PHYTOCHEMICAL, ELEMENTAL AND LD_{50} SCREENING OF AQUEOUS LEAF EXTRACT OF *Annona senegalensis* IN ALBINO WISTAR RATS

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Abstract: Aqueous leaf extract of *Annona senegalensis* was screened for phytochemical and elemental components using standard methods. LD_{50} was evaluated using the arithemetic method. The phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, terpenoids and flavonoids. The elemental analysis revealed the presence of iron (Fe), copper (Cu), magnesium (Mg), lead (Pb), chromium (Cr), cadmium (Cd), cobalt (Co), zinc (Zn) and manganese (Mn) in parts per million concentrations and their values are 7.202, 0.893, 6.637, 0.014, 0.070, 0.057, 0.604, 1.732 and 2.362, respectively. The number of rats used for the $LD_{50}$was 30. Mortality was not observed in any of the doses 100, 200, 400, 800, 1600 except at 3200 mg/kg which produced 100% death of the animals. Thus, the calculated LD_{50} is 2400 mg/kg. The presence of the phytochemicals, elements and the LD_{50} may account to its uses by the natives as traditional medicine and food.

Keywords: *Annona senegalensis*, phytochemical, elements, LD_{50}

Introduction

The genus *Annona* belongs to the family Annonaceae, and species *senegalensis.* It is a specie of flowering plant. The type specimen was collected from Senegal (Orwa *et al*., 2009). The word 'anon' was from Latin being interpreted as yearly produce usually harvested annually. This explains the production habits of fruits of the various species in this genus (Pinto, 2005; Orwa *et al*., 2009).

The *Annona* species are small trees whose heights vary from 5 – 11 m depending on factors such as species, climate, soil and crop management. They may be erect or spreading in habit, with grey-brown bark, often rough and corrugated (Pinto, 2005). The stems of *Annona* species are in general, ferruginous to grayish and tomentose when young, but later become glabrous. With few exceptions, *Annona* species are deciduous. The system has thin lateral and tap roots. The lighter the soil texture, the longer the taproot grows. The taproot of a soursop tree can reach approximately 1.5-1.8 m in depth (Pinto, 2005).

The *Annona spp*. in general grows at a varying of altitudes, and those with the widest adaptation to altitude are also wide in adaptation to latitude. No photoperiod responses have been reported (Cordeiro and Pinto, 2005). Most *Annona* spp. does not adapt to low temperature, however highland species such as Cherimoya, wild soursop and to some extent custard apple are better adapted to cold weather than the lowland soursop and sugar apple (Cordeiro and Pinto, 2005). Wild soursop is adapted to various altitudes being cultivated from 0-1,800 m in Kenya and from 0-2,400 m in other parts of East Africa (FAO, 1983; Anon, 2014). It appears to have adaptation within the range of very low to moderately high rainfall regimes occurring in areas with 600-2,500 mm, while across Africa requirements are far more than 600 mm annual rainfall. It can withstand a relative humidity as low as 44% at midday. The best mean temperatures for wild soursop growth are between 16 and 30°C (FAO, 1983; Anon, 2014). It is often a solitary plant within woodland savannah understory, swamp forests, river banks and former crop land left fallow for an extended period (Anon, 2014).

The plant is used primarily for food by the natives in Africa. The fruit is rich in nutrient, boost food security, foster rural development and support sustainable land care (Anon, 2014). It tends to grow in semiarid to sub-humid regions adjacent to the coast, often, but not exclusively, on coral-based rocks with mostly sandy and loamy soils (Anon, 2014).

The plant has applications in many aspects of human endeavour. The flowers, leaves and fruit are edible and culinary, white fruit pulp has a mild, pineapple-like flavour. Wild Soursop fruits are sold in local markets in Africa. The fruit has a pineapple-like odour and sweet taste (FAO, 1983). It keeps for only a few days. It is used in sherbets, ice creams and for making drinks (FAO, 1988). The flower is added to spice or garnish meals; leaves are eaten by humans as vegetables, or browsed by livestock (Anon, 2014). The leaves are also used to create a general health tonic in the treatment of pneumonia, diseases of the eye, stomach and intestines (Cordeiro *et al*., 2005). The leaves have essential oils with parasiticide, antidiarrhoea, rheumatological and antineuralgic properties (Cordeiro *et al*., 2005). Boiled water infusions of the leaves have anti-spasmodic, astringent and gastric properties (Calzavara and Miiller, 1987; Khan *et al*., 1997), help in treatment of diabetes and gastric upsets (Calzavara and Miiller, 1987) and are used in kidney ailments (Cordeiro *et al*., 2005). The cooked flowers and petals are used for healing eye inflammations; the treatment requires 2-3 washes a day (Calzavara and Miiller, 1987).

Alcoholic leaf extracts of the plant have anti-spasmodic and relaxant activities on the stomach muscles, anti-ulcer activity against indomethacin-induced ulcer and reduces the effect of stress on ulcer induction. These effects are produced by various compounds including flavonoids, alkaloids, tannins and saponins (Cordeiro*et al*., 2005) found in the plant. Moreover, the leaves contain compounds that have insecticidal effects and are used to control insect pests (Abubakar and Abdulrahman, 1998).

The stem bark can be processed to produce yellow-brown dye, insecticide or medicine for treating a wide array of ailments, including parasitic worms in the intestines or flesh (notably guinea worms), diarrhoea, gastroenteritis, lung infections, toothaches and snake bites (Cordeiro *et al*., 2005). The natural gum from the stem bark is used for the treatment of open wounds (Anon, 2014). The stem bark contains 4-ent-kaurenoids that have cytotoxic activity against tumour cell lines (Fatope *et al*., 1996).

The roots are also used in treating several conditions including indigestion, chest colds, cancer, convulsions, venereal disease, diarrhoea, dysentery, fever, filariosis and have anti-neoplastic

and anti-protozoal activities (Fatope *et al*., 1996; Anon, 2014). The seeds have anti-spasmodic and anti-parasitic properties. They contain amyloids oleic acid and steroids (Cordeiro *et al*., 2005).

Immature soursop fruits have medicinal properties against dysentery, cankers, diuretic, scorbutic, anti-thermical processes, skin diseases, rashes, fever, malaria, peptic ulcers, colic and oedema (Khan *et al*., 1997). The peel from immature fruits has constituents that act against atonic dyspepsia, diarrhea and chronic dysentery; it is astringent and provokes vomiting. The acid pulp is used to heal foot parasites and icteric liver diseases. The fruit also has properties that act on the biliary vesicle making it less fragile which enhance flow (Calzavara and Miiller, 1987).

Materials and Methods

Collection and identification of the plant sample

Annona senegalensis leaves were collected from Girei Local Government Area of Adamawa.It was then identified and authenticated by a Taxonomist,Dr. B.T. Kwaga in the Department of Forestry and Wild Life Management of the ModibboAdama University of Technology, Yola. Voucher specimen (PG/15/CHM/008) waskept in the Departmental herbarium (Department of Chemistry). The leaves of the plant were dried under shade, grounded into powder and kept until required.

Experimental animals

Female and male albino rats were obtained from the Laboratory Animal House of the Biochemistry Department, University of Maiduguri and kept in the Postgraduate Veterinary Anatomy Research Laboratory, University of Maiduguri. The rats were given pelletized growers mash 120 g daily (Vital feeds Nigeria Ltd) and water *ad libitum*. Thirty (30) rats were used for acute toxicity study.

Ethical consideration

This research was approved by the Faculty Postgraduate Board Ethics Committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

Plant extraction

The *Annona senegalensis* aqueous leaf extract was obtained using soxhlet extractor and distilled water as the solvent. The extract was evaporated to near dryness on a water bath, weighed and kept at 4°C in refrigerator until required (Evans, 2009c; Zade and Dabhadkar, 2013). The marc which is the chaff of the leaves powder, product of the extraction was kept.

Phytochemical study

The aqueous leaf extract of *Annona senegalensis* was subjected to qualitative phytochemical analysis for detection of the presence of carbohydrates, flavonoids, alkaloids, saponins, glycosides, tannins, terpenes, resin, aloes steroids and anthraquinones using standard methods under room temperature as described by Evans (2009).

Test for carbohydrates

General test (Molisch's test)

Two drops (2) of Molisch's reagent were added to 0.5 g of the extract and thereafter dissolved in 5 ml distilled water. This was followed by addition of 1 ml concentrated tetraoxosulphate (VI) acid (H_2SO4) by the side of the test tube; so that the acid formed a layer beneath the aqueous layer. The mixture of the extract was then allowed to stand for two minutes under room temperature and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers indicated a positive test (Farmsworth, 1966; Evans, 2009a).

Test for monosaccharide (Barfoed's test)

A quantity (0.2 g) of the extract was dissolved in 5 ml distilled water and filtered. One milliliter of the filtrate was mixed with 1 ml of Barfoed's reagent in a test tube and heated on a water bath (60°C) for 2 min. A red precipitate of cuprous oxide was an indication of the presence of monosaccharides (Brain and Turner, 1975).

Test for free reducing sugars (Fehling's test)

Five (5) ml of distilled water was used to dissolve 0.2 g of the extract and filtered. The filtrate was heated (60°C) with 5 ml of equal volumes of Fehling's solutions A and B. Formation of a red precipitate of cuprous oxide (Cu_2O) was an indication of the presence of reducing sugars (Evans, 2009b).

Test for combined reducing sugars

A small quantity (0.2 g) of the extract was hydrolyzed by boiling (>100°C) for 5 min with 5 ml dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. A few drops of Fehling's solution were added to it and then heated (60°C) on a water bath for 2 min. Formation of a reddish-brown precipitate of cuprous oxide was an indication of the presence of combined reducing sugars (Evans, 2009b).

Standard test for ketoses (Salivanoff's test)

Two drops (2) crystals of resorcinol and 2 ml of hydrochloric acid were added to a small quantity of the extract (0.2 g) and the solution was allowed to boil for 5 min. A red colouration was an indication of the presence of ketoses (Vishnoi, 1979).

Test for pentoses

One (1) ml of hydrochloric acid and a little quantity of phloroglucinol (0.2 ml) were added to a small quantity (0.2 g) of the extract. The mixture was heated on a low flame. Appearance of a red colour was an indication of the presence of pentoses (Vishnoi, 1979).

Test for soluble starch

A small quantity (0.2 g) of the extract was boiled (>100°C) for 5 min with 1 ml of 5% potassium hydroxide (KOH), cooled, then acidified with 1 ml of H_2SO_4. A yellow colouration indicated the presence of soluble starch (Vishnoi, 1979).

Test for cardiac glycosides

Salkowski's test

To 0.5 g extract, 2 ml of chloroform tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. Appearance of a reddish-brown colour or yellow at the interphase was an indication of the presence of a steroidal ring (i.e aglycone portion of cardiac glycoside) or methylated sterols (Silva *et al*., 1998).

Liebermann-Burchard test

Steroidal nucleus

To 0.5 g extract, 2 ml acetic acid anhydride was added. When dissolved, it was cooled well in ice. Carefully, concentrated tetraoxosulphate (VI) acid was added; colour development from violet to blue or bluish-green was an indication of the presence of a steroidal ring, that is, aglycone portion of cardiac glycoside (Silva *et al*., 1998).

Test for terpenoids

A small quantity of the extract (0.2 g) was dissolved in ethanol and 1 ml acetic anhydride was thereafter added, followed by the addition of concentrated tetraoxosulphate (VI) acid. A colour change from pink to violet indicated the presence of terpenoids (Silva *et al*., 1998).

Test for flavonoid

Shinoda's test

The aqueous extract of *Annona senegalensis* (0.5 g) was dissolved in 5 ml ethanol, warmed (60°C) and filtered. Three pieces of magnesium chips were added to the filtrate and then followed by a few drops (3) of concentrated hydrochloric acid (HCl). A pink, orange, or red to purple colouration indicated the presence of flavonoids (Markham, 1987).

Ferric chloride test

The extract (0.2 g) was boiled (>100°C) with 5 ml distilled water and filtered. A few drops of 10% ferric chloride solution was added to 2 ml of the filtrate. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group (Evans, 2009b).

Lead ethanoate test

A small quantity (0.5 g) of the extract was dissolved in 5 ml of water and filtered. Three (3) ml of lead ethanoate solution was then added to 5 ml of the filtrate. Appearance of a buff-coloured precipitate indicated the presence of flavonoids (Brain and Turner, 1975).

Sodium hydroxide test

A small quantity (0.2 g) of the extract was dissolved in 5 ml water and filtered, and then 2 ml of 10% aqueous sodium hydroxide was added and it produced a yellow colouration. To this, dilute hydrochloric acid was added; a change of colour from yellow to colourless indicated the presence of flavonoids (Evans, 2009b).

Test for tannins

The extract (0.5 g) was stirred with 10 ml distilled water and filtered. The filtrate was used for the following tests:

- to 2 ml of the filtrate, a few drops of 1% ferric chloride solution were added, occurrence of a blue-black, green or blue-green precipitate showed the presence of tannins.
- a mixture of equal volumes of 10% lead ethanoate was added to 2 ml of the filtrate. Formation of a white precipitate was an indication of the presence of tannins.
- The filtrate of the extract (2 ml) was boiled (>100^{o}C) with 3 drops of 10% HCl and 1 drop of methanol, a red precipitate was taken as an evidence of the presence of tannins (Sofowora, 1993; Evans, 2009).

Test for alkaloids

Preliminary test for alkaloids

A quantity (0.5 g) of the extract was stirred with 5 ml of 1% aqueous HCl on a water bath (60^{o}C) and filtered. Then 3 ml of the filtrate was divided into three test tubes equally. To the first portion, two drops of Dragendorff's reagent was added and the presence of orange-red precipitate indicated a positive result. To the second portion, 1 ml of Mayer's reagent was added, an appearance of buff-coloured precipitate indicated the presence of alkaloids. To the last 1 ml (last portion), two drops of Wagner's reagent was added and a dark-brown precipitate indicated the presence of alkaloids.

Test for resin

Five millitres of copper acetate solution was added to 5 ml of the extract in water. The resultant solution was shaken vigorously and allowed for 5 min to separate. An appearance of a green coloured solution was indicative of the presence of resin (Silva *et al*., 1998).

Test for aloes

To 1 ml of the extract in a test tube, a quantity (0.5 ml) of freshly prepared bromine water was added. Formation of a yellow or orange precipitate indicated the presence of aloes (Evans, 2009b).

Test for saponins

Froth test

Three milliliters (3 ml) of the aqueous solution of the extract was mixed with 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 5 min, allowed to stand for 30 min and observed for honeycomb froth, indicative of the presence of saponin (Evans, 2009).

Haemolysis test

Three millilitres (3 ml) of the extract was mixed with 3 ml of blood. The test tube was stoppered and shaken gently for proper mixing. It was allowed to stand and observed for turbidity, indicative of the presence of saponin (Evans, 2009).

Elemental analysis of the Annona senegalensis leaf

The dried sample of *Annona senegalensis* leaf powder was put in a well labeled crucible and heated in a furnace at 550^{o}C for 3 hours. The hot sample was removed and kept in a desiccator to cool naturally. The sample in the form of ash was digested in a 250 ml beaker with 20 ml of 2 M nitric acid and 10 ml of 35% hydrogen peroxide and heated on a hot plate (>100^{o}C) in a fume cupboard until a clear digest was obtained. After cooling, it was filtered and deionized water was added until it reached 100 ml in a volumetric flask for elemental analysis using atomic absorption spectrophotometer. The elemental concentrations were determined using a Standard Calibration Curve (Sunderman Jr, 1973; Kolthoff and Elving, 1976; Ojo *et al*., 2013).

Acute toxicity study

Aqueous extract of *A. senegalensis* leaf was used for this study. Thirty rats consisting of males and females were randomly divided into 6 groups of five rats each in separate cages. The rats were tagged and housed individually and kept for 7 days in the laboratory to allow them acclimatize. The rats were fed with standard feed (Vital feed, Nigeria) and water *ad libitum*. Group 1 rats served as the control and distilled water was given orally. Groups 2 – 6 rats were treated orally with varying doses of aqueous extract once at 100, 200, 400, 800, 1600 and 3200 mg/kg, respectively. They were observed within 24 h. for signs of toxicity and mortality. Dead rats were posted and histopathology was carried out to determine any pathological changes. The median lethal dose (LD_{50}) was calculated using the arithmetic method of Karbar (1931) as modified by Aliu and Nwude (1982).

$$LD_{50} = \text{apparent least dose lethal to all in a group} - \frac{\sum(a \times b)}{N}$$

Where: N = number of animals in each group; a = dose difference; b = mean mortality

Results and Discussion

The phytochemical analysis of the aqueous extract of *Annona senegalensis* leaf revealed the presence of chemical constituents such as carbohydrates, cardiac glycosides, terpenoids and flavonoids as presented in Table 1.

The aqueous leaf extract of *Annona senegalensis* phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, terpenoids and flavonoids whereas soluble starch, anthraquinone, tannins, phlobotannins, saponins and alkaloids were not present. Carbohydrates metabolism yield glucose. The digestion is completed in the small intestine to the end product, glucose, which is then absorbed into the portal circulation from where it passes to the liver (Nduka, 1999). The presence of carbohydrate validates the use of this plant by the natives as food which gives energy because the end product of carbohydrates' digestion is glucose.

Cardiac glycosides have been utilized in the treatment of congestive heart failure, constipation, oedema and microbial infections (Frantisek, 1991). Terpenoids have antimicrobial, antitumour and antifungal activities (Singh and Singh, 2003). In view of the above, this plant has great therapeutic abilities.

Flavonoids are naturally occurring phenolic compounds in plants. Flavonoids have been shown to possess many pharmacological properties such as: anti-oxidant, anti-inflammatory, anti- cancer activities and anti- microbial effects hence, flavonoids may have a contributory effect to its pharmacological effects the plant possesses (Joy and Kuttan, 1998; Kassuya *et al*., 2003; Adeneye *et al*., 2006). Favonoids as an anti-oxidant, has a rejuvenating effects on cells or tissues, it is anti-aging hence can contribute substantially to the fertility effect of this plant (Nita Bishop, 2003).

The disease fighting potential of flavonoids stems from their ability to reduce inflammation by preventing the release of histamine (which causes allergic symptoms such as congestion). Flavonoids antagonise free radicals, boost immunity, strengthen blood vessels, and increase blood flow, among other actions. Flavonoids are involved in, gene expression, capillary and cerebral blood flow, platelet

aggregation, liver function, enzyme activity and collagen, phospholipid and histamine metabolism (Nita Bishop, 2003). They decrease capillary fragility and are therefore employed in case of hypertension and radiation injuries (Nita Bishop, 2003). Hesperidin is regarded as the most important flavonoids in oranges and has been reported to lower high blood pressure as well as cholesterol in animal studies and also possess strong anti – inflammatory properties (CSIRO, 2004).

The presence of these phytochemicals in the plant as observed in this study might be the reason for its therapeutic uses by the natives meaning that this plant has great potential pharmaceutical industries to explore.

Table 1: Qualitative phytochemical components of the aqueous leaf extract of *Annona senegalensis*

Components	Inference
Carbohydrates	+
Soluble starch	-
Anthraquinones	-
Cardiac glycoside	+
Terpenoids	+
Flavonoids	+
Tannins	-
Phlobatanins	-
Saponins	-
Alkaloids	-

– = absent; + = present

Table 2: Elemental concentration of aqueous leaf extract of the *Annona senegalensis*

Elements	Concentration (mg/kg)	WHO (1996) Standard (mg/kg)
Iron (Fe)	7.202	0.5 – 50
Copper (Cu)	0.893	1 – 3
Magnesium (Mg)	6.637	10 – 20
Lead (Pb)	0.014	–
Chromium (Cr)	0.070	–
Cadmium (Cd)	0.057	10 – 35
Cobalt (Co)	0.604	–
Zinc (Zn)	1.732	15 – 20
Manganese (Mn)	2.362	–

mg/kg = Milligram per kilogram; – = Not present

The result of the elemental screening of the aqueous extract of *Annona senegalensis* leaves is shown in Table 2. The extract contains the following elements iron (Fe), copper (Cu), magnesium (Mg), lead (Pb), chromium (Cr), cadmium (Cd), cobalt (Co), zinc (Zn) and manganese (Mn) in parts per million concentrations and their values are 7.202, 0.893, 6.637, 0.014, 0.070, 0.057, 0.604, 1.732 and 2.362, respectively. Some of the elements found in the extract in this study include: Iron (Fe), copper (Cu), magnesium (Mg), lead (Pb), chromium (Cr), cadmium (Cd), cobalt (Co), zinc (Zn) and manganese (Mn). Their presence in this plant may be responsible for the many therapeutic activities of this plant as claimed by the natives (Shirin *et al*., 2010).

Some of the elements found in the extract are all within the normal range of the WHO standard safety limits (WHO, 1996). Elements such as Pb and Cd are so small in quantity which makes their absorption negligible or impossible for them to produce toxicity (Lalman and McMurphy, 2002).

Iron (Fe) is an essential component in the structure of proteins involved in transportation and utilization of oxygen namely haemoglobin, myoglobin and cytochromes. It is also involved in electron transport chain. Its deficiency includes anaemia, anorexia, reduced growth and increased weight loss (Lalman and McMurphy, 2002). Copper (Cu) is an important cofactor in many enzymes especially those involved in haemoglobin formation, Fe absorption and mobilization, connective tissue metabolism and immune function (Lalman and McMurphy, 2002).

Magnesium (Mg) is known to activate many different enzymes which is essential in energy metabolism, transmission of genetic code, membrane transport and nerve impulse transmission (Lalman and McMurphy, 2002). Cromium (Cr) is implicated in glucose metabolism behaving like insulin. Its presence may be beneficial to diabetic patients (Lalman and McMurphy, 2002).

Cobalt's (Co) primary role is as a building block for vitamin B_{12} which is manufactured in the rumen. Its deficiency includes reduced appetite and reduced disease resistance (Lalman and McMurphy, 2002). Its presence might have helped in enhancing the immune potential of the extract.

Zinc (Zn) has been reported to have beneficial effects on atherosclerotic patients. Subnormal plasma Zn level has been reported in patients with atherosclerosis (Shirin *et al*., 2010). The presence of Zn and Cu in the plant may be correlated with its anticancer property, as both elements are required in growth and proliferation of normal cells. Zinc concentration decreases in cancer patients whereas Cu concentration increases (Shirin *et al*., 2010). It is believed that low concentration of plasma Zn in cancer patients is due to the increased requirement of Zn by cancer tissues (Shirin *et al*., 2010). This seems to be reasonable because of the fact that tumor cells have high rate of DNA synthesis and most of the enzymes involved in the nucleic acid synthesis are Zn dependent (Shirin *et al*., 2010). Deficiencies of these elements may cause different diseases.

Manganese (Mn) is important in bone growth and formation in young animals and in maintaining optimum fertility in female cattle. Its role in metabolism includes its serving as a component of the enzymes pyruvate carboxylase, arginase and superoxide dismutase. Its deficiency includes skeletal abnormalities, low reproductive performance, abortions, still births and low birth weight (Lalman and McMurphy, 2002).

In the above elemental study, it shows that this plant has great potential in management of aneamia, cancer, fertility, growth and development, enhancing immune system, management of diabetes and improving vitality of health.

The result of LD_{50}study is presented in Table 3. There was slight sedation, dyspnea, reduced appetite, coma and eventually death in group six (3200 mg/kg).

In this study, LD_{50}, 3200 mg/kg was the dose that produced 100 % mortality. The calculated LD_{50} of the aqueous leaf extract of *A. senegalensis* administered orally was 2400 mg/kg (Aliu and Nwude, 1982). The rats treated with 100, 200, 400, 800 and 1600 mg/kg doses produced no mortality, suggesting that the plant could be less harmful at those levels. It may therefore be considered nontoxic; although this does not predict the lethal dose in humans or other animals, it however provides a guide for choosing the dose for use in sub-chronic studies. Generally, the smaller the LD_{50} value, the more toxic the substance is and vice versa. These doses 100, 200, 400, 800 and 1600 mg/kg considered to be non-harmful because the higher the LD_{50} the less toxic and the smaller the LD_{50} the more toxic the substance. This agrees with the report of Organization for Economic Cooperation and Development (OECD, 1998) which classifies: very toxic as <5 mg/kg, toxic as >5<50 mg/kg, harmful as >50 < 500 mg/kg and no label as >500 <2000 mg/kg. On the other hand, the report of the Environmental Protection Agency (EPA) (OECD, 1998), United States classifies toxicity as follows: very toxic as ≤ 50 mg/kg, toxic as >50 ≤500 mg/kg, harmful as > 500 - 5000 mg/kg and no label as > 5000 mg/kg. From the above, every substance has to be handled with care. This extract is nontoxic

based on OECD but for EPA (USA) it is harmful (OECD, 1998).

Table 3: Calculation of LD_{50} of aqueous leaf extract of *Annona senegalensis* in rats administered orally

Extract dose (mg/kg)	No. in group	No. of death	%	DD	MD	DD x MD
100	5	0	0	0	0	0
200	5	0	0	100	0	0
400	5	0	0	200	0	0
800	5	0	0	400	0	0
1600	5	0	0	800	0	0
3200	5	5	100	1600	2.5	4000

Thus LD_{50} =

$LD_{50} = \frac{DD\ x\ MDLD_{50}}{N}$ = Apparent least dose lethal to all in a group - $\frac{\sum(a\ x\ b)}{N}$

$LD_{50} = \frac{400}{5}$

= 800

LD_{50} = 3200 – 800 = 2400 mg/kg

LD_{50} = 2400 mg/kg

Where: N = number of animals in each group (N); b = mean mortality (MD); a = dose difference

In conclusion, the presence of the phytochemical and elemental components accounts for its therapeutic values in folkloric medicine by the natives. The acute toxicity study indicated and explained why the natives primarily use it as food because it is less harmful. The natives do not use it only for food but in treatment of various ailments. This study has scientifically provided a base for further researches into this plant.

Conflict of Interest

Authors declare that there is no conflict of interest related to this study.

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