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Chemical composition, antioxidant and antimicrobial activity of the essential oil and methanol extract of the Egyptian lemongrass *Cymbopogon proximus* Stapf

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RESUMEN

Composición química, actividad antioxidante y antimicrobiana del aceite esencial y extractos de metanol de limoncillo egipcio *Cymbopogon proximus* Stapf.

El presente estudio fue llevado a cabo para evaluar las propiedades antioxidantes y antimicrobianas *in vitro* del aceite esencial (Eo) y extractos de metanol de una genuina y endémica planta egipcia, *Cymbopogon proximus* Stapf. La composición química de un hidrodestilado Eo de *C. proximus* fue analizado por GC y GC/MS. Un total de 19 constituyentes representando el 95.47% del aceite fueron identificados; piperitona (72.44%), elemol (9.43%), α-eudesmol (4.34%), limoneno (2.45%) and β-eudesmol (1.26%) fueron los principales componente, que comprenden el 88.92% del aceite. Los resultados de los ensayos antimicrobianos mostraron que el Eo de *C. proximus* inhibió fuertemente el crecimiento de las bacterias ensayadas, aunque no el de las especies de levaduras, mientras que el extracto de metanol tuvo una moderada actividad antibacteriana, pero ninguna actividad anti-*candida*. *Bacillus cereus* and *Salmonella choleraesuis* fueron las más susceptibles con Eo. El tiempo de exposición con Eo para una completa inhibición de la viabilidad celular de *B. cereus* y *S. choleraesuis* fue de 10 min con un 5% y 10 min con 1%, respectivamente. El potencial antioxidante de las muestra fue evaluado usando el método de inhibición del radical libre 2,2-difenil-1- picrilhidrazyil (DPPH). Los extractos de metanol fueron capaces de reducir el radical libre estable DPPH con un IC_{50} de 48.66±3.1 µg/ml. Los resultados presentados aquí pueden sugerir que el Eo y los extractos de *C. proximus* poseen propiedades antioxidantes y antimicrobianas, y además, ellos pueden ser usados como ingrediente en la conservación de alimentos y/o en la industria farmacéutica.

PALABRAS CLAVE: Antimicrobiano – Antioxidante – Composición química – Cymbopogon Proximus – *Eo.*

SUMMARY

Chemical composition, antioxidant and antimicrobial activity of the essential oil and methanol extract of the Egyptian lemongrass *Cymbopogon proximus* Stapf.

The present study was conducted to evaluate the *in vitro* antimicrobial and antioxidant properties of the essential oil (Eo) and methanol extract from a unique, Egyptian endemic plant, *Cymbopogon proximus* STAPF. The chemical composition of a hydrodistilled Eo of *C. proximus* was analyzed by a GC and GC/MS system. A total of 19 constituents representing 95.47% of the oil were identified: piperitone (72.44%), elemol (9.43%), α - eudesmol (4.34%), limonene (2.45%) and β- eudesmol (1.26%) were the main components comprising 88.92% of the oil. The antimicrobial test results showed that the Eo of *C. proximus* strongly inhibited the growth of the test bacteria studied, except for yeast species while the methanol extract had moderate antibacterial, but no anti-*candida* activity. *Bacillus cereus* and *Salmonella choleraesuis* were proven to be the most susceptible against Eo. The exposure time of Eo for complete inhibition of cell viability of *B. cereus* and *S. choleraesuis* were found to be 5 % at 10 min and 1% at 10 min, respectively. The antioxidative potential of the samples was evaluated using methods of inhibition of the free radical 2,2-diphenyl-1- picrylhydrazyl (DPPH) system. The methanol extract was able to reduce the stable free radical DPPH with an IC_{50} of 48.66±3.1 µg/ml. The results presented here may suggest that the Eo and extracts of *C. proximus* possess antimicrobial and antioxidant properties, and therefore, can be used as natural preservative ingredients in food and/or pharmaceuticals.

KEY-WORDS: Antimicrobial – Antioxidant – Chemical composition – Cymbopogon Proximus – *Eo.*

1. INTRODUCTION

Knowledge about the medicinal plants of the Ancient Egyptians came to us through the offerings of the dead found in tombs, the earliest designs and inscriptions on the walls of temples, and the records available in papyri such as the Ebers Papyrus (1500 BC). These show that the Ancient Egyptians were acquainted with a great number of medicinal plants and their products. There are also others which were known to the Egyptians in the days of the Ancient Greeks and Romans. In addition, some medicinal plants were also brought by the Arabs (Sayed, 1980).

The genus *Cymbopogon*, a member of the family Gramineae, is widely distributed in tropical and subtropical regions. Several species yield essential oils used in soap, perfumery and other related industries. *C. citratus* Stapf and *C. flexuosus* Stapf commonly known as lemongrass are native to India and Sri Lanka. Several studies reported antimicrobial activities in lemongrass (*C. citratus*) oil (Onawunmi, 1980, Hammer *et al.*, 1999; Saikia *et al.*, 2001; Appendini and Hotchkiss, 2002; Daferera *et al.*, 2003; Pereira *et al.*, 2004; Leimanna *et al.*, 2009).

C. proximus Stapf is a weed known as Halfa bar that grows in the Egyptian desert. It is highly reputed in Egyptian folk medicine as an effective renal antispasmodic and diuretic agent (Taeckholm, 1974; Boulos, 1984; El-Askary *et al*., 2003). *C. proximus* is an ascending densely tufted perennial grass, common in the hills and rocky grounds of Elba and the sandy coast of the Red Sea on the southern boundaries of Egypt. The entire dried herb has been used for centuries by the Bisharin and Ababda tribes of the Aswan Province in the form of a decoction to produce diuresis, to relieve colicky pains, to help the removal of small stones from the urinary tracts, and as an antipyretic in fevers. A bicyclic sesquiterpene diol, proximadiol with unique antispasmodic properties has been isolated from *C. proximus* leaves (Radwan, 1975). Proximol possesses unique antispasmodic properties as it produces relaxation of the smooth muscle fibers without abolishing the propulsive movement of the tissue (El-Askary *et al*., 2003). The success of proximol in the propulsion of renal and uteric calculi is attributed to this pharmacological characteristic, by which uteric dilation occurs without paralysis and the propulsive waves are preserved. It is quite a safe drug and prolonged use in the recommended therapeutic doses has not shown any side effects. From the current literature, there is not much data concerning the effects of *C. proximus*.

In the present study, we report the composition, the antimicrobial and antioxidant activity of the Eo and methanolic extract obtained from endemic Egyptian *C. proximus* Stapf for the first time.

2. MATERIALS AND METHOD

2.1. Plant samples

For this study fresh plant material was used. The plant was collected during the vegetation period, botanically identified and immediately processed. Specimens were identified at the Botany Department, Faculty of Science, Suez Canal University (Ismailia, Egypt) and voucher specimens were deposited at the Herbarium of the Department of Botany in the cited university.

2.2. Preparation of the methanol extract

The powder form of *C. proximus* (50 g) was extracted with methanol (200 ml X 3 times) at room temperature. The methanol extract was combined and evaporated by a vacuum rotary evaporator at 45°C to the dried powdered form (yield 12.96%, w/w). The resulting extract was then lyophilized and kept in the dark at +4 °C until tested.

2.3. Preparation of essential oil

Eo was obtained using the Clevenger hydro-distillation method. The plant material (about 300 g), was cut into small pieces, and placed in a flask (4 l) together with doubly distilled water (1.5 l). The mixture was boiled for 3 h, the collected Eo was dried with anhydrous sodium sulphate and kept at –18 °C until use.

2.4. GC and GC-MS analysis conditions of the essential oil

GC analysis was performed on a Hewlett Packard 5890 II gas chromatograph equipped with a FID and HP-5ms capillary column (bonded and cross-linked 5%- phenyl-methylpolysiloxane 30 m· 0.25 mm i.d., film thickness 0.25 lm). Injector and detector temperatures were set at 220 and 290°C, respectively. The oven temperature was held at 50°C for 3 min, then programmed to 240°C at a rate of 3°C/min. Helium was the carrier gas, at a flow rate of 1 mL/min. Diluted samples (1/100 in acetone, v/v) of 1.0 lL were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data. GC-MS analysis of the Eo was performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) using a Hewlett Packard 5890 II gas chromatograph equipped with a Hewlett Packard 5972 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, NBS75K library data of the GC-MS system and literature data (Adams, 2001). Alkanes were used as reference points in the calculation of relative retention indexes (RRI).

2.5. Antimicrobial Tests

Microbial strains

The bacterial and yeast strains used in this work are described in Table 2. These microbial strains were isolated from human beings (other than ATCC strains) and belong to the microbiological laboratory collection of the department of microbiology from Suez Canal University, Egypt. Nutrient agar (for bacterial strains) and Sabouraud dextrose agar media (for yeast strains) were inoculated with this suspension of the respective organism and poured into a sterile petri dish.

Disc-diffusion assay

The agar diffusion assay was performed according to the modified Kirby-Bauer disc diffusion method (Robert *et al*., 2003). One ml of each test organism liquid culture was individually suspended in 3 ml of a 0.9% NaCl solution. The Eo and methanol extract were dissolved in 10% dimethylsulfoxide (DMSO) to a final concentration of 30 mg/ml as stock solution and sterilized by filtration through 0.45 µm Millipore filters. Antimicrobial tests were then carried out using 100 µl of suspension containing 10^8 cfu/ml of bacteria

and 10^6 cfu/ml of yeast spread on nutrient agar and Sabouraud dextrose agar media, respectively. The discs (6 mm in diameter) were impregnated with 5 µg of the essential oil and methanol extract, and then placed onto inoculated agar. Negative controls were prepared using the same solvent employed to dissolve the extract. The inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains and 48 h for yeast isolates. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

Micro-well dilution assay of MIC and MBC

The minimal inhibitory concentration (MIC) values of the Eo and methanol extract were studied using the micro-well dilution method for the bacterial strains which were sensitive to Eo and methanol extract in the disc diffusion assay. In brief, the 96-well plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inocula into each well. The inocula of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. One hundred µl aliquot from the stock solutions of the Eo and methanol extract initially prepared at the concentration of 10 mg/ml were added into the first wells. Then, 100 µl from their serial dilutions were transferred into six consecutive wells. The last well containing 195 µl of nutrient broth without the compound and 5µl of the inocula on each strip was used as a negative control. The final volume in each well was 200 µl. The plate was covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker at 300 rpm for 20s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by plating 5µl samples from clear wells on nutrient agar medium. The extract tested in this study was screened twice against each organism. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. MBC (minimum bactericidal concentration) is usually an extension from the MIC, where the organisms quantitatively indicate the minimum concentration when no viable organism appears in the culture.

Cell viability assay for Bacillus cereus and Salmonella choleraesuis

Each of the tubes containing bacterial suspension (approximately 10^6 cfu/mL) of *Bacillus cereus* and *Salmonella choleraesuis* was inoculated with 0.25, 0.5 and 1% concentration of *C. proximus* Eo in 10 mL MHB, and kept at 37°C. Samples for viable cell counts were taken at 0, 5, 10, 15, 20, 40, 60 and 120 min time intervals. The viable plate counts were monitored as followed: 100 µl sample of each treatment was diluted and spread on the surface of MHB agar. The colonies were counted after 24 h of incubation at 37°C. The controls were inoculated without Eo for each bacterial strain with the same experimental condition as mentioned above.

2.6. Antioxidant activity

Determination of total antioxidant capacity

The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acidic pH (Archana *et al*., 2005). The 0.1 mL extract was combined with 3 mL of a reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. This solution was allowed to cool at room temperature and the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

DPPH assay

The hydrogen atoms or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of the purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical diphenylpicrylhydrazyl (DPPH) as a reagent (Burits and Bucar, 2000; Cuendet *et al*., 1997). Fifty microliters of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition of the free radical DPPH in percent (I %) was calculated in the following way:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the essential oil or the extract and the percentage inhibition of free radical formation/percentage inhibition of the DPPH was assayed. Synthetic antioxidant reagents, butylated hydroxyanisole (BHA) and L-ascorbic acid were used as positive controls.

2.7. Statistical Analysis

The variations between experiments were estimated by standard deviations and the statistical significance of changes was estimated using the student's t-test. Only the probability $P \leq 5\%$ was regarded as indicative of statistical significance.

3. RESULTS

3.1. GC-MS analysis of essential oil

The hydrodiztillation of dried *C. proximus* gave a brownish Eo (yields 0.58%, w/w). The identified

compounds, qualitative and quantitative analytical results by GC and GC/MS are shown in Table 1, according to their elution order on a ZB-1 capillary column. The GC–MS analysis of the Eo led to the identification of 19 different components, representing 95.47% of the total oil constituents (Table 1). A total of 19 constituents representing 95.47% of the oil were identified; piperitone (72.44%), elemol (9.43%), α-eudesmol (4.34%), limonene (2.45%) and β-eudesmol (1.26%) were the main components comprising 88.92% of the oil. A portion (4.53%) of the total composition was not identified. The Eo yield from the whole plants of *C. proximus* prepared by the hydrodiztillation method was 0.58% (v/w). GC/MS analysis revealed that the oil contained 19 components in the oil containing piperitone (72.44%).

3.2. Antimicrobial activity

The *in vitro* antimicrobial potential of *C. proximus* Eo and methanol extract against a panel of microorganisms is shown in Table 2. Eo showed moderate *in vitro* antimicrobial activity against all tested bacteria, including Gram positive and Gram negative ones with diameter zones of inhibition 3 to 20 mm, along with MIC and MBC values ranging from 0.25 to 1 µl/ml. Whereas the methanol extract showed less antimicrobial activity. The diameters of inhibition zones found were in the range of 5 to 20 mm, along with MIC and MBC values ranging from 0.5 to 5 mg/ml.

In the comparison of microbial sensitivity to both Eo and methanol extracts, *Bacillus cereus* and *Salmonella choleraesuis* seem to be more sensitive than other infectious pathogens such as *Enterococcus* sp., *E. coli O157:H7* and *Proteus vulgaris*. The results of antimicrobial activity are shown in Table 2. According to the statistical analysis, the gram-positive *B. cereus* was the most sensitive strain, with MIC values ranging from 0.25 µg/ml to 5 µg/ml. No remarkable activity was observed against the the yeast *C. Albicans,* which turned out to be the most resistant strain. Further, the effect on the cell viabilities of *B. cereus* and *S. choleraesuis* demonstrated that exposure of Eo at 0.25, 0.5 and 1% concentration of *C. proximus* Eo had a potential antibacterial effect on the viabilities of *B. cereus* and *S. choleraesuis* strains. The exposure times of Eo for the complete inhibition of cell viability of *B. cereus* and *S. choleraesuis* were found to be 0.5 % at 10 min and 1% at 10 min, respectively (Fig. 1a and b).

3.3. Antioxidant activity

The total antioxidant activity of the *C. proximus* was expressed as the number of equivalents of ascorbic acid (ASE). The antioxidant capacity was estimated from the regression equation derived from

Table 1
Percentage chemical composition of the essential oil Cymbopogon proximus Stapf

Peak No	Compound [a]	KI [b]	Area [c] (%)
1	Limonene	481	2.45
2	α-Pinene	940	0.74
3	Piperitone	1011	72.44
4	δ-3-Carene	1012	0.77
5	α-Terpinene	1019	0.25
6	p-Cymene	1025	0.68
7	1,8-Cineole	1033	0.27
8	(Z)-β-Ocimene	1040	0.48
9	(E)-β-Ocimene	1048	0.25
10	γ-Terpinene	1063	0.12
11	α-Terpinolene	1089	0.07
12	Linalool	1098	0.07
13	Linalyl acetate	1262	0.35
14	β-Bourbobene	1380	0.14
15	β-Elemene	1388	0.82
16	Elemol	1540	9.43
17	γ- Eudesmol	1951	0.54
18	β- Eudesmol	1993	1.26
19	α- Eudesmol	2000	4.34
Oil yield (%) (v/w)			0.58

[a] Components were identified through KI and GC–MS (gas chromatograph coupled with mass spectrometry) and listed according to their elution on HP-5 MS capillary column (30 m). [b]KI: Kovats indexes on HP-Innowax capillary column in reference to C9-C28 n-alkanes (Adams, 2001). [c] The 19 constituents identified represent 95.47 % of the total area.

Table 2
Antimicrobial activity of the essential oil and methanol extract from *of Cymbopogon proximus* Stapf

		Essential oil			Methanol extract		
	Origin	**DD[a]**	**MIC**	**MBC**	**DD**	**MIC**	**MBC**
Gram Positive Bacteria							
Bacillus cereus	ATCC 11778	22	0.25	0.5	20	0.5	1
Bacillus megaterium	Human	9	5	5	5	5	5
Bacillus subtilis	Human	–	–	–	–	–	–
Enterococcus faecium	Human	–	–	–	–	–	–
Enterococcus feacalis	Human	–	–	–	–	–	–
Micrococcus luteus	Human	–	–	–	–	–	–
Serratia marcescens	Human	–	–	–	–	–	–
Staphlococcus aureus	Human	7	2	3	–	–	–
Gram Negative Bacteria							
Bordetella bronchisepta	ATCC 4617	7	5	5	5	5	5
Escherichia coli	Human	10	3	5	5	4	5
Escherichia coli O157:H7	Human	–	–	–	–	–	–
Klebsiella pneumonia	Human	5	5	5	3	5	5
Proteus vulgaris	Human	–	–	–	–	–	–
Pseudomonas aeruginosa	Human	7	5	5	3	5	5
Salmonella choleraesuis	ATCC 19430	20	0.5	1	18	1	1
Yeast							
Candida albicans	ATCC 44831	–	–	–	–	–	–
Candida utilis	Human	–	–	–	–	–	–
Saccharomyces cerevisiae	Human	–	–	–	–	–	–

MIC (minimum inhibition concentration) and MBC (minimum bactericidal concentration) as mg/ml of essential oil or methanol extract; (–) no antimicrobial activity. Values are the average of three trails. [a] Inhibition zone in diameter (mm) around the discs impregnated with essential oil and methanol extract (5 mg/disc).

concentration versus optical density of the sample and ascorbic acid. The extraction by methanol showed equivalents 48.66±3.1 µg dried *C. proximus*. The color of the reaction mixture changes from purple to yellow, and its absorbance at wavelength 517 nm decreases. Table 3 shows the DPPH radical scavenging activities of the essential oil and methanol extract of *C. proximus*. The IC_{50} values were compared with the IC_{50} values of butylated hydroxyanisole (BHA) and ascorbic acid. A lower IC_{50} value indicates greater antioxidant activity. The IC_{50} values of the essential oil and methanol extract were found to be 998.47±67.65 and 48.66±3.1, respectively. The scavenging effects of BHA and ascorbic acid were found to be 2.77 and 3.3 times greater than the methanol extract of *C. proximus*, respectively. The methanol extracts of *C. proximus* showed a highly effective free radical scavenging in the DPPH assay. These extracts exhibited a remarkable antioxidant effect at low concentrations. The essential oil of *C. proximus* was only slightly active.

4. DISCUSSION

In this study it was found that the percentage and piperitone (72.44%) compositions of Eo of *C. proximus* was similar to the Eo obtained from the *C. proximus* leaves of Egyptian origin (El Tahir and Abdel Kader, 2008). The chemical profiles of the Eo from the leaves and buds differ not only in the number of molecules but also in the stereochemical types of molecules extracted. Other components of the oil are known constituents of the plants (El Tahir and Abdel Kader, 2008). The analysis of the oil sample from the Sudanese sample was free of that component (Siddiqui *et al*., 1980). It is most likely that this difference is the result of a misidentification of the Sudanese sample. This could be attributed to several factors such as climate, soil composition, season, edaphic factors, plant organ, age, and vegetative cycle stage (Angioni *et al*., 2006).

The antibacterial activity of the oil of *C. proximus* could, in part, be associated with major constituents such as α-terpinene, linalool, α-pinene and γ-terpinene. These components have been reported to display antibacterial effects (Alessandra *et al*., 2005; Djoukeng et al., 2005). Terpenes were active against bacteria (Djoukeng *et al*., 2005). As described previously by other authors, Eo containing terpenoids are more active against Gram positive bacteria than against Gram negative bacteria (Cosentino *et al*., 1999). In addition, the components in lower amounts

Table 3
Scavenging activity of the DPPH radical of the essential oil and methanol extract from *Cymbopogon proximus* Stapf

Samples	Concentration (µg/mL)	Inhibition (%)	IC50 values (µg/mL)
Essential oil	500	40.63	
	1000	60.94	998.47±67.65
	1500	63.75	
Methanol extract	10	39.38	
	50	59.98	48.66±3.1
	100	89.88	
Butylated hydroxyanisole[a]	10	28.39	
	20	57.03	17.58±0.63
	30	85.11	
Ascorbic acid[a]	10	49.76	14.73±0.55
	20	67.87	

[a] References control.

may also contribute to the antibacterial activity of the oil, probably involving some type of synergism with other active compounds. The NCCLS method (NCCLS, 2000) was used to determine the antibiotic-resistance and sensitivity pattern profile of the antibiotic- resistant bacteria.

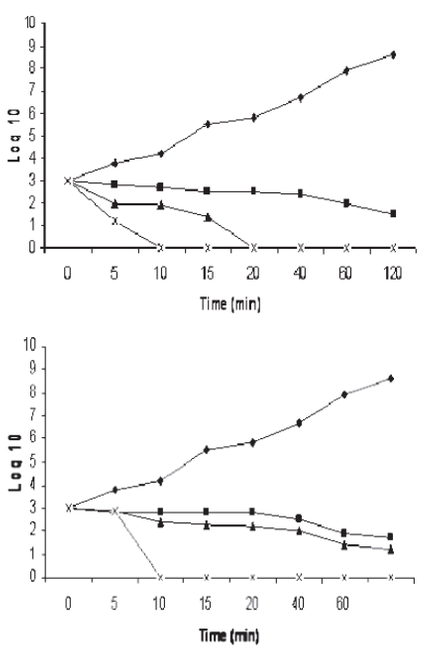

Figure 1
Effect of essential oil from *C. proximus* as 0 (control, ♦), 0.25 (■), 0.50 (▲), and 1% (x) on viability of (a) *Bacillus cereus* and (b) *Salmonella choleraesuis*. Values are the average of three individual replicates (means±S.D). Differences between samples were determined by the Student's t-test and were considered to be significant when p≤0.05.

The DPPH free radical is a stable free radical, which has been widely used as a tool to estimate the free radical-scavenging activity of antioxidants. Antioxidants, upon interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character (Archana *et al*., 2005). The key role of phenolic compounds as antimicrobial and scavengers of free radicals is emphasized in several reports (Rauha *et al*., 2000; Archana *et al*., 2005). Here we propose, for the first time, the use of essential oil and the methanol extract of *C. proximus* as potential antimicrobial and antioxidant sources. According to Borchers, Keen and Geratiwin (Borchers *et al*., 2004), food extracts may be more beneficial than isolated constituents, because other compounds present in the extracts can be change the properties of an individual bioactive component.

In this research, we found that the essential oil and methanol extract of *C. proximus* severely inhibited the growth of food spoilage, food-borne pathogens, and multiantibiotic-resistant bacteria. In addition, the Eo and methanol extract also exhibited a strong scavenging effect on DPPH free radicals. Therefore, from the above results, it can be concluded that the essential oil and methanol extract derived from *C. proximus* could be considered potential alternatives for synthetic bactericides and natural antioxidants for use in the food industry along with their possible applications in the pharmaceutical industry for the prevention or treatment of severe skin diseases caused by emerging antibiotic resistant microorganisms and free radicals. The antioxidative and antimicrobial properties of the essential oils and various extracts from many plants are of great interest in both academia and the food industry, since their

possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones. In this respect, studying the endangered species may be of great interest, since their bioactive properties and secrets could be lost forever if left untapped. Owing to its strong antibacterial and excellent protective features exhibited in antioxidant activity tests, the essential oil and extracts from *C. proximus* could be concluded as a natural source that can be freely used in the food industry as a culinary herb, but, firstly, immediate and necessary measurements should be taken for the protection of this plant species. In conclusion, this study can be considered as the first report on the in vitro antimicrobial and antioxidant properties of the essential oil and methanol extracts prepared from *C. proximus*. I hope that our results introduce a unique natural source which possesses strong antimicrobial and antioxidant substances.

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