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Anti-Mycobaterial Potential of *Crescentia cujete* (Bignoniaceae)

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Abstract: *In the present study antibacterial activity of aqueous and alcoholic extracts of stem bark and leaves of Crescentia cujete L. (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 collected between spring and summer season were dried and extracts was prepared using three portions of the dried powdered bark and leaves. It was soaked separately in 500 ml of distilled water and ethanol (98%) for 72 h and refluxed and filtrates were concentrated under vacuum at 40^{o} C to obtain the dry extracts. 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

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 (Annonymous 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. More than 70% of India's populations still use herbal drugs (Ayurveda, Yoga, Unani, Sidha, Homeopathy and Naturopathy).

Tuberculosis, MTB, or TB (short for *tubercle bacillus*) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* (Kumar *et al*. 2007). Tuberculosis typically attacks the lungs, but can also affect other parts of the body. Tuberculosis is a highly infectious disease with about one third of the world's population including 40 per cent from India estimated to be infected it (Anonymous 2011). India accounts for one third of the global tuberculosis burden of the world. 40% of the Indian population is infected with the TB bacillus. Every day more than 20,000 people get infected with tuberculosis, more than 5,000 people develop TB and more than 1,000 people die of TB in India. Tuberculosis thus continues to be the leading single infection cause of death (Katoch 2004).

Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties (Anonymous 2010). India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for cure of various diseases (Anonymous 2011). So far, few plants have been tested against mycobacteria and a few plants which showed anti-TB activity were *Salvia hypargeia, Euclea natalensis, etc*. (Anonymous 2009, 2010, 2011). Gupta *et*

al.(2010) carried a study to check the antibacterial activity of aqueous extracts of five plants (*Adhatoda vasica; Allium cepa; Aloe vera; Acalypha indica* and *Allium sativum*) against MDR isolates of *M. tuberculosis*, reference susceptible strain *M. tuberculosis* H37Rv as well as fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529).

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 (Alade & Irobi 1993), which possess antibacterial properties (Brantner & Grein 1994, Samy & Ignacimuthu 1998). Medicinal components from plants play an important role in conventional as well as in western medicine. Plant derived medicines have been a part of the evolution of human health-care for thousands of years. Plant based medicines were commonly used in India and China. 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 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 (Binuto & Lajubutu 1994, Binuto *et al*. 2000, Costantino *et al.* 1994, 2003a, b, Park *et al.* 2005, 2006a, 2006b, Rojas *et al.* 2006, Zaveri *et al.* 2007, Omonkhelin et al. 2007, Doughari et al. 2008, Das & Chaudhary 2010, Rinawati 2010). 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 argentia*).

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 *Crescentia cujete* (Bignoniaceae) 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 & METHODS

Plant Material used- Present study was carried out on *Crescentia cujete* plant growing in the Paliwal Park, Agra. Leaves and bark of above mentioned plants were collected between spring and summer season during March to May 2010.

Extract preparation- The plant extracts was prepared using the modified method of 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^{o} 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* 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 MacFarland 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), magesium 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 Dhar *et al.* (1968) and Rasadah & Houghton (1998). 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.

3. RESULTS & DISCUSSION

Antitubercular Potential-The antitubercular potential in the aqueous and ethnol extracts of stem bark and leaves of *Crescentia cujete* 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 *Crescentia cujete* were added on the L-J slants, BacT/ALERT media and extract free control L-J slants after 42 days of incubation at 37^0C 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 *Crescentia cujete* 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.

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

Table1. *Results of anti-tuberculosis assay using aqueous stem bark extract of Crescentia cujete 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
M. tuberculosis H37Rv	42	20	12	53	63
DKU-156	18	06	02	68	94
JAL-1236	70	25	21	60	65
M. fortuitum TCM-1529	02	02	02	00	00

The data in Table 1 shows clearly that addition of water extract of stem bark of *Crescentia cujete* 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 *Crescentia cujete* in BacT/ALERT 3D system: The effect of addition of aqueous bark extract of *Crescentia cujete* 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 Crescentia cujete 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

The results shown in the Table 2 indicate that the addition of aqueous extract of stem bark of *Crescentia cujete* 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 *Crescentia cujete* 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 *Crescentia cujete* in Lowenstein Jensen (L-J) medium: The effect of aqueous extract of leaf of *Crescentia cujete* in Lowenstein Jensen (L-J) medium is shown in Table 3.

Table 3. *Results of anti-tuberculosis assay using water leaf extract of Crescentia cujete 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	61	89
JAL-1236	71	26	23	58	62
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 *Crescentia cujete* 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 *Crescentia cujete* in Middlebrook 7H9 broth in BacT/ALERT 3 D system: The effect of water extract of leaf of *Crescentia cujete* 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 Crescentia cujete 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
M. tuberculosis H37Rv	40	20	10	51	60
DKU-156	18	9	4	65	85
JAL-1236	72	28	21	61	60
M. fortuitum TCM-1529	3	2	3	0	0

It is evident from the results shown in Table 4 that addition of water leaf extract of *Crescentia cujute* 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 *Crescentia cujete* in Lowenstein Jensen (L-J*)* medium: The effect of ethanol extract of stem bark 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 Crescentia cujete 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	61
DKU-156	19	8	3	66	91
JAL-1236	71	26	23	62	64
M. fortuitum 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 *Crescentia cujete* 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 *Crescentia cujete* in Middlebrook 7H9 broth in BacT/ALERT medium: The effect of ethanol extract of stem bark of *Crescentia cujete* 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 Crescentia cujete 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	41	22	11	50	61
DKU-156	19	8	3	66	88
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 6 addition of ethanol stem bark extract of *Crescentia cujete* 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 *Crescentia cujete* in Lowenstein Jensen (L-J*)* medium: The Effect of ethanol extract of leaf of *Crescentia cujete* in Lowenstein Jensen (L-J*)* medium is shown in Table 7.

Table 7. *Results of anti-tuberculosis assay using ethanol leaf extract of Crescentia cujete 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	42	20	10	52	62
DKU-156	20	9	4	65	89
JAL-1236	70	25	21	60	61
M. fortuitum TCM-1529	2	3	3	1	0

It is evident from the results shown in Table 7 that addition of ethanol leaf extract of *Crescentia cujete* 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 *Crescentia cujete* in Middlebrook 7H9 broth in BacT/ALERT in 3D system: Effect of ethanol extract of leaf of *Crescentia cujete* 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 Crescentia cujete 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
M. tuberculosis H37Rv	42	22	11	49	60
DKU-156	18	7	3	67	85
JAL-1236	70	27	22	62	61
M. fortuitum TCM-1529	3		1	0	0

It is evident from the results shown in Table 8 that addition of alcoholic extract of leaves of *Crescentia cujute* 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 various species of Bignoniaceae is shown in Table 9.

Table 9. *Minimum Inhibitory Concentration (MIC) of aqueous and alcoholic extracts of stem bark and leaves of Crescentia cujete.*

Samples	MIC (mg/ml)	
	MDR isolates of *M. tuberculosis.*	
	DKU-156	JAL-1236
Crescentia cujute		
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
Streptomycin	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 *Crescentia cujete* 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. Elelonu *et al.* (2011) have analyzed the chemical constituents of calabash tree (*Crescentia cujete*).

Antimicrobial activity of large number of plants including several members of the family Bignoniaceae has been determined by several workers (Otero *et al*. 2000, Lans *et al*. 2001, Fleischer *et al*. 2003, Kiokias & Gordon 2003, Pizzolatti *et al.* 2003, Martinez & Valencia 2003, Oyedeji *et al.* 2005, Rojas *et al.* 2006, Chauhan & Chauhan 2012). Bamuamba *et al.* (2008) have evaluated the anti-mycobacterial activity of *Olea capensis, Tulbaghia alliacea, Dittrichia graveolens, Leysera gnaphalodes and Buddleja saligna.* They are of ethnopharmacological relevance and used as traditional medicines in the Western Cape Province (South Africa) for anti-mycobacterial activity. Their aim was to assess antimycobacterial activity in plants used in treatment of symptoms of TB, and through activity-guided fractionation of extracts to isolate compounds or mixtures with potential as anti-TB drug leads. Extracts and derived fractions were assayed against strains of *Escherichia coli*, *Staphylococcus aureus*, and *Mycobacterium aurum* A+. Isolated pure compounds were further tested against *Mycobacterium* species *M. avium* ATCC 25291, *M. scrofulaceum* ATCC 19981, *M. microti* ATCC 19422 and *Mtb* H37Rv, and for cytotoxicity against Chinese hamster ovarian cells. Extracts of *B. saligna* and *L. gnaphaloides* exhibited significant anti-mycobacterial activity, primarily associated with the presence of non-cytotoxic triterpenoids oleanolic acid in *B. saligna* and both oleanolic and ursolic acids in *L. gnaphaloides*. It was concluded that anti-mycobacterial activity of extracts of selected plants is consistent with their traditional use. The identification of oleanolic and ursolic acids in these plants, and verification of their activity, underlines the potential for exploring structure-activity relationships of derivatives of these ubiquitous triterpenoids.

In the light of the results of the present study it is concluded that the aqueous extracts of stem bark of some other tree species (*Kigelia africana*, *Jacaranda mimosifolia*, *Millingtonia hortensis*, *Tabebuia argentia*, *Dolichandron* spp. and *Haplophragma* spp.) of the family Bignoniaceae should also be tested for their anti-mycobacterial activity.

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