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Original Article

Phytochemical and Elemental Constituents, Acute Toxicity (LD_{50}) Studies of Aqueous Leaf Extract of *Calotropis procera*

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ABSTRACT

Qualitative phytochemical and elemental analysis and acute toxicity studies of aqueous extract from leaves of *Calotropis procera* were carried out using standard procedures. Phytochemical analysis revealed the presence of carbohydrate, tannins, cardiac glycoside, flavonoids, terpenoid, saponin and saponin glycoside. Elemental analysis revealed the presence of various elements such as Copper lead, nickel, chromium, cadmium, iron and zinc at various concentrations of 0.000335 %, 0.00005 %, 0.000027 %, 0.000014 %, 0.00024 %, 0.00024 % and 0.000022 % respectively. Acute toxicity (LD_{50}) test using the extract in albino rats was calculated to be 774 mg/kg body weight. This finding can explain the beneficial medicinal value of this plant at low doses and toxicity at high doses.

Keywords: *Calotropis procera*, aqueous extract, phytochemicals, elemental constituents, toxicity, albino rats.

INTRODUCTION

Calotropis procera belongs to the Family *Asclepiadaecae* which is an important plant with medicinal properties. It is known by various synonyms in English; calotrope, calotropis, Dead Sea fruit, desert wick, giant milkweed, swallow-wort, mudar fibre, rubber bush, rubber tree, sodom apple) [1]; French (pomme de Sodome, algodón de seda, arbre á soie, coton soie, arbre a soie du Senegal. In Nigeria, it is known by various names eg. In Hausa (tumfafia), Kanuri (kayôu), Igbo (kausu), Yoruba (‘bomubomu) Hindi (madar, akada, akdo, aak); Italian (calotropo); Mandinka (kipapa); Sanskrit (alarka); Somali (boah,bo’ah); Spanish (bomba, algodón extranjero, cazuela); Swahili (mpamba mwitu); Tamil (vellerukku); Tigrigna (dinda, ghindae, akalo); Wolof (faftan), [2][3]. It is found in most parts of the world, especially in warm climate usually growing in dry, sandy and alkaline soils. *Calotropis* is primarily harvested because of its distinctive medicinal properties.

Calotropis procera is a drought-resistant, salt-tolerant weed found along degraded roadsides, lagoon edges and overgrazed pastures. It is native to tropical Africa including Nigeria, Asia and Latin America where the plant is of high socio-economic value. *Calotropis procera* extracr are reported to have antioxidant, antimicrobial and cytostatic properties [4]. The leaf, stem and root are utilized in traditional medicine for treatment of wounds; sores skin diseases, diarrhoea, sinus, fistula and jaundice. Extracts from this plant is reported to relieve stomach pain. The sap is used for treating eye infections, and the bark of the plant is traditionally used for the treatment of coughs, elephantiasis, leprosy and ulcers [5] [6]. The stem is utilize in native roofing of huts and also serve as source of charcoal [7]. Occasionally goats and sheep eat the leaves, but cattle and other livestock avoid it because of pungent smell and oxicity [8] [9]. Some haematological and biochemical changes were reported in rats fed with extracts of *Calotropis procera*. It was proposed that the plant contain potentially useful ethno medicinal compounds. [10].

Phytochemical investigation of *Calotropis procera* root yielded two new compounds identified as urs-18α-H-12, 20 (30) diene-3β-yl acetate (procerursenyl acetate) and n-triacontan-10β-ol (proceranol) [11], along with earlier reported

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triterpenes, triterpenoids, phytosterols, saponins, alkaloids and cardinolides [12] [13] [14].

The aim of this work is to investigate the phytochemical constituents, analyze presence of some elements and their possible toxic or beneficial effect.

MATERIAL AND METHOD

Sample collection: Fresh leaves of *Calotropis procera* were collected on 6th march 2014 early in the morning at the university of Maiduguri campus, Maiduguri, Borno State. The plant was identified and authenticated at the Herbarium of Biological Sciences, University of Maiduguri. The leaves were brought to the Veterinary Pharmacology laboratory and kept for two weeks for air drying under room temperature to reduce the moisture content before processing. The dried leaves were pulverized using pestle and mortar and the powder kept in an air tight container until use.

Plant extraction: Dried sample materials (250 g) were pulverized using wooden mortar and pestle. The sample was transferred into 2 litres round bottom flask and sufficient amount of distilled water was added until it covered the sample. The mixture was refluxed for about 2 hours. The solution was removed and decanted and this procedure was repeated twice using a new solvent each time (distilled water). When the sample was extracted, it was filtered and the filtrate concentrated in hot air oven at 40-50^{0}C, a dark-green substance was obtained after evaporation. It was weighted and transferred into an air tight container for further analysis.

Preliminary qualitative phytochemical analysis: *Calotropis procera* extract was subjected to qualitative chromogenic phytochemical screening according to standard procedure to test for the presence of the following secondary metabolites; alkaloids, carbohydrates, flavonoids, saponins, tannins, glycosides (Cardiac, steroidal), terpenes/terpenoids, fatty acids, resins, aloes, etc. as described by [12] [13] [14], [20] [15] [16] [17] [18].

S**ample digestion for elemental analysis:** Air dried plant material was pulverized into powder. The sample was then put in curable and transferred to a muffle furnace and then heated at 500^{0}C for 3 hours. The sample was then removed and allowed to cool in a desicator. 500 mg of the ashed sample was transferred to 250 ml beaker, and to this, 10 ml of 6 molar hydrochloric acid was added. The beaker was covered and heated for another 15 minutes; the solution was allowed to cool. One milliliter of conc. nitric acid was added and heated to evaporate to dryness. One milliliter solution of 6 molar hydrochloric acid was added again and 10 ml of distilled water was added, it was then heated on a hot plate in a fume cupboard until a clear digest was obtained. The solution was removed for filtration using Whatman's filter paper No.1 into 100 ml volumetric flask up to the 100 ml mark and then transferred into polyethylene bottle for elemental analysis.

Procedure for elemental analysis: The resulting solution from the perchloric acid and nitric acid digestion for the leaf of *Calotropis procera* extract was used for elemental analysis using Atomic Absorption Spectrometry (AAS) with SP/Cr Unicam Model Solar 32 Data Station V7.10 System at the appropriate wave length, temperature and lamp current flow for each element under study, for the determination of Fe, Zn, Cu, Cr, Cd, Nickel and Pb, Flame Emission Spectrophotometry (FES), Galle Kamp (FGA 330) was used for determination of Na,, K and Ca while S and P were determined using u/v Spectrophotometry [19].

Acute toxicity studies: The acute toxicity (LD_{50}) value of the aqueous extract of *Calotropis procera* was determined using standard conventional procedures as described by [21]. This method has two phases which are phases 1 and 2 respectively. Phase 1 required nine animals divided into three groups of three animals per group. Each group of animal were administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals were observed for 24 hours to monitor their behavior and possible mortality. In phase 2, animals were grouped into 4 groups of one animal each and then the aqueous extract are administered at doses to be determined after the phase I and then observed for 24 hours for behavior as well as mortality.

The LD_{50} is calculated by the formula:

$\mathbf{LD_{50}} = \sqrt{D_0} \times D_{100}$

Where:

LD_{50} = lethal dose that kills 50 % of the test population

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

RESULTS

The result of the phytochemical studies revealed the presence of phytochemicals such as carbohydrate, tannins, cardiac glycoside, flavonoids, terpenoids and saponin glycoside (Table 4.1). While elemental analysis showed concentrations of 0.000335 %, 0.00005 %, 0.000027 %, 0.000014 %, 0.00024 %, 0.00024 % and 0.000022 % of cadmium, copper, chromium, iron, lead, nickel and zinc respectively. The LD_{50} was calculated to be 774 mg/kg in albino rats.

Table 1: Phytochemical constituents

Phytochemical	Test	Remark
Carbohydrate	General Molisch Test	+
	Test for free reducing Sugar	-
	Test for Combined reducing sugar	-
	Test for ketoses	-
	Test for pentoses	-
Tannins	Ferric chloride test	+
	Lead acetate test	+
Phlobatannins	Test for phlobatannins	-
Glycosides	Test for anthraquinones	-
	Test for combined anthraquinone	-
Cardiac glycosides	Salkwosky test	+
	Lieberman-Burchard test	+
Flavonoids	Shinoda's test	+
	Ferric chloride test	+
	Lead acetate test	-
	Sodium hydroxide	-
Terpenoids	Test for terpenoids	+
Saponin glycosides	Frothing test	+
Alkaloids	Dragendroff's reagent	-
	Mayer's reagent	-

Key: (+) Present, (-) Absent

Table 2: Result of some elemental analysis

Element	Concentration (%)	Permissible limit % (WHO)
Cadmium (Cd)	0.00024	0.000021
Copper (Cu)	0.000335	0.0003
Chromium (Cr)	0.000014	0.0002
Iron (Fe)	0.00024	0.002
Lead (Pb)	0.00005	0.000043
Nickel (Ni)	0.000027	0.000163
Zinc (Zn)	0.000022	-

Key: WHO; World Health Organization

Table 3: Acute toxicity (ld_{50}) studies of *calotropis procera* extract

GP	Body Wt (g)	Dose mg/kg	Mortality (%)
1	118	10	0
	138		
	180		
2	128	100	0
	136		
	162		
3	130	1000	33.3
	130		
	115		
4	145	600	0
5	136	1000	100
6	162	1600	100
7	217	2900	100

From 200 mg/ml concentration.
Key: GP = Group,
Body Wt: = Body Wt: Body Weight
$LD_{50}=\sqrt{D_0 \text{ x } D_1}$
Where:
D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.
$LD_{50}=\sqrt{600 \text{ x } 1000}$
LD_{50}= 774 mg/kg body weight.

DISCUSSION

The qualitative phytochemical investigation of *Calotropis procera* indicated the presence of carbohydrate, tannins, cardiac glycoside, flavonoids, terpenoid, saponin and saponin glycoside which corresponds with the finding of [22] [11]. These phytochemical compounds have many known therapeutic values for instance glycoside are complex organic substance which hydrolyse to give sugar (glycone) and non-sugar (aglycone) components. Glycosides are also known to exert prolonged physiological action even though they may be poisonous to animal and man [59]. Glycosides are also known to have laxative, diuretic and antiseptic properties [59]. Steroids is a complex organic substance in the presence of adequate diet and sufficient physical activity, a significant increase in muscle mass and strength has been reported to be produced by these steroids [60]. Carbohydrates are complex compound which provide heat and energy for all forms of body activity [61]. Tannins have astringent property which make the intestinal mucosa more resistant and reduced secretion by contracting the gastrointestinal tract [24], this agrees with the report of [4] on the antidiarroeal effect of *Calotropis procera* extract. The anti-inflammatory effects of tannins is reported to control all indication of gastritis, esophagitis, enteritis and irritating bowel [26] which coincides with the report of anti-inflammatory effect of *Calotropis procera* [27] [24] [29] respectively.

Flavonoids are known to relax pre-contracted intestinal smooth muscle and to delay intestinal transit. Flavonoids are reported to possess antibacterial, spasmolytic properties [30]. For instance, plants rich in saponins have immune boosting and anti inflammatory properties [62]. Similarly tannins have been reported to have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby destroying bacterial organism by directly damaging its cell membrane [31]. The antibacterial activities of alkaloids and flavonoids have been reported by a number of authors [32] [33].

The elemental analysis reveals the presence of Iron (Fe) in high concentration (0.00024 %), followed by copper (0.000335 %,) and cadmium (0.00024 %) while chromium, lead, zinc and nickel are in trace quantity. The basic functions performed by these minerals are: as cofactor for biochemical and enzymatic activities in the body tissues, and are equally involved in the maintenance of acid-base balance and regulation of body fluids, they are also involved in transport of gases and in muscle contractions [34] [35]. Mineral elements serve as a source of nutrition to animals; they play a vital role as structural components in cellular processes [36] [37]. The functional roles of the trace elements are described in terms of their role nutritionally or their potential toxicity [63]. Chromium is an essential element for animals and humans. It has been found in nucleoproteins isolated from beef liver and also in RNA preparations [38]. It could play a role in maintaining the configuration of the RNA molecule, because Cr has been shown to be particularly effective as a cross-linking agent for collagen [39]. Cr has also been identified as the active ingredient of the glucose tolerant factor [40], a dietary factor required for the maintenance of normal blood glucose tolerance in the rat. Trivalent chromium is a constituent of ‘‘glucose tolerance factor’’ (GTF), and reported to bind to and activates/potentiates insulin action [40] [35]. Copper is a co-factor in many enzymatic activities including those which provides hair and skin colour, help skin to heal, provides protection from infections and for healthy blood and bones. Iron functions in the formation of haemoglobin which is useful in the transport of oxygen. In cellular respiration, it functions as essential component of enzymes involved in biological oxidation such as cytochromes c, c1, a1, [34]. Fe is an important constituent of succinate dehydrogenase as well as a part of the haeme of haemoglobin (Hb), myoglobin and the cytochromes [41]. Iron is required for proper myelination of spinal cord and white matter of cerebellar folds in brain and is a cofactor for a number of enzymes involved in neurotransmitter synthesis and packaging [42], their uptake and degradation into other iron-containing proteins

which may directly or indirectly alter brain function [43].

Lead is an ubiquitous environmental and industrial pollutant that has been detected in every facet of environmental and biological systems [44]. The manipulation of lead for various uses had caused lead contamination of air, dust and soil [45]. Reports suggest that there is a close relationship between cases of declining reproductive health and environmental pollutants [46], while human exposure to lead continues to be a serious public health problem [47]. The possible effects of the general population of long term, low level exposure to cadmium have been of concern recently [48], because cadmium is pollution from agricultural and industrial activities [49]. Pollution by heavy metals is a serious problem due to their toxicity and ability to accumulate in the biomass [50]. Nickel is an essential element in animals [51]. It has been speculated that nickel may play a role in the maintenance of membrane structure, control of prolactin, nucleic acid metabolism or as a cofactor in some enzymatic activities. It appears that most dietary intakes would provide sufficient amounts of this element [52]. Nickel (Ni) in chick feed containing <0.05 ppm result in increased skin pigmentation of the legs, swollen hocks and thickening of the legs near the joints. These signs were not apparent in chicks fed the same diet supplemented with 3-5 ppm nickel [51]. In a trace element-controlled environment, chicks not fed with nickel supplement feed have shorter, thicker legs, lower haematocrit and plasma cholesterol levels and higher liver cholesterol levels than control chicks. In rats, there is slower growth rate and post-natal mortality. In swine, there is impaired reproduction, abnormal hair coats, and poor growth of offspring as reported [53] [54].

Zinc is distributed widely in plant and animal tissues and occurs in all living cells. It serve as a cofactor and is a constituent of many enzymes like lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, retinine reductase, DNA and RNA polymerase. Zn dependent enzymes are involved in macronutrient metabolism and cell replication [55] [56]. The result of the acute toxicity (LD_{50}) following intraperitoneal administration of *Calotropis procera* aqueous extract in rat at concentration of 200 mg/ml was found to be 774 mg/kg. According to the American Society for Testing and Materials [57], any chemical Substance with LD_{50} estimate less than 2000 mg/kg/oral route but greater than 1000 mg/kg/oral could be considered to be slightly toxic, although [58] considered any compound with an estimated oral LD_{50} equal to or greater than 1000 mg/kg to be safe. Similarly, with intraperitoneal LD_{50} value of 500-5000 mg/kg body weight in toxicity rating are classified as being slightly toxic. Thus the acute toxicity of *Calotropis procera* aqueous extract was 774 mg/kg intraperitoneal route meaning it is slightly toxic according to [58]. That may perhaps be the reason why goats and sheep nibble at the leaves, but cattle and other livestock do not [9].

Conclusion/recommendation: It was concluded that aqueous extract of *Calotropis procera* is toxic and should not be recommended for medicinal purposes at higher doses, but can be medicinally utilized at lower concentration

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