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# PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF ETHANOL EXTRACT OF KIGELIA AFRICANA (LAM) BENTH

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## ABSTRACT

The aim of our investigation was to evaluate the phytochemical and antibacterial activities of Kigelia africana. Phytochemical screening was carried from dried fruit powder followed by extraction with ethanol. Antibacterial activity were tested against the three bacterial strains namely Bacillus subtilis, Staphylococcus aureus, Salmonella typhi and fungal strain were tested against two fungal strains namely Epidermophyton floccusum and Candida albicans using disc diffusion method. The maximum zone of inhibition was seen against S. aureus with 21.1 mm while only mild zone of inhibition was observed in B. subtilis with 18.8 mm and least zone of inhibition was seen in S. aureus with 5.5 mm. In fungal activity the maximum zone of inhibition was seen against C. albicans with 21.1 mm while only mild zone of inhibition were examined in E. floccusum with 15.5 mm and least zone of inhibition was seen in C. albicans with 8.8mm at a concentration of 25µg/ mL respectively. The overall antimicrobial activity assessed from the above results indicates the presence of active constituents in the dried fruit extract and it can be used in pharmaceutical industry.

**KEYWORDS**: Antimicrobial activity, well diffusion method, Kigelia aficana.

## INTRODUCTION

Phytochemicals are non nutritive chemicals produced by plants for their own protection, but they have been found to protect human beings against diseases through recent research. Scientists have identified thousands of phytochemicals, although only small fractions have been studied closely and each one work differently (Yadav *et al.*, 2011). The exploration of

the chemical constituent of the plants and pharmaceutical screening may provide us the basis for developing the lead for development of novel agents.

K. africana is the plant taken up in for study has many medicinal properties due to the presence of numerous secondary metabolites. These compounds include quinines, flavonoids, and steroid constituents (Houghton, 2002).

Plant extracts have great potential as antimicrobial compounds against microorganisms. (Nascimiento *et al*., 2000). Different regions of the world are blessed with different plants and there are several reports of plant extracts on the antimicrobial activity of some of these plants extracts (Hammer *et al*., 1999). Due to the side effects, high cost, unavailability and the resistance developed by pathogenic microorganisms against conventional antibiotics, recently much attention has been paid to plants as well as their compounds that are bioactive against pathogenic organisms. (Gulluce *et al*., 2003). Therefore suitable antimicrobial agents can be used either topically or systematically to prevent infection of wounds and speed up wound healing process. The investigation of certain indigenous plants for their antimicrobial properties is very useful (Khan *et al*., 2003). There is increasing interest in plants as a source of agent to fight microbial diseases and treatment of several infections.

Despite that the medical practice introduces an average of 4-6 antibiotics each year, there is a constant need for new antibiotics because of the inappropriate use of antibiotics in human and veterinary medicine. Unfortunately, it makes certain strains of bacteria and fungi develop ability to produce substance which block the action of antibiotics or change their target to penetrate cells (Zakaria et al., 2010; Saadabi, 2007). These resistant strains of pathogenic microbes are growing and have caused critical problems all over the world. Therefore, the interest towards the development of a new drugs and antimicrobial agents for the treatment of infectious diseases is increasing especially from the plant source (Rajeh *et al*., 2010). Many antimicrobial agents exist, for use against a wide range of infectious diseases. Antibiotics are sometimes associated with adverse effects on hosts which include hyper sensitivity, depletion of beneficial gut, mucosal microorganisms, immuno suppression and allergic reactions. Bacteria have the genetic ability to transmit and acquire resistance to drugs (Soulsby *et al*., 2005). Essential oils and extracts of certain plants have been shown to have antimicrobial effects as well as imparting flavor to foods (Burt Set al., 2004).

## MATERIALS AND METHODS

### Collection of plant material

Healthy fruits of *K.* africana. were collected from Queen mary's college campus Chennai. The healthy plant material was selected. Fruits were washed well with tap water and dried under shade till they dry at room temperature. The dried fruit was made into fine powder. The fine powder was kept in a air tight container and stored.

### Preparation of plant extract

Ten grams of dried fruit powder of K. africana (LAM.) Benth. was extracted separately with 50 mL of ethanol and aqueous for 24h in 3 days. It was soaked overnight at room temperature. The sample was then filtered through whatmann filter paper in a Buchner funnel. The filtered solution was evaporated under vacumm in a rota - vator at $40^0$c to a constant weight and then dissolved in ethanol and aqueous. The solution was stored at $18^0$c until use.

### Phytochemical screening

Chemical tests were carried out on the ethanolic extract of the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

## QUALITATIVE PHYTOCHEMICAL TEST

### Terpenoids

To 2mL of extract 3mL of chloroform and 10% ammonia solution was added. Formation of pink colour indicates the presence of terpenoids.

### Tannins

To 1mL of extract (2mL of 5%) ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

### Saponins

To 2mL of extract 2mL of distilled water was added and shaken in graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponin.

### Quinones

To 1mL of extract 1mL of con $H_2SO_4$ was added. Formation of red colour indicates the presence of quinones.

**Alkaloids**

To 1mL of extract (1mL of 2N) NaOH was added. The yellow colour formation indicates the presence of alkaloids.

**Steroids**

To 1mL of extract equal volume of chloroform is added and subjected with few drops of con. $H_2SO_4$. Appearance of brown ring indicates the presence of steroids.

**Phenols**

To 1mL of extract 2mL of distilled water followed by few drops of 10% $Fecl_2$ was added. Formation of bluish colour indicates the presence of phenols.

**Glycosides**

To 2mL of extract 3mL of chloroform and 10% ammonia solution was added. The formation of pink colour indicates the presence of glycosides.

**Cardiac glycosides**

To 0.5mL of extracts 2mL of glacial acetic acid and few drops of 5% $Fecl_2$ were added. This was under layered with 1mL of con. $H_2SO_4$. The formation of brown ring at the interface indicates the presence of cardiac glycosides.

**Flavonoids**

To each 1mL of extracts (1mL of 2N) was added. The formation of yellow colour indicates the presence of flavonoids.

**ANTIBACTERIAL ACTIVITY**

**well diffusion method**

The dried fruit extract of *K.* africana was tested against bacteria by using *in vitro* agar well diffusion method. Nutrient agar was sterilized by using autoclave, then it is poured in hot petri plates and allowed to get solidify. The wells of desired diameter were made with the help of cork borer. Bacterial suspension of each strain is applied and grown overnight. The fruit extracts was poured in various concentrations with the help of sterile micropipettes by maintaining the aseptic environment. These petri plates were then kept for incubation at $37^0$c for 24hrs. After the completion of incubation period, the zones of inhibition were measured and recorded. The antibacterial activity of *K.* africana fruit extract was performed by against certain bacterial strains of Bacillus subtilis, Staphylococcus aureus *and* Salmonella typhi.

**Preparation of media**

**Nutrient agar**

Peptone - 5gms

Beef extract - 1.5 gms

Yeast extract - 1.5 gms

NacL - 5gms

Agar - 28 gms

Distilled water - 1000 mL

$p^H$ - 7.4 ± 0.2

**Antifungal activity**

**well diffusion method**

The fruit extract of K. africana was screened for antifungal activity using agar well diffusion method (Chung et al., and Perez et al., 1990) with sterile cork borer of size 10mm. The cultures of 24hrs grown inoculums on Potato Dextrose agar were used for inoculation of fungal strain on PDA plates. PDA medium was prepared and poured in petri dishes. The fungal inoculum was introduced to molten PDA by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. The fruit extracts was poured in various concentrations with the help of sterile micropipettes by maintaining the aseptic environment. These petri plates were then kept for incubation at $37^0$c for 72hrs.

**Preparation of media**

**Potato dextrose agar**

Potato - 200 gms

Dextrose - 20 gms

Agar - 20 gms

Distilled water - 1000mL

Final $p^H$ - (7.4± 0.2)

**RESULTS**

**Phytochemical analysis**

The preliminary screening of phytochemical analysis in ethanol extracts of Kigelia africana fruit shows the presence of quinone, alanine, steroids and flavonoids and absence of phenols and glycosides (table 1 and figure 1).

**Antimicrobial activity**

This antibacterial activity of K. africana fruit of ethanol extracts with four concentration of extracts were taken (25, 50, 75, 100 µg/ mL) and tested against three different bacteria (Staphylococcus aureus, Bacillus subtilis, Salmonella typhi). The zone of inhibition of the following was measured in mm. Significant increase in zone of inhibition was observed in increasing the concentration of extracts. The maximum zone of inhibition was seen against S. aureus with 21.1 mm while only mild zone of inhibition were examined in B. subtilis with 18.8 mm and least zone of inhibition was seen in S. aureus with 5.5 mm (Table 2, figure 2) respectively. From the well diffusion method it clearly showed that B. subtilis showed the greater zone of inhibition.

This antifungal activity of K. africana fruit of ethanol extracts with four concentration of extracts were taken (25, 50, 75, 100 µg/ mL) and tested against two different fungi (Epidermophyton floccusum, Candida albicans). The zone of inhibition of the following was measured in mm. Significant increase in zone of inhibition was observed in increasing the concentration of extracts. The maximum zone of inhibition was seen against C. albicans with 18.1 mm while only mild zone of inhibition were seen in C. albicans with 12.2 mm and least zone of inhibition was observed in E. flocusum with 5.5 mm (Table 3, figure 3) respectively. From the well diffusion method it was clear that C. albicans showed a greater zone of inhibition.

**Table 1. Qualitative analysis of phytochemical analysis of ethanol extracts of *K.* africana fruit**.

| S. NO. | PHYTOCHEMICAL TEST | ETHANOL EXTRACT |
|---|---|---|
| 1 | Terpenoid | - |
| 2 | Tannins | - |
| 3 | Saponins | - |
| 4 | Quionone | + |
| 5 | Alkaloids | + |
| 6 | Steroids | + |
| 7 | Phenol | - |
| 8 | Glycosides | - |
| 9 | Cardiac glycosides | + |
| 10 | Flavonoid | + |

**Note: (+) – presence, (-) – absence.**

**Table 2: Antibacterial activity of Ethanolic extract of K. africana fruit. ZI = Zone of inhibition, ZI% = Percentage of inhibition.**

| Bacterial strains | CONCENTRATION OF EXTRACT(µg/mL) | ZONE OF INHIBITION | | POSITIVE CONTROL (AMPICILLIN) (mm) |
|---|---|---|---|---|
| | | ZI (mm) | ZI % | |
| Bacillus subtilis | 25 | 7 | 17.78 | 30 |
| | 50 | 10 | 11.11 | |
| | 75 | 15 | 16.67 | |
| | 100 | 17 | 18.89 | |
| Salmonella typhi | 25 | 12 | 13.33 | 28 |
| | 50 | 15 | 16.67 | |
| | 75 | 17 | 18.89 | |
| | 100 | 19 | 21.11 | |
| Staphylococcus aureus | 25 | 5 | 15.56 | 28 |
| | 50 | 10 | 11.11 | |
| | 75 | 12 | 13.33 | |
| | 100 | 15 | 16.67 | |

**Table: 3. Antifungal activity of ethanolic extract of K. africana fruit. ZI = Zone of inhibition, ZI %= Percentage of inhibition.**

| ORGANISMS | CONCENTRATION OF EXTRACT(µg/mL) | ZONE OF INHIBITION | | POSITIVE CONTROL(HOST CYCLINE 500) |
|---|---|---|---|---|
| | | ZI (mm) | ZI % | |
| Epidermophyton floccusum | 25 | 5 | 15.12 | 15mm |
| | 50 | 5 | 15.25 | |
| | 75 | 5 | 15.84 | |
| | 100 | 5 | 15.05 | |
| Candida albicans | 25 | 10 | 11.11 | 19mm |
| | 50 | 11 | 12.22 | |
| | 75 | 15 | 16.66 | |
| | 100 | 17 | 18.81 | |

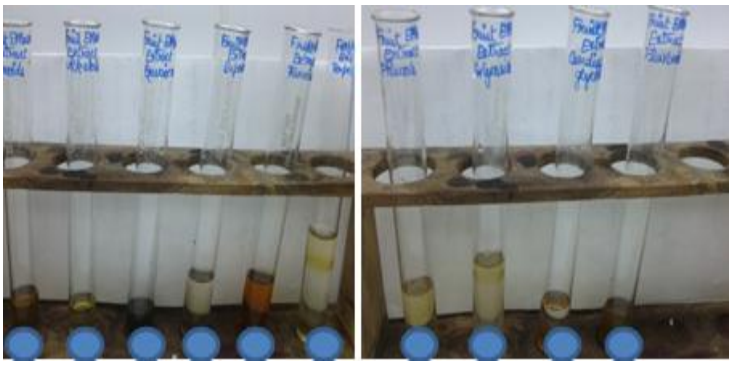
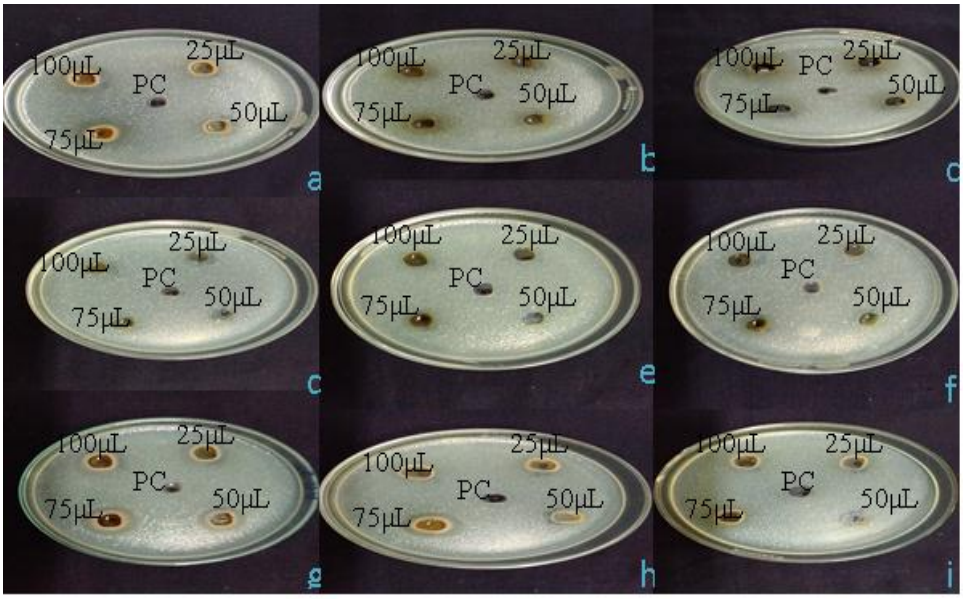


**Figure 1: Phytochemical analysis of ethanolic extract of *K.* africana fruit showing a – steroids, b – alkaloids, c – quinones, d – saponins, e – tannins, f – terpenoids, g – phenols, h – glycosides, I – cardiac glycosides, j – flavonoids.**




**Antibacterial activity of ethanolic fruit extracts of K. Africana A,b,c – Bacillus subtilis, d,e,f – S. typhi, g,h, I – S. aureus.**

## DISCUSSION

### Phytochemical analysis

The crude extract was qualitatively analysed for the presence of terpenoids, tannins, saponins, quinines, alkaloids, steroids, glycosides, cardiac glycosides, phenol and flavonoids. According to Okuda 2005, from the methanol extract of fruit of Kigelia africana showed the presence of glycosides, saponins, steroids and carbohydrates while the absence were observed in alkaloids. According to Shalini et al., 2014 from the methanol extract of Kigelia africana revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, glycosides and sugars. One another study of Efosa et al., 2015 from aqueous, methanol, chloroform and petroleum ether extract of Kigelia africana revealed the presence of glycosides, phenolic compounds, tannins, alkaloids, flavonoids and sugars. The methanol leaf extract of K. africana when tested for phytochemical screening revealed the presence of flavonoids, glycosides, steroids, alkaloids and saponins (Priya et al., 2012). One another study from methanol , chloroform and ethanol leaf extract of K. africana showed the presence of sterols, terpenes, carotenoids, alkaloids, tannins, saponins, coumarins and carbohydrates (Osman et al., 2015).

In the present study from ethanol extract of fruit of Kigelia africana showed the presence of quinone, alkaloid, steroids and flavonoids. Alkaloids and flavonoids act against the fungal diseases mainly in epidermal regions.

- Quinones represent a class of organic aromatic compounds with even number of – CH= groups converted to - C (=O) groups. They show anti tumoral activity, antimicrobial, antiparasitic and against cardiovascular disorders.
- Alkaloids contain basic nitrogen atoms – stimulants, analgestic and antiprotozoal analgesic.
- Flavonoids are polyphenolic molecules, powerful antioxidants with anti inflammatory and immune system benefits.
- Steroids are polycyclic chemical compounds. They are important components of cell membrane and decrease membrane fluidity. They play role as sources of energy, anti inflammatory condition, rheumatoid arthritis and gout.
- Cardiac glycosides increase the output force of heart and increases its pumping strenghth of heart beat.

**Antimicrobial activity**

Herbal remedies play an essential role in traditional medicine in rural areas of South Africa, where these are often the therapeutic treatment of choice. The preparation of herbal medicine which depends on a cultural context may be obtained from healers as already prepared mixtures, or as unprepared raw materials. Although South Africa possesses a rich tradition in the use of medicinal plants and an outstanding floral diversity estimated at 251 220 species of vascular plants (Cracraft and Grifo, 1999).Plant-*based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials is needed as antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects of* synthetic antimicrobials (Iwu et al., 1999).

In the present study antibacterial activities of the crude extracts of ethanol were tested against three pathogenic bacteria and they were compared with standard antibiotic Ampicillin. In ethanol fruit extract of Kigelia africana the average zone of inhibition ranges from (16.1mm to 18.8mm). Highest inhibition was observed against the growth of S. aureus with the zone of inhibition 21.11mm respectively.

According to Amandeep et al., 2013 from the aqueous and ethanol extract of fruit of Kigelia africana against three bacterial sps. aqueous extract showed maximum activity. *vulgare* was observed at 17mm respectively. In ethanolic against S. aureus with 20mm and moderate activity against E. coli with 11mm and least inhibitory effect against P extract the maximum

zone of inhibition was observed *S. aureus* with 16mm and medium effect against *E.coli* with 7mm and least inhibition was observed at 6mm in P. vulgare respectively.

One another study of Efosa et al., 2015 from aqueous, methanol, chloroform and petroleum ether extracts of fruit of Kigelia africana against six bacterial species. Petroleum ether extract showed highest activity against Pseudomonas aerugenosa while other extracts showed no clear zone of inhibition against these two pathogenic bacteria. Chloroform extract showed a dose dependent activity against S.feacalis and S.aureus was found to be more against S.aureus. Methanol extract and aqueous extract were found active against S.aureus and showed higher activity was 50mg/mL. Ciprofloxacin was used as positive control.

The ethanolic extract of K. africana possess antifungal activity against *C. albicans* and water extract showed no activity against the organism (Osahon *et al.*, 2007). The aqueous extract showed no activity indicating that ethanol is better extracting solvent. *C. albicans* have been implicated in the pathology of atopic eczema and psoriasis (Watt & Brayer – Bradwijk 1962).

In the present study antifungal activities of crude extracts of ethanol were tested against two fungal species. In ethanolic extract the maximum zone of inhibition was seen against C. albicans with 18.1mm and moderate zone of inhibition were examined in C. albicans with 12.2mm while least zone of inhibition was seen in E. floccosum with 5.5mm respectively.

## CONCLUSION

- The present study provides valuable information regarding the potential of K. africana as natural source for phytochemical constituents and antimicrobial activities. The potential use of renewable sources offers an excellent nutritional and health package for use in food supplements in nutraceutical formulation and as health food for human consumption. Hence the study concluded that K. africana has a variety of biologically active molecules which can be used as a source of antibiotics. Further study need to be carried out for the purification of active compounds and structural elucidation can be used for drug discovery.
- K. africana is a flowering plant from Bignoniaceae. The fruit is poisonous and strongly purgative. It is usually consumed by drying, roasting or fermentation. The phytochemical and antimicrobial screening reveals that they can be used against skin disorders. The dried powder is also used for coronary disorders.

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