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# Phytochemical screening and comparative antibacterial activity of *Kigelia africana* L. and *Tamarindus indica* L leaf and stem extracts on *E. coli*, *S. aureus* and *P. mirabilis.*

M.I Babangida, D.N, Iortsuun and S.P. Bako
*Department of Biology, Faculty of Life Sciences,*
*Ahmadu Bello University, Zaria-Nigeria*

***Abstract***
*This study was carried out to screen for phytochemical composition and compare the antibacterial activity of Kigelia africana and Tamarindus indica on E. coli, S. aureus and P. mirabilis. The plant materials of Kigelia africana stem bark and leaves were obtained from yankari Game reserve, Bauchi state, Nigeria. The plant materials of Tamarindus indica stem bark and leaves were obtained from Ahmadu Bello University, Zaria. Kaduna State. One hundred (100g) of the plant powder each, was extracted with methanol and chloroform by Soxhlet extraction method. All the extracts were subjected to standard phytochemical screening for the presence or absence of various secondary metabolites. The susceptibility test of the plant extracts on E. coli,S. aureus and P. mirabilis were done using agar well diffusion method. Ciprofloxacin (10µg) was used as control. The phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycoside, steroids and carbohydrates. The antibacterial activity showed that, there was no significant difference between the activity of Kigelia africana and Tamarindus indica on gram positive bacteria (S. aureus), The antibacterial activity differed significantly on gram negative bacteria (E. coli and P. mirabilis) Tamarindus indica stem bark methanol extract has higher activity than Kigelia africana stem bark methanol extract on E. coli. The result also showed that, Kigelia africana stem bark chloroform extract has higher activity than Tamarindus indica chloroform extract.*

***Keywords:*** *Phytochemicals, Antibacterial, Kigelia africana, Tamarindus indica*

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## I. Introduction

Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries (Renisheya *et al.*, 2011). In Pakistan 80% of the population belonging to the rural areas depends on the traditional medicines (Munir *et al*., 2013). Indigenous knowledge of herbal medicine is a big source of the modern knowledge (Kakar *et al*., 2012).Throughout the world plants are used to treat various infectious diseases. They provide natural products that are used against infectious diseases (Walter *et al*., 2011). The concern towards the use of traditional medicine and medicinal plants now-a-days is given much importance in developing countries for the maintenance of good health. The traditionally used rural herbal remedies have been found to be effective against microorganisms (Kone *et al*., 2007). Plant antimicrobials attract more attention since they are generally recognized as safe, and may benefit to human health (Seow *et al*., 2014). Moreover, plant antimicrobials may also add flavor to foods. There have been some extensive searches for potential natural antimicrobials with a broad spectrum of antimicrobial activities that can be used to extend the shelf life of perishable foods (Calo *et al*., 2015).

*Kigelia africana* is a member of the family, Bignoniaceae popularly known as the cucumber or sausage tree because of the huge fruits (average 0.6m in length and 4 kg in weight) ,which hangs from long fibrous stalks (Cragg & Newman, 2001). The leaves are opposite or in whorls of three, 30-50 cm long, and flowers are bell shaped, orange to reddish and 10 cm wide. Individual flowers do not hang down but oriented horizontally (Joffe, 2003).Flowers are bisexual, very large, 2-4.5 cm long, widening and incurving up wards (Grace *et al*., 2002).

The sausage tree is fast growing and can mature in 4 to 5 years. It begins to flower from the age of 6 years. Mature fruits can be found on trees throughout the year (Jackson & Beckette, 2012).Tamarind mostly used as two different varieties, they are sweet and sour. Sweet tamarind is harvested ripe and directly consumed

other side sour tamarind is processed into a range of value-added products (Joshua, 2006). India is world largest producer of tamarind, it is estimated that 300,000 tons are produced annually (El-Siddig *et al*., 2006).

## II. Materials And Methods

**Study area.**

*Kigelia africana* stem bark and leaves were collected from Yankari Game Reserve, Bauchi State, and the GPS coordinate for the collected sample area is 9.7529345N, 10.5114725E.*Tamarindus indica* stem bark and leaves were collected from Ahmadu Bello University, Zaria Kaduna State. The GPS coordinate for the collected sample area is 11.1512N, 7.6546E. The plants were identified at herbarium unit Department of Botany, Ahmadu Bello University,Zaria.

**Authentication of plant materials**

The stem bark and leaves of *Kigelia africana* and *Tamarindus indica* were carefully washed with tap water and rinsed with distilled water. All the plants material were spread in a clean stainless-steel and air dried under shade at room temperature. Dried leaves and bark were crush and ground into coarseand powder using mortar and pestle. The powdered materials were kept in a nylon bag.

**3.2.1 Preparation of the extracts**

This was carried out according to the procedure describe by Sigaroodi *et al*., (2008). One hundred grams (100g) each of dried powdered plant materials were soaked in chloroform and methanol at room temperature for 36hours. The extraction was carried out with solvent under shaking conditions. The respective extracts were then filtered through Whatmann filter paper and aqueous extract was obtained and solvent was removed completely under reduced pressure. Filtered extract was collected in conical flask. The chloroform and methanol extracts were evaporated by rotary evaporator at 45$^{o}$C and the crude extracts were obtained for the determination of antibacterialactivity.

**Phytochemical screening of *Kigelia africana* and *Tamarindus indica***

All the extracts were subjected to standard phytochemical qualitative screening for secondary metabolites as described by Sofowora (2006).

**Preparation of different concentrations of the extract**

Ten 10mg each of the plants (stem bark and leaf) extracts were dissolved in 10ml of distilled water to form two stock solutions of each extract. From the first stock solutions of each extracts, serial dilutions was carried out to prepare 4 different concentrations as,100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively.

**Standardization of inoculum**

The bacterial isolates of *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis* were collected from microbiology laboratory, Department of microbiology, Ahmadu Bello University, Zaria. An over night culture of the bacterial isolates were prepared in nutrient broth. A volume of 0.1 ml of the nutrient broth was emulsified into 20ml of physiological saline, until the turbidity of the suspension of test organism matches with 0.5 Mcfarland turbidity standards $1.5\times10^{8}$cfu/ml (Deeni and Hussain, 1994)

**Susceptibility testing of the extract using agar well diffusion**:

The standardized inucula of the bacterial isolate were streaked on sterilized Mueller hinton agar plates respectively with the aid of sterile wire loop. Four wells were bored on each inoculated agar plate with a sterile cork borer. The well was properly labeled according to different concentrations of the extract prepared which were 100, 50, 25 and 12.5mg/ml respectively. Each well was filled up with approximately 0.2ml of the extract. The inoculated plates with the extract were allowed to stay on the bench for about one hour; this is to enable the extract to diffuse on the agar. The plates were then incubated at 37$^{o}$c for 24 hours. At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of zones was measured using transparent ruler calibrater in millimeter and the result was recorded.

**STATISTICAL ANALYSIS**

Data obtained were subjected T-test to compare the antibacterial activity of *Kgelia africana* and *Tamarindus indica*.

## III. Results and Discusion

**Phytochemical screening of *Kigelia africana* stem bark and leaves, using methanol and chloroform extracts.**

The result of *K. africana* stem bark and leaf methanol revealed the presence of alkaloids, flavonoid, tannins, saponins, cardiac glycoside, steroids and carbohydrate, while anthraquinone was absent. Alkaloids and carbohydrates were present in both *K. africana* stem bark and leaf chloroform extracts, but steroids was present in stem bark chloroform extract, while the other phytochemicals were not present.(Table 4.1).

**Table 4.1: Phytochemical screening of *Kigelia africana* stem bark and leaves extracts.**

| S/N | Phytochemicals | SME | SCE | LME | LCE |
|---|---|---|---|---|---|
| 1 | Alkaloids | + | + | + | + |
| 2 | Flavonoids | + | - | + | - |
| 3 | Tannins | + | - | + | - |
| 4 | Saponins | + | - | + | - |
| 5 | Cardiac glycoside | + | - | + | - |
| 6 | Streriods | + | + | + | - |
| 7 | Anthraquinon | - | - | - | - |
| 8 | Carbohydrates | + | + | + | + |

Key: SME=Stem bark methanol extract, SCE=Stem bark chloroform extract, LME=Leaf methanol extract, LCE=Leaf chloroform extract, +=Present, - =Not present.

The results of phytochemical screening of *Tamarindus indica* stem bark methanol extracts showed the presence of Alkaloids, Cardiac glycoside, Steroids and Carbohydrates, while flavonoids, tannins saponin and anthraquinone were not present. Flavonoids, tannins, saponins, cardiac glycoside, steroid and carbohydrates were present in leaves methanol extract while alkaloids and anthraquinone were not present. Alkaloids, flavonoids, tannins, saponins, cardiac glycoside, steroid and carbohydrate were present in both *T. indica* stem bark and leaves chloroform extracts, while anthraquinone was not present in both. (4.2).

**Table 4.2: Phytochemical screening of *Tamarindus indica* stem bark and leaves extracts.**

| S/N | Phytochemicals | SME | SCE | LME | LCE |
|---|---|---|---|---|---|
| 1 | Alkaloids | + | + | _ | + |
| 2 | Flavonoids | - | + | + | + |
| 3 | Tannins | - | + | + | + |
| 4 | Saponins | - | + | + | + |
| 5 | Cardiac glycoside | + | + | + | + |
| 6 | Streriods | + | + | + | + |
| 7 | Anthraquinon | - | - | - | - |
| 8 | Carbohydrates | + | + | + | + |

Key: SME=Stem bark methanol extract, SCE=Stem bark chloroform extract, LME=Leaf methanol extract, LCE=Leaf chloroform extract, +=Present, - =Not present.

**Comparison between the antibacterial activity of *Kigelia africana* and *Tamarindusindica* on *E. coli, S. aureus* and *P. mirabilis***

This result showed that there was no significant difference P>0.05 between the antibacterial activity of *K. africana* and *T. indica* on *S. aureus*. The antibacterial activity differed significantly P≤0.05 on *E. coli* and *P. mirabilis. Tamarindus indica* SME has higher activity P≤0.05 than *K. africana* SME on *E. coli*, and *T. indica* LME also has higher activity than LME of *K. africana*. The result showed that, the activity of *K. africana* SCE was higher P≤0.05 than *T. indica* SCE on *P. mirabilis*, while the activity of *K. africana* SME, LME and LCE did not differ significantly P>0.05 with that of *T. indica* on *P. mirabilis*. (Table 4.3) **Table 4.3: Comparison between the antibacterial activity of *Kigelia africana* and *Tamarindus indica* on *E. coli, S. aureus* and *P. mirabilis***

| | | Plant species | | |
|---|---|---|---|---|
| Bacteria | Extract | *Kigelia africana* | *Tamarindus indica* | P-value |
| *S. aureus* | SME | 9.00±2.48 | 8.75±1.65 | 0.836 |
| | SCE | 6.50±1.71 | 4.50±1.71 | 0.925 |
| | LME | 3.00±1.92 | 1.75±1.18 | 0.194 |
| | LCE | 4.50±1.71 | 4.50±1.71 | 0.875 |
| *E. coli* | | | | |
| | SME | **7.00±2.08** | **9.25±1.49** | **0.037*** |

| | | | | |
|---|---|---|---|---|
| | SCE | 5.00±2.35 | 3.00±1.29 | 0.161 |
| | LME | **5.00±2.08** | **9.75±2.66** | **0.008*** |
| | LCE | **3.00±1.78** | **5.75±2.17** | **0.076*** |
| *P. mirabilis* | | | | |
| | SME | 6.75±3.40 | 8.00±1.68 | 0.537 |
| | SCE | **11.25±2.39** | **4.00±1.47** | **0.007*** |
| | LME | 6.50±3.28 | 8.75±2.29 | 0.135 |
| | LCE | 4.75±1.70 | 4.25±1.89 | 0.495 |

P. value ≤0.05 is considered significantly different.
Key: SME=Stem bark methanol extract, SCE=Stem bark chloroform extract, LME=Leaf methanol extract, LCE=Leaf chloroform extract.

Plate I. phytochemical screening of *T. indicam.*

plate II. Phytochemical screening of *K. africana*

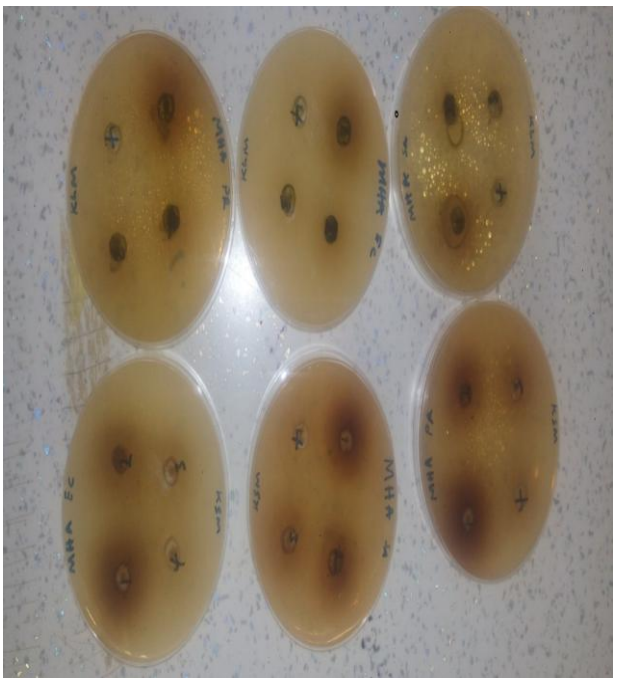

Plate.III Antibacterial activity of *K. africana.*

Plate. IV Antibacterial of *T. indica*

## IV. Conclusion

*Kigelia africana* and *Tamarindus indica* contain significant phytochemicals (secondary metabolites). The Result of this study also showed that, there was no significant difference between the antibacterial activity of *Kigelia africana* and *Tamarindus indica* on *S. aureus* but the antibacterial activity differed significantly on *E. coli* and *P. mirabilis*.

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