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Running Title: Anti-Mycobacterial potential of *Kigelia pinnata* (Bignoniaceae)-Agarwal & Chauhan

# ANTI-MYCOBACTERIAL POTENTIAL OF KIGELIA PINNATA (JACQ.) DC. SYN K. AFRICANA (LAMK.) BENTH. (BIGNONIACEAE)

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**Abstract** Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. The World Health Organization has estimated that almost 9 million new cases and 1.4 million TB deaths in 2011. Medicinal plants offer a great hope for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material. In the present study antibacterial activity of aqueous and alcoholic extracts of stem bark and leaves of *Kigelia pinnata* (Jacq.) DC. syn *K. africana* (Lamk.) Benth. (Tribe-Crescentieae; Family: Bignoniaceae) was tested against MDR isolates DKU-156 and JAL-1236 of *M. tuberculosis*, reference susceptible strain *M. tuberculosis* H37Rv as well as fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529). The leaves and bark were dried and extracts were prepared using distilled water and ethanol (98%). Reference drug susceptible strain *M. tuberculosis* H37Rv as control, multi-drug resistant isolates DKU-156, JAL-1236 and fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529) were used during the present investigation. Antimicrobial assays were performed in Lowenstein Jensen (L-J) medium and Middlebrook 7H9 broth in BacT/ALERT 3D system (Sigma-Aldrich, St. Louis, USA). The aqueous and alcoholic extracts of stem bark and leaves were incorporated in the media. Susceptibility testing of MDR isolates was also performed against streptomycin in the same batch of media for comparison of cfu on drug free controls. The results of the present investigation clearly showed that the aqueous extracts of stem bark were more effective as compared to aqueous and leaf extracts and alcoholic stem bark and leaf extracts.

**Key words:** MDR isolates DKU-156 and JAL-1236 of *M. tuberculosis*, Lowenstein Jensen medium and Middlebrook 7H9 broth in BacT/ALERT 3D system, streptomycin.

## INTRODUCTION

Tuberculosis, MTB, or TB is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* a pathogenic bacterium (Jordao & Vieira 2011, Thaiss *et al.* 2012). Ninety-five percent of TB cases have been produced from underdeveloped countries, 80% of them corresponding to the 15 to 29-year-old group, generating strong socioeconomic problems (WHO 2010, 2011). Furthermore, the lack of treatment adherence has given rise to antibiotic-resistant *M. tuberculosis* strains, the multidrug-resistant TB (MDR-TB), which does not respond to the first-line standard treatment, and the extensively drug-resistant TB (XDR-TB), which occurs when resistance to second-line drugs develops (Zager & McNerney 2008).

Plants have been used in the traditional health care system from time immemorial, particularly among tribal communities. The World Health Organization (WHO) has listed 20,000 medicinal plants globally and about 2000 drugs used are of plant origin (WHO 2009). India's contribution is 15-20%. More than 7,500 species of medicinal plants grow in India which is considered as the botanical garden of the world.

In recent years more attention is being directed towards herbal medicines because these are inexpensive, non-toxic and eco-friendly. There are larger numbers of phyto-pharmaceuticals isolated from plants which are being used in modern medicine. Plants are known to contain innumerable biological active compounds which possess antibacterial properties (Brantner & Grein 1994). Although a large number of plants have been tested for antibacterial properties against gram positive and gram negative bacterial organisms, but only a few have been tested against mycobacteria.

Worldwide, the Bignoniaceae are mostly tropical trees or shrubs comprising of 120 genera and about 800 species (Lohmann 2004). In India the family is represented by 21-25 species found chiefly in western and southern parts and a few are found in Himalayan region (Chauhan 2008). Recent studies have shown that the vegetative parts of several members of the family Bignoniaceae contain a wide variety of chemical compounds (amino acids, phenolics and alkaloids) known to have antimicrobial properties (Binutu & Lajubutu 1994, Binutu *et al.* 1996, 2000, Costantino *et al.* 2003a, Warashina *et al.* 2005, Zaveri *et al.* 2007, Chaudhary *et al.* 2011, Costa-Campos *et al.* 2102). However, they have not been tested for their anti-mycobacterial properties. Chauhan & Chauhan (2012) have shown antimicrobial

activity of some Bignoniaceae (*Adenochalyma alliaceum*, *Jacaranda mimosifolia*, *Millingtonia hortensis*, *Pyrostegia venusta* and *Tabebuia argenticia*).

*Kigelia pinnata* (Jacq.) DC. syn *K. africana* (Lamk.) Benth. (Tribe-Crescentieae; Family: Bignoniaceae) a native of Central Africa and is extensively planted in the tropics. It is a medium sized tree (10-15 m tall) commonly called as sausage tree. It bears showy scarlet coloured flowers with yellow stripes all over India. It is grown as a road side tree in various parts of north India.

In light of the facts enumerated above present study was carried out to check the antibacterial activity of aqueous and alcoholic extracts of stem bark and leaves of *Kigelia pinnata* (Jacq.) DC. syn *K. africana* (Lamk.) Benth. (Tribe-Crescentieae; Family: Bignoniaceae) against MDR isolates of *M. tuberculosis*, reference susceptible strain *M. tuberculosis* H37Rv as well as fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529).

## MATERIALS & METHODS

**Plant Material used-** Leaves and bark of *Kigelia pinnata* plants growing on the road side in the Lajpat Kunj, Civil Lines Agra were collected between spring and summer season during March to May 2010.

**Extract preparation-** The plant extracts was prepared using the modified method after Alade & Irobi (1993). Three portions of the dried powdered samples (bark and leaves) were soaked separately in 500 ml of distilled water and ethanol (98%) for 72 h. Each mixture was refluxed followed by agitation at 200 rpm for 1 h. The filtrates obtained were concentrated under vacuum at 40° C to obtain the dry extracts.

**Mycobacterial strains/isolates-** Reference drug susceptible strain *M. tuberculosis* H37Rv as control, multi-drug resistant isolates DKU-156, JAL-1236 and fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529) were obtained from Mycobacterial Repository Centre, Department of Microbiology and Molecular Biology at National JALMA Institute for Leprosy and other Mycobacterial Diseases (ICMR), Agra.

**Assay protocol-**Antimicrobial assays were performed in Lowenstein Jensen (L-J) medium and Middlebrook 7H9 broth in BacT/ALERT 3D system (Sigma-Aldrich, St. Louis, UAS).

**Lowenstein-Jensen (L-J) medium--** Determination of Colony forming units (cfu) on Lowenstein-Jensen (L-J) - The ten-fold dilution of standard 1 mg/ml *M. tuberculosis* suspension<sup>19</sup> were streaked on L-J medium for determining cfu in the presence and absence of plant extracts. An *M. tuberculosis* suspension of 1 mg/ml is equivalent to MacFarland standard-1<sup>20</sup>. One loopful (6 µl) of this suspension was streaked on the L-J slants using 3 mm external diameter loop. Reagents of L-J media included potassium di hydrogen phosphate anhydrous (Qualigens), magnesium sulphate anhydrous (Qualigens), magnesium citrate (Loba Chemie), L-asparagine (Hi-media, Mumbai), glycerol (Fisher Scientific, Mumbai), and malachite green (Hi-Media, Mumbai).

**Middlebrook 7H9 broth in BacT/ALERT 3D system-** Exposure of mycobacterial suspension (0.2 ml, 1mg/ml) to the millipore (0.22 µm) filtered plant extract (4% v/v) was done for 15 min at room temperature. The resultant mixture was inoculated into Mycobacterial Process (MP) bottles containing Middlebrook 7H9 broth supplemented with reconstitution fluid (Oleic acid, glycerol, & bovine serum albumin) in colorimetric BacT/ALERT 3D system (BioMerieux, France).

**Minimum inhibitory concentration (MIC)--**Minimum inhibitory concentration (MIC) of the aqueous and alcoholic extracts of stem bark and leaves was determined by the method after Andrews (2001). In order to determine the MIC, 2% and 4% v/v concentration of each plant extract was added to LJ medium. The resistance was expressed in terms of the lowest concentration of the plant extract that inhibited all the growth i.e. minimum inhibitory concentration. A parallel set of medium containing different concentrations of the plant extracts was inoculated separately with standard inoculums (4 mg/ml).

Determination of the effect of direct exposure of bacterial suspension to the water extracts of plants was done by counting the CFUs on LJ medium after different *intervals* of exposure: 0.2 ml inoculums of 1 mg/ml suspension of *M. tuberculosis* was added to 0.5 ml plant extract and will be kept for 15 minutes, 2 h, 40 h and 80 h; 600 µl distilled water added after the exposure time of 15 minutes to dilute the extract so that the effective exposure can be controlled for desired duration (15 minutes) of time 30 µl of each was inoculated on LJ slants.

## RESULTS & DISCUSSION

**Antitubercular Potential-**The antitubercular potential in the aqueous and ethanolic extracts of stem bark and leaves of *Kigelia pinnata* was recorded. Average growth and percentage inhibition of *M. tuberculosis* H37Rv, MDR isolates and rapid grower *M. fortuitum* (TMC-1529) by the extracts of stem bark and leaves was observed in Lowenstein Jensen (L-J) and Middlebrook 7H9 broth in BacT/ALERT media. The bark and leaf extracts of *Kigelia pinnata* syn *K. africana* were added on the L-J slants, BacT/ALERT media and extract free control L-J slants after 42 days of incubation at 37°C are described in the following paragraphs in each species studied:

Average growth and percentage inhibition of *M. tuberculosis* H37Rv, MDR isolates and rapid grower *M. fortuitum* (TMC-1529) by stem bark and leaf extracts in distilled water and ethanol of *Kigelia pinnata* added on Lowenstein Jensen (L-J) and BacT/ALERT media and extract free control L-J and BacT/ALERT media slants after 42 days of incubation at 37°C is shown in Tables 1 & 8.

(i) **Effect of water extract of stem bark of *Kigelia pinnata* in L-J medium:** The effect of water extract of stem bark of *Kigelia pinnata* on different *M. tuberculosis* strains in Lowenstein Jensen (L-J) medium is shown in Table 1.

**Table 1- Results of anti-tuberculosis assay using aqueous stem bark extract of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium.**

Isolate code	L-J proportion medium				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
<i>M. tuberculosis</i> H37Rv	42	20	12	53	63
DKU-156	18	06	02	68	94
JAL-1236	70	25	21	60	65
<i>M. fortuitum</i> TCM-1529	02	02	02	00	00

The data in Table 1 shows clearly that addition of water extract of stem bark of *Kigelia pinnata* in L-J medium was effective to a considerable extent in inhibiting the strain DKU-156 followed by JAL-1236 and failed to show any inhibitory activity against *M. fortuitum* TCM-1529. The average growth and percentage inhibition was 94% for MDR isolate DKU-156 and 65% inhibition for another MDR isolate JAL-1236, while for sensitive

*M. tuberculosis* H37 Rv inhibition it was only 63% at 4% v/v concentration in L-J medium by water extract of stem bark.

**ii. Effect of aqueous stem bark extract of *Kigelia pinnata* in BacT/ALERT 3D system:** The effect of addition of aqueous bark extract of *Kigelia pinnata* on Middlebrook 7H9 broth in BacT/ALERT 3D system against *M. tuberculosis* strains is shown in Table 2.

**Table 2- Results of anti-tuberculosis assay using aqueous extract of stem bark of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3D system.**

Isolate code	BacT/ALERT 3D system				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
<i>M. tuberculosis</i> H37Rv	40	18	11	50	62
DKU-156	15	7	3	65	90
JAL-1236	69	23	20	61	66
<i>M. fortuitum</i> TCM-1529	1	3	2	1	1

The results shown in the Table 2 indicate that the addition of aqueous extract of stem bark of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3D system was less effective as compared to that in L-J medium. There was more or less no inhibition against rapid grower *M. fortuitum* (TCM-1529). The effect increased with the increase in concentration and 4% v/v was most effective causing 90% inhibition. The water extract of stem bark of *Kigelia pinnata* added in the Middlebrook 7H9 broth in BacT/ALERT 3 D system caused 90% inhibition in the strain DKU-156 (90%); and only 66% inhibition of JAL-1236 and in *M. tuberculosis* strain H37Rv it was 62%.

**iii. Effect of aqueous leaf extract of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium:** The effect of aqueous extract of leaf of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium is shown in Table 3.

**Table 3- Results of anti-tuberculosis assay using water leaf extract of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium.**

Isolate code	Lowenstein Jensen (L-J) medium.				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
<i>M. tuberculosis</i> H37Rv	41	22	11	50	63
DKU-156	19	8	3	61	89
JAL-1236	71	26	23	58	62
<i>M. fortuitum</i> TCM-1529	3	2	3	0	0

It is evident from the results shown in Table 3 that addition of aqueous leaf extract of *Kigelia pinnata* in L-J medium, caused lesser degree of inhibition against *M. tuberculosis* as compared to that shown by stem bark aqueous extract. There was an average growth and 89% inhibition for MDR isolate DKU-156 and 62% only 62% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 63% at 4% v/v concentration in L-J medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

**iv. Effect of water extract of leaf of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3 D system:** The effect of water extract of leaf of *Kigelia pinnata* on anti-tubercular activity in different strains in Middlebrook 7H9 broth in BacT/ALERT 3 D system is shown in Table 4.

**Table 4- Results of anti-tuberculosis assay using aqueous leaf extract of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3D medium.**

Isolate code	BacT/ALERT 3D system				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
<i>M. tuberculosis</i> H37Rv	40	20	10	51	60
DKU-156	18	9	4	65	85
JAL-1236	72	28	21	61	60
<i>M. fortuitum</i> TCM-1529	3	2	3	0	0

It is evident from the results shown in Table 4 that addition of water leaf extract of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3D medium, caused less inhibition against *M. tuberculosis* as compared to that was recorded in L-J medium. There

was an average growth and percentage inhibition of 85% for MDR isolate DKU-156 and 60% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 60% at 4% v/v concentration in BacT/ALERT 3D system. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

**v. Effect of ethanol extract of stem bark of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium:** The effect of ethanol extract of stem bark of *Kigelia pinnata* on anti-tubercular activity in different strains in Lowenstein Jensen (L-J) medium is shown in Table 5.

**Table 5- Results of anti-tuberculosis assay using ethanol stem bark extract of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium.**

Isolate code	Lowenstein Jensen (L-J) medium				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
<i>M.tuberculosis</i> H37Rv	41	22	11	50	61
DKU-156	19	8	3	66	91
JAL-1236	71	26	23	62	64
<i>M. fortuitum</i> TCM-1529	3	2	3	0	0

It is evident from the results shown in Table 5 that addition of alcoholic extract of stem bark of *Kigelia pinnata* in L-J medium, showed considerable inhibition against *M. tuberculosis*. There was an average growth and percentage inhibition of 91% for MDR isolate DKU-156 and 64% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 61% at 4% v/v concentration in L-J medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

**vi. Effect of ethanol extract of stem bark of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT medium:** The effect of ethanol extract of stem bark of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT medium is shown in Table 6.



**Table 6- Results of anti-tuberculosis assay using ethanol stem bark extract of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3D system.**

Isolate code	BacT/ALERT 3D system				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
<i>M. tuberculosis</i> H37Rv	41	22	11	50	61
DKU-156	19	8	3	66	88
JAL-1236	71	26	23	62	62
<i>M. fortuitum</i> TCM-1529	3	2	3	0	0

It is evident from the results shown in Table 6 addition of ethanol stem bark extract of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3D medium, caused less inhibition against *M. tuberculosis* as compared to that shown by aqueous extract of stem bark. There was an average growth and percentage inhibition of 88% for MDR isolate DKU-156 and 62% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 61% at 4% v/v concentration in this medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

**vii. Effect of ethanol extract of leaf of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium:**

The Effect of ethanol extract of leaf of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium is shown in Table 7.

**Table 7- Results of anti-tuberculosis assay using ethanol leaf extract of *Kigelia pinnata* in Lowenstein Jensen (L-J) medium.**

Isolate code	Lowenstein Jensen (L-J) medium.				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
<i>M. tuberculosis</i> H37Rv	42	20	10	52	62
DKU-156	20	9	4	65	89
JAL-1236	70	25	21	60	61
<i>M. fortuitum</i> TCM-1529	2	3	3	1	0

It is evident from the results shown in Table 7 that addition of ethanol leaf extract of *Kigelia pinnata* in L-J medium caused significant inhibition against *M. tuberculosis*. There was an average growth and percentage inhibition of 89% for MDR isolate DKU-156 and 61% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 62% at 4% v/v concentration in L-J medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

**viii. Effect of ethanol extract of leaf of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT in 3D system:**

Effect of ethanol extract of leaf of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT in 3D system is shown in Table 8.

**Table 8- Results of anti-tuberculosis assay using ethanol leaf extract of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3D system.**

Isolate code	BacT/ALERT in 3D system				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
<i>M. tuberculosis</i> H37Rv	42	22	11	49	60
DKU-156	18	7	3	67	85
JAL-1236	70	27	22	62	61
<i>M. fortuitum</i> TCM-1529	3		1	0	0

It is evident from the results shown in Table 8 that addition of alcoholic extract of leaves of *Kigelia pinnata* in Middlebrook 7H9 broth in BacT/ALERT 3D medium, induced less inhibition against *M. tuberculosis* as compared to that shown in L-J medium by aqueous extract. There was an average growth and percentage inhibition of 85% for MDR isolate DKU-156 and 61% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 60% at 4% v/v concentration in BacT/ALERT RT 30 medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

Thus, it is evident from the foregoing observations that the extract of stem bark in both water as well as in ethanol in both L-J and middlebrook 7H9 broth in BacT/ALERT 3D media was more effective in inhibition of both the MDR isolate, DKU-156 and JAL-1236. However, aqueous extract of stem bark in L-J medium followed by that in Middlebrook 7H9 broth in BacT/ALERT 3D system was the most effective as compared to the aqueous and ethanol extracts of leaves in both the media.

**Minimum Inhibitory Concentration (MIC):** The minimum inhibitory concentration of aqueous and alcoholic extracts of stem bark and leaves of *Kigelia pinnata* is shown in Table 9.

**Table 9- Minimum Inhibitory Concentration (MIC) of aqueous and alcoholic extracts of stem bark and leaves of *Kigelia pinnata*.**

Samples	MIC (mg/ml)	
	MDR isolates of <i>M. tuberculosis</i> .	
	DKU-156	JAL-1236
a. Aqueous stem bark extract	0.15	0.25
b. Aqueous leaf extract	0.25	0.5
c. Alcoholic stem bark extract	0.25	1.0
d. Alcoholic leaf extract	0.5	1.5
<i>Streptomycin</i>	5.5	10.5

It is evident from Table 9 that the aqueous stem bark extract of stem bark and leaves was more effective as compared to the alcoholic extracts.

The results of the foregoing experiments have clearly shown that aqueous and alcoholic extracts of stem bark and leaves of *Kigelia pinnata* have inhibitory effect on all the strains of *Mycobacterium tuberculosis* used in this study. The aqueous extracts of stem bark was more effective as compared to aqueous leaf extracts and alcoholic stem bark and leaf extracts.

Antimicrobial activity of large number of plants including several members of the family Bignoniaceae has been determined by several workers (Fleischer *et al.* 2003, Martinez & Valencia 2003, Jin *et al.* 2005, Rojas *et al.* 2006, Aliyu *et al.* 2009, Dutta & Choudhary 2010, Ejelonu *et al.* 2011, Costa-Compos *et al.* 2012, Chauhan & Chauhan 2012).

The chemical analysis of roots, wood and leaves of *Kigelia africana* has been made. Qualitative tests for the presence of plant secondary metabolites such as carbohydrates,

alkaloids, tannins and saponins using standard procedure (Sofowora 1984). Saponins have been responsible for the antioxidant activities.

A naphthoquinone and lignin have been isolated from the wood of *Kigelia pinnata* by Inouye *et al.* (1981). From the leaves of *Kigelia pinnata* iridoides and from the flowers antocyanes have been isolated and identified. Govindachari *et al.* (1971) have isolated and detected the structure of two new dihydroisocoumarines from *Kigelia pinnata*. The characteristic substances of the inner bark and of the heartwood are naphthochinones, mainly lapachol (3, 6 %), lapachone, its cyclisation product and in lower concentrations (<0.01 %) coumarins and saponins (Wagner *et al.* 1989). Lapachol and lapachone are the biologically most active substances. Akunyili & Houghton (1993) isolated naphthoquinones, hydroisocoumarines, flavonoids and aldehydic iridoids and among them, the naphthoquinones, kigelinole, isokigelinole, pinnatal and isopinnatal from the root and its bark, and the usual plant substances, stigmasterol,  $\beta$ -sitosterol, ferulic acid, the naphthoquinones lapachol, 6-methoxymellein and two new phenolic compounds were also isolated from *Kigelia pinnata*. The antimicrobial activity of *Kigelia pinnata* (Bignoniaceae) has been shown to be due to the presence of iridoids (Akunyili *et al.* 1991, von Poser *et al.* 2000, Gouda *et al.* 2004). In the polar (methanolic) extract of the fruit from *K. africana* verminoside (C<sub>24</sub>H<sub>28</sub>O<sub>13</sub>), an iridoid as a major constituent and among a series of polyphenols verbascoside was isolated (Nyarka *et al.* 2005). Omonkhelin *et al.* (2007) have studied antibacterial and antifungal activities of the stem bark of *Kigelia africana*.

In the light of the results of the present study it is concluded that the aqueous extracts of stem bark of *Kigelia africana* of family Bignoniaceae should further be tested for the principal compounds showing anti-mycobacterial activity.

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