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Original Research Article

Determination of Mineral Content, Cytotoxicity and Anthelmintic Activity of *Syzygium guineense* Fruits

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Abstract: *Syzygium guineense* Willd D.C. (Synonym *Memecylon lopezianum*. A. Chev) is a leafy forest tree of the family Myrtaceae found in many parts of Africa both wild and domesticated. Its fruits and leaves are edible and the fruits are used for treatment of dysentery. Ethanolic extract prepared from fresh seeds was used for cytotoxicity and antihelmintic testing using *Artemia salina* and *Pherithema posthuma* respectively. The dried pulp was analyzed for mineral contents using Delta-Portable X-Ray Fluorescence (DPXRF) with a sensitivity of 10 ppm. The ethanolic extract showed antihelmintic activity in a dose dependent manner giving shorter time of paralysis and death compared to the Albendazole tablets. Brine shrimps results gave the LC_{50} value 9 times higher than the standard drug suggesting absence of toxicity. The fruit pulp contains various amounts of macro elements and micro elements. To our understanding, this is the first study on *S. guineense* fruits on cytotoxicity and antihelmintic activities and mineral analysis. These preliminary findings indicate *S. guineense* fruits as a good source of micro and macronutrients as well as potential antihelmintic agent. The study is ongoing to capture various aspects of isolation and identification of bioactive compounds for drug development and establishment of safety.

Keywords: *Syzygium guineense;* Minerals; Anthelmintic activity and Cytotoxicity activity.

INTRODUCTION

Syzygium guineense (Willd) D.C. synonyms; *Memecylon lopezianum* A. Chev is a tree with edible fruits, belongs to the Myrtaceae family. It is widespread in Sub Saharan Africa, edible organs are the leaves and fruits (the fruit skin and pulp) [1]. The bark is traditionally used to treat stomachache and diarrhea [2]. In Nigeria the plant is used to treat diabetes [3], in Namibia it is used to manage HIV/AIDS opportunistic infections particularly in the treatment of *Herpes zoster* [4] while in Uganda, the plant is used against malaria [5].

Essential oil of leaves consist of caryophyllene oxide (7%), d-cadinene (7.5%), viridiflorol (7.5%), *epi*-α-cadinol (9.8%), α-cadinol (12.7%), *cis*-calamenen-10-ol (14%), citronellyl pentanoate (15.2%), β-caryophyllene (20.1%) and α-humulene (39.5%) [6]. Phytochemical analysis showed the presence of flavonoids, tannins, saponins, carbohydrate, alkaloids and cardiac glycosides [7], arabinogalactan polysaccharides that possessed immunological activities [8].

Testing leaf methanolic extract of *S. guineense* on mice justified oral administration for treatment of snake envenomation [9] while the ethanolic extract exhibited anti-inflammatory and analgesic activities. Regarding antibacterial activity against *Escherichia coli, Bacillus subtilis* and *Shigella sonnei,* arjunolic and asiatic acids showed highest significant antibacterial activity among the isolated triterpenes from leaves [2]. Leaves had demonstrated insecticidal activity against *Melophagus ovinus* (an external parasite of sheep belonging to the family *Hippoboscidae*) [10]. Both ethanolic and aqueous root extracts of *Syzygium guineense* inhibited *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Salmonella typhi* at 20 mg/ml and identified the alkaloids and anthraquinones as the bioactive compounds [1]. In another study, the root bark showed antimycobacterial activity by minimum inhibitory concentration within the range of 800µg/ml to 2000µg/ml [11]. Evaluation of in vivo antihypertensive and in vitro vasodepressor activities of the leaf extract of *Syzygium guineense* exhibited antihypertensive effect that was linked with by dilation of the blood vessels. This confirmed the folkloric antihypertensive use of the plant [12].

Despite of the popular consumption of *Syzygium guineense* fruits in Tanzania, there were no scientific reports regarding its nutritional value, mineral profile / biological tests of edible (fruit pulp) /non edible portion (seeds). Unlike humans, animal consume the whole fruit. Our study focused on mineral analysis, medicinal benefits associated with the gastro-intestinal infection particularly antihelmintic activity and cytotoxicity testing. The current and future results will encourage the use of these fruits for nutritional and medicinal purposes.

MATERIALS AND METHODS

Medicinal uses, Collection and preparation of the sample

Fruits were bought from the local market of Kisutu in Dar es Salaam, Tanzania where sellers reported on the preference of these fruits among diabetic patients in connections with their ability on body sugar regulation. Fruits were washed with clean water to remove dirt then rinsed with distilled water. The fruit pulp was separated from the seeds and the pulp was dried at 45^0C in the oven then crushed to obtain fine powder. The *Syzygium guineense* seeds were blended and 300g was macerated with 400 ml of absolute ethanol for 48 hours. The extraction process was repeated three times to ensure complete extraction. The extract was double filtered by using cotton wool and filter paper and dried on the rotary evaporator at 45^oC**.**

Tested organisms

i. Brine shrimps (*Artemia salina*)
ii. Earthworm (*Pherithema posthuma*)

Standard drugs, chemicals and reagents

Cyclophosphamide was purchased from purchased from Oxoid Ltd (Basingstoke, Hampshire, England). Albendazole tablets purchased from *S*helys Pharmaceutical Company. Dar es Salaam Tanzania, Ethanol (absolute) was supplied by Fluka Chemie GmbH (Sigma-Aldrich®, Zwijnddrecht, Netherlands) and Dimethyl sulfoxide (DMSO) was purchased from Sigma® Poole, Dorset, UK. The Brine Shrimps eggs were purchased from Aquaculture innovations (Grahamstown 6140, South Africa) and sea-salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam Coast.

Equipment

Delta-Portable X-Ray Fluorescence (DPXRF), XRF model: Delta classic, manufactured by InnovX-systems, Inc. Olympus Scientific Solutions Woburn, MA USA.

Mineral content analysis

Mineral content analysis was carried out using Delta-Portable X-Ray Fluorescence with a sensitivity of 10 ppm. The XRF allows the direct analysis of the sample giving the simultaneous measurement of the elements present in the sample. Prepared sample of the powdered drug (10 g) were introduced to the system.

Cytotoxicity testing

Brine shrimp lethality test previously described by Meyer *et al.,* [13] was used to predict the presence of bioactive compounds in the *Syzygium guineense* seed extract. Artificial seawater was prepared by dissolving sea salt (3.8 g) in distilled water to make a concentration of 3.8 g/L and then Brine shrimp eggs were allowed to hatch for 48hours. Stock solution (40 mg/ml) of the ethanolic extract was prepared by dissolving them in dimethyl sulphoxide. Different levels of concentrations (240, 120, 80, 40 and 24 µg/ml) were prepared by drawing different volumes from the stock solutions and then added into vials, each containing ten brine shrimps larvae. The negative control contained brine shrimp larvae, artificial sea water and dimethyl sulphoxide only and the positive control used was cyclophosphamide. The vials were incubated under light for 24 h and the numbers of survivors in each concentration were counted and the percentage mortality was determined.

Antihelmintic activity testing

The anthelmintic activity was carried out on adult earthworm (*Pherithema posthuma*) as described by Nilan's research team [14] with minor modifications. The earthworms of 400 mg to 600 mg were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. The worms were divided into three groups of six adult earthworms. Stock solutions (500mg/ml) of the ethanolic extract and albendazole tablets of 200 mg were prepared separately by dissolving each in distilled water as vehicle. Different of concentrations of 80, 50, 30 and 10mg/ml in normal saline were prepared by drawing different volumes (8, 5, 3 and 1mls) from the stock solutions. Groups of earthworms were released into the petridish containing desired concentration as made above. Group one for control received only normal saline, group two serve as standard, received standard drug Albendazole tablets and group three for ethanolic extract of *Syzygium guineense* seeds. Observations were made for the time taken to cause paralysis and death of individual worms. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli.

RESULTS AND DISCUSSION

Mineral contents: Minerals detected in the present study are shown in Table 1.

Table-1: Mineral contents analysis: Dried fruit pulp of *Syzygium guineense*

Analyte	mg/100g	Analyte	mg/100g
Calcium	20,477	Zinc	nd
Potassium	443	Rubidium	3.2
Phosphorus	8392	Strontium	60.5
Sulphur	1660	Zirconium	18.2
Manganese	8.5	Arsenic	nd
Iron	268.3	Chlorine	nd
Titanium	39.1	* nd = not detected	

The detected essential metals have roles in physiological and biochemical processes possessing therapeutic/prophylactic properties hence maintenance of good health. The mineral analysis of the related species *Syzygium cumini* showed the presence of calcium, phosphorus, iron, potassium and sulphur but devoid of copper, sodium, magnesium, and chlorine [15].

In addition to the essential metals, four trace metals were detected, amongst them, strontium was found in higher quantity. Strontium does not have unique role in human metabolism although it amounts to about 0.32 g in human body. The health benefit of this metal in moderate dietary levels is the promotion of calcium uptake into bones [16]. This could be linked with its ability of retardation of thinning and facilitation of rebuilding and strengthening of new bones [17] by increasing bone mineral density and offering pharmacological effect of reducing fracture risk [18]. Based on these facts, ladies in their menopausal period who are prone to osteoporosis may benefit from eating the fruit pulp. Titanium itself is non-toxic and not rejected by human body while its compounds are used as food and cosmetic additives and medically for implants such as hip and joint replacements due to its bio-inert nature [19]. Although rubidium is not among the essential metals, some evidences suggest its role in reducing free radical pathology and serve as a mineral transporter across defective cell membranes especially in cells associated with aging. Also, having a close physiochemical relationship with potassium and it may have the ability to act as a nutritional substitute [20]. Zirconium has no known biological role, but on average the human body contains 250 milligrams of zirconium, and daily intake is approximately 4.15 milligrams depending on dietary habits [21]. Zirconium and its salts generally have low systemic toxicity and most passes through the gut without being adsorbed, and in case of adsorption it tends to accumulate more in the skeleton than tissues [22]. Regarding the detected trace metals, *Syzygium guineense* edible portion could be regarded safe for consumption.

Cytotoxicity activity

Cytotoxicity activity was expressed in terms of lethality concentration as presented in Table 2. The LC_{50} values obtained from the extract were 9 times higher compared to the standard drug and could be regarded nontoxic according to Meyer *et al.*, [13] defining toxicity when the LC_{50} value is above100µg/ml. However, safety establishment using in vivo animal tests are necessary before recommending the use of seeds as antihelmintic. Along with safety assessment, it is worth testing for various bioactivities since seeds of the related species *Syzygium cumini* had demonstrated several bioactivities including; antimicrobial activity [23], anti-inflammatory and antidiabetic activity [24, 25].

Table-2: Cytotoxicity activity

	LC_{50} (µg/ml)	95% confidence interval (CI)	Retention factor (r^2)	Regression equation
Ethanolic extract	151.43	132.05-173.64	0.970	Y=204.4logx_395.2
Cyclophosphamide	16.36	12.01-22.31	0.995	Y=69.968logx_34.936

Antihelmintic activity

Ethanolic extract of *S. guineense* gave interesting anthelmintic activity in a dose dependent manner for both paralysis and death effects compared to the standard drug (albendazole tablets) as shown in Figure 1 and Figure 2. At the highest tested concentration (80 mg/ml) the time taken to attain 100% paralysis was 12 times shorter compared to standard drug, whereas at lower concentrations (50, 30 and 10mg/ml) difference in the time taken to attain 100% paralysis was not significant compared to the standard drug. At the highest tested concentration (80 mg/ml) the extract caused 100% mortality and the time taken was 26 times shorter compared to the standard drug, Similarly, at lower concentrations (50, 30 and 10mg/ml) 100% mortality was observed and the time taken was 7 times shorter compared to the standard drug.

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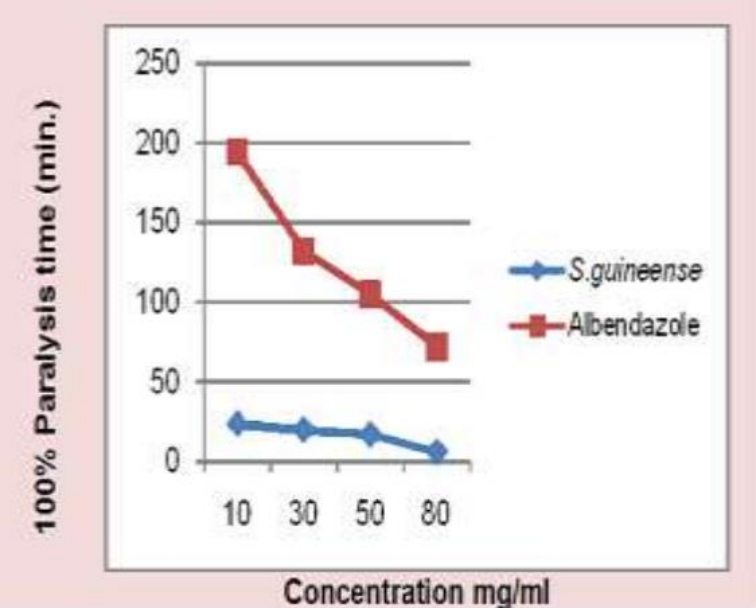

Fig-1: Rate of paralysis

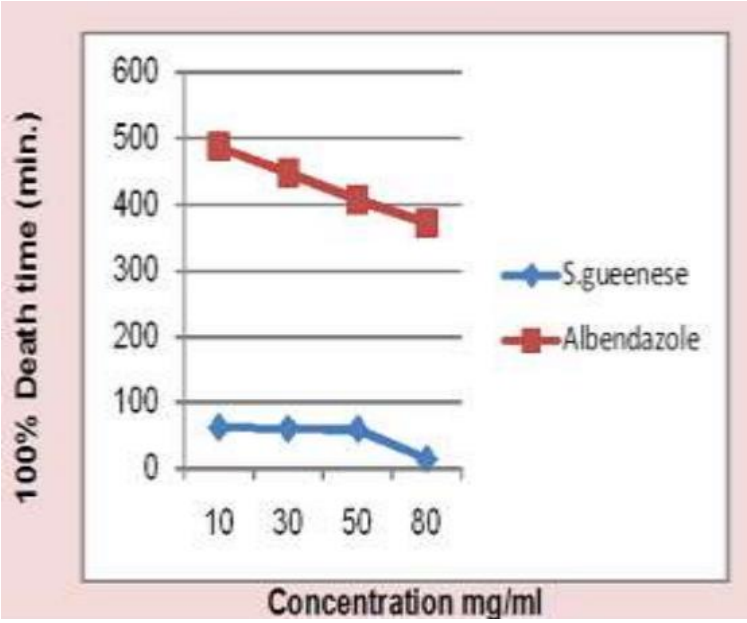

Fig-2: Rate of death

CONCLUSIONS

Our results on *S. guineense* fruit pulps have revealed the presence of both essential and trace metals of health benefits and the non-toxicity and anthelmintic activity of the seed ethanolic extract. The study is on-going focusing on the following; (i) fruit pulp proximate analysis and assessment of its suitability as a functional food since *S. guineense* fruits are trusted as diabetes nutraceutical, (ii) testing of the seed extract against pathogenic worms and isolation/identification of bioactive compounds and, (iii) establishment of safety and the mechanism of action(s).

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