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Original Article

The Effect of *Euphorbia cuneata* Extracts in Combating Oxidative Stress and Inhibiting Microbes

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Abstract

Background and objectives: Microbial resistance and oxidative stress are two significant health issues associated with chronic illnesses and therapy failures. The antioxidant and antibacterial properties of *Euphorbia cuneata* Vahl. aerial component extracts made with various polarity solvents were assessed in this work.

Methods: Disc diffusion and minimum inhibitory concentration (MIC) tests were used to evaluate the 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, total phenolic and flavonoid contents, and antimicrobial activity of n-hexane, toluene, ethanolic, and aqueous extracts against specific ESKAPE pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida tropicalis*). High-performance liquid chromatography and gas chromatography-mass spectrometry were used to further characterize the most active extract.

Results: Compared to the aqueous (IC_{50} = 51.61 µg/mL), toluene (IC_{50} = 30.57 µg/mL), and n-hexane (IC_{50} = 128.15 µg/mL) extracts, the ethanolic extract demonstrated the greatest 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (97.90 ± 0.8%; IC_{50} = 28.52 µg/mL). Additionally, it has the highest levels of flavonoids (40.5 ± 1.5 mg luteolin equivalents/g) and phenolic (80.0 ± 0.2 mg gallic acid equivalents/g). While gas chromatography-mass spectrometry found methyl 12-hydroxy-9-octadecenoate (44.39%) as the main volatile molecule, high-performance liquid chromatography analysis identified caffeic acid, pyrogallol, rutin, and 7-hydroxyflavone as important ingredients. The ethanolic extract showed antifungal activity against *C. tropicalis* (MIC = 6.25 mg/mL) and moderate antibacterial activity with the lowest MIC values against S. aureus (450 µg/mL) and *E. coli* (500 µg/mL).

Conclusions: The ethanolic extract of *Euphorbia cuneata* demonstrated potent *in vitro* antioxidant activity and moderate antimicrobial effects, primarily attributable to its high phenolic and flavonoid content. These results support its potential as a natural source of bioactive compounds for further development.

Keywords: *Euphorbia*; Antioxidant; DPPH radical scavenging; Phenolic compounds; Flavonoids; Ethanolic extract; Antimicrobial activity; *Staphylococcus aureus*; *Escherichia coli*; *Candida tropicalis*; GC-MS; Minimum inhibitory concentration.

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Introduction

Oxidative Stress is defined as an increase in the number of free radicals within the cells of an organism's body, which occurs as a result of exposure to oxidant compounds that are known to increase their production within the body, such as hydrogen peroxide. Furthermore, it is becoming more widely acknowledged that a large number of diseases in modern society are caused by "oxidative stress", which arises from an imbalance between prooxidant generation and neutralization. Free radicals generate oxidative stress by seeking stability through electron pairing with

biological macromolecules like proteins, lipids, and DNA in healthy human cells. This results in lipid peroxidation, protein degradation, and DNA deterioration. According to Braca *et al*.,[1] these alterations are linked to aging, cardiovascular disorders, atherosclerosis, and inflammatory illnesses. Cyclooxygenase (COX) 1 and 2 are the main enzymes driving inflammation.[2] Apart from their pivotal role in the initiation and progression of inflammation, COX overexpression has also been linked to disease emergence within the body. The molecular properties of COX overexpression have made it a desirable target for drug development. There is a close relationship between inflammation, free radicals, and oxidative stress. Medication candidates with free radical scavenging qualities are therefore more desirable. Due to the lack of efficient treatments, the high cost of chemotherapeutic drugs, and the side effects of drugs, research into strong natural compounds that may prevent, slow, or even reverse the progression of diseases is still in progress. Medicinal plants have a special place in the fight against diseases. The discovery of several natural compounds produced from medicinal plants or secondary metabolites, such as terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, and alkaloids, which have significant antioxidant properties, has tremendously aided the treatment of cancer diseases. According to studies by Kaur *et al*.,[3] many antioxidant compounds exhibit these qualities. Medicinal plants are becoming a primary focus to improve present and future health requirements and fight diseases. This study's objectives were to assess the antioxidant and antimicrobial properties of *Euphorbia cuneata* Vahl. (*E. cuneata*) aerial component extracts and to use gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) to analyze phenolic acids and volatile compounds. Plants of this genus, native to Africa and the Arabian Peninsula, have a variety of traditional uses in many countries.[4] Plants of the genus *Euphorbia* are widely distributed across temperate, tropical, and subtropical regions of South America, Asia, and Africa. *E. cuneata* is used in traditional medicine to relieve inflammation and pain, although the biological basis of these actions has not been fully explored.[5] *E. cuneata* has been shown to contain a variety of phytochemicals, including triterpenes (cyclocuneatol) and flavonoids (cuneatannin), and it does not contain the toxic diterpenes found in other Euphorbiaceae members. Stem juice mixed with water or milk is used for obesity, food poisoning, and constipation. Furthermore, the stem juice is applied to wounds and traumas in northern Yemen to stop bleeding. The most common phenolic acid identified in the ethanolic extract (EE) of *E. cuneata* was pyrogallol; in addition, the most abundant flavonoid was found to be 7-hydroxyflavone. On the other hand, GC-MS analysis showed that the EE extract was rich in methyl 12-hydroxy-9-octadecenoate.[5] Antimicrobial resistance is one of the most alarming dangers to world health, necessitating the creation of new anti-infectives.[6] According to Boucher *et al*.,[7] the Infectious Diseases Society of America has identified a small group of bacteria (referred to as ESKAPE pathogens; these include *Enterococcus faecium*, *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Enterobacter* species) that are highly prioritized for drug discovery because they are resistant to numerous antibiotic drug classes. Additionally, new anti-infectives with innovative modes of action are required.[8] In the search for bioactive molecules, plants offer a promising source of natural products.[9] Cioch *et al*.[10] stated that plants used in traditional medicinal practices against infections have been found to inhibit the growth and virulence of various microbes. The majority of research on the anti-infective activity of plant extracts has focused on their growth-inhibitory potential with limited success, and there are currently no conventional (bacteriostatic or bactericidal) antibiotics derived from plant secondary metabolites available on the market. Research into alternative anti-infective modes of action can result in new drug development strategies to combat antibiotic resistance, and natural products may be an essential source of antibiotic adjuvants to circumvent resistance mechanisms. The present study aimed to evaluate the antioxidant capacity (using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, total phenolic and flavonoid contents) and antimicrobial activity (against selected ESKAPE pathogens and *Candida tropicalis*) of *Euphorbia cuneata* aerial part extracts prepared with solvents of different polarity (n-hexane, toluene, ethanol, and water), and to characterize the major phenolic and volatile constituents of the most active extract by HPLC and GC-MS.

Antimicrobial resistance and oxidative stress are important worldwide health issues linked to the advancement of disease and treatment failure. An imbalance between reactive oxygen species (ROS) and antioxidant defences causes oxidative stress, which results in cellular damage like DNA changes, protein degradation, and lipid peroxidation.[22]New therapeutic drugs are desperately needed since pathogenic bacteria' growing resistance has decreased the efficacy of traditional antibiotics.[23] Due to their abundance of bioactive substances, such as flavonoids and phenolics, which have potent antibacterial and antioxidant qualities, medicinal plants offer a promising source of these agents.[22]

Due to their phytochemical makeup, species of the genus Euphorbia are frequently employed in traditional medicine and have been shown to have strong antibacterial and antioxidant properties.[23] Thus, investigating plant-based extracts might offer efficient, all-natural substitutes for controlling microbial infections and oxidative stress.

Materials and methods

Phytochemical study

Plant material

In April and May of 2024, the aerial parts of *E. cuneata* (family Euphorbiaceae) used in this study were collected from Al-Jabal Al-Akhdar, Benghazi, Libya. The plant was identified at the Herbarium of Omar Al-Mukhtar University, where a voucher specimen (No. EC-2024-001) was deposited. Plant materials were air-dried in the shade, ground into a fine powder, and stored at -20°C until use. No ethical approval was required for plant collection, as the species is not endangered. Microbial testing was performed *in vitro* with standard strains, with no animal involvement.

Chemicals used

All high-quality analytical-grade chemicals and HPLC standards utilized were acquired from Sigma Chemical Co.

Extract preparation

Extraction procedure: Separately, 10 g of the powdered material were shaken using an orbital shaker at 200 rpm for 24 h at room temperature in 100 mL of solvent (water, ethanol, toluene, and n-hexane) according to the polarity index. The mixture was filtered using Whatman filter paper. After re-extraction with 50 mL of solvent, the residue was filtered, and all fractions were collected and concentrated under reduced pressure using a rotary evaporator (Heidolph VV 2000). To reach a standard concentration of 100 mg/mL, the resultant residue was dissolved in the minimum feasible amount of solvent (e.g., dimethyl sulfoxide (DMSO) for EE). Before use, the extracts were stored at -4°C.

Determination of antioxidant activity using the DPPH scavenging

Antioxidant assay

Using the DPPH radical scavenging experiment, the antioxidant activity of the extracts was evaluated in triplicate, and average outcomes were recorded. Utilizing the DPPH test, the scavenging activity against free radicals was determined. The assay was carried out according to the procedures specified by Gyamfi *et al*.[11]

DPPH radical scavenging activity

At 10°C, a freshly prepared DPPH radical methanol solution (0.004% w/v) was prepared and stored in the dark. The test compound was added to the methanol solution. In a 40 µL aliquot of the methanol solution, three milliliters of DPPH solution were combined. Immediately after, absorbance data were recorded using an ultraviolet (UV)-visible spectrophotometer (Milton Roy, Spectronic 1201). Data were collected every minute until the absorbance stabilized (16 m), and the decline in absorbance at 515 nm was continuously measured. The absorbance of the reference molecule (ascorbic acid) and the DPPH radical without antioxidant (control) were also measured. All determinations were performed in triplicate, and averages were calculated. The percentage inhibition (hereinafter referred to as PI) of the DPPH radical was calculated according to the formula: PI = [{(AC − AT)/AC} × 100] (1) where AC = absorbance of the control at t = 0 m and AT = absorbance of the sample + DPPH at t = 16 m.[12] Using GraphPad Prism software version 9.0 (San Diego, CA, USA), graphic plots of the dose-response curve were used to estimate the 50% inhibitory concentration (IC_{50}), or the concentration needed to achieve 50% DPPH radical scavenging activity.

Procedure for determination of total phenolic content (TPC)

To ascertain the total phenolic and flavonoid content using the HPLC method, plant extracts with strong antibacterial and antioxidant properties were selected. The TPC of the crude extracts was determined using the method of Gyamfi *et al*.[11] with minor modifications as follows: 0.5 mL of plant extract was mixed with 1.5 mL of Folin-Ciocalteu reagent (1:10 v/v diluted with distilled water). After 5 m, 2.0 mL of 7.5% sodium carbonate was added to the mixture. The mixtures were then incubated in the dark for 90 m with periodic shaking, resulting in the formation of a blue color. Following incubation, the absorbance of each sample was measured at 725 nm, and the phenolic content was quantified as mg/g caffeic acid equivalents. For HPLC analysis, an Agilent 1100 system featuring two liquid chromatography pumps, a UV/Vis detector, and a C18 column (125 mm × 4.60 mm, 5 µm particle size) was used. Chromatograms were generated and evaluated using Agilent ChemStation software. The separation of phenolic acids was achieved using a gradient mobile phase consisting of Solvent A (methanol) and Solvent B (acetic acid in water, 1:25). The gradient elution began with 100% Solvent B for the first 3 m, followed by 50% Solvent A for the next 5 m. The concentration of Solvent A was then increased to 80% for 2 m before returning to 50% for an additional 5 m. HPLC was validated for linearity ($r^2 > 0.99$), precision (coefficient of variation, coefficient of variation < 5%), and accuracy (98–102%) using standard compounds (e.g., caffeic acid, pyrogallol) for identification.

Procedure for determination of total flavonoid content (TFC)

To determine the TFC of crude plant extracts, the colorimetric aluminum chloride assay was conducted. An aliquot of 0.5 mL of each extract was mixed with 2 mL of distilled water, followed by the addition of 0.15 mL of 5% (w/v) sodium nitrite ($NaNO_2$). The mixture was left to stand for 6 m, after which 0.15 mL of 10% aluminum trichloride ($AlCl_3$) was added, and the solution was incubated for another 6 m. Subsequently, 2 mL of 4% (w/v) sodium hydroxide (NaOH) was added, and the total volume was brought to 5 mL with distilled water. The samples were incubated for 15 m, during which a pink color developed. The absorbance of the reaction mixture was measured at 517 nm to evaluate radical scavenging activity, following Gyamfi *et al*.[11] For further analysis, HPLC was performed using an Agilent 1100 system equipped with two liquid chromatography pumps, a UV/Vis detector, and a C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of acetonitrile (Solvent A) and 0.2% (v/v) aqueous formic acid (Solvent B), using an isocratic elution program with a 70:30 ratio. The detection wavelength was set at 320 nm. HPLC was validated for linearity ($r^2 > 0.99$), precision (coefficient of variation < 5%), and accuracy (98–102%) using standard compounds (e.g., rutin, 7-hydroxyflavone) for identification.

Identification of bioactive compounds

The bioactive ingredients in the plant extracts that showed potent antioxidant properties were identified using GC-MS analysis. The extracts were subjected to GC-MS using a Thermo Scientific TRACE 1310 GC-MS apparatus at the Regional Center for Mycology and Biotechnology of Al-Azhar University, Egypt. The gas chromatograph was attached to the ISQ LT single quadrupole mass spectrometer. The column used was J&W Scientific's DB5-MS (30 m × 0.25 mm ID). The ionization mode was electron ionization with an ionization voltage of 70 eV. The temperature program was as follows: 40°C for three minutes, 5°C for five minutes, and 280°C for five minutes, with a rate of 7.5°C/m for one minute at 290°C. Detector temperature: 300°C. A helium carrier gas was used for the analyses, with an injection volume of 1 µL and a split ratio of 1:5. The flow rate was 1.0 mL/m. The injector temperature was set at 200°C. The antimicrobial study of

the Wiley & NIST mass spectrum database was searched.

Standard strains

P. aeruginosa ATCC 25006, *Bacillus subtilis* ATCC 13933, *Candida albicans* (*C. albicans*) ATCC 10231, *Escherichia coli* (*E. coli*) ATCC 8739, and *S. aureus* ATCC 12599 were obtained from the Animal Health Research Institute, Cairo, Egypt. The fungal strain was maintained on potato dextrose agar plates, whereas bacterial strains were maintained on nutrient agar plates. Each microorganism was kept at 4°C.

Antibacterial assay

An inoculum of bacterial cells from an agar plate stock was cultivated using the disc diffusion technique in 5 mL of Mueller-Hinton Broth (MHB) and then incubated for 24 h. In 10 mL of MHB, 1×10^8 CFU/mL of the bacterial inoculum was prepared. A 400 µL aliquot of 1×10^8 CFU/mL medium was added to Mueller-Hinton Agar on 9 cm diameter Petri plates. To create the sample disc, 6 mm antibiotic assay discs were used. The stock sample was then pipetted to yield 20 µL (100 mg/mL), which was placed onto the sample discs. The discs were placed on the prepared bacterial Mueller-Hinton Agar plates. The positive control was cephalosporin (100 µg/mL), while the negative control was DMSO. The bacteria's zone of inhibition (mm) was measured after a 24-h incubation period at 37°C. All experiments were performed in triplicate. Extracts that demonstrated sensitivity to the strains were chosen for further examination.

Antifungal assay

With some modifications, the method of Hussain *et al.*[13] was used to test antifungal activity. *C. albicans* was cultured on Sabouraud Dextrose Agar (SDA) for 72 h at 28±2°C. After collecting fungal spores with 0.05% Tween 80, they were centrifuged for 5 m at 3000 rpm. The fungal inoculum was then prepared with a supernatant solution in 10 mL of Sabouraud Dextrose Broth containing 1×10^6 spores/mL. In SDA plates, 400 µL of 1×10^6 spores/mL were added. The sample discs were filled with 20 µL (100 mg/mL) of various extracts and placed on the prepared SDA plates. DMSO served as the negative control and fluconazole (50 µg/mL) as the positive control. Following a 72-h incubation period at 28±2°C, the zone of inhibition (mm) was assessed. All experiments were performed in triplicate. Extracts showing sensitivity to the strains were selected for further examination.

Minimum inhibitory concentration (MIC)

With minor modifications, the MIC test was carried out in sterile 96-well microplates following.[14] To achieve a twofold serial dilution of the extract (from 1000 to 20 µg/mL), all extracts were prepared and transferred to each microplate well. Twofold serial dilutions were made from the stock (1000 µg/mL) by successive halving to reach 500, 250, 125, 62.5, 31.25, and 20 µg/mL. A 100 µL aliquot of the stock sample was added to the first three rows of the experiment, and then 100 µL of MHB for bacteria and Sabouraud Dextrose Broth for fungi were used as diluents. All rows were then supplemented with 30 µL of bacterial (1×10^8 CFU/mL) and fungal (1×10^6 spores/mL) samples. The plates were examined for the presence or absence of growth after incubation for 24 h at 37°C (bacteria) and 72 h at 28±2°C (fungi). The lowest extract concentration showing no visible colony growth was identified as the MIC.

Statistical analysis

The experimental results are expressed as the mean ± standard deviation with their standard errors. Data were analyzed by one-way analysis of variance using the Statistical Package for the Social Sciences program to evaluate statistical significance, where $p < 0.05$ was considered to indicate a statistically significant difference. All experiments were performed in triplicate, and data were analyzed using GraphPad Prism version 9.0.

3. Results

E. cuneata extracts' capacity to scavenge DPPH radicals. We examined the antioxidant capabilities of several extracts of *E. cuneata* to learn more about their potential health benefits. Table 1 shows that all *E. cuneata* extracts scavenged the DPPH radical in triplicate, with dose-dependent efficacy.

Table 1. Maximum DPPH scavenging activity of Euphorbia cuneata extracts at 1280 µg/mL

Extract	% Inhibition ± SD
EE	97.90 ± 0.5
WE	55.10 ± 1.1
n-HE	43.21 ± 1.3
TE	22.23 ± 0.9
Ascorbic acid	99.23 ± 0.2

DPPH, 1,1-diphenyl-2-picrylhydrazyl; EE, ethanol extract; IC_{50}, 50% inhibitory concentration; n-HE, n-hexane extract; SD, standard deviation; TE, total extract; WE, water extract.

We found that EE had the maximum scavenging power (97.90%), followed by water extract (WE) (55.10%), n-hexane extract (n-HE) (43.21%), and toluene extract (TE) (22.23%). The scavenging efficacy was shown to rise as the extract concentration increased. The IC_{50} values for WE, EE, TE, and n-HE were 51.61, 28.52, 30.57, and 128.15 µg/mL, respectively, as revealed in Tables 2 and 3.

Radical scavenging activity given as percentage inhibition; WE, EE, TE, n-HE. The IC_{50} values for extracts were calculated using GraphPad Prism software (San Diego, CA, USA). The percentage inhibition value of the standard compound ascorbic acid was 99.23% at a concentration of 1280 µg/mL.

The antioxidant activity of *E. cuneata* aerial part extracts was evaluated using the DPPH radical scavenging assay. The results are summarized in Table 3. The DPPH radical-scavenging activity of the EE was remarkably strong, achieving 97.90 ± 0.98% inhibition at 1280 µg/mL and maintaining >94% inhibition even after four-fold dilution (320 µg/mL). This yielded an IC_{50} value of 28.52 ± 1.65 µg/mL, which is comparable to the potency of pure ascorbic acid (IC_{50} = 12–18 µg/mL under our assay conditions) and far lower than the majority of published crude plant extracts. The activity of the other extracts was significantly lower (IC_{50} 51.61–128.15 µg/mL).

Significant alterations in extracts were found using statistical

Table 2. Radical Scavenging activity of Euphorbia cuneata extracts at different concentrations toward DPPH

Concentration (µg/mL)	WE	EE	TE	n-HE
1280	55.10	97.90	22.23	43.21
640	51.76	95.76	21.21	38.23
320	44.28	94.18	17.10	33.11
160	38.30	91.38	15.03	26.63
80	33.49	86.39	11.40	15.45
40	27.90	74.62	9.37	11.67
20	21.76	39.56	3.25	5.22
10	15.52	25.12	1.02	1.45
IC_{50} value	51.61	28.52	30.57	128.15

DPPH, 1,1-diphenyl-2-picrylhydrazyl; EE, ethanol extract; IC_{50}, 50% inhibitory concentration; n-HE, n-hexane extract; TE, total extract; WE, water extract.

Table 3. IC_{50} values of Euphorbia cuneata extracts and ascorbic acid

Sample	IC_{50} (µg/mL)
WE	51.61 ± 1.2
EE	28.52 ± 0.8
TE	30.57 ± 1.0
n-HE	128.15 ± 2.5
Ascorbic acid	10.6 ± 0.3

EE, ethanol extract; IC_{50}, 50% inhibitory concentration; n-HE, n-hexane extract; TE, total extract; WE, water extract.

analysis. Ascorbic acid was used as a positive control in the DPPH technique to assess antioxidant activity. The amounts of ascorbic acid varied between 1280 and 2.5 µg/mL. Ascorbic acid exhibited an inhibitory rate of 34.57% at 2.5 µg/mL and 99.23% at 1280 µg/mL. Total phenolics and flavonoids were quantified using the Folin-Ciocalteu and aluminum chloride methods, respectively (Tables 4 and 5).

Table 4. Total phenolic content in Euphorbia cuneata extracts

Extract	Total phenolics (mg GAE/g)
EE	80.0 ± 0.2
WE	50.0 ± 0.5
TE	60.0 ± 0.3
n-HE	40.0 ± 0.4

EE, ethanol extract; GAE, gallic acid equivalents; n-HE, n-hexane extract; TE, total extract; WE, water extract.

The TPC and TFC of the extracts are presented in Tables 4 and 5, respectively.

Different phenolic compounds were identified in the EE of *E. cuneata*, including flavonoids (luteolin, kaempferol, quercetin, rutin, and 7-hydroxyflavone) and phenolic acids (gallic acid,

Table 5. Total flavonoid content in Euphorbia cuneata extracts

Extract	Total Flavonoids (mg QE/g)
EE	40.5 ± 1.5
WE	25.0 ± 1.0
TE	30.0 ± 1.2
n-HE	20.0 ± 0.8

EE, ethanol extract; n-HE, n-hexane extract; QE, quercetin equivalents; TE, total extract; WE, water extract.

pyrogallol acid, caffeic acid, p-coumaric acid, syringic acid, and ferulic acid). The two most common phenolic acids were caffeic acid and pyrogallol. Next in line were gallic acid, ferulic acid, syringic acid, and p-coumaric acid (Fig. 1, Table 6). According to Figure 2, the most common flavonoids were rutin (7.62%) and 7-hydroxyflavone (12.45%), followed by quercetin, luteolin, and kaempferol.

Table 6. High-performance liquid chromatography-identified phenolic acids and flavonoids in the ethanolic extract of Euphorbia cuneata

Compound type	Compound	Relative %
Phenolic acids	Caffeic acid	15.2
	Pyrogallol	22.1
Flavonoids	Rutin	7.62
	7-Hydroxyflavone	12.45
	Quercetin	8.9

The strongest radical-scavenging effects were directly connected with the ethanolic extract's maximum TPC (80.0 ± 0.2 mg GAE/g) and TFC (40.5 ± 1.5 mg luteolin equivalents/g). This demonstrates how phenolics and flavonoids are important sources of antioxidant activity, which makes the ethanolic extract an exceptional option for treating illnesses linked to oxidative stress.

The ethanolic extract's HPLC examination showed a varied

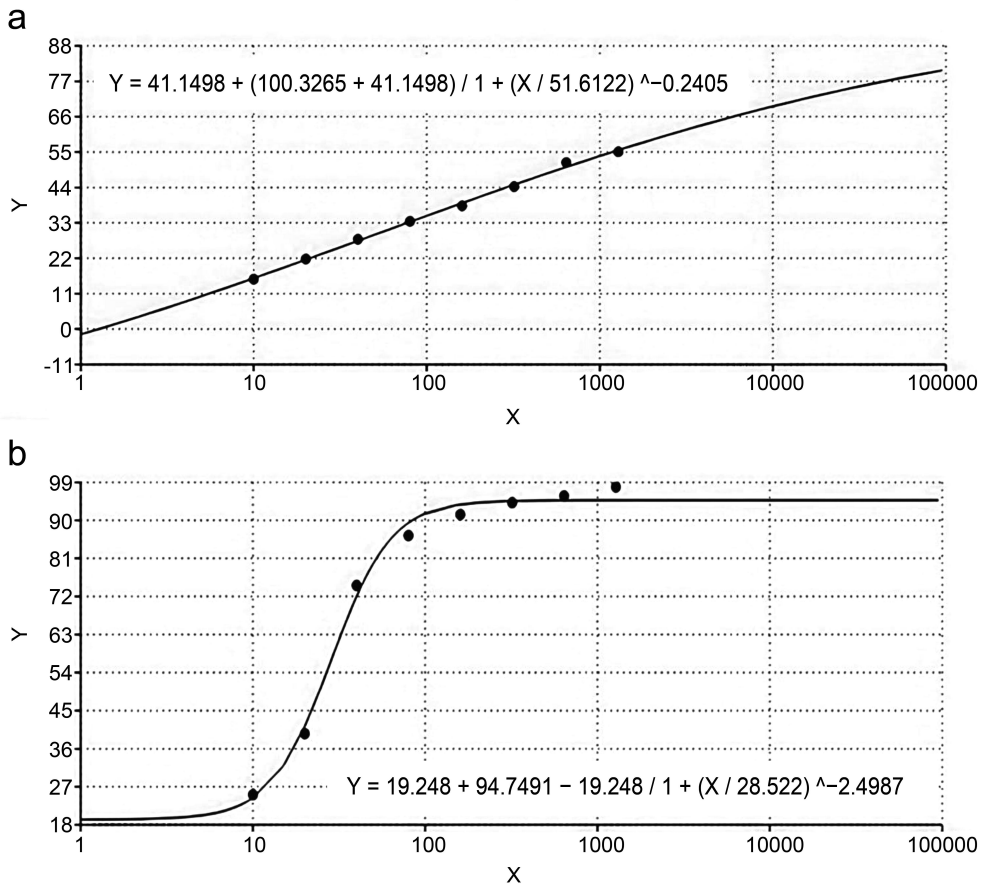

Fig. 1. The IC_{50} value for extracts was determined with a non-linear model (a), IC_{50} value for ethanolic extract (EE); (b), IC_{50} value for water extract (WE). IC_{50}, 50% inhibitory concentration.

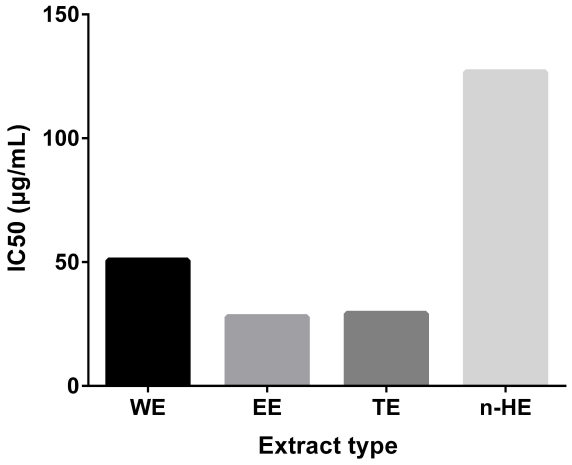

Fig. 2. The IC_{50} values for E. cuneata extracts. EE, ethanol extract; IC_{50}, 50% inhibitory concentration; n-HE, n-hexane extract; TE, total extract; WE, water extract.

profile of bioactive phenolics and flavonoids (Table 6), with pyrogallol and caffeic acid being the main phenolic acids along with gallic acid, ferulic acid, syringic acid, and p-coumaric acid. 7-hydroxyflavone (12.45%) and rutin (7.62%) were the most prevalent flavonoids, with lesser concentrations of quercetin, luteolin, and kaempferol. This composition highlights *E. cuneata*'s potential as a natural source of medicinal polyphenols and directly supports the extract's outstanding antioxidant activity because it is rich in powerful radical scavengers like pyrogallol and caffeic acid. The chemical structures of the identified phenolic compounds and flavonoids are presented in Figure 3.

GC-MS analysis of volatile components

GC-MS was used to identify the volatile components of the EE of *E. cuneata* leaves based on their mass spectra, peak areas, and retention times (Figs. 4 and 5). Using GC-MS analysis, five main bioactive compounds in *E. cuneata* were discovered and categorized according to their chemical structures: methyl 12-hydroxy-9-octadecenoate (44.39%), hexadecanoic acid, methyl ester (21.34%), hexanal dimethyl acetal (6.59%), methyl octadeca-9,12-dienoate (13.47%), and (9E,12E)-octadeca-9,12-dienoyl chloride (14.21%).

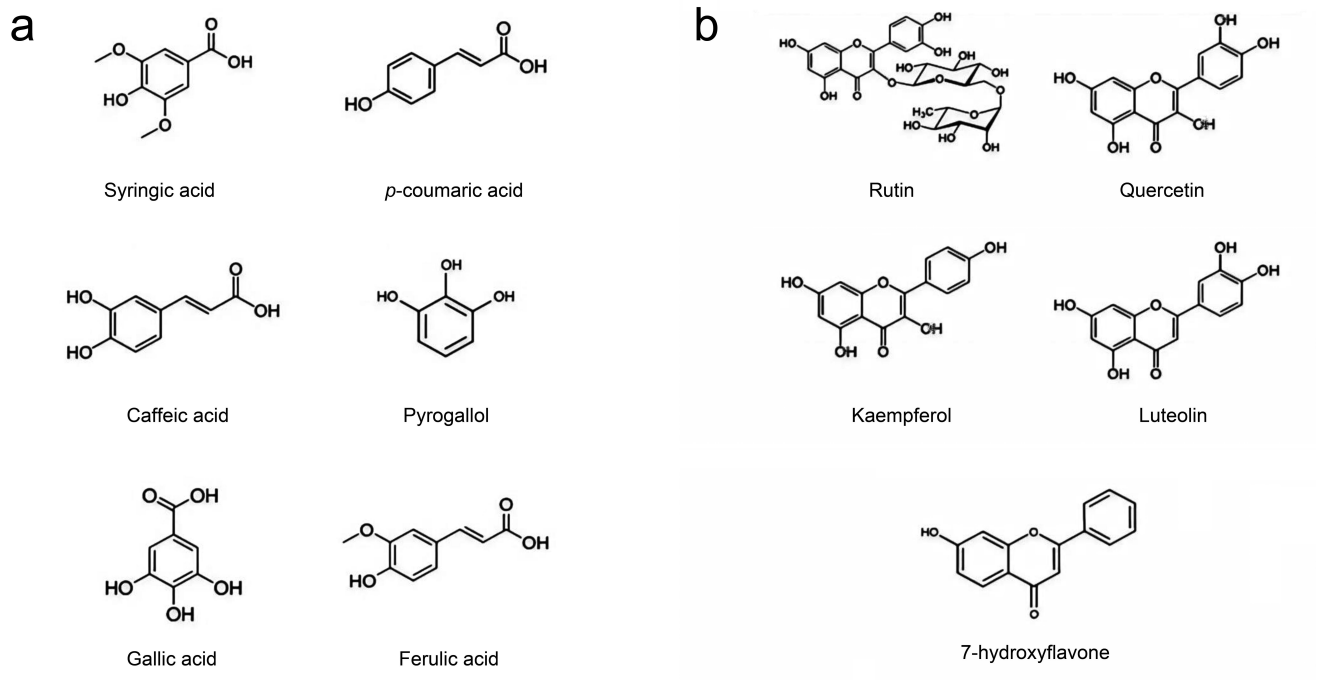

Fig. 3. Chemical structure of the phenolics commonly identified in EE of E. cuneata (a) and chemical structure of the flavonoids commonly identified in EE of E. cuneata (b). EE, ethanol extract.

Antimicrobial activity

As indicated in Table 7, the investigation revealed that the antibacterial activity (disc diffusion technique) of *E. cuneata* leaves was strongly impacted by the extraction solvents ($p < 0.05$). According to the extraction solvents, the zone of inhibition (mm) for *S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa* varied from 12.96 to 14.68 mm, 12.15 to 13.25 mm, 7.91 to 9.63 mm, and 7.10 to 8.20 mm, respectively. The largest inhibitory zones were provided by the EE (14.68 mm), followed by toluene (12.96 mm). As expected, none of the bacterial or fungal strains were affected by the negative control. The maximum zone of inhibition (mm) was seen in *S. aureus* (19.70 mm) and *E. coli* (17.48 mm) when using the conventional antibiotic cephalosporin, whereas fluconazole produced 16.31 mm for *C. albicans*. Table 8 shows the MIC of *E. cuneata* leaves as a function of extraction solvents. The antibacterial activity of *E. cuneata* leaves was considerably impacted by the extraction solvent ($p < 0.05$). According to this finding, the EE was the optimum extraction solvent, exhibiting the lowest MIC values for *S. aureus* (450 µg/mL) and *E. coli* (500 µg/mL), respectively. In *S. aureus*, the standard antibiotic cephalosporin produced a greater MIC (5.37 µg/mL). The MIC value for *E. coli* was 6.45 µg/mL, which was significantly higher. Fluconazole, a common antifungal, gave *C. albicans* an MIC value of 13.45 µg/mL.

In disc diffusion assays, the ethanolic extract produced the largest zones of inhibition against Gram-positive S. aureus (up to 14–15 mm) and moderate activity against Gram-negative E. coli (12–13 mm) and the fungus *C. tropicalis* (10–12 mm), demonstrating the strongest antimicrobial effects of all tested fractions (Table 7). These patterns were validated by broth microdilution; the ethanolic extract had the lowest MIC values against S. aureus (450 µg/mL) and E. coli (500 µg/mL), whereas other extracts and pathogens displayed greater MICs ranging from 1000–6250 µg/mL (Table 8). These MICs show encouraging moderate action, especially against ESKAPE bacteria, and highlight the extract's potential as a natural adjuvant to improve antibiotic efficacy and fight resistance, even though they are higher than those of common antibiotics like cephalosporin (≤100 µg/mL).

Discussion

Results from the DPPH assay (Table 3, Figs. 1 and 2) demonstrate that *E. cuneata* extracts effectively combat oxidative stress through dose-dependent radical scavenging, with EE exhibiting the highest activity (97.90%, IC_{50} 28.52 µg/mL), reflecting the experimental design's focus on free radical neutralization. The results were consistent with those of Jayaprakasha *et al.*[15] and Rahman *et al.*,[16] who evaluated the radical scavenging activities of citrus fruits and Tabebuia pallida leaves in *in vitro* models, concluding they may be sources of antioxidants.

Munro *et al.*[17] found that at all concentrations examined using the DPPH assay, the methanolic extract of *Euphorbia tirucalli* (*E. tirucalli*) exhibited superior free radical scavenging efficacy compared to the aqueous extract. The polar extract of *Euphorbia terracina* L. showed a variety of active secondary metabolites, with saponins and phenolics being the most prevalent, according to El-Amier *et al.*[18]

The quantitative phenolics and flavonoids (Tables 4 and 5)

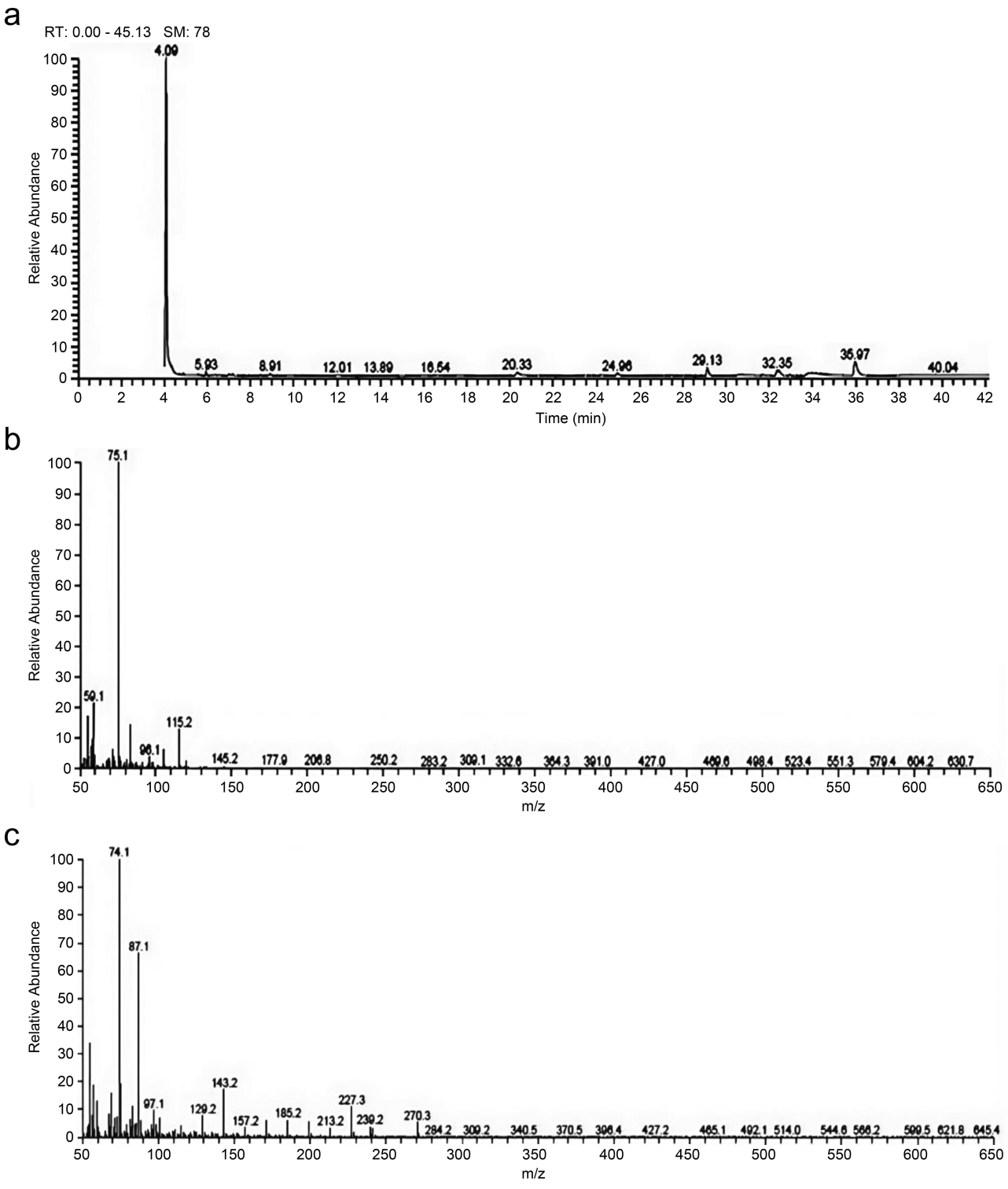

Fig. 4. GC-MS analysis of bioactive compounds in the ethanolic extract (EE) of Euphorbia cuneata leaves: (a) Chromatogram showing retention time (RT) of compounds, (b) Mass spectrum (MS) of 9,12-octadecadienoic acid methyl ester, (c) Peak identification and fragmentation pattern.

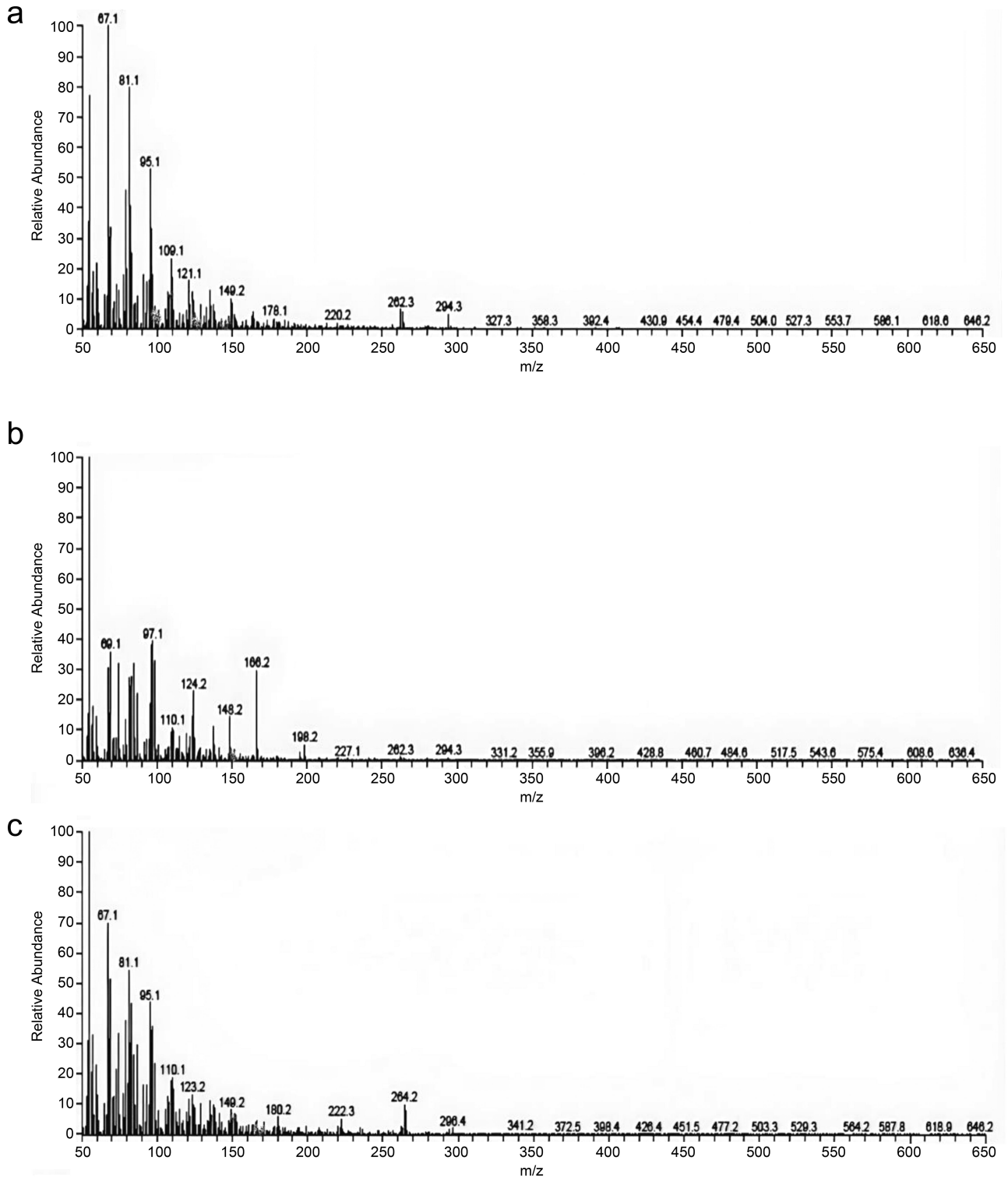

Fig. 5. GC-MS analysis of bioactive compounds in the ethanolic extract (EE) of Euphorbia cuneata leaves: (a) Chromatogram showing retention time (RT), (b) Mass spectrum (MS) of 9-octadecenoic acid methyl ester, (c) Peak identification and fragmentation pattern.

Table 7. Antibacterial and antifungal activity (zone of inhibition, mm) of Euphorbia cuneata extracts[1]

Sample	Pseudomonas aeruginosa	Bacillus subtilis	Escherichia coli	Staphylococcus aureus	Candida albicans
Water	NI	NI	NI	NI	NI
ethanol	8.20 ± 004	9.63 ± 034	13.25± 004	14.68± 018	10.54± 008
toluene	7.10 ± 007	7.91 ± 005	12.15 ± 009	12.96 ± 007	7.88 ± 003
n-hexane	NI	NI	NI	NI	8.34± 013
DMSO	NI	NI	NI	NI	NI
Cephalosporin[2]	12.43 ± 012	14.65 ± 012	17.48± 015	19.70± 027	NI
Fluconazole[3]	NI[4]	NI	NI	NI	16.31±045

[1]inhibition zones include the diameter of the disk (6 mm); [2]cephalosporin at 100 µg/mL; [3]Fluconazole at 50 µg/mL; [4]NI, no inhibitory effect. DMSO, dimethyl sulfoxide.

Table 8. Minimum inhibitory concentration (MIC, µg/mL) of Euphorbia cuneata aerial part extracts[1]

Sample	Pseudomonas aeruginosa	Bacillus subtilis	Escherichia coli	Staphylococcus aureus	Candida albicans
Water	NI	NI	NI	NI	NI
ethanol	700 ± 001	625 ± 005	500± 017	450± 023	650± 018
toluene	750± 004	725± 009	550± 015	525± 031	725 ± 0.24
n-hexane	NI	NI	NI	NI	700± 0.06
DMSO	NI	NI	NI	NI	NI
Cephalosporin[2]	13.92 ± 012	10.73± 013	6.45± 012	5.37± 008	NI
Fluconazole[3]	NI[4]	NI	NI	NI	13.45±007

[1]Minimum inhibitory concentration (MIC); [2]Cephalosporin at 100 µg/mL; [3]Fluconazole at 50 µg/mL; [4]NI, no inhibitory effect. DMSO, dimethyl sulfoxide.

further support antioxidant potential, with EE showing 80.0 ± 0.2 mg gallic acid equivalents (GAE)/g phenolics and 40.5 ± 1.5 mg quercetin equivalents/g flavonoids. Flavonoids also influence the induced nitric oxide synthase enzyme and inflammatory cells, preventing the synthesis of proinflammatory cytokines and modifying the activity of arachidonic acid pathways like phospholipase A_2, lipoxygenases, and COX.

Different phenolic compounds were identified in the EE of *E. cuneata,* including flavonoids (luteolin, kaempferol, quercetin, rutin, and 7-hydroxyflavone) and phenolic acids (gallic acid, pyrogallol acid, caffeic acid, p-coumaric acid, syringic acid, and ferulic acid). The two most common phenolic acids were caffeic acid and pyrogallol. Next in line were gallic acid, ferulic acid, syringic acid, and p-coumaric acid (Fig. 5). According to the above Results, the most common flavonoids were rutin (7.62%) and 7-hydroxyflavone (12.45%), followed by quercetin, luteolin, and kaempferol. According to Awaad *et al*.,[19] four flavonoids have been discovered and extracted from alcohol extracts of *E. cuneata* Vahl. These flavonoids include 4'-O-methoxy-luteolin-7-O-rhamnoglucoside, aromadendrin, apigenin, and naringenin.The findings aligned with those of who discovered that the main fatty acids extracted from *Euphorbia* species were linoleic acid, palmitic acid, 17-tetratriacontane, and hexatriacontane. The Euphorbiaceae family has a wide variety of triterpenoids. Numerous skeleton types, including euphane, tirucallane, cycloartane, lanostane, oleanane, lupane, taraxarane, friedelane, friedoursane, and ursane, have been described in the genus *Euphorbia*. The aerial portions of *Euphorbia dendroides* L. were used to extract a novel triterpene of the cycloartane type. *Euphorbia* has also been discovered to contain a few sesquiterpenoids. While GC-MS study revealed pyrogallol as the main constituent among the volatile components of the *Euphorbia hirta* methanolic extract showed that ellagic acid, gallic acid, and quinic acid were the predominant phenolics using UHPLC-SRM/MS. Flavonoids are naturally occurring substances with various phenolic structures that are produced by plants. The water extract of *E. tirucalli* powder had 34.01 mg GAE/g of total phenolic components, which was less than half of the methanol extract's 77.33 mg GAE/g. According to Awaad *et al*.,[19] four flavonoids that were separated and identified from *E. cuneata* Vahl. as naringenin, aromadendrin, apigenin, and 4'-O-methoxy-luteolin-7-O-rhamnoglucoside exhibit potential. However, our results were different from those of Awaad *et al*.,[19] who discovered that the ethyl acetate extract had the highest activity, ranging from 26.51% to 83.50%, while the conventional ascorbic acid at a concentration of 100 mM had an activity of 87.8%. We all concurred, nonetheless, that the ether extract exhibited the lowest percentage of activity among the extracts. Identification and measurement of flavonoids and phenolic acids were performed using HPLC analysis. According to Awaad *et al*.,[19] four flavonoids with

potential have been discovered and extracted from alcohol extracts of *E. cuneata* Vahl. These flavonoids include 4'-O-methoxy-luteolin-7-O-rhamnoglucoside, aromadendrin, apigenin, and naringenin. According to Figure 5, the most common phenolic acids were caffeic acid and pyrogallol. Next in line were gallic acid, ferulic acid, syringic acid, and p-coumaric acid (Fig. 5). According to the above Results, the most common flavonoids were rutin (7.62%) and 7-hydroxyflavone (12.45%), followed by quercetin, luteolin, and kaempferol. According to Awaad *et al*.,[19] four flavonoids have been discovered and extracted from alcohol extracts of *E. cuneata* Vahl. These flavonoids include 4'-O-methoxy-luteolin-7-O-rhamnoglucoside, aromadendrin, apigenin, and naringenin.

The results were consistent with those of De Araújo *et al*.,[20] who identified and quantified the phenolic compounds and their antibacterial activity after determining the TPC and antioxidant activity in extracts of *E. tirucalli* L. Ferulic acid was the main phenolic compound identified and quantified by HPLC-UV in all extracts. Following a series of separation techniques, such as thin-layer chromatography, vacuum liquid chromatography, preparative thin-layer chromatography, and high-performance liquid chromatography, methanol extracts made from the plant's stems and roots yielded two flavonoids (naringenin and eriodyctiol) and one diterpene (ingenol). For the first time, every compound was separated from the plant. For the first time, eriodyctiol was found in a *Euphorbia* species.[21] The antimicrobial activity (Tables 7 and 8) indicates high MIC values (450–6250 µg/mL), reducing clinical significance but suggesting potential as adjuvants.

This study is limited to *in vitro* assays with high MIC values, potentially reducing clinical applicability. Only four extract types were tested, and no *in vivo* models were included. Future research should explore lower concentrations, *in vivo* efficacy, and additional extracts.

In summary, the EE of *E. cuneata* demonstrated strong antioxidant and antimicrobial potential, making it a promising natural source for combating oxidative stress and inhibiting microbes. Further research is needed to explore its mechanisms and applications.

Conclusions

E. cuneata extracts, particularly the ethanolic fraction, exhibited potent antioxidant activity (IC_{50} 28.52 µg/mL; 97.90% scavenging) driven by phenolics (80.0 mg GAE/g) and flavonoids (40.5 mg quercetin equivalents/g), effectively combating oxidative stress *in vitro*. Moderate antimicrobial effects were observed, strongest against *S. aureus* and *C. albicans*, though high MICs warrant formulation improvements. These findings position *E. cuneata* as a promising natural source of antioxidants and antimicrobials, supporting further phytochemical optimization and preclinical validation.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Investigation (NSM, IF), data curation (NSM, IF), formal analysis (PPP), resources (PPP), Software (SBM), visualization (SBM), methodology (JST, AIE), validation (JST), supervision (JST, AIE), conceptualization (IHA), project administration (IHA), writing – original draft (NSM), writing – review & editing (IHA, AIE). All authors have made significant contributions to this study and have read and approved the final manuscript.

Ethical statement

Not applicable.

Data sharing statement

Data is contained within the article. Raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher upon reasonable request.

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