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Research Article

Investigation of biological interactions in *Euphorbia rigida* extract using molecular docking

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Abstract: In this study, the antioxidant activity, phenolic content, and antimicrobial properties of *Euphorbia rigida* aerial parts methanol extract were investigated. The extract demonstrated significant antioxidant activity with a DPPH radical scavenging activity IC_{50} value of 919.46 µg/mL. The iron chelating activity was characterised by an IC_{50} value of 4.24 mg/mL, with total phenolic content measured at 11.96 mg GAE/g extract DW and total flavonoid content at 26.83 mg QE/g extract DW. The antimicrobial evaluation compared the *E. rigida* aerial parts methanol extract to standard drugs such as Ampicillin, Chloramphenicol, and Ketoconazole. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranged from 12.5 mg/mL to >50 mg/mL. The extract exhibited strong antibacterial effects with MIC and MBC values of 25 mg/mL for *E. coli* and 12.5 mg/mL for *B. cereus*. Additionally, while some antifungal activity was observed against *C. albicans*, it was less effective than Ketoconazole. GG-MS analysis identified Guanosine as the most abundant compound in the extract, accounting for 35.78% of the total area. Molecular docking studies with phosphatidylinositol-specific phospholipase C showed that Guanosine had the strongest binding affinity with a binding energy of -5.0 kcal/mol, forming multiple interactions. Neophytadiene and Dihydroxyacetone exhibited weaker binding affinities and fewer interactions. Toxicity assessments indicated low toxicity for the extract's components, with LD50 values of 2200 mg/kg for Dihydroxyacetone, 13 mg/kg for Guanosine, and 500 mg/kg for Neophytadiene. In summary, the study sought to elucidate the antimicrobial potential and biological interactions of *E. rigida* aerial parts methanol extract.

1. INTRODUCTION

Medicinal plants are those with organs containing substances that affect living organisms. Medicinal plants are considered one of the oldest human achievements for treating diseases. Throughout the development of human civilisations, there has consistently been a close relationship between humans and plants (Jafari-Sales *et al*., 2019). Although many plant species have been identified to date, a plethora of new and valuable plant resources remain to be discovered (Sales, 2020). Though only partially identified so far, these chemical components can serve as drugs and unique starting points to produce pharmaceutical analogues and as

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valuable tools for enhancing our understanding of biological phenomena (Jafari-sales *et al*., 2019). Due to the side effects of antibiotics and the increasing resistance of microorganisms, the use of medicinal plants for combating bacterial infections has become more prevalent (Gholami *et al*., 2019). Due to the increasing problem of antibiotic resistance, attention has shifted towards biologically active compounds obtained from plants. These compounds can potentially serve as new and effective sources of antibacterial and antifungal activities (Erfan & Marouf, 2019; Maiyo *et al*., 2010). The antimicrobial properties of plants are associated with their ability to produce various secondary metabolites with complex structures (Matasyoh *et al*., 2009).

Bacillus cereus is one of the leading etiological agents of toxin-induced foodborne diseases. Its widespread presence in various environments, ability to form spores, and capacity to adapt to different conditions and produce harmful toxins make this pathogen a significant health hazard that should not be underestimated. Food poisoning caused by *B. cereus* can manifest as emetic or diarrheal syndrome. The final harmful effects are not only dependent on the toxins and strains. However, they are also influenced by stress responses, accessory virulence factors, phenotypic characteristics under extrinsic, intrinsic, and specific food conditions, and the host environment (Jovanovic *et al*., 2021).

The genus *Euphorbia* is the largest in the Euphorbiaceae, comprising approximately 2000 species, with 91 species found in Turkey (Özbilgin *et al*., 2012). These plants contain latex and are distinguished by their unique flower structures. *Euphorbia* species include many terpenoid compounds, such as monoterpenes, sesquiterpenes, diterpenes, triterpenes, and steroids. Many of these compounds have been studied for their toxicity and potential therapeutic effects, including antimicrobial, anti-inflammatory, anticancer, and antioxidant properties; some have historically been used as medicines (Gherraf *et al*., 2010).

The primary objective of this study is to comprehensively evaluate the antimicrobial activity of *E. rigida* aerial parts methanol extract. The study aims to determine the extract's effectiveness against various bacterial and fungal pathogens. The extract's antioxidant capacity and phenolic content will also be analysed to assess their contributions to microbial efficacy. Furthermore, the chemical identification of bioactive compounds present in the extract will be performed, and the toxicological profiles of these compounds will be evaluated. In summary, the study seeks to elucidate the antimicrobial potential and biological interactions of *E. rigida* aerial parts methan**ol** extract.

2. MATERIAL and METHODS

2.1. Collection of The Plant

E. rigida specimens were collected from the Asarkale area in Bafra, Samsun, and the entire plant, including both aboveground and underground parts, was harvested in June. Dr. Alper DURMAZ identified the species using the "Flora of Turkey," the latest nomenclature and epithet were confirmed through the POWO (Plants of the World Online) database. The herbarium material was catalogued under accession number OMUB-3184 in the Herbarium of the Biology Department at Ondokuz Mayıs University.

2.2. Plant Extraction

The methanol extract of *E. rigida* aerial parts was dried in an oven at 40°C and then ground into a powder using a blender. The maceration method suggested by (Aytar, 2024) was employed for extraction. The flowers, dried at 40°C, were extracted with methanol at room temperature for two days in a dark environment. The obtained extracts were filtered with filter paper. After filtration, the solvent was evaporated under reduced pressure at 40°C using a rotary evaporator, and solid extracts were stored at 4°C.

2.3. Spectroscopic Analysis of Secondary Metabolites

2.3.1. *Total phenolic content*

Total phenols were measured using the Folin-Ciocalteu method developed by (Singleton *et al*., 1999). Plant extracts were diluted to a concentration of 1 mg/mL. 0.5 mL was taken from these solutions and mixed with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of 7.5% $NaHCO_3$. The Folin-Ciocalteu reagent had been previously diluted tenfold with distilled water. The mixture was incubated at 45 °C for 15 minutes. After the incubation period, the absorbance was measured at 765 nm using a UV spectrophotometer. Total phenol content was determined as gallic acid equivalents dry weight extract (mg GAE/g extract DW), and these values are presented as the means of triplicate analyses.

2.3.2. *Total flavonoid content*

The total flavonoid content was measured following a previously reported spectrophotometric method (Dewanto *et al*., 2002). The procedure was as follows: Extracts of each plant material (1 mL containing 0.1 mg/mL) were diluted with water (4 mL) in a 10 mL volumetric flask. Initially, 5% $NaNO_2$ solution (0.3 mL) was added to each volumetric flask. At the 5 minute, 10% $AlCl_3$ (0.3 mL) was added. At the 6-minute, 1.0 M NaOH (2 mL) was added. Water (2.4 mL) was added to the reaction flask and mixed well. The absorbance of the reaction mixture was read at 510 nm using a UV spectrophotometer.

The total flavonoid content was quantified as quercetin equivalents (QE) per gram of dry-weight extract (mg QE/g extract DW). All measurements were performed in triplicate, and the results were presented as the mean values.

2.4. Determination of Antioxidant Capacity

2.4.1. *DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay*

The DPPH free radical scavenging activity was assessed using a modified method from (Takao *et al*., 1994), with adaptations from (Kumarasamy *et al*., 2007). DPPH (8 mg) was dissolved in methanol (100 mL) to create an 80 µg/mL solution. Plant extract stock solutions (1 mg/mL) were serially diluted. Each diluted extract (2 mL) was mixed with 2 mL of the DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV spectrophotometer.

The DPPH scavenging activity was calculated as follows:

DPPH scavenging activity (% inhibition) = [(A_control - A_sample) / A_control] x 100.

The IC_{50} value, representing the concentration of extract required for a 50% reduction in DPPH concentration, was determined by plotting a concentration curve and performing linear regression analysis. A lower IC_{50} indicates a higher antioxidant capacity. As a reference standard, Butylated Hydroxy Toluene (BHT) was used. All measurements were performed in triplicate, and the IC_{50} value was expressed in µg/mL.

2.5. Determination of Ferrous Ion Chelating Capacity

The ferrous ion chelating capacity of the extract was determined according to the method mentioned in (Dinis *et al*., 1994). Varying concentrations of the extract were mixed with 135 µL of the solvent. 2 mM $FeCl_2$ was added to the solution and incubated for 5 min. After that, five mM ferrozine solution was added and lasted 10 min. After incubation, absorbance was measured at 562 nm by using a spectrophotometer (Thermo Scientific Varioskan Flash) against a blank. Ethylenediaminetetraacetic acid (EDTA) was used as a reference standard. All measurements were performed in triplicate, and the IC_{50} value was expressed in mg/mL.

2.6. Antimicrobial Activity

The Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC), and Minimum Fungicidal Concentrations (MFC) of *E. rigida* aerial parts methanol

extract were determined using sterile 96-well plates according to the Clinical and Laboratory Standards Institute (CLSI) reference methods for bacteria (M7-A7, CLSI, 2018) and yeasts (M27-A3, CLSI, 2008). The test microorganisms included *Staphylococcus aureus* ATCC 25923, *Bacillus cereus*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231. Standard antimicrobial agents, including Chloramphenicol, Ampicillin, and Ketoconazole, were used as controls for bacteria and fungi, respectively. The analyses were conducted in triplicate, and the IC_{50} value was expressed in mg/mL.

2.7. GC-MS Analysis

For gas chromatography-mass spectrometry (GC-MS) analysis following the methodology outlined by (Aytar, 2024). Following this, the samples underwent centrifugation at 3500 revolutions per minute for 10 minutes, and the resulting supernatant was utilised for GC-MS analysis. The GC-MS analysis was conducted using the NIST Standard Reference Database protocol.

2.8. Molecular Docking Studies

Molecules were drawn in the Chem-Draw Ultra 18.0 program, and their minimal energy forms were obtained in the Chem 3D 18.0 program and saved in Mol2 format. The Protein Data Bank was used to record the enzymes (PDB). Phosphatidylinositol-specific phospholipase c (*B.cereus*) (1PTD) (2.60 Å) was chosen and preserved in PDB format. Molecule-enzyme interactions using AutoDock Vina 1.5.7 software and binding energies (kcal/mol) were determined (Trott & Olson, 2010). 2D and 3D visuals are demonstrated by BIOVIA Discovery Studio Visualizer software (Biova, 2019).

2.9. Prediction of Toxicity of Chemicals

The chemical toxicity prediction properties of selected phytocompounds have been evaluated. The 3D structures of two phytocompounds (3,5-Di-tert-butylphenol and 9-Octadecene, (E)-) were saved in PROTOX-II (https://tox-new.charite.de/protox_II/) web servers (Charite University of Medicine, Institute for Physiology, Structural Bioinformatics Group, Berlin, Germany) (Banerjee *et al*., 2018; Salaria *et al*., 2020; Rolta, Salaria, *et al*., 2021; Rolta, Yadav, *et al*., 2021).

2.10. Statistical Analysis

Correlation coefficients (R) were calculated using the CORREL statistical function in MS Excel software to determine the relationship between two variables. Data are expressed as mean ± SD obtained from three separate observations.

3. FINDINGS

In our study, the DPPH assay IC_{50} value of *E. rigida* methanol aerial parts was measured at 919.46 ± 22.51 µg/mL, while the positive control, BHT, showed an IC_{50} value of 230 ± 10 µg/mL; these results indicate that *E. rigida* has good antioxidant activity compared to BHT in terms of DPPH radical scavenging activity. Additionally, the iron chelating IC_{50} value of *E. rigida* methanol aerial parts was measured as 4.24 ± 0.04 mg/mL, while the positive control, EDTA, showed an IC_{50} value of 25.30 ± 4.44 mg/mL; these results indicate that *E. rigida* has stronger iron chelating activity compared to EDTA. *E. rigida* methanol aerial parts contain a total phenolic content of 11.96 mg GAE/g extract DW and a total flavonoid content of 26.83 mg QE/g extract DW (Table 1).

Table 1. DPPH radical scavenging activity, iron chelating activity (IC_{50} (µg/mL) ± *SD*) and total flavonoid content of *E. rigida* aerial parts methanol extract ± *SD** values.

Plant Name	DPPH (IC_{50} µg/mL)	Iron Chelating (IC_{50} mg/mL)	Total Phenolic Compound (mg GAE/g extract DW)	Total Flavonoid Compound (mg QE/g extract DW)
E. rigida methanol aerial parts extract	919.46 ± 22.51	4.24 ± 0.04	11.96 ± 1.10	26.83 ± 3.92
BHT (positive control)	230 ± 10			
EDTA (positive control)		5.30 ± 4.44		

In this study, the antimicrobial activities of *E. rigida* aerial parts methanol extract were compared with standard drugs such as Ampicillin (Amp), Chloramphenicol (C), and Ketoconazole (Keto). The MIC and MBC values of the *E. rigida* aerial parts methanol extract against the tested bacteria are presented in Table 2. The results indicate that the MIC and MBC values range from 12.5 mg/mL to >50 mg/mL. According to the antimicrobial screening results, the MIC values of *E. rigida* aerial parts methanol extract were found to be between 62.5 mg/mL and >125 mg/mL for Ampicillin, between 15.63 mg/mL and >125 mg/mL for Chloramphenicol, and 31.25 mg/mL for Ketoconazole. The methanol extract of *E. rigida* aerial parts demonstrates strong antibacterial effects against microorganisms such as *E. coli* and *B. cereus*, with MIC and MBC values of 25 mg/mL for *E. coli* and a MIC value of 12.5 mg/mL for *B. cereus*. However, the extract shows lower efficacy against pathogens such as *S. aureus* and *P. aeruginosa*. Additionally, while it exhibits some antifungal activity against *C. albicans*, its effectiveness is lower than Ketoconazole.

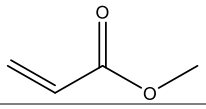
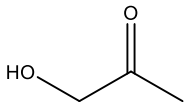
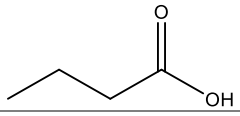
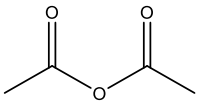
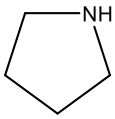
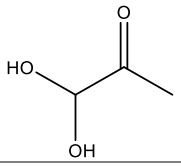
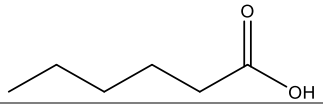
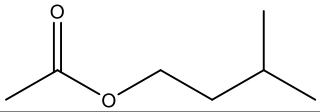
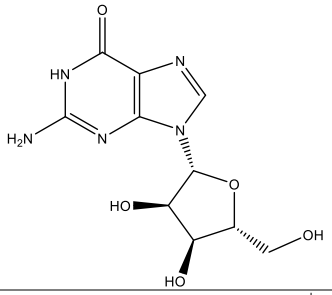
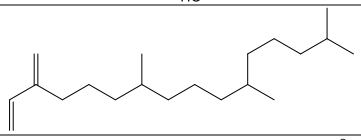
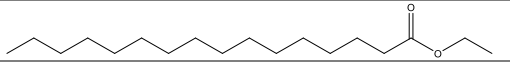
Table 2. MIC, MBC and MFC of the *E. rigida* aerial parts methanol extract and controls (mg/mL).

Microorganisms		Plant Species	Positive Control		
		Euphorbia rigida aerial parts methanol extract	Amp	C	Keto
E. coli ATCC 25922	MIC	25	>125	125	NS
	MBC	25	>125	>125	NS
S. aureus ATCC 25923	MIC	50	62.5	125	NS
	MBC	>50	62.5	>125	NS
B. cereus	MIC	12.5	125	15.63	NS
	MBC	>50	125	15.63	NS
P. aeruginosa ATCC 27853	MIC	50	>125	125	NS
	MBC	>50	>125	>125	NS
C. albicans ATCC 10231	MIC	25	NS	NS	31.25
	MFC	>50	NS	NS	62.5

Amp: Ampicillin; C: Chloramphenicol; Keto: Ketoconazole; NS: Not Studied

The methanol extract of *E. rigida* aerial parts identified various bioactive compounds. The extracts contain 11 bioactive phytochemical compounds with their retention time (RT), concentration (% area), and chemical structure presented in Table 3. The table above presents data from a compound mixture's gas chromatography (GC) analysis. This study analysed the percentage areas of compounds identified in the plant extract. Guanosine was the most abundant compound, accounting for 35.78% of the total area. Dihydroxyacetone followed this at 8.21% and Neophytadiene at 7.75%. Additionally, Pyrrolidine was detected at 4.91%, Hexadecanoic acid, ethyl ester at 4.05%, 1-Butanol, 3-methyl-, acetate at 3.49%, 2-Propenoic acid, methyl ester at 3.13%, Acetic oxide at 2.54%, n-Hexanoic acid at 2.46%, Butanoic acid at 2.43%, and Acetol at 2.48%. These results suggest that Guanosine is the dominant compound in the extract, with other compounds present in notable proportions.

Table 3. The GC-MS analysis results of *E. rigida* aerial parts methanol extract.

No	Retention time (minutes)	Compound Name	Area (%)	Molecular Structure
1	3.508	2-Propenoic acid, methyl ester	3.13	
2	4.163	Acetol	2.48	
3	4.492	Butanoic acid	2.43	
4	4.677	Acetic oxide	2.54	
5	5.098	Pyrrolidine	4.91	
6	7.512	Dihydroxyacetone	8.21	
7	10.660	n-Hexanoic acid	2.46	
8	14.381	1-Butanol, 3-methyl-, acetate	3.49	
9	24.750	Guanosine	35.78	
10	34.503	Neophytadiene	7.75	
11	37.712	Hexadecanoic acid, ethyl ester	4.05	

In this study, the docking analysis of phosphatidylinositol-specific phospholipase C from *Bacillus cereus* (PDB ID: 1PTD) with three different ligands Guanosine, Neophytadiene, and Dihydroxyacetone was conducted to predict their binding interactions and energies (Table 4 and Figure 4).

Table 4. Docking scores and report of predicted interactions of docked conformations phosphatidylinositol-specific phospholipase c (*B.cereus*) (1PTD).

Ligand	Protein	Binding Energy (kcal/mol)	Amino acid	Interacting	Distance
Guanosine	1PTD	-5.0	A: ARG69:HH11 -: [001: O1	Conventional Hydrogen Bond	2.65
			A: ARG69:HH11 -: [001: O4	Conventional Hydrogen Bond	2.43
			A: ARG163:HH21 -: [001: O2	Conventional Hydrogen Bond	1.78
			A: SER236: HG -: [001: N2	Conventional Hydrogen Bond	2.70
			: [001:H1 - A: ASP198:OD2	Conventional Hydrogen Bond	2.26
			: [001:H2 - A: ASP198:OD2	Conventional Hydrogen Bond	1.86
			: [001:H3 - A: ASP198:OD1	Conventional Hydrogen Bond	2.02
			: [001:H4 - A: SER234:O	Conventional Hydrogen Bond	2.57
			: [001:H4 - A: SER234: OG	Conventional Hydrogen Bond	2.76
			: [001:H5 - A: SER234:O	Conventional Hydrogen Bond	1.83
			: [001:H5 - A: SER234: OG	Conventional Hydrogen Bond	2.53
			: [001:H13 - A: GLU117:OE1	Conventional Hydrogen Bond	2.36
			A: SER236:HB1 -: [001: O5	Carbon Hydrogen Bond	2.02
			A: TYR200 -: [001	Pi-Pi Stacked	5.06
			: [001 - A: TYR200	Pi-Pi Stacked	4.57
Neophytadiene	1PTD	-4.5	A: ARG163 -: [001	Alkyl	4.68
			: [001:C11 - A: ARG163	Alkyl	3.46
			: [001:C12 - A: LYS115	Alkyl	3.92
			: [001:C12 - A: ARG163	Alkyl	3.61
			: [001:C20 - A: LYS115	Alkyl	5.46
			A: HIS32 -: [001:C17	Pi-Alkyl	4.41
			A: TRP178 -: [001	Pi-Alkyl	4.38
			A: TRP178 -: [001	Pi-Alkyl	4.52
			A: TRP178 -: [001:C11	Pi-Alkyl	4.32
			A: TRP178 -: [001:C11	Pi-Alkyl	4.42
			A: TYR200 -: [001	Pi-Alkyl	4.28
			A: TYR200 -:[001:C17	Pi-Alkyl	4.74
Dihydroxyacetone	1PTD	-3.8	A: ARG69:HH22 -: [001: O3	Conventional Hydrogen Bond	1.86
			: [001:H6 - A: ASP67:OD2	Conventional Hydrogen Bond	2.70
			: [001:H5 - A: ASP198:OD1	Carbon Hydrogen Bond	2.46

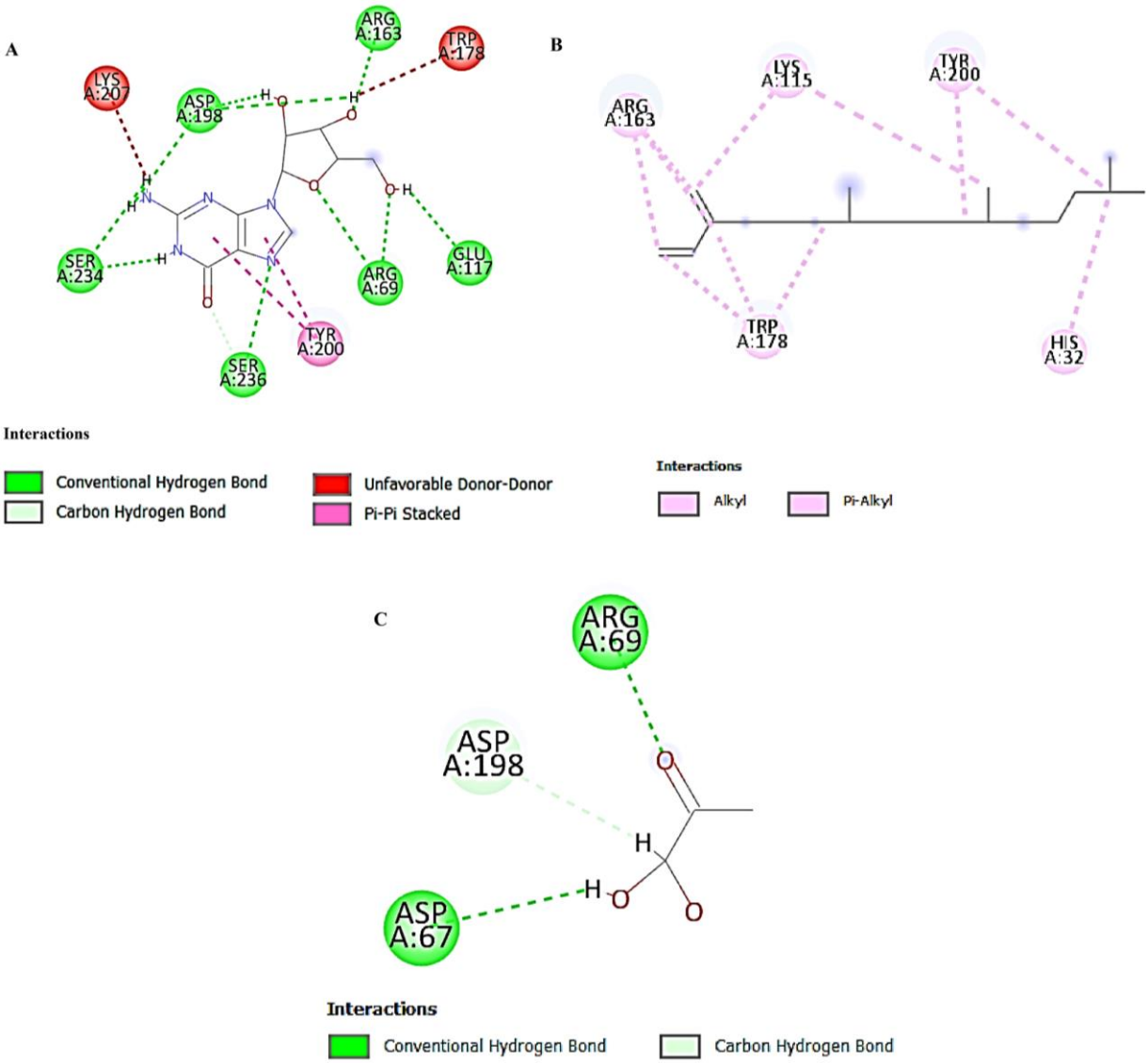


Figure 1. Molecular docking process of A) Guanosine B) Neophytadiene C) Dihydroxyacetone.

Guanosine exhibited the strongest binding affinity, with a binding energy of -5.0 kcal/mol. It formed multiple conventional hydrogen bonds with amino acid residues such as ARG69, ARG163, SER236, and ASP198, along with carbon-hydrogen bonds and Pi-Pi stacked interactions involving TYR200. These interactions suggest a stable binding conformation within the protein's active site (Figure 1).

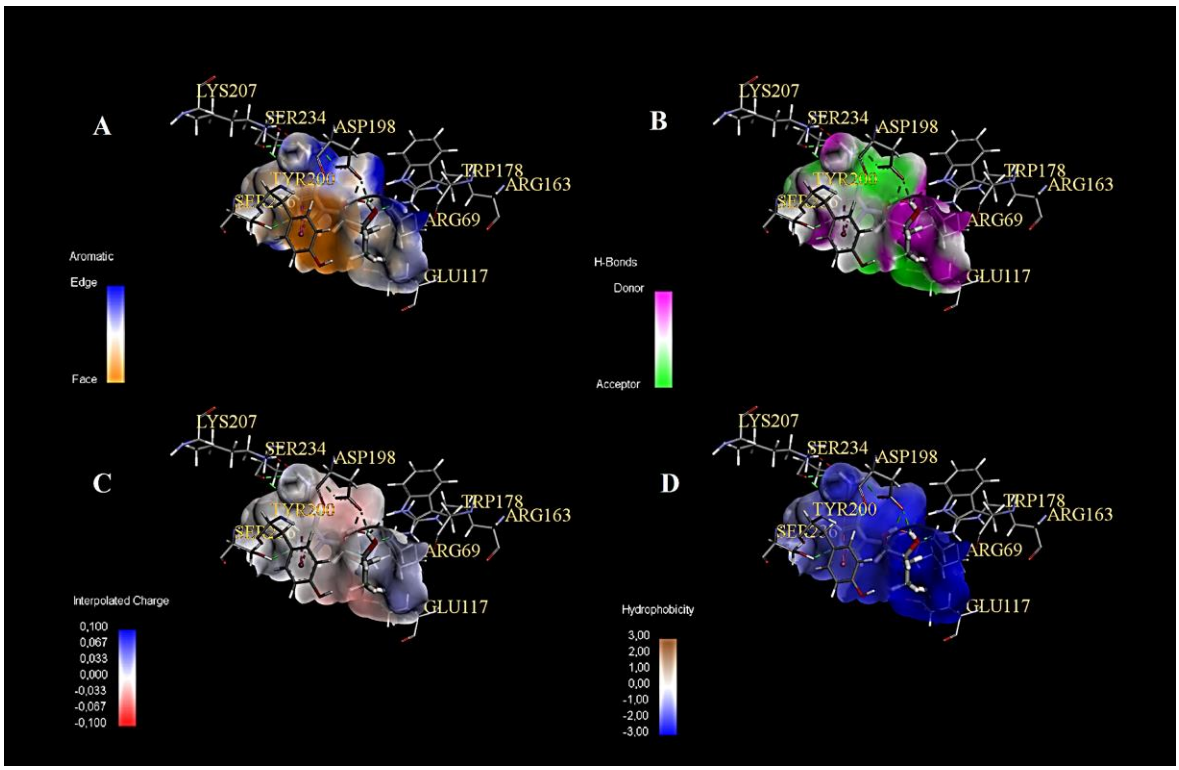


Figure 2. Interaction diagram of Guanosine with phosphatidylinositol-specific phospholipase c (*B. cereus*) (1PTD) in terms of A) Aromatic B) H- bonds C) Interpolated charge D) Hydrophobicity.

Neophytadiene, with a binding energy of -4.5 kcal/mol, primarily interacted through hydrophobic interactions such as alkyl and Pi-alkyl bonds with residues including ARG163, LYS115, HIS32, TRP178, and TYR200. These interactions indicate the ligand's potential to occupy the binding site, though with slightly lower affinity than Guanosine (Figure 2).

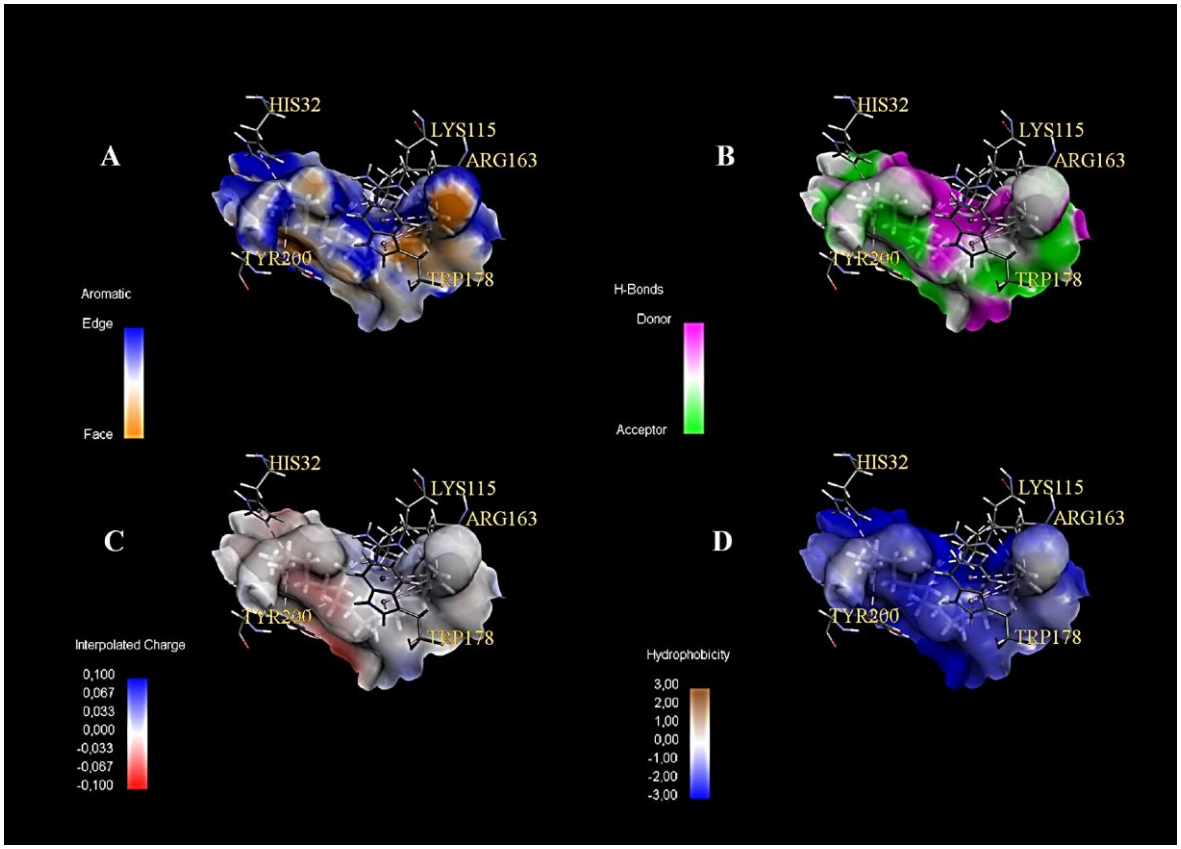


Figure 3. Interaction diagram of Neophytadiene with phosphatidylinositol-specific phospholipase c (*B. cereus*) (1PTD) in terms of A) Aromatic B) H- bonds C) Interpolated charge D) Hydrophobicity

Dihydroxyacetone showed the weakest binding energy at -3.8 kcal/mol. It formed fewer interactions, mainly conventional hydrogen bonds with ARG69 and ASP67 and a carbon-hydrogen bond with ASP198. The lower number of interactions correlates with its relatively reduced binding affinity, suggesting it may not be as effective in occupying the enzyme's active site compared to the other ligands (Figure 3).

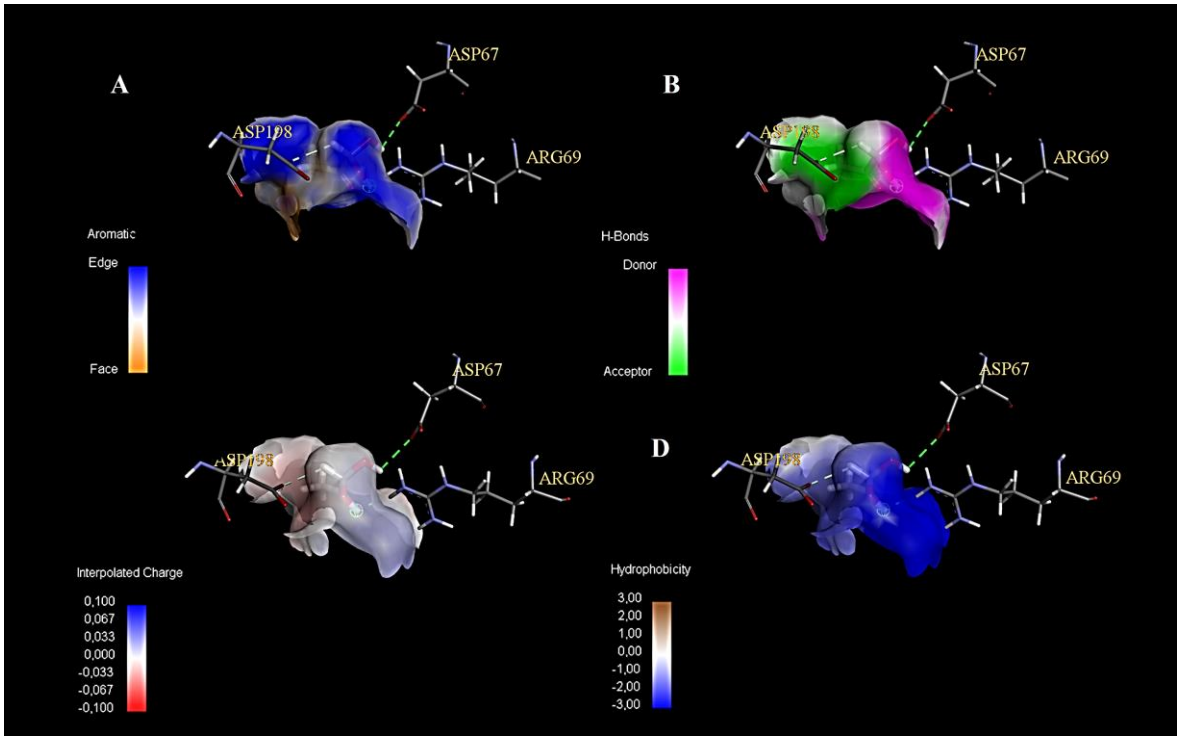


Figure 4. Interaction diagram of Dihydroxyacetone with phosphatidylinositol-specific phospholipase c (*B. cereus*) (1PTD) in terms of A) Aromatic B) H- bonds C) Interpolated charge D) Hydrophobicity

Overall, the results highlight the three ligands' varying binding affinities and interaction profiles, with Guanosine demonstrating the strongest and most diverse interactions with the phospholipase C enzyme.

The acute toxicity profile of the phytochemical components in *E. rigida* extract was extensively analysed, revealing that most of the components exhibit generally inactive toxicity effects (Table 5 and Figure 5). The LD50 values for Dihydroxyacetone, Guanosine, and Neophytadiene were determined to be 2200 mg/kg, 13 mg/kg, and 500 mg/kg, respectively. Correspondingly, the toxicity classifications for Dihydroxyacetone, Guanosine, and Neophytadiene were categorised as class 5, class 2, and class 6, indicating their varied toxicological profiles. When considering biological parameters such as hepatotoxicity, neurotoxicity, immunotoxicity, and cytotoxicity, it was observed that most components displayed inactive profiles. However, Guanosine was identified as active in both neurotoxicity and clinical toxicity assessments, suggesting that this compound may have potential toxic effects that warrant careful evaluation.

Additionally, Guanosine was found to be active in the nrf2/ARE activation and Cytochrome CYP2C9 enzymatic activity, indicating significant effects on these biochemical pathways. Overall, the results indicate that the phytochemical components of *E. rigida* extract largely do not exhibit toxic effects and generally present an inactive profile. Nonetheless, the activity of specific components suggests that further investigations are needed to assess their potential toxicological impacts and interactions thoroughly.

Table 5. Acute toxicity profile values of phytochemical components in *E. rigida* aerial parts methanol extract.

Toxicity Model	Dihydroxyacetone	Probability	Guanosine	Probability	Neophytadiene	Probability
LD_{50} (mg/kg)	2200		13		500	
Toxicity class	5		2		6	
Hepatotoxicity	Inactive	0.95	Inactive	0.73	Inactive	0.79
Neurotoxicity	Inactive	0.93	Active	0.59	Inactive	0.57
Immunotoxicity	Inactive	0.99	Inactive	0.99	Inactive	0.99
Cytotoxicity	Inactive	0.86	Inactive	0.98	Inactive	0.81
Clinical toxicity	Inactive	0.66	Active	0.57	Inactive	0.73
Androgen Receptor (AR)	Inactive	0.99	Inactive	0.99	Inactive	0.99
Estrogen Receptor Alpha (ER)	Inactive	0.98	Inactive	0.98	Inactive	0.98
Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Inactive	0.97	Inactive	0.75	Inactive	1
Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.98	Inactive	0.99	Active	1
Mitochondrial Membrane Potential (MMP)	Inactive	1	Inactive	0.98	Inactive	0.99
Phosphoprotein (Tumor Suppressor) p53	Inactive	0.98	Inactive	0.82	Inactive	1
Thyroid hormone receptor alpha (THRα)	Inactive	0.90	Inactive	0.90	Inactive	0.90
Thyroid hormone receptor beta (THRβ)	Inactive	0.78	Inactive	0.78	Inactive	0.78
GABA receptor (GABAR)	Inactive	0.96	Inactive	0.96	Inactive	0.96
Achetylcholinesterase (AChE)	Inactive	0.95	Inactive	0.73	Inactive	0.79
Pregnane X receptor (PXR)	Inactive	0.92	Inactive	0.92	Inactive	0.92
Voltage-gated sodium channel (VGSC)	Inactive	0.95	Inactive	0.95	Inactive	0.95
Cytochrome CYP1A2	Inactive	0.95	Inactive	0.99	Inactive	0.94
Cytochrome CYP2C19	Inactive	0.93	Inactive	0.88	Inactive	0.94
Cytochrome CYP2C9	Inactive	0.68	Inactive	0.75	Active	0.71
Cytochrome CYP2D6	Inactive	0.90	Inactive	0.92	Inactive	0.77
Cytochrome CYP3A4	Inactive	0.98	Inactive	0.99	Inactive	0.99
Cytochrome CYP2E1	Inactive	0.95	Inactive	0.98	Inactive	0.96

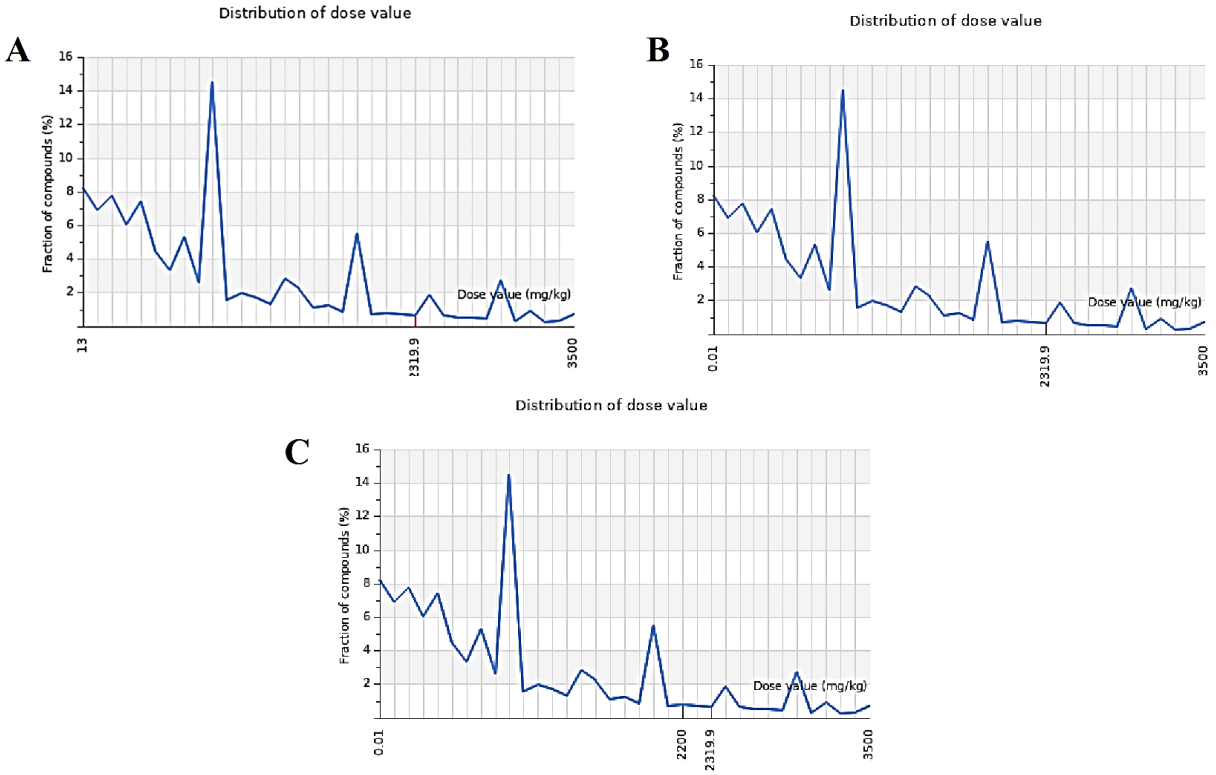


Figure 5. Acute Toxicity Profiles in *E. rigida* aerial parts methanol extract A) Guanosine B) Neophytadiene C) Dihydroxyacetone.

4. DISCUSSION and CONCLUSION

The pharmacological potential of Euphorbia species has attracted considerable attention, primarily due to their diverse bioactive properties. Studies on these plants have examined their antioxidant, antibacterial, and antifungal activities, highlighting potential applications in medical and therapeutic fields. Key parameters such as phenolic content, DPPH radical scavenging activity, iron chelating capacity, flavonoid content, and antimicrobial properties are crucial for assessing the biological activities of *Euphorbia* species.

Our study comprehensively evaluates *Euphorbia* species regarding phenolic content, DPPH radical scavenging activity, iron chelating capacity, flavonoid content, and antimicrobial activities. The detailed analyses of these parameters provide a deeper understanding of the therapeutic potential of *Euphorbia* species. Our results underscore the significance of these plants in health and medicine, establishing a fundamental reference for pharmacological investigations and enhancing the understanding of their potential therapeutic applications.

In the study by Aslantürk *et al*., (2021), the antioxidant activities of *E. rigid*a methanol extract were as follows: At a concentration of 10 mg/mL, the DPPH radical scavenging activity was 32.40% ± 0.004 and the iron chelating activity was 38.43; at 25 mg/mL, the DPPH radical scavenging activity was 34.21% and the iron chelating activity was 42.31%. At 50 mg/mL, the DPPH radical scavenging activity increased to 55.75%, and the iron chelating activity was 45.18% ± 0.017. At 100 mg/mL, the DPPH radical scavenging activity was 78.13%, and the iron chelating activity was 49.60%. At the highest 150 mg/mL concentration, the DPPH radical scavenging activity was 82.40%, and the iron chelating activity was 51.44%.

The study by Aslantürk *et al*. highlights a dose-dependent increase in antioxidant activity with higher concentrations, indicating substantial antioxidant efficacy. In contrast, our study demonstrates significant antioxidant activity with low IC_{50} values for the methanol extract of *E. rigida* aerial parts, suggesting strong antioxidant potential even at lower concentrations.

In the study by Kocazorbaz (2021), the aqueous extract of *E. rigida* leaves contained flavonoids and phenolics at concentrations of 0.086% per gram of leaf and 0.225% per gram of leaf, respectively. These findings indicate that *E. rigida* leaves are a rich phytochemical source of phenolic and flavonoid compounds. Both studies highlight the rich bioactive compound profile of *E. rigida*; however, our findings indicate higher concentrations of phenolics and flavonoids. This difference can be attributed to the variety of extraction methods and solvents used, which can significantly impact the yield of these compounds. The high phytochemical levels identified in our study suggest a strong antioxidant potential and underscore the importance of *E. rigida* for potential applications in health and medicine.

In the study by Ibraheim *et al*., (2013), the ethyl acetate fraction of *Euphorbia aphylla* demonstrated inhibitory activity ranging from 27.7% to 89.4% at concentrations from 10 µg/mL to 250 µg/mL. The alcohol fraction showed an inhibition rate of 77.7% across concentrations from 10 µg/mL to 500 µg/mL. However, the hexane and chloroform fractions exhibited no antioxidant activity at concentrations ranging from 10 µg/mL to 500 µg/mL. Compared to the ascorbic acid standard, the *E. aphylla* fractions exhibited strong antioxidant activity, demonstrating inhibition rates ranging from 47.1% to 99.6% at 10 µg/mL to 250 µg/mL concentrations. In our study, the DPPH radical scavenging activity of the methanol extract from the aerial parts of *E. rigida* was measured, yielding an IC_{50} value. Compared with the study by Ibraheim *et al*., it was found that the ethyl acetate and alcohol fractions of *E. aphylla* exhibit strong antioxidant activity. These findings indicate that both species possess significant antioxidant potential, but their effects may vary depending on specific extraction methods and measurement conditions.

In the study by Al-Ansi *et al*., (2024), the ethyl acetate extract of *E. arbuscula* stem latex exhibited the highest antioxidant activity with an IC_{50} value of 13.55 µg/mL, comparable to that of ascorbic acid, which had an IC_{50} value of 4.09 µg/mL. The chloroform extract followed with an IC_{50} value of 21.87 µg/mL, while the ethanol extract showed an IC_{50} value of 695.33 µg/mL, the butanol extract had an IC_{50} value of 195.17 µg/mL, and the methanol extract was reported to have an IC_{50} value of 463.73 µg/mL. In contrast, the methanol extract from the aerial parts of *E. rigida* demonstrated higher IC_{50} values for DPPH radical scavenging activity. In contrast, the ethyl acetate extract of *E. arbuscula* in Al-Ansi *et al*.'s study exhibited lower IC_{50} values, indicating stronger antioxidant activity. Additionally, the varying IC_{50} values of the methanol, ethanol, butanol, and chloroform extracts highlight that the antioxidant profiles of extracts obtained with different solvents vary. These findings suggest that different species within the same genus or different extracts of the same species can exhibit distinct antioxidant activities, and the choice of solvent significantly influences this activity.

In the study by Zeghad *et al*., (2016), the total phenol content of *E. biumbellata* leaves was reported as 15.13 g/100g GAE in methanol extracts, 14.33 g/100g GAE in water, 15.79 g/100g GAE in methanol-water mixture, and 12.695 g/100g GAE in ethanol. The total flavonoid content of *E. biumbellata* leaves was measured as 5.292 g/100g QE in methanol, 5.006 g/100g QE in water, 7.06 g/100g QE in the methanol-water mixture, and 3.993 g/100g QE in ethanol. For *E. dendroides* seeds, the total phenol content was found to be 3.035 g/100g GAE in methanol, 2.955 g/100g GAE in water, 3.215 g/100g GAE in methanol-water mixture, and 1.84 g/100g GAE in ethanol. The total flavonoid content of *E. dendroides* seeds was determined as 2.846 g/100g QE in methanol, 2.266 g/100g QE in water, 2.98 g/100g QE in a methanol-water mixture, and 1.926 g/100g QE in ethanol.

The methanol extract of *E. rigida* aerial parts shows significant differences in phenolic and flavonoid contents when compared with the findings from the study by Zeghad *et al*. on *E. biumbellata* and *E. dendroides*. The total phenolic content of *E. rigida* methanol extract is lower than *E. biumbellata* and *E. dendroides*, indicating that the methanol extract of *E. rigida* aerial is less rich in phenolic compounds. In contrast, E. rigida methanol extract's total flavonoid content is notably higher than that of the other species. This suggests that the methanol extract

of *E. rigida* aerial parts may be a more significant source of flavonoid compounds. In the study by Basma *et al*., (2011), the DPPH radical scavenging activity of *E. hirta* leaf extract was reported to be 72.96%. The flower extract demonstrated 52.45%, the root extract 48.59%, and the stem extract 44.42% radical scavenging activity. Total phenolic content analysis revealed that the leaf extract contained 206.17 mg GAE/g, the flower extract 117.08 mg GAE/g, the root extract 83.15 mg GAE/g, and the stem extract 65.70 mg GAE/g. Regarding total flavonoid content, the leaf extract had 37.970 mg CEQ/g, the flower extract 35.200 mg CEQ/g, the root extract 24.350 mg CEQ/g, and the stem extract 24.120 mg CEQ/g.

The study by Basma *et al*. (2011) found that the phenolic content of *E. hirta* leaf extract is significantly higher compared to the methanol extract of *E.rigida methanol* aerial parts. This finding indicates that *E. hirta* has a richer phenolic component content. Additionally, the total flavonoid content of *E. rigida* methanol extract is lower than that of *E. hirta* leaf extract but higher than the flavonoid content of *E. hirta* root and stem extracts. In our study, the DPPH radical scavenging activity of *E. hirta* leaf extract demonstrates that both *E. hirta* and *E. rigida* methanol extract from aerial parts have high antioxidant capacities.

These results highlight that different plant species within the same genus can exhibit considerable variability in their phenolic and flavonoid contents. Furthermore, the choice of extraction methods and solvents can significantly influence the extracts' antioxidant activity and component profiles. The data underscore the importance of considering the extraction methods and solvents used, as they play a crucial role in determining plant extracts' antioxidant potential and phytochemical composition. Studies have demonstrated that the methanol extracts of *E. rigida* aerial parts have notable antibacterial and antifungal properties.

In the study by Fred-Jaiyesimi & Abo (2010), the extract of *E. heterophylla* demonstrated significant activity against *S. albus*, *P. mirabilis*, *E. coli, S. typhi*, and *K. pneumoniae*. In this study, the methanol extract of *E. rigida* leaves exhibited strong antibacterial effects against microorganisms such as *E. coli* and *B. cereus*. These findings suggest that *E. rigida* extract could be an effective natural solution for combating infections and holds potential as a treatment option in the healthcare sector. Its antibacterial properties highlight its significance in discovering natural infection prevention and treatment sources.

The methanol extracts of *E. rigida* aerial parts exhibit significant antibacterial and antifungal activities. Fred-Jaiyesimi and Abo demonstrated that extracts of *Euphorbia heterophylla* show substantial efficacy against pathogens such as *S. albus, P. mirabilis, E. coli, S. typhi*, and *K. pneumoniae.* In our study, the methanol extracts of *E. rigida* aerial parts demonstrated strong antibacterial activity against *E. coli* and *B. cereus.*

In the study by Al-Ansi *et al*. (2024), the extracts and pure compounds isolated from the stem latex of *E. arbuscula* exhibited weak or no antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*. In the study by Ibraheim *et al*. (2013), *E. aphylla* ethyl acetate fraction demonstrated significant antibacterial activity, with inhibition zones ranging from 12 mm to 16 mm against *E. coli, P. aeruginosa, B. cereus,* and *M. luteus*. The alcohol fraction was particularly effective against *E. coli* and P. *aeruginosa*, showing inhibition zones of 16 mm and 12 mm, respectively. The chloroform fraction exhibited moderate antibacterial activity, with 14 mm and 13 mm inhibition zones against *E. coli* and *P. aeruginosa*. No antifungal activity was observed in any of the fractions. In contrast, the standard antifungal agent clotrimazole effectively inhibited all tested fungi, with inhibition zones ranging from 18 mm to 34 mm. The standard antibiotic chloramphenicol was effective against all bacterial strains, with inhibition zones ranging from 14 mm to 28 mm. The hexane fraction did not show antimicrobial activity against either bacteria or fungi. Al-Ansi *et al*. found that extracts isolated from the stem latex of *E. arbuscula* exhibited weak antibacterial activity against *S. aureus, E. coli*, and *P. aeruginosa*. Ibraheim *et al*. reported that the ethyl acetate fraction of *E. aphylla* demonstrated significant antibacterial activity against various bacteria, although no antifungal activity was

observed. Our study highlights the robust antibacterial properties of the methanol extracts of *E. rigida* aerial parts.

In the study by Hussain *et al*. (2014), ethanol and methanol extracts of *E. hirta* produced inhibition zones with diameters of 21.15 mm, 22.85 mm, 22.80 mm, and 23.55 mm against *S. aureus*, *E. coli*, and *C. albicans*, respectively. In contrast, ethanol and methanol extracts of *E. thymifolia* exhibited smaller inhibition zones for the same microorganisms, with diameters of 16.84 mm, 18.56 mm, and 20.24 mm against *S. aureus* and 17.84 mm, 19.23 mm, and 20.65 mm against *E. coli*. For C. *albicans, E. hirta* extracts produced inhibition zones of 16.15 mm and 17.90 mm, while *E. thymifolia* extracts yielded zones of 11.49 mm, 12.85 mm, and 14.64 mm. Hussain *et al*. found that the ethanol and methanol extracts of *E. hirta* produced significant inhibition zones against *S. aureus*, *E. coli*, and *C. albicans*. In our research, the methanol extract of *E. rigida* aerial parts exhibited high antibacterial activity against *E. coli* and *B. cereus* compared to standard antibiotics. However, its efficacy against other pathogens was lower, and its antifungal activity against *C. albicans* was less effective than Ketoconazole.

The findings of this study highlight the significant antioxidant and antimicrobial properties of the methanol extract of *E. rigida* aerial parts. The extract demonstrates notable antioxidant potential, as evidenced by its DPPH radical scavenging activity and iron chelating capacity. The total phenolic and flavonoid contents further substantiate the extract's antioxidant efficacy. Antimicrobial assays reveal that the extract exhibits potent antibacterial effects, particularly against *E. coli* and *B. cereus*. However, its effectiveness varies against other pathogens, with lower antifungal activity against *C. albicans* than standard antifungal agents such as Ketoconazole.

Molecular docking analyses indicate that Guanosine, Neophytadiene, and Dihydroxyacetone interact with phosphatidylinositol-specific phospholipase C from *B. cereus*, with Guanosine showing the strongest binding affinity. Guanosine forms a stable binding conformation within the enzyme's active site and exhibits a diverse interaction profile, suggesting its potential as a therapeutic agent. Toxicological evaluations reveal that most of the phytochemical components of *E. rigida* extract show low toxicity. However, Guanosine is identified as having significant toxicological effects, including neurotoxicity. These varied toxicity profiles necessitate further investigation to fully understand the implications of Guanosine’s effects on biological systems. Overall, *E. rigida* aerial parts show promising antioxidant and antimicrobial potential. However, the toxicological concerns, particularly about Guanosine, highlight the need for further research to ensure safety and efficacy for potential therapeutic applications.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

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Emine İncilay Torunoğlu: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing -original draft. **Turan Akdağ:** Supervision, and Validation. Authors may edit this part based on their case.

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