Niger. J. Physiol. Sci. 36 (June 2021): 109 – 114
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full Length Research Article

Anti-Hyperlipidemic and Antioxidant Potentials of Aqueous Leaf Extract of *Telfairia occidentalis* (Hook. F.) in Male Sprague-Dawley Rats

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Summary: In Africa traditional medicine, certain plant leaves are employed in the treatment of metabolic disorders such as dyslipidaemia. *Telfairia occidentalis* is named among Nigerian plants that are under investigation for anti-hyperlipidemic activity. The antihperlipidemic and antioxidant potentials of *Telfairia occidentalis (TO)* aqueous leaf extract were studied in male Sprague- Dawley rats. Twenty-four healthy male Sprague-Dawley rats were grouped into four of six rats thus: Group A (control) received normal saline (10mg/Kg); treated groups B, C and D, received, 50mg/kg; 100mg/kg; and 150mg/kg of *Telfairia occidentalis* aqueous leaf extract for 14 days respectively. At the end of the experiment, serum cholesterol (CHOL), triglyceride (TG), high density lipoprotein (HDL) and low-density lipoprotein (LDL), aspartate amino transferase (AST), alanine amino transferase (ALT), Alkaline phosphatase (ALP) wer assessed. Serum level of creatinine was determined and antioxidants such as reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were investigated. 'Malonaldehyde (MDA) level was also assessed. Results from the current study showed a significant decrease in CHOL and LDL levels at all the doses tested compared with control. *Telfairia occidentalis produced a significant increase in HDL level in all the tested doses compared with control* However, TG was significantly decreased in groups C and D compared with control. *Telfairia occidentalis* aqueous leaf extract produced a significant decrease in AST level in all the tested doses compared with control. ALT level significantly decreased in groups C and D 100mg/kg and 150mg/kg doses compared with control while ALP level significantly increased in all the doses tested compared with control. Creatinine level was significantly decrease in groups B and C compared with control. Results from the antioxidant analysis revealed a significant increase in SOD, GSH and CAT with concomitant reduction in MDA level in all the doses tested MDA's lipid peroxidation when compared with control. The current findings revealed that *Telfairia occidentalis* aqueous leaf extract possesses anti-hyperlipidemic, hepatoprotective effects and improved oxidative balance. However, care has to be taken during its use as it has ability to elevate LDL and activities of liver enzymes at higher dose which may be deleterious to the body system.

Keywords: *Antioxidant, cholesterol, Liver, protein, Sprague-Dawley, fluted pumpkin*

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Manuscript received- August 2020; Accepted- May, 2021

INTRODUCTION

The use of plants in traditional medicine referred to as herbalism or botanical medicine (Evans, 2002) falls outside the mainstream of the Western or Orthodox medicine. It has been estimated that about 75% of the world's population (mainly in the developing countries) rely on traditional medicine as their primary form of health care (Sumner, 2000). The use of traditional medicine in the treatment and management of diseases in the Africa cannot fade away and this could be attributed to the socio-cultural, socio-economic, lack of basic health care and qualified personnel (Eujoba *et al*., 2000).

Plants contain active components such as flavonoids, glycosides, saponins, tannins, etc., which possess medicinal properties that are harnessed for the treatment of different diseases (Chevalier, 2000). The active ingredients for a vast number of pharmaceutically -derived medications contain components originating from phytochemicals. These active substances that contain the healing property are known as the active principles and differ from plant to plant.

Telfairia occidentalis is an edible vegetable plant that belongs to the family Cucurbitaceae. It is a tropical vine grown mainly in West Africa for its vegetable (Akoroda, 1990). In Nigeria, it is known locally as 'Ubong' by Ibibios, 'Ugu' by Igbos and 'Iroko' by Yorubas. The Ghanians refer to it as 'okrobonka' while to the Sierra- Leoneans, it is known as 'Oroko' (Abiose, 1990). The concentration of photosynthesis in leaves makes them rich in protein, minerals and sugar because of their nutritional value leaves is prominent in the diet of many animals, including humans as leaf vegetables (Leaf, 2011). The leaf has high nutritional, medicinal and industrial values being rich in protein 29%, fat 18%, minerals and vitamins 20% (Ndor *et al*., 20130. The aqueous extract of *T. occidentalis* has been shown to be hepatoprotective against garlic-induced oxidative stress (Olorunfemi *et al*., 2005, Oboh *et al*., 2007) while both aqueous and ethanolic extracts have demonstrated hypoglycaemic properties both in normoglycaemic and alloxan-induced diabetic rats (Zhang and Yao, 2002). The haematinic capacity of this plant fresh leaf concoction has a high-value health tonic for impotent men with a cheap and fast remedy for acute anemia (Kayode and Kayode, 2011 Nwozo *et al*., 2004). It was revealed according to Veral *et al*., (2014) that the fluted pumpkin seed shell served majorly as a source of dietary fibre. Also, most cultures consider fruit shells as waste in the strictest sense and therefore avoid their use even in earthnomedicine (Verla *et al.*, 2012). The seeds are cooked and eaten like beans as the shoots and leaves while eaten like vegetables. The leaves contain vitamins and minerals the body needs to stay healthy. The leaves are also a good source of iron (*Telfairia occidentalis* , 2009).

Telfairia occidentalis is a very good vegetable plant, popularly known in Nigeria for its ornamental purposes. It is hard to believe that anyone would ever think of consuming its leaves as a meal. Although, there are claims concerning the medicinal/health benefits of the leaves hidden from the consumers. Moreover, *Telfairia occidentalis* is going to extinction in most countries especially in Nigeria, where limited research work has been done to verify the claims concerning the nutritional-health values. However, in the current study the anti-hperlipidaemic, hepatic and antioxidant effects of *Telfairia occidentalis* in male Sprague-Dawley rats were investigated.

MATERIALS AND METHODS

Plant collection and extraction: *Telfairia occidentalis leaves* were harvested from Itori Ewekoro Local Government Area of Ogun State, Nigeria and the collected samples were identified at FRIN Ibadan Oyo state, Nigeria. The fresh leaves of *Telfairia occidentalis* were air dried until constant weight of 250g was. The dried leaves were powdered using mortar and pestle. 250g of powdered leaves were macerated using distilled water for 48 hours. The extract was filtered and evaporated at 40ºc under reduced pressure. The yield of the dark browned colored dried extract obtained was 38.5g and the weighed extract was stored at 4ºc until use in which case distilled water was used for reconstitution immediately and was given orally to the experimental animals.

Animals: Twenty-four male Sprague-Dawley rats weighing between 150-200g were obtained from the animal house of the College of Medicine of the University of Lagos. They were kept in well-ventilated, hygienic compartments maintained under standard environmental conditions, acclimatized for three weeks before the experiment. They were fed with standard rodent diet and water ad libitum. The experimental procedures used were in accordance with the provisions of the Experimentation Ethics Committee on Animals Use of the College of Medicine of the University of Lagos, Lagos State and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals (2011).

Phytochemical screening: The phytochemical screening of the plant was carried out on dried sample as described by Harbone, (1973) to identify the active components present in *Telfairia occidentalis*

Acute Toxicity test (LD_{50}): The acute toxicity test was carried out as described by Lorke (1983).

Animal treatment and experimental groups: After successful acute toxicity test, the extract was given orally to the rats for two weeks as follows: Group A (Control, 10mg/kg of normal saline), group B (50mg/kg of the extract), group C (100mg/kg of the extract) and group D (150mg/kg of the extract).

Collection of blood sample: Five (5ml) of blood sample was taken by retro-orbital puncture. Blood was allowed to clot for 1 hour at 4^0C then centrifuged at 3,000 rpm for 10 minutes and the serum samples were kept at -20^0C until assayed (Morakinyo *et al*., 2018)

Blood lipids: Serum and liver homogenate lipid levels of triglycerides (TG), Cholesterol (CHOL), low density lipoprotein (LDL), and high density lipoprotein (HDL) after treatment were determined by automatic biochemistry analyzer (Mindray BS-120, Chema Diagnostica, Italy) using diagnostic kits for each, purchased from BioSystems® (S.A Costa Brava of Barcelona, Spain).

Liver and kidney functions: Albumin, alkaline phosphatase (ALP), alkaline amino transferase (ALT) and aspartate amino transferase (AST) were determined using serum samples by an automated analyzer (Mindray BS-120, Chema Diagnostica, Italy). The machine was equally used for the determination of creatinine.

Antioxidant studies

Determination of superoxide dismutase (SOD) activity: Briefly; SOD activity was measured by the inhibition autoxidative capacity of pyrogallol. The SOD activity was evaluated using a spectrophotometer at 420 nm. A calibration curve was constructed using SOD as standard. A 50% inhibition of autoxidation of pyrogallol was defined as one SOD unit (DinizVilela *et al*., 2016)

Determination of reduced glutathione (GSH) activity: The protein content of the samples was initially precipitated by metaphosphoric acid (MPA) at the ratio of 1:1 (homogenate/MPA). The samples were centrifuged at 3000rpm for 10 minutes. The supernatant was collected and mixed with sodium phosphate buffer (0.1M, pH 7.4), containing EDTA (5mM) and ortho-phthaldialdehyde (1 mg/mL in methanol). The mixture was incubated in the dark at room temperature for 15 min and fluorescence was measured at 350 nm (excitation) and 420 nm (emission). A standard curve of GSH (0.001–0.1 mM) was used for linear regression (DinizVilela *et al*., 2016)

Determination of catalase (CAT) activity: Briefly, sample (1ml) was mixed with 49 ml of distilled water to give a 1 in 50 dilution of the sample. The assay mixture contained 4ml of H_2O_2 solution (800 μmoles) and 5ml of Phosphate buffer in a 10ml flat bottom flask. 1ml of properly diluted enzymes preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1ml portion of the reaction mixture was blown into 2ml of dichromate acetic acid reagent at 60s intervals**.** Catalase (CAT) activity was determined by measuring the exponential disappearance of H_2O_2 at 240nm and expressed in units/mg of protein (Aebi, 1984).

Determination of malonaldehyde (MDA) activity: Briefly, the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems, MDA was estimated with the method of Uchiyama and Mihara (1978) which is based on its interaction with thiobarbituric acid (TBA) to form pink complex with absorption at 535nm. Absorbance was read using Microlab 300 recording spectrophotometer (UV 160) in all measurements.

Statistical analysis: All results are presented as the mean and standard error of mean (SEM). Statistical analyses were conducted using GraphPad Prism Software (GraphPad, Inc., La Jolla, CA, USA). Data analyses were performed by one-way analysis of variance (ANOVA) with post hoc Tukey's multiple comparison test. Statistical significance was set at p < 0.05.

RESULTS

Table 1 shows the presence of molisch, tannin, glycosides, terpenoid magnesium chip, flavonoid and fehlings following phytochemical screening. The acute toxicity tests at stages A and B revealed no toxicity at different doses tested except at 5000mg/kg in which there was swollen legs in the group tested. (Table 2 and 3).

Table 1:
Phytochemical screening of the aqueous leaf extract of *Telfairia occidentalis*

Phytochemicals	Present, (+) Strongly present (++)
Molisch	++
Tannin	++
Glycosides	+
Terpenoid	+
Magnesium Chip	++
Flavonoid	++
Fehlings	++

[k]**Table 2:**
Stage A of the acute toxicity (LD_{50}) of the *Telfairia occidentalis* aqueous leaf extract

GROUPS	DOSAGE	MORTALITY
Group B	10	Nil
Group C	100	Nil
Group D	1000	Nil

Table 3:
Stage B of the acute toxicity (LD50) of the *Telfairia occidentalis* aqueous leaf extract

GROUP S	DOSAGE	MORTALITY
Group B	1600	Nil
Group C	2900	Nil
Group D	5000	ST

ST= sign of toxicity

Lipid profile: There was no significant difference (p>0.05) in CHOL level in groups B (27±0.13), C (2.53±0.21) and D (2.55±0.16), compared with control ()2.72±0.192). *Telfairia occidentalis* produced a significant decrease (p<0.05) in TG level in groups C (0.95±0.16) and D (0.70±0.11) compared with control (1.18±0.07). TG also showed a significant decrease in group D (0.70±0.11) compared with group B (1.14±0.13) (p<0.05). HDL showed a significant increase (p<0.05) in groups B (1.56±0.13) compared with control (1.25±0.10). LDL showed a significant increase in groups B (0.62±0.11) and D (0.48±0.10) compared with control (0.83±0.06) (p<0.05) and D (0.48±0.10) significantly decreased compared with group B (0.62±0.11) (Table 4).

Liver functions: The results showed a significant decrease (p<0.05) in AST level in groups C (35.50±3.12) and D (39.25±6.61) compared with control (58.50±4.38) and a significant decrease in group D (39.25±6.61) compared with group B (57.40±7.51) (p<0.05) (Table 5). Table 5 also showed a significant increase (p<0.05) in ALT levels in groups B (25.20±0.65), C (20.00±0.29) and D (17.00±0.24) compared with control (17.67±0.67). Also, *Telfairia occidentalis* produced a significant decrease (p<0.05) in ALT in groups C (20.00±0.29) and D (17.00±0.24) compared with group B (25.20±0.65), and a significant decrease (p<0.05) in ALT in group D (17.00±0.24) compared with group C (20.00±0.29).

Table 4:
Effect of *Telfairia occidentalis* aqueous leaf extract on Cholesterol (CHOL), Triglycerides (TG),high density lipoprotein (HDL) and low density lipoprotein (LDL) levels

Parameters (mmol/l)	Group A	Group B	Group C	Group D
CHOL	2.72±0.192	27±0.13	2.53±0.21	2.55±0.16
TG	1.18±0.07	1.14±0.13	0.95±0.16*	0.70±0.11*#
HDL	1.25±0.10	1.56±0.13*	1.40±0.20	1.40±0.16
LDL	0.83±0.06	0.62±0.11*	0.70±0.07	0.48±0.10*#

Values represent Mean ± SEM. n=6. Significant (*p<0.05 vs. group A, #p<0.05 vs group B)

Table 5:
Effect of *Telfairia occidentalis* aqueous leaf extract on Aspartate amino transferase (AST), Alanine amino transferase (ALT) and alkaline phosphatase (ALP) levels

Parameters	Group A	Group B	Group C	Group D
AST u/l	58.50±4.38	57.40±7.51	35.50±3.12*#	39.25±6.61*#
ALT u/l	17.67±0.67	25.20±0.65*	20.00±0.29*#	17.00±0.24*#α
ALP u/l	68.00±3.98	121.60±4.86*	157.25±16.15*#	111.00±12.43*α

Values represent Mean ± SEM. n=6. Significant (*p<0.05 vs. group A, #p<0.05 vs group B, αp<0.05 vs. group C

Table 5 also showed a significant increase ($p<0.05$) in ALP levels in groups B (121.60±4.86), C (157.25±16.15) and D (111.00±12.43) compared with control (68.00±3.98). Also, *Telfairia occidentalis* produced a significant increase ($p<0.05$) in ALP level in group C (157.25±16.15) and a significant decrease in group D (111.00±12.43) compared with group B (121.60±4.86), with a significant decrease ($p<0.05$) in ALP levels in group D (111.00±12.43) compared with group C (157.25±16.15).

Creatinine: Aqueous leaf extract of *Telfairia occidentalis* displayed a significant decrease ($p<0.05$) in creatinine level in groups B (27.92±0.36). and C (29.95±0.64). compared with control (34.30±0.54). The result also showed a significant decrease ($p<0.05$) in creatinine level in group D (32.38±0.58) compared with groups B (27.92±0.36) and C (29.95±0.64).

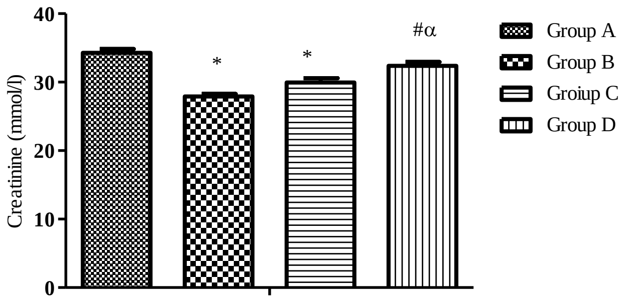

Figure 1:
Effect of *Telfairia occidentalis* aqueous leaf extract on creatinine level. Values represent Mean ± SEM. n=6. Significant (*p<0.05 vs. group A, #p<0.05 vs group B, αp<0.05 vs. group C)

Table 6:
Effect of *Telfairia occidentalis* aqueous leaf extract *on Superoxide dismutase (SOD), reduced glutathione (GSH) and Catalase (CAT) activities*

Parameters	Group A	Group B	Group C	Group D
SOD (mg/ml)	1.11 ±0.03	1.48 ±0.02*	1.47 ±0.02*	1.43 ±0.02*#
CAT (mg/ml)	7.25 ±0.05	10.10 ±0.03*	9.49 ±0.09*	8.32 ±0.07*#α
GSH (µmol/ml)	599.62 ±24.38	835.76 ±19.26*	784.9 0±7.51*#	647.36 ±8.26*#α

Values represent Mean ± SEM. n=6. Significant (*p<0.05 vs. group A, #p<0.05 vs group B, αp<0.05 vs. group C)

Antioxidant enzymes: Assay of antioxidant enzymes showed a significant decrease in SOD in groups B (1.48±0.02), C (1.47±0.02) and D (1.43±0.02) compared with control (1.11±0.03) while group D (1.43±0.02) showed a significant decrease ($p<0.05$) compared with groups B (1.48±0.02), and C (1.47±0.02) (Table 6). There was a significant decrease in CAT in groups B (10.10±0.03), C (9.49±0.09) and D (8.32±0.07) compared with control (7.25±0.05) while group D (8.32±0.07) showed a significant decrease ($p<0.05$) compared with groups B (10.10±0.03) and C (9.49±0.09) (Table 6). *Telfairia occidentalis* aqueous leaf extract a significant decrease in GSH in groups B (835.76±19.26), C (784.90±7.51) and D (647.36±8.26) compared with control (599.62±24.38) while group D (647.36±8.26) showed a significant decrease ($p<0.05$) compared with groups B (835.76±19.26), and C (784.90±7.51) (Table 6). *Telfairia occidentalis* produced a significant decrease in MDA level in group D (8.01±0.05) ($p<0.05$) compared with control (8.74±0.07), groups B (8.53±0.08) and C (8.71±0.05) (Figure 2).

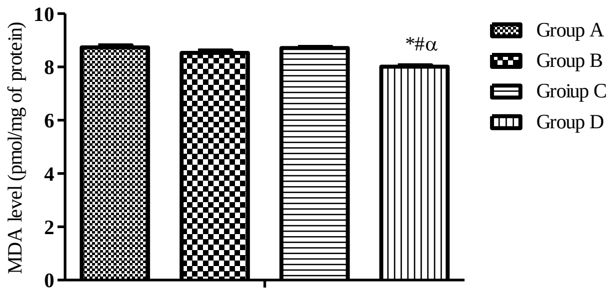

Figure 2:
Effect of Telfairia occidentalis aqueous leaf extract on *Malonaldehyde* (MDA) level. Values represent Mean ± SEM. n=6. Significant (*p<0.05 vs. group A, #p<0.05 vs group B, αp<0.05 vs. group C)

DISCUSSION

Hyperlipidemia is a heterogenous group of disorders characterized by high level of lipids in the bloodstream. It may be caused by disorders of some endocrine glands, kidneys, effects of certain drugs, dietary intake containing high amount of fat, risky life style and ageing (Durrington *et al*., 1988). It is one of the risk factors in development of atherosclerosis (Nwodo *et al*., 2014). In the current study, decreased cholesterol and DL levels were observed which is suggestive suggestive of the hypolipidemic effect of the leaves of *Telfairia occidentalis* . It was also observed that the leaves extract of *Telfairia occidentalis* possesses hypoytriglyceridemic effect with the significant reduction of TG in all the doses tested. The leaf of *Telfairia occidentalis* supplemented in diet has however been reported to lower plasma cholesterol and low density lipoprotein levels (Eseyin *et al*., 2007).

The reduction in the low-density lipoprotein (LDL) level observed in the current study is in agreement with the reports of Ugwu *et al*., (2011) and Onuegbu *et al*., (2015) in which the leaf of *Telfairia occidentalis* produced a decrease in low-density lipoprotein level. This could be as a result of the activity of the fibre content of *Telfairia occidentalis* (Barakat and Mahmond, 2011). Fibre has been reported to decrease LDL by interrupting cholesterol and bile acid absorption and increasing LDL receptor activity (Venkateson *et al*., 2003). Low density lipoprotein is a bad cholesterol which facilitates transport of cholesterol into the cell (Marcel *et al*., 1980) thus, the significant reduction in LDL is suggestive and justifies the cholesterol lowering effect of *Telfairia occidentalis* observed in the current study, thereby, further reducing the risk of metabolic disorder such as obesity and atherosclerosis.

There have been reports on the lipid profile of various plants including *Telfairia occidentalis* and some of which is in accordance with the present study (Harword *et al*., 2005; Venkatesan *et al*., 2003; Vinson *et al*., 1998; Ikeda and Sugano, 1998). Thus, there could be alterations in the concentration of the various lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases. The current study is in agreement with the previous study which reported that it is used as anti-hypercholesterolemic in ethnobotany (Nwozo *et al*., 2004).

In addition, increased alkaline phosphatase observed at 150mg/kg may be suggestive of possible liver exhaustion while at doses 50mg/kg and 100mg/kg, the enzymes activities were not significantly elevated. Literature has shown that the destruction of liver architechture is the principal culprit for the elevation of the liver enzymes. This destruction often occurs in the presence of high amount of toxins and xenobiotics which becomes challenges to the liver (Ejike *et al*., 2008)

Oxidative stress results when the antioxidant system is overwhelmed by the generation of excess reactive oxygen species (ROS) (Halliwell and Gutteridge, 1999). These reactive species like superoxide radical anion (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO^-) cause severe damage to macromolecules, tissues and organs through the process of lipid peroxidation (LPO), protein modification and DNA strand breaks (Sun and Chen, 1998; Zaidi and Banu, 2004). Oxidative stress resulting from the generation of these free radicals is known to contribute immensely to several pathological conditions like aging, cancer, cardiovascular disorder, neurodegenerative diseases among others (Halliwell and Gutteridge, 1999; Abuja and Albertini, 2001).The SOD and GSH properties in this study were investigated because of their synergistic ability to work hand in hand, SOD catalyses the breakdown of superoxide, the most common free radical in the body into oxygen and hydrogen peroxide while GSH catalyses the breakdown of hydrogen peroxide to water.

Furthermore, in all the doses tested leave extract *Telfairia occidentalis* produced a significant reduction of SOD, CAT and GSH suggestive of reduced production of oxidative radicals from the current study. This is in agreement with previous study which reported that the presence of antioxidant and antimicrobial properties, its minerals (especially Iron), vitamins (especially vitamin A and C) and high protein contents were found to prevent oxidative radicals' production (Kayode and Kayode, 2011). This could also be due to the presence of secondary metabolites like tannins, glycosides, saponins, fehlings and terpenoids. A significant reduction was observed in MDA's lipid peroxidation level in all the doses tested suggestive of effective oxidative balance. MDA has been shown to be a biomarker of oxidative stress, excessive production of which has been linked to dyslipideamia and atherosclerosis.

The current study disagrees with Saalu *et al*., (2010) who reported that administration of *Telfairia occidentalis* leaf extract to rat at high dosage caused increased lipid peroxidation. The MDA level observed in this study is however agrees to the report of Ajani and Akinyemi (2016). There have been reports on antioxidant activities of various plants as well, some of which correlates with the present study. For instance, *Pelargonium reniforme* which is used locally for liver disorders, has strong antioxidant activities as a result of its tannin and flanovoid content (Fernandes *et al*., 2004). *Mallotus oppsitifolium*, a Nigerian plant rich in flavonoids has been said to possess antioxidant as well as anti-inflamatory activities (Farombi *et al*., 2001). These strongly agree with the present findings antioxidant activity observed

In conclusion, the results of the present study revealed that leaf extract of *Telfairia occidentalis* possesses anti-hyperlipidemic and antioxidant activities. It is hepato-protective and this is in agreement with its use in folk medicine in combating many diseases in the body. However, since it is dose dependent care has to be taken during therapeutic use as it has potential to increase alkaline phosphatase level.

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