

## Effect of *Lagerstroemia tomentosa* and *Diospyros virginiana* methanolic extracts on different drug-resistant strains of *Mycobacterium tuberculosis*

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### Abstract

*Mycobacterium tuberculosis* (MTB) is the causative agent of tuberculosis. The increasing incidence of multi drug resistance tuberculosis (MDR-TB) and extensively drug resistance tuberculosis (XDR-TB) worldwide highlighted the urgent need to search for alternative antimycobacterial agents. More and more people in developing countries utilize traditional medicine for their major primary health care needs. It has been determined that pharmaceutical plant, *Lagerstroemia tomentosa* and *Diospyros virginiana*, possesses some antibacterial effect. In this study, the antimycobacterial effects of *L. tomentosa* and *D. virginiana* methanolic extracts on sensitive and resistant isolates of MTB were examined. Leaf methanolic extract was prepared using methanol 70%. Sensitivity and resistance of isolates was determined by proportion method. The effects of two different methanolic extract concentrations (20 and 40 µg/ml) of the plants were examined against 6 sensitive and resistant strains of MTB with different patterns of drug resistance. MTB H37Rv (ATCC 27294) was set as control in all culturing and sensitivity testing processes. The results showed that *L. tomentosa* and *D. virginiana* methanolic extracts had weak inhibitory effect on different strains of MTB. The highest percentage of inhibition for *L. tomentosa* and *D. virginiana* was observed 38% and 33.3%, respectively.

**Keywords:** *Lagerstroemia tomentosa*; *Diospyros virginiana*; Methanolic extract; Antimycobacterial effect

### INTRODUCTION

*Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis (TB), is one of the most top killers in the world. In 2011, 8.7 million people afflicted with TB, including 1.1 million cases among people with HIV. It was estimated that 1.4 million people died from TB this year (1). In addition, today, the emergence of resistance to antimycobacterial agents has become an important public health issue in many developing countries.

The increasing incidence of multi drug resistance tuberculosis (MDR-TB) and extensively drug resistance TB (XDR-TB) worldwide highlights the urgent need to search for newer anti-tuberculosis compounds (2). More and more people in developing countries

utilize traditional medicine for their major primary health care needs (3). Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries (4). The scientific experiments which have been carried out on antimicrobial properties of plant components were first documented in the late 19th century (5). Plants have been a source of effective chemotherapeutic agents for various infectious diseases and there is a growing interest in the development of drugs of plant origin. A number of plants have been shown significant in vitro antimycobacterial activities (6). These findings have, therefore, stimulated further research towards the isolation of new antimycobacterial agents from natural products (6). *Lagerstroemia* commonly known as Crape

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myrtle or Crepe myrtle, is a genus of around 50 species of deciduous and evergreen trees and shrubs native to the Indian subcontinent, southeast Asia, northern Australia and parts of Oceania, cultivated in warmer climates around the world. It is a member of the Lythraceae, which is also known as the Loosestrife family (7,8). The extract of *Lagerstroemia speciosa* has shown antinociceptive, antidiarrhoeal and cytotoxic activities and is effective on *Arthrinium sacchari*, *Chaetomium funicola*, Gram positive and Gram negative bacteria (9-11). *L. parviflora* Roxb leaf extract has antipyretic and antitussive activity and is effective against bacterial species such as *Shigella dysenteriae*, *E.coli*, *Streptococcus pneumoniae*, and *Bacillus cereus* (12,13).

*Diospyros virginiana* continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use (14).

Till now antimycobacterial effect of these two plant species have not been determined. In this study the antimycobacterial activity of *L. tomentosa* and *D. virginiana* methanolic extracts on different drug resistant phenotype strains of MTB were evaluated.

## MATERIALS AND METHODS

### *Plant material*

Plant leaves from *L. tomentosa* were obtained from Al-Zohiriya garden, Giza, Egypt in May 2011 and authenticated by Department of Botany, National Research Center (NRC) and Consultant of Plant Taxonomy at the Ministry of Agriculture of Orman botanical garden, Giza, Egypt. Leaves from *D. virginiana* were obtained from the Agricultural Research Center, Giza, Egypt in May 2011 and authenticated by Department of Botany, and NRC.

### *Preparation of the extract*

Five hundred grams of air dried powdered from the leaves of *L. tomentosa* or *D. virginiana* was prepared and extracted separately with 6 liters of methanol 70% at room temperature 3 times until exhaustion by maceration. The extracts were concentrated

under reduced pressure to give 54 and 58 g of crude extract respectively.

### *Phytochemical analysis*

The plant extract was tested for the presence of bioactive compounds according to the following standard tests: Molisch's test for carbohydrates, Shinoda test for flavonoids, Forth test for saponins, Farnsworth test for coumarins, Salkowski 's test for terpenes and sterols, FeCl<sub>3</sub> and Mayer's reagents for detecting of tannins and alkaloids, respectively (15-17).

### *Mycobacterium strains*

A total isolates of MTB species were collected from Tuberculosis Centers of Isfahan. Isolates were subcultured on Löwenstein-Jensen (LJ) media and incubated for 21 days at 37 °C and characterized by conventional methods including staining, colony characteristics, pigmentation, growth temperature and time of growth.

### *Sensitivity evaluation of Mycobacterium tuberculosis*

Drug susceptibility testing was performed using the proportion method with isoniazid (0.2 µg ml<sup>-1</sup>), rifampin (40 µg ml<sup>-1</sup>), streptomycin (4 µg ml<sup>-1</sup>) and ethambutol (2 µg ml<sup>-1</sup>) (18,19). Briefly, the subcultures prepared in Lowenstein-Jensen media and incubated for 21 days. A standard suspension of 10<sup>7</sup> colony forming unit (CFU)/ml (equivalent to # 1 McFarland standard) of MTB isolates was prepared by dispersing of 3 to 5 colonies of Mycobacteria in distilled water. Subsequently, LJ medium without and with subjected antibiotics were prepared and inoculated with 0.2 ml of 10<sup>-2</sup> and 10<sup>-4</sup> dilutions of a McFarland 1.0 standard of each strains of MTB. The inoculated plates were then incubated for 42 days at 37 °C (first reading 28 days, second reading 42 days) and the percentage inhibition of CFU was determined (20). Resistance was defined as growth on drug containing tubes greater than 1% of the growth of drug free control medium for isoniazid, rifampin, ethambutol and 10% for streptomycin (21,22). MTB H37Rv (ATCC

27294) was set as control in all culturing and sensitivity testing processes.

### Determination of antimycobacterial activity

Antimycobacterial effects of *L. tomentosa* and *D. virginiana* on the resistant and sensitive isolates of MTB with two concentrations of the methanolic extract (20 µg/ml and 40 µg/ml) were determined according to the standard procedures (19).

Löwenstein-Jensen medium without and with subjected plants, were prepared and inoculated with 0.2 ml of  $10^{-2}$  and  $10^{-4}$  dilutions of a McFarland 1.0 standard of each isolate of MTB. Rifampin and isoniazid were used as the positive controls whereas water was used as negative control. MTB H37Rv (ATCC 27294) was used in sensitivity testing processes. The percentage of inhibition was determined after 3 to 6 weeks of inoculation by the following formula (23,24):

$$\% \text{Inhibition} = \frac{\text{Number of colony on medium} - \text{Number of colony on medium with extract}}{\text{Number of colony on medium}} \times 100$$

## RESULTS

The results showed that the leaf methanol

extract of *L. tomentosa* contained different compounds as triterpenes, carbohydrates, flavonoids, alkaloids, tannins and coumarins (Table 1). The leaf methanol extract of *D. virginiana* composed of compounds such as carbohydrates, flavonoids, tannins and triterpenes. Both plants were free of saponin (Table 1).

Several isolates of MTB species were identified by conventional biochemical and phenotyping methods. Drug susceptibility testing was performed using proportion method with isoniazid ( $0.2 \mu\text{g ml}^{-1}$ ), rifampin, ( $40 \mu\text{g ml}^{-1}$ ), streptomycin ( $4 \mu\text{g ml}^{-1}$ ) and ethambutol ( $2 \mu\text{g ml}^{-1}$ ) and 6 strains with different patterns of drug resistance were selected for the study. MTB H37Rv (ATCC 27294) was used as control in all processes. In Table 2, the resistance patterns of all MTB isolates are shown.

Antimycobacterial effect of 20 and 40 µg/ml methanolic extract against different drug resistant phenotype isolates of MTB were determined using proportion method. The results showed that *L. tomentosa* and *D. virginiana* extract had weak inhibitory effect against MTB strains. The inhibitory percentages of the extracts are shown in Table 3.

**Table 1.** Phytochemical analysis of *D. virginiana* and *L. tomentosa* methanol leaf extract.

Constituents	<i>D. virginiana</i> methanol leaf extract	<i>L. tomentosa</i> methanol extract leaf
Triterpenes and /or sterols	+	+
Carbohydrates and/or glycosides	+	+
Flavonoids	+	+
Coumarins	-	+
Alkaloids and/or nitrogenous compounds	-	+
Tannins	+	+
Saponins	-	-

+Presence of constituents; -Absence of constituents.

**Table 2.** Drug sensitivity of *Mycobacterium tuberculosis* isolates to rifampin, isoniazid, streptomycin and ethambutol.

Isolate	Rifampin	Isoniazid	Streptomycin	Ethambutol
1	R	R	R	R
2	S	S	S	S
3	R	R	S	S
4	R	R	S	S
5	R	S	S	S
6	R	R	R	S
H37Rv ATCC 27294	S	S	S	S

**R;** Resistant, **S;** Sensitive

**Table 3.** Effect of different concentration of *L. tomentosa* and *D. virginiana* methanol leaf extracts on drug sensitive and resistant *M. tuberculosis*.

Isolates	Percentage of inhibition for 20 µg/ml concentration of extract		Percentage of inhibition for 40 µg/ml concentration of extract	
	<i>L. tomentosa</i>	<i>D. virginiana</i>	<i>L. tomentosa</i>	<i>D. virginiana</i>
1	0	33.3	8.3	18.3
2	0	19.5	21.8	0
3	16.6	16.6	25	25
4	0	0	0	0
5	10	12.7	10.9	5.4
6	35.4	3.2	38	0
H37Rv ATCC 27294	0	20	22	0

## DISCUSSION

The prevalence of MDR strains of MTB is an important reason for the resurgence of TB as a major disease in many parts of the world. There is, therefore, an urgent need for new, inexpensive TB drugs which are effective and with fewer side effects. In many countries, medicinal plants are used by traditional medical practitioners to combat TB. Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries.

Chopra and coworkers reported that the bark of the *L. tomentosa* is considered stimulant and febrifuge, leaves and flowers are used as purgative (29). Kirtikar and Basu suggested that the roots are astringent (30). No previous biological and phytochemical examinations of *L. tomentosa* leaves have been undertaken.

In this study, the antimycobacterial activity of the *L. tomentosa* and *D. virginiana* methanolic extracts (20 and 40 µg/ml) on drug sensitive and resistant MTB strains was determined by proportion method according to standard procedures (18,31). The methanolic extracts of 2 plants showed a weak inhibitory activity against MTB strains (Table 3). This activity for *L. tomentosa* was ranged from very low (8.3 %) to moderate (38 %) on resistance strains of *M. tuberculosis*. Meanwhile, this activity for *D. virginiana* was observed from very low (3.2 %) to moderate (33.3 %) on strains of *M. tuberculosis*. These plants had similar activity on rifampin-isoniazid resistant strain of MTB (16.6 % for 20 µg/ml concentration and 25% for 40 µg/ml concentration). Percentage of inhibition for *L.*

*tomentosa* and *D. virginiana* on H37Rv ATCC 27294 was 22% at 40 µg/ml and 20 % at 20 µg/ml. The leaf extract of *L. tomentosa* was more effective at 40 µg/ml ( $P \leq 0.08$ ) whereas *D. virginiana* was more effective at 20 µg/ml concentration ( $P \leq 0.09$ ) (Table 3). Biological and phytochemical examinations of *D. virginiana* have not been undertaken but activities of other Diospyros species have been evaluated. Clement and Oluremi suggested that *Diospyros bateri* and *Diospyros monbuttensis* have shown strong antibacterial activity against a wide range of gram-positive and gram-negative bacteria (32,33).

Sunity Singh and coworkers have shown that the chloroform extract of root and bark of *Diospyros kaki* had remarkably good antifungal activity against all fungi (34). Renu Gupta and colleagues evaluated anti-tuberculosis activity of *Allium cepa* by proportion method. *Allium cepa* showed 35% inhibition. *L. tomentosa* compared with *Allium cepa* has stronger anti-tuberculosis activity (20). However, further studies on isolation and identification of the effective fractions of these two plants against MTB are warranted.

## CONCLUSION

The results of this study showed that the antimycobacterial effect of *L. tomentosa* plant extract on rifampin, isoniazid and streptomycin resistance strains of MTB is 35.4% for 20 µg/ml and 38% for 40 µg/ml concentration. The results of the study of *D. virginiana* and *L. tomentosa* antimycobacterial effect showed that different concentrations of their methanolic extract (20 and 40 µg/ml) had a weak inhibitory effect on sensitive and

resistant phenotype of MTB. Further studies on isolation and identification of the effective fractions of these two plants against MTB are warranted.

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