

Efficacy of Methanolic Extract and its Fractions of some Tree Species for Potential *in Vitro* Antibacterial Activity

Hussein I.M. Aly¹, Mohamed Z.M. Salem^{1*}, Yousry M. Gohar², Abdel B. El-Sayed¹ and Nader A. Ashmawy³

¹ Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Egypt

² Department of Botany (Division of Microbiology), Faculty of Science (El-Shatby), Alexandria University, Egypt

³ Plant pathology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Egypt

Abstract

The antibacterial activity of methanolic crude extract and its subsequent fractionation into ethyl acetate, *n*-butanol, chloroform and aqueous fractions from wood, bark and leaves of *Ficus benghalensis* L., *Ficus retusa* L., *Dalbergia sissoo* Roxb., *Syzygium cumini* (L.) Skeels, *Populus nigra* L. and *Cupressus sempervirens* L. grown in Egypt was investigated against some pathogenic bacteria. The antibacterial susceptibility test was evaluated by disc-diffusion and minimum inhibitory concentrations methods. The MCE and ethyl acetate fraction were found to possess maximum antibacterial activity. The ethyl acetate and *n*-butanol fractions from *S. cumini* presented good activity against tested bacteria. The wood chloroform fraction from *F. retusa* and *P. nigra* displayed some activity against the tested bacteria. Other trees had different antibacterial activity. The obtained results could be considered adequate for further studies for the isolation and identification of the active chemical components from the extracts for their antibacterial activity. These findings demonstrated that the species had great potential to be used as a bio-resource for natural health products and food preservation.

Keywords: Egyptian trees; antibacterial activity; methanolic extract.

1. Introduction

In Egypt, there are large quantities of wood branches, leaves and bark residues resulting annually from pruning process to street trees such as *Ficus* and *Dalbergia* spp., windbreak and shelterbelts (*Populus*, *Cupressus* and *Casuarina* spp.) and ornamental trees. These residues can be used as a bio-resource for the production of extracts that

may be useful in medicine and chemotherapy or as a source of biologically active compounds for pharmaceuticals.

Nowadays, the medicinal plants are playing a very popular role in health care products. In the food preservation, pharmaceuticals, alternative medicine and natural therapies, the antimicrobial activity of

* Corresponding author: zidan_forest@yahoo.com

these extracts have formed the basis of these products. Furthermore, the extracts of trees can be a very good source of antibiotics against the pathogenic bacteria.

Ficus benghalensis L. is a large evergreen tree found throughout forest tracts of India. It is a popular source of indigenous system of medicine such as Ayurveda. In traditional medicine, the different parts of trees such as stem bark, root bark, aerial roots, vegetative buds, leaves, fruits and latex are used in dysentery, diarrhea, diabetes leucorrhoea and astringent (Patil and Patil, 2010). The previously phytochemical screenings of *F. benghalensis* revealed the presence of saponins, tannins and flavonoids in aqueous and methanolic (MeOH) extract and the methanolic extract had an effect against the growth of some pathogenic bacteria (Manimozhi et al., 2011).

Ficus retusa L. (*F. nitida* Thunb.) or (*F. microcarpa* L.) commonly known as Chinese anyan belongs to family of Moraceae, which is a large evergreen tree possessing few aerial roots. It is distributed throughout Central India and Australia. It is also called as "Indian Laurel Fig". The cytotoxic and antifungal activities of the aerial parts have been reported (Chiang et al., 2005; Taira et al., 2005). Root bark and the leaves boiled in oil form good applications for wounds and bruises. The golden yellow leaves contain high amounts of flavonoids and carotenoids, triterpenoids, fatty alcohol, steroids, coumarins, flavane-4-hydroxybenzoate and isoflavones (Takahashi et al., 2002; Li and Kuo, 1998; Chiang and Kuo, 2003).

Dalbergia sissoo Roxb. (Family: Fabaceae) is commonly known as Indian rosewood found throughout India, Bangladesh, Pakistan and Nepal up to 900 m (Kumar and Kumud, 2010). The extract of *D. sissoo* was reported as, antidysentric (Brijesh et al., 2006), analgesics and antipyretic (Hajare et al., 2000). The bark and wood are bitter, hot and acrid used as aphrodisiac, abortifacient, expectorant, antihelmintic, antipyretic and as a cure for diseases of the blood, leucoderma, dyspepsia and dysentery is mentioned in Ayurveda (Upwar et al., 2011). The wood is good for diseases of the eye, and of the nose, and is used as a cure for scabies and syphilis. A decoction of the leaves is given in the acute stage of gonorrhea. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins, flavonoids,

glycosides (Cardiac glycosides, anthraquinone glycoside and saponin glycosides) and steroids (Kumar and Kumud, 2010; Upwar et al., 2011; Reddy et al., 2008). The stem bark is reported to have a good antioxidant activity (Roy et al., 2011).

Syzygium cumini (L.) Skeels (*Eugenia jambolana* Lam.) belonging to the family Myrtaceae, is a large tree cultivated throughout India for the edible fruits (Black Plum) and is reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components (Banerjee et al., 2005). Leaves have been used in traditional medicine as a remedy for diabetes mellitus in many countries (Texixer et al., 2000). The use of this tree to treat infectious diseases stimulated the investigation of the antimicrobial activity of the methanol and aqueous extract of the leaves against standard and multi drug resistant Gram positive and negative bacteria (Gowri and Vasantha, 2010). Seed extracts showed a good antibacterial activity against multidrug-resistant human bacterial pathogens (Bag et al., 2012).

Populus nigra L. (Family: Salicaceae), native to Europe, southwest and central Asia and northwest Africa, traditionally its leaves are used as a tonic and antiseptic (Al-Hussaini and Mahasneh, 2009). Many bioactive compounds such as phenolic acids, flavonoids and terpenoids have been isolated from *Populus* species (Radoykova et al., 2010; Schnitzler et al., 2010; Dudonné et al., 2011; Zhong et al., 2012). Additionally, the results obtained provided promising baseline information for the potential uses of the extract and flavonoids from this plant as antimicrobial agents to help control plant diseases (Zhong et al., 2012). The flavonoids compounds; pinobanksin, 3,7-dimethylquercetin and pinocembrin were isolated from 50% ethanol extract of *P. nigra* (Adam et al., 2009). Phenolic compounds like caffeic, *p*-coumaric and cinnamic acids also were identified (Dudonné et al., 2011). Although the plant was mentioned in the European folk medicine, in the study of Al-Hussaini and Mahasneh (2011), the ethanolic extract showed very weak activity against bacteria as well as fungi although *Populus* leaves extracts are used in Jordan folk medicine in skin disinfection.

Cupressus sempervirens L., a medium-sized evergreen tree, is an ornamental tree belonging to the Cupressaceae family. It is native to the eastern

Mediterranean region (Ibrahim et al., 2009). Traditionally *C. sempervirens* L. is used for the cure of colds, flu, evils throat, rheumatism and also used as antiseptics and antispasmodics (Chaudhary et al., 2012). It comprises of tannin and used as antipyretic, constipating, diaphoretic and astringent. The methanolic and other extracts were showed good antibacterial activity (Chaudhary et al., 2012; Mothana et al., 2009; Toroglu, 2007). The essential oil of the leaves showed a remarkable antimicrobial activity (Ibrahim et al., 2009).

The cultivated medicinal plants in Egypt received good attention and represent a voluminous addition to the knowledge in the field of medicinal plants. Therefore, the investigations of the antimicrobial within higher plants (timber trees) have become desirable and a very good tool, since the microorganisms have developed resistance to many commercial antibiotics. Therapy of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to numerous antibiotics (Cowan, 1999).

The use of medicinal plants as a source of traditional medicinal methods still plays a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemically active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds (Cowan, 1999). In addition, the natural products of higher plants within the recent years may possess a new source of antimicrobial agents with possibly novel mechanisms of action. When the infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem (Ahmad and Aqil, 2007; Barbour et al., 2004). Furthermore, the different botanical varieties and different geographical origin may affect the quantity and quality of the phytochemical presents in the interested plants (van Vuuren, 2008).

The aim of this study was to evaluate the antibacterial activity of extracts from six timber trees using six standard bacterial strains. The effect of extracts was determined by measuring the minimum inhibitory concentrations (MICs) and the clear inhibition zone around the disc that was contained the tested extract using the disc diffusion

assay. In addition, the phytochemical screenings of the extracts was done.

2. Materials and Methods

Tree Materials

Wood, bark and leaves of *F. benghalensis*, *F. retusa*, *D. sissoo*, *S. cumini*, *P. nigra* and *C. sempervirens* were used in the present study as a source of extracts. The tree specimens were collected from different locations at Alexandria City and vouchered at the Faculty of Agriculture, Alexandria University, Egypt. Samples of wood, bark and leaves were air-dried for at least one week at room temperature after collection and kept away from direct sunlight in paper sacks then grounded into fine powder by a small laboratory mill.

Preparation of Extracts

Samples of 150 g of ground materials were extracted by 1000 mL of 99% methanol for the initial extraction in a Soxhlet extraction system for 8 h. The methanolic crude extract (MCE) was concentrated to a small volume (20 mL) under reduced pressure (40 °C) by rotary evaporator apparatus.

The chloroform fraction (CHCl_3) which contains alkaloids was carried out according to the recommendations of Harborne (1973) and Cannell (1998), where sample of about 1.6 g from the lyophilized MCE was dissolved in 50 mL of 99% methanol and treated with an equal volume of 1% aqueous HCl then 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 dissolved in a few drops of chloroform to obtain the CHCl_3 fr that was containing the precipitated alkaloids.

Simultaneously, samples of about 3.6 g from the MCEs were suspended in distilled water (100 mL) and shaken with an equal volume of ethylacetate (EtOAc). The two layers were separated. The aqueous layer (Aq) was mixed with *n*-butanol (1:1v/v ratio) to afford the *n*-BuOH fr which contains saponins compounds (Ahmad et al., 1990). The previously EtOAc and Aq layers were separated to give the Aq fr and EtOAc fr. The

solvents were completely evaporated at 40 °C, by using rotary evaporator.

The extracts were lyophilized, weighed and stored in sealed vials at 4 °C until use. Extraction yield (% w/w) was calculated as the ratio of the weight of the extract to the weight of the crude plant powder (oven-dry weight). As a consequence, the standard phytochemical methods described previously (Harborne, 1973) were used to test for the presence of flavonoids, alkaloids, phenolic, saponins and tannins.

In vitro Antimicrobial Susceptibility Testing

The biological assays were carried out on the MeOH extract, EtOAc fr, *n*-BuOH fr, Aq fr, and CHCl₃ fr with concentration of 200 µg/mL against the gram positive bacteria; *Bacillus cereus* ATCC 14579 (*B. cereus*), *Bacillus subtilis* ATCC 6633 (*B. subtilis*) and *Staphylococcus aureus* ATCC 6538 (*S. aureus*) and the gram negative bacteria; *Escherichia coli* ATCC 8739 (*E. coli*), *Pseudomonas aeruginosa* ATCC 9027 (*P. aeruginosa*) and *Serratia marcescens* ATCC 13880 (*S. marcescens*). Bacterial strains were provided by the Division of Microbiology, Faculty of Science, Alexandria University, Egypt.

Nutrient agar (NA) medium (g/L, peptone 5.0 g/L, beef extract 3.0 g/L, 0.5 g/L, pH 7.0) was used for maintenance of the tested bacterial organisms. Mueller Hinton agar (MHA) medium (g/L, meat infusion 2.0; casein hydrolysate 17.5; starch 1.5; agar 13.0, pH 7.0) was used in all bioassays applying the disc diffusion method.

The disc-diffusion method was applied to determine the inhibition zones (IZs) of different extracts (Bauer et al., 1996; Elansary et al., 2012) with minor modification as follows: 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. Müller-Hinton agar (MHA) was prepared and autoclaved. Plates were poured to a depth of 5.0 mm and dried for 30 min.

Using sterile cotton swabs, 0.5 mL of the fresh 24-hours old bacterial suspension were spread over the surface of MHA plates, the surface allowed drying. Sterile plain discs of 5 mm diameter

(Whatman filter paper no. 1) were placed on the surface of agar plates and each disc received 20 µL of the concentrated extract dissolved in dimethylsulfoxide (DMSO, E. Merck, Germany) except EtOAc fr was dissolved in propylene glycol. Plates were allowed to stand in a refrigerator at 4 °C for 6 hrs to allow diffusion of the antibacterial agents then incubated at 37 °C overnight. The IZ around the disc was measured and recorded as an average of three replicates.

Determination of Minimum Inhibitory Concentration of Different Extracts

Minimum inhibitory concentrations (MICs) were determined by serial dilution of extracts (4, 8, 16, 32, 56, 128, 256 and 512 µg/mL) beyond the level where no inhibition of growth of test organisms was observed. This was performed in 96-well microplates (Newton et al., 2002) by filling all wells with 50 µL sterile MHB with minor modification. Two wells were used as a 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 Colony-forming unit (CFU)/mL] to each row (except for the sterility control), the micro-plate was covered and incubated at 37 °C at 100% relative humidity overnight. The following morning 50 µL of a 0.2 mg/ml solution of *p*-iodo-nitrotetrazolium 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.

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. Extracts used for the determination of MICs were either solubilized in DMSO or propylene glycol and was made up as a stock solution (512 µg/mL) with distilled water. The MICs were determined as the lowest concentration that completely inhibited macroscopic growth of bacteria. Extracts with a MIC value of <125 µg/mL were considered active.

Statistical Analysis

The results were statistically analyzed using the Statistical Analysis System (SAS, 2001) GLM procedure. Significant differences among treatment means were determined by the least significant

differences ($LSD_{0.05}$) test with a significance level of $P < 0.05$.

3. Results and Discussion

Quantification and Phytochemical Investigation of Extracts

The quantity of MCE extracts and their fractions (EtOAc fr, *n*-BuOH fr, $CHCl_3$ fr and Aq fr) from wood, bark and leaves of six timber trees are presented in Table 1. The tree parts showed to have a different amount of MCE, EtOAc fr, *n*-BuOH fr and Aq fr. By contrast, the $CHCl_3$ fr which contains alkaloids, was not detected in some parts of trees. For example, the leaves and wood of *F. retusa* presented an amount of $CHCl_3$ fr (4.71 and 3.22 g/kg o.d., respectively), whereas the bark didn't contain it.

The phytochemical screenings (Table 2) detected are known to have beneficial importance in medicinal sciences. For instance, flavonoids, steroids, tannins and saponins have been reported for their anti-allergic, anticancer, anti-inflammatory, antimicrobial, antioxidant antifungal and anti-cancer activities, etc. (Cowan, 1999).

Additionally, the previous phytochemical analysis carried out revealed the presence of coumarins, flavanoids, glycosides, phenols, tannins, saponins and steroids in *S. cumini* and didn't show the presence of alkaloids from wood, bark and leaves (Shihabudeen et al., 2010). Moreover, *E. jambolana* was reported to reach in flavonides, saponins and glycoside, volatile oils, gallic and ellagic acid derivatives, tannins and flavonol glycosides (Magina et al., 2009). Also, acylated flavonol glycosides, kaempferol, myricetin and other polyphenols were isolated from *S. cumini* leaves (Mahmoud et al., 2001; Timbola et al., 2002).

In present study the steam bark of *F. retusa* didn't show the presence of alkaloids, whereas the previous results about the preliminary phytochemical investigation of the ethanol extract showed the presence of alkaloids (Kalyan and Kumar, 2012). The leaves of *C. sempervirens* are quite rich in tannins and flavonoids but they are free from alkaloids and low in saponins (Emami et al., 2004). Additionally, previous studies showed that *C. sempervirens* is rich in flavonoids (cupres-

suflavone, amenoflavone, rutin, quercitrin, quercetin and myricitrin) and phenolic compounds (anthocyanidin, catechines flavones, flavonols and isoflavones), tannins, catchol and essential oil (Koriem, 2009; Mazari et al., 2010).

Antibacterial Activity of Different Extracts

The antibacterial activity of different extracts as measured by disc-diffusion and MICs methods (Tables 3–8) showed a highly significant effect ($P < 0.001$) against the growth of the tested bacterial strains. The results of zones of inhibition and MICs are presented in Tables 3-8. The IZs of the different extracts were ranging from 6-25 mm (Table 3), 6-24.53 mm (Table 4), 6-25 mm (Table 5), 6.5-17.5 mm (Table 6), 6.4-25.0 mm (Table 7) and 6-26 mm (Table 8) against the growth of *B. cereus*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, *S. marcescens* and *E. coli*, respectively.

In Table 3, the highest zone of inhibitions against the growth of *B. cereus* was showed by MCE of *P. nigra* wood (25 mm) followed by *S. cumini* wood (19.6 mm) with MIC of 16 and 64 $\mu\text{g/mL}$, respectively. In addition it was highly affected by EtOAc fr from *S. cumini* wood (20 mm) followed by *P. nigra* leaves (19 mm) with MICs of 128 and 64 $\mu\text{g/mL}$, respectively. On the other hand, the *n*-BuOH fr from *C. sempervirens* wood had a highly significant effect ($P < 0.001$) with IZ of 20 mm and MIC of 128 $\mu\text{g/mL}$ from the *n*-BuOH fractions of the studied trees. The previously highest IZs values were higher than the IZ value of the positive control used (15mm with 2 μg clindamycin/disc).

Table 4 presented the mean of IZs as compared to $LSD_{0.05}$ from extracts against *in vitro* growth of *B. subtilis* was high from MCE of *P. nigra* (24.53 mm) and *S. cumini* (22.3 mm) woods with MIC of 32 $\mu\text{g/mL}$. Furthermore, the EtOAc fractions from *F. benghalensis* and *D. sissoo* barks showed the highest IZs (22 and 21.3 mm) with 256 and 64 $\mu\text{g/mL}$, respectively. The *n*-BuOH fr from *S. cumini* leaves showed highly significant effect ($P < 0.001$) against *B. subtilis* (24.6mm) with MIC of 16 $\mu\text{g/mL}$. Moreover, the $CHCl_3$ fr from *P. nigra* wood had an IZ of 20 mm with MIC of 32 $\mu\text{g/mL}$. The previously highest IZs values were higher than the IZ value of the positive control used (17 mm with 30 μg novobiocin/disc).

Table 1

Quantities of different extracts from different parts of timber trees

Tree	Part	Extract (g/kg o.d.) ^a				
		MCE	EtOAc fr	n-BuOH fr	CHCl ₃ fr	Aq fr
<i>Fb</i>	W	27.93	1.28	0.85	0.85	4.36
	B	94.42	19.17	7.32	NA	11.76
	L	89.64	8.88	9.51	NA	54.61
<i>Fr</i>	W	23.71	2.80	6.92	3.22	2.93
	B	59.54	15.35	7.34	NA	10.01
	L	60.52	4.22	5.11	4.71	8.65
<i>Ds</i>	W	43.19	6.59	13.62	0.60	9.05
	B	134.90	21.35	21.04	2.56	42.78
	L	128.79	16.27	22.11	NA	55.98
<i>Sc</i>	W	31.61	6.19	7.81	NA	3.90
	B	159.42	19.22	56.34	NA	17.83
	L	96.65	37.90	7.98	NA	35.59
<i>Pn</i>	W	32.17	15.54	7.56	1.06	3.54
	B	121.24	32.50	45.79	0.57	9.72
	L	174.14	22.45	67.27	NA	28.47
<i>Cs</i>	W	16.50	5.71	0.48	0.39	2.83
	B	89.26	73.92	9.26	NA	1.14
	L	169.74	5.75	17.57	NA	60.83

^a Based on oven dry weigh.

MCE: Methanolic crude extract

NA: CHCl₃ did not extract any components; L: Leaves; B: bark; W: wood*Fb*: *Ficus benghalensis* L., *Fr*: *Ficus retusa* L., *Ds*: *Dalbergia sissoo* Roxb., *Sc*: *Syzygium cumini* (L.) Skeels., *Pn*: *Populus nigra* L., *Cs*: *Cupressus sempervirens* L.**Table 2**

Phytochemical screenings of methanoilc crude extracts

Tree	Part	Phytochemical group				
		Flavonoids	Phenols	Saponins	Alkaloids	Tannins
<i>Fb</i>	W	+	+	+	+	+
	B	+	+	+	-	+
	L	-	+	+	-	+
<i>Fr</i>	W	+	+	+	+	+
	B	+	+	+	-	+
	L	+	+	+	+	+
<i>Ds</i>	W	+	+	+	+	+
	B	+	+	+	+	+
	L	+	+	+	-	+
<i>Sc</i>	W	+	+	+	-	+
	B	+	+	+	-	+
	L	+	+	+	-	+
<i>Pn</i>	W	+	+	+	+	+
	B	+	+	+	+	+
	L	+	+	+	-	+
<i>Cs</i>	W	+	+	+	+	+
	B	+	+	+	-	+
	L	+	+	+	-	+

+: Positive (present)

- : Negative (absent)

Table 5 showed that the MCE from bark and leaves of *S. cumini* had the highest IZs (23 and 25 mm, respectively) with MIC value of 32 µg/mL against the growth of *S. aureus*. The EtOAc fr from *D. sissoo* wood showed good antibacterial activity (22.60 mm) with MIC of 64 µg/mL. In addition, the activity of *n*-BuOH fr was high by *S. cumini* leaves (24 mm) with MIC of 32 µg/mL. The CHCl₃ fr from *D. sissoo* wood showed a good activity (18mm) with MIC of 64 µg/mL. These highest IZs values were comparable with the IZ value of the positive control used (22 mm with 30 µg cephalothin/disc).

The highest antibacterial activity against *P. aeruginosa* from studied trees (Table 6), were reported from the MCE of *P. nigra* leaves and *F. retusa* wood (17.5 and 17.4 mm,) with MICs of >512 and 256 µg/mL, respectively, the EtOAc fr from *S. cumini* (15 mm with MIC of >512 µg/mL) and *F. benghalensis* wood (14 mm with MIC of of >512 µg/mL) woods and the CHCl₃ fr from *F. retusa* wood (13 mm with MIC of >512 µg/mL). All the previous IZs values were lower than the positive control (36 mm with 30 µg tetracycline/disc).

Table 3

Antibacterial activity of different extracts from different tree parts against *B. cereus*

Tree	Part	Extract									
		MCE		EtOAc fr		<i>n</i> -BuOH fr		CHCl ₃ fr		Aq fr	
<i>Fb</i>	W	14.2 ^{f*}	16 [†]	na [*]	>512 [†]	15.6 ^{b*}	>512 [†]	12 ^{b*}	128 [†]	11.5 ^{b*}	128 [†]
	B	14.3 ^f	128	18.6 ^b	>512	12.6 ^d	>512	-	-	8 ^{ef}	>512
	L	12.6 ^{gh}	16	13 ^e	256	10 ^e	>512	-	-	6 ^g	>512
<i>Fr</i>	W	13.7 ^f	128	6.5 ^h	256	9 ^{ef}	>512	6 ^d	256	13 ^a	>512
	B	14 ^f	64	9 ^g	64	8 ^f	256	-	-	8.5 ^e	>512
	L	15.6 ^{de}	>512	13 ^e	256	na	>512	na	>512	6.5 ^g	>512
<i>Ds</i>	W	13.3 ^{fg}	256	10.2 ^{fg}	32	10 ^e	>512	8.5 ^c	>512	7.5 ^f	>512
	B	15.2 ^e	256	12 ^e	>512	14 ^{bcd}	>512	6 ^d	>512	na	>512
	L	na	>512	na	>512	14 ^{bcd}	256	-	-	na	>512
<i>Sc</i>	W	19.6 ^b	64	20 ^a	64	14 ^{bcd}	256	-	-	8 ^{ef}	>512
	B	13.3 ^{fg}	256	18 ^{bc}	64	9.5 ^{ef}	>512	-	-	10.5 ^c	>512
	L	16.2 ^d	64	15.2 ^d	64	14.5 ^{bc}	256	-	-	11 ^{bc}	256
<i>Pn</i>	W	25 ^a	16	10.5 ^f	64	na	>512	11 ^b	64	9.5 ^d	>512
	B	13.8 ^f	128	18.3 ^{bc}	64	na	>512	na	>512	na	>512
	L	17.3 ^c	128	19 ^{ab}	128	10 ^f	>512	-	-	na	>512
<i>Cs</i>	W	18 ^c	64	15 ^d	>512	20 ^a	128	16 ^a	128	8 ^{ef}	>512
	B	15.3 ^{de}	256	10.4 ^f	128	13 ^{cd}	256	-	-	6 ^g	>512
	L	12 ^h	>512	17 ^c	64	14 ^{bcd}	256	-	-	6 ^g	>512
NC (mm)		na									
PC (mm)		15 mm (2 µg clindamycin/disc)									

For legend see Table 1.

Different letters represent statistical differences between the averages of the values. Means with the same letter within the same column are not significantly different at 0.05 level of probability according to LSD_{0.05} test.

*: Values are mean of 3 replicates of IZ including 5 mm diameter of the disc at 200 µg/mL of the concentrated extract

†: MICs values

NC: Negative control; PC: Positive control

na: No activity

-: No extracted material by this solvent

Table 4Antibacterial activity of different extracts from different tree parts against *B. subtilis*

Tree	Part	Extract									
		MCE		EtOAc fr		<i>n</i> -BuOH fr		CHCl ₃ fr		Aq fr	
<i>Fb</i>	W	18.3 ^{c*}	128 [†]	7.2 ^{m*}	256 [†]	16 ^{d*}	>512 [†]	12 ^{d*}	16 [†]	na [*]	256 [†]
	B	12 ^f	64	22 ^a	256	na	>512	-	-	na	>512
	L	12 ^f	256	16.6 ^f	128	na	>512	-	-	na	>512
<i>Fr</i>	W	15.3 ^d	16	7.1 ⁿ	256	12 ^h	16	17 ^c	256	na	256
	B	13.3 ^e	256	13.3 ^j	32	na	>512	-	-	na	>512
	L	13.3 ^e	256	16.6 ^f	128	9 ⁱ	>512	na	>512	6 ^d	>512
<i>Ds</i>	W	12 ^f	256	8.2 ^l	32	15 ^e	256	18 ^b	64	7 ^c	>512
	B	18.3 ^c	64	21.3 ^b	64	na	256	na	>512	na	>512
	L	na	>512	15.6 ^h	128	14 ^f	256	-	-	8.7 ^c	>512
<i>Sc</i>	W	22.3 ^b	32	16 ^g	128	na	>512	-	-	na	>512
	B	17.3 ^c	128	17 ^e	128	na	>512	-	-	12.3 ^a	256
	L	15.6 ^d	128	13.5 ⁱ	32	24.6 ^a	16	-	-	10.5 ^b	128
<i>Pn</i>	W	24.53 ^a	32	7.2 ^m	64	14 ^f	256	20 ^a	32	6 ^d	>512
	B	18 ^c	128	19.6 ^d	32	na	>512	na	>512	na	>512
	L	18 ^c	128	19.6 ^d	64	13.6 ^g	256	-	-	7.7 ^c	>512
<i>Cs</i>	W	18.3 ^c	64	15.6 ^h	128	20.3 ^b	64	17 ^c	128	na	>512
	B	13.6 ^e	256	12.3 ^k	16	17.6 ^c	64	-	-	8 ^c	>512
	L	na	>512	20.3 ^c	32	na	256	-	-	na	>512

NC (mm) na

PC (mm) 17 mm (30 µg novobiocin/disc)

For legend see Tables 1, 3

Table 5Antibacterial activity of different extracts from different tree parts against *S. aureus*

Tree	Part	Extract									
		MCE		EtOAc fr		<i>n</i> -BuOH fr		CHCl ₃ fr		Aq fr	
<i>Fb</i>	W	16.5 ^{f*}	16 [†]	18 ^{*e}	256 [†]	na [*]	>512 [†]	7 ^{l*}	64 [†]	12.3 ^{a*}	16 [†]
	B	9.5 ^m	32	17 ^g	128	10 ^e	>512	-	-	12 ^a	>512
	L	6 ^p	64	16.5 ^h	128	12 ^c	>512	-	-	na	>512
<i>Fr</i>	W	16.3 ^g	>512	14.3 ^k	64	na	256	15 ^b	>512	13 ^a	64
	B	15.3 ⁱ	>512	17 ^g	128	8 ^j	>512	-	-	7.5 ^c	>512
	L	14 ^k	>512	18 ^e	128	9 ^f	>512	8.5 ^e	>512	6 ^d	>512
<i>Ds</i>	W	10 ^l	>512	22.6 ^a	64	12 ^c	>512	18 ^a	64	6.4 ^d	>512
	B	15.4 ^h	64	21 ^b	32	6 ^l	>512	12 ^c	>512	8.5 ^c	>512
	L	7 ^o	>512	17 ^g	128	8.5 ^h	256	-	-	na	>512
<i>Sc</i>	W	17.8 ^e	64	18 ^e	64	11.2 ^d	>512	-	-	9.5 ^b	>512
	B	23 ^b	32	18.5 ^d	64	8.4 ⁱ	>512	-	-	na	>512
	L	25 ^a	32	20 ^c	64	24 ^a	32	-	-	10.2 ^b	32
<i>Pn</i>	W	8.5 ⁿ	>512	18 ^e	64	9 ^f	>512	15 ^b	64	8.4 ^c	>512
	B	16.3 ^g	256	21 ^b	32	na	>512	10 ^d	>512	6 ^d	>512
	L	15 ^j	256	17.5 ^f	64	8.7 ^g	>512	-	-	na	>512
<i>Cs</i>	W	18.7 ^d	64	16 ⁱ	128	7.5 ^k	64	na	>512	na	>512
	B	19 ^c	64	18 ^e	64	14 ^b	256	-	-	6.4 ^d	>512
	L	14 ^k	128	15 ^j	256	na	>512	-	-	na	>512

NC (mm) na

PC (mm) 22 mm (30 µg cephalothin (Keflin)/disc)

For legend see Tables 1, 3

Table 6Antibacterial activity of different extracts from different tree parts against *P. aeruginosa*

Tree	Part	Extract									
		MCE		EtOAc fr		<i>n</i> -BuOH fr		CHCl ₃ fr		Aq fr	
<i>Fb</i>	W	13 ^{d*}	32 [†]	8 ^{c*}	>512 [†]	14 ^{a*}	>512 [†]	na [*]	256 [†]	na [*]	256 [†]
	B	14.2 ^c	32	na	>512	na	>512	-	-	na	>512
	L	14 ^{cd}	64	na	>512	na	>512	-	-	na	>512
<i>Fr</i>	W	17.4 ^a	256	6.5 ^d	>512	na	128	13	>512	8.2 ^a	>512
	B	14 ^{cd}	64	na	>512	na	>512	-	-	na	>512
	L	14 ^{cd}	128	na	>512	na	>512	na	>512	na	>512
<i>Ds</i>	W	na	>512	14.3 ^a	256	na	>512	na	>512	na	>512
	B	na	>512	na	>512	na	>512	na	>512	na	>512
	L	na	>512	na	>512	na	>512	-	-	na	>512
<i>Sc</i>	W	16 ^b	128	15 ^a	>512	na	>512	-	-	na	>512
	B	na	>512	na	>512	na	>512	-	-	na	>512
	L	na	64	na	>512	na	128	-	-	na	256
<i>Pn</i>	W	15.6 ^b	>512	14 ^a	>512	na	>512	na	>512	na	>512
	B	na	>512	na	>512	na	>512	na	>512	na	>512
	L	17.5 ^a	>512	na	>512	13 ^b	>512	-	-	9.3 ^a	>512
<i>Cs</i>	W	na	>512	na	>512	na	>512	na	256	na	>512
	B	na	>512	na	>512	na	>512	-	-	na	>512
	L	na	>512	na	>512	na	>512	-	-	na	>512
NC (mm)		na									
PC (mm)		36 mm (30 µg tetracycline/disc)									

For legend see Tables 1, 3

Table 7Antibacterial activity of different extracts from different tree parts against *S. marcescens*

Tree	Part	Extract									
		MCE		EtOAc fr		<i>n</i> -BuOH fr		CHCl ₃ fr		Aq fr	
<i>Fb</i>	W	18 ^{*c}	16 [†]	14 ^{*f}	>512 [†]	13 ^{*b}	>512 [†]	8 ^{*b}	32 [†]	7.5 ^{*c}	>512 [†]
	B	10 ^e	32	18 ^d	>512	10 ^d	>512	-	-	na	>512
	L	9 ^e	128	na	>512	na	>512	-	-	6.5 ^d	256
<i>Fr</i>	W	17 ^c	64	10 ^h	>512	8.5 ^e	256	15 ^a	>512	8.5 ^c	>512
	B	13.4 ^d	>512	13 ^g	64	6.5 ^f	>512	-	-	7.4 ^{cd}	>512
	L	13 ^d	>512	6.4 ^e	>512	na	>512	7.6 ^b	>512	6.4 ^d	>512
<i>Ds</i>	W	22 ^a	>512	14 ^f	32	11 ^{cd}	>512	na	>512	na	>512
	B	na	>512	na	>512	na	>512	na	>512	na	>512
	L	na	>512	na	>512	na	>512	-	-	na	>512
<i>Sc</i>	W	19.6 ^b	32	19 ^{cd}	64	10 ^d	>512	-	-	na	>512
	B	23 ^a	32	20 ^c	32	na	>512	-	-	na	>512
	L	19.8 ^b	32	15 ^f	128	22.5 ^a	64	-	-	12.4 ^a	128
<i>Pn</i>	W	14 ^d	256	20 ^c	32	10 ^d	>512	15 ^a	32	6.4 ^d	>512
	B	17 ^c	64	18 ^d	64	8.4 ^e	>512	na	>512	na	>512
	L	14 ^d	256	16.4 ^e	128	6.5 ^f	>512	-	-	na	>512
<i>Cs</i>	W	18 ^c	>512	21.4 ^b	>512	13 ^b	256	8 ^b	>512	10.5 ^b	>512
	B	13 ^d	>512	25 ^a	64	12 ^{bc}	>512	-	-	na	>512
	L	na	>512	na	>512	na	>512	-	-	na	>512
NC (mm)		na									
PC (mm)		nt									

For legend see Tables 1, 3

nt: not tested

Table 8Antibacterial activity of different extracts from different tree parts against *E. coli*

Tree	Part	Extract									
		MCE		EtOAc fr		n-BuOH fr		CHCl ₃ fr		Aq fr	
Fb	W	18 ^{e*}	>512 [†]	na [*]	256 [†]	6.2 ^{i*}	>512 [†]	6.1 ^{d*}	32 [†]	9.2 ^{c*}	128 [†]
	B	16.3 ^f	>512	18 ^e	>512	12 ^d	>512	-	-	na	>512
	L	na	128	na	>512	na	>512	-	-	na	>512
Fr	W	10 ⁱ	128	na	128	8.4 ^h	32	6 ^d	>512	8.5 ^c	128
	B	na	128	na	128	na	>512	-	-	na	>512
	L	14 ^g	>512	11.5 ^f	>512	6.4 ⁱ	>512	11 ^b	>512	na	>512
Ds	W	21.3 ^d	32	8.5 ^{de}	16	14 ^b	>512	6 ^d	256	10.5 ^b	>512
	B	16.4 ^f	46	19 ^d	32	8.5 ^h	>512	na	>512	na	>512
	L	na	>512	na	>512	na	>512	-	-	na	>512
Sc	W	16.4 ^f	64	19 ^d	64	10.5 ^f	>512	-	-	na	>512
	B	24 ^c	64	20 ^c	32	15 ^a	256	-	-	12.5 ^a	32
	L	25 ^b	16	10.4 ^g	>512	12.5 ^d	32	-	-	9.4 ^c	64
Pn	W	26 ^a	16	10.2 ^g	32	14 ^b	>512	15.1 ^a	32	6.4 ^d	>512
	B	16.4 ^f	128	21 ^b	32	6.5 ⁱ	>512	8.4 ^c	>512	na	>512
	L	14.6 ^g	256	22.3 ^a	32	11.2 ^e	>512	-	-	na	>512
Cs	W	22 ^d	32	20 ^c	64	9.4 ^g	128	8.4 ^c	64	9.4 ^c	256
	B	17 ^f	46	12 ^f	32	15.3 ^a	128	-	-	na	>512
	L	12 ^h	128	19 ^d	16	13 ^c	256	-	-	9.4 ^c	>512
NC (mm)		na									
PC (mm)		18 mm (2 µg clindamycin/disc)									

For legend see Tables 1,3

In Table 7, the MCE from all parts of *S. cumini* (IZs of 19.6, 23 and 19.8 mm for wood, bark and leaves, respectively) with MIC of 32 µg/mL and EtOAc fr from *C. sempervirens* bark and wood (25 and 21.4 mm, with MICs of 64 and >512 µg/mL, respectively), *S. cumini* bark (20 mm with MIC of 32 µg/mL) and *P. nigra* wood (20 mm and MIC of 32 µg/mL) and the *n*-BuOH fr from *S. cumini* leaves (22.5 mm with MIC of 64 µg/mL) were significantly active ($P<0.001$) against the growth of *S. marcescens*.

Table 8 presented that the MCE from *P. nigra* wood (26 mm with MIC of 16 µg/mL) followed by *S. cumini* leaves and bark (25 and 24 mm, with 16 and 64 µg/mL, respectively), *C. sempervirens* wood (22 mm with MIC of 32 µg/mL) and *D. sissoo* wood (21.5 mm with MIC of 32 µg/mL), the EtOAc fr from leaves and bark of *P. nigra* (22.3 and 21 mm, with MIC of 32 µg/mL), gained the highest activity against the growth of *E. coli*. The values of IZs were lower than the positive control used (18 mm with 2 µg clindamycin/disc).

From the previous results, the MCE from the studied tree species was more active than the other

fractions against all the bacterial strains and the further solvent fractionations of the MCE enhanced the activity particularly in the EtOAc and *n*-BuOH fractions.

The tested extracts from *F. retusa* had a variable degree of antibacterial activity against all of the tested bacteria. However, the MCE of leaves owned the highest activity against the growth of selected bacteria at the lower concentrations. Furthermore, the MCE from the wood and bark was observed to have good activity against the tested bacteria except *E. coli*. The CHCl₃ fr and Aq fr from wood showed good activity against tested bacteria at low concentrations. Within the members of the Moraceae family, the genus *Ficus* is also well documented for its biological activities such as antimicrobial (Al-Fatimi et al., 2007; Changwei et al., 2008). For instance, the extracts from the areal parts and bark of *F. retusa* “variegata” showed mild antimicrobial activity (Sarg et al., 2011). The strong antioxidant and antibacterial activities of *F. microcarpa* bark and leaves extract may be attributed to its high level of phenolic compounds like flavonoids, coumarin and

triterpenoids (Ao et al., 2008). In addition, the plant showed a moderate antioxidant activity and the EtOAc and *n*-BuOH fractions have the high activity (Abdel-Hameed, 2009).

The MCE from wood of *F. benghalensis* showed good activity against the growth of studied bacterial strains at the lower concentrations except *S. aureus*. In addition, the *n*-BuOH fr from wood was observed to have good effect against the bacterial growth. It was noticed the EtOAc fr from the bark had a good efficacy except against *P. aeruginosa*. Moreover, the extracts from the leaves especially EtOAc fr showed slight activity against studied bacteria. The MCE of *F. benghalensis* contains glycoside; 20-tetratriacontene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitosterol-alpha-D-glucose and meso-inositol have been isolated from the bark (Mousa, 1994).

The extracts from *S. cumini* presented good activity against the tested bacteria. The leaves MCE presented a high antibacterial activity followed by wood and bark. In addition, the *n*-BuOH fr from the leaves showed a high antibacterial activity at lower concentrations. Moreover, the EtOAc fr from wood, bark, and leaves and Aq fr from leaves showed good activity at the lower concentrations. Indeed, the EtOAc and *n*-BuOH fractions from *S. cumini* had good activity against the growth of the tested bacteria strains. In addition, the crude plant extracts demonstrated IZ of 18mm against *S. aureus* and further fractionation showed the presence of saponins (*n*-BuOH fr) as the active phytoconstituent (Jasmine et al., 2007). The antimicrobial activity of *S. cumini* might be due to tannins and other phenolic compounds. It is known to be very rich in gallic and ellagic acid polyphenol derivatives (Mahmoud et al., 2001; Chattopadhyay et al., 1998). Additionally, the MCE of *S. cumini* had the highest toxicity against all the bacteria and revealed to show MIC of 0.75 mg/ml against *S. aureus* (Shihabudeen et al., 2010). The extracts of *S. cumini* leaves showed inhibitory activity against the gram negative and positive bacteria and the results suggested a potential application of *S. cumini* leaves for treatment of skin wounds, typhoid and further investigations should be conducted in order to explore their applications (Gowri and Vasantha, 2010).

The EtOAc fr from leaves and bark of *C. sempervirens* and *n*-BuOH fr from wood had the highest antibacterial activity against the growth of tested bacteria at the lowest concentrations except against *P. aeruginosa*. Furthermore, the MeOH extract from wood and bark and *n*-BuOH fr from bark showed good activity against the growth of bacteria except *P. aeruginosa* and *S. marcescens*. It was noticed that the CHCl₃ fr from wood had moderate activity against the tested bacteria at the lower concentrations. Additionally, Mothana et al. (2009) pronounced that the antimicrobial activity of MCE of *C. sempervirens* was observed only against Gram-positive bacteria among them multi-resistant bacteria with inhibition zones >15mm and MIC values <500 µg/mL. Furthermore, the leaves MCE had a significant antimicrobial activity followed by the ethyl acetate and ethanol extracts and the greater inhibitory activity against *E. coli* was possessed by ethyl acetate extract of *C. sempervirens* (Chaudhary et al., 2012).

The MCE and EtOAc fr from the bark and leaves of *P. nigra* had a good antibacterial activity against the growth of the studied bacteria except the bacterium *P. aeruginosa*. Furthermore, the EtOAc and CHCl₃ fractions from wood showed good activity at the lower concentrations against the growth of bacteria except *P. aeruginosa*. Moreover, the EtOAc fr from the wood showed good activity. In the present study, *P. nigra* extracts unexpectedly showed good activity against bacteria and this result might be due to the compounds, 4-hydroxybutanoic acid, 3-hydroxybutanoic acid, phthalic acid, 4-hydroxyhydrocinnamic acid, imidazole, phenetole, benzoic, cis- and trans-*p*-coumaric, ferulic, caffeic and cinnamic acid which were identified previously by GC-MS from the bud exudate of *P. nigra* (Wieslawa et al., 2002).

The EtOAc fr from *D. sissoo* wood was observed to have a good activity at the lower concentrations against the growth of tested bacteria and it was noted that the MCE from bark and *n*-BuOH fr from the leaves showed some activities against the tested bacteria. For instance, it was reported that the decoction of *D. sissoo* leaves contained carbohydrates, proteins, flavonoids, and tannins, phytosterols, glycosides, saponins and alkaloids were absent (Brijesh et al., 2006).

In our study, other fractions (CHCl_3 fr And Aq fr) from the studied trees showed a weak effect against the growth of the tested bacteria and this results could be attributed to the presence of poly and oligosaccharides (water-soluble compounds). For example, the Aq fr from *D. sissoo* leaves showed a weak antibacterial activity and could be related to the presence of polysaccharide isolated previously like L-rhamnose, D-glucuronic acid, D-galactose and D-glucose in different molar ratios (Rana et al., 2009; Rana and Kumar, 2011). The CHCl_3 fr had presented slight activity against tested bacterial strains, however the CHCl_3 fr from *F. retusa* wood and *P. nigra* wood owned a high activity against the tested bacteria and the mechanism of action of alkaloids is attributed to their ability to intercalate with DNA (Phillipson and O'Neill, 1987).

The plants having antibacterial constituents suggested to have enormous therapeutic potential as they could act without any side effect as often found with synthetic antibacterial products. Most antibacterial medicinal plants are more effective against gram-positive than gram-negative bacteria (Srinivasan et al., 2001). However, the current findings show a remarkable activity against gram-negative bacteria (*E. coli* and *S. marcescens*). Furthermore, the most susceptible bacterium was *E. coli* and might be due to its cell wall structure and outer membrane. The most resistance bacterium to the extracts from studied trees was *P. aeruginosa*. Our results suggest that gram-positive bacteria are generally more sensitive to the extracts from studied trees. This was consistent with the previous studies on other species (Arora and Kaur, 1999).

Overall, from the previous results, it was clear that the MCE of wood, bark and leaves exhibited the

strongest bactericidal action against all bacterial strains followed by the EtOAc fr. 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 nonspecific interactions with the proteins (Mason and Wasserman, 1987). Additionally, fractionation of the weakly active crude extracts results in more active antibacterial fractions. For instance, the MCE from *D. sissoo* leaves (Table 4) didn't show any activity against the growth of *B. subtilis* and the EtOAc fr delivered from the MCE had a good activity (15.6 mm of IZ and MIC value of 128 $\mu\text{g/mL}$).

4. Conclusion

In conclusion, the data presented in this study indicated that the *in vitro* activity of the methanolic extract and its fractions from the studied trees against bacteria was varied with the type of microorganism and tree parts used. Furthermore, MIC values though relatively high compared with positive control pure antibiotics may substantiate and validate the medicinal properties of these trees.

The results could be providing justification for the use of these trees in folk medicine to treat various infectious diseases. These results are of interest since they have been obtained with methanol extracts and their subsequent fractions and are not a pure product and it could be considered to have a good potency level. However, further investigation should be carried out on new series of pathogenic microorganisms, in order to validate these trees in the field of medicinal plants.

References

- Abdel-Hameed, E.S. (2009), "Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples", *Food Chemistry*, Vol. 114, pp. 1271–1277
- Adam, M., Berset, C., Kessler, M. and Hamburger, M. (2009), "Medicinal herbs for the treatment of rheumatic disorders- A survey of European herbals from the 16th and 17th century", *Journal of Ethnopharmacology*, Vol. 121, pp. 343–359
- Ahmad, I. and Aqil, F. (2007), "In vitro efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria", *Microbiological Research*, Vol.162, pp. 264–275

- Ahmad, V.U., Perveen, S. and Bano, S. (1990), "Saponin from the leaves of *Guaiaacum officinale*", *Phytochemistry*, Vol. 29, pp. 3287–3290
- Al-Fatimi, M., Wurster, M., Schröder, G. and Lindequist, U. (2007), "Antioxidant, antimicrobial and cytotoxicity of selected medicinal plants from Yemen", *Journal of Ethnopharmacology*, Vol. 111, pp.657–666
- Al-Hussaini, R. and Mahasneh, A.M. (2009), "Microbial growth and quorum sensing antagonist activities of herbal plants extracts", *Molecules*, Vol. 14, pp. 3425–3435
- Al-Hussaini, R. and Mahasneh, A.M. (2011), "Antibacterial and antifungal activity of ethanol extract of different parts of medicinal plants in Jordan", *Jordan Journal of Pharmaceutical Sciences*, Vol. 4, pp. 57–69
- Ao, C., Li, A., Elzaawely, A.A., Xuan, T.D. and Tawata, S. (2008), "Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract", *Food Control*, Vol. 19, pp. 940–948.
- Arora, D.S. and Kaur, J. (1999), "Antimicrobial activity of spices", *International Journal of Antimicrobial Agents*, Vol. 12, pp. 257–262
- Bag, A., Bhattacharyya, S.K., Pal, N.K. and Chattopadhyay, R.R. (2012), "In vitro antibacterial potential of *Eugenia jambolana* seed extracts against multidrug-resistant human bacterial pathogens", *Microbiological Research*, Vol. 167, pp. 352–357
- Banerjee, A., Dasgupta, N. and De, B. (2005), "In vitro study of antioxidant activity of *Syzygium cumini* fruit", *Food Chemistry*, Vol. 90, pp. 727–733
- Barbour, E.K., Al Sharif, M., Sagherian, V.K., Habre, A.N., Talhouk, R.S. and Talhouk, S.N. (2004), "Screening of selected indigenous plants of Lebanon for antimicrobial activity", *Journal of Ethnopharmacology*, Vol. 93, pp. 1–7
- Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1996), "Antibiotic susceptibility testing by a standardized single disk method", *American Journal of Clinical Pathology*, Vol. 45, pp. 493–496
- Brijesh, S., Daswani, P.G., Tetali, P., Antia, N.H. and Birdi, T.J. (2006), "Studies on *Dalbergia sissoo* (Roxb.) leaves: Possible mechanism(s) of action in infectious diarrhea", *Indian Journal of Pharmacology*, Vol. 38, pp. 120–124
- Cannell, J.P.R. (1998), "Natural products isolation: Special problems with the extraction of plants (L.G. Silva, I.S. Lee, A.D. Kinghorn)", Humana press, Totowa, New Jersey, pp. 343–363
- Changwei, A., Anping, L., Abdelnaser, A., Elzaawely, T.D. and Xuan, S.T. (2008), "Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil.", *Food Control*, Vol. 19, pp. 940–948
- Chattopadhyay, D., Sinha, B.K. and Vaid, L.K. (1998), "Antibacterial activity of *Syzygium* species", *Fitoterapia*, Vol. 69, pp. 356–367
- Chaudhary, H.J., Shahid, W., Bano, A., Ullah, F., Munis, F., Fahad, S. and Ahmad, I. (2012), "In vitro analysis of *Cupressus sempervirens* L. plant extracts antibacterial activity", *Journal of Medicinal Plants Research*, Vol. 6, pp. 273–276
- Chiang, Y.-M., Chang, J.-Y., Kuo, C.-C., Chang, C.-Y. and Kuo, Y.-H. (2005), "Cytotoxic triterpenes from aerial roots of *Ficus microcarpa*", *Phytochemistry*, Vol. 66, pp. 495–501
- Chiang, Y.-M. and Kuo, Y.-H. (2003), "Two novel α -tocopheroids from the aerial roots of *Ficus microcarpa*", *Tetrahedron Letters*, Vol. 44, pp. 5125–5128
- Cowan, M.M. (1999), "Plant Products as Antimicrobial Agents", *Clinical Microbiology Reviews*, Vol. 12, pp. 564–582
- Dudonné, S., Poupard, P., Coutière, P., Woillez, M., Richard, T., Mérillon, J.-M. and Vitrac, X. (2011), "Phenolic composition and antioxidant properties of Poplar bud (*Populus nigra*) extract: Individual antioxidant contribution of phenolics and transcriptional effect on skin aging", *Journal of Agricultural and Food Chemistry*, Vol. 59, pp. 4527–4536
- Elansary, H.O., Salem, M., Ashmawy, N. and Yacout, 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
- Emami, S.A., Khayyat, M.H., Rahimizadeh, M., Fazly-Bazzaz, B.S. and Assili, J. (2004), "Chemical constituents of *Cupressus sempervirens* L. cv. *Cereiformis* Rehd. essential oils", *Iranian Journal of Pharmaceutical Sciences*, Vol. 1, pp. 39-42
- Gowri SS, Vasantha K (2010), "Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts", *International Journal of Pharmaceutical Sciences and Research*, Vol. 2, pp. 1569-1573
- Hajare, S.W., Chandra, S., Tandan, S.K., Sarma, J., Lal, J. and Telang, A.G. (2000), "Analgesics and antipyretic activity of *Dalbergia sissoo* Leaves", *Indian Journal of Pharmacology*, Vol. 32, pp. 357-360
- Harborne, J.B. (1973), "Phytochemical methods, A Guide to Modern Techniques of plant analysis", Chapman and Hall, London, Ltd, pp. 49-188
- Ibrahim, N.A., El-Seedi, H.R. and Mohammed, M.M.D. (2009), "Constituents and biological activity of the chloroform extract and essential oil of *Cupressus sempervirens*", *Chemistry of Natural Compounds*, Vol. 45, pp. 309-313
- Jasmine, R., Selvakumar, B.N. and Daisy, P. (2007), "Saponins from *Eugenia jambolana* with antibacterial activity against beta-lactamase producing methicillin resistant *Staphylococcus aureus*", *Research Journal of Medicinal Plant*, Vol. 1, pp. 1-6
- Kalyan, R.E. and Kumar, K.P. (2012), "Anti-secretory activity of ethanolic extract of stem bark of *Ficus retusa* L. in albino wistar rats", *International Journal of Pharmaceutical Sciences and Research*, Vol. 3, pp. 109-112
- Koriem, K.M.M. (2009), "Lead toxicity and the protective role of *Cupressus sempervirens*", *Revista Latinoamericana de Química*, Vol. 37, pp. 230-242
- Kumar, S.M. and Kumud, U. (2010), "Anti-inflammatory Activity of root of *Dalbergia sissoo* (Roxb.) in carrageenan-induced paw edema in rats", *Pharmacognosy Journal*, Vol. 2, pp. 427-430
- Li, Y.-C. and Kuo, Y.-H. (1998), "A monoterpenoid and two simple phenols from heartwood of *Ficus microcarpa*", *Phytochemistry*, Vol. 49, pp. 2417-2419
- Magina, M.D.A., Pietrovski, E.F., Gomig, F., Falkenber, D.B., Cabrini, D.A., Otuki, M.F., Pizzollati, M.G. and Brighente, I.M.C. (2009), "Topical anti-inflammatory activity and chemical composition of the epicuticular wax from the leaves of *Eugenia beaurepaireana* (Myrtaceae)", *Brazilian Journal of Pharmaceutical Sciences*, Vol. 60, pp. 479-87
- Mahmoud, I.I., Marzouk, M.S.A., Moharram, F.A., El-Gindi, M.R. and Hassan, A.M.K. (2001), "Acyated flavonol glycosides from *Eugenia jambolana* leaves", *Phytochemistry*, Vol. 58, pp. 1239-1244
- Manimozhi, D.M., Sankaranarayanan, S. and Sampathkumar, G. (2011), "Phytochemical screening of three medicinally important *Ficus* sp.", *International Journal of Pharmaceutical Research and Development*, Vol. 4, pp. 44-51
- 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
- Mothana, R.A., Gruenert, R., Bednarski, P.J. and Lindequist, U. (2009), "Evaluation of the *in vitro* anticancer, antimicrobial and antioxidant activities of some Yemeni plants used in folk medicine", *Pharmazie*, Vol. 64, pp. 260-268
- Mazari, K., Bendimerad, N., Bekhechi, C. and Fernandez, X. (2010), "Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L.", *Journal of Medicinal Plants Research*, Vol. 4, pp. 959-964.
- Mousa, O., Vuorela, P., Kiviranta, J., Wahab, S.A. and Hiltunen, R. (1994), "Bioactivity of certain Egyptian *Ficus* species", *Journal of Ethnopharmacology*, Vol. 41, pp. 71-76
- Newton, S.M., Lau, C., Gurcha, S.S., Besra, G.S. and Wright, C.W. (2002), "The evaluation of forty-three plant species for *in vitro* antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and

- Sanguinaria Canadensis*”, *Journal of Ethnopharmacology*, Vol. 79, pp. 57–67
- Patil, V.V. and Patil, V.R. (2010), “*Ficus bengalensis* Linn.: An overview”, *International Journal of Pharma and Bio Sciences*, Vol. 1, pp. 1–11
- Phillipson, J.D. and O'Neill, M.J. (1987), “New leads to the treatment of protozoal infections based on natural product molecules”, *Acta Pharmaceutica Nordica*, Vol. 1, pp. 131–144
- Radoykova, T., Nenkova, S. and Stanulov, K. (2010), “Production of phenol compounds by alkaline treatment of poplar wood bark”, *Chemistry of Natural Compounds*, Vol. 46, pp. 807–808
- Rana, V. and Kumar, V. (2011), “Linkage analysis in a trisaccharide from *Dalbergia* by methylation and periodate oxidation methods”, *International Journal of ChemTech Research*, Vol. 3, pp. 483–487
- Rana, V., Kumar, V. and Soni, P.L. (2009), “Structure of the oligosaccharides isolated from *Dalbergia sissoo* Roxb. leaf polysaccharide”, *Carbohydrate Polymers*, Vol. 78, pp. 520–525
- Reddy, R.V.N., Reddy, N.P., Khalivulla, S.I., Reddy, M.V.B., Gunasekar, D., Blond, A. and Bodo, B. (2008), “O-Prenylated flavonoids from *Dalbergia sissoo*” *Phytochemistry Letters*, Vol. 1, pp. 23–26.
- Roy, N., Laskar, R.A., Sk, I., Kumari, D., Ghosh, T. and Begum, N.A. (2011), “A detailed study on the antioxidant activity of the stem bark of *Dalbergia sissoo* Roxb., an Indian medicinal plant”, *Food Chemistry*, Vol. 126, pp. 1115–1121
- Sarg, TM, Abbas, FA, El-Sayed, ZI, Mustafa, AM (2011), “Two new polyphenolic compounds from *Ficus retusa* L. “variegata” and the biological activity of the different plant extracts”, *Journal of Pharmacognosy and Phytotherapy*, Vol. 3, pp. 89–100
- SAS (2001), “Users guide: statistics (Release 8.02)”, Cary, NC: USA: SAS Inst. Inc.
- Scrivivasan, D., Nathan, S., Suresh, T., Perumalsamy, O. (2001), “Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine”, *Journal of Ethnopharmacology*, Vol. 74, pp. 217–220.
- Schnitzler, J.P., Louis, S., Behnke, K. and Loivamaeki, M. (2010), “Poplar volatiles-biosynthesis, regulation and (eco) physiology of isoprene and stressinduced isoprenoids”, *Plant Biology*, Vol. 12, pp. 302–316
- Shihabudeen, M.H., Priscilla, H.H. and Thirumurugan, D.K. (2010), “Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants”, *International Journal of Pharmaceutical Sciences and Research*, Vol. 1, pp. 430–434
- Taira, T., Ohdomari, A., Nakama, N., Shimoji, M. and Ishihara, M. (2005), “Characterization and antifungal activity of gazzyumaru (*Ficus maicrocarpa*) latex chitinase: Both the chitin-binding and the antifungal activities of class 1 chitinase are reinforced with increasing ionic strength”, *Bioscience, Biotechnology, and Biochemistry*, Vol. 69, pp. 811–818
- Takahashi, S., Tamashiro, A., Sakihama, Y., Yamamoto, Y., Kawamitsu, Y. and Yamasaki, H. (2002), “High-susceptibility of photosynthesis to photoinhibition in the tropical plant *Ficus microcarpa* L. f. cv. Golden Leaves”, *BMC Plant Biology*, Vol. 2, pp. 1–8.
- Texixer, C.C., Rava, C.A., Silva, P.M., Melchior, R., Argenta, R., Anselmi, F., Almeida, C.R.C. and Fuchs, F.D. (2000), “Absence of antihyperglycemic effect of jambolan in experimental and clinical models”, *Journal of Ethnopharmacology*, Vol. 71, pp. 343–347
- Timbola, A.K., Szpoganicz, B., Branco, A., Monache, F.D. and Pizzolatii, M.G. (2002), “A new flavonol from leaves of *Eugenia jambolana*”, *Fitoterapia*, Vol. 73, pp. 174–176
- Toroglu, S. (2007), “In vitro antimicrobial activity and antagonistic effect of essential oils from plant species”, *Journal of Environmental Biology*, Vol. 28, pp. 551–559
- Upwar, N., Patel, R., Waseem, N. and Mahobia, N.K. (2011), “Evaluation of anthelmintic activity of *Dalbergia Sissoo* Roxb”, *International Journal of Pharmaceutical Sciences and Research*, Vol. 2, pp. 171–174

-
- van Vuuren, S.F. (2008), “Antimicrobial activity of South African medicinal plants-Review”, *Journal of Ethnopharmacology*, Vol. 119, pp. 462–472
- Wieslawa, M., Marek, D., Tadeusz, H.D. and Karol, B. (2002), “GC-MS and HPLC analysis of phenolic acids Extracted from propolis and from *Populus nigra* bud exudate”, *Chemia analityczna*, Vol. 47, pp. 21–24
- Zhong, L., Zhou, L., Zhou, Y., Chen, Y., Sui, P., Wang, J. and Wang, M. (2012), “Antimicrobial flavonoids from the twigs of *Populus nigra* x *Populus deltoids*”, *Natural Products Research*, Vol. 26, pp. 307–13