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Polyherbal Extracts as Antibacterial Agents: Phytochemical Profiling, MIC Assessment and *in silico* Targeting of Resistance Proteins

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This research explores the use of polyherbal extracts from *Eclipta prostrata*, *E. alba* and *Tridax procumbens* as a potent antibacterial solution, reflecting their significant role in contemporary medicine. Through gas chromatography-mass spectrometry (GC-MS) analysis, 45 distinct chemical compounds were identified in the ethanol extract, with the ethyl acetate extract revealing 35 different compounds. The chemical profiles of these compounds revealed substantial structural diversity and several demonstrated noteworthy antibacterial potential. The minimum inhibitory concentration (MIC) values against various bacterial strains indicated effective antibacterial activity, particularly against *E. coli* and *P. mirabilis*, with MICs as low as 6.25 µg/mL. The study emphasized three compounds, including 6-acetyl-5-(4-fluorophenyl) purine, for their potential in drug development, attributed to their chemical properties such as the ability to form hydrogen bonds and favourable solubility and lipophilicity. These findings underscore the therapeutic potential of polyherbal formulations, highlighting their value in addressing bacterial infections. The docking study demonstrates that 6-(3-fluorobenzylamino) purine exhibits a strong affinity towards ompC, murA and ESBL proteins through various interactions, indicating its potential as an inhibitory agent against drug-resistant proteins in the *Enterobacteriaceae* family. The positive docking scores across different proteins and interaction types underscore the compound's versatility and potential effectiveness in combating antibiotic resistance. Further research could validate these *in silico* findings and explore the clinical applicability of such compounds in treating drug-resistant infections.

Keywords: Polyherbal extract, Bioactive compounds, Lipophilicity, Molecular docking, Antibacterial activity.

INTRODUCTION

Wound infections are the major clinical concerns, intensified by the antibiotic-resistant microorganisms from misuse and overuse of antibiotics, creating an urgent need for alternative therapies [1]. Traditional systems such as Ayurveda and Siddha offer valuable insights for novel drug discovery, particularly through polyherbal formulations with synergistic effects. This approach aims to explore safe, effective and nature-derived antimicrobial strategies to address current challenges in infection management [2,3] and also gaining popularity due to their natural, eco-friendly nature and minimal adverse effects compared to conventional medications [4].

Medicinal plants are rich sources of secondary metabolites with diverse biological activities, including anticancer, antimicrobial, antioxidant and anti-inflammatory properties. However, not all plant species have been thoroughly investigated for their potential bioactive compounds [5,6]. There is an urgent need for robust screening processes to identify new bioactive compounds from medicinal plants. Computational approaches play a vital role in drug discovery, enabling the screening of phytochemicals from medicinal plants for potential pharmaceutical applications. These approaches involve *in silico* prediction models for pharmacokinetics, pharmacology and toxicology [7,8]. While *Eclipta prostrate* [9], *Eclipta alba* [10] and *Tridax procumbens* [11] have been extensively

studied for their medicinal properties, there is still limited literature on certain aspects, such as the antibacterial and antifungal activities of *Eclipta hirta* [12].

In this study, a polyherbal preparation combining *Eclipta prostrata*, *Eclipta alba* and *Tridax procumbens* was evaluated for its ability to inhibit wound-infecting microorganisms. The research further aimed to identify the bioactive compounds in these plants and assess their potential as antibacterial agents using a molecular docking approach against specific target proteins associated with bacterial infections. The chosen targets such as ESBL (extended-spectrum β-lactamase), OmpC (outer membrane protein C) and MurA are critical for bacterial survival, as they play essential roles in cell wall synthesis and bacterial resistance mechanisms. By integrating traditional knowledge of medicinal plants with modern computational methods, this study seeks to identify promising antibacterial compounds and contribute to strategies for combating antibiotic resistance.

EXPERIMENTAL

Preparation of plant extracts: The leaves of *Eclipta prostrata*, *Eclipta alba* and *Tridax procumbens* were collected from the local garden and around 100 g each, cleaned and left to dry in a shady spot for a few days. After drying, the leaves were ground into powder and then stored in dry containers for further process [13].

Polyherbal extraction: The Soxhlet extraction technique was employed to extract bioactive compounds from a ground polyherbal mixture of the studied medicinal plant's leaves in a 1:1:1 ratio. Approximately 50 g of powdered mixture was placed into a cellulose thimble, which allows the solvent to pass through while retaining the solid material. The thimble was positioned in the Soxhlet chamber, which was then connected to a condenser and an extraction flask. The solvent, containing 95% ethanol for polar compounds and ethyl acetate for both polar and non-polar compounds, was heated to its boiling point. The extraction was carried out for 6 h, after that the crude extracts were collected and concentrated using a vacuum rotary evaporator. Ethanol was chosen for its efficiency in dissolving a wide range of polar compounds, while ethyl acetate allowed for the extraction of a broader spectrum of compounds.

Phytochemical analysis: For phytochemical analysis, the standard estimation method was followed. Alkaloids were identified with Mayer's reagent, carbohydrates with Molisch's reagent and sulphuric acid, and glycosides using chloroform and ammonia. Quinones were detected with concentrated sulphuric acid, while cardiac glycosides were confirmed by the formation of a brown ring at the interface with $FeCl_3$ and sulphuric acid. Phenols and tannins were revealed using $FeCl_3$, flavonoids with NaOH, and phytosterols and steroids using chloroform and concentrated H_2SO_4. Phlorotannins were detected with dilute HCl. The appearance of the characteristic colours or precipitates indicated the presence of each respective class of compounds.

GC-MS analysis: A GC Clarus 500 Perkin-Elmer system with an AOC-20i autosampler and a mass spectrometer interface was used to analyze gas chromatography-mass spectrometry (GC-MS). Helium was utilized at a split ratio of 10:1 as the carrier gas, keeping the ion source at 280 ºC and the injector at 250 ºC. The constant flow rate was 1 mL/min and the injection volume were 0.5 µL. The oven was programmed to an initial isothermal at 110 ºC for 2 min, followed by a temperature ramp of 10 ºC/min to 200 ºC, then 5 º/min to 280 ºC and a final isothermal at 280 ºC for 9 min.

Antibacterial susceptibility assay: The antimicrobial activity of the polyherbal extract (PHE) was assessed using the disc diffusion method as described by Bauer *et al*. [14]. Wound pathogens including *E. coli*, *S. aureus*, *P. aeruginosa*, *Proteus vulgaris*, *K. pneumoniae* and *A. baumannii*, were inoculated on Mueller-Hinton agar plates using the swab culture technique, with inoculum adjusted to 0.5 McFarland standard. Sterile discs were loaded with 100 µL of PHE at the desired concentration and placed on the inoculated plates. Plates were incubated at 37 °C for 24 h. Ethanol served as the negative control and ofloxacin (20 µg) as the positive control. Antimicrobial activity was determined by measuring the diameter of the inhibition zone around each disc.

Determination of minimum inhibitory concentration (MIC): To determine the minimum inhibitory concentration, extracts were prepared at 25, 50, 12.5, 6.25 and 100 µg/mL dilutions. Each dilution was inoculated with a standardized bacterial suspension containing approximately 1×10^6 CFU/mL. The inoculated test tubes were incubated at 37 ºC for 24 h. After 24 h of incubation, the test tubes were observed for bacterial growth. Resazurin dye was used to assess microbial viability and the MIC was defined as the lowest concentration showing no visible growth or color change.

Lead-likeliness properties: The desired compounds were retrieved from the PubChem database along with their Canonical SMILES. The generated molecular files of each phytochemical were compared with the NIST library to predict their physico-chemical and pharmacokinetic properties. Subsequently, ADMET (absorption, distribution, metabolism, excretion and toxicity) predictions were performed using tools such as SWISS ADME.

Target protein identification and preparation: The Research Collaborator for Structural Bioinformatics (RCSB) Protein data bank provided the three-dimensional structure of the ESBL (PDB: 6BU3). Initially, a pre-treated HMGR chain was isolated from other chains and ligands and its water molecules (hydrogen bond-free water) were observed crystallographically. The proteins' pre-existing ligands and water molecules were then removed using Pymol software and hydrogen atoms were added and saved in PDB format.

Screening of binding affinity: A structure-based method of drug design was used to anticipate the active site. UCSF Chimera Docking software was used to determine the proteins' binding site coordinates and active sites by molecular docking calculations using Auto-Dock 4.2. The protein Schiff base adducts' binding strength was computed. The docking of phytochemicals and ciprofloxacin (ligands) with the bacterial enzymes was investigated to determine suitable binding energy values.

RESULTS AND DISCUSSION

The qualitative phytochemical profiles of the polyherbal extract (PHE) extracted with ethanol and ethyl acetate is

shown in Table-1. Tannins and sterols were absent in both extracts, whereas carbohydrates, saponins, cardiac glycosides and phlorotannins were present. Moreover, only the ethyl acetate extract contained glycosides, anthraquinones, quinones and anthocyanins.

TABLE-1
QUALITATIVE PHYTOCHEMICAL SCREENING RESULTS OF POLYHERBAL EXTRACT

Test	Ethanol	Ethylacetate
Carbohydrate	+	+
Tannins	–	–
Galactoside	–	+
Alkaloids	+	+
Phenol	+	+
Flavonoid	+	+
Cardiac glycoside	+	+
Glycoside	–	+
Sterol	–	–
Quinone	–	+
Tannins	–	–
Saponins	+	+
Phlobatannins	+	+
Anthocyanin	–	+
Anthraquinone	–	+

GC-MS analysis of the ethanol extract (Fig. 1) revealed 45 distinct compounds matched with the NIST library, each showing unique retention peaks (Table-2). *Bis*(2-ethylhexyl)-phthalate is the predominant compound (20.6%, RT 34.977 min) and has been reported as a potent antimicrobial and larvicidal agent. Other major components included cyclohexasiloxane, dodecamethyl (6.78%, RT 13.979 min) and benzenesulfonic acid, 4-hydroxy (6.73%, RT 6.556 min), observed for strong bioactivity. Moderate levels of phenol, 4,4′-methylene bis (4.86%), 5,5-diethylpentadecane (3.73%), 11-eicosenoic acid (*Z*)-TMS (3.55%) were also detected, along with low concentrations of nonadecane, octadecane, dodecane, hexadecanoic acid, and pentadecanoic acid. Several unique compounds, including phenol derivatives, 2-propyldecahydroquinoline, 6-methylfuro[2,3-*c*]pyrid-5-one, cyclopentane-1,2′(1′H)quinoxaline, (4-hydroxyphenyl)guanidine and oxadiazole, were also identified.

The ethyl acetate extract (Fig. 2) revealed 35 bioactive compounds (Table-3), with cyclopentasiloxane, decamethyl eluting first (RT 10.044 min) and piperidine, 1-(1-cyclohexen-1-yl) eluting last (RT 39.405 min). New compounds such as piperidine, adipic acid, decahydroquinoline, nonadecane and fluoro-4-piperidine were detected. These findings align with previous reports identifying secondary metabolites in *Tridax procumbens* and are consistent with qualitative phytochemical data from related studies.

Screening of inhibitory effect: The PHE efficacy was tested against wound-related pyogenic pathogens like *E. coli*, *P. mirabilis*, *P. aeruginosa*, *A. baumannii*, *K. pneumonia* and *S. aureus* for their antibacterial activity. Zone of inhibition assay was carried out for their antibacterial efficacy. According to MIC analysis, it was found that all of the pathogens were extremely sensitive to 1 mg/mL ethyl acetate extract, but less sensitive to ethanol extract. The polyherbal extract (PHE) exhibited notable antibacterial activity against the tested wound pathogens (Table-4). The highest zone of inhibition was observed against *S. aureus* (17 ± .0001 mm), exceeding that of the standard ofloxacin (14 ± 0.001 mm). *P. mirabilis* was the second-most sensitive strain, with zones of 16 ± 0.0001 mm for PHE and 17 ± 0.0001 mm for the standard. Moderate inhibitory effects were observed against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*. These results are consistent with the previous studies reporting the antibacterial properties of *Tridax* stems [15], attributed to bioactive metabolites such as flavonoids, alkaloids, saponins, tannins and luteolin [16,17].

MIC analysis showed strain-specific variations, with the lowest values recorded for *E. coli* and *P. mirabilis* (6.25 µg/mL) and higher values for *A. baumannii* and *K. pneumoniae* (12.5 µg/mL). In comparison, ofloxacin MIC ranged from 50 to 100 µg/mL, with 25 µg/mL against *P. aeruginosa*. These findings indicate that the PHE possesses significant antibacterial potential, particularly against pyogenic pathogens, supporting earlier reports of its efficacy [18,19].

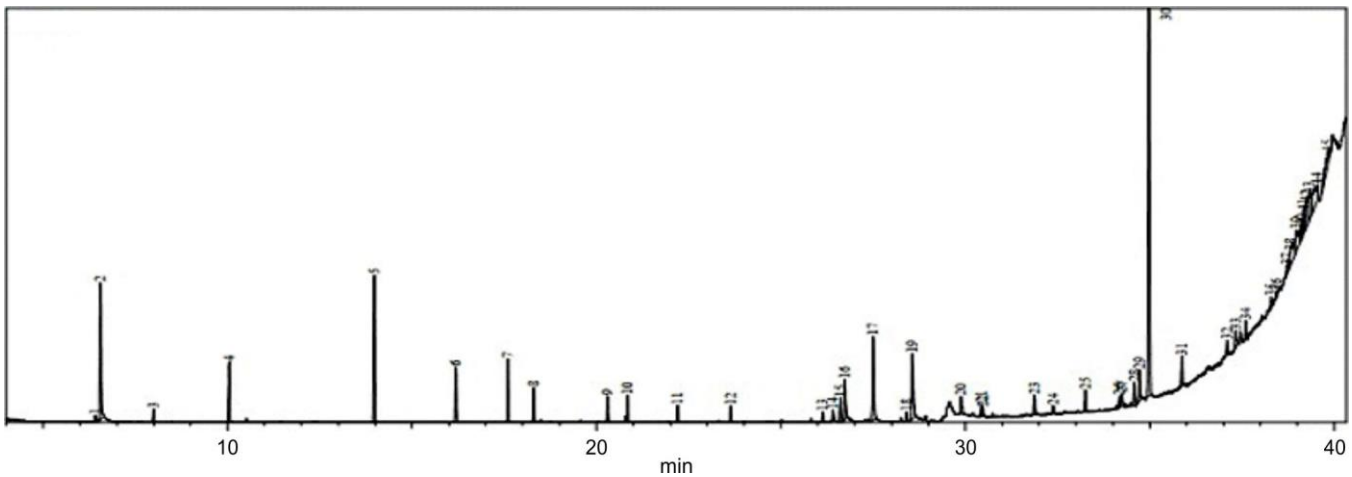

Fig. 1. Gas chromatography–mass spectrometry (GC-MS) spectrum of the ethanol extract showing 45 components with varying retention times. The major peak at a retention time (RT) of 34.977 min (peak 30) represents 20.6% of the total compounds detected

TABLE-2
LIST OF COMPOUNDS ISOLATED FROM ETHANOL EXTRACT OF POLYHERBAL

Compound	Peak	Ret. time	Area (%)
Silicic acid ($Si(OC_2H_5)_4$), tetraethyl ester	1	6.416	0.22
Benzenesulfonic acid, 4-hydroxy-	2	6.556	6.73
Benzaldehyde, 2-hydroxy-	3	7.996	0.54
Cyclopentasiloxane, decamethyl-	4	10.044	2.63
Cyclohexasiloxane, dodecamethyl-	5	13.979	6.78
Octadecane	6	16.188	2.43
Cycloheptasiloxane, tetradecamethyl-	7	17.599	2.82
Octadecane	8	18.298	1.55
Octadecane	9	20.300	1.16
Benzoic acid, 2,4-*bis*(trimethylsiloxy)-, trimethylsilyl ester	10	20.842	1.26
Octadecane	11	22.201	0.71
Cyclohexasiloxane, dodecamethyl-	12	23.643	0.70
3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	13	26.132	0.48
Adduct of diphenylketene with pentamethylcyclopenta-1,3-dienyl	14	26.413	0.44
6-Methylfuro[2,3-*c*]pyrid-5-one	15	26.614	1.21
N-Hexadecanoic acid	16	26.736	2.07
Phenol, 2-[(4-hydroxyphenyl)methyl]-	17	27.500	5.30
Benzoic acid, 2,4-*bis*(trimethylsiloxy)-, trimethylsilyl ester	18	28.411	0.37
Phenol, 4,4'-methylene*bis*-	19	28.559	4.86
Pentadecanoic acid	20	29.882	1.25
Spiro[cyclopentane-1,2'(1'h)-quinoxaline], 3'-(4-morpholinyl)-6',8'-dinitro-	21	30.436	0.56
2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane #	22	30.480	0.42
Spiro[cyclopentane-1,2'(1'h)-quinoxaline], 3'-(4-morpholinyl)-6',8'-dinitro-	23	31.874	0.93
Cyclononasiloxane, octadecamethyl-	24	32.386	0.33
Dodecane, 2,6,10-trimethyl-	25	33.258	1.01
Cyclononasiloxane, octadecamethyl-	26	34.184	0.62
2-(3-Benzoylpropyl)-2-phenyl-1,3-dioxolane	27	34.249	0.78
Dodecane, 2,6,10-trimethyl-	28	34.585	1.16
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	29	34.723	1.77
Bis(2-ethylhexyl) phthalate	30	34.977	20.6
Eicosyl isopropyl ether	31	35.870	1.63
Nonadecane	32	37.100	0.64
Octadecanoic acid, 2,3-di-hydroxypropyl ester	33	37.331	1.06
1,3-Benzenedicarboxylic acid, *bis*(2-ethylhexyl) ester	34	37.603	0.84
Sulfurous acid, 2-propyl tridecyl ester	35	38.285	0.74
Glutaric acid, hex-4-yn-3-yl 3-methylbut-3-en-1-yl ester	36	38.440	0.18
N-(4,6-Dimethyl-2-pyrimidinyl)-n'-(4-hydroxyphenyl)guanidine #	37	38.715	0.93
Succinic acid, 3-methylbut-2-yl 3-methoxyphenyl ester	38	38.810	3.47
Cyclononasiloxane, octadecamethyl-	39	38.975	2.93
(2s,4as,5r,8ar)-5-(Pent-4-en-1-yl)-2-propyldecahydroquinoline	40	39.105	0.63
Benzene, dichlorodimethoxy-	41	39.165	2.31
11-Eicosenoic acid, (z)-, tms derivative	42	39.225	3.55
4-Acetyl-5-(4-fluorophenyl)-5,6-dihydro-4h-imidazo[4,5-*c*][1,2,5]oxadiazole	43	39.320	2.79
5,5-Diethylpentadecane	44	39.539	3.73
3-Pyrrolidinecarboxylic acid, 1-methyl-4-ethyl-2-(1,1,-dimethylethyl)-5-oxo-, (1,1-dimethylethyl) ester	45	39.820	2.89

ADME and pharmacokinetics studies: The bioactive compounds of the polyherbal extract (PHE), identified and matched with NIST database are summarized in Table-5. Based on the molecular weight, solubility, lipophilicity and hydrogen-bonding potential, three compounds *viz*. 4-acetyl-5-(4-fluorophenyl)purine, 6-(3-fluorobenzylamino)purine-[4,5-*c*]-5,6-dihydro-4H-imidazo-oxadiazole and *N*-(4,6-dimethyl-2-pyrimidinyl)-*N'*-(4-hydroxyphenyl)guanidine were chosen as the most promising. These compounds complied with Lipinski's rule of five, exhibiting molecular weights below 500 g/mol (243.24, 248.21 and 257.29), hydrogen-bond donors/acceptors within acceptable limits (1-3 and 4-5), and positive lipophilicity values (1.91, 1.41 and 1.37), indicating good absorption, distribution and drug-likeliness (Table-6). Among 75 identified compounds, these three demonstrated high gastrointestinal absorption and no violations, suggesting safety for human use [20,21]. Moreover, previous studies have also reported the hepatoprotective effects of *E. prostrata*, while *E. alba* extract showed strong antibacterial activity against pyogenic pathogens such as *S. aureus*, *S. typhi* and *E. coli*. Overall, these findings highlight the potential of *E. alba*, *E. prostrata* and *T. procumbens* as herbal therapeutics for managing pyogenic infections, supporting prior reports on their pharmacological benefits for human health [22-24].

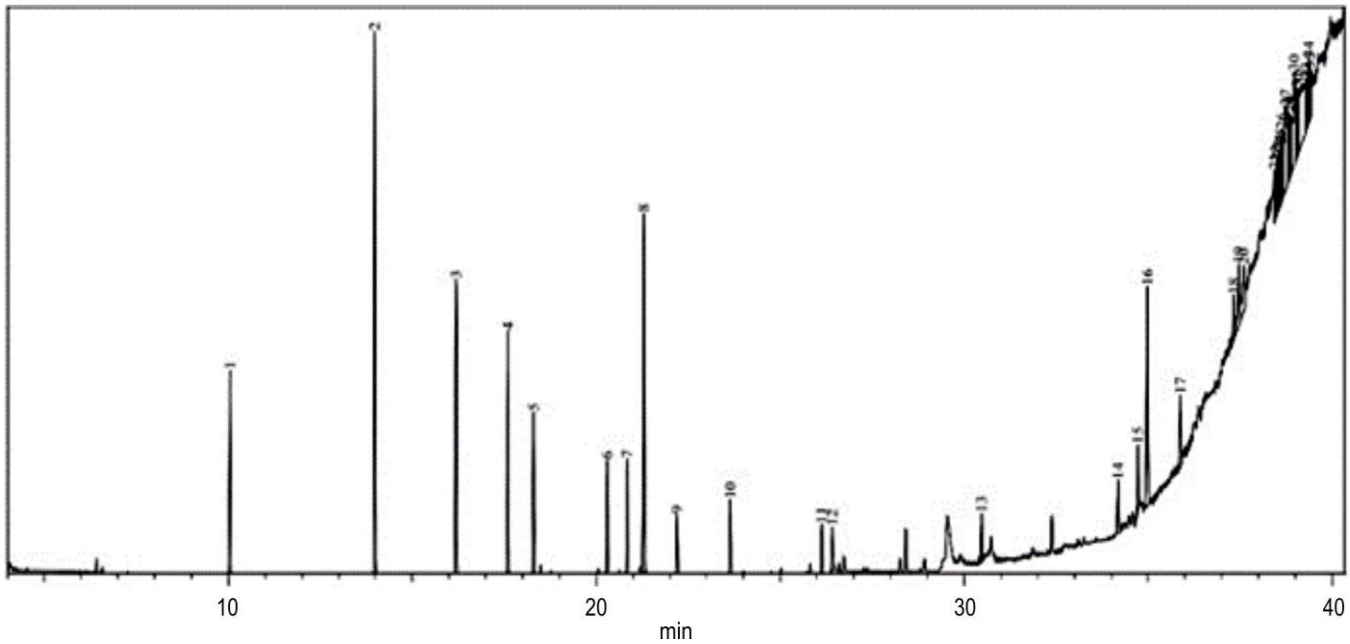

Fig. 2. GC-MS analysis of the ethyl acetate extract showing 35 compounds with varying retention times. The second compound, at a retention time (RT) of 13.977 min, accounts for 11.12% of the total detected compounds

TABLE-3
LIST OF COMPOUNDS ISOLATED FROM ETHYL ACETATE EXTRACT OF POLYHERBAL

Compound	Peak	Ret. time	Area (%)
Clopentasiloxane, decamethyl-	1	10.044	3.93
Cyclohexasiloxane, dodecamethyl-	2	13.977	11.12
Tetradecane	3	16.187	5.91
Cycloheptasiloxane, tetradecamethyl-	4	17.598	4.94
Octadecane	5	18.298	3.3
Octadecane	6	20.3	2.26
Benzoic acid, 2,4-*bis*(trimethylsiloxy)-, trimethylsilyl ester	7	20.842	2.37
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, triethyl ester	8	21.29	7.99
Octadecane	9	22.199	1.2
1,3,3,3-Tetramethyldisiloxanyl tris(trimethylsilyl) orthosilicate #	10	23.641	1.43
2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane #	11	26.132	1.02
3-(Hydroxymethyl)-2-thioxobenzothiazoline	12	26.415	1.1
Cyclononasiloxane, octadecamethyl-	13	30.472	1.03
6-(3-Fluorobenzylamino)purine	14	34.18	1.36
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	15	34.728	1.54
1,2-Benzenedicarboxylic acid	16	34.974	5.48
Cyclononasiloxane, octadecamethyl-	17	35.874	1.98
Octadecanoic acid, 2,3-di-hydroxypropyl ester	18	37.327	1.47
Cyclononasiloxane, octadecamethyl-	19	37.466	2.32
1,4-Benzenedicarboxylic acid, *bis*(2-ethylhexyl) ester	20	37.597	2.05
2-Cyano-2-isopropyl-3-methylbutanoic acid	21	38.43	1.29
Adipic acid, di(4-*tert*-butylphenyl) ester	22	38.485	1.79
Butanoic acid, heptafluoro-	23	38.535	1.51
N-(4,6-Dimethyl-2-pyrimidinyl)-N'-(4-hydroxyphenyl)guanidine	24	38.57	0.84
N-Phthaloyl-o(1)-tosyl-l-phenylalaninol	25	38.6	0.91
1H-Indol-5-ol, 1,2-dimethyl-4-nitro-	26	38.635	2.43
Diethylmalonic acid, monochloride, octadecyl ester	27	38.739	5.66
(2r,4as,5r,8ar)-2,5-Di(pent-4-en-1-yl)decahydroquinoline	28	38.82	1.39
o-(*p*-Chlorophenyl) n-methyl-n-phenylthiocarbamate	29	38.866	1.77
Tetracosamethyl-cyclododecasiloxane	30	38.976	5.2
3-Cyclohexene-1-methanol,. alpha.,4-dimethyl-.alpha.-(4-methyl-3-penten	31	39.045	2.49
Cyclohexanone, 2,4,4-trimethyl-3-(3-methyl-1,3-butadienyl)-, (e)-	32	39.14	6.74
1-(3-Fluoro-4-piperidin-1-yl-phenyl)-butan-1-one	33	39.295	2.24
4-Acetyl-5-(4-fluorophenyl)-5,6-dihydro-4h-imidazo[4,5-*c*][1,2,5]oxadiazole	34	39.37	1.44
Piperidine, 1-(1-cyclohexen-1-yl)-	35	39.405	0.52

TABLE-4
ANTIBACTERIAL ACTIVITY DATA OF POLYHERBAL EXTRACT

Test organisms	Ofloxacin (Standard)		Ethanol extract		Ethyl acetate extract	
	Zone of inhibition (mm)	MIC (µg)	Zone of inhibition (mm)	MIC (µg)	Zone of inhibition (mm)	MIC (µg)
E. coli	17 ± 0.001	100.00	–	–	15 ± 0.020	6.25
P. mirabilis	17 ± 0.001	50.00	–	–	16 ± 0.001	6.25
P. aeruginosa	16 ± 0.001	100.00	–	–	15 ± 0.020	25.00
A. baumannii	15 ± 0.020	75.00	–	–	14 ± 0.024	12.50
K. pneumonia	20 ± 0.001	100.00	–	–	15 ± 0.020	12.50
S. aereus	14 ± 0.001	150.00	–	–	17 ± 0.001	6.25

TABLE-5
PHYSIOCHEMICAL PROPERTIES OF SELECTED LIGANDS

Name of the ligand	No. of H-bond acceptors	No. of H-bond donors	m.w. (g/mol)	Water solubility Log S	Lipophilicity Log Po/w	Class
4-Acetyl-5-(4-fluorophenyl)-5,6-dihydro-4*H*-imidazo-[4,5-*c*][1,2,5]oxadiazole	5	1	248.21	-2.49	1.41	Solubility
N-(4,6-Dimethyl -2-pyrimidinyl)-*N*'-(4-hydroxyphenyl)guanidine	4	3	257.29	-2.60	1.37	Solubility
6-(3-Fluorobenzylamino)purine	4	2	243.24	-3.03	1.91	Solubility

TABLE-6
PHARMACOKINETIC PROFILES AND DRUG-LIKENESS PROPERTIES OF SELECTED LIGANDS

Ligand	BBB permanent	GI absorption	Lipinski	CYP2C19 inhibitor
4-Acetyl-5-(4-fluorophenyl)-5,6-dihydro-4*H*-imidazo[4,5-*c*][1,2,5]oxadiazole	No	High	0	No
N-(4,6-Dimethyl -2-pyrimidinyl)-*N*'-(4-hydroxyphenyl)guanidine	No	High	0	No
6-(3-Fluorobenzylamino)purine	Yes	High	0	No

***In silico* studies:** Three targeted proteins *viz*. ompC (PDB: 3UU2), murA (1NAW) and ESBL (PDB:6BU3) were docked with specific ligands. The binding affinity determined by Auto-Dock 4 and the interaction of 6-(3-fluorobenzylamino)purine with the target protein are represented in Fig. 3. The amino acids interacting with the active sites of the target proteins along with their docking scores are shown in Table-7. The hydrogen-bond formation, π-π interactions and hydrophobic contacts between the ligands and ESBL are taken place, while LYS, VAL and VAL formed hydrogen bonds with ligand atoms C2, C4 and N1, yielding docking scores of -7.696, -6.327 and -6.754 kcal/mol, respectively. LYS also exhibited weak hydrogen bonding and π–π interactions (Fig. 3a). For OmpC, hydrogen-bonded interactions with ASP and LYS showed docking scores of -6.190 and -7.702 kcal/mol. Hydrophobic contacts involving LYS, ASP and GLY with ligand atoms C1, C2, F1 and C4 are shown in Fig. 3b. MurA demonstrated three interaction types (Fig. 3c): ligand atoms N2, N3 and N5 formed hydrogen bonds with PHE and ILE (-6.920 and -8.222 kcal/mol), while ligand atoms C4 and C5 engaged in hydrophobic interactions with ILE and LEU (-5.020 kcal/mol). Moreover, ligand atom C7 formed a weak hydrogen bond with ILE (-8.222 kcal/mol). Overall, several phytochemicals exhibited favourable docking scores with ESBL and other target proteins, particularly those associated with the *Enterobacteriaceae* family, indicating strong binding potential and promising antibacterial activity [25-27].

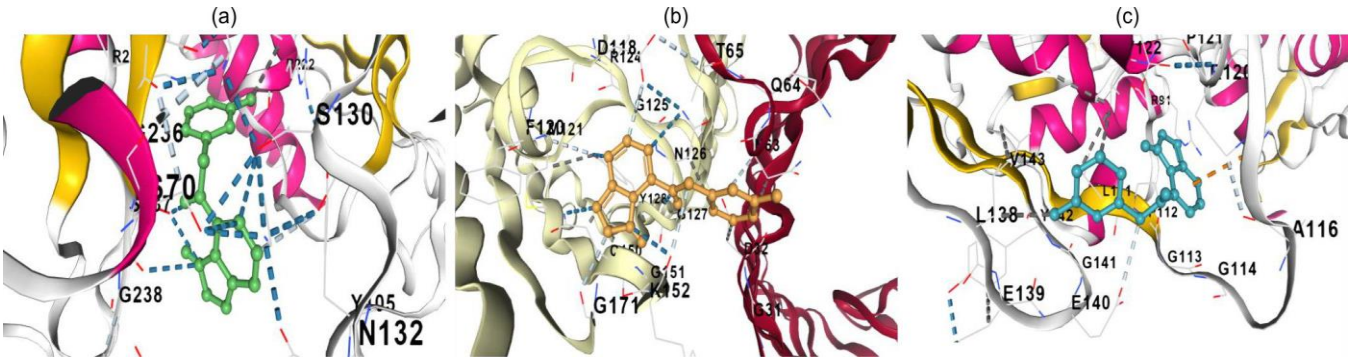

Fig. 3. Docking poses of (a) 1*H*-Purin-6-amine, N-((3-fluorophenyl)methyl)- interaction with ESBL enzyme, (b) 1*H*-Purin-6-amine, N-((3-fluorophenyl)-methyl)- interaction with ompC, (c) Reveals the interaction between 1*H*-purin-6-amine and N-((3-fluorophenyl)-methyl)- with murA revealed hydrophobic contact and weak but stable hydrogen bonding

TABLE-7
PHARMACOLOGICAL POTENTIAL VALUES
OF HIGHLY ABSORBED COMPOUNDS
UNINHIBITED BY CYP2C19 ENZYME

Amino acid	Score Kcal	Type of interaction
ESBL (PDB:6BU3		
LYS	-9.445	Pi pi
		C4
LYS	-11.696	Weak hydrogen bond
		C3
LYS	-7.696	H-bond
VAL	-6.327	C2, C4, N1
VAL	-6.754	
ompC (PDB:3UU2)		
LYS	-10.283	Hydrophobic contact
ASP	-8.3	C1, C2, F1, C4
GL	-4.8	
ASP	-6.190	Hydrogen bond
LYS	-7.702	C2, C4
murA (1NAW)		
ILE	-5.020	Hydrophobic contact
LEU		C4, C5
PHE A30	-6.920	Hydrogen bond
ILE	-8.222	N2, N3, N5
ILE	-8.222	Weak hydrogen bond
		C7

Conclusion

Using polyherbal preparation (PHP), the current work aimed to find unique phytoprinciple ingredients from *E. alba*, *E. prostrate* and *T. prometheus* to investigate their anti-bacterial impact on microorganisms that cause drug-resistant pyogenic infections and infect wounds. The study found that the polyherbal extract (PHE) exhibited strong activity against a wide range of pyogenic pathogens and effectively interacted with key bacterial receptors. Among the GC-MS identified phytochemicals, 6-(3-fluorobenzylamino)purine showed the highest binding affinity and emerged as a promising inhibitor of ESBL, MurA and OmpC proteins. ADME predictions further indicated favorable pharmacokinetic properties for the identified compounds. These findings provide a foundation for developing phytochemical-based therapeutic agents. The strong binding of the selected compounds to the pathogen protein motifs highlights their potential in combating multi-drug-resistant (MDR) infections.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

REFERENCES

1. M. Oliveira, W. Antunes, S. Mota, Á. Madureira-Carvalho, R.J. Dinis-Oliveira and D.D. da Silva, *Microorganisms*, **12**, 1920 (2024); https://doi.org/10.3390/microorganisms12091920
2. H. Kulkarni and U. Ghate, *Int. J. Biomed. Clin. Res.*, **3**, 1 (2025); https://doi.org/10.59657/2997-6103.brs.25.056
3. B. Patwardhan and R.A. Mashelkar, *Drug Discov. Today*, **14**, 804 (2009); https://doi.org/10.1016/j.drudis.2009.05.009
4. M.I. Hutchings, A.W. Truman and B. Wilkinson, *Curr. Opin. Microbiol.*, **51**, 72 (2019); https://doi.org/10.1016/j.mib.2019.10.008
5. S. Lakhera, M. Rana, K. Devlal, I. Celik and R. Yadav, *Struct. Chem.*, **33**, 703 (2022); https://doi.org/10.1007/s11224-022-01882-7
6. U. Anand, N. Jacobo-Herrera, A. Altemimi and N. Lakhssassi, *Metabolites*, **9**, 258 (2019); https://doi.org/10.3390/metabo9110258
7. R. Yadav, R.K. Khare and A. Singhal, *Int. J. Life. Sci. Sci Res.*, **3**, 844 (2017).
8. M.A. Loza-Mejía, J.R. Salazar and J.F. Sánchez-Tejeda, *Biomolecules*, **8**, 121 (2018); https://doi.org/10.3390/biom8040121
9. D. Timalsina and H.P. Devkota, *Biomolecules*, **11**, 1738 (2021): https://doi.org/10.3390/biom11111738
10. R. Jahan, A. Al-Nahain, S. Majumder and M. Rahmatullah, *Int. Schol. Res. Notices*, **2014** 385969 (2014): https://doi.org/10.1155/2014/385969
11. Y. Andriana, T.D. Xuan, T.N. Quy, T.N. Minh, T.M. Van and T.D. Viet, *Foods*, **8**, 21 (2019); https://doi.org/10.3390/foods8010021
12. E.A. Cano, R.E. Afloro, C. Ancheta, E.W. Angeles, M.A. Barrion, N.A. Bico, B.A. Calabon and P.D. Cantor, *Int. J. Biosci.*, **24**, 76 (2024); https://doi.org/10.12692/ijb/24.3.76-92
13. T.V. Surendra, S.M. Roopan, M.V. Arasu, N.A. Al-Dhabi and M. Sridharan, *J. Photochem. Photobiol. B*, **161**, 463 (2016); https://doi.org/10.1016/j.jphotobiol.2016.06.013
14. A.W. Bauer, W.M. Kirby, J.C. Sherris and M. Turck, *Am. J. Clin. Pathol.*, **45(4_ts)**, 493 (1966); https://doi.org/10.1093/ajcp/45.4_ts.493
15. S. Mundada and R. Shivhare, *Int. J. Pharmacol. J. Technol. Res.*, **2**, 2 (2010).
16. J.M. Sasikumar, T. Thayumanavan, R. Subashkumar, K. Janardhanan and P.L. Perumalsamy, *J. Nat Prod Res*, **6**, 34 (2007).
17. R. Tambe, A. Patil, P. Jain, J. Sancheti, G. Somani and S. Sathaye, *Pharm. Biol.*, **55**, 264 (2017); https://doi.org/10.1080/13880209.2016.1260597
18. C. Wiart, S. Mogana, S. Khalifah, M. Mahan, S. Ismail, M. Buckle, A.K. Narayana and M. Sulaiman, *Fitoterapia*, **75**, 68 (2004); https://doi.org/10.1016/j.fitote.2003.07.013
19. S. Karthikumar, K. Vigneswari, and K, Jegatheesan, *Sci. Res. Essays*, **2**, 101 (2007).
20. N. Rana, P. Gupta, H. Singh and K. Nagarajan, *Comb. Chem. High Throughput Screen.*, **27**, 353 (2024); https://doi.org/10.2174/1386207326666230914103714
21. A. Kumar, A.M. Ashif Ikbal, M.A. Laskar, A. Sarkar, A. Saha, P. Bhardwaj and M.D. Choudhury, *Chem. Biodiversity*, **21**, e202401137 (2024); https://doi.org/10.1002/cbdv.202401137
22. L. Jayaraman, S. Shivaji and S. Anandakumar, *Rasayan J. Chem.*, **15**, 676 (2022); https://doi.org/10.31788/RJC.2022.1516754
23. A.L. Dirar, A.Y. Waddad, M.A. Mohamed, M.S. Mohamed, W.J. Osman and M.S. Mohammed, *Int. J. Pharm. Pharm. Sci.*, **8**, 71 (2016); https://doi.org/10.22159/ijpps.2016v8i9.11989
24. M.K. Pandey, G.N. Singh, R.K. Sharma and S. Lata, *J. Appl. Pharm. Sci.*, **1**, 104 (2011).
25. R.K. Sharma, S. Bibi, H. Chopra, M.S. Khan, N. Aggarwal, I. Singh, S.U. Ahmad, M.M. Hasan, M. Moustafa, M. Al-Shehri, A. Alshehri, and A. Kabra, *Evid. Based Complement. Alternat. Med.*, **2002**, 3290790 (2022); https://doi.org/10.1155/2022/3290790
26. U. Rasool, A. Parveen and S.K. Sah, *BMC Complement. Altern. Med.*, **18**, 244 (2018); https://doi.org/10.1186/s12906-018-2312-8
27. M. Masi and J.M. Pagès, *Open Microbiol. J.*, **7**, 22 (2013); https://doi.org/10.2174/1874285801307010022