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Purification and characterization of bioactive compound from *Euphorbia heyneana* Spreng extracts

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ABSTRACT

This study aimed to systematically purify, characterize, and evaluate the phytochemicals of *Euphorbia heyneana* Spreng, showing their potential antibacterial activity at the molecular level and also assessing its cytotoxicity. The purification and identification were achieved through quantitative high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) analysis. The cytotoxicity of the HPLC-purified compounds was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. It has been commonly used as a traditional medicine to treat various ailments. This potent medicinal plant possesses antioxidant, antibacterial, antifungal, diuretic, and anti-ulcerative colitis properties. The major compounds identified in GC-MS analysis of chloroform extract *E. heyneana* Spreng were 10-methylnonadecane (0.190%), Hexadecane, 2,6,11,15-tetramethyl (0.256%), 3-chloropropionic acid, and hexadecyl ester (10.192%). Extract from *E. heyneana* Spreng showed potent antibacterial activity against common pathogenic microorganisms and may be a useful resource for therapeutic uses, particularly in the discovery of novel antimicrobial medicines.

1. INTRODUCTION

Medicinal plants contain various chemical substances which can be used to treat multiple types of human diseases. The chemical compounds of the plants are reported to have antibacterial, antimicrobial, antiviral, and antifungal potentials [1]. From ancient times, the importance of medicinal plants has been recognized. Herbal medicines were used to treat diseases before the availability of synthetic drugs. Plants are employed as therapeutic agents within established systems of traditional medicine, such as Ayurveda, Siddha, and Unani, as well as in less formalized practices, including folk, tribal, and indigenous medicinal traditions. The use of herbal medicine is increasing daily, so screening medicinal plants for their active compounds and understanding innovative mechanisms of action [2, 3] is important. Exploration of *Euphorbia heyneana* Spreng is very important as this plant is very less studied for their medicinal values. The *E. heyneana* Spreng. plant is a prostrate annual herb with up to 15 cm long branches commonly known as chhotidudi and belongs to the family *Euphorbiaceae*. The shape of leaves is clavate or club-shaped, to 8 × 4.5 mm, base circuitously rounded or subcordate, with round apex, edge entire, or saw-like; petiole length measuring 1 mm; while stipules are typically of length 1.5 mm and often have 2–4 linear teeth. The inflorescences are cyathia solitary with cup-shaped involucres; glands four in number. The male flowers are filamentous with few small bracteoles and stamens having a length of 1 mm, and the female flower has a pedicellate type of ovary; minute styles (0.2 mm in length), spreading and bifid to halfway [4]. Fruits, vegetables, medicinal herbs, leaves, flowers, and roots all contain natural bioactive substances called phytochemicals that combine with fibers to protect against diseases [5]. Knowledge of the chemical contents of plants is crucial for discovering therapeutic agents, disclosing novel sources of economically valuable phyto-constituents for synthesizing complex chemical compounds, and finding the significance of folkloric remedies [6]. Presently, various studies have been conducted on medicinal plants that have traditional therapeutic characteristics. The present study aimed to systematically purify, characterizing, and evaluating the phytochemicals of *E. heyneana* Spreng, revealing their potential antibacterial properties with molecular-level insights, while also assessing cytotoxic safety.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Plant samples were collected from different regions of Rohtak (Longitude: 76.606613 Latitude 28.895515) (Haryana) [Figure 1]. For the collection of plant samples, several field surveys were conducted

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at regular intervals in different regions of Rohtak. During the visit, necessary field equipment, e.g. knife, scissors, polythene bags, etc., were used. The required information, such as the botanical name, local name, family name, English name, and habitat, was noted at collection time. The local names of the collected plant samples were recorded with the help of interviews and discussions with the local people. Further identification and authentication of the specimens were done from the Botanical Survey of India, Northern Regional Center 192, Kaulagarh Road, Dehradun 248-195 (Acc. No. 1162).

2.2. Preparation of Plant Extracts

The collected plants/parts were thoroughly rinsed under running tap water and then dried under shade with distilled water. The dried plants/parts were coarsely ground to powder and then filled into a paper thimble made from Whatman filter paper. Using the Soxhlet extraction method, the plant samples were extracted in different solvents (chloroform, ethanol, and petroleum ether). The extracts were stored at −4°C for further use. The chloroform extract was used for the present study [7].

2.3. Microbial Strains

Bacterial strains used in the antimicrobial study were obtained from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India. Gram-positive (G+ve) strains *Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 96) and Gram-negative (G-ve) strains *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 424) were included in this study.

2.4. Screening of Antimicrobial Activity of Plant Extracts

The well-diffusion method described by Magaldi *et al*. with few modifications was used to assess the antimicrobial potency of the plant extract [8]. The nutrient agar plates were prepared under sterile conditions, and 100 µL of each microbial strain was spread separately to form a bacterial lawn. A sterile borer with a diameter of 6 mm was used to create wells in each plate. The designated wells were filled with 50 µL of extract stocks and dilutions (1:1, 1:2, 1:3, and 1:4) and incubated at 37°C overnight. The experiment was repeated three times, and the zone of inhibition was measured and recorded.

2.4. Purification of the Sample by Reverse High-performance Liquid Chromatography (HPLC)

The plant extracts with high antimicrobial activity were purified by reverse HPLC using the C18 column (Agilent 1200 infinity series). The mobile phase comprises chloroform to acetonitrile in the ratio of 60:40 V/V, and this was carried out using the isocratic mode, elution executed at a flow rate of 1 ml/min. The sample was analyzed for 12 min. and perception was done at 260 nm by an ultraviolet photodiode detector. The purified samples were subsequently used for screening in the antibacterial assay.

2.5. Cytotoxicity Studies

In Vitro assay, cytotoxicity activity was checked by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [9]. For testing, Madin-Darby canine kidney (MDCK) cells were washed with phosphate buffer saline and harvested after trypsinization and seed in 96 well plates (5000 cells/well) and then incubated for 24 h at 37°C. Then, the cells were subjected to various concentrations (1, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015, 0.007 mg/mL) of plant extract. Subsequently, the cells were incubated at 37°C with 5% CO_2 for another 24 h. After the incubation period, the medium was removed, and 30 µL of MTT was added to each well in the dark. After 4 h, the resulting formazan crystals were dissolved by adding 100 µL of DMSO. The plate was then kept at room temperature in a dark environment. The absorbance of the samples was measured at 570 nm using a microtiter ELISA plate reader. This entire experiment was repeated two times, and the data were analyzed to determine the cell viability and toxicity by the following formula.

The percentage growth inhibition was calculated by the formula:

$$\%\ cell\ inhibition = 100 - \frac{(At - Ab)}{(Ac - Ab)} \times 100$$

Where At = absorbance value of test compound,

Ab = Absorbance value of blank and Ac = Absorbance value of the control.

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The most potent samples were further characterized by GC-MS analysis [10]. The sample was dissolved in chloroform and injected in a GC-MS-Agilent 7890B GC connected to 5977A MSD, ColumnHP_5MS 5% Phenyl Methyl Silox-60°C–325°C, 30 m × 250 µm × 0.25 µm. Injection mode: Splitless. The following were the GC-MS operating conditions for the analysis: The oven temperature was raised to 280°C and maintained isothermally for 10 min after first reaching 45°C for 2 min and then 140°C at 5°C/min. Helium was employed as the carrier gas at a 1 mL/min rate, and 2 µL of the sample was injected. At 70 eV, the constituent parts of the sample were ionized. The GC operated for 54.0 min. The compounds' structures were subsequently compared with those in the NIST database by searching the NIST14.L library. Retention times and mass spectra were then used to identify substances that were already cataloged in the NIST library (C: \Database\NIST MS2011.L) [11].

2.7. *In Silico* Study of Phytochemical against Microbes

2.7.1. Molecular docking

Molecular docking was carried out using AutoDock4.2.6 (https://autodock.scripps.edu/download-autodock4/) with 100 Lamarckian genetic algorithm runs along with the default parameters. Confirmations with the lowest free binding energies, hydrogen bonds, and the most populated cluster were selected for further analysis. Interaction analyses were carried out using the BIOVIA Discovery Studio visualizer.

3. RESULTS AND DISCUSSION

3.1. Screening of Antimicrobial Activity of Plant Extracts

The present study was conducted with the objectives of carrying out the phytochemical screening and pharmacological evaluation of plant extracts of *E. heyneana* Spreng., extracted with chloroform, petroleum ether, and ethanol [Table 1].

After the primary screening of the crude plant extract, crude plant extracts with antimicrobial activity were selected for purification using reverse-phase HPLC (RP-HPLC). The fraction of the major peak was collected and further analyzed for antimicrobial activity.

Table 1: Assessment of the growth inhibitory potential of the *Euphorbia heyneana* Spreng. plant extracts against bacteria.

Plants	Zone of inhibition (mm)				
	Solvents	***Pseudomonas aeruginosa***	***Escherichia coli***	***Staphylococcus aureus***	***Bacillus subtilis***
Euphorbia heyneana Spreng.	Chloroform	-	16	7	-
	Petroleum ether	-	-	-	-
	Ethanol	8	-	-	-

3.2. Purification of *E. heyneana* Spreng Extract

The chloroform extract of *E. heyneana* Spreng was purified using RP-HPLC and the chromatogram showed three major peaks of NPE. PK1, NPE.PK2, and NPE.PK3 [Figure 2]. The first peak after the injection of the compound at 8.701 min, the peak area was 1840019, and the peak height was 124277. The second peak was after 14.181 min of injecting the sample with an area of 764775, and the peak height was 44571. The third peak was after 15.526 min of injecting the sample with area 485622, and the peak height was 42280 [Table 2]. The area covered by the NPE.PK1 was more in comparison to NPE. PK2 and NPE.PK3, and it was assumed that NPE.PK1 comprises more compounds. These major peaks were collected and further evaluated for antimicrobial activity.

3.3. Antimicrobial Activity of Purified Fraction of *E. heyneana* Spreng

The major three fractions of NPE.PK1, NPE.PK2, and NPE.PK3 were obtained after the purification of *E. heyneana* Spreng extract. These fractions showed varied antimicrobial activity [Table 3]. The NPE.PK2 showed more antimicrobial activity against *P. aeruginosa* with a zone of inhibition of 15 mm diameter. Another fraction of NPE.PK1 showed activity against *P. aeruginosa* only with a 10 mm zone of inhibition. The NPE.PK3 fraction showed activity against *B. subtilis* and *P. aeruginosa* with 10 mm and 5 mm zones of inhibition, respectively. However, no antimicrobial activity was observed against *E. coli* and *S. aureus*.

The purified fractions showed maximum activity against the bacteria *P. aeruginosa* and mild activity against *B. subtilis*; however, no activity was observed against *E. coli* and *S. aureus*. The active fractions were further characterized using the GC-MS technique. In the subsequent sections, these fractions were abbreviated as NPE1 and NPE2.

3.4. Characterization of NPE1 Fraction of *E. heyneana* Spreng

The NPE1 fraction of *E. heyneana* Spreng was analyzed using GC-MS and a chromatogram showed that this fraction consists of 28 different compounds [Table 4]. The compound 3-chloropropionic acid, heptadecyl ester makes a high peak with the largest percent area coverage, i.e., 10.192% at a retention time of 32.041 min [Figure 3]. The second largest percent area coverage was showed by 2-isopropyl-5-methyl-1-heptanol which is 5.791% at retention time 36.072 min, although it also appeared at 34.671 min but percent area coverage was 0.437%. Five compounds with least percent area coverage were 3-Carene, Trichloroacetic acid, hexadecyl ester, 10-Methylnonadecane, Ethanol, 2-(octadecyloxy), and Dodecane, 2,6,11-trimethyl, and their percent area coverage and retention time were 0.169% (9.443 min), 0.181% (45.947%), 0.190% (25.977 min), 0.203% (43.511 min), and 0.237% (20.547 min), respectively. The compound detection in GC-MS is listed in Table 4.

The GC-MS analysis of the chloroform-extracted NPE1 fraction from *E. heyneana* Spreng revealed that it contains 28 compounds of which two compounds had the highest PA. The highest peak area was observed

Table 2: Reverse phase high-performance liquid chromatography analysis of chloroform extract of *Euphorbia heyneana.*

Peaks	Retention time	Area	Height
NPE.PK1	8.701	1840019	124277
NPE.PK2	14.181	764775	44571
NPE.PK3	15.526	485622	42280

Figure 1: *Euphorbia heyneana* Spreng, grown in wild.

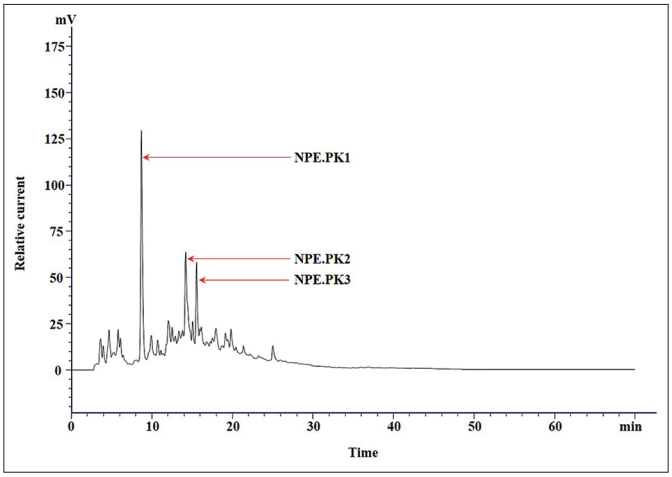

Figure 2: Chromatogram of crude extracts from *Euphorbia heyneana* Spreng chloroform. The chromatogram showed the X-axis (horizontal) which represented retention time (minutes) and Y-axis (vertical) represented relative current (Mv). The time interval between the compound's injections and the compound's detection is described as retention time and plays a significant role in the identification of the compound. The peak area represents the amount of compounds that have passed the detector.

for 3-chloropropionic acid, heptadecyl ester (10.192%) followed by the peak area of 2-isopropyl-5-methyl-1-heptanol (5.791%). Gupta and Kumar [12] reported that the plant extract of *Terminalia arjuna* showed antimicrobial activity against different pathogenic bacteria such as *E. coli*, *P. aeruginosa*, *Raoultella planticola*, *Enterobacter aerogenes*, *B. subtilis*, and *Agrobacterium tumefaciens*. GC-MS analysis of *T. arjuna* extract revealed 3-chloropropionic acid, and heptadecyl ester was one of the major components with a percent area coverage of 3.89%. Another compound 2-isopropyl-5-methyl-1-heptanol was reported to have antimicrobial activity [13].

Table 3: Antimicrobial activity of the purified compounds from chloroform extract of *Euphorbia heyneana*.

Purified compounds	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
NPE.PK1	−	++	−	−
NPE.PK2	−	+++	−	+
NPE.PK3	−	+	−	++

Each "+" sign was equal to 5 mm of the zone of inhibition.

Table 4: Spectral analysis of chloroform extract (NPE1, fraction) of *Euphorbia heyneana* shoot.

Name	RT. Min	MW	SF	PA%
Isobutyl acetate	3.292	116	$C_6H_{12}O_2$	0.358
3-Carene	9.443	136	$C_{10}H_{16}$	0.169
Cyclohexene,1-methyl-5-(1-methylethenyl)-, (R)-	10.136	136	$C_{10}H_{16}$	0.325
1-Hexanol, 2-ethyl-	10.537	130	$C_8H_{18}O$	0.257
1H-Imidazole	12.059	68	$C_3H_4N_2$	0.392
Glycerin	14.556	92	$C_3H_8O_3$	0.811
2,4-Imidazolidinedione, 3-methyl	17.198	114	$C_4H_6N_2O_2$	0.396
Dodecane, 2,6,11-trimethyl	20.547	212	$C_{15}H_{32}$	0.237
Tromethamine	23.895	121	$C_4H_{11}NO_3$	0.957
1-Undecanol	25.709	172	$C_{11}H_{24}O$	0.881
10-Methylnonadecane	25.977	282	$C_{20}H_{42}$	0.190
Hexadecane, 2,6,11,15-tetramethyl	27.353	282	$C_{20}H_{42}$	0.256
3-Chloropropionic acid, heptadecyl ester	32.041	346	$C_{20}H_{39}ClO_2$	10.192
1-Dodecanol, 2-octyl-	33.003	298	$C_{20}H_{42}O$	0.285
Trichloroacetic acid, hexadecyl ester	33.782	386	$C_{18}H_{33}C_{l3}O_2$	0.340
2-Isopropyl-5-methyl-1-heptanol	34.671	172	$C_{11}H_{24}O$	0.437
	36.072	172	$C_{11}H_{24}O$	5.791
1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	37.253	390	$C_{24}H_{38}O_4$	4.171
Terephthalic acid, 4-octyl octyl ester	39.177	390	$C_{24}H_{38}O_4$	3.565
Ethanol, 2-(octadecyloxy)	43.511	314	$C_{20}H_{42}O_2$	0.203
Decanedioic acid, dibutyl ester	43.804	314	$C_{18}H_{34}O_4$	0.378
2-Methyl-Z-4-tetradecane	44.047	210	$C_{15}H_{30}$	0.347
Heptacosyl pentafluoropropionate	45.131	542	$C_{30}H_{55}F_5O_2$	0.362
Trichloroacetic acid, hexadecyl ester	45.947	386	$C_{18}H_{33}C_{l3}O_2$	0.181
Hexanedioic acid, bis (2-ethylhexylester	47.688	370	$C_{22}H_{42}O_4$	0.288
Diisooctyl phthalate	51.584	390	$C_{24}H_{38}O_4$	0.406
Eicosane, 7-hexyl-	52.473	366	$C_{26}H_{54}$	0.454
Hexanoic acid, 2-ethyl-, octadecyl ester	53.922	396	$C_{26}H_{52}O_2$	0.605

3.5. Characterization of NPE2 Fraction of *E. heyneana* Spreng

The NPE2 fraction of *E. heyneana* Spreng was analyzed using GC-MS and a chromatogram showed that this fraction consists of 11 different compounds. The compound 1,4-benzenedicarboxylic acid, bis (2-ethylhexyl) ester was detected at 8 different retention times 27.898, 30.361, 30.93, 32.03, 32.97, 33.857, 36.625, and 36.837 min. The overall percent area coverage by 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester was 51.7% [Table 5]. Terephthalic acid, 4-octyl octyl ester was detected in the NPE2 fraction three times with a cumulative percent area coverage of 42.5%. Their retention time was 10.368, 11.863, and 20.304 min [Figure 4]. Another compound ethyl acetate, n-Propyl acetate, 2,7,7-Trimethylbicyclo 3.1.1 hept-2-en-6-yl acetate, 1-Undecanol, dodecanoic acid, 10-methyl-, methyl ester, Dodecyl acrylate, 9,12-Octadecadienoic acid, methyl ester, trans-13-Octadecenoic acid, methyl ester, Methyl stearate, and their retention time with percentage area coverage were 2.617 (0.367%), 4.55 (0.054%), 11.238 (0.019%), 21.74 (0.02%), 25.209 (0.086%), 29.103 (2.388%), 37.234 (0.135%), 37.34 (0.33%), and 37.81 (0.101%), respectively.

Table 5: GC-MS spectral analysis of chloroform extract (NPE2 fraction) of *Euphorbia heyneana* shoots.

Name	RT. min	MW	SF	PA%
Ethyl acetate	2.617	88	$C_4H_8O_2$	0.367
n-Propyl acetate	4.550	102	$C_5H_{10}O_2$	0.054
Bicyclo[3.1.1]hept-2-en-6-ol, 2,7,7-trimethyl-, acetate	11.238	194	$C_{12}H_{18}O_2$	0.019
1-Undecanol	21.740	172	$C_{11}H_{24}O$	0.020
Dodecanoic acid, 10-methyl-, methyl ester	25.209	228	$C_{14}H_{28}O_2$	0.086
Dodecylacrylate	29.103	240	$C_{15}H_{28}O_2$	2.388
1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	27.898	390	$C_{24}H_{38}O_4$	0.228
	30.361	390	$C_{24}H_{38}O_4$	0.686
	30.930	390	$C_{24}H_{38}O_4$	3.492
	32.030	390	$C_{24}H_{38}O_4$	5.707
	32.970	390	$C_{24}H_{38}O_4$	10.258
	33.857	390	$C_{24}H_{38}O_4$	10.754
	36.625	390	$C_{24}H_{38}O_4$	7.767
	36.837	390	$C_{24}H_{38}O_4$	12.796
Terephthalic acid, 4-octyl octyl ester	34.731	390	$C_{24}H_{38}O_4$	10.368
	35.401	390	$C_{24}H_{38}O_4$	11.863
	36.042	390	$C_{24}H_{38}O_4$	20.304
9,12-Octadecadienoic acid, methyl ester	37.234	294	$C_{19}H_{34}O_2$	0.135
trans-13-Octadecenoic acid, methyl ester	37.340	296	$C_{19}H_{36}O_2$	0.330
Methyl stearate	37.810	298	$C_{19}H_{38}O_2$	0.101

GC-MS: Gas chromatography-mass spectrometry.

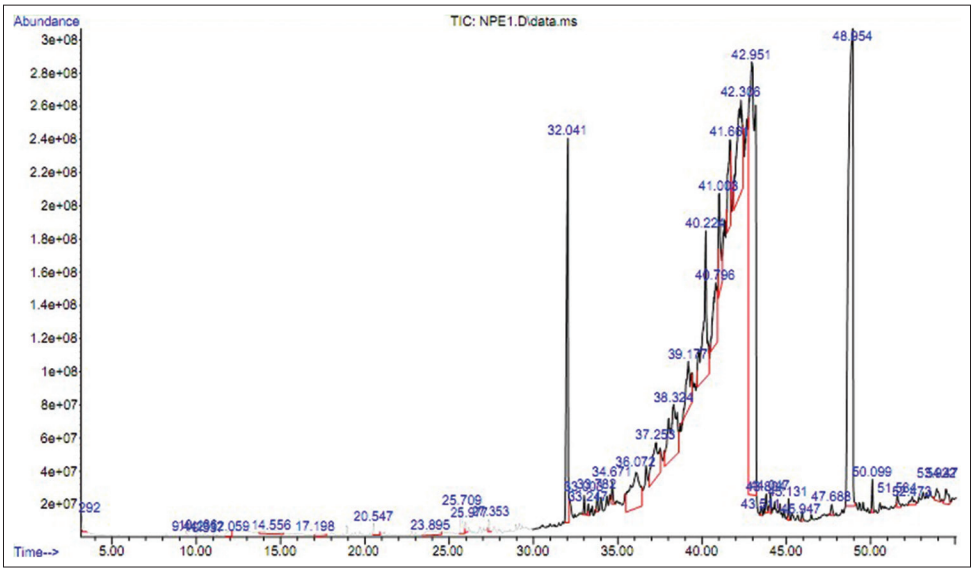

Figure 3: Gas chromatography-mass spectrometry chromatogram for chloroform extract (NPE1 fraction) of *Euphorbia heyneana* Spreng.

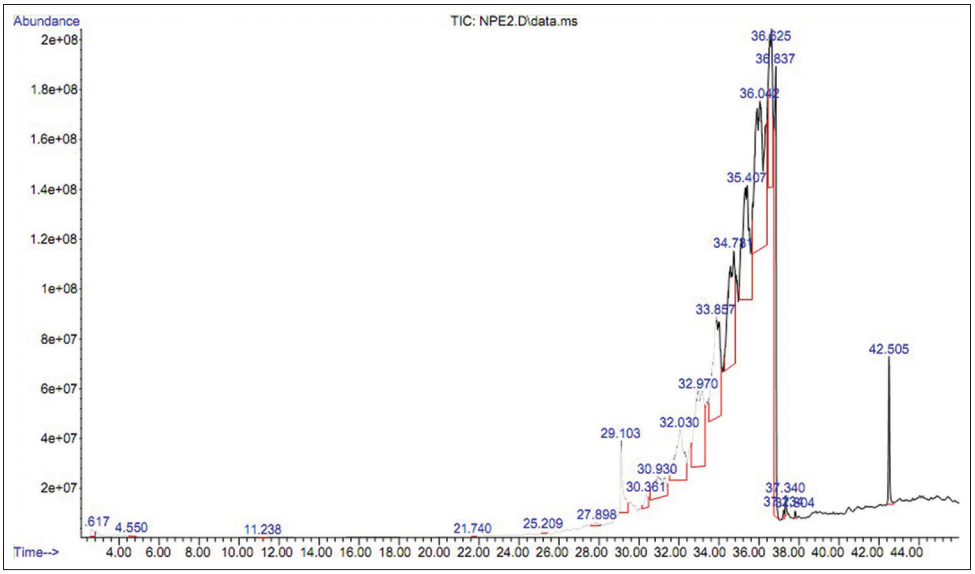

Figure 4: Gas chromatography-mass spectrometry chromatogram for chloroform extract (NPE2 fraction) of *Euphorbia heyneana* Spreng.

The chloroform extracted fraction from the *E. heyneana* Spreng (NPE2) when analyzed with GC-MS, the compound 1,4-benzenedicarboxylic acid, bis (2-ethylhexyl) ester was detected at eight different retention times and their cumulative percent was 51.7%. Valarmathi *et al*. [14] reported antimicrobial activity of the plant extract from *Dryopteris hirtipes* (*Blumze*) *Kuntze* Linn. The GC-MS analysis of the extract demonstrated that the 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester was one of the major fraction of extract with a peak area of 7.16%. In another study by Adebiyi *et al*., 1,4-benzenedicarboxylic acid, bis (2-ethylhexyl) ester was the major component in *Nephrolepis cordifolia* (L) with PA 18.35% [15]. Terephthalic acid, 4-octyl octyl ester was detected in the NPE2 fraction three times with a cumulative percent area coverage of 42.5%. To date, no specific information about the antimicrobial activity of terephthalic acid, 4-octyl octyl ester was found.

3.6. The Minimum Inhibitory Concentration (MIC) of a Plant Extract

The MIC of a plant extract is a crucial parameter in assessing its antimicrobial properties. MIC is defined as the minimum extract concentration at which the visible growth of microorganisms, such as bacteria or fungi is inhibited. This measurement is essential in determining the effectiveness of a plant extract as a potential antimicrobial agent.

The NPE1 and NPE2 fractions of *E. heyneana* Spreng were subjected to evaluation for MIC against tested bacteria. The MIC of the NPE1 and NPE2 fractions were evaluated against *P. aeruginosa*. The minimum concentration at which bacterial growth was inhibited is exhibited in Figure 5. The MIC of both the NPE1 and NPE2 fractions against *P. aeruginosa* was 100 µg/mL.

In the present study, the MIC of NPE1 and NPE2 fractions of *E. heyneana* Spreng against *P. aeruginosa* was also 100 µg/mL.

A similar MIC value was also reported by Perumal and Mahmud [16] that the methanol extract *Euphorbia hirta* L., against *P. aeruginosa* was 125 µg/mL and in the present study, the MIC of chloroform extract of *E. heyneana* Spreng against *P. aeruginosa* was 100 µg/mL. The higher MIC of chloroform extract of the stems of *Euphorbia paralias* was reported by Hlila *et al*. and value reported was 310 µg/mL [17]. The acetone extract of *Cirsium argyracanthum* showed antimicrobial activity against *S. aureus* and MIC reported was 30 mg/mL [18].

3.7. MTT Assay of NPE1 Extract from *E. heyneana* Spreng

The result of the MTT assay of *E. heyneana* Spreng extract NPE1on MDCK cell line exhibited that there was no severe cytotoxic potential against them. The CC_{50} value of NPE1 was 45.6 µg/100 µL. The dose-dependent study showed around 79.5% of cells died at 100 µg/100 µL dose whereas only 1.3% of cells died at 1.6 µg/100 µL dose [Figure 6]. Fifty percent of the cells survived at a dose of 45.6 µg/100 µL.

3.8. MTT Assay of NPE2 Extract from *E. heyneana* Spreng

The MTT assay of NPE2 extract from *E. heyneana* Spreng on the MDCK cell line showed that there was no severe cytotoxic effect of the extract NPE2 on the MDCK cell line. Only 15.1% of the cells survived under 100 µg/100 µL of NPE2. However, at 50 µg/100 µL dose, concentration around 25% of cells survived and at 3.125 µg/100 µL dose, more than 75% of cells survived [Figure 7]. The CC_{50} value of NPE2 was 37.7 µg/100 µL.

The stability of antimicrobial metabolites from plants in terms of cytotoxicity is crucial. As part of the present study on the discovery of bioactive compounds from *E. heyneana* Spreng, wild plants with potential antimicrobial activity were evaluated for their cytotoxicity activity, and the chloroform extract of the *E. heyneana* Spreng was screened in the MTT assay. Concerning the cytotoxicity screening, it was conducted on the MDCK cell line. The determination of cytotoxicity stability of purified antimicrobial metabolites against MDCK cells involves assessing the potency and safety profiles of these compounds, highlighting their potential as effective and safe antimicrobial agents derived from natural sources.

In the present study, the cytotoxicity concentration (CC_{50}) of NPE1 and NPE2 extract from *E. heyneana* Spreng was 456 µg/ml and 377 µg/ml, respectively, against the MDCK cell line. Sunmathi and Sivakumar [19] studied the effect of the ethanolic leaf extract of *Alternanthera philoxeroides* and *Alternanthera sessilis* on the growth of the human osteosarcoma cell line MG-63 [19]. They reported that the extract inhibited cell growth by 67.37% and 47.71%, respectively, at a concentration of 300 µg/ml and the value of the CC_{50} was found to be 249.2 and 314 µg/mL, respectively. Whereas, in the present study, the CC_{50} for the *Alternanthera pungens* against MDCK cell line was 593 µg/mL. The cytotoxic activity of methanolic extract of *C. scabrum* was investigated by Sahli *et al*. against the cell line J774 and WI38 and they reported 50% growth inhibition (CC_{50}) at a dose of 11.53 µg/mL and 29.89 µg/mL, respectively, whereas, in the present study, the CC_{50} for the *Cirsium arvense* against MDCK cell line was 515 µg/mL [20].

3.9. *In Silico* Study of Phytochemical against Microbes

3.9.1. Molecular docking

The molecular docking result revealed that the dihydropteroate synthase exhibited favorable binding affinity with tromethamine. The affinity of tromethamine with dihydropteroate synthase of *S. aureus* was −6.09 kcal/mol, whereas with *E. coli,* the affinity was −6.44 kcal/mol.

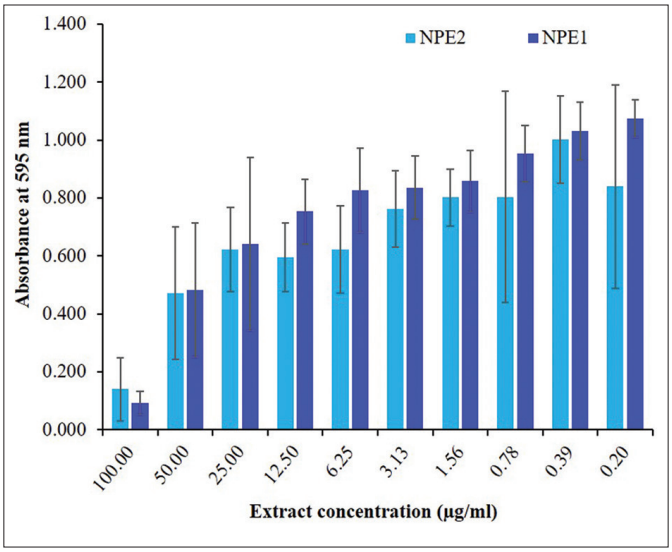

Figure 5: Minimum inhibition concentration (µg/mL) for the chloroform extracts from *Euphorbia heyneana* Spreng.

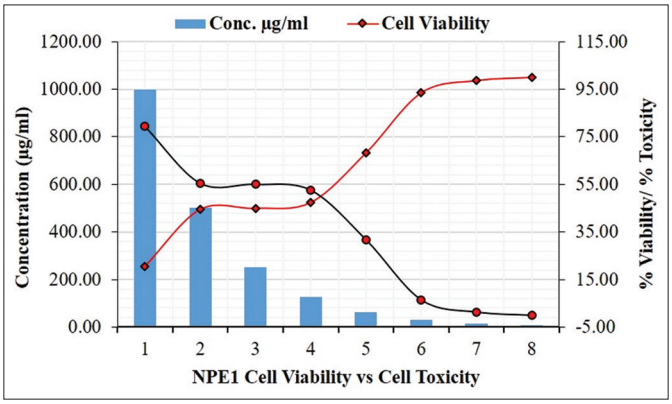

Figure 6: Cell viability against NPE1 extract of *Euphorbia heyneana* Spreng.

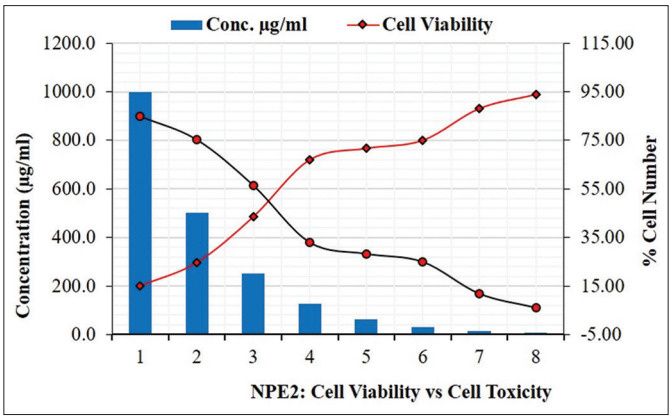

Figure 7: Cell viability against NPE2 extract of *Euphorbia heyneana* Spreng.

However, the binding affinity with 2-isopropyl-5-methyl-1-heptanol and dihydropteroate synthase of *S. aureus* was −6.02 kcal/mol, whereas with *E. coli,* the affinity was –5.15 kcal/mol. In the present study, the Muramyl ligase E (MurE) protein of *E. coli* had a strong binding affinity with only Terephthalic acid, 4-octyl octyl ester (−7.42 kcal/mol). No significant affinity was shown by any ligands other than Terephthalic

acid, 4-octyl octyl ester with MurE protein of Gram-negative and positive bacteria. According to the present study, *S. aureus* and *E. coli* protein DNA gyrase B showed a strong binding affinity with the compounds Tromethamine and Terephthalic acid, 4-octyl octyl ester. The binding energies between DNA gyrase B of *S. aureus* and *E. coli* with tromethamine were −6.23 and −6.80 kcal/mol, respectively, whereas, it was −7.36 and −7.96 kcal/mol, respectively, for DNA gyrase B. In the current study, terephthalic acid, 4-octyl octyl ester had the highest binding affinity with *S. aureus* (−7.24 kcal/mol) and *E. coli* protein transpeptidase (−8.36 kcal/mol). The binding affinity of cyclohexene, 1-methyl-5-(1-methyl ethenyl)-(R) with *S. aureus* transpeptidase was 7.24 kcal/mol while 9,12-octadecadienoic acid had a binding affinity of 6.27 and 6.67 kcal/mol, respectively, with the transpeptidase protein of *S. aureus* and *E. coli*.

Antibacterial activity of the fosfomycin tromethamine in urinary tract infections has been reported by several studies in the urinary tract infection [21, 22]. In a study, the therapeutic benefit of tromethamine was also reported in cystic fibrosis airway disease as it kills the bacteria [23]. The antibacterial activity of 2-isopropyl-5-methyl-1-heptanol was reported by many groups of researchers [24, 25]. The compounds isolated from the *Piper nigrum* contain cyclohexene, 1-methyl-5-(1-methyl ethenyl)-(R) and are reported to be antimicrobial agents [26].

4. CONCLUSION

The extracts derived from the plant *E. heyneana* Spreng demonstrated significant antimicrobial properties against several common pathogenic bacteria. Notably, the NPE1 and NPE2 fractions of *E. heyneana* Spreng effectively inhibited the growth of *P. aeruginosa*, a bacterium commonly associated with hospital-acquired infections and known for its multi-drug resistance. Given the alarming rise of antimicrobial resistance, the findings suggest that *E. heyneana* Spreng holds potential as a valuable resource for medicinal applications, particularly in the development of new antimicrobial agents. However, it is essential to conduct further research to thoroughly characterize and identify the specific compounds responsible for this antimicrobial activity, paving the way for potential therapeutic uses.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

The data supporting the findings of this study are available within the article.

10. PUBLISHER'S NOTE

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11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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