Original Research Article

Chemical characterization and *in vitro* antimicrobial activity of *Caralluma europaea* essential oil and its synergistic potential with conventional antibiotics

ABSTRACT

Aim: This study was carried out to investigate the antioxidant and antimicrobial activities of essential oil from the aerial part of *Caralluma europaea* and to evaluate the synergistic potential between essential oil and antibiotics.

Methodology: The chemical composition, antioxidant, antimicrobial activities and synergetic interaction between antimicrobial agents and essential oil isolated by hydrodistillation from the aerial part of *C. europaea* were evaluated. The chemical composition was analyzed by a Gas chromatography/mass spectrometry (GC/MS) system. Antioxidant activity was measured employing three methods: scavenging of free radical DPPH, reducing power assay and the inhibition of linoleic acid oxidation. The antimicrobial activity of essential oil against microbial strains was qualitatively and quantitatively assessed by the presence or absence of inhibition zones diameters, and MIC values. The *in vitro* association between essential oil and some commercial antibiotics was also investigated.

Results: The GC/MS analysis shows that a total of 21 constituents were identified and the main compounds were Terpinolene (19.5%), α-Terpinene (16.2%) and Linalool (15.3%). Antioxidant study showed that essential oil exhibited antioxidant activity with IC_{50} values ranging from 0.32 mg/ml to 1.45 mg/ml. The result of antimicrobial activity showed that the essential oil had an inhibitory effect against the majority of tested microorganisms except *K. pneumonia* and *P. aeruginosa*. Gram-positive bacteria were found to be more sensitive than Gram-negative ones. Furthermore, essential oil approved an interesting antifungal activity against yeast species. Out of 25 combinations tested 64% showed total synergism, 20% had a partial synergistic interaction and 16% showed no effect. The best synergistic effect was obtained with the combination essential oil-gentamycin.

Conclusion: Our results are of a great importance and suggest that *C. europaea* essential contain bioactive compounds with antioxidant and antimicrobial properties with possible applications in the food and pharmaceutical industries.

Keywords: Caralluma europaea, Essential oil, Chemical composition, Antioxidant activity, Antimicrobial activity, Synergistic effect

1. INTRODUCTION

The toxicity of commercial antioxidants used in the food industry causes actually a really problem. Synthetic antioxidants are unstable, less effective, and cause many side effects [1]. Also many antibiotics have been ineffective due to the rapid development of microbial resistance which led to the emergency of new infection diseases [2]. The emergence of microbial strains resistance is due to the improper and inappropriate use of commercial antibiotics. Therefore, to overcome these problems the search for new antimicrobial and antioxidant natural products continues to draw the attention of many researchers. In fact, medicinal and aromatic plants constitute an alternative and a new potential reservoir of new bioactive compounds [3]. Furthermore, several studies have reported that essential oil showed an interesting microbial activity against a wide range of resistant microbial strains. Also, it has been reported that essential oil and its components of various plants possess a strong antioxidant activity [4].

Recently, the combined use of essential oil and antimicrobial agents is one of the promising strategies to overcome the resistance mechanisms of microorganisms and to minimize undesirable side effect of antibiotics [5]. In previous studies, it has been demonstrated that essential oils obtained from many plants showed a good synergistic interaction with synthetic drugs [5,6]. *Caralluma europaea* (Guss) N. E. Br (Asclepiadaceae) locally known as "ddagmûs" is one of the medicinal plants most commonly used in traditional medicine, distributed in Egypt, Spain, Italy, Libya, Tunisia, Algeria and Morocco [7]. Aerial parts of this medicinal shrub are largely used as powder and mixed with honey to treat diabetes, kyste and goiter [8].

Previous works were reported on the extracts obtained from many species of *Caralluma* (*C. dalzielii*, *C. tuberculata*, *C. umbellate*) for biological activities (antinociceptive, anti inflammatory, antihyperglycemic and hepatoprotective) [9-11]. However, there are few studies on the chemical composition of the genus *Caralluma*. Two studies have reported the chemical composition of essential oils from stems, flowers and fruits of *C. europaea* of Italy [12,13]. To the best of our knowledge, there is no literature report concerning the biological activities of *C. europaea* essential oil and no study has been focused on the antimicrobial synergistic effect between the essential oil and conventional antibiotics. Thus, the current work was undertaken to evaluate the antioxidant and antimicrobial properties of essential oil extracted from aerial part of *C. europaea* against a panel of pathogen microorganisms, to determine its chemical composition and to study the synergistic effects between the essential oil and classical antibiotics.

2. MATERIAL AND METHODS

2.1. Plant material and isolation of the essential oil

The studied plant was collected in March 2014, from Ourika valley (High Atlas of Morocco). The taxonomic identification of plant materials was confirmed by one of the authors (M. Larhsini), and a voucher specimen (CAE 023) was deposited at the Laboratory of Biotechnology, Protection and Valorization of the Plant Resources, Faculty of sciences Semlalia, Marrakech, Morocco. The air-dried aerial parts of plants collected were submitted for 4 hours to water-distillation using a Clevenger-type apparatus. The obtained essential oil was stored in darkness at 4º C until use. The yield percentages, calculated as volume (ml) of essential oil per 100 g of plant dry matter.

2.2. Gas chromatography/mass spectrometry (GC/MS) analysis of essential oils

The essential oil obtained from aerial parts of *C. europaea* was analyzed using GC/MS method (Shimadzu GC/MS-16A gas chromatograph instrument), equipped with a quadruple detector and DB5 capillary column (25m × 3 mm). The injector and detector temperatures were set at 250°C. The column temperature was programmed from 40 to 200 °C at 10 °C/min. 1 µl of oil was injected into GC–MS instrument for analysis. Helium gas was used as carrier gas at flow rate of 1 ml/min. The chemical components of essential oil were identified by comparing their retention indices (RI) and mass fragmentation patterns with those on the stored NIST library (National Institute of Standards and Technology).

2.3. Antioxidant activity

2.3.1. DPPH assay

The stable radical 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used in this spectrophotometric assay to evaluate the scavenging ability of the essential oil [14]. In this reaction, fifty microlitres of samples in methanol (essential oil and synthetic standard antioxidants) was allowed to react with 2 ml of 60 µM methanolic solution of DPPH for 20 min in darkness at room temperature. The decrease in absorbance was measured at 517 nm using a blank containing the same amount of methanol and

DPPH solution. Butylated hydroxytoluene (BHT) and quercetin were used as positive controls. Inhibition of free radical DPPH in percent (I%) was calculated as follow:

$I\% = [(A_{blank} - A_{Sanple}/A_{blank})*100]$

Where A_{blank} is the absorbance of the control, and A_{sample} is the absorbance of the test compounds. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentration of the sample.

2.3.2.β-Carotene/linoleic acid assay

In this assay, the inhibition of conjugated hydroperoxides arising from linoleic acid oxidation was used to determine the antioxidant ability of the essential oil. According to the protocol used by Miraliakbari and Shahidi [15], a stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg of β-carotene was dissolved in 1 ml of chloroform, 25 µl of linoleic acid and 200 mg of tween 40 was added. The chloroform was evaporated under vacuum and 100 ml of distilled water was then added to the residue and mixed to form an emulsion. The samples (essential oil, BHT and quercetine) were dissolved in methanol and 350 µl of each sample solution was added to 2.5 ml of the emulsion. The test tubes were then incubated in a hot water bath at 50°C for 2h, together with a blank contained the same volume of methanol instead of essential oil. After incubation, the absorbencies were measured at 470 nm. The capacity of the essential oil to protect against oxidation of β-carotene was determined as follows:

$I\% = [(A_{sample2h} - A_{blank2h})/ (A_{\text{initial blank}} - A_{blank2h})]*100$

Where $A_{sample2h}$, $A_{\text{blank 2h}}$ are the absorbance values of β-carotene after 2h remaining in the samples and $A_{\text{initial blank}}$ is the absorbance values of β-carotene at the beginning of the experiment. The IC_{50} was calculated from the graph by plotting inhibition percentages against essential oil concentration.

2.3.3. Reducing power assay

The reducing power of the essential oil was determined according to the method of Oyaizu [16]. 1 ml of different concentration of samples (essential oil and control substance) were mixed with phosphate buffer (2.5 ml, 200 mM, PH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid (TCA) were added to the mixture and then centrifuged at 3000 rpm for 10 min. the upper layer solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$). The color formed due to reduction of Fe^{3+} was measured at 700 nm using a spectrophotometer.
The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph by plotting the absorbance at 700 nm against the corresponding sample concentration. BHT and quercetine were used as positive controls.

2.4. Antimicrobial activity

The antibacterial activity of *caralluma europaea* was tested against seven bacteria: Gram positive namely *Staphylococcus aureus* (209 PCIP 53156), *Micrococcus luteus* (ATCC 381), *Bacillus cereus* (ATCC 14579), *Bacillus subtilis* (ATCC 9524), and Gram negative *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (DSM 50090) and a clinically isolated *Klebsiella pneumonia* and Antifungal activity was evaluated using four yeasts: *Candida albicans* (CCMM L4), *Candida kreusei* (CCMML10), *Candida glabrata* (CCMML7) and *Candida parapsilosis* (CCMML18).

2.4.1. Antibacterial activity

Disk diffusion agar method [17] was used to evaluate the antimicrobial activity of *C. europea* essential oil. Petri plates containing 20 ml of Mueller Hinton Agar (MHA) for bacteria, and Sabouraud dextrose agar (SDA) for yeasts were seeded with cultures of microbial strains. Culture suspensions of the tested microorganisms (18-24h growth culture) prepared in sterile saline solution and adjusted to 10^6

colony-forming units (CFU)/ml for bacteria and 1-2 × 10^3 cells for fungal strains. The suspensions were speared MHA and SDA for bacteria and yeasts respectively. Sterile filter paper discs (6 mm in diameter), containing 10 µl of essential oil, were distributed in the agar surface. The Petri plates were placed at 4º C for 4h. Commercial antibiotics and antifungal: cefexime (10µg/disc), ciprofloxacin (5µg/disc), gentamycine (15µg/disc) and fluconazol (40µg/disc) were used as positive controls. Inhibition zones were determined after in incubation of 24 h at 37ºC for bacteria and 48 h at 28 ºC for yeasts.

2.4.2 Determination of Minimal Inhibitory (MIC) and Minimal Microbiocidal Concentrations (MMC)

The MIC values, which represent the lowest essential oil concentration that inhibits the growth of microorganisms, were determined, based on a micro-well dilution method [18]. The essential oil was dissolved in 4% dimethyl sulfoxide (DMSO) and eight dilutions series were prepared and placed in 96-microwells plates. A fresh overnight culture of test strains was used to prepare the cell suspensions in twice concentrated Mueller Hinton Broth (MHB) for bacterial strains and Sabouraud dextrose Broth (SDB) for yeasts to obtain 10^6 colony-forming unit (CFU)/ml and 1-2 × 10^3cells/ml respectively. Each well of microplates included 100 µl of the diluted essential oil and 100 µl of bacterial or fungal suspensions. Then, the microplates were incubated at 37º C for 24h for bacteria and 28º C for 48h for yeasts. Cefexime, ciprofloxacin, gentamicin, and fluconazol used as positive references for bacteria and fungi, respectively. The minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) values were determined by spreading 0.1 ml of clear wells, which did not show any visible growth on MHA, and incubated at 37º C for 24h for bacteria, or in SDA at 28º C for 48h for yeasts. The lowest concentration in which the microorganism was completely killed was taken as MBC for bacteria and MFC for yeasts.

2.4.3. Synergistic interactions

To evaluate the synergistic interaction between *C. europaea* essential oil and some synthetic drugs (cefexime, ciprofloxacin, gentamycin and fluconazole), checkerboard method was used as described by Fadli et al. [6]. Briefly, eight two-fold serial dilutions (CMI to CMI/128) of each antibiotic and the antifungal agent were prepared in sterile distilled water. 50 µl of each dilution of antimicrobial agent were placed in a vertical orientation and mixed with 50 µl of the appropriate concentration of the essential oil at MIC/4. 100 µl of fresh microbial suspension of approximately 10^6 CFU/ml for bacteria and 1-2 × 10^3 cells/ml for yeasts were added to each microwells. The results obtained were determined after incubation at 37º C for 24h for bacteria and 28ºC for 48h for yeasts. The FIC index (Fractional Inhibitory Concentration) was calculated by the method reported by Rosato et al. [5] according to the following formula:

FIC = (MIC_a of the combination/MIC_a alone)/ (MIC_b of the combination/MIC_b alone)

Where MIC_a is the MIC of the essential oil and MIC_b is the MIC of the antibiotic

Total synergism ($FIC_I \leq 0.5$), partial synergism ($0.5 < FIC_I \leq 0.75$), no effect ($0.75 < FIC_I \leq 2$) or antagonism ($FIC_I > 2$) between microbial agents and the studied essential oil was assumed from the values of the FIC index.

2.5. Statistical analysis

All tests were carried out in triplicate and results were expressed as mean ± standard deviation (SD). In antioxidant assays IC_{50} values were reported as mean ± SD.

3. RESULTS

3.1. Chemical composition

The yield of *C. europaea* essential oil obtained by hydrodistillation of dry material was 0.14% (v/w). The oil sample characterized by a typical odour and was analysed by GC-MS, the individual identified components, with their relative percentages, retention index are summarized in Table 1. Twenty-one different components, representing about 97.8% of the total oil, were identified. From the data obtained, the essential oil contained a complex mixture of several compounds which are grouped on the basis of their chemical structures into monoterpenes hydrocarbons, oxygenated monoterpenes, sesquiterpenes hydrocarbons, oxygenated sesquiterpenes, carboxylic acids, aldehydes and alcohols. Terpinolene (19.5%), α-Terpinene (16.2%), linalool (15.3%), hexadecanoïc acid (6.8%), β-Pinene (5.1%) and β-Eudesmol (3.7%) were found to be the major constituents.

Table 1. Chemical composition of the essential oil of *Caralluma europaea* aerial part

Compounds	Percentage	RI[a]
Hexanol	0.9	853
Heptanal	2.1	881
Santolinatriene	3.3	902
Tricyclene	1.1	920
α-Pinene	1.8	933
Camphene	3.3	946
β-Pinene	**5.1**	972
Hexanoic acid	1.4	984
α-Phellandrene	3.1	998
α-Terpinene	**16.2**	1009
Terpinolene	**19.5**	1078
Nonanal	0.7	1082
Linalool	**15.3**	1085
Octanoic acid	3.2	1108
Verbenone	0.8	1183
Nonanoic acid	1.2	1323
Thujopsen	4.1	1432
(Z)-α-Bisabolene	1.5	1495
β-Eudesmol	3.7	1632
Hexahydrofarnesylacetone	2.7	1830
Hexadecanoic acid	**6.8**	1954
Other compounds	2.2	
Monoterpene hydrocarbons	53.4	
Oxygenated monoterpenes	16.1	
Sesquiterpenes hydrocarbons	5.6	
Oxygenated sesquiterpenes	3.7	
Carboxylic acids	12.6	
Carbonylic compounds	2.7	
Alcohols	0.9	
Aldehydes	2.8	
Total	97.8	

[a]RI: retention indices relative to C9-C22 n-alkanes on the DB-5 column.

3.2. Antioxidant activity

The concentrations that led to 50% of inhibition (IC_{50}) are given in the Table 2. The antioxidant activities were compared with that of quercetin and of BHT. The better antioxidant activity was reflected by the lower IC_{50} values.

From results (Table 2) it was revealed that essential oil isolated from *C. europaea* exhibited an antioxidant activity. The lowest IC_{50} was obtained by reducing power assay (IC_{50}= 0.32 ± 0.03 mg/ml), followed by β-carotene-acid-linoleic test (IC_{50}= 1.17±0.019 mg/ml), and the highest IC_{50} value was obtained with DPPH assay (IC_{50}= 1.45 ± 0.019 mg/ml). The antioxidant capacity of reference antioxidants, butylated hydroxytoluene (BHT) and quercetin, was found to be more potent than those of the studied essential oil (IC_{50} values from 0.84 ± 0.04 µg/ml to 2.59 ± 0.07 µg/ml and from 0.95 ± 0.02 µg/ml to 2.62 ± 0.02 µg/ml, respectively).

Table 2. Antioxidant activity of the essential oil isolated from *C. europaea* and positive controls (BHT and quercetine) in DPPH, reducing power and the β-carotene/linoleic acid bleaching assay methods.

Samples	DPPH	Reducing power	β-Carotene-linoleic acid
Essential oil (mg/ml)	1.45 ± 0.019	0.32 ± 0.03	1.17 ± .019
BHT (µg/ml)	2.59 ± 0.07	2.22 ± 0.03	0.84 ± 0.04
Quercetine (µg/ml)	1.98 ± 0.07	2.62 ± 0.02	0.95 ± 0.02

IC_{50} values represent means ± standard deviations for triplicate.

3.3. Antimicrobial activity

The antimicrobial activity of *C. europaea* essential oil was evaluated against Gram-positive (*S. aureus*, *M. luteus*, *B. cereus* and B. *subtilis*), Gram-negative bacteria (*E. coli*, *K. pneumonia*, and *P. aeruginosa*) and against *Candida* strains (*Candida albicans*, *C. glabrata, C. Krusei*, and *C. parapsilosis*). The antimicrobial activity was assessed by evaluating the inhibition zones (IZ) and the determination of MIC values. The results obtained (Table 3) showed that the studied essential oil exhibited an inhibition against all tested strains except *K. pneumonia* and *P. aeruginosa* for Gram-negative bacteria. The inhibition zones diameters ranging from 10 to 18 mm. Gram-positive bacteria were generally found to be more sensitive than Gram-negative ones with inhibition zone diameters ranging from 14.50 to 18 mm. K. pneumonia and *P. aeruginosa* were found to be the most resistant strains. However the usual antibiotics were more potent than the studied essential oil against tested strains.

Table 4 summarized the MIC and MBC values of the essential oil and antibiotics against the tested microorganisms. The essential oil inhibited Gram-positive bacteria at concentrations ranging from 3.75 to 7.5 mg/ml, in contrast Gram-negative bacteria were inhibited with highest MICs values (30 mg/ml), indicating that essential oil has low activity against Gram-negative bacteria.

Essential oil of *C. europaea* showed also an interesting anticandidal activity against Candida strains with inhibition zone diameters and MIC values varying from 12.50 to 17.50 mm and from 1.875 to 3.75 mg/ml, respectively. *C. parapsilosis* was found to be the most susceptible strain with the lowest MIC value 1.875 mg/ml.

It is important to note that the MIC values were often equivalent to MBC, indicating a microbiocidal action of the oil especially on *B. cereus*, *B. subtilis*, *E. coli*, *K. pneumonia*, *P. aeruginosa* and *C.kreusei.*

Table 3. Antimicrobial activity of *C. europaea* essential oil, expressed by diameter of inhibition zone (including the disc diameter, 6 mm).

Strains of bacteria and yeasts	Inhibition zone diameter (mm) of *C. europaea* essential oil and antimicrobial agents				
	Essential oil (10µl/disc)	Cefexime (10µg/disc)	Ciprofloxacin (5µg/disc)	Gentamicin (15µg/disc)	Fluconazole (40 µg/disc)
Gram positive					
S. aureus	18± 0	15.17± 0.17	27.67 ± 0.67	30.0 ± 1	NT
M. luteus	15.50 ±1	25.0 ± 0	29.67 ± 0.88	26.0 ± 0	NT
B. cereus	15.50 ± 0	26.33 ± 0.33	35.67 ± 0.88	40 ± 0	NT
B. subtilis	14.50 ± 0.5	16 ± 1	36 ± 3.5	40 ± 0	NT
Gram negative					
E. coli ATCC	10 ± 0	19.33± 0.88	30 ± 0.58	25.3 ± 0.6	NT
Kl. pneumoniae	NI	10.33± 0.33	7.83 ± 0.93	NI	NT
Ps. aeruginosa	NI	20.17± 0.44	29.67 ± 0.88	20 ± 1	NT
Yeasts					
C. albicans	14.50 ± 0.35				26.50 ± 0.35
C.glabrata	16 ± 0				19 ± 0.58
C.krusei	14.50 ± 0				28 ± 0.76
C.parapsilosis	20.0 ± 0				32.17 ± 0.73

Values represent mean ± standard deviation of triplicate.
NI: no inhibition, NT: not tested

Table 4. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values (mg/ml) of *C. europaea* essential oil and antibiotics.

Microorganisms	Essential oil		Cefexime		Ciprofloxacin		Gentamicin		Fluconazole	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram positive										
S. aureus	7.5	15	0.015	0.062	0.015	0.031	0.008	0.008		
M. luteus	7.5	15	0.031	0.062	0.015	0.062	0.002	0.002		
B. cereus	3.75	3.75	0.062	0.125	0.031	0.125	0.002	0.002		
B. subtilis	3.75	3.75	0.002	0.002	0.002	0.002	0.002	0.002		
Gram negative										
E. coli ATCC	30	30	0.015	0.062	0.008	0.015	0.031	0.031		
Kl. pneumoniae	30	30	1	>1	0.250	>0.250	0.5	0.5		
Ps. aeruginosa	30	30	0.250	>0.250	0.002	0.002	0.125	0.125		
Yeasts										
C. albicans	3.75	7.5							0.031	0.031
C.glabrata	7.5	15							0.500	>0.500
C.krusei	7.5	7.5							0.250	>0.500
C.parapsilosis	1.875	3.75							0.062	0.125

The interaction between essential oil and antimicrobial agents is estimated by calculating the fractional inhibitory concentration of the combination (FIC) index. As can be seen in Table 5, the result of the combined effect between essential oil of *C. europaea* and synthetic drugs (cefexime, ciprofloxacin, gentamycin and fluconazol) revealed that from 25 studied combinations, 16 (64%) showed total synergism, 5 (20%) had a partial synergism, and 4 (16%) had no effect. The best synergistic effect was obtained with the combination of *C. europaea* essential oil and gentamicin in which FIC index ranged from 0.25 to 0.75, and the total synergistic effect obtained with this combination was observed for both Gram-positive and Gram-negative bacteria except *K. pneumonia* which showed a partial synergism (FIC_I= 0.75). The association essential oil-ciprofloxacin exhibited a synergistic effect for both Gram-positive and Gram-negative bacteria with FIC_I varying from 0.28 to 0.51 and from 0.31 to 0.49, and no effect was observed in this association against *K. pneumonia*. This synergy reduced the MIC of ciprofloxacin by 8-32 fold and 8-64 fold respectively for Gram-positive and Gram-negative bacteria. Furthermore, total ($FIC_I \leq 0.5$) partial synergism ($0.5 < FIC_I \leq 0.75$) or no effect ($0.75 < FIC_I \leq 2$) was observed between *C. europaea* essential oil and cefexime, and the FIC_I ranged from 0.31 to 1.29 and from 0.50 to 0.77 respectively for Gram-positive and Gram-negative bacteria.

A good synergism was observed between the studied essential oil and fluconazol against *C. glabrata* and *C. parapsilosis* and decreases the MIC of fluconazol with a gain of 8-32 fold. In contrast, no synergistic effect was observed in the experiment with *C. albicans*.

Table 5. Synergistic interaction between *C. europaea* essential oil and antimicrobial agents against selected bacteria and yeasts.

Microbial strains	CE+Cx		CE+CP		CE+G		CE+ Flu	
	FIC_I	Gain	FIC_i	Gain	FIC_I	Gain	FIC_I	Gain
Gram positive								
S. aureus	1.29[c]	2	0.31[a]	16	0.28[a]	32		
M. luteus	0.31[a]	16	0.51[b]	4	0.37[a]	8		
B. cereus	0.37[a]	8	0.28[a]	32	0.50[a]	4		
B. subtilis	1.27[c]	2	0.28[a]	32	0.37[a]	8		
Gram negative								
E. coli ATCC	0.77[b]	2	0.31[a]	16	0.37[a]	8		
Kl. pneumoniae	0.75[b]	2	1.25[c]	1	0.75[b]	2		
Ps. aeruginosa	0.50[a]	4	0.49[a]	4	0.26[a]	64		
Yeasts								
C. albicans							1.25[c]	2
C.glabrata							0.28[a]	32
C.krusei							1.25[b]	2
C.parapsilosis							0.25[a]	8

FIC_i: Fraction inhibitory concentration index.
CE: *Caralluma europaea*; Cx: cefexime; CP, ciprofloxacin; G, gentamicin; Flu, fluconazole
[a] Total synergism, [b] Partial synergism, [c] No effect

4. DISCUSSION

The phytochemical investigation showed that twenty one compounds were identified in *C. europaea* aerial part belonging to eight different classes. Terpinolene (19.5%), α-Terpinene (16.2%), linalool (15.3%) were the most abundant compounds. A very different composition has been reported previously for stems and fruits of the same species from Italy, the major compounds were hentriacontane, nonacosane and heptacosane [13]. Whereas, another study of the essential oil of *C. europaea* flowers revealed a similar composition, with 41 compounds identified, terpinolene (23.3%), α-Terpinene (19.1%) and linalool (18.4%) were the major compounds [12]. Furthermore, it was observed that the majority of the identified compounds in the Moroccan species have been also detected in the Italian one with differences in percentages. In fact, chemical composition of the essential oil can vary within the same species depending on the geographical location [19]. As reported in the literature, many factors such as the climate, the soil, the plant material and the season in which the plants were collected, the method of preservation and extraction, genetic factors, may be responsible for the variation of the chemical composition of the essential oils [20,21]. Data showed that terpinolene was the main component in the essential oil, which is in concordance with the result previously reported by Formisano et al. [12], who found that terpinolene was the major compounds (23.3 %) in the essential oil isolated from flowers of *C. europaea*.

C. europaea essential oil exhibited an antioxidant activity; this property could be attributed to its high content of monoterpene hydrocarbons (53.4%). In fact, monoterpene hydrocarbons, in particular, terpinolene and α-terpinene possess a very high antioxidant activity. The presence of strongly activated methylene groups in these molecules could be responsible for this activity [22]. Furthermore, several studies reported that monoterpene hydrocarbons, oxygenated monoterpenes, and oxygenated sesquiterpenes are well known to have great antioxidant properties [23,24]. The antioxidant activity of

the studied essential oil could be also attributed to the presence of hexadecanoic acid. Indeed, carboxylic acids (12.6%) have been reported to exhibit antioxidant activity [25]. Therefore, the antioxidant activity of the essential oil could be attributed to a number of these components. The antioxidant and radical scavenging properties of *Caralluma* species extracts are well documented. Our results were similar to that reported by Gujjala et al. [26] who found an efficient activity of hydro-alcholic extract derived from the aerial parts of *C. fimbriata* of India measured by DPPH and reducing power assays.

The studied essential oil showed an antimicrobial activity against the majority of tested microorganisms. This activity can be attributed to the presence of monoterpene hydrocarbons (α-terpinene, α-pinene, β-pinene) and oxygenated monoterpenes. These compounds possess strong antibacterial and antimicrobial activities [27,28]. It has been reported that these chemical components causes perturbations in bacterial cellular integrity resulting in inhibition of respiration and alteration in permeability [29]. Moreover, carboxylic acids have been known to possess antifungal and antibacterial properties [30]. Another important characteristic of essential oil is their hydrophobicity which allowed them to penetrate lipid components of bacterial cell membrane and mitochondria, to disrupt the cell structure and to render them more permeable to critical molecules, which leads to eventual death of the bacteria cells [6,28]. The antimicrobial activity is a consequence of additive or synergistic effects of the chemicals component with each other and minor constituents of the essential oil should be taken into consideration [24].

Gram-positive bacteria were generally found to be more sensitive to *C. europaea* essential oil than Gram-negative ones. These results are in agreement with several studies reporting that essential oils are slightly more active against Gram-positive than Gram-negative bacteria [27,31]. The structure of cell envelope could be responsible for this difference; Gram-negative bacteria have in additional membrane named outer membrane delineating the periplasmic space with the cytoplasmic membrane that restricts diffusion of hydrophobic compounds [32]. The Gram-negative *P. aeruginosa* was the most highly resistant to the tested essential oil. In fact *P. aeruginosa* is well known for its resistance to conventional antimicrobials, due to a very restrictive outer membrane barrier, highly resistant, to synthetic drugs and essential oils [33]. Furthermore, several works reported that *P. aeruginosa,* appear to be least sensitive to the action of essential oils [34,35].

Resistance of bacteria to known antibiotics leads to the emergence of new infectious diseases. Therefore to overcome the resistance mechanisms of bacteria, the interaction of bioactive plant extracts and synthetic drugs is one of the novel strategies [3]. In the present study, synergistic effect between essential oil and antimicrobial agents was studied. As previously reported, several works demonstrate that a good synergistic interaction was found between essential oils or its components of many plants and synthetic drugs [3,5,6,]. Plant extracts combined with conventional antibiotics could inhibit microorganisms by various mechanisms including sequential inhibition of common biochemical pathways and inhibition of protective enzymes [36]. Furthermore, the association between natural and synthetic drug induced a double attack on different target sites of bacteria, this attack lead to an additive or synergistic effect [37].

The antioxidant and antimicrobial activity of *C. europaea* essential oil was comparable to the activity of essential oil obtained from the leaves of *Periploca laevigata* (a plant belonging to the same family) [38].

5. CONCLUSION

The results of this study show that *C. europaea* essential oil exhibited antioxidant and antimicrobial activities and demonstrate that combinations between essential oil and classical antibiotics have synergistic interaction against the majority of microorganisms. The good synergism was obtained with essential oil and gentamycin against Gram-positive and Gram-negative bacteria followed by the combination of essential oil and ciprofloxacin. The association between essential oil and fluconazole showed a synergistic effect against *C. glabrata* and *C. parapsilosis*. Thus, these combinations increase the antimicrobial efficacy of antibiotics and reduce their minimum efficient dose thus minimizing undesirable side effect.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ABBREVIATIONS

C. europaea, *Caralluma europaea*; GC/MS, Gas chromatography/mass spectrometry; DPPH, 2,2-diphenyl-1-picrylhydrazyl ; MIC, Minimal Inhibitory Concentration; *K. pneumonia, Klebsiella pneumonia; P. aeruginosa, Pseudomonas aeruginosa; C. parapsilosis,Candida parapsilosis*; RI, Retention indices; BHT, Butylated hydroxytoluene; IC_{50}, Concentration of 50% inhibition; SD, Standard deviation; MHA, Mueller Hinton Agar; SDA, Sabouraud dextrose agar; CFU, Colony-forming units; MMC, Minimal microbiocidal concentration; MHB, Mueller hinton broth; SDB, Sabouraud dextrose broth; MBC, Minimal bactericidal concentration; MFC, Minimal fungicidal concentration, FICI, Fractional Inhibitory Concentration index; IZ, inhibition zones.