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PHYTOCHEMICAL PROFILING OF *EUPHORBIA DENDROIDES* L. (EUPHORBIACEAE) AND EVALUATION OF ITS ANTIOXIDANT, ANTICANCER, AND ANTIMICROBIAL PROPERTIES

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Abstract

In this study, the phytochemical composition and biological activities of *Euphorbia dendroides* L. extract collected from the western Mediterranean coast of Egypt were investigated. Spectroscopic and chromatographic analyses enabled the identification of major bioactive compounds. The ethyl acetate (EtOAc) extract demonstrated the highest amounts of total phenolics (168.0 mg GAE/g) and flavonoids (71.6 mg QE/g) among all examined extracts. A total of 18 polyphenolic compounds were identified by HPLC, with chlorogenic acid as the predominant phenolic acid and naringenin as the principal flavonoid. The EtOAc extract showed the most potent antioxidant activity (IC_{50} = 2.24 µg/mL), exceeding that of ascorbic acid. Cytotoxic assays indicated a dose-dependent inhibition of cancer cell proliferation, with the dichloromethane (DCM) fraction being the most effective (IC_{50} = 31.42 ± 3.35 for Caco-2, 76.86 ± 1.49 for MCF7, and 76.11 ± 1.09 µg/mL for Panc-1). The EtOAc fraction also showed considerable cytotoxicity (IC_{50} = 90.79 ± 2.13 for Caco-2, 79.86 ± 0.6 for MCF7, and 84.3 ± 1.49 µg/mL for Panc-1). Both fractions were effective in fighting cancer, with a selectivity index value (SI) greater than 2, indicating a possible application in targeted cancer treatments. The extracts demonstrated a strong ability to kill germs, particularly the DCM fraction, which created zones of no growth up to 29 mm against *Staphylococcus aureus* and *Klebsiella pneumoniae*, and a minimum inhibitory concentration (MIC) of 3.9 µg/mL against *S. aureus*. This paper presents the first study to explore the polyphenolic composition and the biological activities of *E. dendroides* extract from Egypt, supporting its potential application in herbal therapy medicine and the development of functional foods.

Key words: *Euphorbia dendroides*; Anti-cancer; Anti-microbial; Antioxidant; Cytotoxicity; polyphenols

Introduction

With over 230 genera and approximately 8,000 taxa, the Euphorbiaceae represents one of the most significant angiosperm families, mainly distributed throughout tropical and subtropical regions (Anon., 2025). This family exhibits considerable morphological diversity, including trees, shrubs, and climbing lianas (Kemboi *et al.*, 2020).

Euphorbia L. constitutes the largest genus within the family, containing more than 2,000 taxa (Kemboi *et al.*, 2020). A distinctive feature of *Euphorbia* species is their latex, which is rich in bioactive compounds with putative health-promoting properties and also serves as an inherent defense mechanism for the plant (Smeriglio *et al.*, 2019).

The taxa of the genus *Euphorbia* have historically been employed in the treatment of microbial infections, inflammation, and other diseases. Recent studies have elucidated the antioxidant properties and cytotoxic activity of these compounds against cancer cells (Kemboi *et al.*, 2020; Nasim *et al.*, 2022).

The *Euphorbia* taxa have been employed in traditional Chinese medicine to provide therapeutic benefits for various conditions, including migraines, wart removal, edema, and rheumatic pain, as well as being used as a piscicide (Ghout *et al.*, 2018).

In addition to its traditional medicinal applications, recent investigations have shown that the genus *Euphorbia* has a broad range of significant biological activities, including cytotoxic, antibacterial, anti-inflammatory, and multidrug resistance-modulating effects (2017; Jannet *et al.*, 2017). Euphorbiaceae is well known for its remarkable chemical diversity, particularly in its isoprenoid constituents. *Euphorbia* taxa are commonly reported to contain diterpenoids and triterpene alcohols in their latex. These diterpenoids showed various core structures, including lathyranes, jatrophanes, ingenanes, tiglianes, and myrsinols (Nielsen *et al.*, 1979; Brooks, 1987). Approximately 650 diterpenes and triterpenes, demonstrating a wide range of biological activities, have been characterized within the genus *Euphorbia*. The therapeutic properties attributed to members of the genus in traditional medicine are attributable to its extensive phytochemical diversity, encompassing essential oils, oxygenated sesquiterpenes, sesquiterpene hydrocarbons,

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macrocyclic diterpenoids, phenolic compounds, and flavonoids (Kemboi *et al.*, 2020; Smeriglio *et al.*, 2021). Phenolic acids and polyphenols, well-known for their powerful antioxidant properties, have been extensively studied for their health benefits, which include anti-inflammatory, anticancer, and neuroprotective activities (Di Lorenzo *et al.*, 2021).

Flavonoids, a significant subgroup of polyphenolic compounds, can help prevent diseases such as cancer, cardiovascular diseases, and diabetes through antimutagenic, anti-inflammatory, and antioxidant properties (Jucá *et al.*, 2020). The biological effectiveness of flavonoids is mainly due to their key structural elements, including aromatic rings and hydroxyl groups (Teles *et al.*, 2018).

E. dendroides is one of the 42 taxa belonging to the genus *Euphorbia* in the flora of Egypt; it typically reaches heights of up to two meters, usually taking the form of a small tree or semi-succulent shrub; it is commonly located along the northwest Mediterranean coasts, mainly on limestone soil, cliffs, and rocks at altitudes of up to 750 meters above sea level; recently, *E. dendroides* has been introduced to several regions around the world, including Australia, southern Argentina, and California (Boulos, 2000, 2008).

While the bioactive properties of various *Euphorbia* species are widely recognized, there is a lack of studies examining the phenolic and flavonoid content of *E. dendroides,* especially those from the Egyptian flora. To fill this gap, the current study quantitatively evaluated the total phenolic and flavonoid contents of different extracts of *E. dendroides*, with a focus on the ethyl acetate fraction, which had the highest concentrations of these compounds. Additionally, HPLC was used to analyze the polyphenolic contents, and the extracts were thoroughly tested for their antioxidant, antimicrobial, anticancer, and anti-inflammatory properties. This research represents the first detailed investigation of the plant *Euphorbia dendroides* from Egypt, contributing to a better understanding of its potential medical applications.

Materials and Methods

Chemicals: HPLC-grade solvents, including water and acetonitrile, were obtained from Thermo Fisher Scientific (US). Trifluoroacetic acid, Folin–Ciocalteu reagent, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5% sodium nitrite ($NaNO_3$), 5% sodium hydroxide (NaOH), and 7% aluminum chloride ($AlCl_3$) were acquired from Sigma-Aldrich (US). Reference standards, including rutin, ascorbic acid, and gallic acid, were supplied by Merck (Darmstadt, Germany). Additional solvents and reagents were procured from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). All experiments were conducted using double-distilled water.

Plant collection: During two field expeditions in March 2023 and April 2024, the plant material was collected from along the western Egyptian Mediterranean coastal area. The specimens of *E. dendroides* have been gathered from El-Salloum (latitude 31°29′25.4″N, longitude 25°13′15.1″E; elevation 31 m above sea level), Matrouh Governorate, by the fifth author (Ahmed K. Osman). The collected plant materials were air-dried and deposited in the herbaria of Sohag University (SHG) and Qena University (QNA) (Fig. 1). Taxonomic identification was done by comparing the specimens' descriptions in the Flora of Egypt with relevant taxonomic literature (Täckholm, 1974; Boulos, 2000).

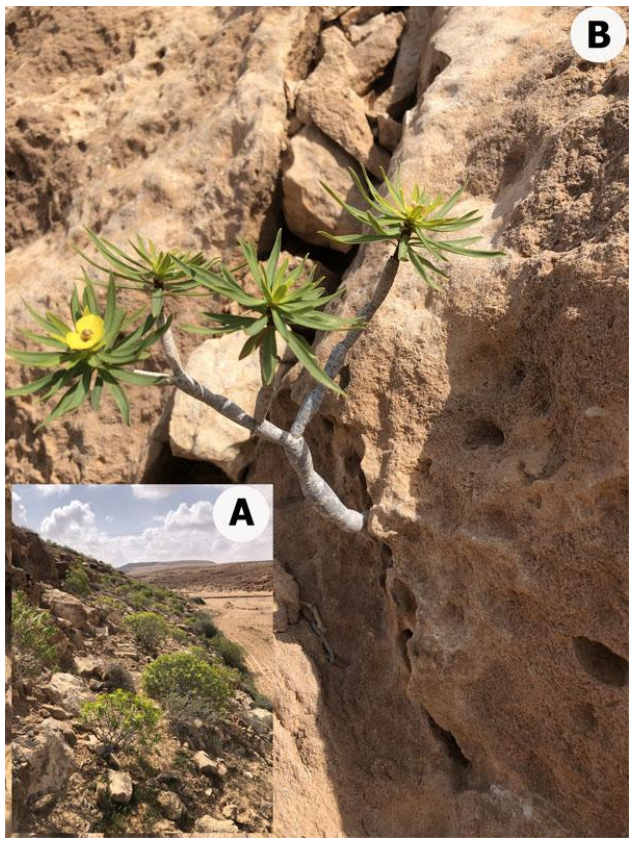

Fig. 1. (A) Habits, vegetative growth, and (B) Enlarged view of *E. dendroides* growing in Egypt.

Phytochemical Extraction and Fractionation: We subjected a total of 50 grams of air-dried and finely powdered aerial parts of *E. dendroides* to maceration in 200 mL of methanol at 25°C, with continuous overnight shaking to guarantee efficient extraction of bioactive constituents. The extraction process was repeated twice under the same circumstances. Using a rotary evaporator, the methanolic extracts obtained were pooled together and concentrated under reduced pressure. In 250 mL of distilled water, six g of the dried methanol extract was dissolved and then subjected to liquid-liquid extraction using dichloromethane (DCM) and ethyl acetate (EtOAc) solvents (each 250 mL, repeated three times). To get the DCM, EtOAc, and water parts, the DCM and EtOAc mixtures were collected separately and then reduced in volume by removing some of the liquid under low pressure.

Determination of the total phenolic content (TPC): The total phenolic content of *E. dendroides* extracts and fractions has been quantified using the Folin-Ciocalteu colorimetric technique. One mL of the extract has been dissolved in two mL of methanol. Then, 500 µL of this solution was mixed with 2.5 mL of Folin-Ciocalteu reagent that was diluted tenfold and 2.5 mL of a 75 g/L sodium carbonate solution. For 10 seconds, the mixture was vortexed, and then it was incubated for 2 hours at 25°C.

Absorbance was measured at 765 nm, with a reagent blank used for reference. The total phenolic content was quantified and expressed as mg of Gallic acid equivalents (GAE) per gram of extract (Ainsworth & Gillespie, 2007).

Quantification of the total flavonoid content (TFC): The aluminum chloride colorimetric technique has been used in determining the total flavonoid content. Inside a 10 mL volumetric flask, one mL of the extract has been dissolved in two mL of methanol. In 25 mL volumetric flasks, solutions of $NaNO_3$, 5% NaOH, and 7% $AlCl_3$ have been prepared.

Inside a sealed glass vial, 200 µL of the extract solution has been mixed with 75 µL of $NaNO_3$ (5%). At room temperature, the mixture was left for 5 minutes, then 1.25 ml of $AlCl_3$ (7%) and 0.5 ml of NaOH (0.5 M) were added. For another five minutes, the mixture has been sonicated and incubated at room temperature.

Using the quercetin standard calibration curve, we estimated the total flavonoid content and expressed it as milligrams of quercetin equivalents (QE) for each gram of dry extract (Chang *et al.*, 2002).

Analysis of polyphenols using HPLC: HPLC analysis was conducted on an Agilent 1260 Infinity system equipped with an Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 µm particle size). The mobile phase consisted of water (solvent A) and a mixture of 0.05% trifluoroacetic acid in acetonitrile (solvent B), flowing at a rate of 0.9 mL/min. The gradient elution was programmed as follows: starting at 82% A at 0 minutes; decreasing to 80% A from 0 to 5 minutes; further dropping to 60% A from 5 to 8 minutes; maintained at 60% A from 8 to 12 minutes; then returning to 82% A from 12 to 15 minutes and held steady at 82% A until 20 minutes. The detection processes were performed using a multi-wavelength detector set at 280 nm, with a 5 µL injection volume per sample. The column temperature was maintained at 40°C to optimize separation and reproducibility. Data acquisition and analysis were performed using Agilent ChemStation software (Agilent Technologies, USA), with compound identification and quantification conducted using authentic reference standards.

Assessment of antioxidant activity using the DPPH radical scavenging method: The free radical scavenging activity of *E. dendroides* extract and fractions has been estimated employing the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. A 0.1 mM DPPH solution was prepared in ethanol. Then, 1 mL of this solution was mixed with 3 mL of the extract solutions at varying concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL), all dissolved in ethanol. After a quick vortex, the mixtures were incubated in the dark at room temperature for 30 minutes. The Milton Roy UV-Vis spectrophotometer was used to determine the amount of light absorbed at 517 nm, evaluating the effectiveness of DPPH radical neutralization using ascorbic acid as a standard antioxidant. All assays were repeated three times to ensure accuracy and reproducibility. The IC_{50} value, which indicates the concentration required to neutralize half of the DPPH radicals, was determined from a dose–response curve plotted on a logarithmic scale. Lower absorbance values indicated more vigorous radical scavenging activity. The following formula has been used to calculate the percentage of inhibition:

$$\text{DPPH scavenging effect (\%)} = (A_0\text{-}A_1/A_0) \times 100.$$

where A_0 = the control reaction absorbance, and A_1 = the absorbance when the test sample or standard occurs.

Cytotoxicity: The (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) MTT assay was done on four cell lines: Caco-2 (colorectal adenocarcinoma), MCF7 (breast adenocarcinoma), PANC-1 (pancreatic carcinoma), and WI-38 (normal lung fibroblasts) to assess the cytotoxic efficiency (Somaida *et al.*, 2020; Anusmitha *et al.*, 2022; Aboelez *et al.*, 2025; Alsirhani *et al.*, 2025). To form a homogeneous monolayer, cells have been seeded in 96-well plates at a density of 1×10^5 cells/mL and incubated at 37°C in a humidified atmosphere with 5% CO_2 for 24 hours. After removing the growth medium, the wash medium was used twice to clean the monolayer. The extracts were serially diluted twice in RPMI medium with 2% serum, and 100 µL of each dilution was applied to the wells. Negative controls consisted of three wells containing maintenance media without any extract.

Six different concentrations of each extract were tested three times, and cell viability was determined as the mean of these replicates. Cytotoxicity was expressed as [100 - cell viability percentage]. The MTT assay measures cellular respiration by quantifying formazan production, which correlates with the number of viable cells. A decrease in viability with increasing extract concentration indicated higher cytotoxic effects.

At 37°C, plates have been incubated and monitored for cytotoxic signs, including disruption of the cell monolayer, cell shrinkage, and granulation. MTT solution (5 mg/mL in PBS) was added at 20 µL to each well, and then the plates were incubated at 37°C in a 5% CO_2 incubator for four hours. After incubation, the liquid was removed, and the formazan crystals were dissolved in 200 µL of DMSO by gentle shaking for 5 minutes to ensure complete dissolution. Optical density was measured at 560 nm, with 620 nm used as a reference wavelength for background correction (Ilaghi *et al.*, 2021; Abd El-Lateef *et al.*, 2024; Alzahrani *et al.*, 2025).

The selectivity index (SI), an essential parameter for anticancer activity, was calculated as the ratio of IC_{50} in normal cells to IC_{50} in cancer cells (SI = IC_{50} normal / IC_{50} cancer) (Calderón-Montaño *et al.*, 2021).

Antimicrobial activity: The antimicrobial properties of *E. dendroides* extract and fractions were evaluated on a range of fungal and bacterial strains, such as *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumoniae* (ATCC 13883), *Candida albicans* (ATCC 10221), and *Aspergillus niger* (ATCC 1015). This study was conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI) protocols. The agar diffusion technique, using Mueller-Hinton agar plates, was employed to evaluate the antimicrobial efficacy of the extracts. Between 20 and 100 µL of each antimicrobial agent or extract solution was placed in wells in the agar, and plates were kept under conditions suitable for the specific microorganisms. Following incubation, the inhibition diameter zones were recorded. The extraction solvent served as the negative control (Alawlaqi *et al.*, 2023; El-Remaily *et al.*, 2024; Ahmed *et al.*, 2025).

Determination of minimum inhibitory concentration (MIC): MIC was determined employing the broth microdilution technique, as outlined in the CLSI M07 and ISO 20776-1 guidelines. By dissolving 10 mg of each extract and fraction in 10 mL of distilled water, stock solutions were prepared to achieve a concentration of 1000 µg/mL. Serial two-fold dilutions were then conducted in broth to produce final concentrations ranging from 125 to 1000 µg/mL. 0.1 mL of each dilution was then dispensed into sterile 96-well microtiter plates.

Bacterial samples were prepared by mixing colonies directly and were adjusted to a 0.5 McFarland standard (approximately 1–2 × 10^8 CFU/mL). They were then further diluted to achieve a final inoculum density of roughly 5 × 10^5 CFU/mL in each well. The plates were incubated at 35 ± 2°C for 16 to 20 hours under standard atmospheric conditions (Qanash *et al.*, 2022; Sharaf *et al.*, 2024).

Evaluation of minimum bactericidal concentration (MBC): The minimum extract concentration that leads to a 99.9% decrease in CFU/mL is related to MBC (Luhata & Usuki, 2021). After determining the MIC, samples from the wells with the MIC and the two concentrations immediately higher than it were spread out to check for live bacteria (CFU/mL). Positive control samples were also plated to determine the initial microbial load.

Results and Discussion

Quantification of total phenolic and flavonoid contents: The total phenolic and flavonoid contents of *E. dendroides* extract and its fractions were measured, as summarized in Table 1. The Folin−Ciocalteu method is an electron transfer-based assay commonly employed for determining total polyphenolic content (Pérez *et al.*, 2023). The results showed that phenolic concentrations ranged between 32.2 and 168 mg gallic acid equivalents (GAE) per gram of extract. EtOAc fraction demonstrated the highest phenolic content (168.0 ± 1.57 mg GAE/g), followed by the total methanolic extract at (72.0 ± 0.45 mg GAE/g), and then the DCM fraction (32.2 ± 0.57 mg GAE/g). Phenolic compounds and polyphenols are well-known for their antioxidant activity and their potential in promoting protein synthesis and nutrient absorption (Platzer *et al.*, 2021).

Table 1 showed that the flavonoid content ranged from 16.9 to 71.6 mg of quercetin equivalents (QE) per gram of dry extract. This was determined using the aluminum chloride colorimetric assay, with quercetin as the reference. The EtOAc fraction had the highest flavonoid content (71.6 ± 1.13 mg QE/g), followed by the total extract (21.3 ± 0.45 mg QE/g) and the DCM fraction (16.9 ± 0.57 mg QE/g). Flavonoids are classified into various subclasses, including flavones, flavonols, flavanols, and flavanones, each of which is associated with distinct biological activities (Shraim *et al.*, 2021).

Those results emphasize that the EtOAc fraction is remarkably rich in both phenolic and flavonoid compounds, which is consistent with findings from preliminary phytochemical investigations. Several factors, including solvent polarity, matrix composition, particle size, and storage conditions, have been reported to influence the efficiency of phenolic extraction (Ghout *et al.*, 2018). Phenolic compounds are responsible for a wide range of biological activities, including antioxidant, antimutagenic, and anticancer effects, and they also play a role in regulating gene expression. On the other hand, flavonoids demonstrate pharmacological potential against microbial infections, inflammation, angiogenesis, arthritis, and various types of cancer (Adil *et al.*, 2024).

This study provides the first comprehensive quantification of total phenolic and flavonoid contents in *E. dendroides* collected from Egypt, revealing that these levels exceed those reported in other *Euphorbia* species (Adil *et al.*, 2024).

Polyphenols HPLC analysis: Further investigation was conducted using RP-HPLC to identify and quantify the detected polyphenols. The EtOAc fraction of *E. dendroides*, with the highest phenolic content, was analyzed using HPLC–UV. We identified and quantified a total of 18 polyphenols, using known reference standards, as presented in the HPLC chromatograms (Fig. 2) and summarized in Table 2. The HPLC analysis of the EtOAc fraction of *E. dendroides* revealed various phenolic compounds, including eight phenolic acids: chlorogenic acid, cinnamic acid, gallic acid, syringic acid, caffeic acid, coumaric acid, ellagic acid, and ferulic acid. Additionally, three simple phenolics were identified (methyl gallate, pyrocatechol, and vanillin), and seven flavonoids from different subclasses, i.e., flavonols (rutin, quercetin, and kaempferol), flavones (apigenin), isoflavones (daidzein), and flavanones (naringenin and hesperetin). The chemical structures of the identified polyphenolic compounds are shown in Fig. 3. Remarkably, chlorogenic acid displayed the highest concentration in phenolic compounds (1552.11 µg/mL), while naringenin showed the highest concentration among the flavonoids (168.66 µg/mL).

Table 1. Quantitative analysis of total phenolics and flavonoids in *E. dendroides* extract and fractions.

Extract	Total phenolic content (mg (GAE)/g)	Total flavonoid content (mg (QE)/g)
Total extraction	72.0 ± 0.451	21.3 ± 0.451
DCM	32.2 ± 0.571	16.9 ± 0.571
EtOAc	168.0 ± 1.573	71.6 ± 1.127

Table 2. Compounds detected in *E. dendroides*, EtOAc fraction, using HPLC, along with their concentrations and retention times.

Peaks no.	Rt	Compounds	Conc.
1.	3.35	Gallic acid	218.11
2.	4.44	Chlorogenic acid	1552.11
3.	5.62	Methyl gallate	100.18
4.	6.10	Coffeic acid	165.74
5.	6.67	Syringic acid	7.52
6.	7.12	Pyro catechol	616.05
7.	8.58	Rutin	5.14
8.	9.02	Ellagic acid	105.23
9.	9.24	Coumaric acid	0.65
10.	10.15	Vanillin	12.62
11.	10.39	Ferulic acid	22.02
12.	10.59	Naringenin	168.66
13.	12.25	Daidzein	12.41
14.	10.72	Querectin	167.82
15.	13.56	Cinnamic acid	8.63
16.	14.65	Apigenin	6.90
17.	15.16	Kaempferol	2.08
18.	15.64	Hesperetin	1.64

Chlorogenic acid is a notable bioactive compound with inflammation-modulating and antioxidant properties, crucial for regulating glucose and lipid metabolism. It is involved in the prevention and management of metabolic disorders, including diabetes, obesity, cardiovascular diseases, cancer, and hepatic steatosis (Tajik *et al.*, 2017). *E. dendroides* contains a diverse array of flavonoid compounds, which were detected in, showcasing extensive biological activities such as antioxidant, antimicrobial, and anti-inflammatory effects (Tijjani *et al.*, 2020). *E. dendroides* specimens from Egypt were found to contain higher levels of polyphenols than those collected from Algeria (Shraim *et al.*, 2021). This study is the first to characterize the polyphenol profile of wild populations of *E. dendroides* in Egypt, underscoring its significance as a valuable botanical resource.

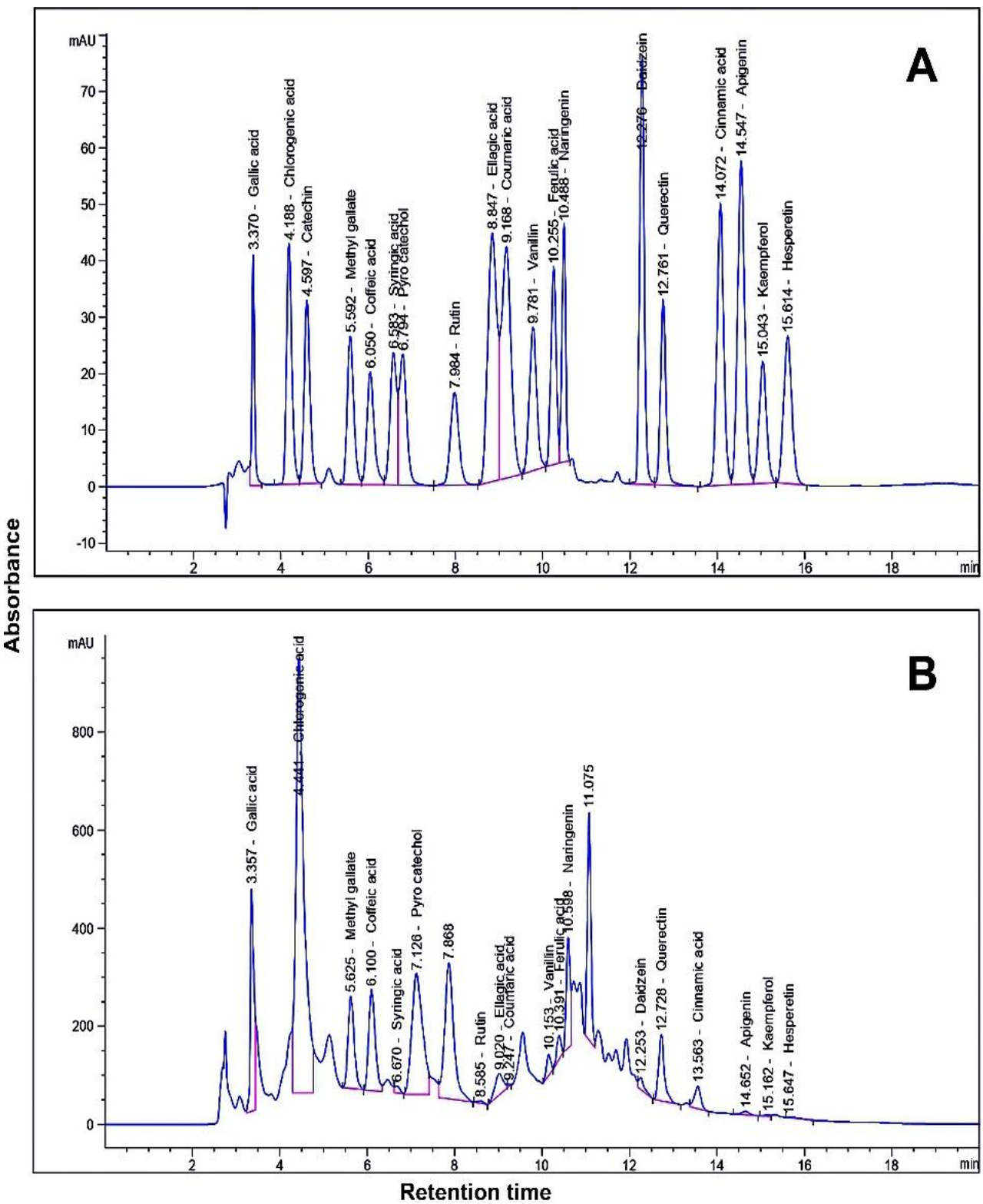

Fig. 2. (A) HPLC chromatogram of standard polyphenols, (B) HPLC chromatogram of polyphenols detected in the EtOAc fraction of *E. dendroides*.

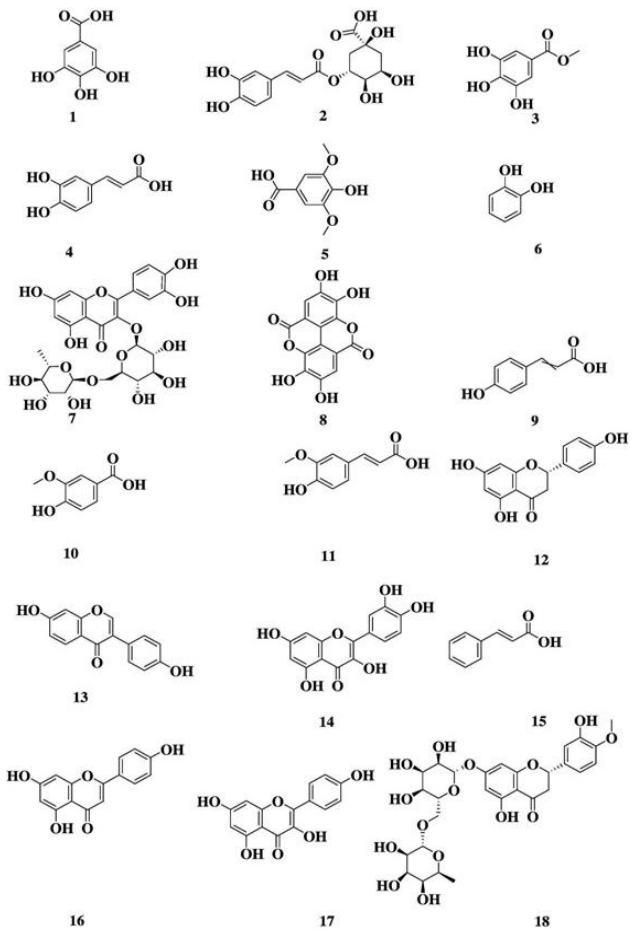

Fig. 3. Chemical structures of polyphenols detected through HPLC analysis of the EtOAc fraction of *E. dendroides*.

DPPH Radical scavenging assay: Antioxidant activity was evaluated using the DPPH radical scavenging assay, a widely accepted method, due to its simplicity, effectiveness, and reliability in determining the radical scavenging capacity of plant-based extracts. The assay depends on the reduction of the stable, purple DPPH radical to a yellow-colored product when it reacts with antioxidant compounds that donate hydrogen atoms or electrons, thus neutralizing the radical species (Aryal *et al.*, 2019).

The *E. dendroides* extract, as well as its DCM and EtOAc parts, showed the ability to neutralize DPPH radicals, with this ability increasing with increasing concentration. As illustrated in Fig. 4, a concentration-dependent enhancement in antioxidant activity was observed. At a concentration of 1 mg/mL, the EtOAc fraction showed the strongest ability to neutralize free radicals (98.4 ± 0.002%), followed by the total extract (94.5 ± 0.006%) and the DCM fraction (85.9 ± 0.004%).

The IC_{50} values presented in Table 3 indicate that the EtOAc fraction has the lowest IC_{50} value (2.24 µg/mL), indicating its potent antioxidant effect. This is followed by the total extract with an IC_{50} of 4.06 µg/mL and the DCM fraction at 24.62 µg/mL. For comparison, the IC_{50} value of the reference antioxidant, ascorbic acid, was 3.45 µg/mL. Remarkably, the EtOAc fraction demonstrated more potent antioxidant activity than ascorbic acid.

Table 3. Antioxidant activity expressed as IC_{50} (µg/mL) for DPPH radical scavenging by *E. dendroides* total extract and fractions.

Extract	IC_{50}
Total extraction	4.06
DCM	24.62
Ethyl acetate	2.24
Stander ascorbic acid	3.45

The differences in antioxidant capacities observed among the *E. dendroides* extracts are likely due to variations in their phenolic and flavonoid contents. The significant antioxidant effect and low IC_{50} value of the EtOAc fraction can be attributed to its high levels of phenolics and flavonoids (Chang *et al.*, 2002). Previous studies have established a linear correlation between total phenolic and flavonoid levels and antioxidant capacity, supporting their role in antioxidant potential (Aryal *et al.*, 2019). The antioxidant properties of those compounds primarily stem from their ability to donate hydrogen atoms, effectively neutralizing reactive free radicals and preventing oxidative damage. Their unique structural properties enhance their ability to scaveng radicals (Aryal *et al.*, 2019).

Cytotoxic activity: Natural products have garnered attention in the search for new anticancer therapies due to their favorable safety profiles and significant therapeutic effects. Herbal medicines are increasingly utilized in the management of many cancers (Wang J.L. *et al.*, 2012). This study evaluated the antiproliferative properties of *E. dendroides* extract and its fractions on three human cancer cell lines, Panc1 (pancreatic), Caco2 (colon), and MCF7 (breast), in addition to the normal lung fibroblast cell line Wi38, which was included in the assessment, using the MTT colorimetric assay. Doxorubicin served as the positive control reference standard. The respective IC_{50} values for the tested samples and the control compound are listed in Table 4. Cell viability was assessed using the MTT assay, which relies on the formation of formazan crystals in proportion to the number of viable cells. Increased extract concentrations were associated with decreased cell viability, indicating enhanced cytotoxic activity (Sundram *et al.*, 2019).

Table 4. IC_{50} and SI values of *E. dendroides* extract and fractions against pancreatic (PANC1), colon (Caco2), breast (MCF7) cancer cells, and normal cells (WI-38).

Cell lines	Total extract		DCM		EtOAc	
	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI
WI38	204.02 ± 1.61	1.00	118.25 ± 2.16	1.00	228.37 ± 3.05	1.00
Caco2	120.29 ± 3.11	1.70	31.42 ± 3.35	3.76	90.79 ± 2.13	2.52
MCF7	121.79 ± 3.47	1.68	76.86 ± 1.49	1.54	79.86 ± 0.60	2.86
PANC1	119.06 ± 1.47	1.71	76.11 ± 1.09	1.55	84.30 ± 1.49	2.71

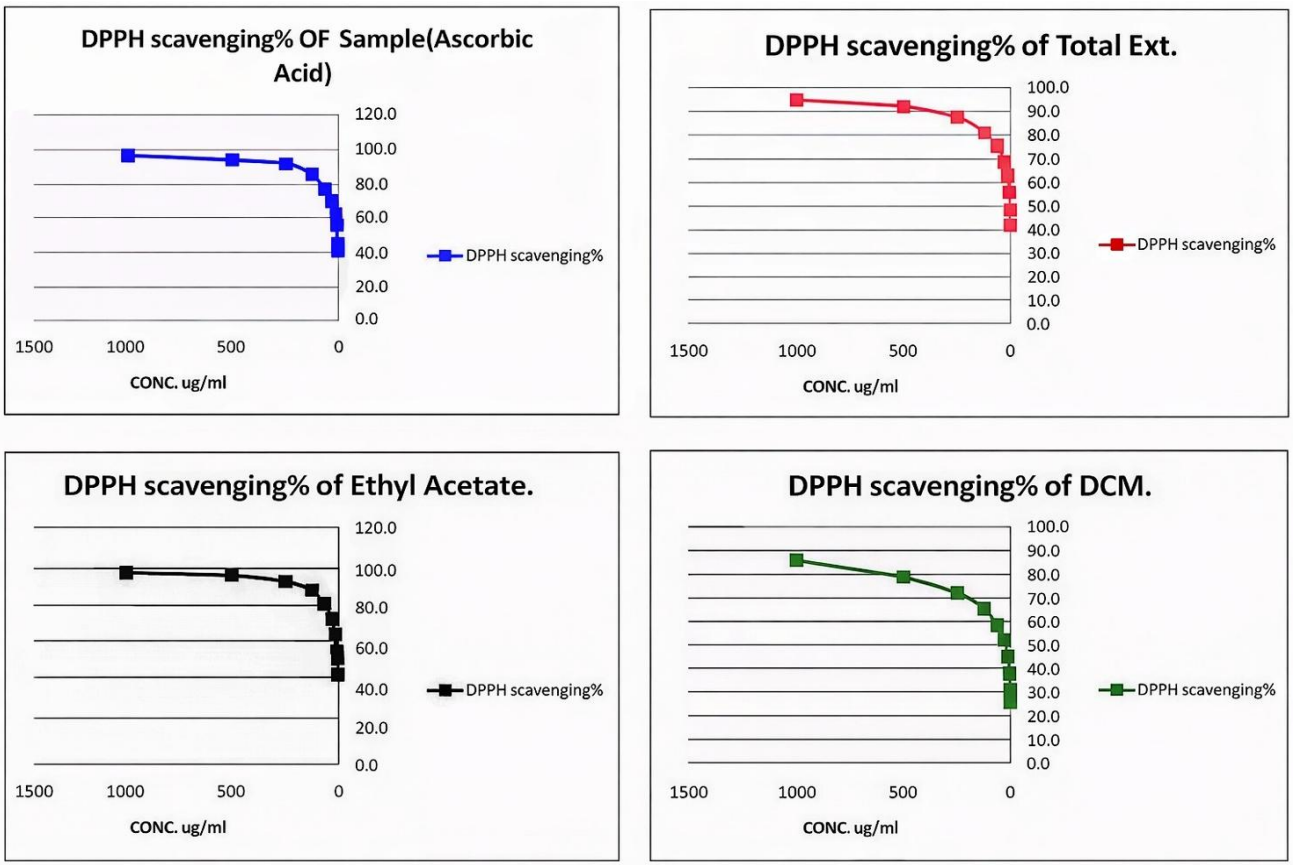

Fig. 4. DPPH free radical scavenging activity of *E. dendroides* extract and fractions compared to ascorbic acid.

The results indicate a concentration-dependent cytotoxic effect for all tested samples with varying levels of potency across the different cancer cell lines. Among the tested samples, the DCM fraction demonstrated the strongest cytotoxicity, as demonstrated by its IC_{50} values: 31.42 ± 3.35 µg/mL against Caco2 cells, 76.86 ± 1.49 µg/mL against MCF7 cells, and 76.11 ± 1.09 µg/mL against Panc1 cell lines. Similarly, the EtOAc fraction showed significant activity, with IC_{50} values of 90.79 ± 2.13 µg/mL for Caco2, 79.86 ± 0.6 µg/mL for Mcf7, and 84.3 ± 1.49 µg/mL for Panc1. The total extract showed average cytotoxic effects, with IC_{50} values of 120.29 ± 3.11 µg/mL for Caco2, 121.79 ± 3.47 µg/mL for Mcf7, and 119.06 ± 1.47 µg/mL for Panc1.

Remarkably, normal cells (Wi38) exhibited reduced sensitivity to the extract and fractions, with IC_{50} values ranging from 118.25 ± 2.16 µg/mL (DCM) to 228.37 ± 3.05 µg/mL (EtOAc). These findings indicate that *E. dendroides* extracts selectively target cancer cells, as evidenced by higher IC_{50} values for Wi38 cells compared to other cancer cell lines.

To evaluate the differential cytotoxicity between normal and cancer cells, the selectivity index (SI) was calculated as shown in Table 4. According to Calderón-Montaño et al. (2021), compounds with an SI higher than 2.00 demonstrate preferential cytotoxicity against cancer cells, indicating potential therapeutic value. The DCM and EtOAc fractions had SI values exceeding 2.00 for several cancer cell lines. Specifically, the EtOAc fraction had SI values of 2.52 for Caco2, 2.86 for Mcf7, and 2.71 for Panc1, while the DCM fraction showed an SI of 3.76 for Caco2. These results point out the advantages of these fractions as selective anticancer agents.

The significant cytotoxic activity exhibited by the DCM and EtOAc fractions is presumed to result from their considerable concentrations of the highly bioactive phenolic and flavonoid constituents. Apoptosis induction, inhibition of cell proliferation, and modulation of key cancer-related signaling pathways have been associated with these compounds (Kopustinskiene *et al.*, 2020; Montané *et al.*, 2020).

The present results are consistent with those of Ghout et al. (2018), who reported that *E. dendroides* extracts from an Algerian origin exhibited substantial antiproliferative effects on C6 glioma cells at a concentration of 250 µg/mL. Similarly, the EtOAc extract of *E. helioscopia* exhibited vigorous cytotoxic activity against SMMC-7721 cells, with an 80.91% inhibition rate at 200 µg/mL over 72 hours (Wang Z.Y. *et al.*, 2012). The observed cytotoxicity and selectivity of *E. dendroides* extracts suggest their potential as natural anticancer agents, justifying further investigation.

Antimicrobial activity: The medicinal importance of plants originates from their wealth of bioactive compounds, which have been extensively recognized for their pharmacological capabilities (Baba & Malik, 2015). With multidrug resistance increasingly emerging as a serious global health threat, considerable research is focused on discovering new antimicrobial agents from natural sources to combat this challenge (Alavi *et al.*, 2023). In this study, we tested how well *E. dendroides* extract works against different germs using the agar well diffusion method, looking at two types of bacteria that stain positively (*S. aureus* and *B. subtilis*), two types that stain negatively (*E. coli* and *K. pneumoniae*), and two types of fungi (*C. albicans* and *A. niger*).

Table 5 and Fig. 5 show the effectiveness of *E. dendroides* extract and its components in inhibiting the growth of four harmful bacteria, as indicated by the varying sizes of the inhibition zones. The total extract of *E. dendroides* showed the largest inhibition zones against *S. aureus* (27 ± 0.40 mm) and *B. subtilis* (23 ± 0.10 mm). Additionally, the EtOAc fraction exhibited significant antibacterial activity, with inhibition zones of 23 ± 0.40 mm against *S. aureus* and 22 ± 0.10 mm against *B. subtilis*. At the same time, the DCM fraction had the most potent effect, especially against *S. aureus* and *K. pneumoniae* (29 ± 0.60 mm and 29 ± 0.10 mm, respectively), and also worked well against *B. subtilis* (25 ± 0.10 mm).

The antifungal activity results indicated that the DCM and EtOAc fractions, as well as the total extract, exhibited zones of inhibition of 25 ± 0.20 mm, 20 ± 0.10 mm, and 18 ± 0.30 mm against *C. albicans*, respectively. However, none of the *E. dendroides* extract or fractions showed detectable antifungal activity against *A. niger* under the test conditions.

This study highlights the DCM fraction as a potent antimicrobial agent, showing significant activity against *K. pneumoniae, S. aureus*, *B. subtilis*, and *C. albicans*. The active constituents in the fraction may disrupt microbial growth and metabolism, thereby inhibiting their proliferation. Generally, Gram-positive bacteria are more vulnerable to antimicrobial agents than Gram-negative bacteria due to the distinct structural composition of their cell walls (Janesha *et al.*, 2020). In Gram-negative bacteria, the outer membrane serves as a hydrophilic permeability barrier, hindering the entry of various biologically active compounds (Bezerra dos Santos *et al.*, 2015). Certain phytochemicals, including flavonoids, tannins, saponins, steroids, alkaloids, and α-bisabolol, have been reported to contribute to antimicrobial activity (Tabanca *et al.*, 2007). Therefore, the phytochemicals present in *E. dendroides* extracts may account for the observed antimicrobial efficacy.

Evaluation of MIC and MBC: The determination of MIC serves as a fundamental technique for quantifying the antimicrobial activity of natural or synthetic compounds. The effectiveness of an antimicrobial agent is inversely related to its MIC value; compounds exhibiting low MICs are considered more effective, while those with high MICs demonstrate limited antimicrobial activity (Adil *et al.*, 2024).

In the present study, the MIC values for the total extract, as well as the EtOAc and DCM fractions of *E. dendroides*, were found to range between 3.90 and 125 µg/mL. Among all tested samples, the DCM fraction exhibited the most potent activity, with an MIC of 3.90 µg/mL against *S. aureus* (Table 6). The differences in how bacteria respond to the treatments are probably due to the natural resistance of the bacteria and the unique chemical properties of the active compounds in the extracts (Chikezie, 2017).

The tested samples also demonstrated bactericidal effects against all pathogenic bacteria tested, with the ratios of MBC to MIC ranging from 1.0 to 4.0. According to Noumedem *et al*., (2013), a sample is considered bactericidal if the MBC/MIC ratio is ≤4.0, whereas a ratio greater than 4.0 indicates bacteriostatic activity. Accordingly, the results verified that the extract and fractions of *E. dendroides* exhibited bactericidal effects against all tested microorganisms.

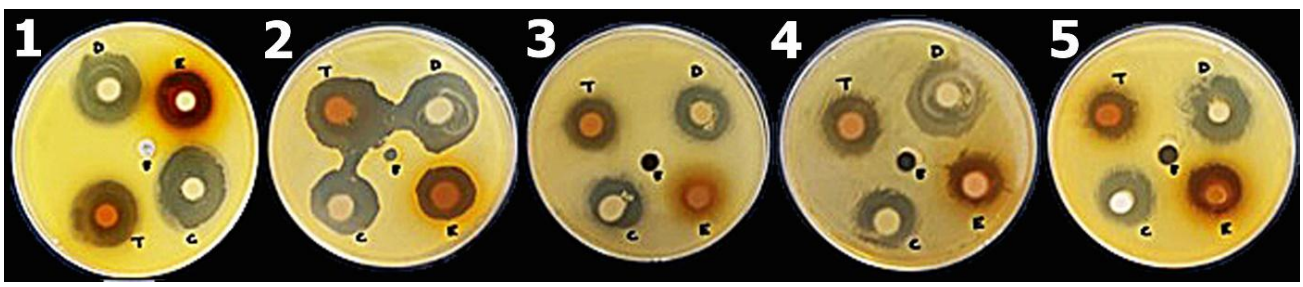

Fig. 5. Antimicrobial activity of *E. dendroides* extract and fractions: MIC and MBC values (µg/mL) against (1*) B. subtilis* (2) *S. aureus* (3) *E. coli* (4) *K. pneumoniae,* and (5) *C. albicans*.

Table 5. Inhibition zones (mm) of *E. dendroides* extract and fractions against various microbial strains.

Microorganism/ Sample	Total extract	DCM	EtOAc	Control
B. subtilis	23 ± 0.10	25 ± 0.10	22 ± 0.10	24 ± 0.10
S. aureus	27 ± 0.40	29 ± 0.60	23 ± 0.40	25 ± 0.60
E. coli	19 ± 0.30	22 ± 0.30	13 ± 0.30	19 ± 0.30
K. pneumoniae	20 ± 0.10	29 ± 0.10	19 ± 0.10	20 ± 0.10
C. albicans	18 ± 0.30	25 ± 0.20	20 ± 0.10	20 ± 0.30
A. niger	NA*	NA*	NA*	18 ± 0.30

* NA = No activity.

Table 6. Antimicrobial activity of *E. dendroides* extract and fractions: MIC and MBC values against different microbial strains.

Microorganism/ Sample	MIC			MBC		
	Total	DCM	EtOAc	Total	DCM	EtOAc
B. subtilis	31.30	15.60	31.30	62.50	62.50	31.30
S. aureus	15.60	3.90	31.30	31.30	7.80	62.50
E. coli	62.50	7.80	125.00	62.50	15.60	125.00
K. pneumoniae	62.50	7.80	62.50	62.50	15.60	62.50
C. albicans	125.00	15.60	125.00	250.00	31.60	125.00

The results of this study validate the traditional use of *E. dendroides* in Ayurvedic medicine for treating infectious diseases. Similar to previous studies on other *Euphorbia* species, comparable antimicrobial activities have been reported. For instance, extracts of *E. hirta* made with methanol and water have been found to inhibit the growth of harmful bacteria, including *Proteus mirabilis, K. pneumoniae, E. coli,* and *Shigella dysenteriae*. Additionally, *E. hirta* exhibited enhanced antibacterial efficacy when exposed to acidic conditions and elevated temperatures. (Abubakar, 2009). Also, the study on *E. parviflora* found that the MIC values ranged from 2.0 to 6.0 µg/mL for various solvent extracts, with the methanolic and chloroform extracts being the most effective against *B. subtilis*, *S. aureus*, and *C. albicans* (Ghout *et al.*, 2018).

Conclusion

This study presents an initial investigation into the polyphenolic composition and bioactivity of *E. dendroides* in Egypt, laying the groundwork for future pharmacological research.

The total extract of *E. dendroides* from Egyptian plants, along with its EtOAc and DCM fractions, exhibited significant biological effects, indicating potential as valuable sources of compounds. The EtOAc fraction contained the highest levels of phenolic and flavonoid compounds, primarily chlorogenic acid and naringenin, which contributed to a strong antioxidant effect, effectively neutralizing DPPH radicals at low concentrations, even more effectively than ascorbic acid. Cytotoxicity tests demonstrated that the extract and its fractions could inhibit the growth of Panc1, Caco2, and MCF7 cancer cells, with both DCM and EtOAc fractions exhibiting selective cytotoxicity, indicating their potential as natural anticancer agents. Furthermore, the DCM fraction proved effective against bacteria and fungi, particularly Gram-positive bacteria and *C. albicans*. These results support the traditional medicinal use of *E. dendroides* and underscore its potential in pharmaceutical applications. Future research should focus on isolating active compounds and confirming the therapeutic benefits of the species.

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