CAHIERS MAGELLANES-NS
Volume 06 Issue 2
2024

ISSN:1624-1940
http://magellanes.com/

HARNESSING THE POWER OF POLYHERBALS: BIOACTIVE COMPOUNDS AGAINST SELECTED MULTIDRUG-RESISTANT BACTERIAL PATHOGENS

Shanmugam K.S[1], Ponmurugan Karuppiah[2] and Ramkumar Lakshmanan[1*]
[1]Department of Microbiology, K.S. Rangasamy College of Arts and Science, Kalvi Nagar, Tiruchengode - 637 215, Tamil Nadu, India.
[2]Department of Botany and Microbiology, College of Science, King Saud University, PO Box 2455, Riyadh,11451, Saudi Arabia.
[*]*Corresponding Author Email:* lakshmananram75@gmail.com

ABSTRACT
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 Spectrum (GCMS) analysis, 45 distinct chemical compounds were identified in the ethanol extract, with the ethyl acetate extract revealing 35 different chemicals. These compounds' profiles suggested diverse chemical compositions, with some exhibiting promising antibacterial properties. 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. This investigation underscores 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
To assess a polyherbal preparation's ability to inhibit wound-infecting microorganisms by combining *Eclipta prostrate, Eclipta alba,* and *Tridax procumbens*. This approach is particularly significant to present clinical scenario concerns over antibiotic resistance. The misuse, over-dosage, and prolonged use of antibiotics result in the prevalence of multidrug resistance in clinical setups. Ayurveda, Siddha is the pioneer of novel drug design. Traditional systems of medicine are gaining popularity due to their natural, environmentally friendly nature and minimal adverse effects compared to conventional medications [1]. Medicinal plants are rich sources of secondary metabolites with diverse biological activities, including antimicrobial, anticancer, antioxidant, and anti-inflammatory properties. However, not all plant species have been thoroughly investigated for their potential bioactive [2-5]. 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*

ISSN:1624-1940
http://magellanes.com/

prediction models for pharmacokinetics, pharmacology, and toxicology [6-9]. While *Eclipta prostrate, Eclipta alba* and *Tridax procumbens* have been studied for their medicinal properties, there's still limited literature available on certain aspects, such as the antibacterial and antifungal activities of *Eclipta hirta*. The current research aims to identify the bioactive compounds present in selected plants and to promote the drug through a molecular docking platform against specific target proteins associated with bacterial infections [10-14]. The targets chosen for molecular docking include ESBL (Extended-Spectrum Beta-Lactamase), OmpC (Outer membrane protein C), and MurA. These proteins are the more possible targets for antibacterial drugs because they are essential for the synthesis of bacterial cell walls. This research combines traditional knowledge of medicinal plants with modern computational methods to identify potential antibacterial compounds, aiming to address the challenge of antibiotic resistance.

MATERIALS AND METHODS

Preparation of Plant Extracts

The leaves of *Eclipta prostrata, Eclipta alba,* and *Tridax procumbens* were collected and around 100 grams each, cleaned and left to dry in a shady spot for a few days. After drying, the leaves were ground into powder. The powdered leaves were then stored in dry containers for further process [15].

Poly-Herbal Extraction

Soxhlet extraction technique was used for extracting compounds from a mixed ground powder of selected medicinal plant materials (polyherbal) with polar and non-polar organic solvents. About 50 g of the polyherbal mix, which is a mixture of selected herbs in a ratio of 1:1:1. This was removed and put inside a thimble. This thimble is typically made of cellulose or some other porous material that allows the solvent to pass through while retaining the solid material. The thimble containing the sample was placed in the Soxhlet chamber. The chamber was then attached to a condenser and an extraction flask. The solvent (95% ethanol) was heated to its boiling point, causing it to evaporate and rise into the Soxhlet chamber. The Soxhlet extraction is carried out for 6 h. Ethanol was chosen as the solvent for isolating polar compounds due to its ability to dissolve a wide range of polar substances. Ethyl acetate was chosen as the solvent for extracting both polar and nonpolar compounds. Soxhlet extraction method was performed to obtain crude extract solutions and was concentrated using a vacuum rotary evaporator.

Phytochemical Analysis

Test for alkaloids, carbohydrates, glycosides, quinones, phenols, tannins, flavonoids

For phytochemical analysis, the standard estimation method was followed. Briefly, two ml of the polyherbal extract (PHE) were taken, and two ml of strong hydrochloric acid (HCl) were added. To the solution, a drop of Mayer's reagent was added. When Mayer's reagent was added, the sample was confirmed to contain alkaloids if a green tint or white precipitate appeared. One ml of Molisch's reagent, two ml of the polyherbal extract (PHE), and a few drops of concentrated sulfuric acid were added. The presence of carbohydrates was indicated by the emergence of a purple or reddish tint. A 10 % ammonia solution, 3 ml of chloroform, and 2 ml of the polyherbal extract (PHE) were added. The presence of glycosides was indicated by a pink appearance. One ml of concentrated sulfuric acid was added to two ml of the polyherbal extract (PHE). Quinones were present as indicated by the production of a red hue. Two ml of glacial acetic acid, 0.5 ml of polyherbal extract, and a few drops of ferric chloride were

ISSN:1624-1940
http://magellanes.com/

mixed in a test tube. In a test tube, content was added to 1 ml of concentrated sulfuric acid. The development of a brown ring at the interface of the line separating the two layers in the test tube was indicative of the presence of cardiac glycosides in the solution. Two ml of pure water, a few drops of a 10% ferric chloride solution, and one ml of the diluted PHE were combined. The presence of phenols was positively correlated with the appearance of a blue or green tint. A solution of 5% ferric chloride was added to two ml of extract. The presence of tannins was indicated by a dark blue or greenish-black appearance. One ml of 2N sodium hydroxide solution was mixed with two ml of PHE extract. The flavonoids were detected by the emergence of a yellow tint. A small amount of strong sulfuric acid was added to the mixture along with around 1 ml of the PHE extract and an equivalent volume of chloroform. The presence of phytosterols was indicated by a bluish-brown ring, while the presence of steroids was shown by a brown ring. About 1 ml of the polyherbal extract was added to a few drops of 2% hydrochloric acid. Red color precipitate indicated the presence of phlorotannins.

GC-MS analysis of Plant extract

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 °C and the injector at 250 °C. The constant flow rate was 1ml/min, and the injection volume was 0.5 µL (EI stands for Electron Ionization, a common ionization technique in mass spectrometry). The oven's temperature programming was 110 °C for two min of start heating (isothermal), followed by 10 °C/min increases to 200 °C, 5 °C min increase to 280 °C, and a final isothermal at 280 °C for nine min.

Antibacterial susceptibility assay

The method proposed by Bauer et al. (1966) [16] for determining the antimicrobial properties of botanical extracts using the disc diffusion method. Using the swab culture approach, wound test pathogens such as *E. coli*, *S. aureus, P. aeruginosa, Proteus vulgaris, K. pneumoniae,* and *A. baumannii* were inoculated on Mueller-Hinton (MH) agar plates. The inoculum concentration was adjusted to 0.5 McFarland optical density. A concentration of mg/ml of the PHE was prepared, and 100 µL of the prepared PHE extract was loaded onto sterile discs. The loaded discs with the PHE extract were then placed on the surface of the MH agar plates inoculated with the test pathogens. For 24 h, the agar plates were incubated at 37 °C. Ethanol served as the negative control and Ofloxacin (20 µg) as the positive control. The zone of inhibition surrounding each disk was measured following the incubation. The diameter of the clear zone around the disc indicated the antimicrobial activities of the PHE extract against the test pathogens. The results were interpreted based on the size of the inhibition zone.

Minimum Inhibitory Concentration (MIC) determination

To determine the minimum inhibitory concentration, extracts were prepared at 25, 50, 12.5, 6.25, and 100 µg/ml dilutions. Each dilution is inoculated with a standardized bacterial suspension containing approximately 1×10^6 colony-forming units (CFU) per 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 is a dye commonly used to assess microbial viability. The lowest concentration of the extract at which no visible growth or metabolic activity (indicated by lack of color change with resazurin) of the bacteria was observed after the incubation period.

ISSN:1624-1940
http://magellanes.com/

Lead-likeliness properties

PubChem database (www.ncbi.nlm.nih.gov/pubchem), searching for the desired compounds, and retrieving their Canonical SMILES. The NIST library was matched with the produced molecular files of each phytochemical compound to anticipate the compounds' various pharmacokinetic and physicochemical features. Then, using tools such as SWISS ADME, Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) prediction was performed.

Target protein identification and preparation

The Research Collaborator for Structural Bioinformatics (RCSB) Protein data bank (www.rcsb.org) 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 the ligand's 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 bacterial enzymes was investigated to determine suitable binding energy values.

RESULTS AND DISCUSSION

Phytochemical analysis by GC-MS

Using a qualitative approach, the phytochemical profile of the polyherbal extract (PHE) made with ethanol and ethyl acetate is presented in Table 1. Tannins and sterols were absent from both solvent extracts, but the presence of carbohydrates, tannins, cardiac glycoside, saponins, and phlorotannins was confirmed. Additionally, only the ethyl acetate extract included glycosides, quinone, anthraquinones, and anthocyanins.

Table 1. Analyzing the phytochemical profile of a polyherbal extract qualitatively

Test	Ethanol	Ethylacetate
Carbohydrate	+	+
Tannins	-	-
Galactoside	-	+
Alkaloids	+	+
Phenol	+	+
Flavonoid	+	+

ISSN:1624-1940
http://magellanes.com/

Cardiac glycoside	+	+
Glycoside	-	+
Sterol	-	-
Quinone	-	+
Tannins	-	-
Saponins	+	+
Phlobatannins,	+	+
Anthocyanin	-	+
Anthraquinone	-	+

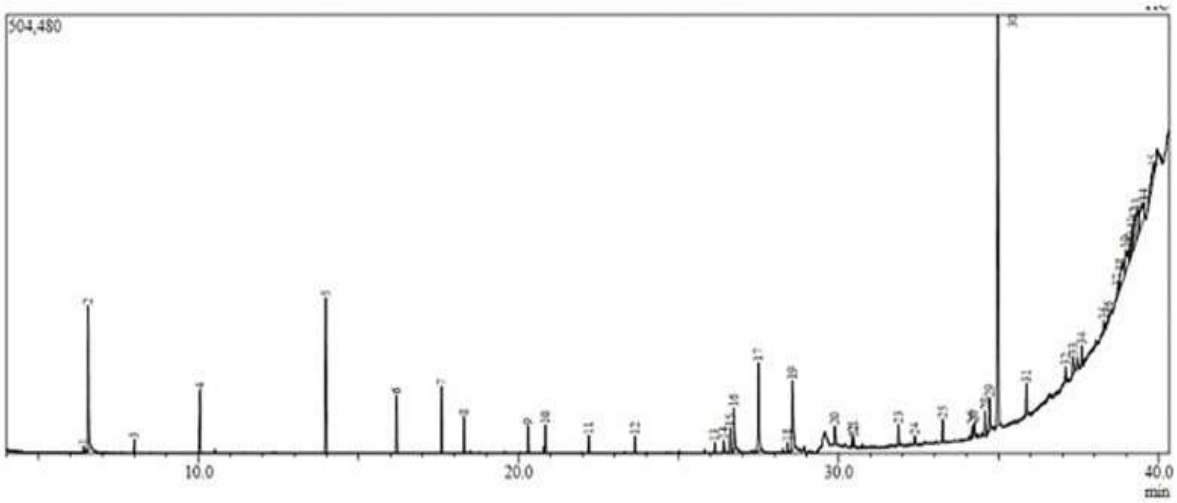

Figure 1. Gas chromatography analysis of the mass spectrum of an ethanol extract reveals that 45 components have varying retention times. The major Peak was obtained with an RT of 34.977 min for Peak 30 which is found to be 20.6% of the total compound

Figure 1 shows the results of the ethanol extract GCMS study. 45 distinct components were found and matched with NIST, each of which had a distinct retention peak. Table 2 lists the chemicals that were identified. Bis (2-ethylhexyl) phthalate was the most prevalent compound and was reported (New 1) to be a potent antimicrobial larvicidal complex (20.6% RT 34.977 min).

ISSN:1624-1940
http://magellanes.com/

Table 2. Compounds extracted with ethanol (NIST library)

Name of the NIST-matched compound	**Peak**	**Ret. time**	**Area (%)**
Silicic acid (h4sio4), 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	20.3	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	0.7
3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	13	26.132	0.48

ISSN:1624-1940
http://magellanes.com/

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	27.5	5.3
Benzoic acid, 2,4-bis(trimethylsiloxy)-, trimethylsilyl ester	18	28.411	0.37
Phenol, 4,4'-methylenebis-	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	30.48	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

ISSN:1624-1940
http://magellanes.com/

Eicosyl isopropyl ether	31	35. 87	1. 63
Nonadecane	32	37. 1	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. 44	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. 81	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. 32	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. 82	2. 89

It was succeeded by Cyclohexasiloxane, dodecamethyl-(RT 13.979 min and 6.78 %), and Benzenesulfonic acid, 4-hydroxy (RT 6.556/6.73%), which was described as a strong bioactive substance from cyanobacteria. Phenol, 4,4'-methylene bis (4.86%), 5,5-diethylpentadecane (3.73%), 11-

ISSN:1624-1940
http://magellanes.com/

eicosenoic acid, (z)-, and TMS (3.55%) were among the bioactive principles that were detected at moderate levels. Low concentrations of bio-compounds such as nonadecane, octadecane, dodecane, hexadecanoic acid, and pentadecanoic acid were discovered. Numerous unusual substances were present in the extract, such as phenol derivatives, 2-propyldecahydroquinoline, 6-methylfuro [2,3-c] pyrid-5-one, cyclopentane-1,2'(1'h)-quinoxaline, 4-hydroxyphenyl) guanidine, and oxadiazole.

The two extracts' GCMS spectra showed the presence of several metabolites. Table 1 lists all 35 bioactive principles that were filtered out of the polyherbal extract. Figure 2 shows the GC-MS spectrum of an ethyl acetate extract that was examined for the phytochemical principles of specific plants. Cyclopentasiloxane, decamethyl, is the first component to elute at RT 10.044 min, while piperidine, 1-(1-cyclohexen-1-YL), is the last molecule to elute at RT 39.405 min. Table 3 displays the presence of new chemicals such as piperidine, adipic acid, decahydroquinoline, nonadecane, and fluoro-4-piperidine. Ikewuchi and associates (2015)[17] reported about six phytochemicals. Wang *et al.,*[18] identified aqueous and methanolic leaf extracts of *Tridax procumbens* and isolated eight secondary metabolites. The findings of Sawant and Godghate (2013) [19] are consistent with the qualitative phytochemical data.

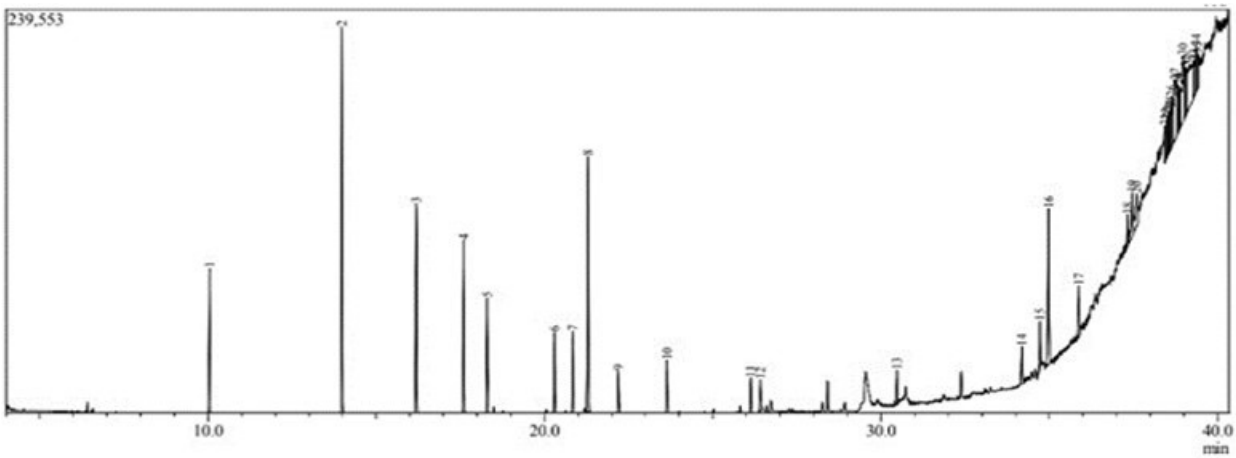

Figure 2. Ethyl-acetate extract, showing mass spectrum with 35 compounds having different retention times under Gas chromatography. The second compound showed a peak with RT of 13.977 min covered about 11.12%

Table 3. Compounds extracted with ethyl acetate (NIST matched)

Name of the NIST-matched compound	Peak	Ret.time	Area (%)
Cyclopentasiloxane,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

ISSN:1624-1940
http://magellanes.com/

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

Screening of the Inhibitory Effect of PHE and Np

The PHE efficacy was tested against wound-related pyogenic pathogens like *E.coli, P. mirabilis, P. aeruginosa, A.baumannii, K. pneumonia,* and *S. a*ureus for their antibacterial activity. Zone of inhibition assay was carried out for their antibacterial efficacy. According to MIC analysis, it was discovered that all of the pathogens were extremely sensitive to 1 mg/ml ethyl acetate extract, but less sensitive to ethanol extract. This could be because the bioactive chemical and pathogenic receptors did not interact

ISSN:1624-1940
http://magellanes.com/

very well. When it came to *S. aureus,* the extract's greatest zone of inhibition was measured at 17±0.001 mm, which was intermediate when it came to Oflaxacin (14±0.001 mm). *P. mirabilis*, which measured 16±0.001 mm by PHE and 17±0.001 mm by the standard, was the second-most sensitive strain. Moderately intermediate bacteria included *E. coli, P. aeruginosa*, *K. pneumonia,* and *A. baumannii*. The inhibitory zone is measured in mm (Table 4). The findings of this study are consistent with those of earlier investigations. Indigenous people have traditionally used *Tridax* stem in their folk medicines due to its antibacterial qualities [20] (Mundada and Shivhare, 2010). The majority of metabolomics, including flavonoids, alkaloids, saponins, tannins, and luteolin, are responsible for the antimicrobial properties of plant compounds [21–22] (Sasikumar et al., 2007; Tambe et al., 2017). The MIC results showed variation in the extract's inhibitory concentrations for every bacterial strain. It was discovered that certain plant extracts had MIC values that were lower than the reference. The lowest mean inhibitory concentration was measured at 6.25 µg/ml for *E. coli* and *P. mirabilis*, and it was 12.5 µg/ml for *A. baumannii* and *K. pneumoniae*. Compared to polyherbal extract, the standard Oflaxacin MIC ranges from 50 to 100 µg/ ml, whereas a high value of 25 µg/ml is recorded against *P. aeruginosa*. The fact that our results demonstrated significantly greater activity against pyogenic infections supported the findings of Wiart et al. [23] (2004) and Karthikumar et al. [24] (2007) previous reports.

Table 4. Antibacterial effect of polyherbal extract

Test organisms	Ofloxacin		Ethanol		Ethyl Acetate	
	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±0.02	6.25
P. mirabilis	17±0.001	50.00	-	-	16±0.001	6.25
P. aeruginosa	16±0.001	100.00	-	-	15±0.02	25.00
A. baumannii	15±0.02	75.00	-	-	14±0.024	12.50
K. pneumonia	20±0.001	100.00	-	-	15±0.02	12.50
S. aereus	14±0.001	150.00	-	-	17±0.001	6.25

ADME and Pharmacokinetics of PHE

The bioactive principle's properties of PHE are elicited through NIST-matched are given in Table 5. Based on their molecular weight, solubility, lipophilicity, and potential as H-bond donors and acceptors, three compounds were selected. Specifically, 4-Acetyl-5-(4-fluorophenyl) and 6-(3-fluorobenzylamino) purines[4,5-c]-5,6-dihydro-4h-imidazoOxadiazole (1,2,5) and N-(4,6-dimethyl-2-pyrimidinyl) together of the chemicals discovered, -N'-(4-hydroxyphenyl) guanidine was shown to be the most effective. These compounds' physiochemical characteristics adhered to "Lipinski's rule of five." The range of the hydrogen bond donor and acceptor is 1-3 and 4-5, respectively, which is less than 10 and 5. By having molecular weights of 243.24, 248.21, and 257.29, respectively, the compound is less than 500 g/mol and may be transported, dispersed, and adsorbed more readily than heavier molecules cannot. The lipophilicity of the compound is 1.91, 1.41, and 1.37, in that order. To be stable in a lipid media and to be considered a potential medicine, a compound's lipophilicity value must be positive and less than 5. The pharmacokinetics and drug-likeliness characteristics of ligands are displayed in Table 6. Of the 75

ISSN:1624-1940
http://magellanes.com/

compounds, three are considered safe for human consumption because of their good gastrointestinal absorption and lack of violations25–26. Additionally, past studies have demonstrated the hepatoprotective benefit of *E. prostrate*. In the end, it was discovered that the *E. alba* leaf extract possessed strong antibacterial capabilities against pyogenic infections. Documentation was done on the outcomes of tests conducted on *E. alba*'s ethanol extract against *S. aureus*, *S. typhi*, and *E. coli*. The results of this study showed and underlined the possibility of promoting *E. alba, E. prostrata,* and *T. procubans* as herbal drugs that provide superior treatment against pyogenic infections and illnesses [25] (Lavanya et al., 2022). Previous research has validated this outcome, which may be used for the benefit of human well-being [26, 27] (Pandey et al., 2011; Dirar et al., 2016).

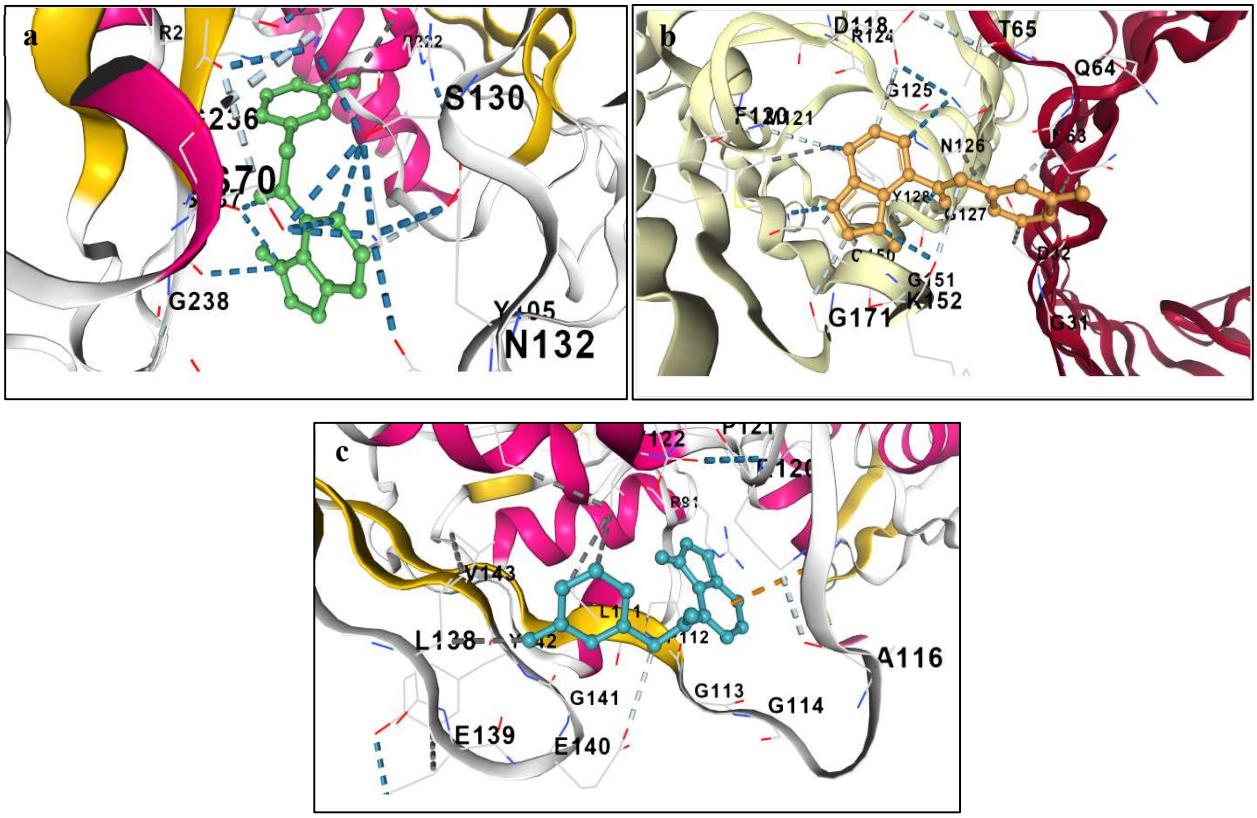

Figure 3. (a) Shows the 1H-Purin-6-amine, N-((3-fluorophenyl)methyl)- interaction with ESBL enzyme, (b) Shows 1H-Purin-6-amine, N-((3-fluorophenyl)methyl)- interaction with ompC, (c) Reveals the interaction between 1H-Purin-6-amine and N-((3-fluorophenyl)methyl)- with murA revealed hydrophobic contact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

In silico design on Drug-resistant protein

Three targeted proteins—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 Figure 3. Table -7 lists the amino acid that interacts with the active site and the docking score. The research focused on the hydrogen bond formation, Pi-Pi contact, and hydrophobic interaction that occurred between Ligand and ESBL. LYS, VAL, and VAL formed hydrogen bonds with the ligand atoms C2, C4, and N1, with docking scores of

ISSN:1624-1940
http://magellanes.com/

-7.696, -6.327, and -6.754 Kcal/mol, in that order. Additionally, LYS had a weak hydrogen bonding interaction and Pi-Pi (Fig. 3a). The hydrogen-bonded docking score with OmpC for ASP and LYS residues was -6.190 and -7.702, respectively. Fig. 3b illustrates the hydrophobic contact that was detected between LYS, ASP, and GLY with ligand atoms C1, C2, F1, and C4. The receptor MurA forms three different interactions with different amino acids (Fig. 3c). With PHE and ILE, the ligand atoms N2, N3, and N5 formed hydrogen bonds, and the expected docking scores were -6.920 and -8.222 kcal. While ligand atoms C4 and C5 demonstrated hydrophobic interaction with ILE and LEU (-5.020 kcal), ligand atom C7 demonstrated a weak hydrogen bond with ILE (-8.222 kcal). Numerous phytochemicals with ESBL and on protein, especially with the Enterobacteriaceae family, produced positive docking scores [28-30] (Masi and Pages, 2013; Rasool et al., 2018; Sharma et al., 2022).

Table 5. Physiochemical properties of ligands

Name of the ligand	Number of H-bond acceptors	Number of H-bond donors	Molecular weight (g/mol)	Water solubility Log S	Lipophilicity Log Po/w	Class
4-Acetyl-5-(4-fluorophenyl)-5,6-dihydro-4h-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. Exploring Pharmacokinetic Profiles and Drug-Likeness Properties of Ligands

Name of the ligand	BBB Permanent	GI Absorption	Lipinski	CYP2C19 inhibitor
4-Acetyl-5-(4-fluorophenyl)-5,6-dihydro-4h-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

Table 7: Demonstrates how different bond types are represented and how an amino acid interacts with a ligand. It was discovered that the docking scores of murA, ompC, and ESBL formed excellent hydrogen bond formation. For atoms with stable hydrogen bonds, the docking scores varied from - 6 to - 8.

ISSN:1624-1940
http://magellanes.com/

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

CONCLUSION

Using polyherbal preparation, the current work aimed to find unique phytoprinciple ingredients from *E. alba, E. prostrate,* and *T. prometheus* to investigate their antibacterial impact on microorganisms that cause drug-resistant pyogenic infections and infect wounds. It was discovered that PHE was a powerful, wide range of pyogenic pathogens that interacted with bacterial receptors. Among the several phytochemicals examined by GC-MS compound 6-(3-fluorobenzylamino), purine was shown to have a greater binding affinity with particular ligands and to be a potential inhibitor of ESBL, murA, and ompC protein. Moreover, ADME prediction demonstrated advantageous ADME characteristics for the components that were found. Our findings provided a foundation for the development of phytochemical substances as novel drugs. The suggested phytochemical's ability to bind with the pathogen's protein motifs has demonstrated its effectiveness in fighting multidrug-resistant (MDR) infections.

REFERENCES

1. Hutchings MI, Truman AW, Wilkinson B. Antibiotics: past, present and future. CurrOpinMicrobiol. 2019;51(3):72-80. doi: 10.1016/j.mib.2019.10.008, PMID 31733401.
2. Lakhera S, Rana M, Devlal K, Celik I, Yadav R. A comprehensive exploration of pharmacological properties, bioactivities and inhibitory potentiality of luteolin from Tridax

ISSN:1624-1940
http://magellanes.com/

procumbens as anticancer drug by in-silico approach. Struct Chem. 2022;33(3):703-19. doi: 10.1007/s11224-022-01882-7. PMID 35106037.
3. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21(5):559. doi: 10.3390/molecules21050559, PMID 27136524.
4. Mir MA, Hamdani SS, Sheikh BA, Mehraj U. Recent advances in metabolites from medicinal plants in cancer prevention and treatment. Curr Immunol Rev. 2019;15(2):185-201. doi: 10.2174/1573395515666191102094330.
5. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites. 2019;9(11):258. doi: 10.3390/metabo9110258, PMID 31683833.
6. Yadav R, Khare RK, Singhal A. Qualitative phytochemical screening of some selected medicinal plants of Shivpuri district (MP). Int. J. Life. Sci. Sci Res. 2017;3:844-7.
7. Loza-Mejía MA, Salazar JR, Sánchez-Tejeda JF. F. In silico studies on compounds derived from Calceolaria: phenylethanoid glycosides as potential multitarget inhibitors for the development of pesticides. Biomolecules. 2018;8(4):121. doi: 10.3390/biom8040121, PMID 30360548.
8. Trott O, Olson AJ. Auto Dock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61. doi: 10.1002/jcc.21334, PMID 19499576.
9. Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J Comput Aided Mol Des. 2010;24(5):417-22. doi: 10.1007/s10822-010-9352-6, PMID 20401516.
10. Ogueke CC, Ogbulei JN, Okoli IC, Anyanwu N. Antimicrobial activities and toxicological potentials of crude ethanolic extracts of Euphorbia hirta. J Am Sci. 2007;3:11-6.
11. El-Mahmood AM. Antibacterial activity of crude extracts of Euphorbia hirta against some bacteria associated with enteric infections. J Med Plants Res. 2009;3:498-505.
12. Naqash SY, Nazeer RA. Anticoagulant, antiherpetic and antibacterial activities of sulphated polysaccharide from Indian medicinal plant Tridax procumbens L. (Asteraceae). Appl Biochem Biotechnol. 2011;165(3-4):902-12. doi: 10.1007/s12010-011-9307-y, PMID 21671053.
13. Ram AJ, Bhakshu LM, Raju RRV. In vitro antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. J Ethnopharmacol. 2003;90:353-7.
14. Sandeep BP, Nilofar SN, Chandrakant SM. Review on phytochemistry and pharmacological aspects of Euphorbia hirta Linn. J Pharm Res Health Care. 2009;1:113-33.
15. Surendra TV, Roopan SM, Arasu MV, Al-Dhabi NA, Sridharan M. Phenolic compounds in drumstick peel for the evaluation of antibacterial, hemolytic and photocatalytic activities. J Photochem Photobiol B. 2016;161:463-71. doi: 10.1016/j.jphotobiol.2016.06.013, PMID 27318603.
16. Bauer H, Paronetto F, Burns WA, Einheber A. The enhancing effect of the microbial flora on macrophage function and the immune response: a study in germfree mice. The Journal of experimental medicine. 1966 Jun 1;123(6):1013-24.
17. Ikewuchi CC, Ikewuchi JC, Ifeanacho MO. Phytochemical Composition of Tridaxprocumbens Linn Leaves: potential as a Functional Food. Food Nutr Sci. 2015;06(11):992-1004. doi: 10.4236/fns.2015.611103.

ISSN:1624-1940
http://magellanes.com/

18. Wang H, Li N, Zhu C, Shi S, Jin H, Wang S. Anti-complementary activity of two homogeneous polysaccharides from Eclipta prostrata. Biochem Biophys Res Commun. 2017;493(2):887-93. doi: 10.1016/j.bbrc.2017.09.126, PMID 28951216.
19. Sawant RS, Godghate AG. Preliminary phytochemical analysis of leaves of Tridax procumbens Linn. Int J Sci Environ Technol. 2013;2(3):388-94.
20. Mundada S, Shivhare R. Pharmacology of Tridax procumbensa weed [review]. Int. J. Pharmacol. J Technol Res. 2010;2(2):1391-4.
21. Sasikumar JM, Thayumanavan T, Subashkumar R, Janardhanan K, Perumalsamy PL. Antibacterial activity of some ethnomedicinal plants from the Nilgiris, Tamil Nadu, India. J. Nat Prod Res. 2007;6(1):34-9.
22. Tambe R, Patil A, Jain P, Sancheti J, Somani G, Sathaye S. Assessment of luteolin isolated from Eclipta alba leaves in animal models of epilepsy. Pharm Biol. 2017;55(1):264-8. doi: 10.1080/13880209.2016.1260597, PMID 27927066.
23. Wiart C, Mogana S, Khalifah S, Mahan M, Ismail S, Buckle M et al. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Penisular Malaysia, Fitoteropia. 2004;75(1):68-73.
24. Karthikumar S, Vigneswari K, Jegatheesan K. Screening of antibacterial and antioxidant activities of leaves of Eclipta prostrata (L.). Sci Res Essays. 2007;2(4):101-4.
25. Lavanya J, Subhashini S, Shanmugam A. Phytochemical screening, cytotoxic activity and molecular docking studies of Eclipta alba leaves extract against oral cancer. RasayamJ. Chem. 2022;15(1):676-85.
26. Dirar AI, Waddad AY, Mohamed MA, Mohamed MS, Osman WJ, Mohammed MS et al. In silico pharmacokinetics and molecular docking of three leads isolated from Tarconanthus camphoratus L. Int J Pharm Pharm Sci. 2016;8(5):71-7.
27. Pandey MK, Singh GN, Sharma RK, Lata S. Antibacterial activity of Eclipta alba (L.) Hassk. J Appl Pharm Sci. 2011:104-7.
28. Sharma RK, Bibi S, Chopra H, Khan MS, Aggarwal N, Singh I, et al. In silico and in vitro Screening Constituents of Eclipta alba leaf extract to leveal antimicrobial Potential. Evid Based Complement Alternat Med. 2022;17:3290790.
29. Rasool U, S P, Parveen A, Sah SK, S H. Efficacy of Andrographis paniculata against extended spectrum β-lactamase (ESBL) producing E. coli. BMC Complement Altern Med. 2018;18(1):244. doi: 10.1186/s12906-018-2312-8, PMID 30176904.
30. Masi M, Pagès JM. Structure, Function, and Regulation of Outer Membrane Proteins Involved in Drug Transport in Enterobactericeae: the OmpF/C – TolC Case. Open Microbiol J. 2013;7:22-33. doi: 10.2174/1874285801307010022, PMID 23569467.