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Anti-Mycobacterial potential of *Tabebuia aurea* (Manso) Benth & Hook (Bignoniaceae)

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Abstract
Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. The World Health Organization has estimated 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 *Tabebuia aurea* (Manso) Benth. & Hook. (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.

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

1. Introduction
Tuberculosis is a common and lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* a pathogenic bacterium (Jordao and Vieira, 2011; Thaiss *et al.,* 2012) [21, 27]. 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) [33, 34]. 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 and McNerney, 2008) [35].
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) [31]. 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 and Grein, 1994) [9]. 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) [23]. 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) [11]. 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 (Binotu and Lajubutu, 1994; Binotu *et al.,* 1996; 2000; Costantino *et al.,* 2003a; b; Warashina *et al.,* 2005; Zaveri *et al.,* 2007; Chaudhary *et al.,* 2011; Costa-Campos *et al.,* 2102) [6, 7, 8, 10, 13, 14, 31, 36]. However, they have not been tested for their anti-mycobacterial properties. Chauhan and Chauhan (2012) [12] have shown antimicrobial activity of some Bignoniaceae (*Adenochalyma alliaceum*, *Jacaranda mimosifolia*, *Millingtonia hortensis, Pyrostegia venusta* and *Tabebuia argentea*).

Tabebuia aurea a native of Paraguay is commonly known as golden bell or Caribbean trumpet tree, the largest genus of the family Bignoniaceae with 293 species. *Tabebuia aurea* (Manso) Benth. & Hook. is 5-8 meter tall deciduous tree grown in the gardens and avenues for its beautiful dark yellow flowers and foliage.

Present study was carried out to record the antibacterial activity of aqueous and alcoholic extracts of stem bark and leaves of *T. aurea* against MDR isolates of *M. tuberculosis*, reference susceptible strain *M. tuberculosis* H37Rv as well as fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529).

2. Materials and methods

2.1 Plant Material used

Leaves and bark of. *Tabebuia aurea* were collected from Hotel Marina, Hariparvat and the Botanical gardens, R.B.S. College, Khandari Farm, Agra between spring and summer season during March to May 2011 and 2012.

2.2 Extract preparation

The plant extracts was prepared using the modified method after Alade and Irobi (1993) [3]. 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 ^{o}C to obtain the dry extracts.

2.3 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.

2.4 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).

2.5 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* suspension19 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 Mac Farland standard-120. 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).

2.6 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).

2.7 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) [5]. In order to determine the Minimum inhibitory concentration (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.

3. Results and Discussion

Average growth and percentage inhibition of *M. tuberculosis* H37Rv, MDR isolates and rapid grower *M. fortuitum* (TMC-1529) by stem bark and leaf extracts of *T. aurea* in distilled water and ethanol added on Lowenstein Jensen (L-J) and BacT/ALERT 3D system and extract free control L-J and BacT/ALERT 3 D system slants after 42 days of incubation at 37 ^{0}C is shown in Tables 1-8

3.1 Effect of water extract of stem bark of *Tabebuia aurea* in L-J medium

The Effect of aqueous stem bark extract of *Tabebuia aurea* in L-J medium is shown in Table 1.

Table 1: Results of anti-tuberculosis assay using water stem bark extract of *Tabebuia aurea* in Lowenstein Jensen (L-J) medium.

Isolate code	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
M. tuberculosis H37Rv	42	20	12	53	65
DKU-156	18	6	2	68	92
JAL-1236	70	25	21	65	68
M. fortuitum TCM-1529	2	2	2	0	0

It is evident from the results shown in Table 1 that by addition of aqueous stem bark extract of *Tabebuia aurea* there was an average growth and percentage inhibition of 92% for MDR isolate DKU-156 and 68% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 65% at 4% v/v concentration in L-J medium by

water extract of stem bark. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

3.2. Effect of water extract of stem bark of *Tabebuia aurea* in BacT/ALERT 3D system

The effect of addition of bark extract of *Tabebuia aurea* in aqueous on Middlebrook 7H9 broth in BacT/ALERT 3D system is shown in Table 2.

Table 2: Results of anti-tuberculosis assay using aqueous extract of stem bark of *Tabebuia aurea* 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
M. tuberculosis H37Rv	40	18	11	50	62
DKU-156	15	7	3	65	90
JAL-1236	69	23	20	61	66
M. fortuitum TCM-1529	1	3	2	1	1

It is evident from the results shown in Table 2 that addition of aqueous extract of stem bark of *Tabebuia aurea* in Middlebrook 7H9 broth in BacT/ALERT 3D medium caused inhibition to considerable extent against *M. tuberculosis.* There was an average growth and percentage inhibition of 90% for MDR isolate DKU-156 and 66% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 62% at 4% v/v concentration in BacT/ALERT 3D medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

3.3 Effect of aqueous extract of leaves of *Tabebuia aurea* in Lowenstein Jensen (L-J) medium

The Effect of aqueous extract of leaves of *Tabebuia aurea* in Lowenstein Jensen (L-J) medium is shown in Table 3.

Table 3: Results of anti-tuberculosis assay using aqueous leaf extract of *Tabebuia aurea* 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
M. tuberculosis H37Rv	41	22	11	50	63
DKU-156	19	8	3	66	90
JAL-1236	71	26	23	62	65
M. fortuitum TCM-1529	3	2	3	0	0

It is evident from the results shown in Table 3 that addition of aqueous leaf extract of *Tabebuia aurea* in L-J medium there was an average growth and percentage inhibition of 90% for MDR isolate DKU-156 and 65% 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).

3.4 Effect of water extract of leaf of *Tabebuia aurea* in Middlebrook 7H9 broth in BacT/ALERT medium

The effect of water extract of leaf of *Tabebuia aurea* in Middlebrook 7H9 broth in BacT/ALERT medium is shown in Table 4.

Table 4: Results of anti-tuberculosis assay using ethanol leaf extract of *Cresc Dolichandrone falcata* in Middlebrook 7H9 broth in BacT/ALERT 3D system

Isolate code	BacT/ALERT3D system				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
M. tuberculosis H37Rv	41	22	11	50	63
DKU-156	19	8	3	66	89
JAL-1236	71	26	23	62	62
M. fortuitum TCM-1529	3	2	3	0	0

It is evident from the results shown in Table 4 that addition of aqueous leaf extract of *Tabebuia aurea* in Middlebrook 7H9 broth in BacT/ALERT 3D medium caused significant inhibition against *M. tuberculosis.* There was an average growth and percentage inhibition of 89% for MDR isolate DKU-156 and 62% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 63% at 4% v/v concentration in BacT/ALERT 3D medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

3.5 Effect of ethanol extract of stem bark of *Tabebuia aurea* in Lowenstein Jensen (L-J*)* medium

The Effect of ethanol extract of stem bark of *Tabebuia aurea* in Lowenstein Jensen (L-J*)* medium is shown in Table 5.

Table 5: Results of anti-tuberculosis assay using aqueous stem bark ex extract of *Tabebuia aurea* in Lowenstein Jensen (L-J) medium.

Isolate code	L-J proportion method				
	Mean cfu on media			% Inhibition	
	Control	Plant extract		Plant extract	
		2% v/v	4% v/v	2% v/v	4% v/v
M. tuberculosis H37Rv	42	20	12	53	65
DKU-156	18	6	2	68	92
JAL-1236	70	25	21	65	68
M. fortuitum TCM-1529	2	2	2	0	0

It is evident from the results shown in Table 5 addition of aqueous stem bark extract of *Tabebuia aurea* in L-J medium caused significant inhibition against *M. tuberculosis.* There was an average growth and percentage inhibition of 92% for MDR isolate DKU-156 and 68% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 65% at 4% v/v concentration in L-J medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

3.6 Effect of ethanol extract of stem bark of *Tabebuia aurea* in BACT/ ALERT 3D system: The effect of ethanol extract of stem bark of *Tabebuia aurea* in BACT/ ALERT 3D system is shown in Table 6.

Table 6: Results of anti-tuberculosis assay using ethanol bark extract of *Tabebuia aurea* 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
M. tuberculosis H37Rv	42	20	12	53	65
DKU-156	18	6	2	68	92
JAL-1236	70	25	21	65	68
M. fortuitum TCM-1529	2	2	2	0	0

It is evident from the results shown in Table 6 that addition of ethanol extract of stem bark of *Tabebuia aurea* there was an average growth and percentage inhibition of 92% for MDR isolate DKU-156 and 68% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 65% at 4% v/v concentration in BacT/ALERT 3D medium by water extract of stem bark. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

3.7 Effect of ethanol extract of leaves of *Tabebuia aurea* in L-J medium

The effect of addition of bark extract in water on L-J medium showed significant inhibition against *M. tuberculosis* is shown in Table 7.

Table 7: Results of anti-tuberculosis assay using ethanol leaf extract of *Tabebuia aurea* 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
M. tuberculosis H37Rv	41	22	11	50	63
DKU-156	19	8	3	66	90
JAL-1236	71	26	23	62	65
M. fortuitum TCM-1529	3	2	3	0	0

It is evident from the results shown in Table 7 that addition of alcoholic extract of leaves of *Tabebuia aurea* in L-J medium, water extract of leaf showed significant inhibition against *M. tuberculosis.* There was an average growth and percentage inhibition of 90% for MDR isolate DKU-156 and 65% 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).

3.8 Effect of ethanol extract of leaves of *Tabebuia aurea* in BacT/ ALERT 3D system

The Effect of ethanol extract of leaves of *Tabebuia aurea* in BacT/ ALERT 3D system is shown in Table 8.

Table 8: Results of anti-tuberculosis assay using ethanol extract of leaf of *Tabebuia aurea* 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
M. tuberculosis H37Rv	40	18	11	50	62
DKU-156	15	7	3	65	88
JAL-1236	69	23	20	61	66
M. fortuitum TCM-1529	1	3	2	1	1

It is evident from the results shown in Table 8 that addition of alcoholic extract of leaves of *Tabebuia aurea* in Middlebrook 7H9 broth in BacT/ALERT 3D medium caused significant inhibition against *M. tuberculosis.* There was an average growth and percentage inhibition of 88% for MDR isolate DKU-156 and 66% inhibition for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37 Rv inhibition was 62% at 4% v/v concentration in BacT/ALERT 3D medium. There was no inhibition against rapid grower *M. fortuitum* (TCM-1529).

3.9 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of aqueous and alcoholic extracts of stem bark and leaves of *Tabebuia aurea* is shown in Table 9.

Table 9: Minimum Inhibitory Concentration (MIC) of aqueous and alcoholic extracts of stem bark and leaves of *Tabebuia aurea* against the MDR isolates DKU-156 and JAL-1236 of *Mycobacterium tuberculosis* (MIC is expressed as the lowest concentration of the extract that causes 99% inhibition of mycobacterium growth. All assays were run in duplicate and streptomycin (0.5 µg/mL, Sigma) was utilized as positive controls.

Samples	MIC (mg/ml)	
	MDR isolates of *M. tuberculosis.*	
	DKU-156	JAL-1236
a. Aqueous stem bark extract	10.25	10.75
b. Aqueous leaf extract	12.50	11.05
c. Alcoholic stem bark extract	17.25	13.25
d. Alcoholic leaf extract	15.15	14.50
Antibiotic Streptomycin	5.5	10.55

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

Antimicrobial activity of several members of the family Bignoniaceae has been determined by several workers (Fleischer *et al.,* 2003 [19]; Martinez and Valencia, 2003 [24]; Jin *et al.,* 2005 [20]; Rojas *et al.,* 2006 [26]; Aliyu *et al.,* 2009 [4]; Dutta and Choudhary, 2010 [17]; Ejelonu *et al.,* 2011 [10], Costa-Compos *et al.,* 2012 [16]; Chauhan and Chauhan, 2012 [12]; Agrawal and Chauhan 2015a; b) [1].

The growth-inhibiting activity of dried inner bark (taheebo) *Tabebuia impetiginosa* constituents against *Helicobacter pylori* ATCC 43504 was examined using paper disc diffusion and minimum inhibitory concentration (MIC) bioassays by Park *et al*. (2006) [25]. The activity of the isolated compounds was compared to that of the commercially available anti-*Helicobacter pylori* agents, amoxicillin, metronidazole, and

tetracycline. The biologically active components of dried inner bark of *Tabebuia impetiginosa* were characterized by spectroscopic analysis as 2-(hydroxymethyl) anthraquinone, anthraquinone-2-carboxylic acid, and 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (lapachol). With the paper disc diffusion assay 2- (hydroxymethyl) anthraquinone exhibited strong activity against *Helicobacter pylori* ATCC 43504 at 0.01 mg/disc. Anthraquinone-2-carboxylic acid, lapachol and metronidazole were less effective, exhibiting moderate anti-*Helicobacter pylori* activity at 0.1 mg/disc. Amoxicillin and tetracycline were the most potent compounds tested, displaying very strong activity at 0.005 mg/disc. 2-(Hydroxymethyl) anthraquinone exhibited moderate activity at this dose. Tetracycline still had strong activity at 0.001 mg/disc while amoxicillin had little activity at this dose. In the MIC bioassay, 2-(hydroxymethyl) anthraquinone (2_g/mL), anthraquinone-2-carboxylic acid (8 _g/mL), and lapachol (4 _g/mL) were more active than metronidazole (32 _g/mL) but less effective than amoxicillin (0.063 _g/mL) and tetracycline (0.5 _g/mL). The anti-*Helicobacter pylori* activity of seven 1,4-naphthoquinone derivatives (structurally related to lapachol), 1,4-naphthoquinone, 5,8-dihydroxy-1,4-naphthoquinone (naphthazarin), 2-methyl-1,4-naphthoquinone (menadione), 2-hydroxy-1,4-naphthoquinone (lawsone), 5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin), 5-hydroxy-1,4-naphthoquinone (juglone), and 2,3-dichloro-1,4-naphthoquinone (dichlone) was also evaluated using the paper disc assay. Menadione and plumbagin were the most potent compounds tested with the later still exhibiting very strong activity at 0.001 mg/disc. Menadione, juglone and tetracycline had strong activity at this low dose while the latter two compounds and amoxicillin had very strong activity at 0.005 mg/disc. Lawsone was unusual in that it had very strong activity at 0.1 and 0.05 mg/disc but weak activity at doses of 0.01 mg/disc and lower. Naphthazarin, lapachol and dichlone had similar activities while metronidazole had the lowest activity of all compounds tested. These results may be an indication of at least one of the pharmacological actions of taheebo.

According to Wagner *et al.* (1989) [30], the quinone pattern of the heartwood **(**80.85 % of the trunk) differs from that of the inner bark. Lapachol (orange needles mp 137-139 °C) is the major constituent of the heartwood together with other anthraquinones, while furanonaphthoquinones only occur in the inner bark (32). ß-Lapachone, crystallising with yellow needles (3, 4- dihydro 2, 2-O-dimethyl-2H naphtho-/1, 2-b/pyran-5, 6-dione) is a biologically very active compound. In an investigation from the year 2006 thirteen new phenolic glycosides could be found. Most of them have a glycosyl unit, esterified by a benzoic acid derivative. In a further comprehensive study about the constituents of the *Tabebuia impetiginosa* bark twelve compounds were evaluated, four iridoid glycosides, one phenylethanoid glycoside, five phenolic glycosides, one lignan and seven known compounds. Warashina *et al.* (2004) have isolated two aglycone moieties of the isocoumarin glycosides, four iridoid glycoside, two lignin glycoside, three phenyl ethanoid glycosides, and eight phenolic glycosides from the bark of *Tabebuia impetiginosa*.

The distribution and decarboxylation of iridoids in various members of the family Bignoniaceae including *Tabebuia* species have been described by von Poser *et al*. (1997, 2000). Two cyclopentene dialdehydes showing anti-inflammatory activity were isolated from the bark of *T. impetiginosa* (Koyama *et al*., 2000) [22]. The *Tabebuia impetiginosa* dried inner bark derived materials, particularly 2-(hydroxymethyl) anthraquinone, needs further study as potential *Helicobacter pylori* eradicating agents or lead compounds.

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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