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Phytochemical Components Analysis of Methanolic Stem Bark Extract of Warbugia Ugandensis on Atherosclerotic Lesions in Aortic Tunica Intima of New Zealand Rabbits Upon Induction of Atherosclerosis.

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Abstract

Background-*Warbugia ugandensis also known as green heart has a wide variety of use, the common one being in atherosclerosis. Phytochemical are plant products that may be harvested and used in manufacture of new drugs. The aim of this study was to analyze phytochemical components of Warbugia ugandensis, later on determine its acute oral toxicity and summarize by analyzing its histomorphological benefits.*

Methodology- *This was a posttest only true experimental design in which a total of 18 pure bred New Zealand rabbits were used. W. ugandensis stem barks were harvested and prepared for use in the present study. Several tests were done to assess presence of phytochemical components in stem bark extract of Warbugia Ugandensis. Necessary ethical documentation was acquired prior to start of experiment.*

Results- *on phytochemical component analysis of methanolic extracts, it was established cardiac glycosides were absent, alkaloids and anthraquinones were very low, saponins, phenols and tannins were moderately present while flavonoids and phytosteroids were highly present. It was observed that 1600mg upto 5000mg of W. Ugandensis extracts had normal activity with no mortality being recorded therefore no acute oral toxicity.*

Conclusion- *Flavonoids, tannins, phenols, saponins and Phyto-steroids are present in methanolic bark extract of W. ugandensis and therefore, are useful antioxidant and anti-inflammatory components.*

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I. Introduction

Warbugia ugandensis is a native tree of East Africa origin, commonly referred to as greenheart. It has a diversity of uses, including atherosclerosis (Orwa et al., 2009). The genus *Warbugia*, is a member of cinnamon family Canellaceae which has been described as Africa's panacea. It is an evergreen tree that can grow up to thirty meters tall and seventy centimeters in diameter. It has a variant bark which is sometimes smooth or crusty, light green and a rounded crown. The leaves grow to 3-15 cm by 1.4-5 cm and appear alternately on stems and have dotted glands on their surface with no stipules (Orwa et al., 2009). The flowers are kidney-shaped and appear either lonely or in small 3-4- flowered cymes. Flowering occurring in the early part of wet season, fruiting taking place in late part of wet season and fruit may remain on the tree for quite a long time. In Kenya, *W.* ugandensis flowers in December-January with sown in May.

W. ugandensis does well in coastal rainforests at altitudes of 100-2200m with a mean annual rainfall of 1000-1500 mm (Orwa et al., 2009).

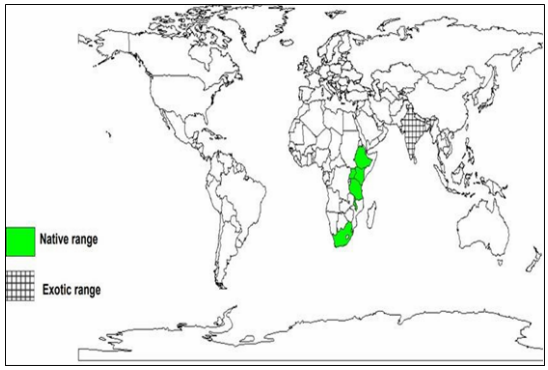

Figure 1 : Species of Warbugia ugandensis distribution across Africa and other regions (Orwa et al., 2009)

Phytochemicals Composition of Bark extract of *W*. ugandensis bark extract

These are natural products which are rich in chemical components produced by plants. These chemicals include; Alkaloids, tannins, flavonoids, triterpenes, steroids and saponins. They denote an important hoard for discovery of new drug compounds. Studies by (Khera et al., 2020) suggest that *W. ugandensis* has amazing anti-inflammatory and antioxidant activities, particularly with discovery of a new aryl naphthalene lignan amide. Extracts of *W. ugandensis* have powerful antioxidant and anti-inflammatory effects, mainly due to the presence of adequate terpenoids, drimane and coloratan type sesquiterpenoids. In addition to ugadensial, waburganal, mukaadial and other secondary metabolites such as tannins, flavonoids, saponins steroids and mannitol (Chung et al., 2019).

It has been noted that flavonoids are advantageous to the cardiovascular system. This is due to its antioxidant and anti-inflammatory properties, which result from its ability to reduce oxidation of low-density lipoproteins, thereby improving lipid profiles and also inducing vasodilation and regulating apoptotic processes in the endothelium (Ciumărnean et al., 2020). In addition, studies by (Fernández-Friera et al., 2017) showed that in rabbits with hypercholesterolemia, the accumulation of lipids was reduced after administration of flavonoids. Studies by (Carlson et al., 2008) concluded that tannins have potential for various health-promoting activities, particularly antioxidant, antitumor, cardio protective, anti- inflammatory and antimicrobial activity. As such, the study seeks to explore anti- atherosclerotic properties of *W. ugandensis* on aortic tunica intima.

Manifestation of Atherosclerosis

Atherosclerosis is a chronic inflammatory vascular wall disease which has been known to target tunica intima, the innermost histological layer of blood vessels. This condition is featured by accumulation of oxidized low-density lipid-C in macrophages located within tunica intima. Moreover, it presents with migration and proliferation of smooth muscle cells into tunica intima thus interfering with histomorphological organization of the aorta (Marchio et al., 2019).

II. Materials And Method

Sample Collection

The Stem barks was collected randomly with the assistance of a plant taxonomist having experience in *W. ugandensis* from Mount Kenya forest ensuring sustainable debarking (Khumalo, 2007). A voucher specimen of *W. ugandensis* bark, flower, and fruit was deposited at JKUAT botanical herbarium, and a voucher number obtained for each plant.

Figure 2*:* Shows Warbugia Ugandensis barks.

Sample Preparation

In order to remove dirt and soil, stem barks were washed, then cut into smaller pieces which were air dried at room temperature (25°C) for 2-3 weeks out of direct sunlight. The air-dried pieces were ground into powder using an electric grinder, weighed and preserved at 4°C in watertight plastic bags until extraction.

Figure 3: ***W. ugandensis*** **coarse powder**

Extraction of *W. ugandensis*

1000 grams of each plant powder were soaked in 2 litres of 75% methanol at room temperature for one day (24 hours) and then filtered out with Whatman No. 1 filter paper, after which solvent evaporation was achieved by vacuum evaporation using a rotary evaporator (Rotavapor R100, Buchi) to get the bark extract. The respective extracts were stored in air tight bijou bottles at -20 °C until use. Calculation of percentage yield of bark extract.

$$Percentage\ yield = \frac{Weight\ of\ plant\ after\ extraction}{Weight\ of\ plant\ before\ extraction} \times 100$$

Qualitative determination of phytochemicals in *W. ugandensis*

Test for Alkaloids

Crude powder extract was dissolved in 2 N HCl, filtered and filtrate divided into four parts to be used for the following alkaloid tests (Uma et al., 2014):

a) **Dragendroff's test:** This involved use of potassium bismuth iodide solution which was applied in 2ml of filtrate. A red-orange precipitate did not form indicating absence of alkaloids.
b) **Mayer's test:** Here, few drops of potassium mercuric iodide was added to the 2ml filtrate and there was no formation of yellow precipitate hence no presence of alkaloids.
c) **Wagner's test:** Iodine in potassium iodide was used for the 3rd filtrate. In the event no reddish or brownish precipitate formed then it signified absence of alkaloids.
d) **Hager's test:** Saturated picric acid solution was employed for the 4th precipitate which did not yield yellow precipitate hence absence of alkaloids.

Test for Flavonoids

Lead acetate test: Formation of a yellow precipitate on treating the extract with lead acetate solution indicated presence of flavonoids.

Shinoda's test: This test was done by treating extracts with magnesium and concentrated HCl. Presence of red and orange-red colors indicated flavanone and its absence respectively.

Test for Tannins

Braymer's test: The aqueous extract of the crude dry powder was treated with 10% alcoholic FeCl3 to yield a blue-black color indicating presence of tannins.

Test for Steroids and triterpenoids

Liebermann-Burchard test: this test was evaluated by adding 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid after dissolving extract sample in 2ml of chloroform in a dry test tube. A sequence of color changes from red, to blue and finally bluish green was observed indicating presence of a steroidal nucleus while color change to red- purple indicated presence of triterpenoid nucleus.

Test for Saponins

Foam test: Crude dry powder of extract was dissolved in 2ml of distilled water then briskly shaken and allowed to stand for 10 minutes. Formation of froth which lasted 10 minutes indicated moderate saponins.

Test for Cardiac glycosides

0.5g of the extract was hydrolyzed with mineral acid (dilute sulphuric acid) of 20ml in a boiling bath for 3 minutes then filtered. Three drops of a strong lead sub-acetate solution were added to the filtrate solution. A mixture of 5ml chloroform and filtrate were well shaken in a separating funnel. The lower organic layer was separated in two crucibles to test for lactones and deoxy sugars, the other two test tubes of extract were tested for Liebermann's and Baljet.

Keller-Killian Test

Chloroform was evaporated to dryness, then 0.4ml Glacial acetic acid with a trace of Ferric chloride was added down the side of the tube, followed by 0.5ml concentrated Sulphuric acid. There was absence of red-brown color at the junction, upper acetic blue denoting absence of deoxy sugar.

Kedde Test

Chloroform was evaporated to dryness followed by the addition of 1 drop of 90% alcohol and 2 drops of 2% of 3.5-dinitrobenzoic acid. This solution was alkalinized by adding 20% sodium hydroxide (NaOH). Absence of violet/purple color for lactones portrayed no lactones.

Test for Anthraquinone

Borntrager test

0.5g of the extract was dissolved in 5ml of dilute hydrochloric acid and boiled for 2-3 minutes, filtered then transferred in a separating funnel. It was thereafter extracted with 5ml of chloroform.

5ml of lower organic layer was put in a test tube and 4 drops of ammonia added. Observation was made on the basis development of rose pink to red color in the ammoniacal layer which demonstrated moderated amount.

Ethical approval

Ethical approval from the Animals Ethics and Research Committee for conducting the study was obtained from Jomo Kenyatta University Agriculture Technology Institutional Scientific and Ethics Review Committee **(JKU/ISERC/02316/0891**). National Commission for Science, Technology and Innovation, approval number **NACOSTI/P/23/28152** granted permission to conduct the research. Animals were handled in accordance with established University of Nairobi Biology Animal House handling guidelines.

III. Results

Qualitative phytochemicals, present in crude methanolic extract of *W. ugandensis*.

In order to determine qualitative phytochemicals, screening test as per the protocols for presence of antioxidants and anti-inflammatory components was done. It was observed that Alkaloids and cardiac glycosides were absent while Anthraquinones were of low amount in crude methanolic extracts of W. ugandensis. Flavonoids and Phyto-steroids were high while Phenols and Anthraquinones were absent while tannins, phenols and saponins were moderate in crude methanolic extracts of *W. ugandensis*. The aqueous extract showed that phenols, anthraquinones, and cardiac glycosides were absent, while alkaloids, tannins, and phytosteroids were in low traces. Flavonoids were found to be moderately present in the aqueous extract (Table 1).

Table 1: Phytochemicals in methanolic and aqueous extracts.

Phytochemicals	Methanolic extract	Aqueous extract
Alkaloids	+	++
Flavonoids	+++	++
Tannins	++	+
Phyto-steroids	+++	+
Phenols	++	Absent
Saponins	++	+
Cardiac glycosides	Absent	Absent
Anthraquinones	+	Absent

KEY: +++=Highly present, ++=Moderately present, +=Traces/low

IV. Discussion

Phytochemicals are natural products rich in chemical components produced by plants and represent an important cache for discovery of new drug compounds (Khera et al., 2020). Discovery of a new aryl naphthalene lignan amide suggest that *W. ugandensis* has remarkable antioxidant and anti-inflammatory effects (Khera et al., 2020). Anti-inflammatory and antioxidants chemicals are vital in prevention of lesions and enhancing human body infection defense systems by reactivating the ability to counter infections (Halliwell, 2011).

In the present study, flavonoids, tannins, saponins, phenols and Phyto-steroids were present and this concurred with studies done by (Ngugi, 2020) on effects of *W. ugandensis* in treatment of Asthma. It was observed that levels of flavonoids were high and moderate in both methanolic and aqueous extracts respectively. This observation is similar to that of (Okello et al., 2023) which demonstrated that the levels of flavonoids and phenols were high.

It was also noted that Phyto-steroids were present in methanolic extract of *W. ugandensis* which concurs with the findings of a previous study (Das et al., 2018) in which the researchers showed that Phyto-steroids defend inflammation by pulling down the pro-inflammatory mediators. Again, it was observed that Tannins were moderate denoted by results from brammers test which yielded a blue-black color in methanolic extracts of *W. ugandensis.* These findings are similar to (Carlson et al., 2008) concluded that tannins have the potential for various health-promoting activities, particularly antioxidant, antitumor, cardio protective, anti-inflammatory and antimicrobial activity. phenols and saponins were moderately present while anthraquinones were in very low in amounts as indicated in the phytochemical testing under methodology which concurs with (Bayram et al., 2012).

In the present study it was observed that cardiac glycosides were absent which is in tandem with findings of (Morsy, 2014) who points out that cardiac glycosides are typically found in small levels in plants, which may have an impact on their isolation. However, these findings are contrary to the works of (Bakir Çilesizoğlu et al., 2022; Oyewole & Akingbal, 2011; Prabasheela et al., 2015) who found out that cardiac glycosides were present in most plants as they played a role both antimicrobial and anti-heart failure role when administered to individuals or used in diet. it can be postulated that the levels in cardiac glycosides were absent in *W. Ugandensis* as compared to other studies and plants of similar family due to time differences in conducting the studies and other studies were conducted on leaves as compared to present study that was done on barks. Part of plant where phytochemicals were done plays have a variant presence. According to the findings, saponin, anthraquinone, alkaloids were present and cardiac glycoside phytochemicals was absent in *W. ugandensis* bark extract made from both aqueous and Methanol. This was in line with a study conducted by (Abuto et al., 2018), in which the bark of *W. ugandensis* was extracted using methanol and examined using GC-MS (gas chromatography-mass spectrometry) to identify changes in the profiles of plant chemicals from various communities in the Kenyan Rift Valley, they deduced from this research that *W. ugandensis* had high concentrations sesquiterpenoids with Low amounts of other types of chemicals, like and Phytosterols, phenolics, and tocopherols.

V. Conclusion

Flavonoids, tannins, phenols, saponins and Phyto-steroids are present in methanolic bark extract of *W. ugandensis* and therefore, are useful antioxidant and anti-inflammatory components. These components are postulated to have had a positive benefit as evidenced on the subsequent experiments. Therefore, there is need for further studies to evaluate the possibility of including these components in some of the drugs being manufactured to take care of atherosclerosis.

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Declaration Of Conflict Of Interest
No funds were received for this research therefore the authors are fully responsible for its entirety

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