PRELIMINARY STUDIES ON THE PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF THE METHANOL EXTRACT OF *Kigelia africana* LEAVES AND STEMS AGAINST *Candida albicans* AND *Salmonella typhi*

Y. M. MUSA

Pre- ND Department, Federal Polytechnic, P. M. B. 0231, Bauchi.

ABSTRACT
*Kigelia africana* leaf and stem samples (150g) were extracted with Methanol solvent (250cm$^3$) at 60$^\circ$C for 8 hours. The extracts were phytochemically screened for the secondary metabolites present and also subjected to antimicrobial test against *Salmonella typhi* and *Candida albicans*. The results showed that the extract of *Kigelia africana* leaves contains tannins, flavonoid, saponin and glycoside. Also, the extract was able to inhibit the growth of the two microorganisms with a minimum inhibitory concentration of 10mg/ml for both *Candida albicans* and *Salmonella typhi*.

INTRODUCTION
All parts of plants have been found to have chemical compositions that vary from the type of the plant and from location to location since from time immemorial (1). *Kigelia africana* (or K. africana) belongs to the family Bignoniaceous. Its common names include sausage tree in English, Pandoro (Yoruba) and Maman giwa (Hausa). It is a tree growing up to 20m tall or more. The fruit is eaten by several species of mammals, including baboons, bush pigs, savannah elephants, giraffes, hippopotami, monkeys and porcupine. *Kigelia africana* plant is widely distributed in South, central and West Africa (2). *K. africa* grows along water courses, in riverside fringes, alluvial and open wood land, high rainfall, savannah, and in rain forest. It occurs on loamy or clay soils and sometimes on rocky soil (3). *Kigelia africana* like other plants is traditionally used in the treatment of some ailments. *Kigelia africana* plant has been reported for its activity against salmonella typhi and proteus vulgaris. The wood extracts posses’s antimalaria activity against drug- resistant strains of plasmodium falciparium superior to chloroquine and quinine (4). The ethanolic extract of the stem bark was examined to show strong analgesic and anti-inflammatory mediators which probably accounted for the analgesic and anti-inflammatory properties (5). The dried fruit and bark extract has been established to be a strong pain reliever when administered on painful joints, back and rheumatism (6). The anti-inflammatory activity of verminoside, from *K. africana* was also carried out. It shows significant anti-inflammatory effects (7). *K. Africana* is widely used to treat gynecological disorders. Aqueous preparation of the roots, fruits and flowers are administered orally or virginally pastry while the breast fruit and the bark are used to promote breast development in young women or in contrast to reduce swelling and mastitis of breast.

NATURAL PRODUCTS AND PHYTOCHEMISTRY
Natural products are organic compounds that are formed by living systems. Naturally occurring compounds may be divided into three broad categories. This includes those compounds which occur in all cells and play a central role in the metabolism and reproduction of those cells. These compounds include the nucleic acids and the common amino acids and sugars. They are known as primary metabolites (8). Secondly, there are the high-molecular-weight polymeric materials such as cellulose, the lignins and the proteins which form the cellular structures. Finally, there are those compounds that are characteristic of a limited range of species. These are the secondary metabolites. Most primary metabolites exert their biological effect within the cell or organism that is responsible for their production. Secondary
metabolites, on the other hand, have often attracted interest because of their biological effect on other organisms. The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites. It has been estimated that over 40% of medicines have their origins in these natural products.

Phytochemical surveys can reveal natural products that are “markers” for botanical and evolutionary relationships. Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases (9). It is estimated that 5000 individual phytochemicals have been identified in fruits, vegetables, and grains. They are otherwise called the secondary metabolites. The phytochemicals vary in distribution within the plant parts, as well as in their occurrence within plant species (10). Free radicals are associated with various physiological and pathological events such as inflammation, aging, mutagenicity and carcinogenicity. Antioxidants are free-radical scavengers which provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and subsequent lipid peroxidation, protein damage and DNA strand breaking. Therefore, there is a need for isolation and characterization of natural antioxidants having less or no side effects, for use in foods or medicines to replace synthetic antioxidant (11).

Various chemical investigations have been carried out on K. Africana and it has been found to contain so many classes of compounds like the polyphenols. Sherad et al (2012) reported that the bark of Kigelia africana contains some chemical substances like carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides and the fruit contains vermonosides (12).

Test for antimicrobial activity
Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. For example, the use of bearberry (Arctostaphylos uva-ursi) and cranberry juice (Vaccinium macrocarpon) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (Melissa officinalis), garlic (Allium sativum) and tee tree (Melaleuca alternifo-lia) are described as broad-spectrum antimicrobial agents (13). In agar dilution methods, solutions containing a specific number of microbes are placed on a nutrient-rich agar plate and inoculated with varied concentrations of an antimicrobial agent. The plate is incubated for around 24 hours and the turbidity, growth sediment or amount of microbial colonies present is assessed. Microbes are classified as susceptible, intermediate or resistant to antimicrobial activity. The aim of agar dilution is to determine the smallest concentration of antimicrobial agents, such as antibiotics, disinfectants or preservatives, required to destroy or prevent microbe growth, which is called the minimum inhibitory concentration (MIC). MIC is measured in milligrams per liter, and values obtained are used to measure the effectiveness of antimicrobial agents.

This work is a preliminary study on the chemical composition of the leaves and stem of Kigelia africana for the possible understanding of the substances present and their effect on two microorganisms: Candida albicans and Salmonella typhi.

MATERIALS AND METHODS
Preparation of the Sample
Kigelia africana leaves and stems were collected from Tafawa Balewa Local Government Area of Bauchi state in Nigeria and were dried at room temperature for Seven days and ground into powder with the used of ceramic mortar and pestle and sieved using laboratory sieve of 212µm aperture.

EXTRACTION
The sample prepared (30g) was poured into a porous thimble and placed in the soxhlet extractor chamber. It was refluxed for Eight hours with Methanol (300cm³) until the extraction was achieved. The extract was then evaporated at 60°C on a water bath to remove traces of the solvent. A yield of 12% was obtained from the sample.

PHYTOCHEMICAL SCREENING
The crude extract obtained was partitioned between n- hexane/Chloroform/Methanol in the ratio 1:1:2 to separate the various secondary metabolites present. The solvents from the fractions were evaporated and
the Methanol fraction was screened for the secondary Metabolites present using methods described by Sofowora (1984); and Trease and Evans (1978).

**Test for Alkaloid**

A 1% HCl was added to the *Kigelia africana* (3g) extract in a test tube. Brown precipitate indicated the presence of alkaloids.

**Test for Tannins**

The extract of *Kigelia Africana* (6.5g) was mixed with distilled water (20cm³) and was boiled on a water bath and filtered in a test tube. Few drops of Iron (III) Chloride (1%) were added. A brownish green or blue-black colouration observed indicated the presence of tannin.

**Test for Flavonoids**

Dilute Ammonia (5cm³) followed by few drops of concentrated TetraoxoSulphate (VI) acid was added to the aqueous extract in a test tube. The appearance of yellow colour which disappeared upon further standing indicated the presence of flavonoid.

**Test for Cardiac Glycoside**

The extract of *Kigelia Africana* (6.5g) was dissolved in Chloroform (2cm³) and few drops of concentrated TetraoxoSulphate (VI) acid were added. Reddish brown steroidal ring indicated the presence of cardiac glycoside.

**Test for Saponin**

The extract of *Kigelia Africana* (0.5g) was shaken with water (2cm³?) in a test tube. The Frothing which persisted on warming indicated the presence of saponin.

**ANTIMICROBIAL ACTIVITY TEST**

The antimicrobial activity was carried out using standard agar dilution method as described by Bauer et al (1966).

**PREPARATIONS OF MEDIA**

Nutrient agar (168g) was weighed and dissolved in distilled water (60cm³) in a 250ml conical flask. The solution mixture was warmed on a water bath for 15 minute to ensure that undissolved crystals of the nutrient agar were properly dissolved. After dissolving the excess crystal, the flask was plugged with cotton wool and autoclaved at 121ºC for 15 min.

**PREPARATION OF CULTURE PLATES.**

Two plastic disposable Petri dishes were used. They were washed and oven dried at 50ºC for ten minutes for sterility. The plates were then removed and arranged on a bench that has been disinfected. On the bench pouring was made near a Bunsen flame to avoid contamination by some unwanted microorganisms. About 20ml of the sterilized media was poured into each Petri dish and kept to gel and solidify. They were transferred into an oven and left overnight to ensure that there were no contaminants before inoculation.

**ANTIMICROBIAL ACTIVITY TEST**

The antimicrobial activity was carried out using standard agar dilution adopted by porcs plate techniques as described by.

**PREPARATIONS OF MEDIA**

Nutrient agar (168g) was dissolved in distilled water (60cm³) in a 250cm³. The solution was at 60ºC for 15 minutes after which it was autoclaved at 121ºC for 15 minutes.

**PREPARATION OF CULTURED PLATES.**

Two Petri dishes were oven dried at 50ºC for 10mins. They were arranged on a disinfected bench. The prepared media (20cm³) was poured into the Petri dishes and kept to solidify. The plates were then left to stand overnight.

**PROCEDURE**

The two Petri dishes containing the nutrient agar were seeded with the test organism *Candida albicans* and *Salmonella typhi* by the spread techniques and left for 30 minute to dry. Then the sterilized hole borer of 0.6mm was used to bore eight hole on the plate as well as the control. Solutions of the *Kigelia Africana*
extract were prepared in distil water. The concentration of the solutions poured into the hole was 10%w/v, 20%w/v and 50%w/v and the control contains only Chloramphenicol solution (1mg/ml). The plates were examined for zones of inhibition of the microorganisms measured and expressed in millimeters.

RESULTS AND DISCUSSIONS
The result for the extraction gave a pale green extract when the *Kigelia Africana* was extracted with Methanol as solvent. The extract showed the presence of tannin, saponin, flavonoid and cardiac glycoside as shown in the table below.

**Table 1: Preliminary phytochemical screening of dried leaves and stem of *K. africana***

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycoside</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = present, – = not present

**Antimicrobial Activity**
The methanol extracts of *Kigelia africana* were found to be active against the test organisms (*Candida albicans* and *Salmonella typhi*) with varying mean zones of inhibition.

**MINIMUM INHIBITORY CONCENTRATION**

The results for the antimicrobial activity of the Methanol extract against *Candida albicans* and *Salmonella typhi* are as shown in table 2.

**Table 2: The Mean zones of growth inhibition for the Antimicrobial activity of methanol leaf and stem extract of *K. africana* by agar diffusion method against *Candida albicans* and *Salmonella typhi***

<table>
<thead>
<tr>
<th>EXTRACT</th>
<th>Mean zones of inhibition of test organisms (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Mg/mL)</td>
<td><em>Candida albicans</em></td>
</tr>
<tr>
<td><strong>K. a Leaf</strong></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>00.00</td>
</tr>
<tr>
<td>10</td>
<td>15.00</td>
</tr>
<tr>
<td>20</td>
<td>18.00</td>
</tr>
<tr>
<td>50</td>
<td>22.00</td>
</tr>
</tbody>
</table>

**K. a Stem**
The organic extracts showed greater activity than aqueous extract because most of the antibacterial principles were either polar or non-polar and were extracted only through the organic solvent medium (John Britto, 2001). It was reported that methanol was a better solvent for the consistent extraction of antimicrobial substances from medicinal plants when compared to other solvents such as aqueous, ethanol, chloroform and hexane (Lin et al., 1999. Present observation suggested that the organic solvent extraction method was suitable to verify antibacterial activity. This agrees with the studies of Krishna et al. (1997).

The antibacterial activity of plant extracts can be attributed to the combined bioactivity of so many metabolites. A number of phytochemicals have been studied for their antibacterial activity which are potentially useful against infectious diseases. The chemical structure of the antimicrobial agents found in higher plants belong to the most common classes of higher plant secondary metabolites such as flavonoids (Watchter et al., 1999).

From the results of this study, it can be observed that Kigelia africana showed maximum antibacterial activity against Candida albicans and Salmonella typhi with a minimum concentration of 10mg/ml and hence this plant can be used to discover bioactive natural products that may be used for the development of new pharmaceuticals.

REFERENCES


