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Biological Activity of Extracts from *Morus alba* L., *Albizzia lebbeck* (L.) Benth. and *Casuarina glauca* Sieber Against the Growth of some Pathogenic Bacteria

Mohamed Z.M. Salem[1*], Hussein Aly[1], Yousry Gohar[2] and Abdel-Wahab El-Sayed[1]

[1]Department of Forestry and Wood Technology, Faculty of Agriculture (El–Shatby), Alexandria University, Egypt

[2]Botany Dept., Microbiology Section, Faculty of Science (El-Shatby), Alexandria University, Alexandria, Egypt

Abstract

Different extracts from sapwood, heartwood and bark from *Morus alba*, *Albizzia lebbeck* and *Casuarina glauca* were used as an antibacterial agent against the growth of some pathogenic bacteria. Methanol (80%) was used for the extraction and ethyl acetate, *n*-butanol, chloroform and water were used for the subsequent partitioning. The *in vitro* antibacterial activity was performed by disc diffusion and 96-well micro-plates methods. The antibacterial activity was compared with standard antibiotics. The ethyl acetate fraction (EtOAc fr) of wood and bark exhibited the strongest antibacterial activity against the studied bacterial strains. The most active extracts were observed by *M. alba*. The extracts of *A. lebbeck* and *C. glauca* showed a weak to moderate activity against the tested bacterial strains. These results are of interest since they have been obtained with methanol extract and its fractions dissolved into different solvents, and are not a pure product. It could be considered that the extracts of *M. alba*, *A. lebbeck* and *C. glauca* have a good potency level of antibacterial activity. The obtained results might be considered sufficient for further studies about the isolation and identification of active principles compounds especially from *C. glauca*.

Keywords*: Morus alba, Albizzia lebbeck, Casuarina glauca, sapwood, heartwood, antibacterial activity, methanol extract, bark*

1. Introduction

Natural products are gaining a revitalized attention in medical community and their therapeutic uses are gradually increasing. As many synthetic drugs have revealed serious side effects. Therefore, a better strategy is to look for natural substances with strong pharmacological action and less cytotoxicity. In the last few years much attention was directed to the potential health promoting properties of phytochemical compounds (Kartal, 2007).

Morus alba L., known as white mulberry, is a short-lived, fast-growing, small to medium sized

* Corresponding author: zidan_forest@yahoo.com

tree, native to northern China and is widely cultivated and naturalized elsewhere. Mulberry trees especially *M. Alba* is a widely found plant in Egypt. It is a wild plant available all over the year and found in a large amount in Beni-Suef governorate mainly in Beni-Suef villages (Hussein et al., 2010). *M. alba* has garnered great attention for its antioxidative and antidiabetic effects and is an important ingredient of herbal tea (Lokegaonkar and Nabar, 2011). Recent studies have shown *M. alba* has antioxidant, antibacterial, antiviral and anti-inflammatory properties (El-Beshbishy et al., 2006; Chung et al., 2003). The plant is reported to contain the main active principles phytoconstituents like; tannins, phytosterols, sitosterols, saponins, triterpenes, flavanoids, benzofuran derivatives, morusimic acid, anthocyanins, anthroquinones, glycosides and oleanolic acid as the (Chen et al., 2005). Some phenolic compounds (flavonoids, stilbenes and 2-arylbenzofurans) have been reported from *M. Alba* and have been known to show antimicrobial (Yogisha and Raveesha, 2009).

Albizzia lebbeck (L.) Benth., is a deciduous tree belonging to family Fabaceae with an unarmed deciduous tree of 12-21 m, compound leaves, flat oblong fruits, round cream colored seeds and grow wild (Hussain et al., 2008). The bark is anthelmintic and used in the treatment of inflammation, bronchitis, toothache and leprosy (Rashid et al., 2003) and mainly used in dental infections. Previously studies showed that the plant contains phenolic glycosides, alkaloids, terpenoids, steroids, tannins, saponins, anthraquinones, flavonos and other phenolics (Rahul et al., 2010).

Casuarina glauca Sieber, commonly known as the swamp she-oak, is a species of the family Casuarinaceae. *Casuarina* species are tropical and subtropical trees native to Australia, southeastern Asia and islands of the western Pacific Ocean. Among other uses, *Casuarina* trees are widely used as windbreaks for rehabilitating and stabilizing dunes, as ornamental trees and for timber and firewood production (El-Lakany, 1983). Most of the studies on the antibacterial activity of the family Casuarinaceae were done using *C. equisetifolia*, and the recent report has demonstrated antibacterial activity of a *C. equisetifolia* against a variety of bacterial species (Ahsan et al., 2005). Additionally, previous studies reported that the polyphenols and condensed tannins (casuarictin, casuarinin and casuariin) were found in the branchlets of *C. glauca* (Shimokawa et al., 1991; Okuda et al., 1983).

In the present study the methanolic extract and its partitioning into different solvents of sapwood, heartwood and bark of *Morus alba*, *Albizzia lebbeck* and *Casuarina glauca* were evaluated as antibacterial agents against the growth of *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* ATCC 13880 and *Agrobacterium tumefaciens* ATCC 33970. The suggested chemical components from the most active fractions were analyzed using GC-MS.

2. Materials and Methods

Tree Material

The wood and bark of *M. alba*, *C. glauca* and *A. lebbeck* used in the present study were collected from different locations at Alexandria City, Egypt. Plant vouchers were identified and stored for deposition at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University.

Preparation of Extracts

Different parts of *M. alba*, *C. glauca* and *A. lebbeck* were air-dried under room temperature for at least one week, grounded to fine powder, placed on polyethylene bags and kept away from moisture in a dried place until use. Powdered plant materials (50 g) were extracted with 500 mL of methanol (80%) for 8 hrs using Soxhlet extractor apparatus at 40 °C and the solvent was evaporated under reduced pressure. The extracts were lyophilized, weighed and stocked in sealed vials at 4 °C until use. The weight of the extract was calculated per one kilogram of oven-dry weight of plant material (g/kg o.d.).

The methanol (MeOH) extract from each part was subsequently partitioned using separating-funnel into ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH) and water (Aq). These fractions were separated with solvent/solvent group separation procedure used by the USA National Cancer Institute separation procedure (Aly et al., 2012; Suffness and Douros, 1979).

On the other hand, samples of about 3.6 g from the lyophilized MeOH extract were dissolved in 50mL of 99% MeOH and treated with an equal volume of 1% aqueous HCl and the alkaloids were precipitated by drop-wise addition of 10% NH_4OH. The precipitate was collected by centrifugation (5000 rpm at 4 °C for 30 min) and washed with 1% NH_4OH. The residue was then dissolved in a few drops of chloroform ($CHCl_3$) to obtain the precipitated alkaloids (Harborne, 1973). The solvents were completely evaporated at 40 °C by rotary evaporator.

The MeOH extracts were subjected to qualitative phytochemical analysis for the presence of alkaloids, tannins, Phenols, saponins, steroids and flavonoids (Harborne, 1973).

Antimicrobial Assay

Bacterial Strains

Three gram-positive bacteria; *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538; three gram-negative bacteria: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Serratia marcescens* ATCC 13880 were used in the present study. The previous human pathogenic bacteria were supplied from the Division of Microbiology, Faculty of Science, Alexandria University, Egypt. Furthermore, the Gram-negative bacterium; *Agrobacterium tumefaciens* ATCC 33970, a plant pathogen was provided by the Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt.

Disc Diffusion Assay

Kirby-Bauer method was used for disc diffusion method (NCCLS, 1997), with minor modification. Culture medium was used to maintain *A. tumifaciens* was (g/L, beef extract 5.0, yeast extract 1.0, peptone 5.0, sucrose 5.0 and $MgSO_4$ 0.4, pH 7.2). Nutrient agar (NA) medium was used for maintenance of the other bacterial test organisms. Mueller Hinton Agar (MHA) medium was used for screening the antibacterial activities applying the disc diffusion method and then it was prepared and autoclaved.

The MHA plates were prepared by pouring about 15 mL of melted media into sterile Petri dishes to a depth of 5.0 mm and allowed to solidify for 5 min. From a pure culture; five colonies of the organism to be tested were transferred with a wire loop to a test tube containing 4 ml of Mueller Hinton broth (MHB). All five colonies had similar morphology and were picked successively. The tubes were incubated at 37 °C for 2 to 5 h to produce a moderately cloudy suspension.

Using sterile cotton swabs, 0.5 mL of fresh 24-hours old bacterial suspension were spread over the surface of MHA plates, the surface allowed drying. Sterile plain discs of 4 mm diameter (Whatman filter paper no. 1) were placed on the surface of agar plates and each disc was loaded with 30 µL of the concentrated extract (200 µg/mL) dissolved in dimethylsulfoxide (DMSO, E. Merck, Germany) except EtOAcfr was dissolved in propylene glycol. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 h. Negative control was prepared using respective solvent. Clindamycin (2 µg/disc), novobiocin (30 µg/disc), cephalothin (30 µg/disc) and tetracycline (30 µg/disc) were used as positive controls with the respective bacteria. At the end of incubation, the inhibition zone (IZ) formed around the disc was measured in millimeter with transparent ruler. These studies were performed as an average of triplicate.

Minimum Inhibitory Concentrations (MICs) Assay

The extracts were subjected to a serial dilution (4, 8, 16, 32, 56, 128, 256 and 512 µg/mL). This was performed in 96-well micro-plates (Eloff, 1998; Elansary et al., 2012) by filling all wells, with 50µL sterile MHB with minor modification. Two wells were used as sterility and growth control respectively with the sterility control containing only Oxoid® MHB, whilst the growth control containing both MHB as well as test organism. After adding 50 µL of the bacterial suspension (10^5 CFU/mL) to each row (except for the sterility control), the micro-plate was covered and incubated overnight at 37 °C at 100% relative humidity. The following morning 50 µL of a 0.2mg/mL solution of *p*-iodonitrotetrazolium violet (INT, Sigma-Aldrich) was added to each well and the plate was returned to the incubator for at least half an hour to ensure adequate color development.

For the determination of MICs values; the HME, *n*-BuOH fr, $CHCl_3$ fr and Aq fr were dissolved in

DMSO and EtOAc fr was dissolved in propylene glycol. The stock solution was made up of a concentration of 512 µg/mL using distilled water. Inhibition of the growth was indicated by a clear solution or a definite decrease in color reaction. This value was taken as the MIC of the extract or the lowest concentration that completely inhibited macroscopic growth of bacteria. Extracts with a MIC value of <125 µg/mL were considered active.

Antibiotic Resistance of Test Bacterial Strains

Antibiotic sensitivity of test strains was determined using the standard disc diffusion against a number of antibiotics. The efficacy of antibiotics per disc was as follows, clindamycin (2 µg/disc), novobiocin (30 µg/disc), cephalothin (30 µg/disc), and tetracycline (30 µg/disc).

Chemical Constituents of Fractionated Extracts by Gas Liquid Chromatography/Mass Spectrometry (GC-MS) Apparatus

Accordingly, identification of the chemical constituents of fractionated extracts were made using HB5890 gas liquid chromatography (GLC) coupled with HB5890B series mass spectrometer (MS) at Central Lab. Unit in High Institute of Public Health, Alexandria, Egypt. The gas liquid chromatography was equipped with a split less injector at 280 ºC, and a flame ionization detector (FID) was held at 300 ºC using helium as a carrier gas. Samples were separated on a capillary column (30 m long, and 0.25 mm internal diameter) HP-5 (Avondale, PA, USA) of 0.25 µm film thickness. The temperature of the gas chromatograph column was programmed from 80 ºC to 100 ºC at a heating rate of 15 ºC/minute, and then increased to a maximum final temperature of 310 ºC at a heating rate of 5 ºC/minute, holding the maximum final temperature for a residence time of 10 minutes. The temperature of ion source in the mass spectrometer was held at 200ºC. All mass spectra were recorded in the electron impact ionization (EI) at 70 electron volts. The mass spectrometer was scanned from m/z 40 to 410 at a rate of two scans per second. An integrator automatically calculated peaks areas. Neither internal nor external chemical standers were used in this chromatographic analysis. Interpretation of the resultant mass spectra were made using a computerized library searching program, and by studying the fragmentation pattern of such compound which resulted from mass spectrometry analysis.

3. Results and Discussion

Quantification and Phytochemical Analysis of Extracts

The quantity of HME and its fractions (EtOAc, *n*-BuOH, $CHCl_3$ and Aq) from sapwood, heartwood, and bark of three tree species are presented in Table 1. The tree parts showed to have different amounts of HME, EtOAc, *n*-BuOH and Aq. By contrast, the $CHCl_3$ which contains alkaloids was not detected in some parts of trees. For example, sapwood of *M. alba* had 4.73 g/kg o.d. of $CHCl_3$, whereas heartwood and bark didn't contain it. The phytochemical analysis of HME, presented in Table 2, revealed the presence of alkaloids, steroids, saponins, tannin and steroids.

Antibacterial Activity of Extracts

The most effective extracts are shown in underline values (Table 3). The values of MICs of different extracts against the growth of *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. marcescens* and *A. tumefaciens* are presented in Tables 4-6. The HME had shown better antibacterial activity compared to its partitioning. Among the partitions, the EtOAc had the highest activity against the tested bacteria. As shown in Table 3, the HME and EtOAc from heartwood and HME of bark from *M. alba* had the highest activity against the growth of *B. cereus* with IZs 21, 25 and 22 mm, respectively and these values were over the antibiotic used (17mm with clindamycin).

The most effective extracts against the growth of *B. subtilis* was presented by *M. alba*; heartwood (HME with 22.3 mm and EtOAc with 19.6 mm), sapwood (HME with 23 mm and EtOAc with 20mm) and bark (25.3 mm with HME), heartwood of C. *glauca* (*n*-BuOH with 17.8 mm).

Furthermore, these values had higher inhibition zone than positive control used (17 mm with novobiocin).

Table 1

Quantities of different extracts from *M. alba*, *C. glauca* and *A. lebbeck*

Timber tree	Part	Extract (g/kg o.d.)[a]				
		HME	**EtOAc fr**	***n*-BuOH fr**	**$CHCl_3$ fr**	**Aq fr**
M. alb	Heartwood	97.00	67.53	3.78	-	10.29
	Sapwood	57.89	14.50	5.82	4.73	3.74
	Bark	169.04	97.96	12.25	-	0.40
C. glauca	Heartwood	64.38	3.01	3.03	1.35	15.74
	Sapwood	24.74	4.15	2.41	0.34	6.36
	Bark	168.47	7.12	54.19	9.63	50.46
A. lebbeck	Heartwood	81.23	17.94	29.04	5.52	18.97
	Sapwood	48.37	2.89	22.64	-	5.90
	Bark	130.48	17.18	60.38	2.90	13.75

[a] Based on oven dry weigh.
(-): No extracted material by this solvent

Table 2

Qualitative phytochemical analysis of methanol extracts

Timber tree	Part	Phytochemical group					
		Flavonoids	**Phenols**	**Saponins**	**Alkaloids**	**Tannins**	**Steroids**
M. alba	Heartwood	+	+	+	-	+	-
	Sapwood	+	+	+	+	+	+
	Bark	+	+	+	-	+	+
C. glauca	Heartwood	+	+	+	+	+	+
	Sapwood	+	+	+	+	+	-
	Bark	+	+	+	+	+	+
A. lebbeck	Heartwood	+	+	+	+	+	+
	Sapwood	+	+	+	-	+	+
	Bark	+	+	+	+	+	+

+ Detected.
- Not detected.

Some of the extracts from *M. alba* showed a good activity against the growth of *S. aureus* and higher than or equal to the reference antibiotic (22 mm with cephalothin) used (Yogisha and Raveesha 2009). The activity was higher in heartwood (HME with 22.3 mm and EtOAc with 22 mm), sapwood (HME with 23 mm) and bark (HME with 22 mm). Additionally, the EtOAc from heartwood, sapwood and bark from *M. alba* had the highest effect against the growth of *P. aeruginosa* with IZs of 12.3, 11 and 10 mm, respectively, whereas these IZs were lower than the values from the antibiotic used (36 mm with tetracycline). Furthermore, the extracts from *M. alba* (heartwood HME with 20.1mm and EtOAc from sapwood and bark with 17 and 20 mm, respectively) showed good activity against the growth of *S. marcescens*.

The most effective extracts against the growth of *E. coli* were presented by *M. alba* (the HME and EtOAc from heartwood with 25.3 and 20.3 mm and sapwood with 20.3 and 21 mm, respectively, and bark HME with 21.3 mm). On the other hand, the highest activity was shown by the leaves HME from *C. glauca* (26.3 mm). These IZs were lower than the values from the antibiotic used (18 mm with clindamycin). Additionally, the HME and EtOAc from heartwood with 20 and 18 mm and bark with 21 and 17.4 mm, respectively, from *M. alba* presented the highest activity against the growth of *A. tumefaciens*.

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Table 3
Antibacterial activity of different extracts from *M. alba*, *C. glauca* and *A. lebbeck*

Tree species	Bacterial strain	IZ (mm) Heartwood					Sapwood					Bark					Negative control [b]	Positive Control [c]
		HME	EtOAc	*n*-BuOH	$CHCl_3$	Aq	HME	EtOAc	*n*-BuOH	$CHCl_3$	Aq	HME	EtOAc	*n*-BuOH	$CHCl_3$	Aq		
M. alb	*B. cereus*	21	25	12.5	-	na	17.6	16.3	na	na	8	22	15.2	na	-	na	na	12
C. glauca		na	9.5	7	na	na	9	9.5	8	na	na	8	na	na	na	7		(CC)
A. lebbeck		13.4	na	13	na	na	na	na	na	-	na	na	6.5	9	na	8		
M. alb	*B. subtilis*	22.3	19.6	16.3	-	na	23	20	6	na	na	25.3	15.2	8	-	7	na	17
C. glauca		na	9.6	17.8	13	na	12	7.4	17	na	6	9.6	9	8	12	na		(NV)
A. lebbeck		12.3	na	9	na	na	na	11	na	-	na	16.3	7.1	8	na	na		
M. alb	*S. aureus*	23	22	6.4	-	12.5	23	16.3	na	na	na	22	19	10.5	-	na	na	22
C. glauca		na	13	17	13	na	10.6	na	14.2	na	na	na	8.5	na	na	na		(KF)
A. lebbeck		9.6	NA	na	na	na	na	na	na	-	na	9	15.3	8	na	10		
M. alb	*P. aeruginosa*	8	12.3	na	-	na	na	11	na	na	na	na	10	na	-	na	na	36
C. glauca		na	NA	na	na	na	na	na	na	na	na	na	na	na	na	na		(TE)
A. lebbeck		7	NA	na	na	na	8	na	na	-	na	9.6	8	na	na	na		
M. alb	*S. marcescens*	20.1	15	9.4	-	na	na	17	na	na	na	8.4	20	na	-	na	na	n.s.
C. glauca		na	na	14.6	na	na	11.2	na	12.4	na	na	na	na	na	na	na		
A. lebbeck		na	na	na	na	na	na	na	na	-	na	7	na	na	na	na		
M. alb	*E. coli*	25.3	20.3	7.4	-	na	20.3	21	na	na	na	21.3	14.2	na	-	na	na	18
C. glauca		na	7.5	12.2	na	na	na	6.2	12.2	na	na	9.5	na	na	na	na		(CC)
A. lebbeck		12.5	na	6.4	na	na	9.6	na	na	-	na	na	na	na	na	na		
M. alb	*A. tumefaciens*	20	18	9	-	na	16.3	21	na	6.2	na	21	17.4	8	-	na	na	n.s.
C. glauca		na	8.2	13	12.5	na	na	8	na	7	na	10.3	7	na	na	na		
A. lebbeck		15.2	13	na	na	na	na	na	na	-	na	na	7.5	11	na	na		

a. Values are mean of 3 replicates including the 5 mm diameter of disc at 200 µg/mL of the concentrated extract.
b. Negative control was prepared using respective solvent
c: CC (2 µg clindamycin/disc), NV (30 µg novobiocin/disc), KF (30 µg cephalothin (Keflin)/disc), TE (30 µg tetracycline/disc).
na: Not active at 200 µg/mL concentration.
(-): No extracted material by this solvent
n.s.: not tested

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Table 4

Minimum inhibitory concentrations (MICs) values of extracts from *M. alba* against seven strains of bacteria

Part	Extract	MIC (μg/mL)						
		B. subtilis	***B. cereus***	***S. aureus***	***P. aeruginosa***	***S. marcescens***	***E. coli***	***A. tumefaciens***
Sapwood	HME	16	32	32	>512	>512	16	64
	EtOAc	16	32	32	128	32	16	16
	n-BuOH	>512	>512	>512	>512	>512	>512	>512
	$CHCl_3$	256	>512	>512	>512	>512	>512	128
	Aq	>512	>512	>512	>512	>512	>512	>512
Heartwood	HME	16	32	16	256	32	16	32
	EtOAc	16	16	16	128	32	16	32
	n-BuOH	64	128	64	>512	256	64	256
	$CHCl_3$	-	-	-	-	-	-	-
	Aq	>512	>512	>512	>512	>512	>512	>512
Bark	HME	16	32	32	>512	256	16	64
	EtOAc	16	16	16	8	16	16	16
	n-BuOH	256	>512	256	>512	>512	>512	256
	$CHCl_3$	-	-	-	-	-	-	-
	Aq	>512	>512	>512	>512	>512	>512	>512

Table 5

Minimum inhibitory concentrations (MICs) values of extracts from *A. lebbeck* against seven strains of bacteria

Part	Extract	MIC (μg/mL)						
		B. subtilis	***B. cereus***	***S. aureus***	***P. aeruginosa***	***S. marcescens***	***E. coli***	***A. tumefaciens***
Sapwood	HME	64	256	128	>512	64	>512	>512
	EtOAc	256	256	>512	>512	>512	>512	256
	n-BuOH	32	256	64	>512	128	256	256
	$CHCl_3$	>512	>512	>512	>512	>512	256	256
	Aq	>512	>512	>512	>512	>512	>512	>512
Heartwood	HME	>512	>512	>512	>512	>512	>512	>512
	EtOAc	64	128	1 28	>512	>512	128	256
	n-BuOH	64	256	32	>512	1 28	64	32
	$CHCl_3$	>512	>512	64	>512	>512	64	256
	Aq	>512	>512	>512	>512	>512	>512	>512
Bark	HME	256	64	>512	>512	>512	256	128
	EtOAc	>512	>512	256	>512	>512	>512	256
	n-BuOH	>512	>512	>512	>512	>512	>512	>512
	$CHCl_3$	>512	>512	>512	>512	>512	>512	>512
	Aq	>512	>512	>512	>512	>512	>512	>512

Table 6

Minimum inhibitory concentrations (MICs) values of extracts from *C. galauca* against seven strains of bacteria

Part	Extract	MIC (µg/mL)						
		B. subtilis	***B. cereus***	***S. aureus***	***P. aeruginosa***	***S. marcescens***	***E. coli***	***A. tumefaciens***
Sapwood	HME	>512	>512	>512	256	>512	128	>512
	EtOAc	128	>512	>512	>512	>512	>512	128
	n-BuOH	>512	>512	>512	>512	>512	>512	>512
	$CHCl_3$	-	-	-	-	-	-	-
	Aq	>512	>512	>512	>512	>512	>512	>512
Heartwood	HME	32	256	32	64	>512	<0.5	>512
	EtOAc	>512	>512	>512	>512	>512	>512	>512
	n-BuOH	>512	64	128	>512	>512	256	128
	$CHCl_3$	>512	>512	>512	>512	>512	>512	>512
	Aq	>512	>512	>512	>512	>512	>512	>512
Bark	HME	16	>512	32	16	32	>512	>512
	EtOAc	32	256	32	256	>512	>512	256
	n-BuOH	256	256	256	>512	>512	>512	32
	$CHCl_3$	>512	>512	>512	>512	>512	>512	>512
	Aq	256	>512	128	>512	>512	>512	32

The MICs presented in Tables 4-6 were viewed in bold and underline. Table 4 observed that the HME from heartwood and EtOAcfr from sapwood, heartwood, and bark of *M. alba* had the highest antibacterial activity against all of tested bacterial strains at the lower concentrations (MIC around 16 µg/mL). In addition, it was found that the HME from bark and *n*-BuOH from heartwood had a good antibacterial effect on the growth of tested bacteria except *P. aeruginosa*.

Our results revealed that the MeOH extract from *M. alba* had a wider range of activity on the test organisms with high zone of inhibition when compared to the standard antibiotics (Omidiran et al., 2012). From the previous results, the ethanolic extracts from *M. alba* exhibited moderate antimicrobial activities, whereas the aqueous extracts showed poor antimicrobial properties (Wang et al., 2012) and the ethanol with methanol fraction highly inhibited the growth of *S. aureus* (Moorthy and Boominathan, 2011). Additionally, the alcoholic extract of the stem was found to be more effective with all the tested bacteria (Babu and Ammani, 2009). Moreover, the maximum antibacterial activity of methanolic extract was shown by M. *alba* (Koppula et al., 2010).

The extracts from *A. lebbeck* showed a variety of antibacterial activity. The HME from heartwood showed good activity at lower MICs values ranged between 32 and 64 µg/mL. The methanol extract of bark exhibited good activity against *E. coli* (Uma et al., 2009). Other reports found that, only 100 mg /mL concentration or ethanol extract of *A. lebbek* displayed antibacterial activity against *E. coli* and this activity may be due to presence of flavone derivatives (Khan et al., 2010). On the other hand, the total glycosides, cardenolide glycosides, anthraquinone glycosides isolated from the stem bark revealed antimicrobial activity against the test cultures of some bacterial strains. The mechanism of action of the active principles showed that the glycosides caused leakage of cytoplasmic constituents (Ganguli and Bhatt, 1993).

Furthermore, it has also been shown that *A. lebbeck* has moderate activity against *B. subtilis* and *E. coli* (Acharyya et al., 2009) with MIC ranging from 0.125 to 32 mg/mL.

Data from Table 6 presented that the extracts from *C. glauca* had a weak activity against all the tested bacteria. Among them, the HME and EtOAc from heartwood displayed good activity against the growth of the tested bacteria. The antibacterial activity could be related to polyphenols and

condensed tannins (casuarictin, casuarinin and casuariin) that were found in the branchlets of *C. glauca* (Shimokawa et al., 1991; Okuda et al., 1983).

These results are of interest since they have been obtained with methanol extracts and were partitioned into some solvents, and are not a pure product. It could be considered to have a good potency level and it is possible to conclude that methanolic extracts of *Morus alba*, *Albizzia lebbeck* and *Casuarina glauca* had different level of antibacterial activity. The obtained results might be considered sufficient for further studies for the isolation and identification of the active principles especially from *Casuarina glauca*. Furthermore, it was cleared that the extracts from heartwood were well known in phytochemical studies and antibacterial screening of the extracts, and could represent an interesting perspective for significant complete inhibiting of bacterial (Lee et al., 2000).

Of the investigated 7 bacterial strains, the more sensitive microorganisms were *B. cereus*, *E. coli* and *S. aureus*, while the most resistant ones were *Pseudomonas* spp. (Gómez-Estaca et al., 2010). These results could be attributed to the cell wall lipopolysaccharides of gram-negative bacteria, which may prevent active components from reaching the cytoplasmic membrane (Ouattara at al., 1997).

The strains which presented the biggest inhibition zones (diffusion method) are not always the most sensitive (value of MIC was lower) because the diameter of IZs does not reflect the antibacterial activity of a compound (Hernández et al., 2005). Moreover, the IZ values could be affected by the solubility of the oil, the diffusion range in the agar, the evaporation (it can affect the dose), etc. (Cimanga et al., 2002).

Overall, the ethyl acetate fractions from *C. glauca* heartwood and *M. alba* bark had the highest antibacterial activates, so that these fractions were subjected to GC-MS analysis to obtain the suggested chemical compounds as shown below.

Table 7

Suggested chemical compounds present in ethyl acetate fraction extracted from *C. glauca* heartwood by using GC-MS.

Compound	Retention time (min)	MW[A]	Area %[B]
Formic acid,1-methylethyl ester	3.78	88	25.46
Propene 3,3,3-D3	3.88	45	9.81
1,2-Benzenediol or Pyrocatechol	4.73	110	13.73
n-Pentanal	7.26	86	2.76
Unknown	7.37	169	4.02
2,5-Dimethoxyamphetamine	8.20	196	6.34
Benzyl benzoate	8.38	212	23.94
1,2-Benzenediol, [4-(1-hydroxy-2-methyl amino) ethyl]-,(R)	8.65	183	1.63
2,4-(1H,3H)-Pyrimidinedione, 5-nitro	9.38	157	5.90
Benzeneethanamine, 2-fluoro- beta	9.70	185	6.41

(A): Molecular Weight of the compound.
(B): Based on total areas of the identified peaks.

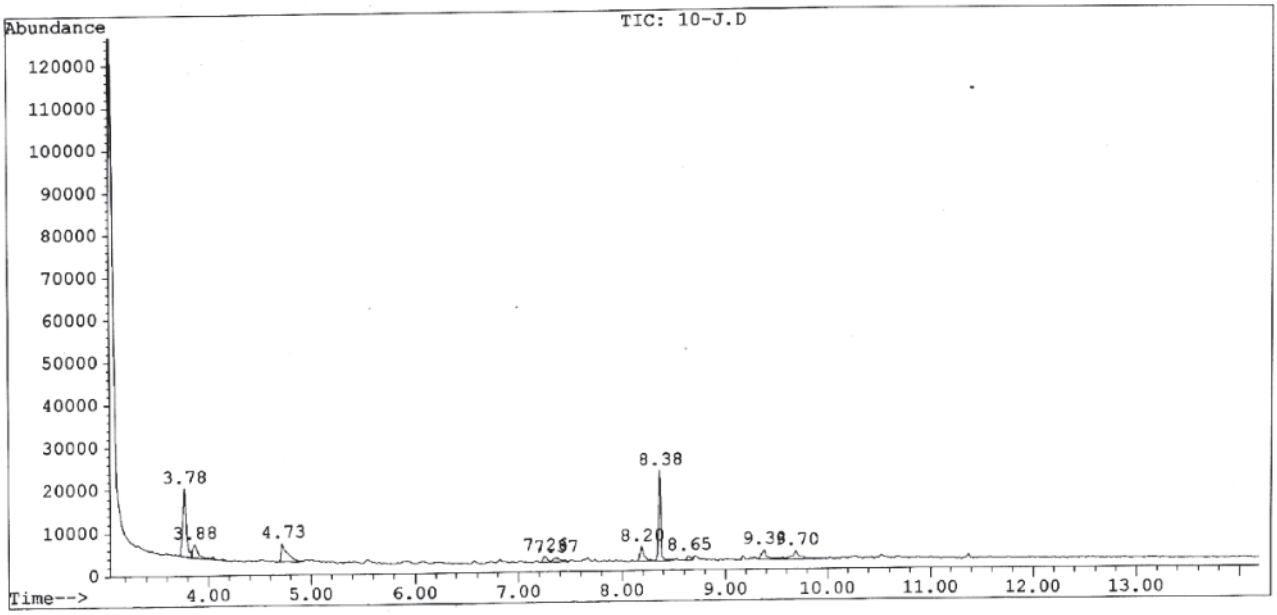


Figure 1: Chromatogram of chemical components presents in ethyl acetate fraction extracted from *C. glauca* heartwood.

Table 8

Suggested chemical compounds present in ethyl acetate fraction extracted from *M. alba bark* by using GC-MS.

Suggested compound	Retention time (min)	MW[A]	Area %[B]
1,3,8,10-Tetraoxaperlo (3,4-cd: 9,10cd) dipyran	3.87	392	1.11
Resorcinol or 1,3-benzenediol	5.22	110	34.40
Unknown	5.54	154	2.59
Cyclobutanol	5.73	72	5.22
Unknown	8.64	44	2.82
2-Heptanol,6-amino-2-methyl	8.91	145	4.45
2-Octanamine	9.13	129	2.34
L-Alanine,N- (N-acetyl-L-alanyl)-butyl ester	9.38	258	4.87
2-Amino-1-propanol	9.87	75	5.65
Heptaminol	10.68	145	2.59
Xanthatin,8-[4-[(aminocarbonyl) methoxy] phenyl]-1,3-dipropyl	11.61	385	5.87

(A): Molecular Weight of the compound.
(B): Based on total areas of the identified peaks.

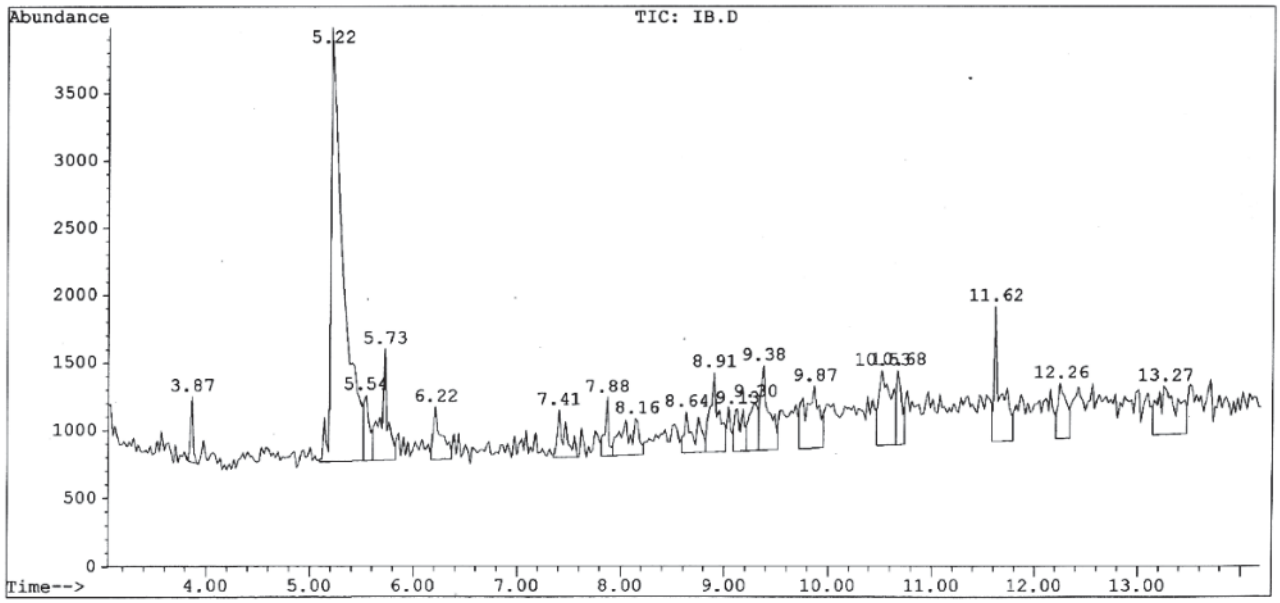


Figure 2: Chromatogram of chemical components presents in ethyl acetate fraction extracted from *M. alba* bark.

Suggested Chemical Components in the Ethyl Acetate Fraction Extracted from *C. glauca* Heartwood

C. glauca is the most common species cultivated in different environmental conditions in Egypt. That is widely used as windbreaks, shelterbelts, and roadsides. Table 7 and Figure 1 are showing the chemical compounds in the ethyl acetate fraction from *C. glauca* heartwood and had 10 components. Formic acid, 1-methylethyl ester was the highest component in the EtOAc fr. from the heartwood of *C. glauca* with the yield (25.46%).It was also found that the EtOAc fr. contains the benzyl benzoate in a high value (23.94%) and 1,2-benzenediol or pyrocatechol in a yield of 13.73%.

Suggested Chemical Components of The Ethyl Acetate Fraction Extracted From *M. Alba* Bark

The suggested chemical components of the ethyl acetate fraction from the bark of *M. alba* had 11 components (Table 8 and Figure 2). The highest yield was found for resorcinol or 1,3-benzenediol (34.40%). The acid hydrolysis of this component was associated with wide range of biological activities against the growth of *E. coli* and *B. subtilis* (Ujita and Malik 2002). It was also found that there were some components in slight concentrations such as, xanthatin,8-[4-[(amino carbonyl) methoxy] phenyl]-1,3-dipropyl in a yield of 5.87%, 2-amino-1-propanol (5.65%), cyclobutanol (5.22%), L-alanine, N-(N-acetyl-L-alanyl)-butyl ester (4.87%) and 2-heptanol, 6-amino-2-methyl (4.45%).

4. Conclusion

Timer trees represent a rich source of antimicrobial agents. Thus interest has been restored recently in the present investigation of some trees grown wildly in Egypt to identify novel active phytochemicals that might lead to new classes of microbial drug development. Among the antimicrobial activity of the partitions (ethyl acetate, *n*-butanol, chloroform and water), the polar partition (Ethyl acetate) appeared to be the most interesting because of its activity against several microorganisms. Among the bacteria, the Gram-negative bacterium *P. aeruginosa* was highly resistant to extracts treatments followed by the other Gram-negative *S. marcescens*. *P. aeruginosa* had not shown any recognizable response to the treatments with *n*-BuOH, $CHCl_3$ and Aq and had small inhibition diameters with the extract samples. The EtOAc fraction of wood and bark exhibited the strongest bactericidal action against all bacterial strains, and the mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-

specific interactions with proteins (Mason and Wasserman, 1987).

The use of crude extracts from *Morus alba, Albizzia lebbeck* and *Casuarina glauca act* as antibacterial material or might be of great value for pharmaceutical industry. Indeed further work should be done to elucidate main chemical compounds responsible for the activity and its value for human and plant health.

References

Acharyya, S., Patra, A. and Bag, P.K. (2009), "Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multi-drug resistant *Vibrio cholera*", *Tropical Journal of Pharmaceutical Research,* Vol. 8, pp. 231–237.

Ahsan, M.R., Islam, K.M., Haque, M.E. and Mossaddik, M.A. (2009), "In vitro antibacterial screening and toxicity study of some different medicinal plants", *World Journal of Agricultural Sciences,* Vol. 5, pp. 617–621.

Aly, H.I.M., Salem, M.Z.M., Gohar, Y.M., El-Sayed, A.B. and Ashmawy, N.A. (2012), "Efficacy of methanolic extract and its fractions of some tree species for potential *in Vitro* antibacterial activity", *International Journal of Agricultural and Food Research,* Vol. 1 No. 1, pp. 12-27.

Babu, K.S. and Ammani, K. (2009), "Antimicrobial activity of *Morus alba* L.", *Asian Journal of Bio Science,* Vol. 4, pp.124–126.

Chen, C.-C., Liu, L.-K., Hsu, J.-D., Huang, H.-.P, Yang, M.-Y. and Wang, C.-J. (2005), "Mulberry extract inhibits the development of atherosclerosis in cholesterol fed rabbits", *Food Chemistry,* Vol. 91, pp. 601–607.

Chung, K.O., Kim, B.Y., Lee, M.H. Kim, Y.R., Chung, H.Y., Park, J.H. and Moon, J.O. (2003), "In-vitro and in-vivo anti-inflammatory effect of oxyresveratrol from *Morus alba* L.", *Journal of Pharmacy and Pharmacology,* Vol. 55, pp. 1695–700.

Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., Totté, J., Pieters, L., Vlietinck, A.J. (2002), "Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo", *Journal of Ethnopharmacology,* Vol. 79, pp. 213–220.

Elansary, H.O., Salem, M.Z.M., Ashmawy, N.A. and Yacout, M.M. (2012), "Chemical composition, antibacterial and antioxidant activities of leaves essential oils from *Syzygium cumini* L., *Cupressus sempervirens* L. and *Lantana camara* L. from Egypt", *Journal of Agricultural Science,* Vol. 4, pp. 144-152.

El-Beshbishy, H.A., Singab, A.N., Sinkkonen, J. and Pihlaja, K. (2006), "Hypolipidemic and antioxidant effects of *Morus alba* L. (Egyptian mulberry) root bark fractions supplementation in cholesterol-fed rats", *Life Sciences,* Vol. 78, pp. 2724–33.

El-Lakany, M.H. (1983), "A review of breeding drought resistant *Casuarina* for shelterbelt establishment in arid regions with special reference to Egypt", *Forest Ecology and Management,* Vol. 6, pp. 129–137.

Eloff, J.N. (1998), "A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria", *Planta Medica,* Vol. 64, pp. 711–713.

Ganguli, N.B. and Bhatt, R.M. (1993), "Mode of action of active principles from stem bark of *Albizzia lebbeck*", *Indian journal of experimental biology,* Vol. 31, pp. 125–29.

Gómez-Estaca, J., López de Lacey, A., López-Caballero, M.E., Gómez-Guillén, M.C. and Montero, P. (2010), "Biodegradable gelatinechitosan films incorporated with essential oils as antimicrobial agents for fish preservation", *Food Microbiology,* Vol. 27, pp. 889–896.

Harborne, J.B. (1973), "Phytochemical methods" London. Chapman and Hall, Ltd. pp. 49–188.

Hernández, T., Canales, M., Avila, J.G., García, A.M., Martínez, A., Caballero, J., Romo de Vivar, A. and Lira, R. (2005), "Composition and antibacterial activity of essential oil of *Lantana achyranthifolia* Desf. (Verbenaceae)",

Journal of Ethnopharmacology, Vol. 9, pp. 551–554.

Hussain, M.M., Rahman, M.S., Jabbar, A. and Rashid, M.A. (2008), "Phytochemical and biological investigations of *Albizzia lebbeck* Benth", Boletin *Latinoamericano* y del *Caribe* de Plantas Medicinales y *Aromaticas,* Vol. 7, pp. 273–278.

Hussein, MS.h., El-Tawil, O.S., Yassin, N.E.-H. and Abdou, A. (2010), "The protective effect of *Morus Alba* and *Calendula Officinalis* plant extracts on carbon tetrachloride- induced hepatotoxicity in isolated rat hepatocytes", *Journal of American Science,* Vol. 6, pp. 762–773.

Kartal, M. (2007), "Intellectual property protection in the natural product drug discovery, traditional herbal medicine and herbal medicinal products", *Phytotherapy Research,* Vol. 21, pp. 113–119.

Khan, M.T.J., Ahmad, K., Alvi, M.N., Noor-ul-Amin, Mansoor, B., Saeed, M.A., Khan, F.Z. and Jamshaid, M. (2010), "Antibacterial and irritant activities of organic solvent extracts of *Agave americana* Linn., *Albizzia lebbek* Benth. *Achyranthes aspera* Linn. and *Abutilon indicum* Linn - A Preliminary Investigation", *Pakistan Journal of Zoology,* Vol. 42, pp. 93–97.

Koppula, S., Ammani, K., Bobbarala, V. and Bramhachari, P.V. (2010), "Antibacterial activity screening of few medicinal plants from the Southern Region of India", *Journal* of *Pharmacy Research,* Vol. 3, pp. 2453–2456.

Lee, S.S., Lee, H.J., Choi, D.H., Hishiyama, S. and Katom, A. (2000), "Studies on biological activity of wood extractives (IV). Antimicrobial and antioxidative activities of extractives from the heartwood of *Morus bombycis* and synthesized congeneric stilbenoids", *Journal of the Korean Wood Science and Technology,* Vol. 28, pp. 70–77.

Lokegaonkar, S.P. and Nabar, B.M. (2011), "In vitro assessment of the antiplaque properties of crude *M. Alba* leaf extract", *Journal of Herbal Medicine and Toxicology,* Vol. 5, pp. 71–77.

Mason, T.L. and Wasserman, B.P. (1987), "Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds", *Phytochemistry,* Vol. 26, pp. 2197–2202.

Moorthy, V. and Boominathan, M. (2011), "Comparative antimicrobial activities of *Morus alba* crude extract and fraction against *Staphylococcus aureus*", *International Journal of Institutional Pharmacy and Life Sciences,* Vol. 1, pp. 48–56.

NCCLS (1997), "National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Disk Susceptibility Tests", Sixth Edition: Approved Standard M2-A6. NCCLS, Villanova, PA.

Okuda, T., Yoshida, T., Ashida, M. and Yazaki, K. (1983), "Tannins of *Casuarina* and *Stachyurus* species. I: Structures of pendunculagin, casuarictin, strictinin, casuarinin, casuariin, and stachyurin", *Journal of the Chemical Society. Perkin transactions I,* Vol. 1, pp. 1765–1772.

Omidiran, M.O., Baiyewu, R.A., Ademola, I.T., Fakorede, O.C., Toyinbo, E.O., Adewumi, O.J. and Adekunle, E.A. (2012), "Phytochemical analysis, nutritional composition and antimicrobial activities of white mulberry (*Morus alba*)", *Pakistan Journal of Nutrition,* Vol. 11, pp. 456–460.

Ouattara, B., Simard, R.E., Holley, R.A., Piette, G.J.P. and Bégin, A. (1997), "Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms", *International Journal of Food Microbiology* Vol. 37, pp. 155–162.

Rahul, C., Pankaj, P., Sarwan, S.K. and Mahesh, J.K. (2010), "Phytochemical screening and antimicrobial activity of *Albizzia lebbeck*", *Journal of Chemical and Pharmaceutical Research,* Vol. 2, pp. 476–484.

Rashid, R.B., Chowdhury, R., Jabbar, A., Hasan, C.M. and Rashid, M.A. (2003), "Constituents of *Albizzia lebbek* and antibacterial activity of an isolated flavone derivative", *Saudi Pharmaceutical Journal,* Vol. 11, pp. 52–56.

Shimokawa, H., Nonaka, G., and Nishioka, I. (1991), "Tannins and related compounds from *Casuarina glauca* Sieber" Proc. 111th Annual Meeting of the Pharmaceutical Society of Japan, vol. 2, Tokyo, p. 147.

Suffness, M. and Douros, J. (1979), "Drugs of plant origin, p. 73–125. In: V.T. De Vita and H. Bush (eds.). Methods in cancer research. Vol XVI. New York Academic Press, New York.

Ujita, N.S. and Malik, M.S. (2002), "Synthesis and antimicrobial studies of phenylenebis (oxy-acetic acid hydrazides) and their derivatives", *Journal of Ethnopharmacology*, Vol. 12, pp. 308–312.

Uma, B., Prabhakar, K., Rajendran, S., Sarayu, Y.L. (2009), "Antimicrobial Activity of *Albizzia Lebbeck* Benth against Infectious Diarrhoea", *The Internet Journal of Microbiology*, Vol. 7, pp. 31.

Wang, W., Zu, Y., Fu, Y. and Efferth, T. (2012), "In vitro antioxidant and antimicrobial activity of extracts from *Morus alba* L. leaves, stems and fruits", Am. J. Chin. Med., Vol. 40, pp. 349–56.

Yogisha, S. and Raveesha, K.A. (2009), "In-vitro antibacterial effect of selected medicinal plant extracts", *Journal of Natural Products*, Vol. 2, pp. 64–69.