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Original Article
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Antimicrobial and Anti-Inflammatory Potential of *Euphorbia paralias* (L.): a bioprospecting study with phytoconstituents analysis

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Objectives: The phytochemicals in the aerial parts of *Euphorbia paralias* (also known as Sea Spurge) and their anti-inflammatory and antimicrobial activities were investigated.
Methods: The methanolic extract was characterized using GC-MS and HPLC techniques. The anti-inflammatory feature was estimated through a Human Red Blood Cell (HRBC) membrane stabilization technique, while the antimicrobial feature was evaluated by the disc diffusion agar technique, minimum bactericidal concentration, and minimum inhibitory concentration (MIC) *via* micro-broth dilution method.
Results: The GC/MS results demonstrated the existence of various phytochemicals, such as n-hexadecenoic acid, cis-11-eicosenoic acid, and methyl stearate, recognized for their anti-inflammatory and antibacterial features. The similarity of the phytochemical composition with other *Euphorbia* species emphasizes the genus-wide similarity. The anti-inflammatory activity exhibited a noteworthy inhibitory effect comparable to the reference drug indomethacin. The extract's antimicrobial potential was tested against a range of microorganisms, demonstrating significant action against Gram-positive bacteria and *Candida albicans*. The quantification of total phenolics and flavonoids further supported the therapeutic potential of the extract.
Conclusion: The methanolic extract from *E. paralias* emerges as a successful natural source of important active constituents with potential applications as anti-inflammatory and antimicrobial agents. This research provides a first step to valorize *Euphorbia paralias* insights as a source of worthwhile phytochemicals that have potential applications in the pharmaceutical industry.

Keywords: *Euphorbia paralias*, euphorbiaceae, phytochemical analysis, anti-inflammatory, antimicrobial

INTRODUCTION

Euphorbia paralias belongs to the family Euphorbiaceae, assessed for its production of phenolics, glycosides, and active metabolites. Flourishing in maritime sands, this species is commonly found along the coast, spanning the Mediterranean basin and some European countries. *Euphorbia* species, including *E. paralias*, have been described in the literature for their importance in treating gastrointestinal diseases such as diarrhea [1] and liver diseases [2]. Additionally, *E. paralias* has shown anticancer potential against pancreatic, colon, and lung cancers, besides antioxidant activity [3].

Inflammation is a crucial biological response induced by immune system cells; however, overexpression of inflammatory

mediators released by immune cells destroys healthy tissues [4]. Current anti-inflammatory drugs, particularly non-steroidal anti-inflammatory drugs, may cause peptic ulcers [5], renal toxicity, and electrolyte imbalance [6]. Phytochemicals have been explored for their anti-inflammatory activities in preventing tissue damage [4].

Infectious diseases are caused by microorganisms [7] and are a major cause of morbidity and mortality in patients; therefore, the treatment of infectious diseases is crucial. Several factors affect the treatment of infectious diseases: for instance, antimicrobial drug resistance, for example, methicillin-resistant *Staphylococcus aureus* [8]; and antimicrobial adverse effects [9], such as tetracycline-induced growth inhibition in children and deposition of tetracyclines in bones and teeth [10]. Another class of antibiotics, fluoroquinolones, are associated with tendinopathies, cardiotoxicity, and neurotoxicity [11].

Plant phytochemicals are a source of effective anti-inflammatory and antimicrobial compounds. *Euphorbia helioscopia* extract has been investigated for its antimicrobial activity [12]. Therefore, *E. paralias* extract may also have antimicrobial effects, but with reduced potential for the development of bacterial resistance and adverse effects.

MATERIALS AND METHODS

1. Methanolic crude extract

The aerial parts of *E. paralias* were gathered from the coast of North Egypt, and the preparation of crude *E. paralias* methanolic extract was carried out according to our previous research [3].

2. Gas chromatography–mass spectrometry

An ISQLT single-quadrupole mass spectrometer was connected to a Thermo Scientific TRACE 1310 gas chromatograph (Thermo Fisher Scientific; Waltham, MA, USA). The column was a DB5-MS, inner diameter of 0.25 mm, and length of 30 m (J&W Scientific; Folsom, CA, USA); the ionization voltage was 70 eV, and an electron ionization mode was applied. The temperature program was: 40℃ for 3 min, 280℃ for 5 min, 5℃/min to 290℃ (maintained for 1 min), and static at 7.5℃/min thereafter. The injected volume of the *E. paralias* methanolic extract was 1 μL. The temperature of the injector was 200℃ and that of the detector was 300℃. The flow rate of the carrier gas was 1 mL/min. The Wiley and NIST mass spectral databases were used to identify the chemical compounds in the *E. paralias* methanolic extract [13].

3. Anti-inflammatory activity

1) Human red blood cell membrane stabilization

Red blood cell and lysosomal membranes share the same characteristics regarding membrane lysis and content release when exposed to phenylhydrazine, methyl salicylate, heat, or hypotonic medium. Indomethacin inhibits the lysis of lysosomal membranes, and the release of lysosomal enzymes from lysosomes. Therefore, the human red blood cell (HRBC) membrane stabilization technique was applied to estimate the anti-inflammatory properties of *E. paralias*, by comparing the ability of *E. paralias* versus indomethacin (positive control) to prevent HRBC membrane lysis in a hypotonic solution [14].

2) Preparation of erythrocyte suspension

A total of 3 mL of fresh whole blood was drawn into heparinized tubes from a volunteer who had not used any painkillers for 2 weeks before the trial. To ensure the absence of hematologic diseases a complete blood count was performed. Thereafter, the tubes were centrifuged for 10 min at 3,000 rpm. Normal saline was added to solubilize the red blood cell pellets. Both the supernatant and normal saline volumes were equal. An isotonic buffer solution (1.15 g of disodium phosphate, 9 g of sodium chloride, 0.2 g of 10 mM monosodium phosphate, and pH 7.4 in 1 L of distilled water) was used to reconstitute the dissolved pellets of red blood cells as 40% v/v suspension. Finally, this suspension was subjected to an anti-inflammatory assay [15, 16].

3) Hemolysis triggered by hypotonicity

The *E. paralias* methanolic extract (1 mg) was dissolved in 5 mL of hypotonic solution. A series of extract concentrations (100 to 1,000 μg/mL) was prepared and added to duplicate pairs (per dose) of centrifuge tubes. Additionally, 1 mg of extract was dissolved in 5 mL of isotonic solution, and the extract doses (100-1,000 μg/mL) were added to duplicate pairs (per dose) of centrifuge tubes. A total of 5 mL of the hypotonic solution and 5 mL of indomethacin (200 μg/mL) were added to the reference tubes. Each tube was loaded with 100 μL of the previously prepared erythrocyte suspension and mixed. The mixtures were centrifuged at 1,300 rpm for 3 min and incubated for 1 hour at 37℃. The supernatant (hemoglobin) was decanted, and the

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absorbance (optical density; OD) was measured using a Milton Roy Spectronic spectrophotometer at 540 nm [15, 16]. The hemolysis inhibition percentage in the presence of the extract was computed using the following equation:

$$\% \text{ inhibition of hemolysis} = 1 - \left(\frac{OD2 - OD1}{OD3 - OD1} \right) \times 100\%$$

where OD_1 = test sample absorbance in isotonic solution; OD_2 = test sample absorbance in hypotonic solution; and OD_3 = control sample absorbance in a hypotonic solution. The sample dose needed to prevent 50% of red blood cells from hemolyzing under the test conditions is known as the IC_{50} value.

4. Antimicrobial activity

1) Microbiologic media and microbial strains

The antimicrobial activity of the test samples was investigated against a panel of microorganisms, including bacteria and a yeast strain. The bacterial strains included *Bacillus subtilis* (ATCC 6633), *S. aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 10541), *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (ATCC 13883), and *Salmonella typhi* (ATCC 6539). Additionally, antifungal activities were evaluated using *Candida albicans* (ATCC 10221). Muller-Hinton broth, Sabouraud's dextrose agar, and trypticase soy yeast broth were obtained from Himedia Ltd (Mumbai, India). All microbial strains were attained from the ATCC (Rockville, MD, USA). Gentamicin and fluconazole, in the form of sterile soluble solutions, were acquired as pharmaceutical preparations from a community pharmacy as Garamycin (Schering-Plough; Kenilworth, NJ, USA), and fluconazole (Pfizer; New York, NY, USA), respectively.

2) Agar well diffusion

Investigation of the antimicrobial properties of the *E. paralias* methanolic extract employed the agar well diffusion method. Muller-Hinton agar plates were the testing medium for the tested bacterial strains, while Sabouraud's dextrose agar was used for *C. albicans*. After surface inoculation with the test microorganisms, holes were aseptically created in the agar plates, giving wells with diameters of 6-8 mm, using a sterile Cork-Borer tube. Subsequently, 50 μL of the methanolic extract was introduced into each well. The agar plates were then incubated under conditions tailored to the specific requirements of each test microorganism. The inoculum suspension was adjusted to achieve semi-confluent growth on the agar plate surface, and plates were inoculated within 15 min of this adjustment. Following even streaking of the entire dried agar surface in three different directions, the agar was allowed to dry for no more than 15 min. The incubation time was set to 16-18 hours for the test microorganisms. The diameter of the inhibition zone surrounding each well was then obtained at the point of prominent growth reduction. In cases of insufficient growth at the recommended times, the agar plates were re-incubated and re-evaluated, thus ensuring comprehensive and accurate assessment of the antimicrobial activity of the methanolic extract [17]. Suitable positive-control agents were used for each test microorganism: gentamicin for bacterial species; and fluconazole for fungal species.

3) Minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the *E. paralias* methanolic extract were measured using a sterile 96-well plate broth microdilution method, following the standard procedure outlined in CLSI ISO 20776-1. The 96-well plates were sterile, optically clear plates with either round or conical bottoms, each containing a final volume of 200 μL. For preparing the microdilution plates, intermediate twofold dilutions of the *E. paralias* methanolic extract were volumetrically prepared in sterile Muller-Hinton broth (Sabouraud's dextrose broth for the yeast strain). The dilutions were performed in a laminar airflow cabinet to ensure aseptic conditions [17].

For the preparation of inoculum, the technique of direct colony suspension was used. Isolated colonies were chosen from an 18- to 24-hour agar plate using a suitable growth medium for each test strain. The suspension was adjusted to attain turbidity equivalent to a 0.5 McFarland standard, resulting in an inoculum containing about 1 to 2 × 10^8 colony-forming units (CFU)/mL. The adjusted inoculum suspension was diluted in sterile water or broth within 15 min to reach a final inoculum concentration of approximately 5 × 10^5 CFU/mL [17]. Each well of the microdilution tray was inoculated with 5 μL of prepared inoculum suspension. Inoculated microdilution 96-well plates were incubated at a controlled temperature of 35 ± 2℃ for 16-20 hours in an ambient air incubator. To prevent desiccation, each plate was securely sealed with a well-fitting plastic cover before incubation [17].

The lowest dose of antimicrobial that completely prevents growth of the target microorganism in the microdilution wells is known as the MIC. The MIC values were estimated through visual examination for the presence or absence of growth, aided by appropriate viewing devices as required. The validity of each test was confirmed by observing acceptable growth (turbidity) in the growth-control wells [17].

MBC values were determined by sampling wells where no visible growth was observed after incubation at a suitable temperature. The presence of viable cells was then assessed. The concentration at which no colonies were detected was considered the MBC value [17].

4) Antibiofilm activity

The antibiofilm assay was executed through a microtiter plate assay to assess the impact of *E. paralias* methanolic extract on biofilm formation by the test microorganisms. In each well of the 96-well polystyrene flat-bottom plates, 300 μL of inoculated fresh trypticase soy yeast broth with a final concentration of 10^6 CFU/mL was dispensed. The cultures were then incubated at 37℃ for 48 hours and exposed to previously determined sublethal concentrations (75%, 50%, and 25% of the MBC). Control wells were also incorporated, including wells with medium only, without extracts, and with methanol only. Post-incubation, supernatant removal, and thorough washing of each well with sterile double-distilled water were performed to eliminate the planktonic cells. The plates were allowed to complete drying of the remaining liquid before staining. Subsequently, the formed biofilm was stained with a diluted concentration of aqueous solution of crystal violet (0.1%), and the staining time was set for 15 min. After staining, excess dye was eliminated from each plate by washing it three times with sterile distilled water. The crystal violet dye bound to the walls of each 96-well plate was solubilized by adding a volume (250 μL) of 95% ethanol to each well. Following incubation for 15 min, optical densities were recorded by a microplate reader at a wavelength of either 570 or 600 nm [17]. The biofilm inhibition ability of the sample was then determined using the following formula:

$$\text{Biofilm inhibition (\%)} = \left(1 - \frac{\text{Sample absorbance} - \text{Blank absorbance}}{\text{Control absorbance} - \text{Blank absorbance}}\right) \times 100$$

5. Statistical analyses

The data are presented as mean ± standard deviation (SD). The data for anti-inflammatory activity of the *E. paralias* extract and the indomethacin positive control were compared using an unpaired t-test. The level of statistical significance is indicated by: $^{ns}p > 0.5$; $^{**}p > 0.01$; and $^{****}p > 0.001$. The hemolysis inhibition percentage, MIC to MBC ratio, and the percentage of biofilm inhibition were computed using an Excel spreadsheet version 2019 (Microsoft; Redmond, WA, USA).

RESULTS

1. Gas chromatography–mass spectrometry

The results for various chemical compounds in the *E. paralias* methanolic extract are shown in Table 1 and Fig. 1. These compounds belonged to different chemical classes: monosaccharide derivatives, organic aromatic compounds, monoterpenoids, fatty acids and fatty acid esters, flavonoids, and miscellaneous compounds. The compound with the highest relative intensity was the fatty acid n-hexadecanoic acid (41.20%), followed by the fatty acid methyl esters methyl stearate (7.99%) and methyl hexadecanoic acid (7.59%). The *E. paralias* methanol extract also contained small amounts of other fatty acid esters: for example, glycidyl palmitate (3.19%); 9,12,15-octadecatrienoic acid methyl ester (0.47%); and hexadecanoic acid 2,3-dihydroxypropyl ester (0.51%). In contrast, the lowest percentage was for the fatty acid cis-11-eicosenoic acid (0.45%). Also, the plant extract contained the triglyceride triacetin (5.21%). Further, the analysis showed the existence of the vitamin C derivative l-(+)-ascorbic acid 2,6-dihexadecanoate (0.53%). Organic compounds such as cinnamaldehyde (2.57%) and cinnamaldehyde dimethyl acetal (1.18%) were also detected in the *E. paralias* extract. Other components such as the flavonoid 4H-1-benzopyran-4-one (1.84%), aryl aldehyde 2-furancarboxaldehyde (3.17%), (Z)-3-phenylacrylaldehyde (4.55%), and the fatty acid amid 9-octadecenamide (2.17%) were also found in the *E. paralias* extract.

2. Anti-inflammatory activity

The *E. paralias* methanolic extract revealed membrane stabilization for HRBCs compared with indomethacin. At concentrations of 800 μg/mL of *E. paralias* extract and indomethacin,

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Table 1. Compounds characterization in methanol extract by GC-MS

No.	Chemical compounds	Molecular weight (g/mol)	Molecular formula	Category	Retention time (min)	Relative abundance %
1	Glucuronamide	193	$C_6H_{11}NO_6$	Monosaccharide derivative	6.12	0.51
2	2-Furancarboxaldehyde (furfural)	96	$C_5H_4O_2$	Aryl-aldehydes	23.17	3.17
3	(Z)-3-Phenylacrylaldehyde	132	C_9H_8O	Miscellaneous compounds	24.48	4.55
4	Cinnamaldehyde	132	C_9H_8O	Organic aromatic compound	25.07	2.57
5	Triacetin	218	$C_9H_{14}O_6$	Triglyceride	28.7	5.21
6	4-Hexen-1-ol,5-methyl-2-(1-methylethenyl)-(Lavandulol)	154	$C_{10}H_{18}O$	Monoterpenoid alcohol	30.91	1.2
7	Cinnamaldehyde dimethyl acetal	178	$C_{11}H_{14}O_2$	Organic aromatic compound	31.42	1.18
8	9,12,15-Octadecatrienoic acid, methyl ester	292	$C_{19}H_{32}O_2$	Fatty acid methyl ester	34.1	0.47
9	4-(2,4,4-Trimethyl-cyclo hexa-1,5-dienyl)-but-3-en-2-one	190	$C_{13}H_{18}O$	New chemical entity	40.09	0.83
10	Hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$	Methyl esters of fatty acid	51.41	7.59
11	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	Long-chain fatty acids	53.15	41.2
12	l-(+)-Ascorbic acid 2,6- dihexadecanoate	652	$C_{38}H_{68}O_8$	Vitamin C derivatives	56.15	0.53
13	Methyl stearate	396	$C_{27}H_{56}O$	Fatty acid methyl ester	57.74	7.99
14	Cis-11-Eicosenoic acid	310	$C_{20}H_{38}O_2$	Long-chain fatty acids	60.24	0.45
15	Hexadecanoic acid 2,3-dihydroxypropyl ester	330	$C_{19}H_{38}O_4$	Fatty acid ester	61.62	0.51
16	Glycidyl palmitate	312	$C_{19}H_{36}O_3$	Fatty acid ester	63.21	3.19
17	9-octadecenamide	281	$C_{18}H_{35}NO$	Fatty amid	64.71	2.17
18	4H-1-benzopyran-4-one	164	$C_9H_6O_2$	Flavonoids	70.39	1.84

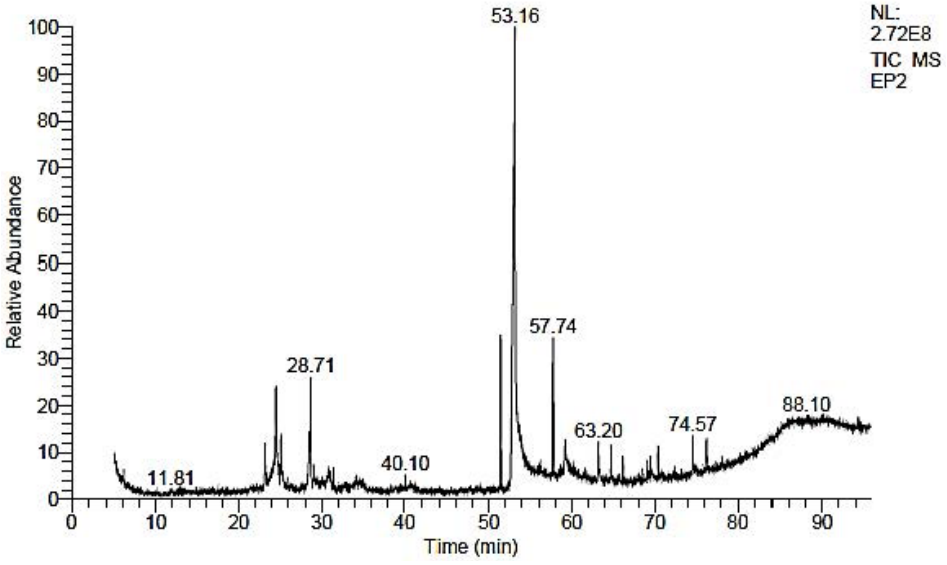

Figure 1. The GC-MS chromatogram of *E. Paralias* methanolic extract.

the hemolysis inhibition percentages were 96.3 ± 0.007% and 96.6 ± 0.004%, respectively; therefore, there was no statistically significant difference between the anti-inflammatory effects of *E. paralias* and indomethacin (Table 2 and Fig. 2). This indicates that *E. paralias* may have anti-inflammatory activity.

3. Antimicrobial activity

1) Disc agar diffusion assay

The antimicrobial activity of *E. paralias* was investigated via the disc diffusion agar assay (Fig. 3), which revealed distinct

Table 2. Hemolysis inhibition by *E. paralias* and indomethacin

Concentrations (μg/mL)	Hemolysis inhibition by *E. paralias* (%)	Hemolysis inhibition by indomethacin (%)
1,000	98.9 ± 0.004^{ns}	98.6 ± 0.004
800	96.3 ± 0.007^{ns}	96.2 ± 0.004
600	92.9 ± 0.006**	94.6 ± 0.004
400	89.9 ± 0.002****	93.4 ± 0.003
200	85.1 ± 0.001****	92.5 ± 0.005
100	80.9 ± 0.004****	91.6 ± 0.003

These results are represented as mean ± SD. ^{ns}p > 0.5; **p > 0.01; ****p > 0.001 shows significant changes in relation to indomethacin. A *t*-test was applied to compare the *E. paralias* and positive control indomethacin.

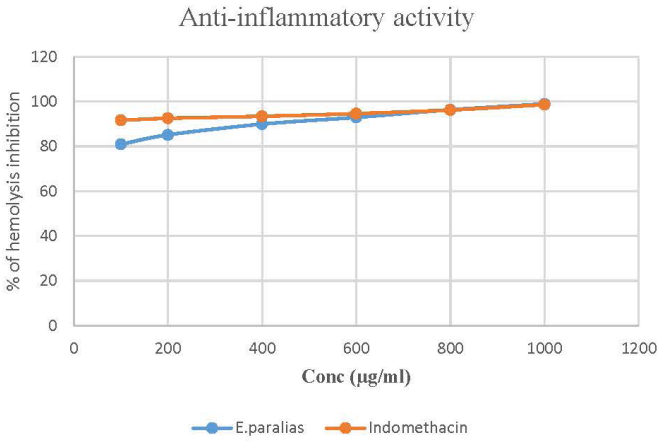

Figure 2. Percentage of hemolysis inhibition at different concentrations of *E. paralias* and indomethacin.

patterns of susceptibility among the tested strains. The Gram-positive species *E. faecalis* and *B. subtilis* displayed notable susceptibility, with observed zones of inhibition of 36 mm and 33 mm, respectively. In comparison, *S. aureus* exhibited a slightly smaller zone of inhibition (19 mm). *E. coli* and *K. pneumoniae* showed moderate susceptibility, with inhibition zones of 18 mm and 21 mm, respectively. *S. typhi* demonstrated a significant zone of inhibition, measuring 26 mm. Additionally, the yeast *C. albicans* displayed an inhibition zone of 19 mm.

2) MIC to MBC ratio

Fig. 4 shows the MBC/MIC ratios for *E. paralias* methanolic extract against the tested microorganisms. *E. coli* had a ratio of 1.0, while the *E. paralias* extract displayed varying levels of efficacy against different microorganisms. For instance, *E. faecalis* had a ratio of 3.5, *B. subtilis* 2.5, *S. aureus* 2.0, *K. pneumoniae* 1.0, and *S. typhi* 2.0.

3) Antibiofilm activity

The antibiofilm activity of the *E. paralias* extract is depicted in Fig. 5. At 75%, 50%, and 25% MBC, biofilm inhibition ranged from 85-95%, 75-90%, and 65-80%, respectively, for different bacteria. The *E. paralias* extract demonstrated substantial antibiofilm activity across all tested concentrations and bacterial strains (Supplementary Materials).

DISCUSSION

In the current study, gas chromatography–mass spectrometry (GC-MS) identified several classes of phytochemicals in the *E. paralias* extract with potential anti-inflammatory and antimicrobial properties. The major constituent in the *E. paralias* extract was n-hexadecenoic acid, a fatty acid associated with anti-inflammatory and antibacterial activity [18]. Another fatty acid found in the extract and known to possess antimicrobial properties was cis-11-eicosenoic acid [19]. The fatty acid methyl ester 9,12,15-octadecatrienoic was also identified and reported to possess anti-inflammatory activity [20]. Methyl stearate, a fatty acid ester with anti-inflammatory and antimicrobial effects, was also detected in the extract [21], as was hexadecanoic acid methyl ester [22, 23]. Further, hexadecanoic acid 2,3-dihydroxypropyl ester, a fatty acid ester with antibacterial effects [24], and 9-octadecenamide, a fatty amide with anti-inflammatory and antimicrobial activity [25], were identified. Cinnamaldehyde, which possesses anti-inflammatory and antimicrobial activity, was also identified [26, 27], as were 2-furancarboxaldehyde [28] and (Z)-3-phenylacrylaldehyde [29], which have antimicrobial effects. Additionally, l-(+)-ascorbic acid 2,6- dihexadecanoate has been associated with anti-inflammatory and antibacterial properties [30, 31]. However, the monoterpenoid alcohol 4-hexen-1-ol, 5-methyl-2-(1-methylethenyl)-acetate, and the flavonoid 4H-1-benzopyran-4-one have not yet been biologically assessed for therapeutic potential.

The results of the HRBC membrane stabilization assay demonstrated that the *E. paralias* methanolic extract (versus indomethacin) had anti-inflammatory properties at 800 μg/mL. These data are consistent with an anti-inflammatory study in which *Euphorbia cuneate* methanol extract 1,000 μg/mL showed considerable HRBC membrane stabilization [32]. Additionally, the anti-inflammatory activity of *Euphorbia retusa* was investigated in carrageenan-induced paw edema in mice:

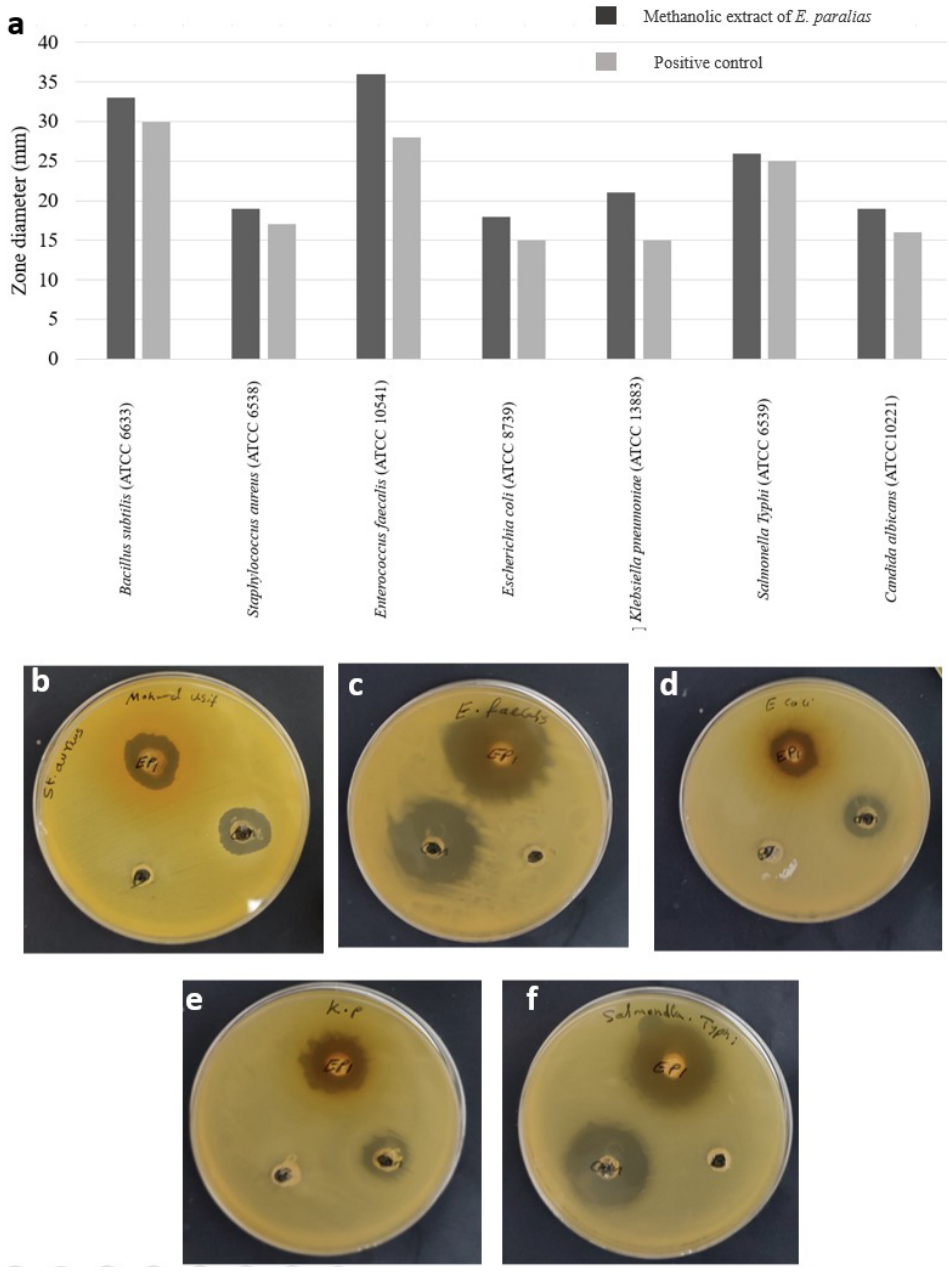

Figure 3. Antimicrobial activity of the methanolic extract from *E. paralais* against various gram-positive bacteria, gram-negative bacteria, and a yeast test microorganism. Zones of inhibition, measured in millimeters, are displayed with corresponding standard deviations (a). Selected examples (b-f) include *S. aureus, E. faecalis, E. coli, K. pneumoniae*, and *E. faecalis*. The experiment was conducted using the disc agar method, with each plate containing a test well (methanolic extract), a negative control well, and a positive control well (gentamicin for bacterial species and fluconazole for *C. albicans*) for comparative.

the inflammatory mediator malondialdehyde was significantly decreased in paw tissues, and the activity of superoxide dismutase, an antioxidant enzyme, was increased in paw and liver tissues compared to paracetamol [33]. Therefore, this study may support our anti-inflammatory findings for *E. paralias*. The 9,12,15-octadecatrienoic acid methyl ester and hexadecanoic acid methyl ester were identified in *Euphorbia dracunculoides* by GC-MS analysis [34]. These compounds were also detected by the GC-MS analysis in our study (Table 1). *E. dracunculoides* also significantly decreased carrageenan-induced paw edema in rats compared to diclofenac sodium (positive control) [34]. Therefore, the same identified compounds in *E. dracunculoides* and *E. paralias*, and the anti-inflammatory effect of *E. dracunculoides*, may support the potential anti-inflammatory effect of *E. paralias* in this study.

The *E. paralias* extract had significant antimicrobial features, particularly against Gram-positive bacteria. In disc diffusion assays, clear inhibition zones were observed for *E. faecalis* and *B. subtilis*, while *S. typhi* also showed noteworthy inhibition (26 mm). The MBC/MIC ratios indicated varied bactericidal/bacteriostatic effects. *E. coli* demonstrated a potent MBC/MIC ratio of 1.0, indicating nearly identical inhibitory and killing

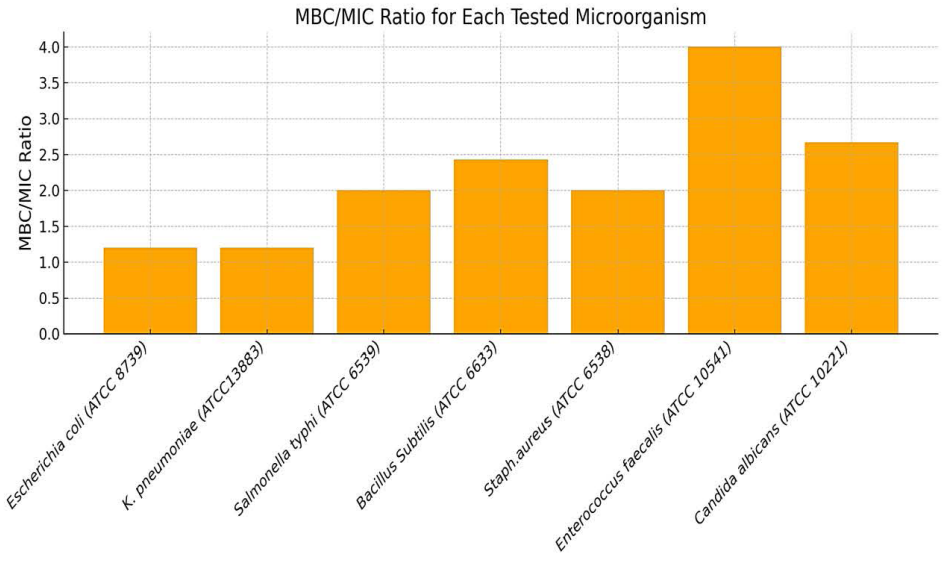

Figure 4. MBC/MIC ratio recorded for the *E. paralias* methanolic extract against tested bacterial and fungal strains. Each reading for the MBC or MIC shown in the figure represents the average of duplicate experiments.

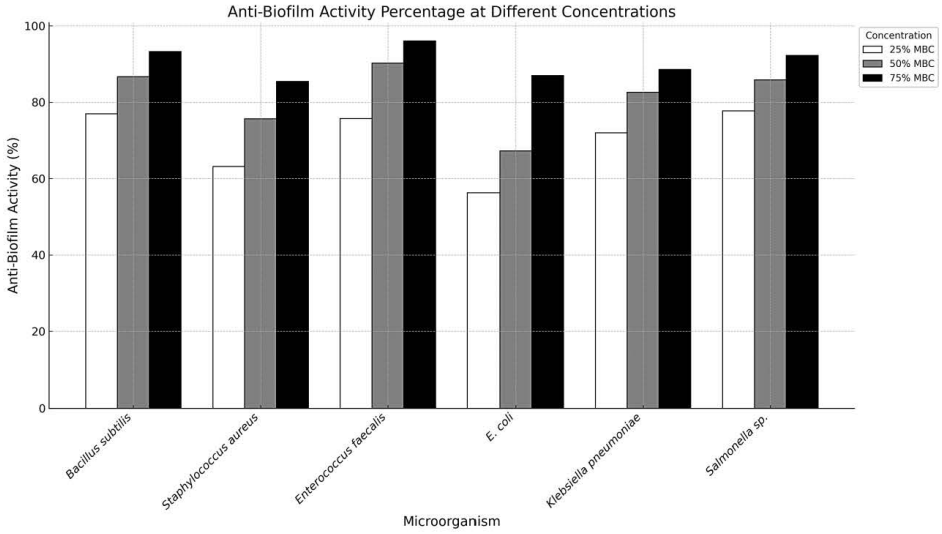

Figure 5. The percentage of inhibition of three different concentrations of methanolic extract of *E. paralias* against each of the test microorganisms used in the antibiofilm activity experiment. Each reading shown in the figure is the average of duplicate experiments.

concentrations. Conversely, *E. faecalis* (3.5) and *B. subtilis* (2.5) had higher ratios, suggesting bacteriostatic effects.

Further, our results suggested diverse bactericidal efficacy among the test microorganisms. *K. pneumoniae* and *E. coli* exhibited remarkable susceptibility, with an MBC/MIC ratio near 1.0, indicating similar concentrations for killing and inhibition. In contrast, *C. albicans* presented the highest ratio, at 4.0, signifying a fourfold greater antibiotic concentration for complete eradication versus growth inhibition. *E. faecalis* and *S. aureus* also had elevated ratios, indicating moderate resistance and probably requiring a greater concentration of the *E. paralias* methanolic extract to achieve higher suppression. Intriguingly, *S. typhi* had an intermediate ratio.

Notably, the extract demonstrated consistent antibiofilm activity across several bacterial strains (at 75%-25% MBC), underscoring the extract's potential as an effective antibacterial agent. These findings align with previous research on the Euphorbiaceae family. Amtaghri et al. [35] reported diverse antimicrobial and antifungal activity for *Euphorbia* spp., and these authors also reported the use of members of this genus (e.g., *Euphorbia rayleana*) in traditional medicine. Studies on *Euphorbia hirta* and *Euphorbia fusiformis*, by Natarajan et al. [36] and Sudhakar et al. [37], respectively, showed that antimicrobial activity varied according to the species, extraction solvents, and plant parts studied. Additionally, studies using the dilution method reported diverse MIC values (0.125 to 1

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mg/mL) for various extracts of *E. hirta* against bacterial strains [38]. Interestingly, a study conducted by Hlila et al. [39] in 2017 demonstrated that the antibacterial actions of chloroform and acetone extracts of *E. paralias* gathered from north Tunisia were significant against *Pseudomonas aeruginosa* and *B. subtilis*; and a methanolic extract of *E. paralias* also showed antimicrobial efficacy, particularly against the Gram-positive bacterium *B. subtilis*; therefore, these findings may suggest that *E. paralias* contains a diverse range of compounds that could be effective against microorganisms.

CONCLUSIONS

E. paralias methanolic extract was analyzed by GC-MS and high-performance liquid chromatography techniques, revealing a rich composition of phytochemicals with potential anti-inflammatory and antimicrobial properties. Notable compounds, such as n-hexadecenoic acid, cis-11-eicosenoic acid, and methyl stearate, were identified, and are known for their anti-inflammatory and antibacterial effects. The identified compounds aligned with those reported in other *Euphorbia* spp., confirming the consistency of phytochemical composition within the genus. The HRBC membrane stabilization assay substantiated the anti-inflammatory potential of the extract, supporting conventional uses for pain and inflammation management. Overall, this study provides valuable knowledge on the anti-inflammatory and antimicrobial activity, and potential therapeutic applications, of *E. paralias*.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPLEMENTARY MATERIALS

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