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ORIGINAL ARTICLE

Chemical composition and bioactivity of the volatile oil from leaves and stems of *Eucalyptus cinerea*

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Abstract

Context: *Eucalyptus cinerea* F. Muell. ex Benth. (Myrtaceae) is a medium-sized tree cultivated in Egypt.
Objective: First, to determine the chemical composition of the volatile oil of the juvenile leaves and stems of *E. cinerea* to identify its chemotype. Second, to study the *in vivo* antioxidant activity and *in vitro* antimicrobial activity of the studied volatile oils against selected Gram-positive, Gram-negative bacteria, yeast, and mycelia fungi.
Materials and methods: The volatile oil was prepared by hydrodistillation and then identified by GC/MS analysis. Broth microdilution and agar dilution methods were applied for determining the MIC. The antioxidant activity was studied by determination of glutathione level in blood of alloxan-induced diabetic rats.
Results: The yield of the volatile oil hydrodistilled from the juvenile leaves and stems of *E. cinerea* was 4.5 and 0.5%, respectively. 1,8-Cineole was the major identified oxygenated monoterpenoid (84.55% and 60.15% in the juvenile leaves and stems, respectively). The antibacterial activity of the oil of the juvenile leaves was more potent against all the tested organisms than that of the stems. The (MIC) of volatile oil of the juvenile leaves against *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus faecalis*, *Candida albicans*, and *Aspergillus flavus* were 5.2, 5.6, 4, 4.8, and 12.8 μg/ml, respectively. Also, the juvenile leaves' oil was more active as an antioxidant than that of the stems. They restored glutathione level by 33.7 ± 1.1 and 29.6 ± 0.7 mg/dl, respectively, compared with vitamin E (35.9 ± 1.2 mg/dl) which was used as a reference.
Discussion and conclusion: Results suggest that the volatile oil is 1,8-cineole chemotype. Moreover, the oil of the juvenile leaves of *E. cinerea* might find usefulness as a therapeutic agent following further development.

Keywords

1,8-Cineole, antimicrobial, antioxidant, chemotype, *Eucalyptus cinerea* F. Muell. ex Benth., GC/MS, glutathione, MIC, Myrtaceae

History

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Introduction

Volatile oils are complex mixtures of biologically active substances used as flavoring agents and constituted a number of commercial products. Currently, their importance is more prominent due to the increasing demand of food, cosmetics, and pharmaceutical industries. Recent scientific literature revealed the antimicrobial, antifungal, and antioxidant potential of volatile oils. In the view of the multiple applications of volatile oils, their characterization based on their chemical profiles, is of great importance.

Eucalyptus L' Hér. is a large genus of the family Myrtaceae. It comprises about 900 species and subspecies (Brooker & Kleinig, 2004). Although it is a native of Australia, it is found in almost all parts of the world due to human introduction. It is now cultivated in many tropical, sub-tropical, and even sub-temperate countries (Batish et al., 2006).

Eucalyptus is generally grown for its wood, which is primarily used in making cellulose pulp and second for boards and panels (Brooker & Kleinig, 2004). *Eucalyptus* species are well-known for their volatile oils. These have been widely used in the perfumery and fragrance industries, and are among the world's top-traded oils (Batish et al., 2008). Volatile oil from *Eucalyptus* species is used as an antiseptic, antipyretic, and analgesic since ancient times (Brooker & Kleinig, 2004). It is reported to possess a wide range of biological activities including antimicrobial, fungicidal, insect-repellant, fumigant, pesticidal, and acaricidal activity (Batish et al., 2008).

Eucalyptus cinerea F. Muell. ex Benth. (Silver Dollar Gum, Argyle Apple and Mealy Stingybark) is a small to medium-sized tree (Bailey, 1976).

Reviewing the current literature, no reports were found dealing with the chemical composition and bioactivities of the volatile oil of the juvenile trees of *E. cinerea* cultivated in Egypt. There have been previous investigations on the mature

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trees of the same plant by (El-Ahmady et al., 2007; Emara & Shalaby, 2011). This work investigates the main constituents of the volatile oil of the juvenile leaves of *E. cinerea* F. Muell. ex Benth., in order to define its chemotype and to find out its probable antimicrobial and antioxidant effects.

Materials and methods

Plant material

The juvenile leaves and stems of *E. cinerea* were collected during October 2009 from El-Salheya, Sharqia Governorate, Egypt. The plant was kindly identified by Dr. Eve Lucas, Science team leader (Myrtaceae), Kew Garden, UK. A voucher specimen (EC-2008-52) was kept in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Microbial strains

A series of bacterial and fungal strains (available in stock culture of Micro-analytical Centre, Faculty of Science, Cairo University, Cairo, Egypt) were used for antibiotic sensitivity testing comprising Gram-positive bacteria [*Bacillus subtilis* (ATCC 6051), *Streptococcus fecalis* (ATCC 9790), *Staphylococcus aureus* (ATCC 6538)], and Gram-negative bacteria [*Klebsiella pneumonia* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 9027), *Neisseria gonorrhoeae* and *Escherichia coli* (ATCC 8739)], filamentous fungi [*Aspergillus flavus* (ATCC 15517)] and yeast [*Candida albicans* (ATCC 10231)].

Animals

Adult male albino rats, weighing 130–150 g body weight (BW) were obtained from the animal house of the National Research Centre, Giza, Egypt. They were kept under the same hygienic conditions and were fed by the basal diet recommended by the American Institute of Nutrition (AIN, 1977); a semi-purified diet that contained (g/kg): 200 casein, 555 sucrose, 100 cellulose, 100 fat blends (cholesterol and lard, 1:5), 35 vitamin mix, and 35 mineral mix, for 1 week before the experiment. Water was supplied *ad libitum.* The room temperature was maintained at $22 \pm 2\,^{\circ}C$, well ventilated, relative humidity, and with a 12 h cycle of light and dark. All experimental procedures were conducted in accordance with internationally accepted principles for laboratory animal use and care, and were approved by the Ethics Committee of the National Research Centre (No. 9-031) in accordance with recommendations for the proper care and use of laboratory animals (NIH Publication No. 80-23; revised 1978).

Reference drugs and kits

Vitamin E: (DL-α-tocopheryl acetate), Pharco Pharmaceutical Co., available in the form of soft gelatinous capsules, each containing 400 mg Vit. E. The Biodiagnostic glutathione kit, Wak-Chemie Medical, Steinbach, Germany, was used for the assessment of antioxidant activity. Norfloxacin (Sigma Pharmaceutical Industries, Monofiya, Egypt) and amphotricin B (Bristol-Myers Squibb, Switzerland) were also used.

Table 1. Percentage yield, physical characters and physical constants of the volatile oil of the leaves and stems of *Eucalyptus cinerea*.

Item	Juvenile leaves	Stems
Percentage yield[a]	4.5	0.5
Color	Faint yellow	Yellow
Odor	Camphor like	
Specific gravity	0.953	0.961
Refractive index	1.458	1.461
Solubility in EtOH 95%	1:3	1:3

[a]Results are mean of three determinations, calculated on fresh weight basis.

Preparation of the volatile oil

Fresh samples of the juvenile leaves and stems (500 g each) of *E. cinerea* were directly subjected to hydrodistillation (HD) in a Clevenger-type apparatus for 4 h (Saber et al., 1984). The oil obtained was dried over anhydrous sodium sulfate and stored in a refrigerator until analysis.

Determination of physical constants of the volatile oil

Percentage yield, color, odor, specific gravity, and refractive indices were determined according to the Egyptian pharmacopeia method (Saber et al., 1984). Results are displayed in Table 1.

GC/MS analysis of the volatile oils

Each volatile oil (0.1 µl) prepared from the juvenile leaves and stems of *E. cinerea* was separately injected into Agilent 6890N GC system, model 6890 (Palo Alto, CA), fitted with an Agilent mass spectroscopic detector (MSD), model 5937, as well as a 30 m long, cross-linked 5% phenyl methyl polysiloxane (HP-5 MS, Hewlett Packard, Palo Alto, CA) fused-silica column (I.D. 0.25 mm, film thickness 0.25 µm). The initial temperature was 60 °C, kept isothermal for 2 min, then ramp to 240 °C at 3 °C/min, and finally to 325 °C which was kept isothermal for 10 min. The ion source temperature was 200 °C and the interface temperature was 250 °C. The carrier gas was ultra-high purity helium adjusted at a flow rate of 0.1 ml/min. Ionization energy was 70 eV, and the scan range was 30–550 amu at a rate of (3.99 scans/s).

The constituents of the oils were identified by comparing their retention times, Kovat's indices, and mass fragmentation patterns with those of available reference samples, GC/MS database, spectrometric electronic libraries (Wiley138, NIST, NBS and comparing with the published data (Adams, 2005). Kovat's retention indices of the compounds were calculated relative to the retention times of a series of *n*-paraffin hydrocarbons with a logarithmic scale (Jenning & Shibamoto, 1980). Relative percentages were calculated from the total ion chromatograms by the computerized integrator. The results of GC/MS analysis are recorded in Table 2.

Antimicrobial activity

The agar disc diffusion method was adopted according to CLSI guidelines (2009). The volatile oils of the juvenile leaves and stems were tested by impregnating sterile discs of (Whatman filter paper, No. 3, 5 mm diameter) in 10 µl of the volatile oils. Standard discs of norfloxacin and amphotericin B served as positive controls.

Table 2. Constituents identified by GC/MS analysis of the volatile oil of the juvenile leaves and stems of *Eucalyptus cinerea*.

No.	R_t	Constituent	K.I.	Percentage	
				Juvenile leaves	Stems
1	5.72	**α-Pinene**	**939**	**2.90**	**8.00**
2	7.08	β-Pinene	979	0.02	–
3	7.48	β-Myrecene	991	0.04	0.15
4	8.07	α-Phellandrene	1003	0.10	0.58
5	8.77	p-Cymene	1023	0.47	0.25
6	8.98	**Limonene**	**1027**	**1.50**	**1.19**
7	9.18	**1,8-Cineole**	1031	**84.55**	**60.15**
8	9.27	β-*trans*-Ocimene	1037	–	0.30
9	9.66	β-*cis*-Ocimene	1052	0.09	–
10	10.12	γ-Terpinene	1064	0.89	0.75
11	11.86	β-Linalool	1099	0.07	0.24
12	13.58	Pinocarveol	1141	0.08	–
13	15.41	Terpinene-4-ol	1179	0.79	0.87
14	16.16	**α-Terpineol**	**1187**	**2.14**	**8.55**
15	18.08	β-Citral	1318	–	0.21
16	19.45	α-Citral	1343	–	0.35
17	21.95	Methyl geranate	1325	0.25	0.94
18	23.1	**Terpinyl acetate**	**1354**	**4.90**	**7.70**
19	33.16	Globulol	1585	0.22	**1.89**
Total identified constituents				99.01%	92.12%
Hydrocarbons					
Monoterpenes				6.01	11.22
Sesquiterpenes				–	–
Total hydrocarbons				6.01	11.22
Oxygenated compounds					
Monoterpenes				92.78	79.01
Sesquiterpenes				0.22	1.89
Total oxygenated compounds				93.00	80.90

The significance for the bold is for emphasis and to draw attention to the high values with their corresponding compounds.

Table 3. The antibacterial activity of the volatile oil of the juvenile leaves and stems of *E. cinerea*.

Microorganism	Diameter of zone of inhibition (mm) (%, potency relative to standard drug)		
	Oil (Juvenile leaves) (%)	Oil (stems) (%)	Ref. standard Norfloxacin (%)
Bacillus subtilis	15 (71.42)	13 (61.9)	21 (100)
Staphylococcus aureus	17 (65.38)	13 (50)	26 (100)
Streptococcus faecalis	18 (90)	13 (65)	20 (100)
Escherichia coli	16 (69.57)	10 (43.47)	23 (100)
Pseudomonas aeruginosa	16 (72.73)	12 (54.54)	22 (100)
Neisseria gonorrhea	16 (64)	13 (52)	25 (100)

The discs were then placed onto the surface of the plates containing the solid bacterial medium (Mueller–Hinton agar) or the fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the tested microorganisms. The plates were incubated at 37 °C for 25 h in case of bacteria and at 25 °C for 48 h in case of fungi. After incubation, the inhibition zones were recorded in mm. Diameters less than 5 mm indicated no effect. The experiment was done in triplicate. The results are recorded in Tables 3 and 4.

Determination of the minimum inhibitory concentration (MIC)

Stationary phase cultures of bacteria were prepared at 37 °C and used to inoculate fresh 5.0 ml culture to an OD_{600} of 0.05.

Table 4. Antifungal activity of the volatile oil of the juvenile leaves and stems of *E. cinerea*.

Microorganism	Diameter of zone of inhibition (mm) (%, potency relative to standard drug)		
	Volatile oil of leaves (%)	Volatile oil of stems (%)	Ref. standard amphotericin B (%)
Candida albicans	16 (76.19)	11 (52.38)	21 (100)
Aspergillus flavus	14 (73.7)	10 (52.63)	19 (100)

The 5.0 ml culture was then incubated at 37 °C until an OD_{600} was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6×10^5 CFU/ml. Serial dilutions from the volatile oil of the leaves (0–128 µl/ml) were prepared and mixed with 5.0 ml of the standardized bacterial suspension then added to the plates and incubated for 24 h at 37 °C. The colony forming units (CFU) were counted for each dilution (NCCLS: M7-A4, 2000).

Broth microdilution method

The MIC was determined by a whole-cell assay in a 96-well microtiter format. *Candida* cells with an initial cell optical density at 600 nm (OD_{600}) of 0.001 in sabouraud dextrose broth (SDP, Difco, Iowa City, IA) medium were inoculated with serial dilutions (0–120 µl/ml) of the volatile oil SDP medium. Growth inhibition was measured by determining the OD_{600} at 48 h. The lowest concentration of the oil that led to an OD_{600} of ≤ 0.010 was determined as the MCF of the tested sample. The concentration that causes 90% inhibition MIC_{90} was also measured (Wu et al., 2003).

Inoculum preparation

A. flavus was stored frozen at −70 °C, passed twice on potato dextrose agar (PDA) at 35 °C prior to susceptibility testing. Inocula were prepared as described in the NCCLS M38-A document (NCCLS, 2002). The cultures were grown on PDA slants at 35 °C for 7 d. To prepare conidial inocula, cultures were flooded with sterile 0.85% saline containing 0.025% Tween 20 and gently probed with a pipette tip. The resulting suspension was vortexed, heavy particles were allowed to settle for 3–5 min, and the upper layer was adjusted to a transmittance of 80–82% by using a spectrophotometer (wavelength, 530 nm). The stock suspension of *Aspergillus flavus* contained mostly conidia. These non-germinated conidial inoculum suspensions were diluted 1:100 in a RPMI 1640 medium for testing by the agar dilution method.

Agar dilution method

The oil samples were serially diluted in molten medium equilibrated at 50 °C (RPMI 1640 medium) with 2% glucose to achieve final concentrations: (0–320 µl/ml). One milliliter was added to each well in a 24-well plate with a flat bottom and allowed to solidify. The centre of each well was inoculated with 10 µl of the conidial suspension. Drug-free growth control was included. MIC was determined after 48 h at 35 °C. MICs were defined as the lowest oil concentration that had granular appearing micro-colonies of growth instead of filamentous radiating colonies on solid agar.

Determination of the median lethal dose (LD_{50})

The LD_{50} of the volatile oil was estimated according to Karber's (1931) procedure.

Antioxidant activity

The antioxidant activity of the volatile oil obtained from the juvenile leaves and stems was calculated by determination of glutathione level in blood of alloxan-induced diabetic rats. The method of Beutler et al. (1963) was adopted, using vitamin E as a positive control. The method depends on the fact that both protein and non-protein SH-groups react with Ellman's reagent [5,5-dithiobis-(2-nitrobenzoic acid)] to form a stable yellow color of 5-thio-2-nitrobenzoic acid, which can be measured at 412 nm. In order to determine glutathione level in blood, precipitation of protein SH-groups was necessary before the addition of Ellman's reagent.

Precipitating solution was prepared by dissolving 1.67 g of *m*-phosphoric acid, 0.2 g of ethylene-diamine-tetracetic acid disodium salt (EDTA), and 30 g sodium chloride in 100 ml bidistilled water. This solution is stable for 3 weeks at 4 °C. A fine precipitate of EDTA may be formed and does not interfere. The addition of EDTA is only necessary when water supplies contain appreciable concentrations of metallic ions.

Adult male albino rats (weighing 130–150 g) were divided into five groups (six animals each). One group was kept as a negative control; while for the other groups, *Diabetes mellitus* was induced according to the method described by Eliasson and Samet (1969) in which a single dose of 150 mg alloxan/kg BW was injected intra-peritoneal in each animal followed by an overnight fast.

A group of rats were kept untreated. Another group received the reference drug (vitamin E, 7.5 mg/kg BW), while the other two groups received the volatile oil of the juvenile leaves and stems (100 mg/kg BW) of *E. cinerea*. The rats were kept for 1 week before the determination of glutathione in blood. Blood samples (0.1 ml each) were hemolyzed by the addition of 0.9 ml bidistilled water. To the hemolysates, 1.5 ml of the precipitating solution were added, mixed, and allowed to stand for 5 min. Centrifugation at 3000 rpm was carried out for 15 min. To 1 ml of the resulting supernatant, 4 ml phosphate solution (0.3 M) was added followed by Ellman's reagent. The optical density was measured within 5 min at 412 nm using a Shimadzu double beam spectrophotometer (Shimadzu Corporation, Tokyo, Japan) (UV-150-02). A blank solution was prepared with 4 ml phosphate solution (0.3 M), 1 ml dilute precipitating solution (3:2), and 0.5 ml Ellman's reagent. To 1 ml standard glutathione solution, 4 ml phosphate solution (0.3 M) and 0.5 ml Ellman's reagent were added and the optical density was measured at 412 nm against blank containing 1 ml bidistilled water instead of the standard solution. Blood glutathione was calculated by the following equation:

$$\text{GSH (mg/dl)} = \frac{\text{Absorbance of the sample} \times 3.75 \times 2.5 \times 100}{\text{Absorbance of the standard} \times 1000 \times 0.1}$$

Statistical analysis

Results were statistically analyzed using Student's *t*-test (Snedecor & Cochran, 1980) and compiled in Table 5.

Table 5. *In vivo* antioxidant effect of the volatile oil of the juvenile leaves and stems of *E. cinerea*.

Group	Blood glutathione level (mg/dl)	Relative potency
Control	36.3 ± 1.4	–
Diabetic non-treated	21.4 ± 0.5[a]	–
Diabetic + vitamin E (7.5 mg/kg)	35.9 ± 1.2	1
Diabetic + leaves volatile oil (100 mg/kg)	33.7 ± 1.1	0.94
Diabetic + stems volatile oil (100 mg/kg)	29.6 ± 0.7	0.82

Results are the average of three determinations.
[a]Statistically significant from control group at $p < 0.01$.

Results

The percentage yield of the volatile oil of *E. cinerea* cultivated in Egypt obtained from the juvenile leaves and the stems was 4.5% and 0.5%, respectively, on a fresh weight basis. Table 1 shows that the physical properties as well as the physical constants were almost the same in the two oil samples.

Results of GC/MS analysis of the volatile oils, obtained from the juvenile leaves and stems of *E. cinerea*, Table 2, were qualitatively similar but they differed quantitatively. Sixteen compounds were identified representing 99.01% and 92.12% of the volatile oil of the leaves and stems, respectively.

Both oils were rich in oxygenated compounds (93.00% and 80.90% in leaves and stems, respectively). 1,8-Cineole was the major identified oxygenated monoterpene (84.55% and 60.15% in the leaves and stems, respectively).

α-Terpineol was detected in the stem oil (8.55%) in a concentration higher than that of the leaves (2.14%). Meanwhile, terpinyl acetate was also found in the stem oil in a relatively higher amount (7.70%) than that of the leaf oil (4.9%). Globulol was higher in the stem oil (1.89%) than that of the leaves (0.22%). In contrast, α- and β-citral were only detected in the stem oil.

Other oxygenated compounds were detected in relatively small quantities as methyl geranate (0.25% and 0.94%) and terpinene-4-ol (0.79% and 0.87%) in the oils of the leaves and stems, respectively.

The percentage of hydrocarbons reached 6.01% and 11.22% of the total composition in both juvenile leaves and stems oils, respectively. They were represented by 8 and 7 monoterpenes in the volatile oil of the juvenile leaves and stems, respectively.

α-Pinene was the major identified hydrocarbon in the stems (8.00%), however, it amounted to 2.9% in juvenile leaves oil. Limonene was detected in a relatively higher amount in the juvenile leaves' oil than that of the stems (1.5% and 1.19%).

The juvenile leaves' oil showed higher antibacterial effect against the tested organisms than that of the stems, Tables 3 and 4. The volatile oil of the juvenile leaves exhibited pronounced activity against *Streptococcus faecalis* followed by *Pseudomonas aeruginosa* and *Bacillus subtilis*as regard to Norfloxacin (Table 3). It also showed moderate antifungal activity against *Candida albicans* and *A. flavus* followed by the oil of the stems which exhibited weaker activity using Amphotericin B as a reference antifungal, Table 4.

Table 6. Minimal inhibitory concentration (MIC) of the volatile oil of the juvenile leaves of *E. cinerea*.

	MIC (μl/ml)				
Sample	*Escherichia coli* (G^-)	*Pseudomonas aeruginosa* (G^-)	*Streptococcus faecalis* (G^+)	*Candida albicans*	*Aspergillus flavus*
Eucalyptus cinerea leaf oil	5.2	5.6	4	4.8	12.8

Table 6 revealed that the MIC of the volatile oil of the juvenile leaves recorded 5.2, 5.6, 4, 4.8, and 12.8 μl/ml against *E. coli*, *P. aeruginosa*, *S. faecalis*, *Candida albicans* and *A. flavus*, respectively.

In the present study, LD_{50} of both volatile oil samples; juvenile leaves and stems reported safety up to maximum doses of 1000 and 1020 mg/kg BW, respectively. Antioxidant results recorded in Table 5 revealed that the reduced level of blood glutathione was greatly restored by the volatile oil (100 mg/kg BW) of both juvenile leaves and stems of *E. cinerea*. The volatile oil of the juvenile leaves was more powerful (potency = 94%) than that of the stems (potency = 82%).

Discussion

The percentage yield of the volatile oil of the juvenile leaves of *E. cinerea* cultivated in Egypt was higher than that obtained from the stems. Meanwhile, their physical properties and physical constants were almost the same for the two sample oils.

Juvenile leaves of *E. cinerea* under investigation gave the highest yield of volatile oil (4.5%) followed by that obtained from Tunisia (3.90 %, Elaissi et al., 2011). However, the yield of both volatile oils was higher than that of Morocco (0.26%, Zrira et al., 2004) and India (2.89%, Babu & Singh, 2009). This could be attributed to environmental factors.

The present work would help in chemotaxonomical characterization of *E. cinerea* together with the morphological features. Furthermore, the detection of 1,8-cineole as the major constituent in the oils under investigation is in accordance with reported literature, confirming the identity of the Egyptian *Eucalyptus* species.

Natural drugs could present an interesting approach to limit the emergence and spread of microorganisms, which currently are difficult to treat. Volatile oils have been shown to be potential agents in the treatment of infections, and are safe in terms of human and animal health. Although the majority of the volatile oils are classified as Generally Recognised As Safe (GRAS) (Kabara, 1991), their use in food and as preservatives or in pharmaceutical preparations, is often limited, since effective antimicrobial doses may exceed organoleptically acceptable levels. Therefore, there is an increasing demand for accurate knowledge of the MIC of the volatile oils to enable a balance between the sensory acceptability and the antimicrobial efficacy (Lambert et al., 2001). In this context, the antimicrobial activity and determination of the MIC of both oils under investigation was carried out. Minimum inhibitory concentrations are important to confirm the resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrews, 2001). A lower MIC is an indication of a better antimicrobial agent.

The volatile oil of the juvenile leaves revealed higher antimicrobial activity against the selected Gram-positive and Gram-negative bacteria as well as potent antifungal activity; accordingly its MIC was determined. The MIC of the leaves' oil recorded low values: 5.2, 5.6, 4, and 4.8 μl/ml against *E. coli*, *Pseudomonas aeruginosa*, *S. faecalis* and *Candida albicans*, respectively, and moderate value (12.8 μl/ml) against *A. flavus*. These results evidenced that the volatile oil of the juvenile leaves possesses potent antimicrobial activity against pathogenic or opportunistic strains to aquaculture or human.

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease, and even altitude sickness (Baillie et al., 2009; Prabhat et al., 1995). They are also used as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline (Dabelstein et al., 2007). Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress, and may damage or kill cells (Sies, 1997). The volatile oils are reputed for their antioxidant activity. In that regard, the *in vivo* antioxidant activity of the volatile oil of both juvenile leaves and stems of *E. cinerea* was studied. Results revealed significant antioxidant activity for both samples; however, the volatile oil of the juvenile leaves was more powerful than that of the stems. This may be due to higher content of 1,8-cineole in the leaf oil. Our laboratory finding results suggest the use of these oils as food preservatives.

Conclusion

This study has shown that volatile oil of *E. cinerea* F. Muell. ex Benth. cultivated in Egypt possesses rather a significant activity against different microorganisms, including human pathogens, food poisoning and spoilage bacteria, and blastomycete opportunistic fungus *C. albicans*. These results suggest the potential use of *E. cinerea* volatile oil in the treatment of infectious diseases such as scarlet fever, upper respiratory infections, and food poisoning in addition to its use as a natural anti-oxidant agent.

Declaration of interest

The authors declare no conflict of interest.

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