

Antimicrobial and Antioxidant Activities of Stem Bark Extracts of Different Ornamental Trees

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Abstract:

Aqueous, ethanol, methanol and acetone stem bark extracts from nine tree species; *Pinus halepensis*, *Leucaena leucocephala*, *Ficus sycomorus*, *Quercus ruber*, *Albizia lebbbeck*, *Terminalia arjuna*, *Bauhinia variegata*, *Cassia fistula* and *Kegelia africana* growing in Assiut, Egypt were investigated for their antimicrobial activity against three bacterial and three fungal species *in vitro* as well as their antioxidant activity using DPPH radical scavenging. Patterns of inhibition varied with the plant extract, the solvent used for extraction, and the organism tested. Acetone extract of *K. africana* gave the maximum significant inhibitory effect against the three bacterial strains. *Bacillus cereus* was the most inhibited bacteria, followed by *Erwinia carotovora*. The maximum antifungal activity was noticed in different extracts of *C. fistula* against the three fungal strains; *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani* with significant differences comparing with the other extracts. Samples extracted by acetone and ethanol, respectively, showed significant increment in antibacterial and antifungal activities over either methanol or aqueous ones, which recorded the minimum inhibition. The methanol extract of both *K. africana* and *B. variegata* showed the strongest inhibition of DPPH radical activity. The EC₅₀ reached its lowest values with acetone extract of *A. lebbbeck* followed by ethanol and acetone extracts of *P. halepensis*.

Keywords: Antibacterial activity, Antifungal activity, Antioxidant EC₅₀, DPPH assay, Stem bark

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Introduction:

Synthetic pesticides, which have been universally considered for long time as the most efficient solution to control plant diseases, may enter the food chain posing a significant human health and environmental hazards. This has highlighted the need for the use of alternative compounds that are environmentally friendly and safe to humans. Plant secondary metabolites, such as essential oils and plant extracts are known to possess insecticidal, antifungal, acaricidal, antibacterial and cytotoxic activities. These plant-derived products are used medicinally in different countries and are a source of many potent and powerful drugs and antimicrobial agents (Srivastava et al., 1996; Tepe et al., 2004). The resurgence of interest in effective, safe, natural products has prompted researchers in different parts of the globe to screen and apply plant extracts in different discipline including plant pathology (Daferera et al., 2000).

Plant pathogens are of serious concern, as they cause huge damage to economic crops. *Fusarium spp.*, are well known for their pathogenicity causing reduction in growth in a variety of host plants (Ahmad et al., 1998). Fusarium wilt of cumin, caused by a soil-and seed-borne vascular wilt pathogen *Fusarium oxysporum* f. sp. *Cumini* is a devastating disease that occurs in major cumin growing areas of the world (Mehta et al., 2012; Pappas and Elena, 1997). *F. solani* and *R. solani* are the most important soilborne fungal pathogens, which develop in both cultured and non-cultured soils, causing the symptoms of damping off and

root rot diseases to wide range of vegetable and crop plants including tomato (Abu-Taleb et al., 2011). In the current study, *Bacillus cereus* and *Streptomyces scabies* were used as model organisms for studying Gram-positive bacteria, while *Erwinia carotovora* was used as a model of gram-negative bacteria. Common scab is a serious disease of potatoes and other root and tuber crops, affecting the quality and market value of these crops. The disease is caused by gram-positive soil bacteria of the genus *Streptomyces* (Wanner, 2007). The enterobacterial plant pathogen *Pectobacterium* (formerly *Erwinia carotovora*) causes soft rot diseases in monocot. and dicot. host plants in at least 35% of angiosperms. In potato, *Pectobacterium* causes wilt, soft rot, and blackleg and affects plant health during field production and storage (Ma et al., 2007; Perombelon, 2002).

Antimicrobial activities of plant oils and extracts in particular have formed the basis of many applications in agriculture including natural pest, disease and weed control (Callaway and Aschehoug, 2000). Antimicrobial compounds from plants may inhibit the growth of microorganisms by different mechanism than those presently used. The search for new antimicrobial agents is even more urgent as the resistance to drugs by microorganisms has increased because of their genetic ability to transmit and acquire resistance to drugs used as therapeutic agents (Eloff, 1998; Hammer et al., 1999).

Oxidative damage caused by superoxide, hydroxyl and peroxy radicals is a troublesome issue causing low density lipoprotein and im-

portant cells and organs, as well as food systems. Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts or isolated products from plant origin rather than looking for synthetic ones (McClements and Decker, 2000). Recent works have focused on polyphenolic compounds such as flavonoids, anthraquinones, anthocyanidins and xanthenes which are present quite commonly in the bark of many tree species of the higher plants (Siddhurajua *et al.*, 2002). Consequently, many previous studies have attempted to shed light on the antioxidant and antimicrobial activities of some tree species. However, scientific evidence of the antimicrobial and the antioxidant effects of many of them has not been investigated yet.

The main objective of the current study was to investigate the potential antimicrobial activity of aqueous, ethanol, methanol and acetone stem bark extracts of nine tree species against some bacterial and fungal strains and to determine their DPPH free radical scavenging activity.

Materials and Methods:

Plant materials:

The current study was conducted during 2014 season at the Laboratories of Ornamental Plants and Landscape Gardening and Plant Pathology Departments, Assiut University. Stem bark samples were collected from nine tree species, namely *Albizia lebbeck* L., *Bauhinia variegata* L., *Cassia fistula* L., *Ficus sycomorus* L., *Kegelia africana* Lam., *Leucaena leucocephala* Lam., *Pinus halepensis* Miller, *Quercus ruber* L. and *Terminalia arjuna* Roxb. Trees

were locally grown and authenticated at the Experimental Farm of Ornamental Plants and Landscape Gardening Department, Faculty of Agriculture, Assiut University, Egypt. Collected samples were shredded into small pieces, air-dried and pulverized into fine powder.

Extraction:

A sample of fifty grams powder of the nine plant species was macerated in 500ml of four solvents (Aqua, Ethanol, Methanol and Acetone at 40% concentration) for 72 h at room temperature under constant shaking. The macerate was filtered with Whatman No. 1 filter paper and the residue was further macerated twice with the same solvent overnight and filtered. The filtrates obtained from each extraction were combined and kept in tightly stoppered bottle in a refrigerator (2-4 °C) as crude extracts. For the DPPH assay, the solvent was evaporated from the crude extract to dryness under reduced pressure (rotary evaporator Heidolph VV2000) and the residue was freeze-dried (Freeze-dryer Telstar-LyoQuest Plus-55).

Antimicrobial activity screening:

The antimicrobial activity of the plant extracts was screened against three bacterial species; *Erwinia carotovora* subsp. *Carotovora jones*, *Streptomyces scabies* Thaxter and *Bacillus cereus* Frankland & Frankland and three fungal species; *Fusarium oxysporum* f. sp. *cumini*, *Fusarium solani* (Mart.) Sacc. and *Rhizoctonia solani* Kuhn. These microbial species were isolated from the soil and naturally infected plants collected from farmer fields in Assiut Governorate, Egypt. *E. carotovora* and *S.*

scabies were isolated from *Potato* plants and its tubers while *B. cereus*, a non-plant pathogenic species, was isolated from soil rhizosphere of potato plants by serial dilution method. *F. oxysporum* was isolated from cumin roots; *F. solani* and *R. solani* were isolated from tomato seedlings. Bacterial isolates were identified by a series of morphological, biochemical as well as physiological characteristics using the standard characterization procedure of Skinner and Lovelock (1979), Sneath *et al.* (1986) and Buchanan and Gibbon (2001). Identification of the isolated fungi was carried out on 5-12 days old culture using the morphological and microscopic characteristics of mycelium and spores according to Booth (1977), Pitt (1979), Domsch *et al.* (1980), Sivansen (1984), Agrios (1997) and Lucas (1998). The antibacterial and antifungal tests were conducted according to the following procedures.

***In vitro* Antibacterial Activity:**

Crude extract solutions were filtered through a 0.22 μm sterile filter (PES Syringe filter). The antibacterial activity of the extracts solution was determined in accordance with the agar-well diffusion method described by Irobi *et al.* (1994). The bacterial isolates were first grown in a nutrient sucrose (NS) liquid medium for 24 h at $30\pm 2^\circ\text{C}$ before use and standardized to 10^6 CFU/ml. 150 μl of the standardized cell suspensions were spread on a nutrient sucrose agar (NSA) plates. Wells were then bored into the agar using a sterile 7 mm diameter cork borer. Approximately 100 μl of the crude extract were introduced into each well, allowed to

stand at room temperature for about 2 h and then incubated at $30\pm 2^\circ\text{C}$. Controls were set up in parallel using the solvents that were used with each extract. Plates were incubated for 24 h. After this period, it was possible to observe inhibition zone. Overall, cultured bacteria with halos equal to or greater than 7 mm were considered susceptible to the tested extract. Streptomycin sulfate salt with concentration of 50 $\mu\text{g/ml}$ was used as positive controls.

***In vitro* Antifungal Activity:**

The fungal isolates were allowed to grow on a Potato dextrose agar (PDA) at $26\pm 2^\circ\text{C}$ for five days until they sporulated. The antifungal activity was determined by the paper disc diffusion method (Garg and Jain, 1988) with some modifications. Sterilized 9 cm-Petri dishes containing 10 ml of PDA medium (pH 7) supplemented with Streptomycin sulfate salt 50 $\mu\text{g/ml}$ were used. Plates were inoculated with 7 mm-disks of each fungus obtained from 5 days old cultures on a side of each plate. Subsequently, one equal filter paper discs saturated with extract solution (75 μl) was placed at opposite sides on surface of each inoculated plate. Plates were incubated at $26\pm 2^\circ\text{C}$. Four replicates were used for each treatment. Observations of inhibition of the tested fungi were recorded after incubation period when the control plates reached its maximum growth and covered the plate surface. Discs with the solvent used for dissolution were used as negative control. The diameter of mycelial growth was measured using a ruler, to determine the mycelial growth inhibition area. After measuring all the replicates, the aver-

ages of the treatments were calculated according to the following formula:

Percentage of mycelial growth inhibition = $\frac{A - B}{A} \times 100$

Where: (A) is the colony diameter of pathogen alone (control); (B) is the colony diameter of pathogen with extract solution.

Antioxidant activity (DPPH radical scavenging assay):

The antioxidant activity of the plant materials was assayed by employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich) radical scavenging assay (Shikanga *et al.*, 2010). The method is based on the reduction of DPPH as a stable free radical having an odd electron, which gives a maximum absorption at 517 nm (purple colour). Each sample was prepared through dissolving 10 mg of the plant extract in 10 ml methanol absolute using Vortex and water bath. The final aliquot (1000 µg/ml) was filtrated. Five dilutions were prepared from each extract; 1000, 500, 250, 125, 62.5 µg/ml (three replicates). Each diluted extract (0.2 ml) was added to DPPH solution (1.8 ml of 0.1 mM DPPH in methanol). The reaction mixture was incubated at 25°C for 30 min, after which the absorbance was measured at 517 nm. The DPPH is reduced to DPPH-H when it reacts with the antioxidants and, consequently, the absorbance decreased resulting in decolourisation (yellow colour) with respect to the number of electrons captured. Methanol (0.2 ml) instead of the plant extract was added to the mixture and served as the control. The DPPH radical scavenging activity of each plant extract was calculated as the inhibition percentage.

Inhibition % of DPPH free radical activity = $\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$

Linear regression analysis was used to determine the best-fitting straight line from which the EC₅₀ values were determined using SPSS Statistics 16.0.

Statistical analysis:

The results were analyzed using ANOVA means differences were compared using LSD at 5% according to Gomez and Gomez (1984).

Results:

***In vitro* Antimicrobial activity:**

The possible antibacterial activity of 36 stem bark crude extracts from nine tree species were investigated comparing with Streptomycin sulfate salt as a standard antibiotic. According to the results presented in Table 1, the tested extracts showed significant antibacterial activity against one or more of the bacterial strains tested. The extracts exhibited variable antibacterial activity depending on the solvent used for extraction (Aqua, Ethanol, Methanol and Acetone) and the bacterial strain tested. The three bacterial strains involved in the current study were proved susceptible to all of the 36 extracts studied showing inhibition zones more than 7mm.

Data indicated that acetone extract of *K. africana* recorded the best activity against the three bacterial strains comparing with all other extracts with inhibition zone diameter between 16.37 and 25mm. These values are considered high relative to the mean inhibition zones for positive control (Streptomycin sulfate salt) which were 31.37, 29.5 and 33.37 mm with *E. carotovora*, *S. scabies*

Table (1): The antimicrobial activity of various stem bark extracts against different microbial strains.

Plant species	Solvent	Inhibition zone (mm) of the microbial species					
		Bacterial strains			Fungal strains		
		<i>E. carotovora</i>	<i>S. scabies</i>	<i>B. cereus</i>	<i>F. oxysporum</i>	<i>F. solani</i>	<i>R. solani</i>
<i>A. lebbbeck</i>	Aqua	15.75 de	14.50 cd	16.75 de	47.49 q	46.66 q	42.49 l
	Ethanol	16.00 de	14.50 cd	16.00 ef	56.10 hij	64.99 a	54.16 fgh
	Methanol	14.25 gh	14.25 cd	15.25 fgh	49.71 op	61.94 bc	51.94 i
	Acetone	17.12 c	14.75 c	17.75 c	54.99 jk	58.60 efg	55.82 cde
<i>B. variegata</i>	Aqua	10.25 lmn	9.50 nop	11.75 kl	49.99 op	52.22 kl	38.32 n
	Ethanol	14.00 gh	11.00 ijk	11.75 kl	56.93 ghi	64.71 a	48.04 j
	Methanol	12.00 i	10.25 klmn	12.50 jk	53.05 lm	59.72 de	54.16 fgh
	Acetone	14.50 gh	12.25 gh	15.00 ghi	52.49 mn	54.44 i	43.33 kl
<i>C. fistula</i>	Aqua	12.00 i	13.25 ef	14.25 i	48.60 pq	48.60 o	37.49 n
	Ethanol	15.62 def	10.75 ijkl	15.25 fgh	66.10 a	59.16 ef	62.77 a
	Methanol	15.50 ef	12.25 gh	15.75 fg	54.99 jk	51.94 lm	53.33 ghi
	Acetone	16.50 cd	12.75 fg	17.00 cd	58.60 def	64.71 a	62.77 a
<i>F. sycomorus</i>	Aqua	9.25 opq	9.25 opq	11.25 lm	47.49 q	49.16 no	22.22 q
	Ethanol	12.25 i	10.50 jklm	11.75 kl	59.72 d	62.22 bc	31.94 o
	Methanol	10.50 lm	9.75 mnop	12.00 jkl	58.04 efg	61.11 cd	29.44 p
	Acetone	14.75 fg	12.25 gh	15.00 ghi	52.22 mn	54.16 i	32.22 o
<i>K. africana</i>	Aqua	11.50 ijk	10.25 klmn	12.25 jk	51.11 no	52.49 jkl	48.05 j
	Ethanol	14.25 gh	12.75 fg	17.00 cd	63.60 c	63.05 b	52.77 hi
	Methanol	13.75 h	11.50 hi	14.37 hi	54.44 kl	56.38 h	54.71 defg
	Acetone	19.12 b	16.37 b	25.00 b	63.33 c	57.49 gh	56.10 cd
<i>L. leucocephala</i>	Aqua	8.75 pq	7.25 st	8.12 r	48.60 pq	48.88 o	54.99 def
	Ethanol	9.50 nop	8.50 qr	9.50 pq	57.49 fgh	61.94 bc	58.88 b
	Methanol	8.37 q	7.50 st	8.87 qr	49.99 op	56.10 h	54.44 efg
	Acetone	11.00 jkl	8.00 rs	11.25 lm	53.05 lm	52.49 jkl	54.99 def
<i>P. halepensis</i>	Aqua	9.75 mno	7.50 st	10.00 nop	48.04 q	46.93 pq	41.94 lm
	Ethanol	11.75 ij	9.00 pq	11.75 kl	63.33 c	58.05 fg	54.44 efg
	Methanol	11.62 ijk	9.00 pq	11.12 lm	59.16 de	53.88 ij	49.44 j
	Acetone	14.50 gh	14.25 cd	15.37 fg	54.44 kl	53.60 ijk	52.77 hi
<i>Q. ruber</i>	Aqua	8.75 pq	7.50 st	9.75 opq	44.44 r	46.10 q	44.44 k
	Ethanol	10.75 kl	9.75 mnop	11.75 kl	64.16 bc	58.60 efg	56.66 c
	Methanol	10.25 lmn	9.75 mnop	10.75 mn	54.99 jk	49.16 no	51.94 i
	Acetone	11.50 ijk	9.75 mnop	10.50 mno	55.82 ijk	50.55 mn	53.33 ghi
<i>T. arjuna</i>	Aqua	11.00 jkl	10.00 lmno	12.75 j	55.55 ijk	48.32 op	40.55 m
	Ethanol	16.50 cd	13.75 de	17.25 cd	65.55 ab	52.49 jkl	58.60 b
	Methanol	16.25 cde	12.50 fg	17.00 cd	58.04 efg	57.49 gh	58.88 b
	Acetone	17.00 c	11.25 ij	17.75 c	54.44 kl	61.38 c	54.71 defg
Positive control	Streptomycin (for bacteria)	31.37 a	29.50 a	33.37 a	-	-	-
Negative cont.	Aqua	7.00 r	7.00 t	7.00 s	0.00 s	0.00 r	0.00 r
	Ethanol	7.00 r	7.00 t	7.00 s	0.00 s	0.00 r	0.00 r
	Methanol	7.00 r	7.00 t	7.00 s	0.00 s	0.00 r	0.00 r
	Acetone	7.00 r	7.00 t	7.00 s	0.00 s	0.00 r	0.00 r
LSD 0.05		0.89	0.93	0.90	1.40	1.44	1.63

* Means within the same column followed by different letters are significantly different ($P \leq 0.05$) based on LSD.

and *B. cereus*, respectively. Regarding aqueous extracts, the best results were noticed in *A. lebbeck* with the three bacterial stains (15.75, 15.5 and 16.75 mm). The highest results of the ethanol extracts were in *T. arjuna* (16.5, 13.75 and 17.25 mm), *A. lebbeck* (16.0, 14.5 and 16 mm) and *C. fistula* (15.62, 10.75 and 15.75 mm). Results of methanol extracts were highest in *T. arjuna* (16.25, 12.5 and 17.0 mm) and *C. fistula* (15.50, 12.25 and 15.75 mm). With regard to the acetone extracts, the best results were in *K. africana* (19.12, 16.37 and 15.0 mm), *A. lebbeck* (17.12, 14.75 and 17.75) and *T. arjuna* (17.0, 11.25 and 17.75) with the three bacterial strains, respectively. Generally, acetone extracts have the best antibacterial activity comparing with aqueous, ethanol and methanol ones with the inhibition zone ranging from 11.0 to 19.12 mm with *E. carotovora*, 8.0 to 16.37 mm with *S. scabies* and 10.50 to 25.0 mm with *Bacillus cereus*. The solvents could be put in a descending ordered according to their antibacterial activity as follows; acetone, ethanol, methanol and aqua.

Among the tested extracts, the maximum antifungal activity was noticed in different extracts of *C. fistula* against the three fungal strains. Ethanol extracts of *T. arjuna* showed the next inhibition values (66.55 and 58.60mm) against *F. oxysporum* and *R. solani*, respectively. Ethanol extracts of *A. lebbeck* and *B. variegata* recorded significant antifungal activity against *F. solani* with growth inhibition 64.99 and 64.71mm, respec-

tively. In most cases, the ethanol extracts exhibited the highest inhibition zones between 44.49 and 66.10mm with *F. oxysporum*, 46.10 and 64.99mm with *F. solani* and 22.22 and 62.77 with *R. solani*.

Antioxidant activity:

Results obtained indicated that various stem bark extracts of the nine tree species tested exhibited varied antioxidant activity as presented in Table 2. Inhibition % of DPPH free radical scavenging activity of the five serial dilutions tested in addition to the EC₅₀ are also shown in Table 3. At 1000 µg/ml concentration, the antioxidant activity of the thirty-six extracts tested ranged from 43.39 to 98.85%. Extracts of *C. fistula*, *F. sycomorus*, *L. leucocephala*, *P. halepensis* and *Q. ruber* exhibited their highest antioxidant activities with acetone ranging from 90.02 to 97.04%. Ethanol extracts of *A. lebbeck* and *T. arjuna* proved superior comparing with the other solvents with antioxidant activities of 88.06 and 96.12%, respectively. Among all the tested extracts, the methanol extract of both *K. africana* and *B. variegata* showed the strongest inhibition of DPPH radical activity recording 98.85 and 96.70%, respectively.

The EC₅₀ reached its lowest values with *A. lebbeck* acetone extract (4.42 µg/ml) followed by ethanol and acetone extracts of *P. halepensis* (6.38 and 6.46 µg/ml) confirming their powerful antioxidant activity.

Table (2): DPPH free radical scavenging activity of various stem bark extracts (mean \pm SD).

Plant species	Solvent	Dilutions				
		1000 μ g/ml	500 μ g/ml	250 μ g/ml	125 μ g/ml	62.5 μ g/ml
<i>A. lebbbeck</i>	Aqua	0.221 \pm 0.001	0.234 \pm 0.001	0.266 \pm 0.002	0.536 \pm 0.003	0.585 \pm 0.012
	Ethanol	0.104 \pm 0.001	0.216 \pm 0.001	0.230 \pm 0.001	0.264 \pm 0.001	0.299 \pm 0.001
	Methanol	0.163 \pm 0.001	0.202 \pm 0.002	0.329 \pm 0.002	0.607 \pm 0.001	0.706 \pm 0.001
	Acetone	0.145 \pm 0.015	0.167 \pm 0.001	0.206 \pm 0.003	0.251 \pm 0.001	0.267 \pm 0.001
<i>B. variegata</i>	Aqua	0.400 \pm 0.001	0.656 \pm 0.002	0.745 \pm 0.001	0.800 \pm 0.001	0.809 \pm 0.001
	Ethanol	0.037 \pm 0.001	0.043 \pm 0.002	0.053 \pm 0.001	0.065 \pm 0.001	0.425 \pm 0.001
	Methanol	0.029 \pm 0.002	0.043 \pm 0.002	0.063 \pm 0.001	0.200 \pm 0.001	0.460 \pm 0.001
	Acetone	0.035 \pm 0.001	0.044 \pm 0.001	0.056 \pm 0.002	0.091 \pm 0.001	0.324 \pm 0.001
<i>C. fistula</i>	Aqua	0.365 \pm 0.003	0.410 \pm 0.001	0.637 \pm 0.001	0.680 \pm 0.001	0.745 \pm 0.002
	Ethanol	0.050 \pm 0.001	0.074 \pm 0.002	0.096 \pm 0.001	0.315 \pm 0.002	0.480 \pm 0.001
	Methanol	0.042 \pm 0.001	0.046 \pm 0.001	0.050 \pm 0.001	0.170 \pm 0.001	0.584 \pm 0.001
	Acetone	0.026 \pm 0.001	0.040 \pm 0.001	0.045 \pm 0.001	0.305 \pm 0.001	0.443 \pm 0.001
<i>F. sycomorus</i>	Aqua	0.491 \pm 0.006	0.625 \pm 0.006	0.655 \pm 0.006	0.768 \pm 0.010	0.801 \pm 0.002
	Ethanol	0.091 \pm 0.001	0.120 \pm 0.001	0.440 \pm 0.002	0.680 \pm 0.001	0.727 \pm 0.001
	Methanol	0.092 \pm 0.004	0.097 \pm 0.001	0.421 \pm 0.002	0.635 \pm 0.001	0.750 \pm 0.001
	Acetone	0.086 \pm 0.001	0.093 \pm 0.002	0.094 \pm 0.002	0.430 \pm 0.001	0.633 \pm 0.001
<i>K. africana</i>	Aqua	0.194 \pm 0.001	0.265 \pm 0.001	0.556 \pm 0.001	0.693 \pm 0.003	0.761 \pm 0.002
	Ethanol	0.064 \pm 0.002	0.300 \pm 0.001	0.480 \pm 0.001	0.650 \pm 0.001	0.751 \pm 0.002
	Methanol	0.010 \pm 0.001	0.011 \pm 0.006	0.165 \pm 0.001	0.338 \pm 0.010	0.677 \pm 0.002
	Acetone	0.041 \pm 0.001	0.302 \pm 0.001	0.352 \pm 0.001	0.437 \pm 0.001	0.702 \pm 0.002
<i>L. leucocephala</i>	Aqua	0.471 \pm 0.015	0.611 \pm 0.006	0.685 \pm 0.006	0.735 \pm 0.006	0.801 \pm 0.002
	Ethanol	0.155 \pm 0.001	0.354 \pm 0.002	0.600 \pm 0.001	0.712 \pm 0.002	0.784 \pm 0.002
	Methanol	0.268 \pm 0.010	0.521 \pm 0.012	0.707 \pm 0.002	0.756 \pm 0.001	0.791 \pm 0.002
	Acetone	0.080 \pm 0.001	0.240 \pm 0.002	0.562 \pm 0.001	0.712 \pm 0.001	0.777 \pm 0.002
<i>P. halepensis</i>	Aqua	0.079 \pm 0.008	0.090 \pm 0.002	0.086 \pm 0.001	0.106 \pm 0.002	0.413 \pm 0.002
	Ethanol	0.080 \pm 0.002	0.082 \pm 0.003	0.095 \pm 0.003	0.099 \pm 0.002	0.276 \pm 0.002
	Methanol	0.088 \pm 0.010	0.080 \pm 0.002	0.095 \pm 0.021	0.094 \pm 0.002	0.297 \pm 0.001
	Acetone	0.075 \pm 0.001	0.079 \pm 0.002	0.093 \pm 0.002	0.104 \pm 0.002	0.266 \pm 0.002
<i>Q. ruber</i>	Aqua	0.098 \pm 0.010	0.111 \pm 0.001	0.155 \pm 0.001	0.543 \pm 0.001	0.705 \pm 0.001
	Ethanol	0.231 \pm 0.006	0.240 \pm 0.006	0.310 \pm 0.001	0.340 \pm 0.001	0.430 \pm 0.001
	Methanol	0.131 \pm 0.001	0.140 \pm 0.002	0.271 \pm 0.001	0.370 \pm 0.001	0.641 \pm 0.001
	Acetone	0.087 \pm 0.001	0.218 \pm 0.020	0.256 \pm 0.001	0.291 \pm 0.001	0.554 \pm 0.001
<i>T. arjuna</i>	Aqua	0.426 \pm 0.001	0.633 \pm 0.002	0.735 \pm 0.001	0.772 \pm 0.001	0.800 \pm 0.001
	Ethanol	0.034 \pm 0.001	0.120 \pm 0.001	0.284 \pm 0.001	0.533 \pm 0.001	0.628 \pm 0.010
	Methanol	0.073 \pm 0.001	0.103 \pm 0.001	0.445 \pm 0.001	0.552 \pm 0.001	0.577 \pm 0.001
	Acetone	0.074 \pm 0.001	0.174 \pm 0.001	0.595 \pm 0.001	0.643 \pm 0.001	0.766 \pm 0.001

Table (3): Inhibition % of DPPH free radical scavenging activity of various stem bark extracts

Plant species	Solvent	Dilutions					EC ₅₀ µg/ml
		1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	
<i>A. lebbeck</i>	Aqua	74.58	73.08	69.32	38.25	32.64	158.80
	Ethanol	88.06	75.12	73.50	69.59	65.51	14.15
	Methanol	81.22	76.69	62.06	30.07	18.66	213.17
	Acetone	83.33	80.76	76.31	71.04	69.24	4.42
<i>B. variegata</i>	Aqua	53.92	24.42	14.17	7.80	6.76	1135.00
	Ethanol	95.78	95.05	93.89	92.47	51.08	37.50
	Methanol	96.70	95.08	92.78	77.00	47.00	57.08
	Acetone	95.93	94.93	93.55	89.52	62.67	24.03
<i>C. fistula</i>	Aqua	57.91	52.76	26.61	21.66	14.17	609.62
	Ethanol	94.24	91.47	88.94	63.75	44.70	69.05
	Methanol	95.12	94.70	94.28	80.41	32.72	70.74
	Acetone	97.04	95.39	94.82	64.86	48.96	8.86
<i>F. sycomorus</i>	Aqua	43.39	28.03	24.58	11.52	7.72	1086.00
	Ethanol	89.52	86.18	49.27	21.70	16.24	225.19
	Methanol	89.36	88.82	51.50	26.84	13.56	214.56
	Acetone	90.13	89.29	89.21	50.46	27.04	110.53
<i>K. africana</i>	Aqua	77.69	69.47	35.94	20.20	12.37	334.32
	Ethanol	92.67	65.44	44.70	25.15	13.44	264.96
	Methanol	98.85	98.69	80.95	61.06	22.04	110.13
	Acetone	95.28	65.21	59.41	49.65	19.09	177.53
<i>L. leucocephala</i>	Aqua	45.70	29.57	21.12	15.36	7.76	1042.00
	Ethanol	82.14	59.25	30.88	17.97	9.68	374.28
	Methanol	69.12	39.94	18.59	12.90	8.91	630.86
	Acetone	90.75	72.31	35.22	17.97	10.52	294.61
<i>P. halepensis</i>	Aqua	90.86	89.67	90.09	87.83	52.42	26.97
	Ethanol	90.75	90.51	89.06	88.56	68.20	6.38
	Methanol	89.86	90.75	89.09	89.21	65.78	7.53
	Acetone	91.36	90.90	89.25	88.02	69.32	6.46
<i>Q. ruber</i>	Aqua	88.71	87.21	82.14	37.44	18.82	150.77
	Ethanol	73.35	72.31	64.29	60.83	50.46	47.12
	Methanol	84.95	83.83	68.82	57.41	26.19	124.52
	Acetone	90.02	74.88	70.55	66.47	36.18	90.39
<i>T. arjuna</i>	Aqua	50.96	27.11	15.32	11.10	7.83	1237.00
	Ethanol	96.12	86.18	67.28	38.59	27.65	145.90
	Methanol	91.59	88.13	48.73	36.37	33.49	161.22
	Acetone	91.47	79.95	31.45	25.92	11.75	265.08

Discussion:

Due to the anticipated antimicrobial activity of plant extracts, the current study was conducted to find out the potential antimicrobial activity of thirty-six crude extracts of nine tree species extracted using four different solvents. Results showed broad antibacterial and antifungal activities of the tested extracts against various bacterial and fungal strains tested. This variation was strongly affected by the plant species, the solvent and the susceptibility of the microbial strains. According to results, the highest antibacterial activity was recorded in acetone extract *K. africana*. These results are in accordance with those obtained by Desai *et al.* (1971). They attributed the high antibacterial activity displayed by acetone extract of *K. africana* to the presence of kigelin, β -sitosterol, 3-dimethylkigelin, ferulic acid and iridoids which were isolated from its bark. The antimicrobial action of the extracts may be attributed to astringent nature of the phenolic constituents including tannins and other polyphenols present in the extracts (Taguri *et al.*, 2004). The high to moderate results obtained by stem bark extract of *A. lebeck* were further supported by the findings of (Salem *et al.*, 2013). They also found that the flavonoid to phenolics ratio was 0.072 bark extract. The plant extracts revealed the presence of alkaloids, saponins, carbohydrates, proteins and tannins with the absence of steroids in methanol extract and alkaloids in aqueous extract.

According to the growth inhibition of bacterial and fungal strains tested, ethanol and methanol gave

appreciable results which could be explained upon the ability of them to enhance the extraction of a large quantity of secondary metabolites due to their polarity, concentration or nature (Tekwu *et al.*, 2012). The plant methanol extract was found to be a potent antibacterial and antifungal agent by many authors such as Malla *et al.* (2014), Agyare *et al.* (2013) and Bharti *et al.* (2006). It is evident from results that susceptibility of pathogens to plant extracts depends upon plant species (Bagwan, 2001), solvent used for extraction and extract concentration, as well as the organism tested (Abou-Jawdah *et al.*, 2002; Kumaran *et al.*, 2003).

Most of the tested extracts revealed promising antioxidant activities which are reflected by the elevated DPPH radical scavenging ability. The stem bark of most of the tested plant species was found to be a rich source of phyto-constituents such as polyphenolic compounds having immense antioxidant potential (Malla *et al.*, 2014; Siddhurajua *et al.* 2002). The antioxidant activities of extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins, and phenolic diterpenes which play an important role in protecting oxidative damage from free radicals (Ayoola *et al.*, 2008; Nayak *et al.*, 2009; Okuda, 2005). Phenolic compounds are powerful chain breaking antioxidants having anticancer, anti-diabetic, anti-aging properties and prevention of cardiovascular diseases (Dixon *et al.*, 2005; Shahidi and Wanasundara, 1992). The free radical scavenging activity of the plant extract is related to their hydrogen or

electron-donation abilities and the confirmation of antioxidant compound of the extracts (Abdul-Hafeez *et al.*, 2014).

Conclusions:

Application of stem bark crude extracts as bactericides and fungicides is an effective and safe technique. Results revealed that stem bark extracts of most of the plant species tested could be recommended as antibacterial and antifungal agents. In addition, most of the tested extracts were found to have antioxidant activity confirming their value. This suggests further experiments to focus on *in vitro* assessment of other biological activity for the most anticipated extracts.

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الفعالية المضادة للميكروبات والمضادة للأكسدة لمستخلصات من قلف الساق لأشجار زينة مختلفة

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الملخص:

أجريت الدراسة الحالية على تسعة أنواع شجرية نامية بمشغل الزينة بكلية الزراعة - جامعة أسيوط - مصر، وهي الصنوبر الحلبي، اللبوسينيا، الجميز، البلوط الأحمر، اللبخ، الترماليا، خف الجمل، الخيار شمبر والمشطورة، حيث تم تجهيز مستخلصات من قلف الأشجار باستخدام أربعة مذيبات وهي الماء والإيثانول والميثانول والأسيتون. وبلغ عدد المستخلصات التي تم تجهيزها 36 مستخلص، تم اختبار فاعليتها المضادة للميكروبات معملياً ضد ثلاث سلالات بكتيرية وثلاث أنواع فطرية. كما تم إجراء اختبار الفاعلية المضادة للأكسدة لتلك المستخلصات باستخدام طريقة تثبيط الجذور الحرة بنثائي الفينيل بكريل هيدرازيل DPPH. وأظهرت النتائج تبايناً واضحاً في التأثير المثبط تبعاً لنوع المستخلص ونوع المذيب المستخدم وكذلك النوع الميكروبي المختبر. أظهر مستخلص الأسيتون لشجرة المشطورة أقصى تأثير ضد مثبت الأنواع البكتيرية الثلاثة بفارق معنوي مقارنة بباقي المستخلصات، حيث كانت بكتريا *Bacillus cereus* هي الأكثر تأثراً، تلتها *Erwinia carotovora*. في حين لوحظ أعلى نشاط مضاد للفطريات عند معاملة الأنواع الفطرية الثلاثة: *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani* بمختلف مستخلصات شجرة الخيار شمبر بفارق معنوي مقارنة بباقي المستخلصات. وبشكل عام فإن العينات المستخلصة بأي من الأسيتون أو الإيثانول أظهرت أفضل تأثير مضاد للبكتريا أو الفطريات، في حين تم تسجيل أقل تأثير مثبط عند المعاملة بالمستخلصات المائية. كما أظهر مستخلص الميثانول لكل من المشطورة وخف الجمل أعلى فعالية مضادة للأكسدة في اختبار DPPH. وقد سجل أقل متوسط للجرعة الفعالة EC₅₀ في مستخلص الأسيتون لشجرة اللبخ يليه مستخلصي الإيثانول والأسيتون لشجرة الصنوبر الحلبي.