



Short Communication

Chemical Composition and Acute Toxicity Studies on the Aqueous Extract of *Combretum dolichopentalum* Leaf in Swiss Albino Mice

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Abstract

Studies on the phytochemical and macronutrient composition of the leaves of *Combretum dolichopentalum* were carried out. The qualitative phytochemical composition shows the presence of alkaloid, flavonoid, saponin, tannin, steroid, glycosides and resins. The quantitative phytochemical composition shows that it contains 5.765 ± 0.002 mg/100g tannin, 80 ± 0.003 mg/100g flavonoids, 0.033 ± 0.003 mg/100g cyanide, 8.717 ± 0.003 mg/100g alkaloids, 17.86 ± 0.002 mg/100g saponins and 6.969 ± 0.005 mg/100g steroid. The macronutrients obtained include 2.101 ± 0.003 mg/100g soluble carbohydrate and 200.0 ± 0.002 mg/100g reducing sugar. The result of acute toxicity (LD_{50}), showed *C. dolichopentalum* leaf extract to be lethal at doses 3000 and 5000 mg/kg body weight. This study revealed that *C. dolichopentalum* is a rich source of phytochemical and reducing sugar, which if adequately processed will not only offer chemoprotective benefits to its users, but could also serve as a good source of nutrient.

Keywords: *Combretum dolichopentalum*, phytochemical, macronutrient, antioxidant, acute toxicity.

Introduction

The importance of biological, chemical and pharmacological evaluation of plant derived agents used in the treatment of human ailments has been increasingly recognized in the last decades¹. This is due to the presence of bioactive constituents in plants such as alkaloids, tannins, flavonoid, saponins etc^{2,3,4}. Studies have been carried out on possible intervention of herbal preparations with diabetic and arthritic conditions in experimental animals^{5,6}. *Combretum dolichopentalum* (family - *combretaceae*) is widely distributed and used from Guinea to Southern Nigeria and Cameroon. It is commonly known as sun-birds wine. In Nigeria the locals of Umuahia call it mmanyana n'zeza, Edo's call it Okoso. *C. dolichopentalum* is used for the treatment of stomach ache, pains, gastrointestinal disorders, such as dysentery, passage of bloody stool, diarrhea and stomach ulcer in and around Ogwa and Mbaise in Imo State Nigeria⁷. *C. dolichopentalum* is also used as antibiotics and for treatment of stomach upset by nursing mothers after delivery in Mbaise in Imo state of Nigeria. This study aims to determine the fundamental and the scientific bases for the use of *C. dolichopentalum*. This was achieved by quantifying the phytochemical and the macronutrient constituents and also estimation of the acute toxicity (LD_{50}) value. This is vital because of the increasing demand for medicinal plants and plant products as alternative to orthodox medicines especially in developing nations. It is hoped that this study will enhance interest in them.

Material and Methods

Plant sample collection and separation: *C. dolichopentalum* leaves were collected from the premises of the University of Nigeria, Nsukka, Enugu State. They were authenticated by Mr. A. Ozioko of the Bioresource Development and Conservation Program (BDCCP), research centre in Nsukka. The fresh leaves were air dried for 2 weeks and ground with a grinding mill. Packaged in air-tight glass jar and stored in at room temperature until analysis was carried out.

Phytochemical Test: The presence of alkaloids, flavonoids, tannins, saponins, cyanides, glycosides, resins and steroids were determined qualitatively^{8,9}. The concentrations of the phytochemicals were determined quantitatively⁸

Macronutrient constituent: Macronutrient composition of the leaf extract for soluble carbohydrate, reducing sugar, fats and oil were estimated quantitatively¹⁰.

Animals for Acute Toxicity Studies: The animals used in this acute toxicity study (LD_{50}) were Swiss albino mice (22-28 g) of either sex obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized at the animal house of the Department of Biochemistry, University of Nigeria, Nsukka for seven days before the experiments. The mice were maintained *ad libitum* on water and growers mash (Pfizer Feeds, Aba, Nigeria). The acute toxicity test of *C. dolichopentalum* extracts was carried out by a modified

method acute toxicity testing¹¹, to define the range of lethal dose and safe dose for the extract. Swiss albino mice were grouped into 6 with three mice per group. They were treated orally with 250, 500, 1000, 2000, 3000 and 5000 mg/kg body weight respectively of the *C. dolichopentalum* extract. The animals were then observed over a 24 hr period for nervousness, dullness, in-coordination and or death. Oral acute toxicity dose was calculated using geometric mean of LD₅₀

Statistical Analysis: The results obtained were presented as mean \pm standard deviation

Results and Discussion

The result of the qualitative phytochemical composition of *C. dolichopentalum* is shown in table 1. It showed the presence of flavonoids, alkaloids, saponins, steroids, glycosides and resins in the leaves. Phytochemicals are known secondary metabolites of plants which exhibit diverse pharmacological and biochemical effects on living organisms¹². These secondary metabolites in *C. dolichopentalum* contain appreciable concentrations of flavonoid, saponins, alkaloid and saponins as shown in table 2. It contains Tannin, 5.765 \pm 0.002 mg/100g; flavonoids, 8.0 \pm 0.003 mg/100g; alkaloids, 8.717 \pm 0.003 mg/100mg; Saponins, 17.86 \pm 0.02 mg/100mg; steroids, 6.969 \pm 0.005 mg/100g; and 0.033 \pm 0.003 mg/100g cyanide. Phytochemicals have been associated with antimicrobial activities and numerous physiological activities in mammalian cells in various studies¹³⁻¹⁵. The content of alkaloid and flavonoid could be the reason for its use in the treatment of stomach ache, pains and gastro intestinal disorders. These two phytochemicals have been shown to have anti-inflammatory and analgesic effects¹⁶. The use of the leaves for the treatment of diarrhea and stomach ulcer in and around Ogwa in Imo State in Nigeria, suggest a combined effect of alkaloid, and tannin content. Tannin, a polyphenol have been shown to exhibit antimicrobial actions and alkaloids, a parasitic repelling effect¹⁷. Caution is usually taken in the consumption of plant material, with very high concentration of alkaloids and tannins because they could inhibit certain mammalian enzyme activities such as those of cyclic- AMP. Alkaloids also affect glucagons and thyroid stimulating hormones¹⁸. The concentrations of these phytochemicals in *C. dolichopentalum* are below levels found in some plant materials, which required further treatment to reduce their phytochemical concentration before use^{15,19}. The benefits derived from *C. dolichopentalum* use by nursing mothers after delivery may be linked to the desire to eliminate or reduce oxidative stress arising from pregnancy and labour. Flavonoid which have been shown to be a constituent of *C. dolichopentalum* are powerful antioxidant compounds and have also been reported to have antiviral, antiallergic, anti-platelet, antitumour and anti-inflammatory properties^{20,21}. Saponins are known to have hypocholesterolemic activities²² and are thus useful in controlling cholesterol level. A

decrease in serum cholesterol concentration reduces the metabolic burden that would have been placed on the liver during metabolism²³. The cyanide content also support the claim that *C. dolichopentalum* could be consumed without pretreatment. This is indicated by the very low concentration of cyanide (0.033 \pm 0.003 mg/100g), this is also less than the permissible limits of 10-20 mg/100g²⁴. Hydrogen cyanide (a product of cyanide) has been shown to be extremely toxic to a wide spectrum of organisms, due to its ability to link with metals (Fe²⁺, Mn²⁺, Cu²⁺) that are functional groups of many enzymes. Thus, inhibiting processes like the reduction of the oxygen in the cytochrome respiratory chain, electron transport in photosynthesis and the activities of enzymes like catalase and oxidase^{25,26}. Hydrogen cyanide can be significantly reduced by boiling, heating and soaking²⁷. The micronutrient constituent of *C. dolichopentalum* showed it contains 200.0 \pm 0.002mg/100g reducing sugar, 2.101 \pm 0.003mg/100g soluble carbohydrate as shown in table 2. *C. dolichopentalum* extract also showed a trace amount of protein. This high content of reducing sugar suggests that *C. dolichopentalum* leaves may be used as a source of carbohydrate (disaccharide and monosaccharide). Carbohydrates provide energy to the cells of the body, particularly the brain, the only carbohydrate dependent organ in the body²⁸. Fats and oil were not detected.

Table – 1
Result of qualitative phytochemical composition of *C. dolichopentalum* extract

Phytochemical	Extract
Alkaloid	+++
Flavonoid	+++
Saponin	+++
Tannin	++
Acidic compound	-
Steroid	++
Glycoside	++
Resins	++

Bioavailability key; - = not detected, + = present in low concentration, ++ = present in moderately high concentration, +++ = present in very high concentration

Table – 2
Result of macronutrient composition of *C. dolichopentalum* extract

Macronutrient	Extract
Fat and oil	-
Protein	+
Carbohydrate	++
Reducing sugar	+++

Bioavailability key: - = not detected, + = present in low concentration, ++ = present in moderately high concentration, +++ = present in very high concentration

Table – 3
Result of quantitative phytochemical composition of
C. dolichopentalum

Sample	Concentration
Soluble carbohydrate	2.101 ± 0.003 mg/100g
Tannin	5.765 ± 0.002 mg/100g
Flavonoids	8.0 ± 0.003 mg/100g
Cyanide	0.0334±0.003 mg/100g
Steroid	6.969 ± 0.005 mg/100g
Glycoside	1.0914±0.003 mg/100g
Alkaloids	8.717± 0.003 mg/100g
Saponin	17.86 ± 0.002 mg/100g
Reducing Sugar	200.0±0.002 mg/100g

Values are means ± standard deviation of triplicate determinations.

Table – 4
LD₅₀ of aqueous extract of *C. dolichopentalum*

Group(n=3)	Plant Extract (mg/kg body weight)	Dead Birds
1	250	0
2	500	0
3	1000	0
4	2000	0
5	3000	0
6	5000	0

Geometric mean of LD₅₀ calculated as Square root of the highest non-lethal dose multiplied by the least lethal dose.

LD50 = Highest non-lethal dose x Least lethal dose

$$= \sqrt{2000 \times 3000}$$

$$= \sqrt{2449.49 \text{ mg/ kg body weight}}$$

Acute toxicity study is an important test in the toxicological investigation of an unknown substance. The index of acute toxicity is the LD₅₀, though not regarded as a biological constant^{11,29}. The acute toxicity (LD₅₀) value of aqueous extract of *Combretum dolichopentalum* leaves was calculated to be 2449.49mg/kg body weight. The result of the oral acute toxicity (LD₅₀), studies showed *C. dolichopentalum* leaf extract to be lethal at doses 3000 and 5000 mg/kg body weight, an indication that the LD₅₀ of the plant is less than 3000mg/kg. This result places *C. dolichopentalum* in category 5 (> 2000mg/kg ≤ 5000mg/kg) according to the globally harmonized system of classification and labeling of chemicals³⁰.

Conclusion

This research has revealed that *C. dolichopentalum* is a rich source of phytochemical and reducing sugar. This indicates that the leaves of *C. dolichopentalum* will offer medicinal, chemoprotective and nutritional benefit to its users.

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