FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 8, August, 2025, pp 322 – 329
DOI: https://doi.org/10.33003/fjs-2025-0908-3927

SPECTROSCOPIC ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *EMILIA PRAETERMISSA* DICHLOROMETHANE FRACTION

Beauty Ighomena and *Emmanuel E. Odion

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

*Corresponding authors' email: emmanuel.odion@uniben.edu

ABSTRACT

The search for better antibacterial agents from plants has resulted in the identification of molecules with existing analogues. *Emilia praetermissa* is an herbaceous plant that is traditionally used to treat bacterial skin infections and diarrhea. This study aimed to identify compounds in partially purified *E. praetermissa* and investigate their antibacterial activity. Gradient elution chromatography was used to achieve partial purification of the compounds. Gas chromatography–mass spectrometry and Fourier transform infrared spectroscopy were used to identify the phytochemicals from the eluent of interest (EP1). The agar well diffusion plate protocol enabled the assessment of the susceptibility profile of the eluents (EP1-EP11) (10 mg/mL) against clinical isolates: *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus*. EP1 revealed fourteen compounds, belonging to alkane, amide, butylphenol, phthalate, triazole and pericyclic compounds. The functional groups identified include O-H, N-H, C-H and C-C, which combine with the data from the GC–MS. Susceptibility testing revealed maximum inhibition of S. aureus (18±0.11) mm, B. subtilis (12±0.12) mm and *E. coli* (14±0.20) mm by various eluents EP1-EP11, excluding EP4, EP5 and EP10.The results show that the identified compounds inhibit the pathogenic bacteria responsible for inducing skin infections and diarrhea, confirming their traditional use.

Keywords: *Emilia praetermissa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis,* Gradient elution

INTRODUCTION

Emilia praetermissa Milne Redhead (Asteraceae) is an herbaceous plant that originated in West Africa and is widely distributed on other continents, such as the Caribbean and Asia (Chung et al., 2009; Graveson, 2016; POWO, 2020), where it was initially misidentified as *Emilia forsbegii* (Chung et al., 2009). It is habitually known for its invasive nature in both cultivated and fallow lands. According to reports locally, *E. praetermissa* is very effective in the treatment of internal and external wounds, bacterial diarrhea, and respiratory infections and in preventing newborn skin infections (Lisowski, 1997).

Bacteria have been implicated in several skin infections and diarrheal-related diseases (Collins et al., 2022). These entities are microscopic, ubiquitous, single-cell organisms that are broadly classified as gram-positive or gram-negative (Sizar et al., 2025). Gram-positive bacteria are characterized by a thick peptidoglycan cell wall, whereas gram-negative bacteria possess a thin peptidoglycan wall with a cell membrane (Shugar and Baranowska, 1954). Many of these bacteria can be found within and outside the body, where they have either beneficial or harmful effects. Some examples of gram-positive bacteria include *Staphylococcus aureus* and *Bacillus subtilis*, whereas gram-negative bacteria include *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* (Tripathi et al., 2025).

S. aureus are cocci measuring 1 µm in diameter and are distinguished from other species in the genus by their coagulase-positive nature, salt leniency and regular hemolysis (Foster, 1996). It has been implicated in several forms of infection, ranging from superficial skin lesions to deep seat and nosocomial infections. It has also been reported to cause food poisoning through enterotoxin release into foodstuff and toxic shock syndrome into the blood through the release of super antigens (Easmon and Adlam, 1983; Foster, 1991; Foster and McDevitt, 1994). *S. aureus* colonizes the skin and nasal passage while exerting its virulence by releasing surface proteins that promote the colonization of host tissue (Bhakdi and Tranum-Jensen, 1991). The release of capsules and immunoglobulin-binding protein A promotes phagocytosis and toxin release, which damage host tissues (Lyon and Skurray, 1987; Provost et al., 1995). *E. coli* are rods with a facultative anaerobic nature capable of respiratory and fermentative metabolism. In humans, it inhibits the gastrointestinal tract, where it forms part of the normal microbiota (Prevost et al., 1995; Blount, 2015). It exists singly or in pairs, measuring 1–3 µm × 0.–0.7 µm in size and is considered to be non-spore-forming and non-acid fast (Campbell and Reece, 2002). Although many strains of *E. coli* are considered safe, some serotypes may cause diarrhea when consumed with food or drink; others may cause anaemia, while infections may be observed in some body organs and tracts (Campbell and Reece, 2022). Virulence may be acquired in *E. coli* by bacteriophage and plasmid formation, with pathogenicity islands encoding virulence factors and transporons within a genome (Basavaraju and Gunashree, 2023).

The search for better antibacterial agents has necessitated the study of plant materials because of their believed safety, low untoward effects and cost effectiveness. Extracts from plants are known to contain phytochemicals with similar or better analogues than existing drugs. The antibacterial nature of the leaf extract of *E. praetermissa* was established in a previous study by our group, and GC–MS analysis was also used to obtain a list of identified phytocompounds with likely antibacterial activities (Ighomena et al., 2025). This study intends to narrow down the identified compounds by partial purification of the dichloromethane fraction from the extract that has previously shown antibacterial activity. Thus this research aims to identify compounds with antibacterial potential from the leaves of E. praetermissa.

MATERIALS AND METHODS

Collection, Identification and Preparation of the Plants

Fresh aerial parts of E. praetermissa were collected in October within the hours of 7:00 am - 8:30 am at the University of

Benin, Ugbowo Campus, with geo-references at latitude 60 23' 30"N and longitude 50 36' 30"E. Identification and authentication were performed by Professor Emmanuel Aigbokhan of the Plant Biology and Biotechnology Department. The herbarium voucher number was UBH-E407, while further authentication was performed by uploading the picture to Google image service. A specimen of E. praetermissa was placed in the herbarium for prospective reference. The leaves were carefully separated from the stem and air dried away from direct sunlight for a duration of 25 days. The sample was reduced to a fine powder with the aid of an electric milling machine, and an airtight container was used to store the pulverized sample until further work was carried out.

Extraction and Partial Purification

Extraction was performed via maceration using 2x2.5 L of methanol and 400 g of the powdered sample. This setup was left for 48 h with stirring at intervals of 12 h; this mixture was subsequently decanted into a 3 L beaker and passed through size 1 filter paper. A rotary evaporator under reduced pressure, maintained at a temperature of 50°C was used to concentrate the filtrate, and the weight of the extract obtained was noted (35.00 g) and subsequently kept in a refrigerator at 4°C until use.

The crude extract (26.00 g) was adsorbed to 25 g of silica gel (mesh size 60–120 µm) until it became powdery, after which it was transferred into a column (measuring 5 cm × 50 cm) packed with silica gel (200 g). N-hexane (600 mL) was isocratically used to elute the column, and the eluent was collected. This process was repeated by gradient elution using n-hexane-dichloromethane at different ratios: 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. Each variation in solvent mixture resulted in a total volume of 300 mL and a total of 292 test tubes used to collect the eluent from the column. These samples were observed to have different colours ranging from yellow to deep green and pale green. The eluents were bulked together based on TLC profile into eleven groups (EP1-EP11), though crystals were observed in test tubes labelled 41--67 (EP1); hence, they became our focus. The white crystals were subjected to gas chromatography–mass spectrometry (GC–MS) and Fourier transform infrared (FTIR) spectroscopic analysis, and the antibacterial potential of the eluents (EP1--EP11) was evaluated.

GC–MS analysis

This analysis was carried out via coupled GC–MS with a triple-axis detector and a 10 µL syringe autosampler. The GC system (7890A) was an Agilent USA model, while the MS was an inert MSD 5675C. A capillary column from Agilent (19091-433HP-5Ms) treated with 5% phenyl, 95% methyl polysiloxane and measuring 30 m (length), 0.2 µm (internal diameter), and 250 µm (thickness) was utilized for chromatographic separation, while helium was the carrier gas. The ion source and interface temperatures were set at 250°C and 300°C, 16.2 psi as the pressure and a 1:50 split ratio with a 1 µl injector in split mode, while 280°C was the injection temperature. The temperature of the column was started at 50°C for 2 min and adjusted at a rate of 20°C/min to 100°C. This mixture was elevated to 250°C at 20°C/min and maintained for 5 min. While acquiring data, software from the manufacturer was utilized to control the system, and compound qualification was achieved by mass spectra comparison with data from previous research and NIST with minimum similarity index of 90 % (Odion et al., 2024).

FTIR Spectroscopy

Functional group determination in EP1 was performed via a Perkin Elmer 3000 MX spectrometer. This process involves mixing a few crystals of EP1 with potassium bromide and reducing them to a fine homogenous powder in a mortar (agate). This formed an appropriate pellet following the application of a pressure of 6350 kg. The spectra were recorded following scanning in the wavenumber range between 4000 cm-1 and 650 cm-1. The resolution was set at 4 cm-1, and the scan per spectrum was 32 cm-1. Analysis of the infrared spectra was performed via WIN-IR Pro Version 3.0, with a sensitivity of the peak at 2 cm-1.

Antimicrobial activity

Preparation of media and agar plates

A total of 38 g of Mueller–Hinton agar was added to 1 L of distilled water in a conical flask, and this mixture was heated to 100°C while stirring. The mixture was covered with foil paper and autoclaved for 15 min at 121°C. The mixture was allowed to cool to 45°C and subsequently dispensed (30 mL) under a sterile lamp into Petri dishes, while the plates were allowed to set and solidify. The samples were dried in a hot air oven at 50°C for 10 minutes to remove excess surface moisture.

Collection and preparation of bacterial cultures

The drug-resistant clinical isolate of the bacteria used for this study was obtained from the University of Benin Teaching Hospital and was cultured overnight (24 h at 37°C) in sterile nutrient broth by inoculation. The bacteria used for this study included *S. aureus, K. pneumoniae, E. coli, B. subtilis* and *P. aeruginosa*. Identical colonies from these plates were inoculated into sterile nutrient broths. These mixtures were allowed to incubate for 12 h and subsequently adjusted with the MacFarland standard (0.5%) to obtain an inoculum size of 108 cfu/mL. These concentrations were further adjusted to 106 cfu/mL by diluting the inoculum to 1:100.

The modified agar well diffusion method was used in the susceptibility test; this was achieved by thoroughly mixing 200 µL of standard inoculum with 30 mL of sterile Mueller-Hinton agar, which was allowed to solidify. A sterile cork borer (8 mm in diameter) was used to cut the wells into the agar plate. The base of each well was sealed with molten Mueller-Hinton agar to create a uniform and stable condition for proper diffusion and interaction of the fraction or eluent against the bacteria (Golus et al., 2016). This was achieved using a calibrated micropipette with a rubber teat, 10 mg/mL of the eluent (EP1--EP11) was delivered into each well. The plates were allowed to stand for 30 min before being incubated upside down at 37°C for 24 h. Inhibition zone diameters (measured in millimetres) of each eluent were measured and recorded. A similar protocol was repeated for the control, ciprofloxacin (0.5 mg/mL) (Babaiwa et al., 2017).

Statistical Analysis

The antimicrobial test was performed in triplicate, and the data are presented as the means ± standard errors of the means. The data were analysed via one-way analysis of variance. A post hoc study was performed via Tukey analysis, and the level of significance was set at $p \leq 0.05$ (***), using version 5 of the GraphPad Prism.

RESULTS AND DISCUSSION

Following extraction, the crude extract was adsorbed onto silica gel, and different solvent ratios (dichloromethane--hexane) were used to elute the prepared column. The eluents were collected in test tubes (Table 1), which were subsequently bulked (EP1--EP11) together on the basis of thin-layer chromatographic profiles (Table 2).

Table 1: Partially Purified Crude Extract of Emilia Praetermissa

Solvent Mix	Solvent Ratio	Volume (mL)	Test Tubes
Hexane	100	600	1-40
Hexane-dichloromethane	90:10	300	41-67
Hexane-dichloromethane	80:20	300	68-91
Hexane-dichloromethane	70:30	300	92-118
Hexane-dichloromethane	60:40	300	119-142
Hexane-dichloromethane	50:50	300	143-169
Hexane-dichloromethane	40:60	300	170-193
Hexane-dichloromethane	30:70	300	194-221
Hexane-dichloromethane	20:80	300	220-242
Hexane-dichloromethane	10:90	300	243-269
Dichloromethane	100	300	270-292

Table 2: Coding of Eluents from Test Tubes

Coding	Test Tube
EP1	41-67
EP2	68-80
EP3	81-96
EP4	97-111
EP5	112-143
EP6	144-157
EP7	158-197
EP8	198-224
EP9	225-240
EP10	241-268
EP11	269-292

Susceptibility Test

The antibacterial potential of the eluents was evaluated against five bacterial species, *B. subtilis*, *E. coli*, *K. pneumoniae, S. aureus* and *P. aeruginosa*, via the agar well diffusion method previously described by (Babaiwa et al., 2017). Ciprofloxacin was utilized as the reference drug at 0.5 mg/mL. *E. coli, B. subtilis* and *S. aureus* were susceptible to 10 mg/ml EP1-EP4, EP6-EP9 and EP11, respectively (Table 3). The zone of inhibition was 12±0.12 mm (EP4) for B. subtilis, ranging from 12 mm to 14 mm (EP1-EP3, EP6-EP9 and EP11) for *E. coli* and 14 mm to 18 mm (EP1-EP3, EP6-EP9 and EP11) for *S. aureus*. The effects of the EP2, EP3, EP7 and EP10 strains tested against *E. coli* were significant compared with those of ciprofloxacin, with a level of significance at $p \leq 0.05$. Similarly, EP2, EP6 and EP11 were significantly different at $p \leq 0.05$ compared with the standard drug with *S. aureus. B. subtilis* showed susceptibility to EP4, while resistance was observed against other eluent. *E. coli* and *S. aureus* were observed to be susceptible to EP1-EP3 and EP6-EP11 eluents while EP4, EP5 and EP10 were observed to be resistance. Maintaining similar concentrations, resistance was observed against *P. aeruginosa* and *K. pneumoniae* via EP1 - EP11.

Unpublish study for our Laboratory revealed the susceptibility of *P. aeruginosa* to dichloromethane fraction of *E. praetermissa*. Further purification of the fraction in this study (EP1 to EP11), showed activities against *S. aureus, B. subtilis* and *E. coli* while *P. aeruginosa* was resistance. This could be due to the synergistic or additive effects of the dichlorormethane fraction, which could have been loss during the partial purification process. Also resistance may have been observed in gram-negative bacteria, but susceptibility seen with *E. coli* may indicate that these eluents possess specific related antibacterial actions. The specific mechanism by which these genes confer resistance could involve efflux pump action, permeability barrier induction, mutational or recombination changes in target enzymes and alterations in targets with decreased affinity for drug compounds (Gitau et al., 2018). Additionally, resistance could hypothetically be due to virulence (Boraschi, 2022; Fantone and Boraschi, 2022). The susceptibility of *S. aureus, E. coli* and *B. subtilis* to these eluents could imply their use in the treatment of skin infection and diarrhea caused by these bacteria.

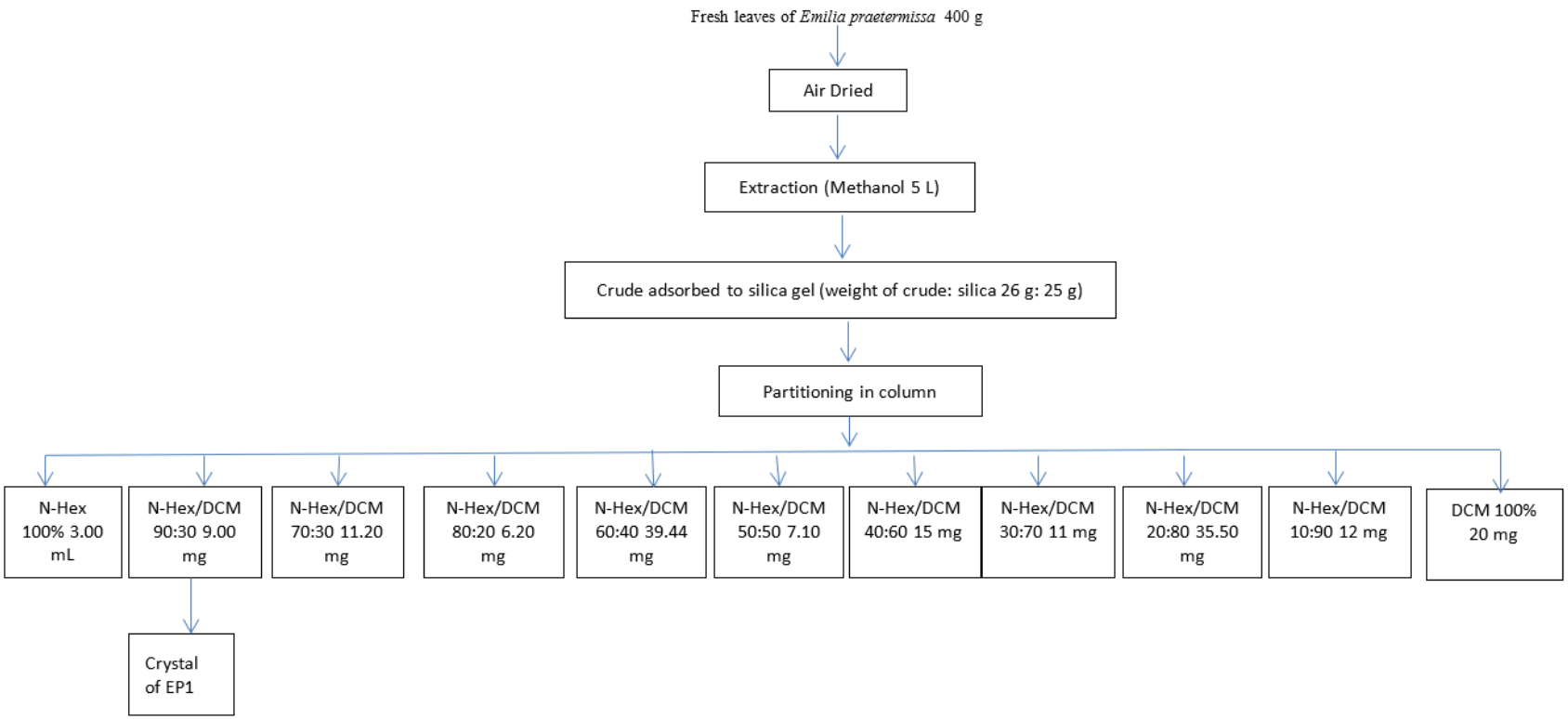

Figure 1: Fractional Purification of Antibacterial Compounds from the Crude Emilia Praetermissa

Table 3: Antibacterial Activity

Bacteria	EP1	EP2	EP3	EP4	EP5	EP6	EP7	EP8	EP9	EP10	EP11	Ciprofloxacin
Escherichia coli	14±0.20	13±0.11	12±0.11a	NZ	NZ	13±0.10	14±0.10a	13±0.11	13±0.12	NZ	13±0.00a	13±0.11
Klebsiella pneumoniae	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	22±0.20
Pseudomonas aeruginosa	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	30±0.14
Staphylococcus aureus	16±0.10a	18±0.11a	15±0.21	NZ	NZ	18±0.10a	18±0.10a	18±0.08a	18±0.05a	NZ	14±0.20a	26±0.12
Bacillus subtilis	NZ	NZ	NZ	12±0.12	NZ	NZ	NZ	NZ	NZ	NZ	NZ	30±0.22

Keywords: NZ = No zone of inhibition; zones of inhibition were measured in millimetres. Significant at aP>0.05, compared to control (n=3)

FTIR Analysis

The functional groups in the EP1 eluent were determined from the absorption spectrum from the FTIR data. The absorption spectrum is provided in Figure 2, while a summary of the absorption range and specific functional groups in EP1 are provided in Table 4. The main functional groups include the O–H bond of the hydroxyl group and the N–H medium bond of the secondary amine and amide. C-H and C-C stretches were observed for saturated alkanes and rings. The C=C bonds and C=C stretches are indicative of alkene and alkyne groups. The H-C=O stretch for the aldehydric group and overtones was observed at 1919.60 cm-1, although it was weak. Towards the end of the group frequency region, N-O symmetric stretching and C-O stretching were observed, representing nitrile and aldehydric, amide and ester groups, respectively.

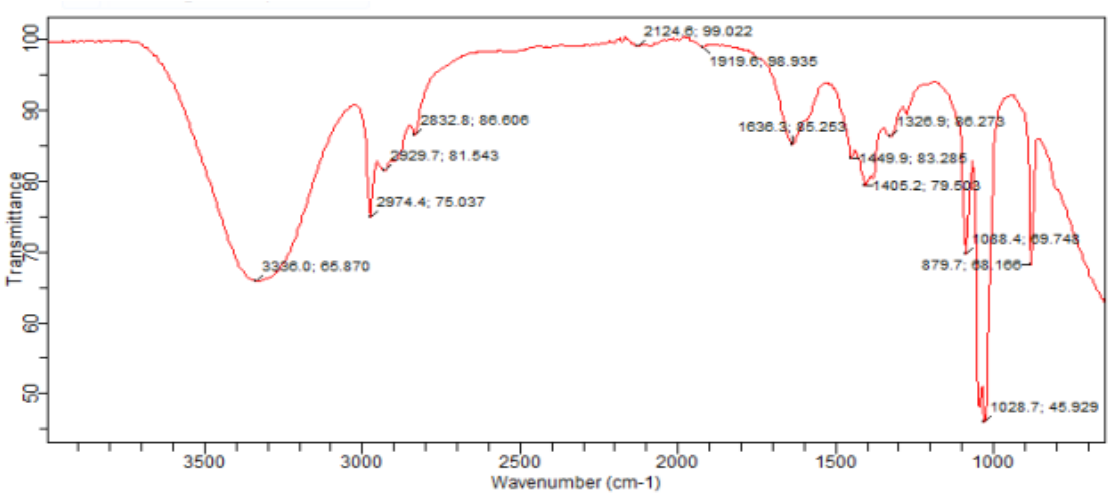

Figure 2: FTIR spectra of EP1

Table 4: FTIR Spectra Interpretation

S/N	Wavenumber (Reference)	Wavenumber (Test Sample)	Functional group assignment	Inference
1	3650-3200	3336.00	O-H, N-H	N-H medium, H-bonded
2	3000-2850	2974.40	C-H	C-H stretching bond
3	3000-2850	2929.70	C-H	C-H stretching bond
4	2830-2695	2832.80	H-C=O	H-C=O stretch
5	2260-2100	2124.60	-C=C-	C=C stretch
6	2000-1665	1919.60		Overtones, weak from this range
7	1680-1620	1636.30	C=C	C=C bonds
8	1500-1400	1449.90	C-C	C-C stretch in ring
9	1360-1290	1326.90	N-O	N-O symmetric stretch
10	1260-1050	1088.40	C-O	C-O stretch
11	1300-1000	1028.40	C-O	C-O stretch
12	1000-650	879.70	=C-H	=C-H bend

GC–MS analysis

The chromatogram of EP1 revealed fourteen compounds (peaks) (Figure 3), with retention times ranging from 4.792 min to 19.944 min. The prominent compounds were observed at 9.319 min, 9.719 min, 13.662 min, 13.902 min, and 19.944 min, with a total percentage area of 82.70%, while the individual percentage areas were as follows: 15.74%, 7.90%, 5.52%, 46.92%, and 6.62%, respectively.

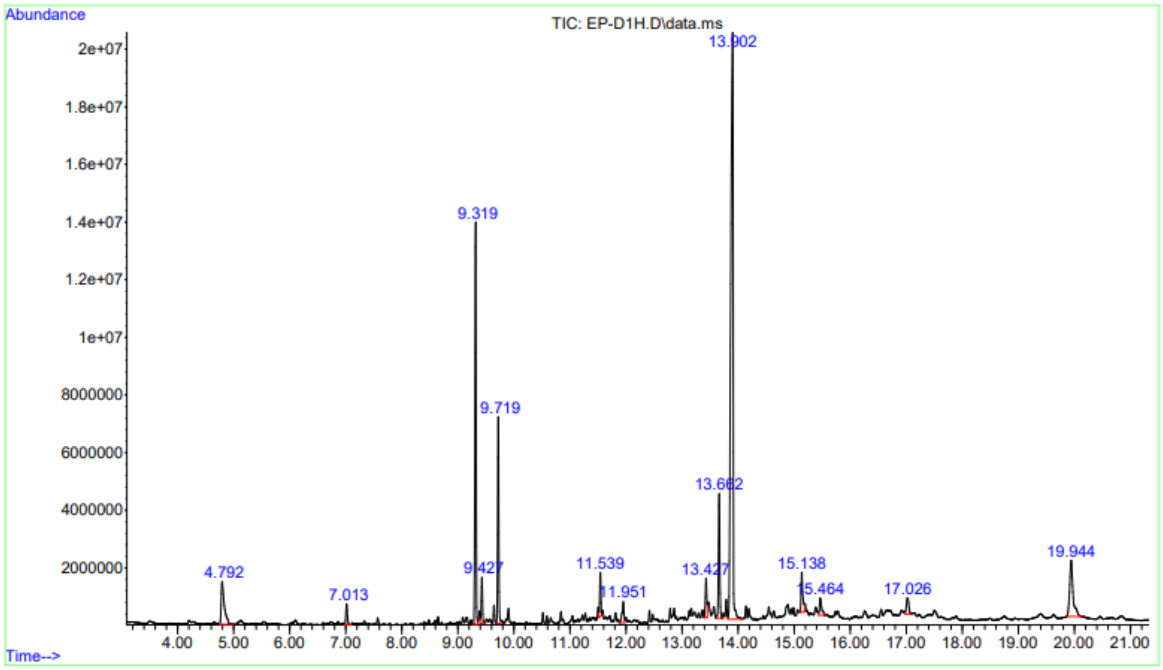

Figure 3: Chromatogram of EP1

These fourteen compounds are mainly triazole, straight-chain alkanes, epoxide, amide and phthalate (Table 5). The prominent compounds are 2-ethyl-2H-benzotriazole (4.51%), N-(3-Allyl-2-oxo-2,3-dihydro-1,3-b enzothiazol-6-yl) acetamide (5.52%), bis(2-ethylhexyl) phthalate (6.62%), 2,4-di-tert-butylphenol (7.90%), (4aS)-3,5,5,9-tetramethyl-1,2,5,6,7,8-hexahydro-4a,8-epoxybenzo[7]annulene (15.74%) and di-sec-butyl phthalate (46.92%). These findings indicate a high level of phthalates in EP1 (53.54%). In a previous study, bis-(2-ethylhexyl) phthalate was shown to have antibacterial activity against *E. coli* and *S. aureus* (Craig et al., 2006). In contrast, di-sec-butyl phthalate, which is isolated from the whole plant of Begonia malabarica (Begoniaceae), has shown antibacterial potential against *K. pneumoniae, P. aeruginosa* and *E. coli* at 100 mg/mL (Javed et al., 2022). 2,4-Di-tert-butylphenol purified from the Streptomyces species KCA1 from Phyllanthus niruri inhibited *S. aureus* and *E. coli* at 0.78 µg/mL and 50 µg/mL (Shobi and Viswanathan, 2018). These findings contradict those of this study, especially with respect to the inhibition of *K. pneumonia* and *P. aeruginosa*.

Table 5: Compounds Identified from EP1 from the Retention time, Percentage area and Molecular Weight

S/N	Retention Time (min)	Percentage Area	Base peak	Molecular weight	Compound
1	4.792	4.51	91.0	147.0	2-ethyl-2H-Benzotriazole
2	7.013	1.09	57.0	268.5	Nonadecane
3	9.319	15.74	161.0	218.3	(4aS)-3,5,5,9-Tetramethyl-1,2,5,6,7,8-hexahydro-4a,8-epoxybenzo[7]annulene
4	9.427	1.87	57.0	212.4	Pentadecane
5	9.719	7.90	191.0	206.3	2,4-Di-tert-butylphenol
6	11.539	1.77	57.0	280.7	Heptacosane
7	11.951	1.34	57.0	240.5	Heptadecane
8	13.427	1.85	57.0	280.7	Heptacosane
9	13.662	5.52	231.0	248.3	N-(3-Allyl-2-oxo-2,3-dihydro-1,3-b enzothiazol-6-yl) acetamide
10	13.902	46.92	149.0	278.3	Di-sec-butyl phthalate
11	15.138	2.33	57.0	282.5	Eicosane
12	15.464	1.23	57.0	282.5	Eicosane
13	17.026	1.31	57.0	296.6	Heneicosane
14	19.944	6.62	149.0	390.6	Bis(2-ethylhexyl) phthalate

Savelli et al., (1988) reported the use of derivatives of 2-ethyl-2H-benzotriazole as antimicrobial agents, whereas Nuvole et al. (1989) reported the susceptibility of *E. coli* to acidic derivatives of benzotriazole. This compound is made of a benzene ring fused to a triazole; this triazole ring possesses antimicrobial activity, which can be partially or completely lost following annulation of the triazole ring at any point Nuvole et al.,(1989). Pentadecane, heptadecane, eicosane, heneicosane and nonadecane, with a percentage area of 12.79%, are straight alkanes; some have previously been reported in E. coccinea (Sanna et al., 1992) to have antimicrobial activity (Mihigo et al., 2015). Derivatives of nonadecane have been reported to have moderate antibacterial effects against *E. coli, S. aureus, P. aeruginosa* and *B. subtilis*

(Marrero et al., 2010; Begum et al., 2016). The presence of these compounds in the eluent EP1 may be responsible for the antibacterial activity observed and could be synergistic in action against these bacteria.

CONCLUSION

Gradient eluents from hexane-dichloromethane of E. praetermissa were shown to possess antibacterial activity against selected clinical isolates. Antibacterial activity was observed could be correlated to the presence of long-chain alkanes, benzotriazole, phthalate and butylphenol derivatives in the EP1 eluent. It is likely due to their individual effect, synenergistic or additive effects. Thus providing support for its traditional use in the treatment of skin infection and diarrhea, since some of the bacteria involved in these conditions are inhibited. A major limitation in this study is the presence of phthalate, a documented plasticizer with documented antibacterial property. This study has pave way for further search to elucidate the mechanism by which the extract exert it effects on the different bacteria isolates, with isolation and characterization of the compounds responsible for this effect.

ACKNOWLEDGEMENT

The authors wish to especially thank Mr. Wilfred Aisagbonbuomwan, a laboratory staff member of the Department of Pharmaceutical Microbiology, for assisting with the microbial tests.

REFERENCES

Babaiwa, U.F., Erharuyi, O., Falodun, A. and Akerele, J.O. (2017). Antimicrobial Activity of Ethylacetate Extract of Citrullus lanatus Seeds. Tropical Journal of Pharmaceutical Research. 16 (7): 1631-1636. https://doi.org/10.4314/tjpr.v16i7.23.

Basavaraju, M. and Gunashree, B.S. (2023). Escherichia coli: An Overview of Main Characteristics [Internet]. Escherichia coli - Old and New Insights. IntechOpen. http://dx.doi.org/10.5772/intechopen.105508.

Begum, I.F., Mohankumar, R., Jeevan, M. and Ramani, K. (2016). GC–MS Analysis of Bio-Active Molecules Derived From Paracoccus pantotrophus FMR19 and The Antimicrobial Activity Against Bacterial Pathogens and MDROs. Indian Journal of Microbiology 56:426–32. https://doi.org/10.1007/s12088-016-0609-1.

Bhakdi, S. and Tranum-Jensen, J. (1991). Alpha-toxin of Staphylococcus aureus. Microbiology Review. 55:733.
Blount, Z.D. (2015). The Unexhausted Potential of E. coli. eLife 25(4):e05826. https://doi.org/10.7554/eLife.05826.

Boraschi D. (2022). What Is IL-1 for? The Functions of Interleukin-1 Across Evolution. Frontier in Immunology. 13:872155. https://doi.org/10.3389/fimmu.2022.872155.

Campbell, N.A. and Reece, J.B. (2002). Biology. San Francisco: Pearson Education Incooperation.

Chung, K-F., Ku, S-M., Kono, Y and Peng, C-I. (2009). Emilia praetermissa Milne-Redh. (Asteraceae) - A Misidentified Alien Species in Northern Taiwan. Taiwania 54 (4), 385-390.

Collins, J.P., Shah, H.J., Weller, D.L., Ray, L.C., Smith, K., McGuire, S., Trevejo, R.T., Jervis, R.H., Vugia, D.J., Rissman, T., Garman, K.N., Lathrop, S., LaClair, B., Boyle, M.M., Harris, S., Kufel, J.Z., Tauxe, R.V., Bruce, B.B., Rose, E.B., Griffin, P.M. and Payne, D.C. (2022) Preliminary Incidence and Trends of Infections Caused by Pathogens Transmitted Commonly Through Food - Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2016-2021. Morbidity and Mortality Weekly Report 71(40):1260-1264.

Craig, R., Rayner, C.R. and Munckhof, W.J. (2006). Antibiotics Currently used in The Treatment of Infections Caused by Staphylococcus aureus. Internal Medicine Journal 35.2(2):S3-16. https://doi.org/10.1111/j.1444-0903.2005.00976.x.

Easmon, C.S.F. and Adlam C. (1983). Staphylococci and staphylococcal infections. Vols 1 and 2. Academic Press, London.

Fantone, J.C. (1985). Mechanisms of Chemotactic Factor Stimulation of Polymorphonuclear Leukocytes: Modulation by Prostaglandins. In: Higgs, G.A., Williams, T.J. (eds) Inflammatory Mediators. Satellite Symposia of the IUPHAR 9th International Congress of Pharmacology. Palgrave Macmillan, London. https://doi.org/10.1007/978-1-349-07834-9_12.

Foster, T.J. (1991). Potential for Vaccination Against Infections Caused by Staphylococcus aureus. Vaccine 9:221.

Foster, T.J. and McDevitt, D. (1994). Molecular basis of adherence of staphylococci to biomaterials. p. 31, In Bisno AL, Waldvogel FA (eds): Infections Associated with Indwelling Medical Devices, 2nd Edition. American Society for Microbiology, Washington, D.C.

Foster, T. (1996). Staphylococcus. In: Baron S, Editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 12. https://www.ncbi.nlm.nih.gov/books/NBK8448/

Gitau, W., Masika, M., Musyoki, M., Museve, B. and Mutwiri T. (2018). Antimicrobial Susceptibility Pattern of Staphylococcus aureus Isolates from Clinical Specimens at Kenyatta National Hospital. BMC Research Notes. 11(1):226. https://doi.org/10.1186/s13104-018-3337-2.

Golus, J., Sawicki, R., Widelski, J., Ginalska, G. (2016).The agar microdilution method – a new method for antimicrobial susceptibility testing for essential oils and plant extracts. Journal of Applied Microbiology. https://doi.org/10.1111/jam.13253.

Graveson, R. (2016). Plants of Saint Lucia: A Pictorial Flora of Wild and Cultivated Vascular Plants.

Javed, M.R., Salman, M., Tariq, A., Tawab, A., Zahoor, M.K., Naheed, S., Shahid, M., Ijaz, A. and Ali, H. (2022). Antibacterial and Larvicidal Potential of Bis-(2-Ethylhexyl) Phthalate from Lactiplantibacillus plantarum. Molecules 25; 27(21): 7220. https://doi.org/1010.3390/molecules27217220.

Kaper, J., Nataro, J. and Mobley, H. (2004). Pathogenic Escherichia coli. Nature Reviews. Microbiology. 2:123-140. https://doi.org/10.1038/nrmicro818.

Lisowski, S. (1997). Le genre Emilia (Cass) Cass. (Asteraceae) dans la flore de Guinée (Afrique Occidentale).

Bulletin du Jardin Botanique National de Belgique 66:201-206.

Lyon, B.R. & Skurray, R. (1987). Antimicrobial Resistance in Staphylococcus aureus: Genetic Basis. Microbiology Reviews 51:88.

Marrero, J., Rodrı´guez, I.I. and Rodrı´guez, A.D. (2010). The Natural Products Chemistry of the Gorgonian Genus Pseudopterogorgia (Octocorallia: Gorgoniidae) University of Puerto Rico, San Juan, PR, USA. Elsevier. 407.

Mihigo, S.O., Tekayela, M., Masesane, I.B. and Sichilongo, K. (2015). Preliminary GC–MS Profiling and Antibacterial Activity Investigation of Emilia coccinea (Sims) G. Don (Asteraceae). International Journal of Chemistry and Aquatic Sciences 1(2) 31-36.

Naeim, H., El-Hawiet, A., Abdel Rahman, R.A, Hussein, A., El Demellawy, M.A. and Amira M.. (2020). Antibacterial Activity of Centaurea pumilio L. Root and Aerial Part Extracts Against Some Multidrug Resistant Bacteria. BMC Complement Medicine and Therapies 20, 79. https://doi.org/10.1186/s12906-020-2876-y.

Nuvole, A., Sanna, P., Paglietti, G., Juliano, C., Zanetti, S. and Cappuccinelli P. (1989). 1,2,3-triazolo[4,5-f]quinolines. II. Preparation and antimicrobial evaluation of 6-ethyl-6,9-dihydro-1(2)(3)-R-1(2) (3)H-triazolo [4,5-f]quinolin-9-one-8-carboxylic acids as anti-infectives of the urinary tract (1). Farm Science 44:619–632.

Odion, E.E., Ogboru, R.O. and Ighene M.O. (2020). Identification of Compounds in Elaeis guineensis Fruits using GC–MS. Dhaka University Journal of Pharmaceutical Sciences 19(2):153-159. https://doi.org/10.3329/dujps.v19i2.50631.

POWO. (2020). Plants of the World Online. In: Plants of the World Online, London, UK: Royal Botanic Gardens, Kew.

Prevost, G., Couppie, P., Prevost, P., Gayet, S., Petiau, P., Cribier, B., Monteil, H. and Piemont, Y. (1995). Epidemiological Data on Staphylococcus aureus Strains Producing Synergohymenotropic Toxins. Journal of Medical Microbiology (4):237-45. https://doi.org/10.1099/00222615-42-4-237.

Sanna, P., Carta, A., Paglietti, G., Zanetti, S. and Fadda G. (1992). 1,2,3-Triazolo[4,5-h]quinolines. III. Preparation and Antimicrobial Evaluation of 4-ethyl-4,7-dihydro-1(2)H Triazolo [4,5-h]quinolin-7-one-6-carboxylic acids as Anti-infectives of The Urinary Tract. Farm Science 47:1001–1019.

Savelli, F., Pau, A., Boido, A. and Sparatore F. (1988). Aminoalkyl Derivatives of Benzotriazole and Naphthotriazole. Bollettino Chimico Farmaceutico 127:144–147.

Seenivasan, A., Manikkam, R., Kaari, M., Sahu, A.K., Said, M. and Dastager S.G. (2022). 2,4-Di-tert-butylphenol (2,4-DTBP) Purified from Streptomyces sp. KCA1 from Phyllanthus niruri: Isolation, characterization, and antibacterial and anticancer properties. Journal of King Saud University - Science 34, 5, 102088. https://doi.org/10.1016/j.jksus.2022.102088.

Shobi, T.M. and Viswanathan, M.B.G. (2018). Antibacterial Activity of di-butyl phthalate Isolated from Begonia malabarica. Journal of Applied Biotechnology and Bioengineering 5(2): 97–100. https://doi.org/10.15406/jabb.2018.05.00123.

Shugar, D. and Baranowska, J. (1954). Studies on the Gram Stain; The Importance of Proteins in The Gram Reaction. Acta Microbiologica Polonica 3(1):11-20.

Sizar, O., Leslie, S.W. and Unakal, C.G. (2025). Gram-Positive Bacteria. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 1-5. https://www.ncbi.nlm.nih.gov/books/NBK470553/

Tripathi, N., Zubair, M. and Sapra, A. (2025). Gram Staining. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.1-7. https://www.ncbi.nlm.nih.gov/books/NBK562156/