Potential antibacterial and antifungal activity of *Kigelia africana* fruit

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**Abstract**
This study aimed to evaluate the potential of *Kigelia africana* (Bignoniaceae) extracts as antimicrobial under laboratories conditions. The tested bacteria include *Staphylococcus aureus*, *ß- haemolytic streptococcus*, *Escherichia coli* and *Salmonella enteridis* were investigated by disc diffusion method. The fungi were *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium* spp., *Trichophyton mentagrophytes* (Human strain), *Trichophyton mentagrophytes* (Animal strain), *Trichophyton schoenledini*, *Trichophyton verrucosum* and *Scopulariopsis brevicaulis*. These fungi were tested by agar incorporation technique. The aqueous extract was prepared by freeze - dried method while the organic solvent extracts were prepared by Soxhlet apparatus. The results revealed that the aqueous and methanol extracts were effective against *Candida albicans* and *Trichophyton mentagrophytes*, respectively. The petroleum ether extract at highest concentration exhibited activity against *ß- haemolytic streptococcus*. In addition, Petroleum ether extract inhibited the growth of *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* spp. Moreover, petroleum ether extract reduced diameter of growth of *Trichophyton schoenledini*, and exhibited fungicidal effect against *Trichophyton mentagrophytes*. However, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteridis*, *Aspergillus fumigatus*, *Trichophyton verrucosum* and *Scopulariopsis brevicaulis* were not susceptible to any extract. The minimum inhibitory concentrations of petroleum ether extract against *A. flavus*, *A. niger*, *Pen. spp.*, *T. mentagrophytes* (Animal strain) were 125, 150, 150 and 3.13 mg/ ml, respectively. According to these results, the petroleum ether extract of *K. africana* extracts could be considered as a potential source of natural product with bactericstatic, fungistatic and fungicidal properties.


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1. Introduction

1.1 Antimicrobial Drug resistance
Pathogenic bacteria and fungi are the major cause of infectious diseases. The success of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of the microorganisms (Jeyachandran and Mahesh, 2007). Emergence of microbial resistance is one of the major problems nowadays; thus there have been tremendous efforts towards finding new chemicals, especially herbs, for the development of new antimicrobial drugs. However some medicinal plants play an important role as antibacterial and antifungal.

1.2 Search for discovery of a drug from medicinal plants
On the other hand, nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been prepared from natural sources. Many of these preparations are based on the uses of these agents in traditional medicine (Saini et al., 2009). Therefore, it is necessary to search for an alternative medicine with potential antibacterial and antifungal activities, cheap and affordable. *Kigelia africana* is one of the highly valuable ethno medicinal plants belonging to the family Bignoniaceae and its vernacular name is Um Shutoor (Sudan); Cucumber tree, Sausage tree (English). The plant fruit showed some antibacterial activity (Grace et al., 2002;
Jeyachandran and Mahesh, 2007) plus antifungal properties (Saeed, 2009). *K. africana* was selected on the basis of its traditional uses and biological activities to evaluate the possibility for novel pharmaceuticals having antibacterial and antifungal properties. *K. africana* has been used by traditional healers to treat a wide range of skin ailments like, fungal infections, psoriasis, eczema. In the folk medicine, the fruits are used as dressing for ulcers, purgative and increase the flow of milk in lactating women. In West and Central Africa unripe fruit is used as a dressing for wounds, and treatment of hemorrhoids and rheumatism (Saini et al., 2009). Additionally, the pharmacological activities of the plant include antibacterial, antifungal, antineoplastic, analgesic, and anti-inflammatory, anti-malarial, central nervous system stimulant, antiprotozoal, anti-diarrheal and cosmetic.

2. Objective of Research

This study is justified to search for discovery of a drug from natural products to solve the problem of antimicrobial drug resistance. Treatment with herbal medicine might provide medical and economic benefit.

3. Materials and Methods

3.1 Plant collection

Fruits of *K. africana* (Lam.) Benth in Hook., Fl. Nigrit.: 463 (1849) were collected from the banks of the Blue Nile River in Khartoum State. The plant part was identified and authenticated at the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan. The voucher specimen has been deposited in the herbarium museum of the Institute. The fruit was air dried in the shade, coarsely powdered and kept in polythene bags.

3.2 The Microorganisms

Clinical isolates of *Staphylococcus aureus* (Staph. Aureus), *β*-haemolytic *Streptococcus*, *Escherichia coli* (E.coli) and *Salmonella enteridis* (*Salmonella* enteritis) were isolated and identified at Bacteriology Department. Reference strains of *Candida albicans* (C. albicans) (ATCC 7596) and *Aspergillus niger* (A.niger) (ATCC 9763) were obtained from Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI). Clinical isolates of *Aspergillus flavus* (A.flavus), *Aspergillus fumigatus* (A.fumigatus), *Penicillium* spp.(Pen.spp.), *Trichophyton metagrophytes* (T.metagrophytes), Human strain, *Trichophyton metagrophytes* (T.metagrophytes, Animal strain), *Trichophyton schoenleinii* (T. schoenleinii), *Trichophyton verrucosum* (T.verrucosum) and *Scopulariopsis brevicaulis* (S.brevicaulis) were isolated and identified at Mycology Department, Veterinary Research Institute, Khartoum, Sudan.

3.4 Extraction Procedure

Extraction of the plant was carried out according to the method described by Harbone (1984).

3.4.1 Preparation of aqueous extract

The coarsely powdered fruit (100 g) was soaked in 500 ml of hot distilled water, and left to cool down with continuous stirring at room temperature. The extract was then filtered through Whatman No. 1 filter paper and frozen at (-10°C). Frozen extract was dried using freeze-drier (Trivac, USA) till powdered extract obtained. Yield percentage was calculated by the formula below:

\[
\text{Yield percentage} = \frac{\text{Weight of extract obtained}}{\text{Weight of plant sample}} \times 100
\]

3.4.2 Preparation of organic solvents extracts

The coarsely powdered fruit (100 g) was extracted successively with 500 ml each of petroleum ether and methanol (w/v) using Soxhlet apparatus. Extraction was carried out for about 4 hours for petroleum ether and 8 hours for methanol. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally, the extracts were poured into Petri dishes and left at room temperature till complete dryness, and the yield percentage was calculated.

3.5 Antimicrobial bioassay

The antibacterial, and antifungal activities were examined by the disc diffusion, agar diffusion and incorporation method, respectively.

3.5.1 Testing for antibacterial activity

The bacterial strains preserved in nutrient agar at 4°C were revived in nutrient broth and incubated at 37°C overnight to contain 10^6 C.F.U. / ml. Nutrient agar and blood agar plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into three segments. A loop full of diluted culture was spotted with a standard loop that delivers 10 µl on the surface of segment. Sterile filter paper discs (Whatman No.1, 6 mm diameter) were impregnated with approximately 10 µl of each plant extract concentration. The impregnated discs (5.00, 4.00, 3.00, 2.50, 2.00, 1.50, 1.25 mg/ disc) were air-dried at room temperature, and thereafter placed on the surface of inoculated agar plates. Simultaneously, Ciprofloxacin at concentration of 5 µg/ disc was used as positive control. Water, methanol and petroleum ether were used as negative control plus infected untreated control. The plates were incubated in the upright position at 37°C for 18 hours. Three replicates were carried out for each extract against each of the test
organisms. The diameters of the resultant growth inhibition zones were measured in mm, and the mean values were tabulated.

**Table 1:** Yield Percentage of aqueous, methanol and petroleum ether extracts of *Kigelia africana* fruit

<table>
<thead>
<tr>
<th>Extract</th>
<th>Weight of dry fruit (g)</th>
<th>Weight of extract (g)</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100</td>
<td>17.015</td>
<td>17.015</td>
</tr>
<tr>
<td>Methanol</td>
<td>100</td>
<td>25.264</td>
<td>25.264</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>100</td>
<td>2.146</td>
<td>2.146</td>
</tr>
</tbody>
</table>

**Table 2:** Diameter of inhibition zone of *K.africana* petroleum ether extract and ciprofloxacin against some pathogenic bacteria

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P.E. extract (5.0 mg/disc)</td>
</tr>
<tr>
<td><em>S.aureus</em></td>
<td>0.0</td>
</tr>
<tr>
<td><em>B-haemolytic streptococcus</em></td>
<td>15.0</td>
</tr>
<tr>
<td><em>S.enteridis</em></td>
<td>0.0</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>0.0</td>
</tr>
</tbody>
</table>

P.E. = Petroleum ether

**Table 3:** Diameter of growth inhibition zone of water and petroleum ether extracts of *K.africana* fruit against some pathogenic fungi

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Extract</th>
<th>Extract concentration (mg/ml)</th>
<th>Growth inhibition zone (mm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C.albicans</em></td>
<td>Water</td>
<td>500</td>
<td>15.33±4.16</td>
<td>85.17</td>
</tr>
<tr>
<td></td>
<td>Nystatin</td>
<td>0.21</td>
<td>18.00±1.73</td>
<td></td>
</tr>
<tr>
<td><em>A.flavus</em></td>
<td>Petroleum ether</td>
<td>500.0</td>
<td>20.00±0.00</td>
<td>73.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400.0</td>
<td>12.67±0.58</td>
<td>46.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300.0</td>
<td>11.67±0.58</td>
<td>42.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250.0</td>
<td>11.67±1.53</td>
<td>42.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200.0</td>
<td>11.00±0.00</td>
<td>40.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150.0</td>
<td>9.67±0.58</td>
<td>35.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125.0</td>
<td>9.33±0.58</td>
<td>34.14</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>1.28</td>
<td>27.33±2.50</td>
<td></td>
</tr>
<tr>
<td><em>A.niger</em></td>
<td>Petroleum ether</td>
<td>500.0</td>
<td>12.00±0.00</td>
<td>83.74</td>
</tr>
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<td></td>
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<td>400.0</td>
<td>11.33±0.58</td>
<td>79.06</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>10.33±0.58</td>
<td>72.09</td>
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<tr>
<td></td>
<td></td>
<td>250.0</td>
<td>10.00±0.00</td>
<td>69.78</td>
</tr>
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<td></td>
<td></td>
<td>200.0</td>
<td>8.67±0.58</td>
<td>60.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150.0</td>
<td>7.00±0.00</td>
<td>48.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125.0</td>
<td>0.00±0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>1.28</td>
<td>14.33±0.78</td>
<td></td>
</tr>
<tr>
<td><em>Pen.spp.</em></td>
<td>Petroleum ether</td>
<td>500.0</td>
<td>24.00±1.00</td>
<td>88.89</td>
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<td></td>
<td></td>
<td>400.0</td>
<td>20.67±0.58</td>
<td>76.56</td>
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<tr>
<td></td>
<td></td>
<td>300.0</td>
<td>17.33±0.58</td>
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</tr>
<tr>
<td></td>
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<td>250.0</td>
<td>16.00±0.00</td>
<td>59.26</td>
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<tr>
<td></td>
<td></td>
<td>200.0</td>
<td>14.67±0.58</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>150.0</td>
<td>12.33±0.58</td>
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<tr>
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<td></td>
<td>125.0</td>
<td>0.00±0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>1.28</td>
<td>27.00±0.00</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD of zone of inhibition (mm); inhibition zones are the mean of three replicates.
Figure 1: Sporulation of A. favus (Untreated control), and sterile hyphae (white arrow) after 72 h exposure to methanol extract of K. africana at concentration of 500 mg/ml (50 mg/well), Lactophenol cotton blue’s stain (x400)

Figure 2: Growth inhibition zone of petroleum ether extract of K. africana fruit against Penicillium spp. at concentration of 250 mg/ml (25 mg/well), K (Ketoconazole).

Figure 3: Fungicidal effect of petroleum ether extract of K. africana fruit at concentration of 3.13 mg/ml against T. men (Animal strain).

Figure 4: Growth inhibition of petroleum ether extract of K. africana fruit against T. men (Human strain) at concentration of 12.5 mg/ml.
3.5.2 Testing for antifungal activity

3.5.2.1 The cup-plate agar diffusion method

The cup-plate agar diffusion method as described by Perez et al. (1990) was adopted with some minor modifications to assess the antifungal activity of the plant extracts. Overnight broth cultures were adjusted to yield approximately 1.0x10^7 C.F.U./ml for yeast. For molds, suspension of mature spores were obtained by gentle washing of the surface of solid media with 0.05% (v/v) solution of Tween 80® (Hi Media Laboratories limited, Munbi-400 086, India), and the resulting suspension was adjusted to 1.0x10^6 C.F.U./ml. Molten sterile dextrose agar (20 ml) was poured into each Petri dish. The agar plates were left to set at room temperature. A loop full of diluted culture (10 µl) was delivered on the surface of the agar. The fungal suspension was spread on the surface of the agar. In each plate, 4 cups (6 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Cups were filled separately with 100 µl of each extract concentration of 500, 400, 300, 250, 200, 150 and 125 mg/ml; Nystatin and Ketoconazole as positive controls of *C.albicans* and molds, respectively. The final concentrations of each extract per well were 50, 40, 30, 25, 20, 15 and 12.5 mg/well. The plates were left at room temperature for two hours, and then incubated in the upright position at 37°C for 2-3 days. The experiment was replicated three times. The diameters of inhibition zones were measured in millimeter using a transparent well calibrated ruler, averaged and the mean values were tabulated. The percentage of inhibition was calculated as follows:

*Percentage of inhibition= (Diameter of inhibition zone of extract)/ (Diameter of inhibition zone of antibiotic) x100.*

The antifungal susceptibility of the extracts and the standard drug Ketoconazole was determined by using agar incorporation technique in Sabouraud’s Agar Medium supplemented with Chloramphenicol (50 µg/ml) and Cycloheximide (500 µg/ml). A volume of 0.5 ml from each extract concentration of 500, 400, 300, 250, 200, 150 and 125 mg/ml was added separately to test plates to give final concentrations of 12.5, 10.0, 7.5, 6.25, 5.0, 3.75 and 3.13 mg/ml, respectively. The test and the control plates were inoculated with the organism. All plates were incubated at 25°C for 3-7 days except for Dermatophytes which were incubated for up to 2 weeks at the same temperature (Warnock, 1989). The negative controls (water, methanol and petroleum ether) were run simultaneously.

**Statistical analysis**

Calculations were carried out in triplicate with their mean values and standard deviations by using SPSS version 16.
4. Results

4.1 Yield Percentage of the extracts
The yield percentages (w/w) of aqueous, methanol and petroleum ether extracts of K. africana fruits were presented (Table 1).

4.2 Effect of the extracts on bacteria
In the current study, petroleum ether extract was the only extract which had antibacterial activity. It was potent against β-haemolytic Streptococcus at concentration of 5.00 mg/ disc with 15.0 mm diameter of growth inhibition zone. The diameter of growth inhibition zone of Ciproloxac (5 µg/disc) against Staph. aureus, β-haemolytic Streptococcus, Salm. enteridis and E. coli (Table 2).

4.3 Effect of extracts on fungi
The aqueous extract of K. africana fruit exhibited activity only against C. albicans at the highest concentration with growth inhibition zone of 15.33 ± 4.16 mm using Nystatin as a reference drug (Table 3). On the other hand, the methanol extract reduced the diameter of growth of T. mentagrophytes at concentration of 12.50 mg/ml. Moreover, treatment of A. flavus with methanol extract at concentration of 500 mg/ml (50.0 mg/ well) resulted in sterile hyphae (Fig. 1) compared with negative control where heavy sporulation was observed (Fig. 1). Additionally, Petroleum ether extract exhibited activity against A. flavus, A. niger, and Pen. spp. (Fig. 2).

The diameters of growth inhibition zones and inhibition percentage were illustrated (Table 3). Moreover, the petroleum ether extract of the fruit reduced the diameter of growth (one colony) of both strains of T. mentagrophytes at all concentrations compared with negative control (multiple colonies) (Fig. 3, 4). The petroleum ether extract also reduced the diameter of growth of T. schoenleinii at all concentrations resulting in cotton colony at concentrations (6.25- 3.13 mg/ml) and moist colony at concentrations (7.5-12.5 mg/ml). The moist colony was illustrated (Fig. 5).

4.5 Minimum inhibitory concentrations
Minimum inhibitory concentration (MIC) of petroleum ether extract against A. flavus, A. niger, pen. spp. and T. mentagrophytes (Animal strain) was 125, 150,150 and 3.13 mg/ml, respectively.

This study confirmed that K. africana fruit extracts should be useful as a potential source of natural antimicrobial agent. Furthermore, phytochemical analysis could be performed to identify novel compounds that are responsible for these biological properties.

5. Discussion
The current study indicated that both tested G-positive and G-negative bacteria were not susceptible to any extract of K. africana fruit except β-haemolytic Streptococcus which responded only to petroleum ether extract at the highest concentration. The pattern of inhibition varied with the plant parts, the solvents used for the extraction and the tested organism (Saini, 2009). Susceptibility differences between bacteria may be due to the structural differences in the cell wall outer membrane of the Gram-negative bacteria. The Gram-negative cell wall outer membrane appears to act as a barrier to many substances including antibiotics (Tortora et al., 2001). The outer lipopolysaccharide membrane serves as impermeable barrier against most drugs (Ghosh et al., 2016). The findings of this study were similar to the results noted by Grace et al., (2002) who found that aqueous extract of the fruit had no antibacterial activity while ethyl acetate extract of K. africana fruit and stem bark showed similar antibacterial activity. The aqueous and methanolic extracts of the fruit were effective against bacteria and fungi (saeed, 2009) confirmed the present results. However, the current results are in contrast to Arkhinov et al. (2014) who found that Gram-negative and Gram-positive bacteria were approximately equally susceptible to K. africana fruit extracts. This could be due to the habitat and the time of collection of the plant, solvents used for extraction, and / or the type of the tested microorganisms. Additionally, Saini et al. (2013) found that aqueous and ethanolic extracts had significant activity against S. aureus and E. coli.

The antimicrobial activity of K. africana may be due to the presence of numerous secondary metabolites. Previous reports have attributed the antimicrobial activity of the fruit extract of the plant to caffeic acid and kigelinone (Binutu et al., 1996). Moreover, flavonoids and iridoides have been isolated from the fruits and leaves of the plant (Gouda et al., 2003, 2006). Furthermore, a mixture of three fatty acids exhibiting antibacterial effects was isolated from the acetyl acetate extract of the fruits. Palmitic acid was the major compound in this mixture which possessed antibacterial activity (Saini, 2009). The methanol and/or petroleum extracts inhibit the growth of some molds which was observed by clear inhibition zone or reduced growth diameter. The extent of the inhibition of the fungi was related to the concentration of the plant extract, and reduction in growth could be characteristic of fungistatic action. These findings support out the findings of a previous study which has reported that K. africana solvent extracts had broad spectrum antifungal activity (Arkhipov et al., 2014).
Conclusion

The results of this study partially validate the traditional usage of *K. africana* fruit extracts to treat microbial diseases. The plant exhibited wide antifungal spectrum compared to antibacterial activity. Thus, it could be a promising natural antifungal agent. Further study is required to characterize the antimicrobial extracts.

Research Highlights

- Screening of aqueous, methanolic and petroleum ether extract has been done against some pathogenic bacteria and fungi.
- Aqueous and organic solvent extracts were prepared using freeze-drier and Soxhlet apparatus, respectively.
- MIC of petroleum ether extract against *A. flavus, A.niger, Pen.* spp. and *T. mentagrophytes* (animal strain) was 125, 150, 150 and 3.13 mg/ml, respectively.
- The finding may help to use the plant as antifungal agent.

Limitations

*In vivo* study to confirm these results is needed. Phytochemical analysis of the most active extract and its toxicity is necessary.

Recommendations

It was suggested that the future work is, necessary to elucidate the whole picture of the studied extracts including: Since the petroleum ether extract is the most potent antimicrobial in this study, biological directed fractionation, is recommended followed by isolation, structure determination and identification of active compounds.

*In vivo* study to confirm these results is important.

Toxicity of effective extract is needed.

Funding and Policy aspects this type of research could lead to industrial development; financial funding is needed for a national project to go further.

Authors’ Contribution

This work was carried out in collaboration between all authors. The first author, Dr. Hayat Mahgoub planned and conducted the study under the guidance of Professor Hassan Elsubki (fourth author). Dr. Elham Abdelbasit (second author) and Dr. Yahia Ali (third author) managed the mycology and bacteriology work, respectively.

Competing of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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