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# Detection and Identification of Metronidazole in Kigelia Africana Fruit Growing in Sudan

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## Abstract

Kigelia Africana is a locally growing tree used in alternative medicine. Studies have shown that the tree is used in blood and circulatory system disorders, and has a wide activity ranging from anticancer, antimicrobial, anti-malarial, anti-inflammatory and antidiarrheal. The present study was carried out to detect the presence of some phytochemical groups and metronidazole in the fruit. The Phytochemical screening of the fruit revealed the presence of tannins, steroids, terpenoids and alkaloids. The HPLC analysis of the alkaloid extract of the fruit showed the presence of metronidazole by comparison with a standard reference. The antidiarrheal property associated with the plant could be due to the presence of metronidazole. The presence of metronidazole in the fruit of Kigelia Africana has not been reported before in literature and /or iridoids which have been reported to be present in the fruit. This plant has great potential to be developed as drug by pharmaceutical industries but before recommending its use in modern system of medicine, clinical trials are to be done.

**Keywords:** Kigelia africana; Antidiarrheal; Metronidazole; Anticancer; Antimicrobial; Phytochemical screening

## Introduction

The term medicinal plants include various types of plants used in herbalism (herbology or herbal medicine).The word herb has been derived from the Latin word “herba” and old French word “herbe”. Nowadays, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or root, as well as non-woody plant. Earlier, the term “herb” was only applied to non-woody plant, including those that come from trees and shrubs. Medicinal plants are used as medicine, food, flavour, or perfume and also in certain spiritual activities. Treatment with medicinal plants is considered very safe as there is no or minimal side effects [1]. The golden fact is that, use of herbal treatment is independent of any age groups and the sexes. In Sudan, we have broad selection of medicinal plants with great potential of medicinal substances to the good of mankind [2]. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterial [3]. Natural products, either as pure compounds or as standardized plant extract, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants. Kigelia Africana is an important medicinal plant [4]. The many uses of K. Africana in therapy of physical and magi co-religious or spiritual complaints indicate that this tree is a valuable and popular source of traditional medicine. K. Africana is used in treatment of primary and secondary infections, and as a disinfectant the unripe fruit is used as vermifuge and as treatment for hemorrhoids and rheumatism. It

can be used orally as enema in treating children stomach ailments usually worms and for dressing wounds. The present work is aimed at studying the Phytochemical and antimicrobial properties of Kigelia Africana.

## Materials and Methods

### Materials

### Plant material

The plant material (fresh K. Africana fruits) was brought from Medani (Jazera state).

### Chemicals and solvents

Ethanol, Methanol, Acetone, Glacial acetic acid, Potassium iodide, Bismuths nitrate, Acetic acid, Toluene, Distilled water, Chloroform, Ethyl acetate, Petroleum ether and Ammonia.

### Reagents

Dragendoroff`s reagent, Mayer`s reagent, Fehling`s reagent, Wagner`s reagent and Ferric chloride.

### Equipment's

Glassware, Capillary tubes, Conical flask, Funnels, Graduated measuring cylinder, Separator funnel, Beakers, Mortar and pestle, Rack and holder, Peppite and Petri dish.

### Apparatus and instruments

Oven –Baird and Tat lock, Water bath, Melting point apparatus, HPLC instrument, IR –Shimadzu.

## Methods

### Preparation of plant material

The plant material of Kigelia Africana was cleaned, freed from dust and foreign material, and then dried under shade and finally powdered using an electrical house-hold spice grinder.

### Phytochemical screening of Kigelia Africana fruit

In order to study chemical composition of kigelia Africana fruit four extractions were prepared namely, chloroform, ethanol, methanol, and acetone [5-17].

## Test for Alkaloids

The powdered plant material (30g) was weighed into a beaker. It was moistened with concentrated ammonia solution and allowed to stand for 20 minutes. 150 ml of chloroform were added; the mixture was stirred for 10 minutes and filtered through a plug of cotton wool. The meal was washed twice with 15 ml chloroform and the washings were added to the filtrate. The combined filtrate was evaporated to dryness on a water bath. The residue was cooled and dissolved in 20 ml chloroform. The chloroform extract was transferred to a separating funnel and shaken with 75 ml of 5% sulphuric acid. The two layers were allowed to separate. The chloroform (lower) layer was drained off and discarded. 10 ml of chloroform was further added, shaken, drained off and discarded until the acid layer was colourless the acid layer was made alkaline with concentrated ammonia solution and extracted with 20 ml of chloroform. The extract was retained and evaporated to dryness. The residue was dissolved in 10 ml of ethanol and the following tests were carried out (Evans, 1989). To 1 ml of the methanol extract in separate test tubes, 2-3 drops of the following reagents were added

- **Wagner's reagent:** light brown to brownish precipitate indicates the presence of alkaloids.
- **Mayer's reagent:** white or creamy white precipitate indicate the presence of alkaloids
- **Dragendoroff's reagent:** orange or orange-yellow precipitate indicate the presence of alkaloids

### Test for anthraquinone and their derivatives

The plant material (2.0 g) was extracted with 10 ml of benzene and filtrated. 5 ml of 10% ammonia solution was added to the filtrate and shaken. The presence of a pink, red or violet colour in the ammoiniacal (lower layer) phase indicates the presence of free hydroxyanthraquinone. For the anthraquinoneglycosides.2.0 g of powered material was boiled with 10 ml of dilute sulphuric acid and filtered while hot. The filter was shaken with 5ml of benzene. The benzene layer was separated and to it, 3 ml of 10% ammonia solution were added and mixture shaken. A pink, red or violet colouration in the lower layer shows the presence of anthraquinone glycosides (Sofowora, 1993).

## Test of Saponin Glycoside

### Frothing test

The powdered material (0.5 g) was shaken with 5 ml of water in the test tube for 30 seconds. A persisted froth suggests the presence of the saponin (Brain and turner, 1975).

## Test of the Steroids and Terpenoids

### Salkowski` test

The powdered drug (0.5g) was extracted with 5 ml of chloroform and filtered into a clean and dry test tube. 2ml of concentrated sulphuric acid were carefully added down the side of test tube to form a lower layer .a reddish brown colour at the interface indicates the presence of steroidal ring (Sofowora, 1993).

## Test for the Tannins

### Ferric chloride test

The powdered fruit (2.0 g) was extracted with 10 ml of water and filtered. To 2 ml of the filtrate in a test tube two drops of ferric chloride solution were added. A green or greenish black precipitate indicates the precipitate shows the presence of hydrolysable tannins (Evans, 1989).

### Extraction of metronidazole

20 tablets of Metronidazole of 500mg were grounded by mortar and pestle and added acetone and set in water bath. Then filtered and left to evaporate, pale yellow crystals were formed.

### Metronidazole identification from extraction

Melting point test was done on a melting point apparatus and infra-red spectroscopy was done on a Shimadzu IR instrument.

### Strengths and limitations

The limited references, and few local studies that have been done on the Kigelia Africana, the present study will provide significant information on the strains of this plant growing in Sudan.

### Dissemination of results

This study results will be disseminated through presentation to Nile College staff and students and publication via biomedical journals. Results and discussion.

## Results

The Phytochemical screening of the fruits gave the results shown in below (Table 1).

***Table 1:*** *Phytochemical screening of Kigelia africana fruit.*

| Test | Result | color |
|---|---|---|
| **Alkaloids(Wagner`s reagent )** | +ve | Brown |
| **Alkaloids** (Mayer reagent) | +ve | Yellow |
| **Alkaloids (Dragendoroff's reagent)** | +ve | Orange |
| **Anthraquinone** | -ve | - |
| **Saponin** | -ve | - |
| **Tannins** | +ve | Greenish black |
| Key: +ve- present, -ve-not present | | |

## Characterization of Metronidazole

The identity of Metronidazole extracted from tablets (Figure 2) was confirmed by the melting point (159-163o C) and its infra-red spectrum as shown in Figures 3 and 4 and retention time of the sample and standard on HPLC chromatograms (Figures 1-4).

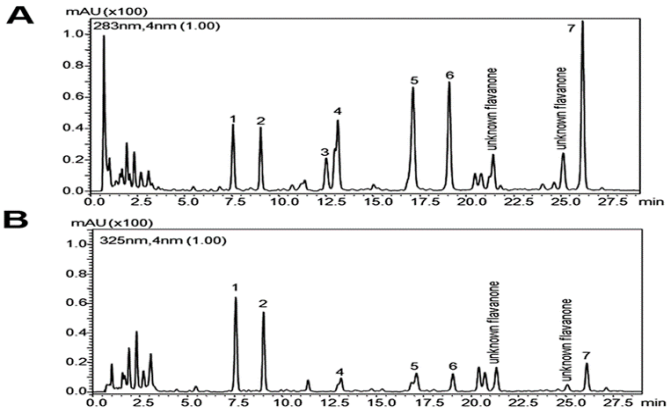


***Figure 1:*** *HLPC Chromatogram of Metronidazole sample A and standard B.*

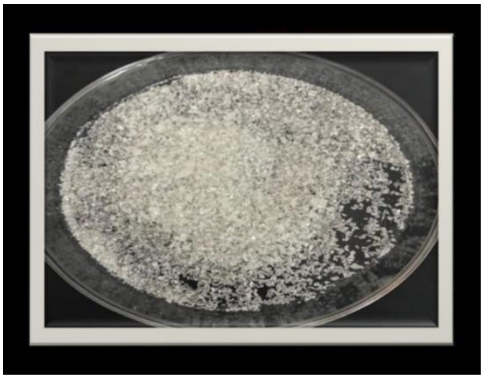

***Figure 2:*** *Metronidazole extracted from Nilozol tablets.*

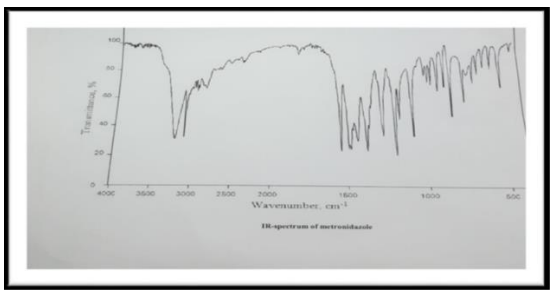

***Figure 3:*** *IR-spectrum of metronidazole STD.*

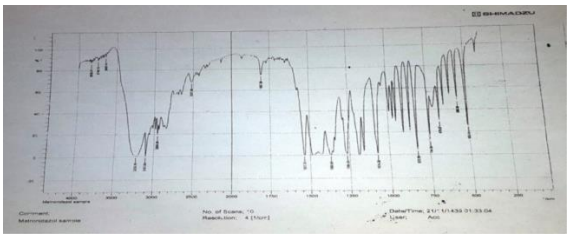

***Figure 4:*** *IR-spectrum of metronidazole STD.*
*HPLC of metronidazole compared to sample of kigelia africana: Metro std 400ppm.*

## Discussion

This study revealed the presence of steroids, anthraquinone, terpenoids and alkaloids. These results are in agreement with those reported. HPLC of the alkaloid extract of the plant showed the presence of Metronidazole which is significant in the treatment of diarrhea. The presence of Metronidazole in Kigelia pinnata (synonym of K. Africana) has been reported in literature. The antidiarrheal property associated with Kigelia Africana could be due to presence of Metronidazole.

## Conclusion

The Kigelia Africana fruits were found to contain alkaloids, steroids, tannins, terpenoids and anthraquinone. The alkaloid extract was found to contain Metronidazole. The presence of Metronidazole in Kigelia Africana fruit has not been reported before in literature.

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