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Research Article

Analgesic, Phytochemical And Toxicological Investigations Of Ethanol Extract Of The Leaves Of *Kigelia Africana* (Lam.) Benth (Family *Bignoniaceae*)- Sausage Tree

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ABSTRACT

The plant of *Kigelia africana* had been in use from time immemorial by the people of Ogidi in Idemili Local Government area of Anambra State, Nigeria to treat pyrexia, analgesia and infectious diseases. For example the decoction of the plant is used to treat wounds, running stomach (diarrhea), aches and pains as well as fever. This investigation was carried out to ascertain the truth of this claim.

The leaves of *Kigelia africana* was collected and dried at ambient temperature and pulverized. Exactly 200g of the powdered drug was extracted with 400ml of ethanol using the cold maceration technique for 24hours with occasional shaking. This was filtered and the procedure repeated with the marc. The combined filtrates were concentrated under reduced pressure with rotary evaporator. The preliminary phytochemical tests were carried out using standard methods. The analgesic was conducted using hot plate method. The acute toxicity test of the extract was determined using the Lorke's method.

The leaves of *Kigelia africana* exhibited analgesic property. Alkaloids, flavonoids, saponins, tannins, proteins, steroids and carbohydrates were found. Toxicity test showed that the extract was safe at the dose of 1000mg/kg.

Preliminary studies support the claim that the leaves of *Kigelia Africana* possesses, analgesic properties.

KEYWORDS: *Kigelia Africana*; hot plate method; Lork's method.

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INTRODUCTION

Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria[17]. Even the World Health Organization (WHO, 2002)[36] is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs (WHO Traditional Medicine Strategy, 2002). However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonised by majority of allopathic medical practitioners as it is considered to have no scientific basis[28]. This work is therefore a preliminary work to prove that there is scientific evidence to the use of leaves of *Kigelia Africana* in the treatment of pyrexia.

One major problem of herbal medicine practice is that there is no official standard and / or local monograph. In Nigeria, the Federal Government has urged the federating states to set up traditional medicine boards to license and regulate the practice of herbal practitioners under the supervision of ministries of health[4].

Many medicines including reserpine, ergotamine, vincristine, and vinblastine are of herbal origin[5]. About one quarter of the present prescription drugs dispensed by community pharmacies in the United States contain at least one active principle originally derived from plant materials[39].

KIGELIA AFRICANA (Bignoniaceae)
Common Names: Sausage Tree, Cucumber Tree

CLASSIFICATION

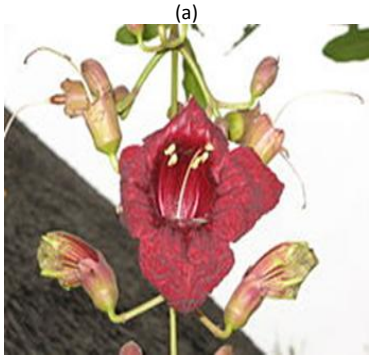
KIGELIA AFRICANA (Lam.) Benth.

- **Kingdom**: Plantae-Plants
- **Subkingdom:** Tracheobionta-Vascular plants
- **Super-division:** Spermatophyta-Seed plants
- **Division:** Magnoliophyta-Flowering plants
- **Class:** Magnoliopsida-Dicotyledons
- **Subclass:** Asteridae
- **Order:** Scrophulariales
- **Family:** Bignoniaceae - Trumpet-creeper family
- **Genus:** Kigelia DC. - Sausage tree
- **Species:** *Kigelia africana* (Lam.) Benth.- Sausage Tree

Synonyms

Some synonyms are still accepted by a few horticulturists as distinct species, but botanical studies agree that the genus contains only one

(a)



(b)

Figure 1(a) & (b). Shows leaf and flower in Kolkata, West Bengal, India.

Figure 2.Bark in Kolkata, West Bengal, India.

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species[23].

- *Bignonia* africana Lam. (basionym)
- *Tecoma africana* (Lam.) G.Don
- *Crescentia pinnata* Jacq.
- *Kigelia pinnata* (Jacq.) DC.
- *Kigelia abyssinica* A.Rich.
- *Kigelia aethiopica* Decne.

Kigelia Africana
(Lam.) Benth.
Name derivation
Kigelia is based on an African name and africana means from Africa. The genus Kigelia has one species and occurs only in Africa[12]. The genus name comes from the Mozambican Bantu name, kigeli-keia, while the common names sausage tree and cucumber tree refer to the long, sausage-like fruit[18]. Its name in Afrikaans Worsboom also means Sausage Tree, and its Arabic name means "the father of kit bags"[29].

Other vernacular names
AFRIKAANS: Worsboom.
ARABIC: Abu shutor, Abu sidra, Um mashatur, Um shutur.
FRENCH: Saucissonnier.
GERMAN: Leberwurstbaum.
SPANISH: Arbol De Las Salchichas.
IGBO: Ogilisa ofia.

Constituents

- The bark contains only a bitter principle and tannic acid.
- Studies have yielded naphthaquinones, iridoids, fatty acids, norviburtinal, sterols, lignans, terpenoid, flavonoids, and volatile constituents.
- Study isolated pinnatal in a root bark extract.
- Fruit extract yielded alkaloids, glycosides, terpenoids, flavonoids, tannins, saponins, and reducing sugars.
- A flavanol glycoside has been isolated from the fruits.
- Study isolated kigelin as a major constituent of the plant from the root heartwood.
- Stigmasterol and lapachol have been isolated from the roots.
- Kigelin, ß-sitosterol, 3-dimethyl kigelin and ferulic acid have been isolated from the bark[2].

Uses
The genus Kigali has one species and only occurs in Africa along river banks. The plant is used as an abortifacient. It is used for the treatment of the side effects of parturition (childbirth), relieves an inflamed spleen (splenitis) and contains anti-inflammatory and antimicrobial properties[11]. Traditionally made beer (muratina) from its fruit extract is bathed in a bath by children with measles for treatment. Either its crushed dried fruits or its fresh fruits are used in ethnomedicine to treat ulcers, sores and syphillis. Its fruit contains antibacterial properties[32]. A decoction made from its roots is drunk to treat gastrointestinal problems. Its leaf extract is applied onto wounds to treat them. A decoction made from its bark is drunk for relief from headaches, rheumatism, treat epilepsy and venereal diseases. A decoction made from its leaf is drunk to treat malaria. A juice extract from its fruit is used for treating wounds. A mixture made from mixing ash harnessed from roasting its leaf together with honey is used in treating high blood pressure. In the cosmetic industry, its fruit extracts are used in making beauty, anti skin aging and skin ointment products that are used against eczema and psoriasis due to its antimicrobial properties[1]. Its roots are used in the dye or colourant industry to make related products due to its bright colour extracts. Its fruit is traditionally used to make traditional beer commonly known as "muratina" by the Kikuyu people of Kenya. Its fresh fruit is poisonous and cannot be directly eaten. The mode of propagation is by seed.

Growth
It is a tree growing up to 20 m tall. The bark is grey and smooth at first, peeling on older trees. It can be as thick as 6mm on a 15cm branch[8]. The wood is pale brown or yellowish, undifferentiated and not prone to cracking[6]. The tree is easily propagated from the fresh seed sown in river sand in September, or from truncheons. Protect young plants from frost. Plant in full sun, add lots of compost and mulch well. Water moderately. It is relatively pest-free[24].
Fast-growing and frost-tender, this tree has a rather invasive root system, so keep it clear of buildings, paving, pools, etc. Position it with care - a falling fruit can severely damage a parked vehicle! Despite this, it is said to be a popular shade and street tree in tropical Africa and Australia. Plant alongside rivers and dams on farms and game farms. It is also suitable for large estates and municipal parks. It tolerates temperatures ranging from about 4°C to 40°C.
The tree is evergreen where rainfall occurs throughout the year, but deciduous where there is a long dry season. The leaves are opposite or in

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whorls of three, 30–50 cm long, pinnate, with six to ten oval leaflets up to 20 cm long and 6 cm broad; the terminal leaflet can be either present or absent. The flowers (and later the fruit) hang down from branches on long, flexible stems (2-6 meters long). Flowers are produced in panicles; they are bell-shaped (similar to those of the African Tulip Tree but darker and more waxy), orange to reddish or purplish green, and about 10 cm wide. Individual flowers do not hang down, but are oriented horizontally. Some birds are attracted to these flowers and the strong stems of each flower make ideal footholds. Their scent is most notable at night indicating that they are adapted to pollination by bats, which visit them for pollen and nectar. They also remain open by day, however, and are freely visited by many insect pollinators, particularly large species such as carpenter.The fruit is a woody berry from 30–100 cm long and up to 18 cm broad; typically it weighs between 5 and 10 kg, and hangs down on long, rope-like peduncles[10]. The fruit pulp is fibrous and pulpy, and contains numerous seeds. It is eaten by several species of mammals, including Baboons, Bushpigs, Savannah Elephants, Giraffes, Hippopotamuses, monkeys, and porcupines. The seeds are dispersed in their dung. The seeds are also eaten by Brown Parrots and Brown-headed Parrots, and the foliage by elephants and Greater Kudu[23,37]. Introduced specimens in Australian parks are very popular with cockatoos.The trees are also found in large numbers in Ingraham Institute NH-24 campus, Ghaziabad Uttar Predesh in India. Whether it is the same species has not yet been verified. N. 1972. Trees of southern Africa, Balkema, Cape Town.

BOTANY

African sausage tree is a wide spreading, deciduous, about 10 meters in height. Leaves are alternate and odd pinnate. Leaflets are opposite, ovate to elliptic-ovate, 8 to 16 cm long and pointed or blunt at the tip. Flowers are red, nocturnal, and borne in panicles on very long, pendulous pedicels. Calyx is 2.5 to 3 cm long, unequally 5-toothed, or lobed. Corolla is 10 to 12 cm long, the tube is rather slender and the limb, broadly bells-shaped, somewhat curved, and 5-lobed. Fruit is hard, greyish-brown, scurfy, large, sausage-shaped, oblong or oblong-cylindric, 20 to 30 centimeters in length, indehiscent, and hanging on very long and fibrous peduncles[26].

Ecology: It is one of the first trees to flower in the Kruger Park in early spring (August to October) - on a recent visit, after a prolonged, dry winter, the tree was 'alive' with insects and birds. We saw, in a short space of time, Black, Scarletchested and Whitebellied Sunbirds, Blackheaded Oriole, Sombre and Blackeyed Bulbuls, Masked Weaver, Brownheaded Parrot and Grey Lourie (which eats flowerbuds)[3]. Young monkeys sank their small faces deep into the flowers to get at the nectar, and duiker, kudu and impala were eating the fallen flowers. Elephant and kudu occasionally browse the leaves, and baboons, monkeys, bushpigs and porcupines eat the fruit. Epauletted fruit bats are thought to pollinate the flowers and Charaxes butterflies also visit the tree[27].

AIMS AND OBJECTIVES

i. To investigate the anti-pyretic, activities of Ethanolic extract of *Kigelia Africana* leaves.
ii. To verify the local use of the plant leaves.
iii. To identify the phytochemical constituents responsible for this claim.

MATERIAL AND METHODS

DRUG, CHEMICALS AND SOLVENTS

Paracetamol (Emzor Nigeria), Aspirin tablets (Emzor Nigeria),Tween-80, Potassium bismuth iodide solution (Dragendorff's reagent), Solution of iodine in potassium iodide (Wagner's reagent), potassium mercuric iodide solution (Mayer's reagent), Millon's reagent, α -Napthol in ethanol (Molisch's reagent), Sulphuric acid (H_2SO_4), Ammonium hydroxide (NH_4OH), Sodium hydroxide (NaOH), Ferric chloride, Olive oil, Fehling's solution I, Fehling's solution II, Potassium hydroxide (KOH), Picric acid (Hager's reagent), lead acetate, Aluminium chloride, Nitric acid, Distilled water, Analytical grade N-Hexane (BDH), Analytical grade ethanol (Sigma).

MATERIALS

Test tubes, test tube rack, syringe and needle (1ml, 2ml, 3ml),Electronic weighing balance(Gulfes Mediqaland scientific, England), measuring cylinder, conical flask, beakers(10ml, 25ml, 50ml, 500ml capacities) Milling machine (Thomas Laboratory Mill, UK) glass rod, hand gloves, hot plate , Rotary Evaporator, Water bath, Muslim cloth, cornical flasks, oven, Whatman (No 1) filter paper, glass funnels

METHODS

COLLECTION AND IDENTIFICATION

The fresh leaves of *Kigelia Africana* were obtained from Ogidi, Idemili North Local Government Area

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of Anambra State in December 2013, during the dry season and were identified by Mr Ozioko, a Taxonomist with the Bio source Development and conservation program (BDCP), Nsukka, Enugu State, Nigeria. The plant leaves were air-dried for 2 weeks in the Pharmacognosy laboratory. They were milled and 200g of the powdered plant material was obtained.

PREPARATION OF ETHANOL EXTRACT

200g of the powered plant sample was macerated in 400mls of ethanol (analytical grade) for 48 hours, after which it was filtered with muslin cloth and further filtered using Whatman (No 1) filter paper. The procedure was repeated with the marc and filtered to obtain more phyto-constituents. The filtrates were combined and concentrated with the rotary evaporator at 45°C.

PHYTOCHEMICAL ANALYSIS

The test carried out was based on procedures outlined by Harbourne (1973)[19] and Evans (1996)[13].

TEST FOR CARBOHYDRATES

Molisch's test

To 0.5g of the plant extract 2ml of distilled water was added, boiled and filtered. To the filtrate few drops of α- naphthol solution in ethanol (Molisch reagent) was added and concentrated sulphuric acid was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrates.

TEST FOR SAPONINS

To 20ml of distilled, 0.5g of the extract was added and boiled on a hot water bath for 2 minutes and filtered. The filtrate was allowed to cool and was used for the following tests.

a. **Frothing test**
5ml of the filtrate was diluted with 15ml of distilled water and shaken vigorously. Formation of stable froth indicates the presence of saponins
b. **Emulsion test**
c. To the frothing solution was added 2 drops of olive oil and the contents shaken vigorously. The formation of emulsion indicates the presence of saponins

TEST FOR TANNINS

To 0.5g of the extract, 20ml of water was added, boiled and, filtered and used for the following tests.

a. **Ferric chloride test**
To 3ml of the filtrate, few drops of ferric chloride were added. Formation of a greenish black precipitate indicated the presence of tannins.
b. **Lead acetate test**
To 3ml of the filtrate was added lead acetate solution. Formation of precipitate indicated the presence of tannins.

TEST FOR FLAVONOIDS

10ml of ethyl acetate was added to 0.2g of the extract and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate was used for the following tests.

a. **Ammonium test**
4ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layers were allowed to separate. A yellow colour in the ammoniacal layer indicated the presence of flavoniods.
b. **1% Aluminium chloride solution test**
4ml of the filtrate was shaken with 1ml of 1% aluminium chloride solution and the layers were allowed to separate. The formation of yellow colour in the aluminium chloride layer indicates the presence of flavonoids.

TEST FOR REDUCING SUGAR

5ml of a mixture of equal volume of Fehling's solution I and II was added to 5ml of the extract and then heated on a water bath for 5 minutes. A brick red precipitate shows the presence of reducing sugar.

TEST FOR PROTEIN

0.5g of the extract was extracted with 20ml of distilled water, filtered and the filtrate was used for the following tests.

a. **Millon's test**
To a little portion of the filtrate in a test tube, two drops of millon's reagent was added. A white precipitate indicated the presence of proteins.
b. **Xanthoproteic test**
5ml of the filtrate was heated with few drops of concentrated nitric acid. A yellow colour which changed to orange on addition of an alkali (dilute sodium hydroxide) indicated the presence of protein.

TEST FOR STERIODS AND TERPENOIDS

9ml of ethanol was added to 1g of the extract and refluxed for a few minutes and filtered. The filtrate

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was concentrated to 2.5ml on a boiling water bath. 5ml of hot water was added to the concentrated solution, the mixture was allowed to stand for 1 hour and the waxy matter was filtered off. The filtrate was extracted with 2.5ml of chloroform using separating funnel.

To 0.5ml of the chloroform extract in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface showed the presence of steroids.

Another 0.5ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

TEST FOR ALKALOIDS

20ml of 3% sulphuric acid in 50% ethanol was added to 2g of the extract and heated on a boiling water bath for 10 minutes, cooled and filtered. 2ml of the filtrate was tested with a few drops of Mayer's reagent (Potassium mercuric iodide solution), Dragendorff's reagent (Bismuth potassium iodide solution), Wagner's regent (iodo-potassium iodide solution) and Picric acid solution (1%). Alkaloids give a milky precipitate with mayer's reagent; reddish brown precipitate with wagner's reagent; yellowish precipitate with picric acid and brick red precipitate with dragendorff's reagent.

ANIMALS

White albino rats (150-250kg) of either sexes obtained from the animal house of the Department of Pharmacology and Toxicology of Madonna university Elele Campus, Rivers State were used for this study. All the animals were housed under standard environmental conditions with free access to food and water.

ACUTE TOXICITY TEST

The LD_{50} was carried out using the method employed by Lorke (1983).

It involves a total of thirteen (13) rats. This test was carried out in two phases. Phase one employed a total of nine(9) rats, they were grouped into three(3) ,i.e. Three(3) rats per group, Group one received 10mg/kg of the extract, Group two received 100mg/kg, while Group three received 1000mg/kg. All the administration was by intra-peritoneal route. The animals were constantly monitored for the next four hours, then intermittently for the next 6hr, and then over a period of 24hr, the numbers of dead animals were noted. From the result of the first phase, the second phase was carried out. In this phase a total of four (4) rats were used they were grouped into four (4) groups of one rat per group. Group 1 received 1600mg/kg; Group 2 received 2900mg/kg, Group 3 received 5000mg/kg, Group 4(Control) received 1ml of Tween 80. The animals were monitored for another 24hr for any death.

ANALGESIC ACTIVITY

The analgesic activity was carried out using hot plate method of test. A total of 15 albino rats were employed. They were grouped into five(5) with three(3) rats per group. Each rat was placed individually on a hot plate at a temperature of 40±1°C. The time at which animal started licking its paw or showing signs of discomfort was noted and was taken as a normal endurance duration, after that the animals were treated as follows: Group 1(Control) received 0.5ml of Tween 80, Group 2 received 150mg/kg of paracetamol, Group 3 received 400mg/kg of the extract, Group 4 received 200mg/kg of the extract, group 5 received 100mg/kg of the extract. Readings were taken at 30, 60, 90, 120min, after oral administration of drugs, the duration of time each animal in each group can stay comfortably on the hot plate taken, then the negative control Group(1) was compared with other groups for significance in analgesic activity using analysis of variance(ANOVA).

STATISTICAL ANALYSIS

All procedures were carried out in triplicates and the results expressed as ±Standard error of mean (SEM). Differences in observation were determined by analysis of variance (ANOVA) using Dunnette analysis method

RESULTS

Tannins	(+ve)
Flavanoids	(++ve)
Carbohydrates	(++ve)
Saponins	(+ve)
Protein	(+ve)
Alkaloids	(++ve)
Steroids	(++ve)

Table 1. Phytochemical analysis of ethanolic extract of *Kigelia Africana*.

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Table 2. Acute toxicity test for ethanolic extract of *Kigelia Africana*.

Phase	Doses	Number of death.
1	10mg/kg	0/3
	100mg/kg	0/3
	1000mg/kg	0/3
2	1600mg/kg	1/1
	2900mg/kg	1/1
	5000mg/kg	1/1
Control	1ml of tween 80	0/1

The LD_{50} values were calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of consecutive doses for which 0% and 100% survival rates were recorded.

LD_{50}=√ (1000×1600) =1264.9

Table 3. Analgesic study of Ethanol extract of *Kigelia Africana.*

Group	Agent & dose	Initial time(**sec**)	**30min**	**60min**	**90min**	**120min**
Group 1 Control	0.5ml /kg of Tween 80	3.0±0.07	3.4±0.02	3.4±0.02	2.5±0.12	2.4±0.12
Group 2 Standard	Paracetamol (150mg/kg)	3.0±0.02	3.5±0.00	7.0±0.00	6.5±0.02	5.0±0.02
Group 3	Ethanolic extract (400mg/kg)	3.0±0.00	3.3±0.02	4.8±0.00	5.6±0.02	6.6±0.10
Group 4	Ethanolic extract (200mg/kg)	3.1±0.02	3.4±0.05	3.8±0.02	4.0±0.40	4.6±0.00
Group 5	Ethanolic extract (100mg/kg)	3.0±0.00	3.1±0.02	3.5±0.02	3.6±0.00	3.6±0.00

DISCUSSION

Many reviews and articles reporting the biological activities of flavonoids[30,20], anthraquinones, polyphenols and phenols, and tannins[33], have been published in recent years. Several phenol compounds have been identified and isolated from plants and they have shown promising bacterial inhibiting properties against specific and broad spectrum of cultured as well as clinical bacterial strains, including Methicillin-Resistant Staphylococcus aureus (MRSA), and multi-drug resistant bacteria. The presence of alkaloids has been shown to demonstrate biological activity[15].

Alkaloids, phenols, flavonoids and glycosides have a number of biological activities and strong antibacterial potentials[38]. Alkaloids have exhibited promising activity against *H. pylori*[16] and a number of other bacterial strains[31,21,22]. The Result of phytochemical screening showed abundance of flavanoids, steriods, alkaloids, carbohydrate and moderate availability of protein, saponins. tannins in the ethanol extract of *Kigelia Africana* and some of this secondary metabolites such as flavonoids have been reported to be responsible for analgesic and anti-inflammatory properties[35]. The extract also showed a significant elongation in hot plate reaction time ($p<0.0001$) when compared with the standard drug paracetamol (150mg/kg) giving a greater effect than standard paracetamol. The dose of 400mg/kg is far more effective than the standard drug – paracetamol.

CONCLUSION

The ethanol leaves extract exhibited analgesic activity, hence its use by the local community in Ogidi of Anambra State, Nigeria as analgesic drug.

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