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Research article

Novel *Erodium glaucophyllum* (L.) Aiton growing in arid environment: Phytochemical characterization, antimicrobial, antioxidant, and anticancer potential ☆

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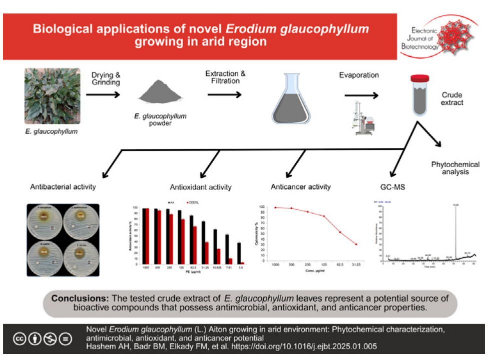
GRAPHICAL ABSTRACT

Novel *Erodium glaucophyllum* (L.) Aiton growing in arid environment: Phytochemical characterization, antimicrobial, antioxidant, and anticancer potential



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ABSTRACT

Background: The misuse of antimicrobial agents resulted in a global serious health concern, namely antimicrobial resistance. Also, traditional bioactive compounds are associated with undesirable adverse effects. This study aimed to first assess the phytochemical analyses and biological activities of *Erodium glaucophyllum* (L.) Aiton from the arid region.

Results: The gas chromatography-mass spectroscopy analysis of *Erodium glaucophyllum* leaves crude extract revealed the presence of 36 biologically vital compounds, with 8 main compounds being identified. Docosenamide, hexadecanoic acid, thiocarbamic acid, hentriacontane, β-sitosterol, quinoline, and oleic acid were among the most significant compounds. Docosenamide was the most prevalent compound, comprising 45.3%. The phytochemical analysis revealed the presence of a diverse array of chemical compounds, such as carbohydrates, polyphenols, flavonoids, and tannins. In moderate

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Chlorogenic acid
Docosenamide
Erodium glaucophyllum
Phytochemicals
Plant leaves

concentrations, saponins, glycosides, quinones, proteins, and amino acids were present. Additionally, alkaloids, steroids, diterpenes, and cardiac glycosides were identified in trace amounts. Also, chlorogenic acid was the dominant with 69.14% among other phenolic compounds. The antimicrobial results of the tested extract showed promising activity against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium,* and *Pseudomonas aeruginosa*, with minimum inhibitory concentration of 31.25, 15.62, 15.62, and 62.5 μg/ml, respectively. Furthermore, the extract demonstrated potent antioxidant activity, with an EC50 of 51.7 μg/ml, and anticancer activity against MCF-7 malignant cell line, with an IC50 of 58.4 μg/ml.
Conclusions: The tested crude extract of *Erodium glaucophyllum* leaves represents a potential source of bioactive compounds that possess antimicrobial, antioxidant, and anticancer properties.
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1. Introduction

Bioactive compounds are naturally occurring substances that have physiological effects on living organisms [1]. Antimicrobial agents represent the conventional bioactive substances that are essential for the field of medicine [2,3,4]. The major challenge facing healthcare workers in developing countries is the emergence and spread of multidrug-resistant (MDR) pathogens [5]. MDR microbial phenotypes have emerged from the overuse and misuse of antimicrobial medications. Given the growing difficulty of treating infections, this has grown to be a serious worldwide health concern [6]. Additionally, many traditional bioactive compounds can cause adverse effects, ranging from mild to severe. For example, chemotherapeutic agents often lead to debilitating side effects, including nausea, hair loss, and immunosuppression [7]. Accordingly, the rise of drug resistance and the drawbacks linked to conventional chemicals have sparked a rising curiosity in investigating alternative bioactive substances [2]. Also, the implementation of safe and more effective therapeutic alternatives is essential for preventing the spread of antimicrobial-resistant bacteria [8]. Additionally, employing an appropriate treatment strategy, after identification of the causative infectious agent, significantly saves the patients' lives and reduces the treatment costs [9].

Natural scientists and ancients have long been respected the antimicrobial properties of plants. Today, the priority of many researchers is the study of traditional medicine plants to achieve new antimicrobial alternatives [10,11]. Plant-derived bioactive chemicals consist of a diverse array of chemical classes, such as polyphenols, alkaloids, flavonoids, terpenes, and others [12]. Coastal regions, open forests, and rocky slopes are among the Mediterranean habitats where *Erodium glaucophyllum* is commonly found. It can survive in dry and arid climates and grows well in well-drained soils. Because of its thick roots, the plant can efficiently reach water sources and survive in areas with little rainfall [13]. The potential utility of *E. glaucophyllum* in various industries, including horticulture, traditional medicine, agri-food, pharmaceuticals, and cosmetics, represents a unique economic benefit. This plant contains numerous bioactive antioxidant components such as tannins, flavonoids, and polyphenols, making it an excellent candidate for natural antioxidant production. *E. glaucophyllum*'s antibacterial and antioxidant properties further contribute to its economic value by offering potential applications in the pharmaceutical, conventional medicine, and dietary supplement industries [14]. The *E. glaucophyllum* has ecological value as it contributes to ecosystem stability and supports biodiversity. Pollinators such as bees and butterflies are attracted to its blossoms, aiding in the reproduction of this plant species and other related flora. Furthermore, a variety of small organisms find refuge and residence within the leaves of the plant [14]. The *E. glaucophyllum* has potential as a natural antioxidant source. The plant extract is capable of scavenging free radicals and providing protection against oxidative stress due to the presence of flavonoids, polyphenols, and other phytochemicals. Therefore, *E. glaucophyllum* is useful in various areas, including nutrition, medicine, and the creation of products containing antioxidants [15].

This study aimed to collect *E. glaucophyllum* from aid region, Ain-Sokhna-Alqattamiya road, Suez, Egypt. Also, to assess phytochemicals of the crude extract of *E. glaucophyllum* leaves (CEEGLs) qualitatively and quantitatively, moreover, to unveil the different biological activities of the CEEGL.

2. Materials and methods

2.1. Plant material

Erodium glaucophyllum (L.) Aiton from Ain-Sokhna-Alqattamiya road, Suez, Egypt was collected (Fig. 1). The plant utilized in this study was identified by Prof. Dr. Abdou Marie Hamed at Ecology Lab., Botany and Microbiology Dep., Faculty of Science, Al-Azhar University, Cairo, Egypt. Experimental research and field studies involving plants, including the gathering of plant specimens, adhere to applicable institutional, national, and international regulations and legislation.

Fig. 1. Photo of the collected *E. glaucophyllum* (L.) Aiton.

2.2. Preparation of the crude extract of *E. glaucophyllum*

Twenty grams of dried *E. glaucophyllum* were extracted with 100 ml of ethyl acetate as the solvent. The solution was permitted to remain for 72 h at ambient temperature and subsequently filtered using Whatman No. 2 filter paper. Following the filtration process, the resulting liquid was subjected to evaporation and allowed to dry at ambient temperature. The crude extract was then kept at 4°C until needed for further analysis [16].

2.3. Gas chromatography–mass spectroscopy of the CEEGL

According to the study by Abdelaziz et al. [17], the metabolites present in *E. glaucophyllum* extracts were examined, enumerated, and identified using gas chromatography-mass spectroscopy (GC-MS) Model ISQ 7000. The identified components' spectra were compared to the reference spectra stored in the WILEY 09 (Wiley, New York, NY, USA) and NIST 11 libraries to determine their identities [18]. Furthermore, identify the chemical formula, molecular weight, and name of any compounds found in the *E. glaucophyllum* extracts through this analytical approach.

2.4. Phytochemical analyses

2.4.1. Qualitative phytochemical analysis

All the test samples were exposed to phytochemical investigation to figure out the presence of synthetic constituents as indicated by Trease and Evans [19], Harborne [20], and Sofowara [21].

2.4.2. Quantitative determination of phytochemicals

2.4.2.1. Determination of total phenolics. Fifty microliters of the *E. glaucophyllum* extract were diluted with deionized water to obtain a final volume of 1 ml. Thereafter, 0.5 mL of Folin-Ciocalteu reagent was incorporated into the mixture and allowed to incubate for 5 min at 25°C. Subsequently, 2.5 ml of a saturated Na_2CO_3 solution was introduced, and the mixture was incubated for 40 min at 25°C in the absence of light. The absorbance at 725 nm was quantified utilizing a calibration curve with gallic acid as the standard [22].

2.4.2.2. Determination of total flavonoids. Distilled water (dH_2O) was added to 0.5 ml of *E. glaucophyllum* extract to make it 1 ml in total. After that, 150 μL of a 5% $NaNO_2$ solution in water was added, and the mixture was left to sit for 5 min. After that, 150 μL of a dilute 10% solution of $AlCl_3$ was added, and the mixture was left to sit for 6 min. After that, 2 ml of a 4% aqueous NaOH solution was added, and distilled water was used to make the total amount 5 mL. After that, the mixture was left alone for 15 min without being touched. At a wavelength of 510 nm, absorbance was recorded with rutin dissolved in methanol as the standard [23].

2.4.2.3. Determination of total tannins. One half milliliter of *E. glaucophyllum* extract and 0.5 ml of dH_2O were combined with 0.1 g of polyvinyl poly pyrrolidone at 0°C. The solution was subsequently incubated at 4°C for a duration of 4 h, followed by centrifugation for 10 min. Subsequently, 0.5 mL of 1 N Folin-Ciocalteu reagent was mixed with 0.1 ml of the non-tannin phenolic fraction from the sample in each test tube, which was then brought to a total volume of 1 ml with dH_2O. Following a 5 min incubation at room temperature, 2.5 ml of a 5% Na_2CO_3 solution was introduced and subsequently kept in the dark for 40 min, tannic acid was utilized as the reference and assessed at 725 nm.

2.4.2.4. Determination of total flavonols. The sample of *E. glaucophyllum* was filled with 2 ml of $AlCl_3$ aqueous and 6 ml of CH_3COONa aqueous. Incubation of the mixture was placed at a temperature of 20°C for a period of two hrs. At 440 nm, the absorbance of the samples was determined. A calculation was performed to determine the total flavonol content, and the results were reported as mg of rutin equivalent per g of dry weight (mg RTE/g DW).

2.4.2.5. Determination of total steroids. Dried *E. glaucophyllum* (5 g) was boiled for 30 min in 50 ml of HCl_{aq}, and then, the solution was filtered. A total of 50 ml of $CH_3COOC_2H_5$ was added to the filtrate, mixed well, and the $CH_3COOC_2H_5$ layer was recovered. The layer fraction of $CH_3COOC_2H_5$ was subjected to drying for a duration of 5 min at a temperature of 100°C using a steam bath. The extraction of steroids involved heating the dried fraction with concentrated amyl alcohol. The steroid content was determined and reported as mg per 100 g of the dry weight of the plant sample [20].

2.4.2.6. Determination of total alkaloids. *E. glaucophyllum* (1 g) was mixed with 70% ethanol and glacial acetic acid and left for 6 h before filtration and then precipitate the alkaloids by slowly adding concentrated ammonia solution drop by drop. The filtered alkaloids are dried in an oven at 70°C. The content of alkaloids is then calculated and expressed as mg per 100 g of the dry weight of *E. glaucophyllum* [24].

2.4.2.7. Determination of total saponins. Fifty grams of dried *E. glaucophyllum* were mixed with 25 ml of 20% ethanol, and the mixture was kept at 55°C for 4 h. The liquid was filtered, and 5 ml of diethyl ether was added. The layer of diethyl ether was taken away, but the layer of water was kept. Thirteen milliliters of butanol were added to the layer of water that was still there, making two separate levels. It was possible to separate the butanol layer. The butanol layers were mixed with 5 ml of a 5% sodium chloride solution to make a precipitate. The mixture was then dried, measured, and given as mg per 100 g of dry *E. glaucophyllum* [25].

2.4.2.8. High-performance liquid chromatography for determination of polyphenols. A device from the Agilent 1260 series was used for the high-performance liquid chromatography (HPLC) analysis. A Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 μm) was used for the separation. At a flow rate of 0.9 ml/min, the mobile phase was made up of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). As explained below, the mobile phase was progressively created in a linear gradient: 11–18 min (60% A); 18–22 min (82% A); 22–24 min (82% A); 0–1 min (82% A); 1–11 min (75% A); and 0–18 min (82% A). At 280 nm, the multi-wavelength detector was measured. For every sample solution, a 5 μL injection volume was set. The temperature of the column was kept constant at 40°C.

2.5. Antimicrobial activity

The initial qualitative screening for antibacterial efficacy of CEEGL was evaluated following the agar well diffusion technique via determination of the inhibition zone diameter (IZD) caused by the extract as outlined by Elkady et al. [26] using dimethyl sulfoxide (DMSO) as a solvent with no antibacterial activity. The activity was assessed against the representative reference strains with notable antimicrobial-resistant phenotypes including Gram-positive *Bacillus subtilis* ATCC 6051 and *Staphylococcus aureus* ATCC 25923, as well as Gram-negative *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 27853. Then, the quantitative CEEGL antibacterial activity was assessed using broth microdilution technique for determination of its minimum inhibitory concentration (MIC) against the tested strains as described by Elkady et al. [27] with some modification. Briefly, each tested strain suspension equivalent to 0.5 McFarland standard was prepared. The tested extract was serially diluted in a 96-well microtiter plate using tryptic soya broth (TSB) (Oxoid, Basingstoke, UK) followed by addition of the prepared bacterial suspension. The plates were then incubated for 18 h at 37°C. The lowest concentration of the tested CEEGL showing non-observable bacterial growth was recorded as the MIC. All antimicrobial test procedures were conducted under aseptic conditions in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines [28,29,30,31,32].

2.6. Antioxidant activity

The CEEGL's antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique [33]. In this assay, the colored DPPH stable free radical combined with the tested plant extract with possible antioxidant activity. This combination consequently resulted in a measurable decolourization of the DPPH with different degrees proportional to the antioxidant activity of the tested extract [34]. The crude extract's capacity to scavenge the DPPH radicals was investigated at concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/ml. The percentage of antioxidant activity was calculated in correlation with ascorbic acid (AA) to ascertain the DPPH scavenging activity of various concentrations of CEEGL, as outlined in **Equation 1:**

$$\text{Antioxidant activity}\,\% = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100 \qquad (1)$$

2.7. Cytotoxicity and anticancer activity

The cytotoxicity experiment was performed according to the MTT procedure established by Van de Loosdrecht et al. [35]. The MCF-7 and Wi-38, sourced from ATCC, were utilized to evaluate the cytotoxic or anticancer effects of CEEGL, respectively. The measured OD of the cells at 560 nm was utilized to calculate cell viability and inhibition % [4], following **Equation 2** and **Equation 3**, respectively:

$$\text{Viability}\,\% = \frac{\text{Test OD}}{\text{Control OD}} \times 100 \qquad (2)$$

$$\text{Inhibition}\,\% = 100 - \text{Viability}\,\% \qquad (3)$$

3. Results and discussion

3.1. GC–MS of the CEEGL

The GC–MS analysis confirmed the presence of 36 compounds of biological importance (Table 1, Fig. 2). The top 8 compounds, in terms of peak area percentage, were considered major compounds and are as follows: Docosenamide (45.3%), Heptadecyne, 17-chloro (4.29%), Hexadecanoic acid, methyl ester (4%), Thiocarbamic acid, N,N-dimethyl,S-1,3-diphenyl-2-butenyl ester (2.64%), Hahnfett (2.58%), ç-Sitosterol (2.58%), Quinindoline (2.26%), and Oleic Acid (2.1%). The compounds indicated in the plant extract are linked to a variety of behavior. Antimicrobial, antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, antiviral, antidiabetic, and insecticidal qualities are some of these properties.

The first major compound was Docosenamide (45.3%). Many studies have proved that docosenamide (Z) is present in natural sources, including a variety of plants and animals. It is most frequently detected in vegetable oils, especially mustard and rapeseed oils, corn, sunflower, and soybean oils. Furthermore, docosenamide is present in the waxes of various plants and in some animal fats, such as fish oil [36]. Docosenamide exhibited significant inhibitory effects against various pathogenic microorganisms. It demonstrated strong inhibitory activity against the Gram-positive bacteria *Staphylococcus epidermidis* and *S. aureus*, as well as the Gram-negative bacteria *Enterobacter aerogenes* and *Klebsiella pneumoniae*. Furthermore, it displayed inhibitory action against the fungus *Candida krusei* [37]. Research also suggests that it may possess anti-inflammatory qualities, which could be relevant for skin health. Given its biological attributes, Z holds the potential for medicinal applications and food additives [36].

Heptadecyne is characterized by its hydrophobic nature, making it useful in drug delivery systems to improve the solubility and bioavailability of hydrophobic drugs. It can act as an intermediate in the synthesis of more complex molecules, including pharmaceutical active compounds [38]. Heptadecyne, 17-chloro, has anticancer activity and can inhibit tumor growth and induce apoptosis in cancer cells. It also exhibits antimicrobial action against various bacteria and fungi [39,40]. Hexadecanoic acid, methyl ester, is a saturated fatty acid used in pharmaceuticals, nutrition, cosmetics, antioxidant, pesticide and antimicrobial applications [41]. Also, Shaaban et al. [42] reported that Methyl hexadecanoate demonstrated antibacterial activity against *S. aureus* W35, *P. aeruginosa* D31, *K. pneumoniae* DF30, and *K. pneumoniae* B45 through disruption of bacterial cell membranes. The thiocarbamic acid functional group features a sulfur atom that is double-bonded to a carbon atom, along with two organic substituents attached thus thiocarbamic acid derivatives are applied as pesticides, fungicides, pharmaceuticals [43]. Sitosterol is a phytosterol that has antioxidant, anti-inflammatory, and antibacterial activity specially against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [44]. Sitosterol is recorded as anti-inflammatory, anticancer, antidiabetic, immune modulator agent [45]. Quinindoline is a heterocyclic compound with antimicrobial, anticancer, and anti-inflammatory effects [46]. Oleic acid is a monounsaturated fatty acid with anti-inflammatory, antimicrobial, antioxidant, anticancer, and preservative effects [17,47]. The mechanism of action of oleic acid depends mainly on metabolic regulation [48].

3.2. Phytochemical analysis

The phytochemical analysis of CEEGL (Table 2, Table 3) revealed the presence of various chemical compounds. Flavonoids, polyphenols, tannins, and carbohydrates were found in abundant quantities. Our results are similar to other studies that proved the presence of these compounds in *E. glaucophyllum* [13,14]. These chemicals have been linked to a number of health benefits, such as their ability to fight cancer, reduce inflammation, fight germs and fungi, protect the heart, and fight free radicals [84,85]. Tannins can bind to proteins and other organic compounds, leading to the formation of precipitates and reducing the permeability of cell membranes. Tannins can reduce intestinal inflammation and promote healing [86,87]. Carbohydrates are essential for sustaining bodily functions and providing fuel for various metabolic processes. They are also important in the food industry [88].

Saponins, glycosides, quinones, proteins, and amino acids were present in moderate amounts. Proteins and amino acids, the building unit of body, play crucial roles in various physiological processes. Quinones are known for their diverse biological activities, including antimicrobial and antioxidant properties [45].

The presence of cardiac glycosides, steroids, alkaloids, and diterpenes is present in small quantities. These compounds have bioactive properties in the pharmaceutical field. Steroids and

Table 1
GC–MS of CEEGL.

No.	Compound name	RT (min)	Peak area %	Activity	Reference
1	Hexadecatrienoic acid, methyl ester butyl	33.16	0.44	Antimicrobial activity and Cardioprotective	[49,50]
2	Baimuxinal	33.66	0.16	Antimicrobial	[51]
3	Cyclopentane acetic acid, 3-oxo-2-pentyl-, methyl ester	36.10	1.03	Antibacterial and anticancer	[52]
4	Octadecanoic acid, 9,10-epoxy-, isopropyl ester	36.97	1.33	Antioxidant and anticancer	[53]
5	1-Hexadecanol, 2-methyl	39.15	0.41	Antibacterial and antifungal	[54]
6	Octanal, 2-(phenylmethylene)	40.46	0.31	Anticancer	[55]
7	Oleic Acid	34.12	0.26	Antimicrobial, antioxidant, and apoptotic	[56]
8	Stephamiersine	43.70	0.28	Antibacterial and antifungal	[57]
9	Isopropyl myristate	43.93	1.67	Antibacterial	[58]
10	Quinindoline	44.06	2.26	Antibacterial	[59]
11	7-Heptadecyne, 17-chloro	44.65	4.29	Antimicrobial and insecticidal	[40]
12	1-Propyl-2-methyl-7-methoxy-5 h,6h-pyrido[3,4–b] Indole	45.37	0.42	Antiviral, anti-inflammatory, anti-tubercular, anticancer, anti-HIV, antioxidant, antimicrobial, antidiabetic, antimalarial, and anticholinesterase	[60]
13	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	45.88	0.28	Antibacterial	[61]
14	Hexadecanoic acid, methyl ester	47.12	4.00	Antioxidant and antimicrobial	[42,62]
14	Octadecanoic acid, 4-hydroxy-, methyl ester	48.84	0.98	Antiviral and antimicrobial	[63]
15	Oleic acid	49.65	2.10	Antimicrobial	[64]
16	Doconexent	52.22	0.21	Antimicrobial	[65]
17	Undec-10-ynoic acid, tetradecyl ester	52.96	0.68	Insecticidal	[66]
18	Heptadecanoic acid, 16-methyl-, methyl ester	53.34	0.23	Anticancer	[67]
19	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)	53.85	0.40	Antibacterial	[59]
20	9-Octadecenamide	60.51	0.24	Anti-inflammatory and antibacterial	[68]
21	Tert-Hexadecanethiol	61.50	0.21	Antioxidant and antibacterial	[69]
22	2-,2-Dibenzylethanol	62.56	0.67	Antiproliferative or antitumoral, antimicrobial, and anti-inflammatory	[70]
23	Erucic acid	62.82	0.23	Antimicrobial	[71]
24	7,8-Epoxylanostan-11-ol, 3-acetoxy	64.72	0.41	Antimicrobial	[72]
25	Spiro[2.3]hexane-5-carboxylic acid,1,1-diphenyl-, methyl ester	64.97	0.96	Antimicrobial	[73]
26	Cyclodecasiloxane, eicosamethyl	65.15	0.34	Antibacterial and antioxidant	[74]
27	Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester	65.47	2.64	Antimicrobial	[75]
28	Trilinolein	71.94	1.02	Antimicrobial	[76]
29	13-Docosenamide, (Z)	72.19	45.3	Antibacterial and antifungal	[37]
30	Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)	72.86	0.98	Anti-diabetic	[77]
31	Hahnfett	75.63	2.58	Antioxidant effect and antifungal	[78]
32	Isochiapin b %2	76.00	0.37	Anti-insect, antimicrobial, antioxidant, and anticancer	[79]
33	Cholestan-5-ol-6-one	76.24	0.90	Antimicrobial	[80]
34	ç-Sitosterol	83.73	2.58	Antimicrobial	[81]
35	Iso-allocholate	85.12	1.17	Antimicrobial	[82]
36	Stearic acid, 3-(octadecyloxy) propyl ester	85.54	0.19	Antioxidant and antimicrobial	[83]

alkaloids possess diverse biological activities, including anti-inflammatory, analgesic, and cytotoxic properties. Diterpenes exhibit a wide range of biological activities in medicinal applications.

3.3. Determination of polyphenols using HPLC

The antioxidant properties and prospective health benefits of polyphenols, a diverse group of naturally occurring compounds found in plant extracts, are well-known [89]. These compounds play a crucial role in plant defense mechanisms, helping to protect against pathogens and environmental stress. Polyphenols in human nutrition have garnered attention for their capacity to mitigate oxidative stress, diminish inflammation, and perhaps decrease the risk of chronic diseases, including cardiovascular disease and cancer [89,90]. In the current study, HPLC was used to determe the quantities of different phenolic compounds as illustrated in Table 4. Results revealed that chlorogenic acid was the dominant with 69.14% among other phenolic compounds (Fig. 3). Moreover, the quantity of chlorogenic acid in CEEGL extract was 43385.63 μg/ml. Also, other phenolic compounds such as gallic acid, syringic acid, and ellagic acid were detected with 2640.01, 1874.67 and 5193.28 μg/ml, respectively.

Chlorogenic acid is a significant polyphenol predominantly located in coffee, fruits, and vegetables, renowned for its various biological actions. It demonstrates potent antioxidant qualities, aiding in the neutralization of free radicals and the reduction of oxidative stress within the body [91]. Furthermore, it possesses anti-inflammatory properties that may enhance cardiovascular health by lowering blood pressure and cholesterol levels. Subsequent research suggests that chlorogenic acid may have antibacterial characteristics, contributing to the protection against specific infections [92].

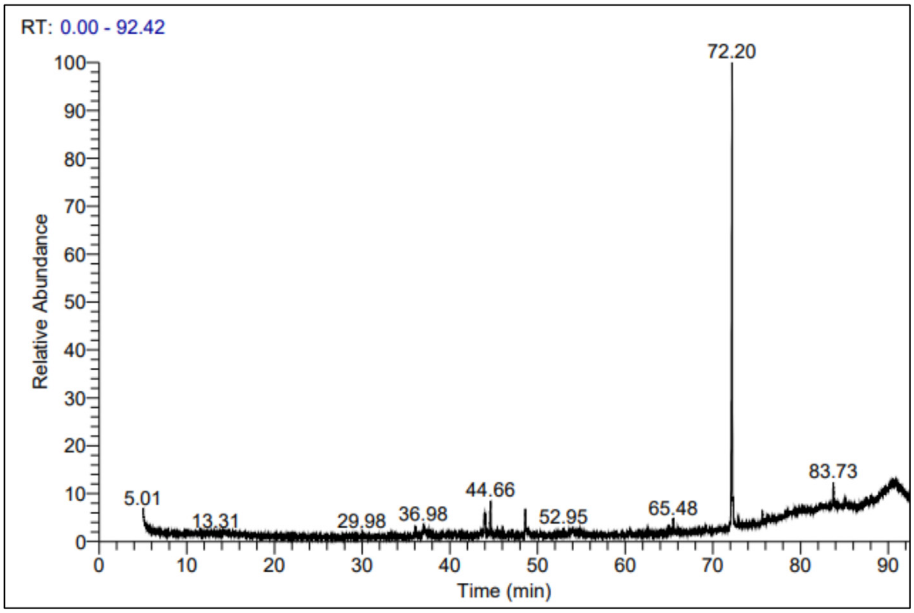

Fig. 2. GC–MS of CEEGL.

Table 2
Preliminary phytochemical screening of *E. glaucophyllum.*

No.	Test	Results
1	Flavonoids	+++
2	Cardiac glycosides	+
3	Alkaloids	+
4	Steroids	+
5	Saponins	++
6	Polyphenols	+++
7	Anthraquinones	–
8	Glycosides	++
9	Diterpenes	+
10	Quinones	++
11	Tannins	+++
12	Phlobatannins	–
13	Gums	–
14	Anthocyanins	–
15	Carbohydrates	+++
16	Proteins and amino acids	++

Table 3
Quantitative phytochemical analysis of *E. glaucophyllum.*

Parameters	Results
Total flavonoids	176.10 ± 1.40 (mg RTE/g DW)
Total flavonols	88.27 ± 0.99 (mg RTE/g DW)
Total phenolic acids	259.58 ± 1.92 (mg GAE/g DW)
Total tannins	126.07 ± 1.27 (mg GAE/g DW)
Total alkaloids	0.83 ± 0.12 (mg/100 g DW)
Total saponins	2.22 ± 0.19 (mg/100 g DW)
Total steroids	1.04 ± 0.09 (mg/100 g DW)

3.4. Antimicrobial activity

Natural products provide an important platform for screening antimicrobial alternatives that could be safely effective in controlling the worldwide increase in antibacterial resistance [93]. The secondary metabolites, including terpenoids, phenolics, flavonoids, and alkaloids produced by many plant species, could exhibit broad-spectrum antimicrobial activity [94]. In our study, the antibacterial activity of CEEGL towards various bacterial strains was assessed via agar well diffusion and broth microdilution assays for determination of its IZD and MIC values, respectively. The large IZD (Fig. 4), in a range of 22.0 ± 0.90–26.43 ± 0.60 mm (Table 5), illustrated the strong *in vitro* antibacterial activity of our CEEGL. An obvious effect, in comparison with the undetectable IZD of amoxicillin/clavulanic (30 μg) antimicrobial standard, was observed against the tested *B. subtilis* ATCC 6051, *S. aureus* ATCC 25923, *S. typhimurium* ATCC 14028, and *P. aeruginosa* ATCC 27853 bacterial strains. The study carried out by Abdelkebir et al. [95] reported high *E. glaucophyllum* crude extract bioactivity level against Gram-positive and Gram-negative pathogenic bacterial species. Also, in a relatively similar study [13], the IZD caused by *E. glaucophyllum* crude extract against a wide range of pathogenic Gram-positive and Gram-negative bacteria was at the range of 7–15 mm. Additionally, in Bakari et al. [96] study, the flower extract of *E. glaucophyllum* revealed a powerful antibacterial activity in a range of 15.2 ± 0.2 mm for Gram-positive *S. aureus* ATCC 6538 and *B. subtilis* JN 934392 to 32.2 ± 0.2 mm for Gram-negative *S. Enteritidis* ATCC43972. Moreover, endophytic *Aspergillus terreus* extract exhibited promising antifungal activity toward fungi causing mucormycosis [97]. Quantitatively, our recorded *E. glaucophyllum* crude extract MIC was in the range of 15.62–62.5 μg/ml against the tested strains (Table 5). Han et al. [98] reported that *E. glaucophyllum* extract reveals inhibitory activities towards *S. aureus* and *E. coli* with MICs of 3.16 and 2.5 mg/ml, respectively. The different reported microbial susceptibility patterns to the extract, in the form of variable IZD and MIC, could be explained by different microbial structures as well as, the variable active constituent concentration in the extract depending on extraction method, used solvent, and the investigated part of the plant. Also, our low determined MIC reflect the prominent antibacterial activity of the *E. glaucophyllum* crude extract against a wide range of bacterial strains. This possible broad-spectrum activity of *E. glaucophyllum* crude extract against Gram-positive and Gram-negative bacterial species could be associated with its saponins, flavonoids, and tan-

Table 4
Different phenolic compounds in CEEGL extract using HPLC.

Phenolic acid name	RT	Area (mAU*S)	Area %	Conc (µg/ml)
Gallic acid	3.558	452.35458	6.6682	2640.01
Chlorogenic acid	4.283	4690.52832	69.1438	43385.63
Methyl gallate	5.373	229.76515	3.3870	856.49
Coffeic acid	5.894	138.98938	2.0489	821.71
Syringic acid	6.239	435.71100	6.4229	1874.67
Ellagic acid	7.189	581.84833	8.5771	5193.28
Coumaric acid	8.676	93.24339	1.3745	224.91
Vanillin	9.285	17.93205	0.2643	47.89
Ferulic acid	9.587	20.58087	0.3034	83.47
Naringenin	10.152	53.46299	0.7881	347.60
Rosmarinic acid	11.776	15.92885	0.2348	115.15
Daidzein	15.915	2.79647	0.0412	12.68
Querectin	17.565	26.80905	0.3952	129.33
Cinnamic acid	19.286	23.77504	0.3505	29.49

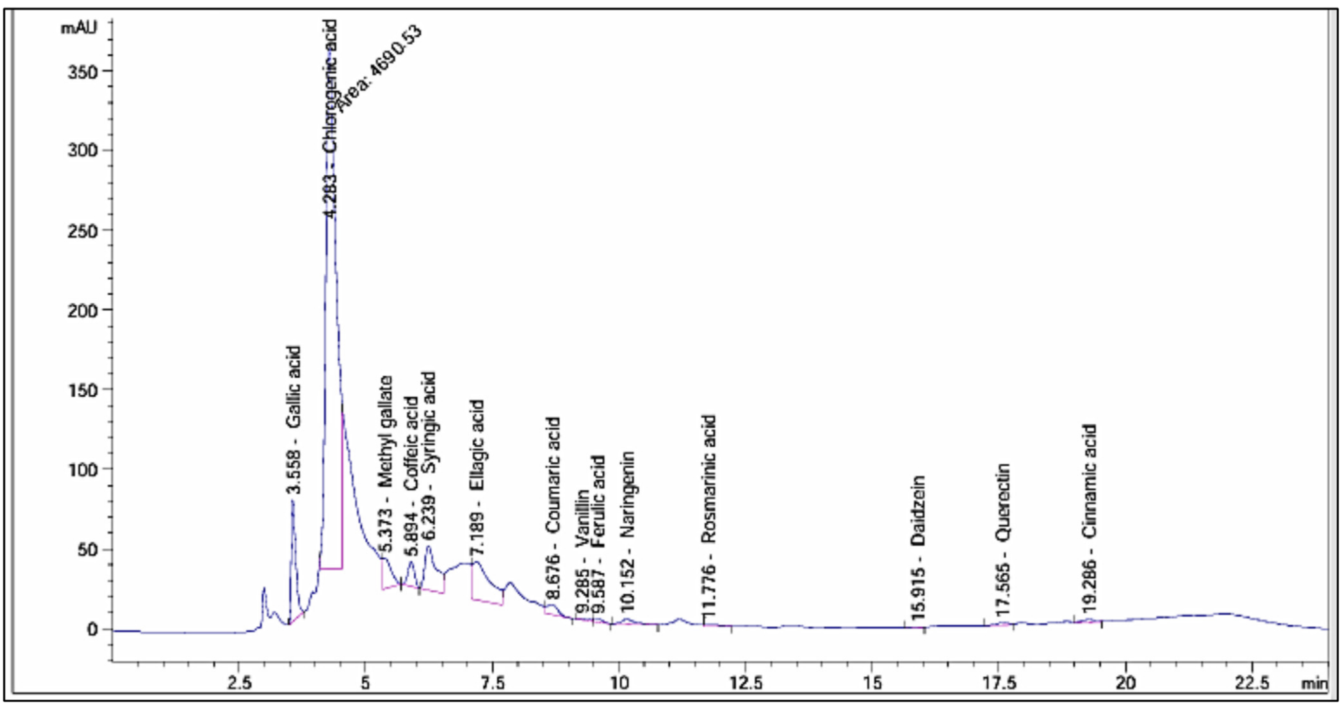

Fig. 3. Chromatograms of phenolic compounds from the extract of CEEGL.

nins, catechin, and gallic acid phenolic active constituents that are identified as an effective inhibitor for pathogenic bacteria.

3.5. Antioxidant activity

Phenolic compounds are the most detected natural secondary metabolites with biological activity and quick ability to accommodate the free radicals and subsequent cells protection from oxidative damage [99]. The strong free radical scavenging activity of medicinal plants, in comparison with commonly used fruits and vegetables, is correlated to the presence of significantly high levels of phenolic compounds [100]. Our findings showed the direct correlation between CEEGL concentration and its DPPH scavenging activity, in comparison with equal ascorbic acid concentration. The maximum DPPH scavenging activity (100%) was recorded at a concentration of 1000 µg/ml. Additionally, the ascorbic acid and CEEGL concentrations that achieve 50% antioxidant activity (EC50) were 6.7 µg/ml and 51.7 µg/ml, respectively (Fig. 5). Since the antioxidant activity is classified as either very strong with EC50 < 50 µg/ml, strong with 50 ≤ EC50 < 100 µg/ml, moderate with 100 ≤ EC50 < 150 µg/ml, or low with EC50 > 150 µg/ml [101], our results indicate the strong antioxidant ability of the obtained *E. glaucophyllum* crude extract. Similarly, Bouaziz et al. [102] reported very strong DPPH free radical scavenging capacity *E. glaucophyllum* extract with EC50 = 0.44 µg /ml. Likewise, the related Hamza et al. [103] Tunisian study recorded the *E. glaucophyllum* very strong antioxidant effect with DPPH scavenging activity of 20.29 ± 72.64 µg/ml in comparison with 5.18 ± 71.98 for ascorbic acid. Interestingly, Barba et al. [104] reported the catechin-phenolic compound-based molecules as one of the main active constituents in *E. glaucophyllum* extracts that confers its biological antioxidant activities. Our results revealed the presence of high percentage of antioxidant constituents and consequently the significance of *E. glaucophyllum* as a potential natural resource for nutrient and pharmaceutical antioxidants and subsequently controlling some health disorders.

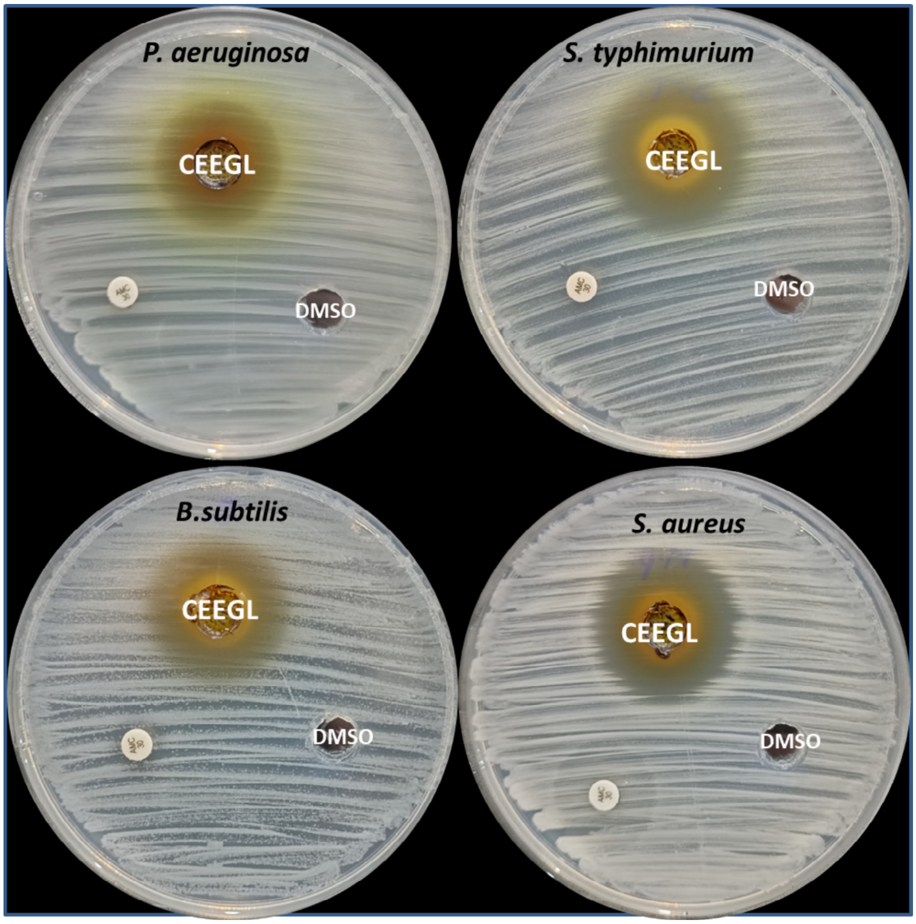

Fig. 4. The diverse antibacterial activity of CEEGL (2000 μg/ml), showed by agar well diffusion assay, against *P. aeruginosa* ATCC 27853, *S. typhimurium* ATCC 14028, *B. subtilis* ATCC 6051, and *S. aureus* ATCC 25923. PE; plant extract, AMC; amoxicillin/clavulanic (30 μg) and DMSO; dimethyl sulfoxide.

Table 5
Antibacterial activity of CEEGL towards selected standard strains.

Antibacterial effect Bacterial strains	IZD[a] (mm)			MIC (μg/ml)
	DMSO	PE	AMC (30 μg)	CEEGL[b]
P. aeruginosa ATCC 27853	ND	22.0 ± 0.90	ND	62.5
S. typhimurium ATCC 14028	ND	26.16 ± 1.26	ND	15.62
B. subtilis ATCC 6051	ND	24.5 ± 0.50	ND	31.25
S. aureus ATCC 25923	ND	26.43 ± 0.60	ND	15.62

[a] IZD; inhibition zone diameter, MIC; minimum inhibitory concentration, DMSO; dimethyl sulfoxide, PE; plant extract, AMC; amoxicillin/clavulanic, and ND; not detected.
[b] CEEGL; crude extract of *E. glaucophyllum* leaves.

3.6. Cytotoxicity and anticancer activity

The majority of anticancer agents may cause serious undesirable effects as they could interfere with nucleic acid replication and cell division in both cancerous and regular cells. *Erodium* L'Herit is one of the major genera of family Geraniaceae, comprising 74 species that is mainly present in the Mediterranean region. This genus affects the vital cellular processes, namely apoptosis and senescence, and some species were shown to be cytotoxic towards different cell lines [105]. In our research, the cytotoxic effect of different CEEGL concentrations, in the range of 31.25 μg/ml–1000 μg/ml, on Wi-38 normal cell line verified its low cytotoxicity outcome. Fortunately, the cell viability was increased upon exposure to decreased concentrations of the tested crude extract with an elevated IC50, 192.4 μg/ml (Fig. 6). On the malignant side, exposure of the MCF-7 to different CEEGL concentrations, in the range of 31.25 μg/ml–1000 μg/ml, demonstrated its important anti-proliferative effect. The cell line viability percentage was decreased upon exposure to increased CEEGL concentrations with a relatively low IC50, 58.4 μg/ml (Fig. 7). In a similar study, the *E. glaucophyllum* crude extract, as one of Qatari medicinal plants, attenuated the growth of breast cancerous MDA-MB-231 cell line. The results showed the inducible cell death effect in comparison with the normal human dermal neonatal fibroblast control cells that were healthy with regular cellular attachment [106]. In another related cytotoxicity study, the significant anticancer activity was demonstrated by the low IC_{50} (31.2 μg/ml) of their tested extract [107]. Accordingly, the *E. glaucophyllum* medicinal plants could represent a precious source for naturally derived pharmacologically effective anticancer ingredients.

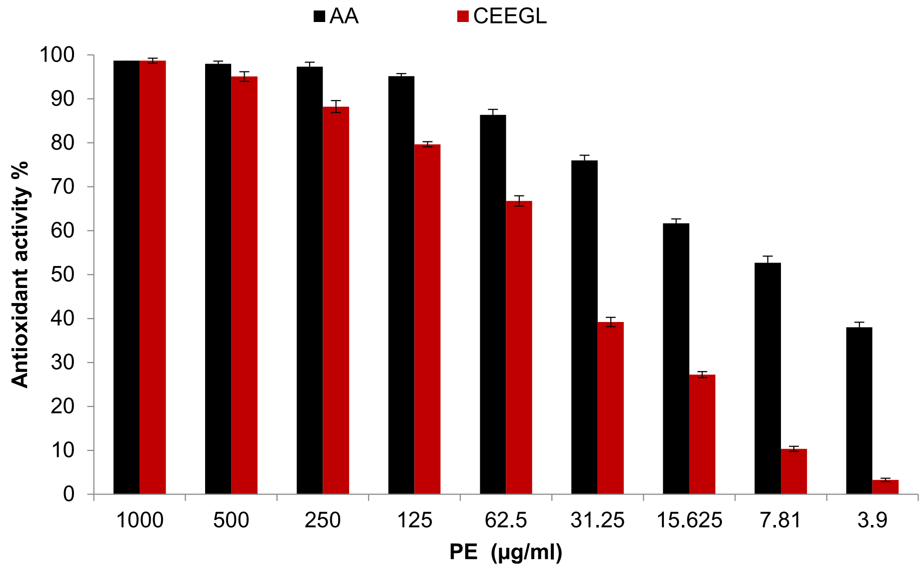

Fig. 5. DPPH scavenging ability of CEEGL at different concentrations in relation to the corresponding concentration of ascorbic acid as a positive control.

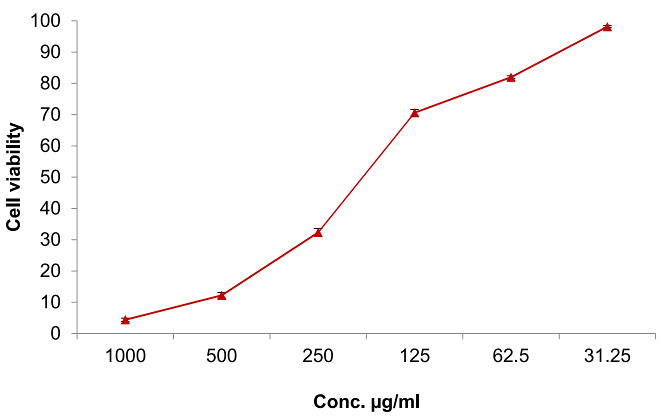

Fig. 6. Cytotoxicity of CEEGL at different concentrations on Wi-38 normal cell line.

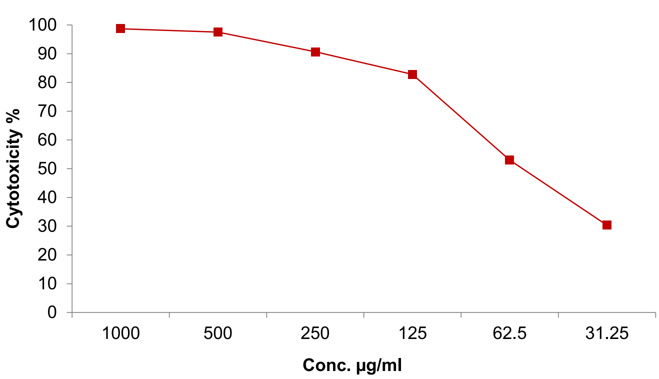

Fig. 7. Anticancer activity of different concentrations of CEEGL against MCF-7 malignant cell line.

4. Conclusions

In the current study, *E. glaucophyllum* was collected from arid region in Suez, Egypt; and evaluated for different biological activities. The GC–MS analysis of CEEGL revealed 36 compounds, including 8 major ones like Docosenamide (45.3%). Phytochemical analysis of the extract identified flavonoids, polyphenols, tannins, carbohydrates, saponins, glycosides, quinones, proteins, amino acids, cardiac glycosides, steroids, alkaloids, and diterpenes. The obtained CEEGL exhibited promising antimicrobial activity against *B. subtilis, S. aureus, S. typhimurium*, and *P. aeruginosa* with MICs ranging from 15.62 to 62.5 μg/ml. Additionally, it demonstrated strong antioxidant activity (EC50 = 51.7 μg/ml) and anticancer activity (IC50 = 58.4 μg/ml) against the MCF-7 cancerous cell line. Finally, future investigation of *E. glaucophyllum* crude extract could reveal biological molecules that represent possible hopeful candidates as bioactive compounds for antioxidants, anticancer, and antibacterial agents for medical applications.

CRediT authorship contribution statement

Amr H. Hashem: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bahaa M. Badr:** Writing – review & editing, Writing – original draft, Validation, Methodology, Funding acquisition. **Fathy M. Elkady:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Mostafa A. Abdel-Maksoud:** Writing – review & editing, Methodology, Funding acquisition. **Abdulaziz Alamri:** Writing – review & editing, Software, Methodology. **Mohamed A. El-Tayeb:** Writing – review & editing, Validation, Funding acquisition, Formal analysis. **Bushra H. Kiani:** Writing – review & editing, Resources, Data curation. **Amer M. Abdelaziz:** Writing – review & editing, Writing – original draft, Validation, Methodology.

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Declaration of competing interest

The authors declare no competing interests.

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Supplementary material

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Data availability

No data was used for the research described in the article.

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