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ANTIMICROBIAL POTENTIAL OF LEBANESE CEDAR EXTRACT AGAINST HUMAN PATHOGENS AND FOOD SPOILAGE MICROORGANISMS.

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ABSTRACT: *Water and methanolic extracts of the leaves, stems, and pulp of Lebanese Cedar (Cedrus libani), were assayed for antibacterial and antifungal properties against human and food spoilage pathogens namely: Klebsiella pneumonia, MRSA, ESBL E. coli, Listeria monocytogenes, and Candida albicans using disk diffusion method. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) were evaluated. The bacterial and fungal strains tested showed a various degree of sensitivity represented by inhibition zone diameter: Klebsiella pneumonia (27mm), MRSA (27mm), ESBL Escherichia coli (20mm), and Candida albicans (21mm). Listeria monocytogenes showed the strongest inhibition zone 70 and 37mm with methanolic and water leaves extracts respectively. The MIC, MBC, and MFC were between 5- 200 µl/ml, and 300 µl/ml respectively. Time-kill curve showed a fast and sharp antimicrobial activity. The efficacy of killing by Lebanese cedar extract was essentially the same in light and dark. Phytochemical analysis of Lebanese cedar extract explored the presence of terpenoids, flavenoids, glycosides, phenols, saponins and traces of tannins. Electron microscopy examination of cells treated with Lebanese cedar extracts showed a great variation in the cell structure*.

KEYWORDS: Antimicrobial , Lebanese Cedar, Human Pathogens, Food Spoilage Microorganisms

INTRODUCTION

Many plants are essential in human health care, both in self-medication and in national services (Gupta et al., 2011). The World health Organization (WHO) concluded that 4 billion people, 80 percent of the world population, use herbal drugs for healthcare (Fransworth et al., 1985). Essential oils from spices and herbs are the most promising natural antimicrobials, because they do not cause microbial resistance due to the diversity of mechanisms of action. They are generally recognized as safe for human consumption without limitations on intake and commonly accepted by consumers (Dobre et al., 2011). The harmful side effects and high cost of the other forms of treatment and their non availability to the poor populations, who live in remote areas, are also the reasons for the demand for herbal medicine (Tamilarasi & Ananthi, 2012)

Lebanon Cedar (*Cedrus libani* A. Rich.) is significant from the historical, cultural, aesthetic, scientific, and economic perspectives (Boydak, 2003). *Cedrus libani* are widely used as traditional medicine in Lebanon for treatment of different infectious diseases (Loizzo et al., 2008). It was recently described that cones and leaves of *Cedrus libani* possess antimicrobial activity (Digrak et al., 1999), anti-ulcerogenic remedies and anti-*Helicobacter pylori* activity (Yesilada., 1999). Results revealed that *C. libani* resins are highly effective against tested

micro-organism by preventing their growth to a greater extent (Kizil et al., 2002). Saab et al. (2011) reported that *C. libani* seeds could be suggested as food supplements and/or health herbal products or starting point of in-depth pharmaceutical treatments development for human chronic myelogenus leukemia. Foodborne diseases are a major health concern that can have severe impact on Society (Singh et al., 2013). The World Health Organization (WHO) has estimated that 2.2 million deaths occur each year because of diarrheal diseases. Data from WHO show that diarrheal illnesses are a significant cause of mortality in children under five years old in six world regions (Dewaal et al., 2009).
The aim of the present study is to evaluate the efficacy of Lebanese Cedar extracts against most common human pathogens and food spoilage microorganisms.

MATERIALS AND METHODS

Plant material
Three different parts of Lebanese cedar tree were collected in plastic bags from Barouk Mountains of Lebanon at the altitude 1500 m above sea level. Leaves, stems and pulp parts of *C. libani* were washed with water, dried at 45 °C for 6 h, and then were crushed into powders with a mixer (Zeng et al., 2012).

Aqueous extraction
The powdered samples (100 g) were added to 2000 ml of distilled water with continuously stirring at room temperature for 24 h. Thereafter, the extract was filtered and condensed using a rotary evaporator at 45 °C under vacuum. Then, the dried extract was stored at 4 °C prior to further analysis (Zeng et al., 2012).

Methanolic extraction
Extraction was performed as described by Samie et al. (2005) with minor modifications. 50 g of each ground plant material was soaked in 500 ml of methanol for up to 72 h with frequent shaking. Then, samples were filtered twice using Whattman No.1 filter paper and evaporated to dryness (semi-solid residues) under reduced pressure at 40°C. The extracts were stored in sterile glass bottles. After filter sterilization using Sartorius Stedim, biotech 0.20µm bacterial filter.

Test organisms
Four different human pathogens and food spoilage bacterial strains were used throughout the present work. Two gram positive strains namely: MRSA and *Listeria monocytogenes*, and two gram negative strains namely: ESBL *E.coli* and *Klebsiella pneumonia* were kindly provided by Ain W Zain hospital and American University Hospital, and identified phenotypically as described in Beregy's Manual of determinative bacteriology (Buchanan & Gibbson, 1974). The bacterial strains were all maintained on nutrient agar medium at 4 °C with monthly transfer on fresh media.One yeast strain namely *Candida albicans* was kindly provided from Elias Hrawi Govermental Hospital and maintained on Sabouraud – Dextrose agar slants and stored at 4°C with monthly transfer on fresh media.

Inoculum preparation for antimicrobial susceptibility testing
Inocula were prepared directly by suspending colonies grown overnight on agar plate directly in nutrient broth medium. Slowly, with agitation, add 9.9 ml of 1% chemically pure sulfuric acid, and 0.1 ml of 1.175% aqueous solution of barium chloride ($BaCl_2.H_2O$) to make a total

10 ml per tube. The suspended barium sulfate precipitate corresponds approximately to 1.5×10^8 CFU/ml (Madigan et al., 2006). The 0.5 MacFarland standard which is commercially available, provides an optical density comparable to the density of a bacterial suspension of 1.5×10^8 CFU/ml. If the bacterial suspension initially does not match with the standard's turbidity, the suspension may be diluted, or supplemented with more organisms, as needed (Mahon et al., 1998). From a freshly prepared culture of each isolate (16 to 24 hrs old nutrient agar), 4 to 5 colonies were emulsified in 5ml 0.85% saline solution to achieve a turbid suspension. Matching turbidity using the unaided eye is by holding the bacterial suspension and McFarland tubes side by side and viewing them against a black – lined background (Mahon et al., 1998).

Disk diffusion susceptibility testing

Antimicrobial activity was carried out using a disc-diffusion method (Murray et al., 1995). Petri dishes were prepared with 10 ml of sterile Mueller Hinton Agar. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10min. The loaded plant extract discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The plates were incubated for 24 h at 37°C. Zones of inhibition were recorded in millimeters and the experiment was repeated twice.Standard antibiotic discs, recommended by CLSI, which were suitable for microorganisms, were placed as positive controls (Kose et al., 2010). For bacterial pathogens, Gentamycin (CN-10μg), and Ciprofloxacin (CIP-5μg) were used as positive control for obtaining comparative results. Imipenem (IPM - 10 μg), and Amikacin (AK - 30 μg), were used as a positive control for ESBL *E.coli*. For fungal pathogens, Ketonazole (0.4g/ml), and Nystatin (0.5g/ml) were prepared and delivered to Sabourraud – Dextrose agar plates

Germination curve assay for *Candida albicans*

For the germination assay, fresh cells of *Candida albicans* (3×10^6) CFU/ml were resuspended in peptone - yeast extract - glucose (PYG) medium and incubated at 37°C with gentle agitation (160 rpm) on a rotary shaker. At various time intervals, 10μl aliquots were removed and the numbers of germinated cells were assessed by hemocytometer counting. The percent of germination was calculated and graphed against time of incubation in PYG broth. Cells were counted at a magnification of ×400, and a total of 200 cells per field were counted. The counting was done three times, and the mean value of these three independent counts was used to obtain the percent of germination (Mavanathu et al., 1999).

Broth microdilution method

The most promising extracts were tested for MIC by dilution method. This test was performed in sterile 96-well microtechniques (Ellof, 1998). The microtechniques were applied in 96-well microtiter plates with U-shaped wells (Ellof, 1998). The cultures under test were diluted in Müller – Hinton broth at a density adjusted to 0.5 McFarland turbidity. The final inoculum was 1.5×10^8 CFU/ml of microbial cultures. After the addition of inocula, trays were covered, incubated at 37°C for 24 h. MICs were determined according to NCCLS guidelines (National Committee for clinical laboratory standard). The experiment was repeated three times. The MIC is the lowest concentration of Lebanese cedar extract that inhibited the growth of the test strain in the wells by visual reading and by the growth inhibition on macro-plates as described by Ellof (1998). The minimum bactericidal concentration (MBCs) of the tested Lebanese cedar extract was determined by inoculating the MIC dilution into Müller – Hinton agar plates and incubated at 37 °C for 18 h (Rota et al., 2008). The lowest concentration not exhibiting bacterial growth was recorded as the minimal bactericidal concentration (MBC). The

experiment was repeated three times. The minimum fungicidal concentrations (MFCs) of the tested extracts were determined by subculturing 10 µl from each well of the microtitre tray, inoculating onto SDA and incubating aerobically at 35°C (Hammer et al., 2003).

Time kill curve

The selected Lebanese cedar extracts that showed a bactericidal effect against the selected microorganisms were used, and time – kill curve was plotted. A 16- h culture was harvested, the suspension was adjusted using the McFarland standard and was then further diluted in saline 0.85% to achieve approximately 1.5×10^8 CFU/ml. The selected Lebanese cedar extracts were added to aliquots of 1ml Müller Hinton broth in amounts that would achieve the bactericidal concentrations for the selected bacteria followed by the addition of 1ml of the inoculum. Further samples were taken from each tube to monitor growth by measuring the absorbance (optical density) at 600nm wavelength at time intervals (0, 2, 4,6,8,12,18 and 24h) (Yin et al., 2002).

For *Candida albicans,* based on the data obtained from MIC and MFC for the Lebanese cedar extracts, concentration was chosen corresponding to 1 × MFC. Germinated and ungerminated cells of *Candida albicans* were incubated in 1ml of PYG broth at 37°C for 24h in the presence of a negative control (without extract). Further samples were taken at time intervals (2, 4,6,8,12,18 and 24h) for viable counts which were carried out by serial dilution of samples by 10 fold in sterile distilled water and plating on SDA. Results were estimated according to log values (Hammer et al., 2002).

Effect of light on the efficacy of killing

The Lebanese cedar extracts were tested for their killing efficacy with and without light (Hudson et al., 2011). Aliquots of suspension were mixed with the extracts in transparent sterile plastic tubes and either exposed to light or covered with aluminum foil (dark reaction) for a period of 60 min with incubation at room temperature (20°C) on a rocker platform (Sharma et al., 2008).

Transmission Electron Microscope

On the basis of MIC, MBC, MFC values and Time-Kill curve data, methicilin – resistant *Staphylococcus aureus,* was treated with Lebanese cedar stem extract (50µl/ml) , *Klebsiella pneumonia* was treated with Lebanese cedar stem extract (100 µl/ml), ESBL *Esherichia coli* was treated with Lebanese cedar leaves extract (50 µl/ml), *Listeria monocytogenes* was treated with Lebanese cedar leaves extract (5 µl/ml), and *Candida albicans* was treated with Lebanese cedar stem extract (300 µl/ml). Freshly taken samples were fixed using a universal electron microscope fixative as described by McDowell & Trump, (1976) Series dehydration steps were followed using ethyl alcohol and propylene oxide. The samples was then embedded in labeled beam capsules and polymerized. Thin sections of cells exposed to extracts were cut using LKB 2209-180 ultra-microtome and stained with a saturated solution of urinyl acetate for half hour and lead acetate for 2 min (McDowell & Trump., 1976).The procedure was applied to extract-exposed cells. Electron Micrographs were taken using a Transmission Electron Microscope (JEM-100 CX Joel), at the Electron Microscope Unit, Faculty of Science, Alexandria University, Egypt.

Phytochemical analysis

Test for Flavonoids (Ammonia test)
1 ml of the extract was taken in the test tube and ammonia solution was added (1:5) followed by the addition of conc. sulphuric acid. Appearance of yellow color and its disappearance on standing indicates the positive test for flavonoids.

Test for Glycosides (Keller Kiliani test)
5 ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1 ml of conc. sulphuric acid.
Formation of brown ring at interface confirms the presence of glycosides.

Test for Phenols (Ferric chloride test)
0.5 ml of the extract was added with few drops of neutral ferric chloride (0.5%) solution. Formation of dark green color indicates the presence of the phenolic compounds.
Test for Saponins (Froth test)
1 ml of the extract was taken in a test tube and distilled water (2 ml) was added to it. The test tube was then kept in boiling water bath for boiling and was shaken vigorously. Existence of froth formation during warming confirms the presence of saponins.

Test for Steroids: (Libermann - Burchard's test)
2 ml of acetic anhydride was added to 0.5ml of the extract and then added 2 ml of conc. sulphuric acid slowly along the sides of the test tube. Change of colour from violet to blue or green indicates the presence of steroids.

Test for Tannins (Ferric chloride test)
1 ml of the extract was added with 5 ml of distilled water and kept for boiling in hot water bath. After boiling, sample was cooled down and to this 0.1% ferric chloride solution was added. Appearance of brownish green or blue black coloration confirms the presence of tannins.

Test for Terpenoids (Salkowski test)
5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3ml of conc. sulfuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

RESULTS AND DISCUSSION

In the present investigation, a comparative study was done to evaluate the antimicrobial potential of Lebanese cedar extract against human pathogens and food spoilage microorganisms. Three different extracts of different parts of Cedrus libani (leaves, stem, and pulp) were treated using aqueous and methanolic extraction. Methanolic extracts were found to be more potent than aqueous extracts (Plate 1 and Fig 1).

Leaves methanolic extracts were found to be highly active against all the tested bacteria with the highest antibacterial activity *against Listeria monocytogenes* with inhibition zone 70mm, but did not show an antifungal activity against *Candida albicans*. These results coincide with that reported by Digrak et al. (1999) who noticed that there are no antifungal effects for all the tested extracts.

Cedrus deodara oil at the concentration of 150 µg/disc showed zone of inhibition against *A. fumigatus* but at the same concentration did not show any antifungal activity against *C. albicans* (Parveen et al., 2010).

Stem methanolic extract was very active against all pathogens under investigation particularly *Listeria monocytogenes*, MRSA, ESBL *E.coli, Klebsiella pneumonia* and *Candida albicans*, with average inhibiton zones 20, 27, 20, 26 and 21mm respectively. The methanolic pulp extract showed moderate activity against MRSA of average inhibition zone (20mm) and low antimicrobial activity against other pathogens.The activity of Lebanese cedar extracts is strong in comparison to some commonly used bactericides and fungicides that showed inhibition zone diameters varying from 20 to 35mm (Figure 2).

Lekgari, (2010) reported that different solvents can be used for extraction, depending on the kind of phytochemicals that are targeted for extraction. This may lead to conclude that the Lebanese cedar extracts that have the effective component of the extract is methanol- ethanol soluble and water insoluble.

The results of the present investigation agree with that obtained by Derwich et al. (2010) who reported that the essential leaves of *Cedrus altantica* were active against *Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis, Bacillus sphericus* and *Staphylococcus intermedius*. Hudson et al. (2011) reported that Cedar leaf oil extracted by steam distillation from western red cedar showed a bactericidal rather than bacteriostatic effect against Gram positive bacateria namely: *Bacillus subtilis, Streptococcus pyogenes* and *Enterococcus faecalis* and Gram negative bacteria namely: *Acinetobacter baumann*i, *Hemophius influenza*, *Salmonella enteritidis* and *Escherichia coli*. Water-soluble extract from pine needles of *Cedrus deodara* showed a significant antibacterial activity against the tested food-borne pathogens including *Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Bacillus subtilis* and *Bacillus cereus* (Dewaal et al., 2009).

The aqueous Lebanese leaves extract did not show an antifungal activity against *Candida albicans*. On the contrary, it was reported by Hudson et al. (2011) that Cedar leaf oil obtained by steam distillation from Western red cedar inactivated the medically important yeast *Candida albicans* and the filamentous mold *Aspergillus niger*.The minimum inhibitory concentration MIC and minimum bactericidal concentration MBC ranged from 5-300µl/ml, whereas the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were 5-200 µl/ml and 300 µl/ml respectively (Table 1-2 and Plate 2).

MIC determination by broth dilution methods is mainly used for determining the potency of some bioactive plant extracts. A classification of plant materials based on MIC results was proposed where a chemical with MIC up to 500µl/ml is said to be a strong inhibitor, MIC between 600 and 1500 µl/ml is viewed as moderate inhibitor while that with MIC above 1600 µl/ml is considered as a weak inhibitor (Aligianis et al., 2001). The MIC results obtained by the present study reveal that Lebanese cedar extracts could be considered as strong inhibitors. In the present study, the growth time kill curves of the cells of *Listeria monocytogenes* treated with the Lebanese methanolic leaves cedar extracts epressed as OD_{600} decreased sharply and rapidly. Cells were killed after 4 h, while the growth curve (untreated cells) of control increased at a faster rate, and then kept stable until the end (Fig 4a). Also time kill curves showed that upon using Lebanese methanolic stem extract the OD_{600} of ESBL *E.coli*, MRSA

and *KLebsiella pneumonia* decreased sharply and the bacteria were killed after 4, 10 and 12 h respectively. However, the OD_{600} of control increased at a faster rate, and then kept stable As for *Candida albicans*, time kill curves showed that upon using Lebanese methanolic stem extract, the number of colonies for the germinated cells of *Candida albicans* was sharply reduced after 12 h while that of ungerminated cells of *Candida albicans* was reduced after 20 h (Fig 4e).

Ethnomedical, phytochemical and bioactivity studies have been performed on different parts of *C. libani* employed as human and veterinary remedies, as leaves, stems, seeds, crude oleoresins (a sort of tar) from cones, woody carved stems and roots (Yusuf et al., 2008). Interesting results have been reported about antimicrobial, larvicidal and antiviral properties (*Herpes symplex* virus type 1, HSV-1) of alcoholic extracts of all *C. libani* crude drugs and of essential oils hydrodistilled from seeds: in all these reports, bioactivities have been related to the abundance of terpene compounds (Kizil et al., 2002; Yilmaz et al., 2005). Understanding of the phytochemical constituents is important for exploration of the authentic effectiveness of the plant (Razia et al., 2013). In the present study phytochemical analysis of the Lebanese cedar extracts was done to explore their composition. The results revealed the presence of terpenoids, flavenoids, glycosides, phenols, saponins and traces of tannins. These results agree with the results obtained by Devmurari, (2010) who reported that the phytochemical studies of *Cedrus deodara* revealed the presence of alkaloids, glycosides flavonoids, triterpenoid, tannins, proteins and fixed oil.

In particular, the terpenoid substances are the secondary metabolites that characterize *C. libani* derived products (Kizil et al., 2002; Yilmaz et al., 2005; Loizzo et al., 2008).
Terpenoids, which are GRAS (generally recognized as safe) have been found to inhibit the growth of cancerous cells, decreases tumor size, decrease cholesterol level and also decrease micro-organism concentration (Sylvestre et al., 1995; Kumar et al., 2004). Limonene was the main compound in *Cedrus libani* (22.7%).This terpene is used as an antimicrobial inhibitor in the food industry (Tumen et al., 2010).

Saab et al. (2011), reported that the chloroform extracts of Cedrus libani seeds evidenced the predominant presence of terpenes, the monoterpenes α- and β- pinene were the most abundant. Enantiomers of α- pinene, β-pinene, limonene and linalool have a strong antibacterial activity (Dorman & Deans, 2000).Electron microscopy examination of the treated cells showed that the main targets of cinnamon and green tea extracts were cell wall and cell membrane of treated pathogens. The cells exhibited notable alterations in the cell wall and cell membrane. The cytoplasmic volume decreased with structural disorganization, drastic shape changes and loss of integrity. Plasma membrane seemed to be irregular, dissociated from cell wall, invaginated and associated with the formation of abnormal structures. Some cells lysed, ruptured, and shrank with morphological alterations. (Figur 6; a, b, c, d and e).The marked effect of the extract components might have conferred lipophilic properties and the ability to penetrate the plasma membrane (Knobloch et al., 1989).

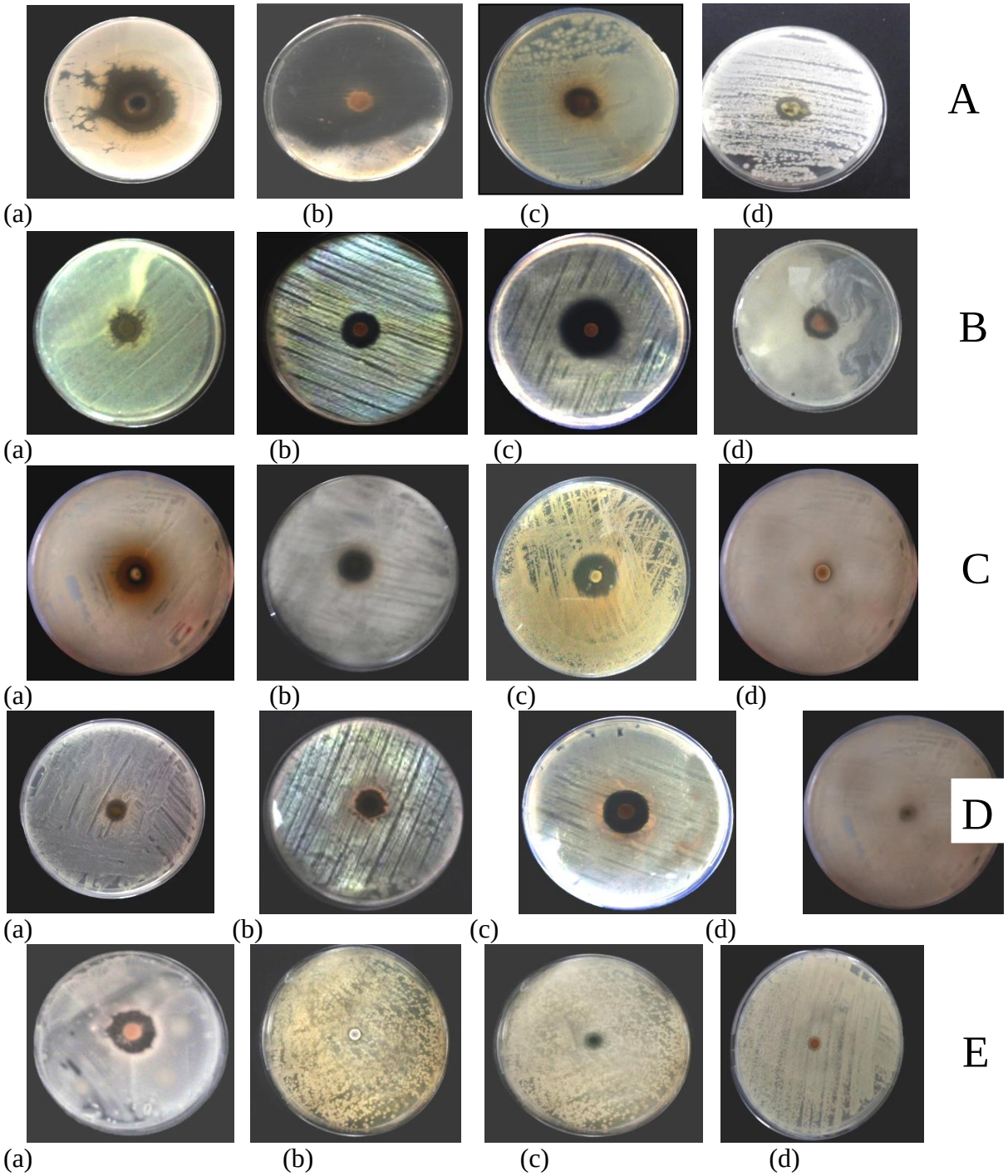

Plate 1: Antimicrobial activity of Lebanese cedar extracts against human and food borne pathogens (A: *Listeria monocytogenes;* B: *MRSA;* C:*Klebsiella pneumonia;* D: ESBL *E.coli and* E: *Candida albicans)*
(a): aqueous leaves extract; (b): methanolic leaves extract; (c): methanolic stem extract; (d): methanolic pulp extract

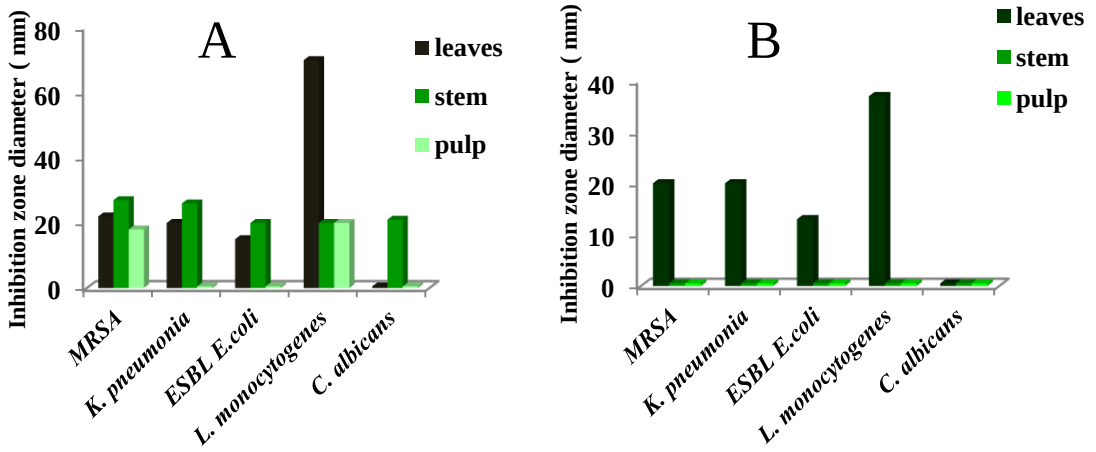

Figure 1: The inhibition zone measurement of methanolic Lebanese cedar extract (A), and aqueous Lebanese cedar extract (B) against human and food borne microorganism

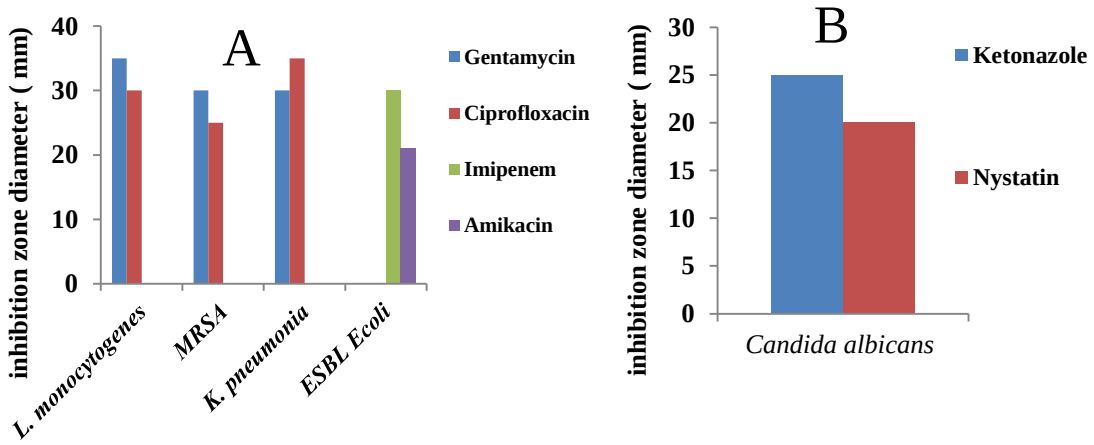

Figure 2: Inhibition zone diameter (mm) of Lebanese cedar extracts against the selected bacteria versus common bactericides (A), and (B) against the selected fungi versus common fungicides.

Table 1: Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) of the selected Lebanese cedar extracts against the selected pathogens

Microorganism	Lebanese Cedar extract	MIC(µl/ml)	MBC (µl/ml)
Listeria monocytogenes	methanolic leaves extract	5	5
MRSA	methanolic stem extract	50	50
Klebsiella pneumonia		100	100
ESBL *E. coli*	methanolic stem extract	50	50
	methanolic stem extract		

Table 2: Minimum fungicidal concentration (MFC) against germinated and ungerminated cells of *Candida albicans*

Microorganism	Lebanese Cedar extract	MIC (µl/ml)	MFC (µl/ml)
Candida albicans (ungerminated cells)	Methanolic stem extract	200	200
***Candida albicans* (ungerminated cell)**	**Methanolic stem extract**	**300**	**300**

(a) (b) (c) (d) (e) (f)

Plate 2: Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) against the selected pathogens.
(a) Lebanese Methanolic leaves extract against *Listeria monocytogenes* ; (b) Lebanese methanolic stem extract against MRSA; (c) Lebanese methanolic stem extract against *Klebsiella pneumonia*; (d) Lebanese methanolic stem extract against ESBL *E.coli*; (e) Lebanese methanolic stem extract against ungerminated cells of *Candida albicans*; (f)Lebanese methanolic stem extract against germinated cells of *Candida albicans*.

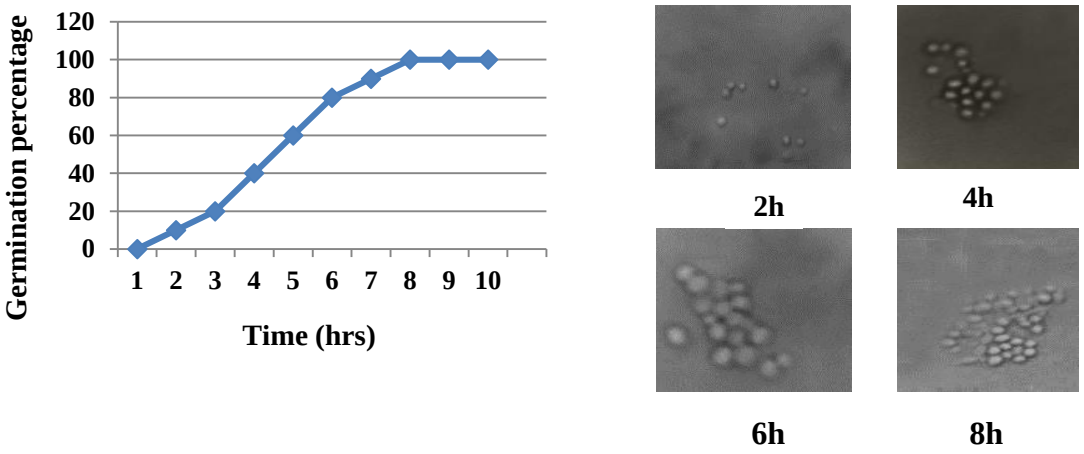

Figure 3: Germination growth curve of *Candida albicans* growing on PYG medium at different time intervals.

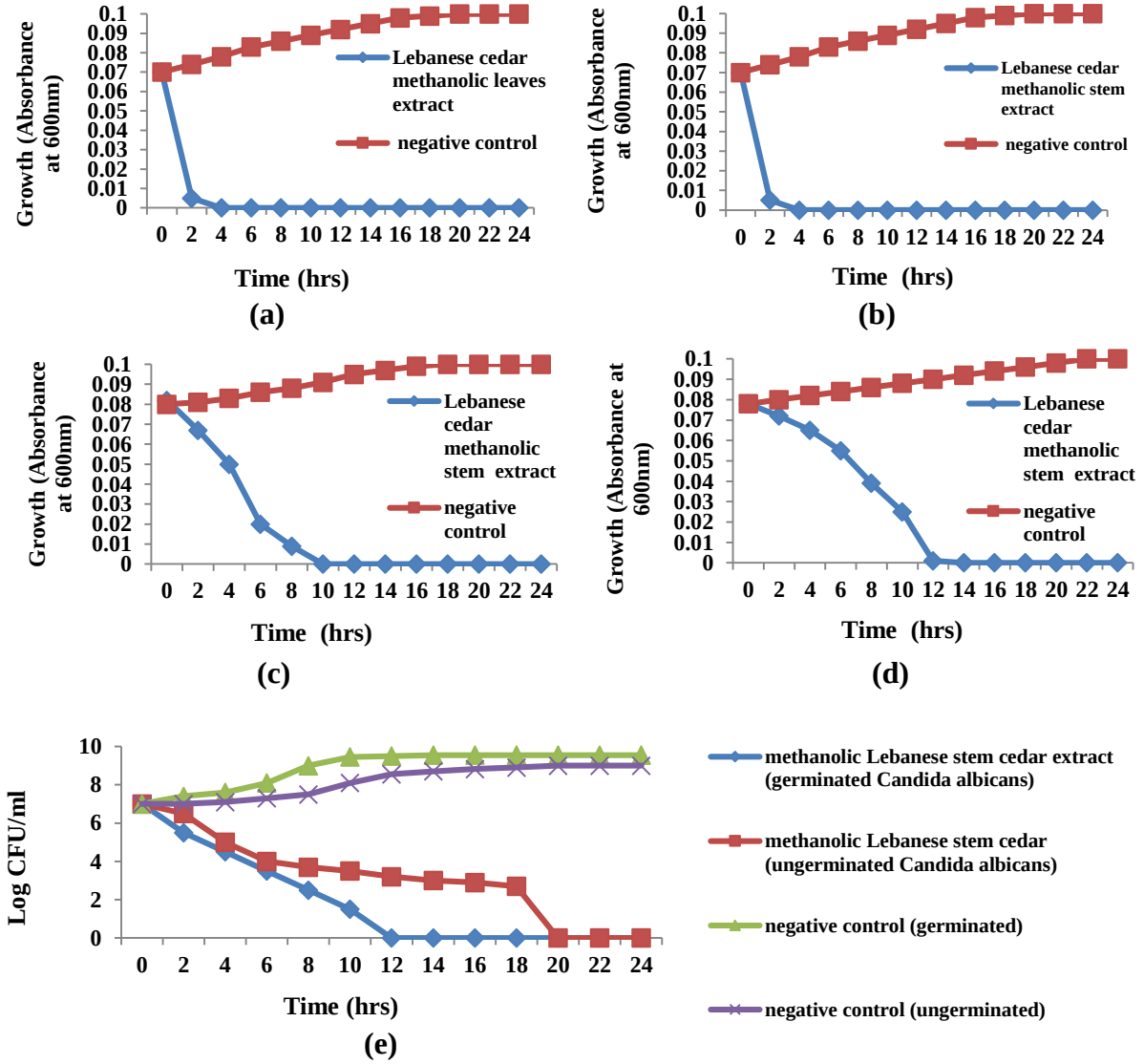

Figure 4: Time-kill curve of the most potent Lebanese cedar extracts against the selected pathogens in comparison to a negative control. (a) *Listeria monocytogenes*; (b) ESBL *E.coli*; (c) MRSA; (d) *Klebsiella pneumonia;* (e) germinated cells of *Candida albicans* and ungerminated cells of *Candida albicans*

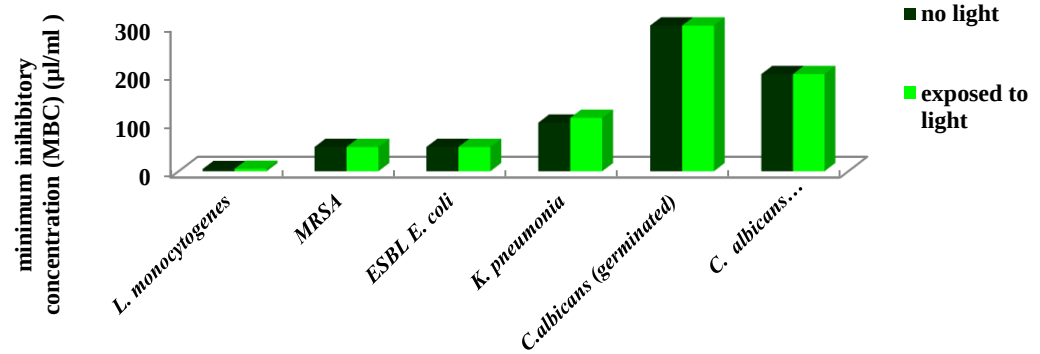

Figure 5: Effect of light on the efficacy of killing of Lebanese cedar extract against the selected pathogens

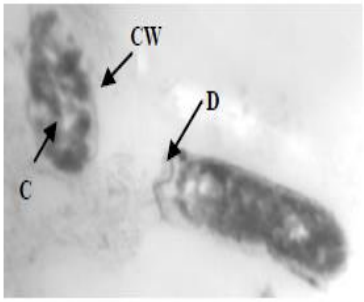

Figure 6a: TEM micrograph of *Listeria monocytogenes* treated with Lebanese cedar methanolic leaves extract (C: cytoplasm, CW: cell wall, D: damage)

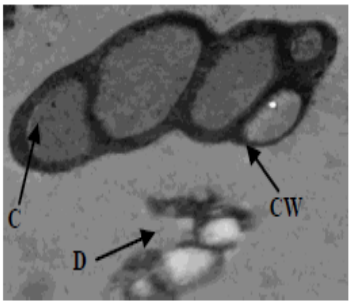

Figure 6b: TEM micrograph of MRSA treated with Lebanese methanolic stem extract (C: cytoplasm, CW: cell wall, D: damage)

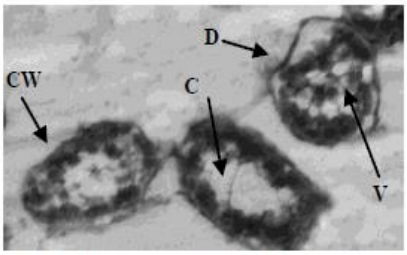

Figure 6c: TEM micrograph of *K. pneumonia* treated with Lebanese methanolic stem extract (C: cytoplasm, CW: cell wall, D: damage)

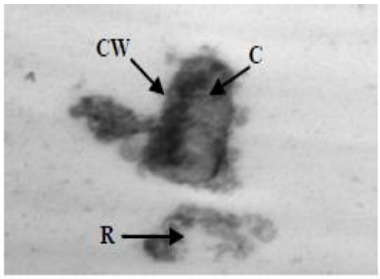

Figure 6d: TEM micrograph of ESBL *E.coli* treated with Lebanese methanolic stem extract (C: cytoplasm, CW: cell wall, R: rupture)

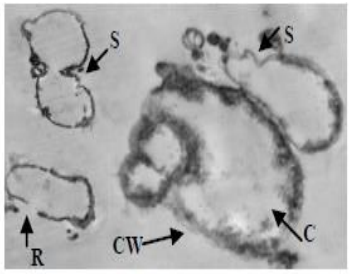

Figure 6b: TEM micrograph *Candida albicans* treated with Lebanese methanolic stem extract (C: cytoplasm, CW: cell wall, S: shrinkage, R: Rupture)

Conclusion

The Lebanese cedar extract particularly the methanolic extract, obtained from different parts of the tree (leaves, stem and pulp), showed an antimicrobial effect against human pathogens and food spoilage microorganisms which suggests that it could be considered as a safe antimicrobial agent.

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