissus Leiocarpus
Anogeissus leiocarpus is a tall deciduous tree which belong to the family Combretaceae and up to about 30m with high grey, yellowish, scaly and when old blackish bark. Slash yellow, flamed, secreting dark coloured gum. Twigs fin and weeping, brown, pubescent. Leaves solitary or alternate and typically arranged two on one side, two on the opposite side, ovate 4-17cm long, short petioled, base acute, tip mucronate, 4-8 lateral nerve. Under side of leaf slightly pubescent. Flowers during rainy season, yellow-green to creamy-white, with red calyx, and long stamina globose heads on 5-10mm long petioles in the leaf axles, sometimes in clusters. The Fruits are small cone-like, dark brown heads, breaking up easily into numerous two-winged seeds and remains on the tree for long time (Aliyu, 2006). The plant is widely distributed in the sahel savanna and also in forest savannah zone. It is widely spread in some of the western and eastern state of Nigeria, particularly in Kano state. The common name of the plant is African birch and the Hausa (vernacular) name is “marke” (Aliyu, 2006 and Ghanzamfar, 1989).

1.2 Ethnomedicinal Use/Medicinal Application Of The Plant
The plant is used in the treatment of Fevers, antihelminthic (Iwu, 1993). Diarrhoea, wound dressing laxative, tonic, aphrodisiac, dermatological disorders and ulcers (Maydell, 1990). The part used are the stem, bark, root or leaves, and also used for treatment of ascaricida, gonorrhea, blood clot, asthma, coughing and tuberculosis (Mann et al, 2003). The leaves of the plant are used in the treatment of skin disease, diabetics (Dweek, 1996).

2. MATERIALS AND METHOD
2.1 Collection of Plant Sample
The leaves of Anogeissus leiocarpus were collected in November 2010, from Kofar Arewa in Gwarzo local government area, Kano State, Nigeria. The plant was identified and air dried under shade, in the Department of Biological Sciences, Bayero University Kano. After which the leaves were grounded into fine powder using mortal and pistil.

2.2 Extraction Procedure
250g of the sample was percolated with 750ml of ethanol for two weeks. The percolate was then decanted; filtered and concentrated using rotary evaporator at 40\(^0\) C to yield the crude extracts and were labeled F0, F02, F03, F04, F05, and F06 respectively.
2.3 Partitioning of The Plant Sample

Partition is the process of separating parts of a substance by mixing liquids of different polarities in which the parts of the substance will solubilize and hence separate themselves according to their polarities. The solvents used in order of increasing polarity are ethanol, petroleum ether, n-hexane, ethyl acetate, chloroform and methanol. Maceration / partition were then carried out firstly on petroleum ether. 80ml of Pet-ether was added to the ethanol extract and stirred to dissolve the pet-ether soluble part of the extract in solution, the residue from pet-ether was decanted into an empty beaker and was labeled as F0, further maceration was carried out with n-hexane in order to remove fatty acid and esters, where the residue was labeled F0, this procedure was done to both ethyl acetate, chloroform and methanol which were labeled F0, and F0 respectively.

3. RESULT AND DISCUSSION

In the partitioning process, different solvents were used, hence different fractions were obtained.

Table 1: Physical Characteristics of the Extract and Fractions

<table>
<thead>
<tr>
<th>ANOGEISSUS LEIOCARPUS</th>
<th>SOLVENTS</th>
<th>TEXTURE</th>
<th>COLOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Crystalline</td>
<td>Green</td>
<td></td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>Oily</td>
<td>Yellowish green</td>
<td></td>
</tr>
<tr>
<td>N-Hexane</td>
<td>Oily</td>
<td>Green</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Crystalline</td>
<td>Green</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>Crystalline</td>
<td>Green</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>Crystalline</td>
<td>Green</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Phytochemical Screening Of Extracts And Fractions

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Reducing sugars</th>
<th>Steroids</th>
<th>Tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Petroleum Ether</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ether</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Hexane</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Methanol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + = Present
- = Absent

Table 3: brine shrimp lethality test on plant extract/fractions of A. leiocarpus

Acute toxicity study: Determination of lethal concentration (LC50)

<table>
<thead>
<tr>
<th>S/NO</th>
<th>FRACTION</th>
<th>LC50 VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>0.0018mg/ml</td>
</tr>
<tr>
<td>2</td>
<td>Petroleum ether</td>
<td>4.07mg/ml</td>
</tr>
<tr>
<td>3</td>
<td>Normal hexane</td>
<td>3.37mg/ml</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl acetate</td>
<td>0.0017mg/ml</td>
</tr>
<tr>
<td>5</td>
<td>Chloroform</td>
<td>18.58mg/ml</td>
</tr>
<tr>
<td>6</td>
<td>Methanol</td>
<td>2.26mg/ml</td>
</tr>
</tbody>
</table>
4 DISCUSSION

The Brine shrimp larvae were allowed to stand for 24 hours for signs of inaction or death which result from the toxic nature of the plant extract. It was found that ethanol and ethyl acetate fractions with LC50 value of 0.0018mg/ml and 0.0017mg/ml respectively were extremely toxic because at a very low concentration of 10mg/ml, less number of deaths was recorded. Thus, the LC50 value was calculated. Acute toxicity of Brine shrimp larval to *A. Leiocarpus* is summarized in table 3. In the present study toxicity was evaluated at concentrations of 10, 100 and 1000mg/ml to determine the LC50 ranges. Also, control solutions were conducted to confirm the accuracy of the test. The result indicate that the LC50 of *A. Leiocarpus* is about 0.0017-18.58mg/ml which is due to the driving factors of natural products such as flavonoids, steroids, tannins etc. 75% motility was observed at 24-hours in 10mg/ml i.e. the lowest concentration tested. The most toxic fraction is ethyl acetate with LC50 of 0.0017mg/ml i.e. more than 10mg/ml at 24-hours test and the least toxic fraction is in chloroform with an LC50 of 18.58mg/ml.

However, concentrations were found to be very toxic at higher concentrations (1000mg/ml) and become less toxic as the concentration decreases. Ethyl acetate fraction (F04) with LC50 0.0017mg/ml and Ethanol fraction (F01) with LC50 0.0018mg/ml were found to be extremely toxic to the Brine shrimps, with a 24-hours LC50 of <1mg/ml, whereas petroleum ether (F02) with LC50 of 4.07mg/ml, N-Hexane (F03) with an LC50 value of 3.37mg/ml and Methanol (F06) with LC50 of 2.26mg/ml were found to be very toxic with 24-hours LC50 of 1-10, lastly chloroform (F05) of 18.58mg/ml was found to be moderately toxic (Jin-Seok, 2005). Natural products Alkaloids, Flavonoids, Steroids, Tannins and Reducing sugar were screened on all fractions which are secondary metabolites and active components of many drugs found in plant.

5 CONCLUSION

Considering the experiment carried out on the leaves of *Anogeissus leiocarpus* against different solvents, some fraction shows extreme toxicity, while others show very high toxicity, where as only one fraction shows moderate toxicity to brine shrimps. Also, the brine shrimps larvae mostly die as the concentration become higher and higher, therefore it is concluded that if this plant is to be use as tea substitute, it must be use in lower concentration, which may not be harmful because when the leaves were boiled, the taste was like that of tea and possessed some certain characteristics of tea. However, this shows that if the plant is used in higher concentration it may be toxic.

**Recommendations**

It is recommended that further research should be carried out on other parts of the plant such as the stem, bark and seeds.

It is recommended that antifungal and antibacterial activities should be tested on the plant extracts.

It is recommended that the plant leaves should be percolated with water to confirm whether the toxicity was caused by percolating the leaves with ethanol.

**REFERENCES**