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Phytochemical and Acute Toxicity Assessment of *Syzygiumguineense* (Willd.) DC. and *Acacia hockii* De Wild. Traditionally used for Female Contraception/fertility regulation in Baringo County, Kenya

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ABSTRACT

A boiled stem bark mixture of Syzygiumguineense and Acacia hockii is traditionally used by the Tugen community in Baringo County, Kenya, for fertility regulation in women. This study aimed to validate the safety and efficacy of these plants through phytochemical screening and acute oral toxicity tests. Standard phytochemical tests were employed to identify the compounds present in the stem bark extracts. Acute toxicity was evaluated following the Acute Toxicity Class model based on the OECD 423 Guideline (2001). The study was conducted between April and July 2024 at the Department of Chemistry & Biochemistry for sample extraction and phytochemical analysis, and the Department of Biological Sciences, University of Eldoret, for toxicity testing.

The stem bark extracts of both plants contained tannins, saponins, flavonoids, glycosides, alkaloids, terpenoids, steroids, and phenols, according to phytochemical screening conducted using established procedures. Rats were used in acute oral toxicity experiments, with each plant extract administered at a set dose of 2000 mg/kg body weight. The rats were split into test and control groups. Over the course of 14 days, signs of toxicity and death were noted every day, and body weights after fasting were recorded once a week.

The fact that no deaths or changes in physiological indicators were noted during the trial suggests that the LD50 is higher than 2000 mg/kg. Furthermore, the rats fed with the extracts showed a consistent increase in their fasting body weights when compared to the control group ($p = 0.05$). This study concludes that the ethnobotanical use of Syzygiumguineense and Acacia hockii is safe based on acute toxicity results. The phytochemical compounds present may be responsible for the plants' pharmacological effects in traditional medicine. However, further long-term toxicity studies are recommended to fully assess the toxicological profile and reinforce the clinical significance of these findings.

Keywords: *Syzygiumguineense, Acacia hockii, phytochemical, acute toxicity, Stem bark*

1.0 Introduction

The oldest known type of medical treatment is found in plants. For up to 80% of people worldwide, herbal therapy is still the major form of medical care today [2]. Usually, a particular plant part such as the root, leaves, fruit, flowers, or seeds, is utilized in customary formulations or as a standalone source of active ingredients when creating pharmaceutical goods. Plants are the source of many contemporary pharmaceuticals; around 25% of prescription medications contain at least one active component derived from plant material [21]. Medicinal plants are integral to healthcare across all cultures and have consistently played a key role in global health systems. In most developing countries, herbal treatments are culturally embedded and often represent the predominant healing method. These remedies are widely accepted due to their considerable efficacy, economic affordability, and, in many cases, their accessibility as the only available option [26]. Numerous studies highlight the crucial role medicinal plants play in primary healthcare delivery in Kenya, where a significant portion of the population relies on traditional medicine, similar to many developing nations, especially those in sub-Saharan Africa [30].

While almost any substance can be harmful at certain doses, lower doses may present no harmful effects. Between these extremes, a range of effects can occur, from subtle long-term toxicity to immediate lethal outcomes [29]. Phytotoxins are a broad class of harmful substances that are produced by plants and are believed to have evolved as defense mechanisms against herbivores including insects and mammals [15]. A lot of chemicals identified as toxic are found in plants that are part of the human diet [28]. Traditional healers from the Arror sub-tribe of the Tugen people in Baringo County, Kenya, frequently use a mixture of *Syzygiumguineense* and *Acacia hockii* stem barks as herbal contraceptives or antifertility treatments for women.

Syzygiumguineense (Willd) D.C (Myrtaceae), commonly known as "Lomoiywo" in Tugen and "water berry" in English, can grow as a tree, shrub, or fire-adapted subshrub, depending on its environment. When it grows as a tree, it typically reaches heights of 10 to 15 meters, though some can grow as tall as 25 meters. The tree has a dense, rounded crown and a broad, fluted trunk. When a tree is younger, its bark is smooth; as it ages, it becomes rough and dark.

Its broad, angular stems and drooping branches. The young leaves start off purple-red and mature into a dark green, with shiny, smooth surfaces and long, rounded tips attached to short, grooved stalks. The flowers are notable for their white, prominent stamens, arranged in dense clusters about 10 centimeters wide, emitting a sweet fragrance that attracts numerous insects [1].

This species is widespread across sub-Saharan Africa and thrives in the wooded savannahs and tropical forests of the continent. Traditionally, various parts of *S. guineense* are used in the treatment of numerous ailments, including malaria. The fruits and bark are commonly used to treat dysentery and diarrhea, while infusions made from the leaves, fruits, or bark are used to manage hypertension. Studies have demonstrated that the methanol extract of the bark exhibits antihypertensive, vasodilatory, analgesic, anti-inflammatory, antimalarial, and antidiabetic effects. The bark is also traditionally used to address gastrointestinal issues and diarrhea [25]. The twigs and leaves possess anthelmintic properties, particularly against hookworms. Furthermore, the water extracts from the fruits and bark have shown activity against bacteria that cause diarrhea. A study in Cameroon highlighted the antioxidant properties of the leaves and their positive impact on oxidative stress. See figure 1.

Figure 1: Syzygiumguineense tree

Acacia hockii, a member of the Fabaceae family, is a species commonly used in traditional medicine across Kenya. Known as "White thorn acacia" and locally referred to as Tilatil in the Tugen community, this species is prevalent throughout East Africa. It can grow as a multi-stemmed shrub, reaching heights of 2-4 meters, or as a small tree up to 6-7 meters tall, occasionally spreading to 9 meters in width. The bark varies from red-brown to greenish or greenish-brown, and rarely, pale-yellow. The thorns are up to 2 cm long, short, straight, and spinescent stipules. Each of the 1–11 pairs of pinnae on the leaves has 9–29 pairs of leaflets. The axillary, pedunculate heads of bright yellow or orange flowers have a diameter ranging from 5 to 12 mm. The slender, reddish-brown pods can have a straight or crescent form. The smooth, elliptic, olive-brown seeds have dimensions of 5-7 x 3-4 mm. The plant is well-known for its many therapeutic applications, which include gout, dropsy, swellings, oedema, and pain relief [9]. It is also a vermifuge. It has been used to treat abscesses and stomach aches in Kenya in addition to treating malaria.Children with fever in Tanzania are given a boiling bark infusion, while in Uganda, a root decoction is used to treat tuberculosis-related illnesses and hookworms [16].

Figure 2: Acaciahockii tree

Despite their widespread traditional use, the safety and phytochemical compositions of the stem barks of *S. guineense* and *A. hockii* have not been extensively researched. Therefore, it is crucial to establish safety and quality assurance protocols to guarantee the provision of high-quality medicinal plant materials. The scientific evaluation of the safety and toxicity of *S. guineense* and *A. hockii* is a crucial component of this investigation. The investigation's findings are intended to fill in knowledge gaps about these species and offer more proof in favor of future research on the toxicity profiles of herbal remedies derived from these plants. In light of this, the study's goal is to investigate the phytochemical makeup and acute toxicity of methanol extracts of *S. guineense* and *A. hockii's* stem barks.

1.2 Objectives of the Research

The aim of the research was to assess the toxicity and phytochemical content of stem bark extracts from *Syzygiumguineense* and *Acacia hockii* in order to give scientific evidence in support of their ongoing use.

1.3 Justification of the Study

In developing nations, women frequently face barriers to accessing modern contraception, especially in rural areas [24]. Furthermore, there is a belief that contemporary contraceptives have negative side effects, which makes women and their partners feel insecure. This frequently leads to the abandonment or sporadic use of family planning techniques [3]. As a result, women in these areas are increasingly using natural contraception. Evaluating the toxicity and effectiveness profiles of these herbal therapies can foster acceptance and increase self-assurance. Toxicology knowledge is essential for preventing hazardous exposures and for offering treatments when poisoning occurs. In the meanwhile, phytochemical screening is crucial to pharmaceutical research in order to identify plants with medicinal potential [18]. To validate their traditional use, it is crucial to assess the phytochemical content and toxicity profiles of *Acacia hockii* and *Syzygiumguineense*.

2.0 Materials and Methods

2.1 Plant collection and identification

A local herbalist made the initial in-person identifications of the medicinal plants. Freshly harvested from the vicinity of Barbarchun village in Baringo County

[0°46'35.3"N 35°49'24.5"E], Kenya, the stem barks of Syzygiumguineense and Acacia hockii were cut off the tree trunks using a panga in October 2023. The various plant pieces, including the stem barks, were packed separately in plastic bags and shipped. The plant materials were recognized and certified by a taxonomist from the University of Eldoret, and they were given the voucher numbers Muh/Zyg/21/08 and Muh/Ach/21/19. The University of Eldoret'sDepartment of Biological Sciences herbarium is where the specimens are kept. After being cleared of debris and sliced into small pieces, the stems were allowed to dry for a few weeks in the shade. An electric mill (Disk Mill FFc-23, China) was then used to grind the stems into a fine powder.

2.2 Sample Extraction

Maceration was used to extract 100 grams of powdered stem barks from *S. guineense* and *A. hockii* [27, 13]. 100 grams of each stem bark were extracted by soaking them in 500 milliliters of methanol for 72 hours in a 1-liter conical flask. The mixture was then filtered, and a rotary evaporator [EL 30, type AG CH-9230, Germany] was used to evaporate the solvent at 40°C. The resultant extracts were subsequently dried in an oven at 40°C for 24 hours to guarantee total solvent removal. The concentrates were then placed in airtight glass jars and refrigerated at 4°C until they were required.

2.3 Experimental Animals

The female Wistar rats (*Rattusnorvegicus*), weighing roughly 240–260 g, were obtained from the University of Eldoret's Department of Biological Science animal house. The rats were acclimated for two weeks before the trial began. They always had access to commercially prepared food and water. The housing was kept at a temperature of 25 ± 2 °C and had a 12-hour light/dark cycle. The Guide for Care and Use of Laboratory Animals [8] contains established public health rules that were followed when handling and caring for the animals.

2.4 Preparation of Administration Doses

The Organization for Economic Cooperation and Development's guidelines were followed in the production of the doses used in this investigation. In order to create a stock solution that contained 2000 mg/kg body weight and could be given to a 100 g rat, the following formula was used, as described by [7]:

Animal dose (mg/kg b.wt) = animal weight (g)/1000(g) × chosen dosage

The Organization for Economic Cooperation and Development recommends reconstituting fifty milligrams in 0.2 milliliters of physiological saline [vehicle]. Based on the individual fasting weights of the rats utilized, an 8-milliliter stock solution of crude methanol stem bark extracts from S. guineense and A. hockii was serially diluted to obtain doses of 2000 mg/kg body weight.

2.5 Phytochemical Screening

The phytochemical components of the stem bark extracts from *Acacia hockii* and *Syzygiumguineense*were identified using qualitative techniques, in accordance with the protocols outlined in [16, 14].

2.5.1 Tannins Test

Five milliliters of distilled water were added to each extract to dilute it to a concentration of around 0.5 grams. Then, 1 milliliter of the solution was mixed with 4 drops of neutral ferric chloride (5 percent). An intense green hue indicated the presence of tannins.

2.5.2 SaponinsTest

One gram of each extract was diluted with distilled water to a total volume of 20 ml and shaken in a graduated cylinder for 15 minutes. A foam layer of 1 cm indicated the presence of saponins.

2.5.3 Test for Flavonoids

Each extract was treated with three drops of sodium hydroxide in two milliliters. There were flavonoids present since a yellow tint appeared and vanished when a few drops of diluted hydrochloric acid were added.

2.5.4 Glycosides Test

Two milliliters of acetic acid and two milliliters of chloroform were added to each extract after they had been hydrolyzed with diluted hydrochloric acid. Concentrated sulfuric acid was progressively added to the combinations after they had cooled. The green coloration indicated the presence of glycosides.

2.5.5 Test for Alkaloids

Each extract was mixed with five milligrams of 1% aqueous hydrochloric acid, boiled in a water bath, and then filtered. Two milliliters of Wagner's reagent (iodine in potassium iodide) were combined with one milliliter of the filtrate. Alkaloids were present because a reddish-brown precipitate appeared.

2.5.6 AnthraquinonesTest

After shaking two milliliters of each diluted extract and an equal amount of benzene, the organic layer was separated. The ammonia layer turned pink upon the addition of an equal volume of diluted ammonia solution, signifying the presence of anthraquinones.

2.5.7 Test for Terpenoids

Three milliliters of strong sulfuric acid were carefully placed along the test tube's side, after five milliliters of each crude stem bark extract and two milliliters of chloroform were combined. The reddish-brown hue at the interface verified the presence of terpenoids.

2.5.8 Steroids Test

Two milliliters of chloroform were combined with one gram of each extract, and one milliliter of strong sulfuric acid was poured around the test tube's walls. The presence of steroids was detected by the emergence of a red tint.

2.5.9 Phenols Test

We combined one gram of each stem bark extract with water. Drop by drop, ferric chloride solution was added. Phenol was recognized by the appearance of red, blue, green, or purple hue.

2.6 Acute Oral Toxicity Study

The experiment was conducted using normal female rats that were not pregnant or nulliparous, in accordance with OECD test guideline 423 (2002). The animals were randomized into three groups (n=3) at random prior to dosing. For five days, they were housed in their cages to help them get used to the lab environment. Overnight, food was not given, but water was freely available. The second and third groups received 2000 mg/kg body weight of methanolic extracts from the stem barks of *Albiziahockii*and*Syzygiumguineense*, respectively, while the first group served as the negative control (untreated). Gastric gavage was used to provide these extracts orally.

Food was avoided for an extra three to four hours following the administration of the extracts. Every day over the next 14 days, clinical observations were conducted to keep an eye out for any indicators of toxicity and mortality, paying particular attention to the first four hours. Once a day, visual cage-side examinations were carried out to evaluate any alterations in the skin, hair, eyes, mucous membranes, nasal passages, and the autonomic and central nervous systems of the animals. On days 1, 7, and 14, the animals were fasted for an entire night before their fasting body weights were measured. For a maximum of 14 days, mortality rates and toxicology-related symptoms connected to the stem bark extracts were also observed.

2.7 Statistical Analysis

Data from the acute toxicity effects on body weights were collated, displayed as Mean ± Standard Error of the Mean (SEM), and subjected to analysis of variance (ANOVA) in a Microsoft Excel spreadsheet. The statistical significance was evaluated using a p = 0.05 significance level. Both quantitative and qualitative evaluations of the acute oral toxicity data were carried out in compliance with OECD (2002) Guideline No. 423 [4].

3.0 Results and Discussion

3.1 Phytochemical composition of *Syzygiumguineense and Acacia hockii* stem-bark extracts

The qualitative phytochemical screening, summarized in Table 1, revealed that the methanolic extracts contained tannins, saponins, alkaloids, flavonoids, glycosides, terpenoids, phenols, and steroids, while anthraquinones were absent in both extract types. The traditional use of *S. guineense* and *A. hockii* as female anti-fertility agents is supported by the presence of alkaloids, saponins, and flavonoids. Alkaloids are believed to contribute to anti-fertility effects through various mechanisms, such as inhibiting ovulation, disrupting the oestrus cycle, inducing post-coital antifertility effects, causing abortion, affecting endocrine functions, preventing implantation, and promoting embryo resorption [11]. Saponins exhibit abortifacient, anti-zygotic, and anti-implantation effects, while flavonoids have demonstrated anti-zygotic, blastocytotoxic, and anti-implantation properties [22].

In addition to their reproductive effects, these phytochemicals' presence in the stem bark extracts of S. guineense and A. hockii suggests further pharmacological properties. Tannins are linked to wound healing, anti-inflammatory, antioxidant, antimicrobial, cardioprotective, anti-diabetic, and anti-obesity effects [17]. Terpenoids have been associated with antimicrobial, anti-cancer, antinociceptive, antispasmodic, hepatoprotective, and anti-inflammatory properties [11]. Flavonoids are beneficial in preventing coronary heart disease, reducing oxidative stress, and controlling growth because of their antioxidant, free radical scavenging, hepatoprotective, antibacterial, anti-inflammatory, anti-cancer, and antiviral properties [19]. Saponins have demonstrated cardioprotective, anti-diabetic, anti-cancer, and immune-stimulating effects [23]. Alkaloids, on the other hand, have been associated with anti-cholinesterase, antioxidant, anxiolytic, anti-inflammatory, and antidepressant properties, particularly in the treatment of conditions like Alzheimer's disease [6].

Table 1: Phytochemical composition of S. guineense and A. hockii stem-bark extracts

Phytochemical tested	*S. guineense* stem bark extract	*A.hockii* stem bark extract
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Glycosides	+	+
Alkaloids	+	+
Anthraquinones	-	-
Terpenoids	+	+
Steroids	+	+
Phenols	+	+

Key: + = present; - = absent

3.2 Acute oral toxicity Study of *S. guineense and A. hockii* stem bark extracts

Acute toxicity studies indicated that the LD50 of methanolic extracts from the stem barks of *S. guineense* and *A. hockii* exceeded 2000 mg/kg. These results are consistent with previous research on medicinal plants from the Myrtaceae [12] and Fabaceae [20] families, which also demonstrated no acute toxic effects. The extracts of *S. guineense* and *A. hockii* are categorized under category 5 of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which denotes low toxicity. They are categorized as somewhat dangerous (Category III) by the WHO [5]. As indicated in Table 2, rats given methanolic extracts of S. guineense and A. hockii at a dose of 2000 mg/kg body weight did not exhibit any unfavorable physical or behavioral alterations.

Table 2: Effects on physical and behavioral characteristics of a single dosage of bark extracts from S. guineense and A. hockii stem

Observation	Control	*Syzygiumguineense* 2000 mg/kg b.wt	*Acacia hockii 2000mg/kg*b.wt
Changes in respiration	-	+	+
Alterations in circulation	-	-	-
Changes in fur and skin	-	-	-
Stiffling power	+	+	+
Sound reaction to contact	+	+	+
Response to touch	+	+	+
Transportation	+	+	+
Urinating	+	+	+
Defecation	+	+	+
Diarrhoea	-	-	-
Reflex of righting	+	+	+
Sluggishness	-	+	+
Sedatives	-	-	-
Shivers	-	-	+
Seizures	-	-	-
Death toll	-	-	-

Key: +=present -=absent

Table 3 shows the outcomes of the weekly body weight changes for each of the rat groups. The untreated control group and the treatment groups did not differ in weight changes in a way that was statistically significant. Both the methanolic stem bark extracts of *Acacia hockii*and*Syzygiumguineense*appear to be safe for usage based on the doses used in the field.

Table 3: Effects of Acacia hockii and Syzygiumguineense stem bark extracts in methanol on treatment and control rats' body weights during an acute oral toxicity investigation.

Parameters	Doses	Mean±SEM	Significance
	Control	149.3±5.63	
Body weight initial week	*S.guineense*- 2000mg/kg	146.83±3.14	0.91
	A. hockii- 2000mg/kg	145.33±3.04	
	Control	154.90±7.65	
Body weight week 1	*S. guineense*- 2000mgkg	151.23±3.08	0.89
	A. hockii- 2000mg/kg	151.78±5.68	
	Control	166.93±9.30	
Body weight week 2	*S.guineense*- 2000mg/kg	165.73±5.49	0.93
	A. hockii- 2000 mg/kg	163.10±6.80	

The values are given as mean±SEM for n = 3/group.

Conclusions

A phytochemical examination of the aqueous and ethanolic extracts of the*V. hockii* showed the presence of flavonoids, alkaloids, terpenoids, phenols, sterols, tannins, and cardiac glycosides. Similarly, various phytochemicals were detected in the stem bark extracts of *S. guineense* and *A. hockii*, suggesting their potential in pharmaceutical development. Acute oral toxicity tests, conducted using a single dose of 2000 mg/kg body weight of methanol stem bark extracts from *Sguineense* and *V. hockii* in rats, showed no visible toxicity symptoms or mortality throughout the observation period. Thus, the stem barks of *Syzygiumguineense* and *Acacia hockii* contain key phytochemicals relevant to natural contraception. Additionally, no mortality or adverse clinical effects were observed in the acute oral toxicity studies conducted over the short study duration.

Recommendations

Short-term usage of Syzygiumguineense and Acacia hockii as a traditional method of female contraception is safe. Studies on chronic toxicity are also required to ascertain its long-term effects. The presence of phytochemical components lends credence to its continued application in conventional medicine. Research needs to be conducted to quantify its phytochemical constituents.

Authors' Contributions

Richard T. Kiptisia: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Software, Writing - original draft, Writing - review & editing.
Anastasia Nandwa: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Software, Writing - review.

Competing Interest

The writers have declared that they have no conflicting interests.

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