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Quantitative Phytochemical Investigation, Antibacterial Potency, and Drug-ability Assessment of Three Indian Medicinal Plants Leaf Extracts Using Bioinformatics Tools

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Abstract: Natural regimens have long-held ethnomedicinal values, serving as primary sources for mainstream medicine. Therefore, scientists are paying more attention to studying the biological activity of existing plant species in organized ways to select potent bioactive metabolites to use for specific therapeutic purposes. This study used the same approach to find potent antibacterial phytoconstituents in three well-known Indian medicinal plants: *Psidium guajava* L., *Syzygium cumini* L. and *Punica granatum* L. In the earlier study, methanolic leaf extracts of the above plant extracts were more effective than n-hexane extracts against biofilm and drug-resistant pathogenic bacteria. Accordingly, we selected methanolic crude extracts for gas chromatography-mass spectrometry (GC-MS) to identify the phytoconstituents presented. In addition, we added a few reported candidates from the above plant extracts for molecular docking studies against four bacterial targets. For molecular docking studies, we retrieved all phytoconstituents or ligands from the PubChem database and bacterial target proteins from the protein data bank using PyRx 0.8-AutoDock 4.2 software. Furthermore, we used various bioinformatics and chemoinformatics tools to examine the investigated phytoconstituents physicochemical properties, toxicity, and drug-ability profiles. Out of the 30 GC-MS report-derived candidates from three plants, P5 from *P. guajava*, P18 from *S. cumini*, and P21 from *P. granatum* had the potent binding ability with bacterial targets. In the same way, out of the 30 reported candidates, P39, P43, and P56 from three plants, along with amikacin, showed strong binding against the same bacterial target. Both sets of candidates showed favorable physicochemical and toxicity profiles; however, all GC-MS-derived and few reported candidates exhibited negative drug-likeness. The study reveals that these crude extracts have antibacterial properties because they contain both GC-MS and existing phytoconstituents. The study starts with a crude extraction and then uses bioinformatics to choose two possible antibacterial candidates, ursolic acid and punicacortein A. This platform could be useful for finding an antibacterial agent that works specifically on a specific target. To sum up, the study encourages the isolation of more bioactive candidates from different Indian medicinal plants and uses bioinformatics tools to speed up the selection of strong leads that can speed up the process of making antibacterial drugs within limited resources.

Introduction

In the 21st century, antibiotic resistance is an emerging critical global health challenge, posing a threat to the effectiveness of commonly recommended antibiotics and their ability to combat bacterial infections (GBD 2019; Ranjbar and Alam, 2022; WHO, 2023a). Primarily, the misuse and overuse of antibiotics, not only in humans, but also in animals, and agriculture, accelerated the development of antibiotic-resistant progeny, creating a scenario where once-treatable infections become more

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difficult to manage (Ranjbar and Alam, 2022; WHO, 2023a). The consequences of antibiotic resistance include increased morbidity, mortality, prolonged illness, and higher healthcare costs (Aslam et al., 2018; GBD, 2019; Ranjbar and Alam, 2022; WHO, 2023a). Generally, both Gram-negative (GN) and Gram-positive (GP) pathogens are becoming more prevalent in our surroundings, leading to a continuous increase in morbidity and unexpected mortality by bloodstream infection (Gandra et al., 2019; et al., 2019; Frickmann Howden et al., 2023). Therefore, we urgently need newer antibacterial therapeutic strategies to combat this growing public health crisis due to antibiotic-resistant strains from both GN and GP in the form of biofilm-producing pathogens, methicillin-/ vancomycin-resistant *Staphylococcus aureus* (V/MRSA), and multidrug-resistance (MDR) pathogens (GBD 2019; Ranjbar and Alam, 2022; WHO, 2023a).

As available antibiotics and antibacterials become ineffective against emerging drug-resistant bacterial strains, researchers are continuously exploring various alternative and complementary sources. In this context, ethnomedicines are widely recognized for their effectiveness in controlling such infections due to their multipotency, additive antioxidant potency, reduced side effects/toxicity, cost-effectiveness, and the slower rate at which bacterial strains succumb to natural regimens compared to current antibiotics (Bhattacharjee, 2021; Ghosh et al., 2022; De et al., 2023; Rai and Sharma, 2024; Darro and Khan, 2024; Dey-Ray et al., 2024). There is growing recognition of the value of integrating traditional and modern approaches in the health care and wellness sector in the form of 'integrative medicine' or 'complementary and alternative medicine (Banerjee et al., 2014; WHO-2023b; Sahoo et al., 2022a; Park et al., 2019; Acharya et al., 2021; Sarkar et al., 2021). Researchers have looked into the antibacterial properties of many traditional medicines so far, and India's wide range of plant species has shown some that show promise (Aqil and Ahmad, 2007; Maiti et al., 2010; 2011; Swain and Padhy, 2015; Vaou et al., 2019; Chassagne et al., 2021; Balkrishna et al., 2022). Researchers also aim to validate the antibacterial properties of well-known ethnomedicinal practices scientifically passed down through generations. Research continues to investigate the mechanisms and efficacy of traditional remedies, with the goal of integrating valuable ethnomedicinal knowledge into modern healthcare practices.

With the advanced instrumental and technology facilities, researchers are using high-throughput platforms to identify bioactive candidates from crude extracts. We are aware that various phytochemical classes, such as alkaloids, terpenes, steroids, flavonoids, phenols, glycosides, etc., are present in the crude extract. However, isolating the bioactive constituents for mainstream therapeutic applications is more crucial. Advanced facilities like gas chromatography–mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), high-resolution mass spectrometry (HRMS), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), column chromatography, etc. help us to isolate and characterize bioactive candidates (Capriotti et al., 2021; Barthwal and Mahar, 2024). In addition, researchers are increasingly using various bioinformatics, or computational biology, tools to explore the target specific bioactivity, predict toxicity, and assess the drug-ability of any desired drug candidates (Swain et al., 2022a; Sahoo et al., 2022b; Sarkar et al., 2024). Moreover, researchers utilize these tools to delve into the detailed biology, molecular mechanics, drug stability, and kinetic behaviours of a therapeutic candidate and its target enzyme interaction (Swain et al., 2022a; Sahoo et al., 2022b). Based on our previous *in vitro* data and available resource facility, we selected methanol solvent-derived leaf extracts of three Indian medicinal plants, namely, *Psidium guajava* L., *Syzygium cumini* L., and *Punica granatum* L. to identify the antibacterial agent after GC-MS study along with a literature search. Furthermore, we used bioinformatics tools to verify the target-specific potency, toxicity, and drug-ability profile of the selected phytoconstituents. The present study proposes a more ingenious and cost-effective approach to identifying potential antibacterial candidates from the aforementioned medicinal plants in order to accelerate the discovery of phytochemical-based antibacterial drugs.

Materials and Methods

Quantitative phytochemical analyses using GC-MS

Based on our previous antibacterial reports, we selected methanolic leaf extracts from three medicinal plants (*P. guajava*, *S. cumini* and *P. grantum*) for quantitative phytochemical analyses using GC-MS. We prepared plant samples according to the previous protocol (Konappa et al., 2020). The GC-MS study was carried out with a Turbo Mass Spectrophotometer (USA) model Clarus 590 Gas Chromatography/Clarus SQ 8S Mass Spectrometer (with a liquid auto sampler) for 35 minutes. We then analysed the identified mass spectra using the NIST (National Institute of Standards and Technology) library as a reference dataset by comparing retention times, peak areas, and mass spectral patterns (Konappa et al., 2020; Ralte et al., 2022).

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Retrieval of chemical structure and their physiochemical properties

Through GC-MS analyses, we found a lot of phytoconstituents in the crude extracts. However, we chose ten phytoconstituents from each GC-MS report based on their high-intensity spectra to use in further computer-based analyses. Since GC-MS gives us some basic information about the amounts of low-molecular-weight phytoconstituents, we also included ten high-molecular-weight phytoconstituents from the plants listed above that had already been found or reported (through column chromatography or other method) in an extensive literature search. This was done so that we could compare them and choose the best antibacterial candidates. The *P. guajava*-derived phytochemicals through GC-MS are named PG_GCMS_1-10, and those recorded from literature are named PG_LR_1-10. Similarly, GC-MS-derived phytochemicals of *S. cumini* (SC_GCMS_1-10) and from literature (SC_LR_1-10) were recorded. For GC-MS-derived phytochemicals *P. grantum*, PG_GCMS_1-10 and literature-based candidates are named PG_LR_1-10 for better understanding and recognition of the antibacterial potency. We have recorded the physicochemical profiles (molecular weight, molecular formula, hydrogen bonds, topological surface area, XlogP values, along with molar refractivity or MR and bioavailability or BA profiles) of GC-MS-derived candidates along with selected existing candidates from PubChem (https://pubchem.ncbi.nlm.nih.gov/), ChemSpider (https://www.chemspider.com/) and also predicted using the tool, SwissADME (http://www.swissadme.ch/). The Simplified Molecular Input Line Entry System (SMILES) notations of both GC-MS-derived and literature-based selected phytoconstituents to be used in further bioinformatics analyses or investigation.

Bacterial target protein selection, retrieval and preparation for docking study

We conducted the entire computational work on a Linux-Ubuntu 16.04 LTS workstation using several cheminformatics software packages (Swain et al., 2022a; Sahoo et al., 2022b). Generally, ligand and target structure are essential to performing the molecular docking study. In the present study, selected phytoconstituents (GC-MS-derived and literature-based) are our ligands. To investigate the antibacterial activity of our phytoconstituents, we need to target specific proteins. Based on the bacterial strains used in the *in vitro* study, we have selected four bacterial targets as follows: the biofilm-associated target enzyme, poly-beta-1,6-N-acetyl-D-glucosamine N-deacetylase (PgaB); 3-oxoacyl-ACP reductase (FabG) of *K. pneumoniae*; *Pseudomonas* quinolone signal A (PqsA) of *P. aeruginosa*; and pyruvate kinase of methicillin-resistant *S. aureus* (PyK-MRSA). We retrieved all ligand structures from PubChem with individual CID numbers (Table 2) and the standard antibiotic amikacin for our study. We also retrieved all four bacterial target proteins from the protein data bank with individual PDB IDs: PgaB (PDB ID: 3VUS), FabG (PDB ID: 6T77), PqsA (PDB ID: 5OE3), and PyK-SA/MRSA (PFB ID: 3T05), respectively (https://www.rcsb.org/).

To get a reliable binding energy or docking score (kcal/mol.), we optimized and minimized both ligand and protein structure and saved them in PDB file format before the docking study. We performed the molecular virtual screening-cum-molecular docking study using the PyRx 0.8 and AutoDock 4.2 software (Swain et al., 2022a; Sahoo et al., 2022b; Swain and Hussain, 2022). In summary, each ligand generated ten docking poses (kcal/mol.) against each target and selected the best pose based on the lowest binding energy produced between them. Furthermore, we visualized the protein-ligand 3-D and two-dimensional (2-D) molecular interactions with targets using the software BIOVIA-DSV-2019 (Swain et al., 2022a; Sahoo et al., 2022b).

Toxicity and drug-ability profile prediction

After biological activity analyses or investigations, the toxicity profile plays a crucial role in the early stages of drug discovery. Higher toxicity often led to the withdrawal of active candidates from early phases of clinical trials, despite their higher therapeutic value. Previously, researchers studied toxicity profiles using various *in vitro* and *in vivo* models, which are more resource-consuming methods. However, through advanced computational tools, we may predict possible toxicity profiles on any candidates by comparing with huge training datasets (Sahoo et al., 2021; Swain and Hussain, 2022). Using the ProTox tool (http://tox.charite.de/protox_II/), we have predicted hepatotoxicity (HT), carcinogenicity (CG), cytotoxicity (CT), toxicity class (TC), and lethal dose (LD_{50} in mg/kg) for all selected phytoconstituents, and standard antibiotic amikacin (Sahoo et al., 2021; Swain and Hussain, 2022).

The drug-ability profile, also known as the drug-likeness score, is another advanced prediction by bioinformatics tool that assumes the drug suitability profile as per the chemical structure of a desired candidates. Usually, drug-likeness depend on many parameters, including physiochemical, solubility, bioavailability, toxicity, pharmacokinetics, and more. This parameter plays a crucial role when choosing drug-

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Table 1. The phytochemicals from *P. guajava* were chosen for this study from GCMS reports (PG_GCMS_1-10) and existing reports (PG_LR_1-10), *S. cumini* (SC_GCMS_1-10) and SC_LR_1-10), and *P. grantum* (PG_GCMS_1-10 and PG_LR_1-10).

Sl. No.	*Psidium guajava* L.		*Syzygium cumini* L.		*Punica grantum* L.	
	From GC-MS report	From existing reports	From GC-MS report	From existing reports	From GC-MS report	From existing reports
1.	Allyl n-octyl ether	Apigenin	Cyclohexane, 1-methyl-4-(1-methyl ethyl)-,cis-	β-Sitosterol	1,3,5-tris (cyclo hexyl)pent-1-ene	Brevifolin
2.	N-Pentane, 2-cyclohexyl-5-[1-cycloazapropyl]-	Catechin	Oxalic acid, cyclohexylmethyl ethyl ester	Ellagic acid	Cyclohexane, 1,1'-(2-propyl-1,3-propane diyl)bis-	Catechin
3.	1,3-Cyclo pentanediol, cis-	Ellagic acid	2-Propenoic acid, undec-10-enyl ester	Epifriedelanol	Bicyclo[3.1.1]h eptan-2-one, 6,6-dimethyl-	Cinnamic acid
4.	Oxirane, [(tetra-decyloxy)methyl -	Gallic acid	Trans-1-methyl-2-nonyl-cyclohexane	Gallic acid	2,5-Furandione, 3-dodecyl-	Ellagic acid
5.	Cyclohexanone , 4-methyl-	Guaijaverin	1,1'-Bicycloheptyl	Kaempferol	Cyclopentane, 2-(1-hydroxy-2-propyl)-1,3-dimethyl-	Punicic acid
6.	Carbonic acid, octyl prop-1-en-2-yl ester	Kaempferol	E-9-Tetradecenoic acid	Myricetin	(2,4,6-Trimethyl cyclohexyl) methanol	Punicacortein A
7.	Cyclopentane-1,2-diol	Luteolin	1,3-Benzodioxol-2-amine, hexa hydro-n,n-dimethyl-	Myricitrin	5,14,23-Octadeca trien-14,15-diol	Punigluconin
8.	1-Heptanol, 2,4-dimethyl-,(2s,4r)-(-)-	Quercetin	1,4-Dioxaspiro [5.5]undecan-2-one	Myristic acid	2-Dodecen-1-yl(-) succinic anhydride	Quercetin
9.	4-Octanol, 7-methyl-, acetate	Ursolic acid	Cyclohexane, 1-ethyl-4-methyl-, trans-	Petunidin 3-gentiobioside	Cyclopentanecar boxamide, n-(2-fluorophenyl)-	Tercatain
10.	1-Heptanol, 2,4-dimethyl-, (r,r)-(+)-	Vanillic acid	Ricinoleic acid	Quercetin	1,1':3',1"-Tercyclopentane, 2'-dodecyl-	Vanillic acid

able chemicals from a large group to further study in the lab. We used the MolSoft tool (https://www.molsoft.com/) to predict the drug-likeness score of each phytochemical. Overall, the results help to get some prior information to select some desired lead candidates to accelerate the phytochemical-based antibacterial drug discovery process (Swain et al., 2022a; Swain and Hussain, 2022).

Results and Discussion

Quantitative phytochemical analyses using GC-MS

Three medicinal plants (*P. guajava*, *S. cumini* and *P. grantum*) underwent a quantitative phytochemical study using GC-MS on their methanol leaf extracts. Although there are huge number peaks observed in GC-MS spectra (**Figure S1**), only ten phytoconstituents (PG_GCMS_1-10, SC_GCMS_1-10, and PG_GCMS_1-10) were selected from each plant extra based on higher RT, peak area, and volume as presented in **Table 1**. Overall, because all three are well-known medicinal-cum-edible plants, we have discovered a higher number of alcohol, benzoic, and phenolic derivatives with a lower molecular

Table 2. Recorded physicochemical profiles of selected GM-MS-derived phytoconstituents and selected existing phytoconstituents, including standard amikacin.

Sl. No.	GC-MS derived phytoconstituents							Sl. No	Existing phytoconstituents from literature						
	MW	XlogP	HA	HD	MR	TPSA	BA		MW	XlogP	HA	HD	MR	TPSA	BA
P1	170.29	4.1	1	0	55.60	9.23	0.55	P31	270.24	1.7	5	3	73.99	90.90	0.55
P2	195.34	4.3	1	0	67.19	3.01	0.55	P32	290.27	0.4	6	5	74.33	110.38	0.55
P3	102.13	-0.2	2	2	26.36	40.46	0.55	P33	302.19	1.1	8	4	75.31	141.34	0.55
P4	270.45	6.4	2	0	83.89	21.76	0.55	P34	170.12	0.7	5	4	39.47	97.99	0.55
P5	112.17	1.4	1	0	33.85	17.07	0.55	P35	434.35	0.4	11	7	104.19	190.28	0.55
P6	214.30	4.7	3	0	62.08	35.53	0.55	P36	286.24	1.9	6	4	76.01	111.13	0.55
P7	102.13	-0.1	2	2	26.36	40.46	0.55	P37	286.24	1.4	6	4	76.01	111.13	0.55
P8	144.25	3.1	1	1	46.54	20.23	0.55	P38	302.24	1.5	7	5	78.03	131.36	0.55
P9	186.29	3.5	2	0	56.28	26.30	0.55	P39	456.70	7.3	3	2	136.91	57.53	0.55
P10	144.25	3.1	1	1	46.54	20.23	0.55	P40	168.15	1.4	4	2	41.92	66.76	0.55
P11	140.27	4.5	0	0	48.07	0.00	0.55	P41	414.71	9.3	1	1	133.23	20.23	0.55
P12	241.26	2.6	4	0	55.45	52.60	0.55	P42	302.19	1.1	8	4	75.31	141.34	0.55
P13	224.34	5.2	2	0	69.75	26.30	0.55	P43	428.37	10.1	1	1	135.36	20.23	0.55
P14	224.43	8.1	0	0	76.91	0.00	0.55	P44	170.12	0.7	5	4	39.47	97.99	0.55
P15	194.36	6.8	1	0	65.18	0.00	0.55	P45	286.24	1.9	6	4	76.01	111.13	0.55
P16	226.36	5.1	2	1	70.71	37.30	0.85	P46	318.24	1.2	8	6	80.06	151.59	0.55
P17	171.24	1.3	3	0	46.21	21.70	0.55	P47	464.4	0.5	8	12	111.2	207	0.17
P18	170.21	1.4	3	0	43.56	35.53	0.55	P48	228.37	5.3	2	1	71.18	37.30	0.55
P19	126.24	4.3	0	0	43.26	0.00	0.55	P49	641.55	-2.0	17	11	147.17	281.82	0.55
P20	298.46	5.7	3	2	91.10	57.53	0.85	P50	302.24	1.5	7	5	78.03	131.36	0.55
P21	316.56	10.1	0	0	105.9	0.00	0.55	P51	196.20	1.7	4	1	51.64	55.76	0.55
P22	250.46	8.7	0	0	84.41	0.00	0.55	P52	290.27	0.4	6	5	74.33	110.38	0.55
P23	138.21	1.6	1	0	41.09	17.07	0.55	P53	148.16	2.1	2	1	43.11	37.30	0.55
P24	266.38	6.1	3	0	77.92	43.37	0.55	P54	302.19	1.1	8	4	75.31	141.34	0.55
P25	156.27	3	1	1	49.23	20.23	0.55	P55	278.43	4.9	2	1	88.99	37.30	0.55
P26	157.21	3	1	1	49.24	20.23	0.55	P56	634.45	-0.8	18	12	142.09	321.66	0.55
P27	420.71	11.1	2	2	138.4	40.46	0.55	P57	802.56	1.0	23	14	180.36	405.49	0.55
P28	266.38	5.1	3	0	77.92	43.37	0.55	P58	302.24	1.5	7	5	78.03	131.36	0.55
P29	207.24	2.5	2	1	57.83	29.10	0.55	P59	772.57	1.0	21	13	177.14	360.35	0.55
P30	374.69	12.7	0	0	125.5	0.00	0.55	P60	168.15	1.4	4	2	41.92	66.76	0.55
Std.	585.60	-7.9	17	13	128.9	331.9	0.55	Std.	585.60	-7.9	17	13	128.99	331.94	0.55

*, used as standard antibiotic (amikacin); H-A, hydrogen bond acceptors; H-B, hydrogen bond donor; MR, molar refractivity; MW, molecular weight; TPSA, topological polar surface area; BA, bioavailability.

weight of 100 to 375 g/mol. Along with the GC–MS candidates, we picked ten more phytoconstituents from earlier studies (PG_LR_1–10, SC_LR_1–10, and PG_LR_1–10) to look for possible antibacterial in each plant extract. The existing candidates, which were primarily isolated through column chromatography and other high-throughput techniques, exhibited a comparatively higher molecular weight (> 800 g/mol.). Noted that, out of thirty existing or reported candidates from there plants, six phytochemicals (catechin, ellagic acid, gallic acid, kaempferol, quercetin, vanillic acid) were found common in all three plants (Table 1). In addition, existing candidate bioactivity profiles have a significantly higher index than GC-MS-derived candidates, according to the investigation reports. Despite our inability to identify the candidates through GC-MS analyses, the presence of a higher number of phenolic classes of candidates in the above leaf extracts confirms their higher antibacterial potency and lower toxicity profiles.

Retrieval of chemical structure and their physiochemical properties

The physicochemical profiles of GC-MS-derived and phytochemicals selected from existing reports were well documented in Table 2. Physicochemical profiles, also called the Lipinski rule (RO5), are a set of parameters

Table 3. Recorded molecular docking score (kcal/mol.) of selected GM-MS-derived and selected existing phytoconstituents including amikacin against four putative bacterial target enzymes from four pathogenic bacteria.

Sl. No.	GC-MS derived phytoconstituents					Sl. No.	Existing phytoconstituents from literature				
	EC_PgaB (3VUS)	KP_FabG (6T77)	PA_PqsA (5OE3)	SA_PyK (3T05)	DL		EC_PgaB (3VUS)	KP_FabG (6T77)	PA_PqsA (5OE3)	SA_PyK (3T05)	DL
P1	-3.9	-3.7	-5.1	-4.8	-1.28	P31	-7.3	-8.2	-8.9	-8.6	0.39
P2	-5.2	-4.9	-6.1	-4.9	-0.45	P32	-7.4	-8.0	-8.4	-7.7	0.64
P3	-4.5	-4.3	-4.9	-4.7	-1.48	P33	-7.5	-8.4	-8.6	-8.5	-1.11
P4	-4.4	-4.8	-5.2	-4.6	-1.30	P34	-6.5	-6.0	-6.6	-5.9	-0.22
P5	-4.5	-5.0	-5.9	-5.3	-1.72	P35	-7.8	-8.4	-8.7	-8.2	0.93
P6	-4.7	-4.5	-5.2	-5.2	-1.25	P36	-7.3	-8.0	-8.9	-8.2	0.50
P7	-4.0	-4.1	-5.0	-4.3	-1.46	P37	-8.0	-8.3	-8.9	-8.1	0.38
P8	-4.4	-4.4	-5.4	-4.9	-1.31	P38	-7.5	-8.2	-8.2	-7.9	0.52
P9	-4.7	-4.7	-5.6	-5.3	-0.09	P39	-7.5	-9.5	-8.2	-9.1	0.66
P10	-4.6	-4.5	-4.5	-5.1	-1.31	P40	-6.1	-5.6	-6.7	-6.1	-0.18
P11	-4.8	-4.9	-6.0	-6.2	-1.51	P41	-6.6	-8.3	-8.4	-6.6	0.78
P12	-5.6	-5.6	-6.4	-5.8	-0.61	P42	-7.5	-8.4	-8.6	-8.5	-1.11
P13	-4.3	-4.0	-5.4	-3.6	-1.64	P43	-8.0	-9.3	-8.8	-8.3	-0.57
P14	-4.8	-5.3	-6.1	-4.9	-1.02	P44	-6.5	-6.0	-6.6	-5.9	-0.22
P15	-5.9	-4.9	-5.8	-6.8	-1.65	P45	-7.3	-8.0	-8.9	-8.2	0.50
P16	-4.8	-5.4	-5.5	-5.0	-0.30	P46	-7.4	-8.3	-8.8	-8.0	-0.24
P17	-4.9	-5.5	-5.7	-6.0	-1.04	P47	-4.6	-5.1	-5.5	-4.7	-0.54
P18	-5.8	-6.1	-6.2	-6.7	-1.22	P48	-5.1	-4.9	-6.9	-7.2	0.41
P19	-4.4	-4.9	-5.0	-5.8	-1.26	P49	-7.5	-8.2	-8.2	-7.9	0.52
P20	-4.8	-5.7	-5.8	-4.9	-0.36	P50	-6.6	-8.3	-8.4	-6.6	-0.98
P21	-6.7	-8.3	-8.2	-8.3	-0.30	P51	-5.4	-5.4	-5.6	-5.6	0.64
P22	-5.7	-6.7	-6.5	-6.7	-0.77	P52	-7.4	-8.0	-8.4	-7.7	-1.17
P23	-5.1	-5.6	-6.7	-5.4	-1.12	P53	-5.8	-6.0	-6.2	-6.4	-1.11
P24	-5.0	-5.8	-6.6	-5.1	-1.10	P54	-7.5	-8.4	-8.6	-8.5	-0.30
P25	-5.2	-5.3	-4.9	-5.8	-1.09	P55	-5.3	-6.1	-6.1	-5.3	0.28
P26	-5.3	-5.1	-5.8	-5.8	-1.29	P56	-8.1	-9.4	-9.8	-9.1	0.26
P27	-2.9	-1.9	-2.3	-2.6	-0.46	P57	-6.9	-6.9	-6.8	-7.5	0.52
P28	-5.6	-5.5	-7.0	-5.1	-1.14	P58	-7.5	-8.2	-8.2	-7.9	0.62
P29	-5.9	-6.8	-6.6	-7.1	0.12	P59	-9.0	-9.2	-9.1	-8.9	-0.18
P30	-6.7	-7.4	-5.9	-6.4	-1.04	P60	-6.1	-5.6	-6.7	-6.1	0.39
Std.	-8.8	-9.8	-9.7	-8.3	1.03	Std.	-8.8	-9.8	-9.7	-8.3	1.03

used in modern drug discovery platforms to choose active oral candidates. They are based on molecular weight (≤ 500 g/mol.), XlogP (≤ 5), number of hydrogen bond acceptors (≤ 10), donors (≤ 5), and tPSA (≤ 142 Å) profiles. In addition, we have added the molar refractivity (MR: 30 to 130) and bioavailability (BA: > 0.55) parameters towards selecting the ideal lead candidates (Table 2). As all GC-MS derivatives have a lower molecular weight, most of the candidates followed the ideal RO5, whereas existing candidates have a higher molecular weight and deviated from the RO5 rule. Not only do most of these candidates have a molecular weight exceeding 500 g/mol., but they also have an XlogP value that exceeds the ideal threshold of less than 5. However, the standard antibiotic amikacin, as well as most of the marketed drugs, do not follow the RO5 condition, indicating that a higher emphasis on the RO5 may eliminate more potential candidates (Sahoo et al., 2021; Swain and Hussain, 2022). Therefore, this predefined parameter provides more relevant information and guides the selection of better oral drug candidates for increased experimental success. Nonetheless, there is always a negotiation between potency and physicochemical parameters, as nanoformulation-type platforms are available to improve the physicochemical parameters of any desired drug candidates. Additionally, the predicted BA-score showed that all phytoconstituents with a suitable profile scored at 0.55, while P16 and P20 scored significantly higher at 0.85 than the others (Table 2).

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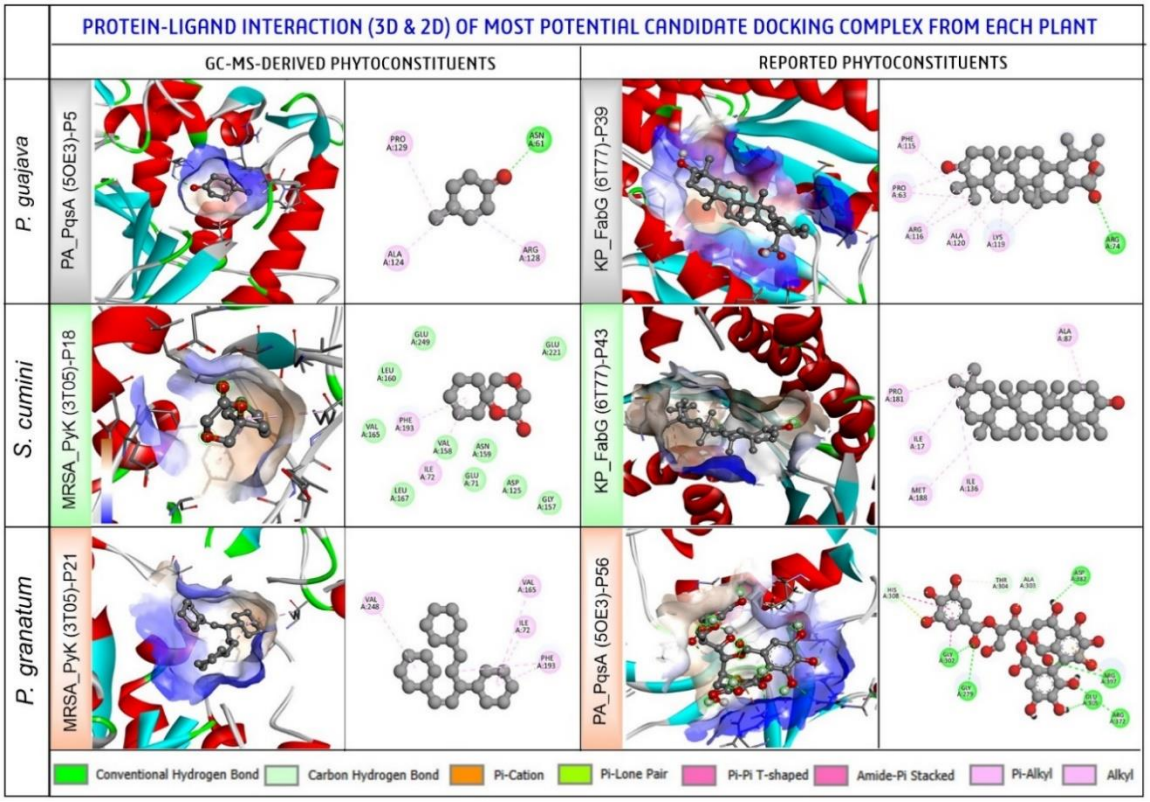

Figure 1. Three-dimensional and two-dimensional protein-ligand interactions of the three most potent docking complexes from GC-MS and reported phytoconstituents from each plant. Individual 3D and 2D interaction figures using BIOVIA-DSV software

Bacterial target protein selection, retrieval and preparation for docking study

Both the GC-MS-derived and previously reported candidates' molecular docking scores with standard antibiotics against four bacterial target, are presented in Table 3. The docking score showed that the GC-MS-derived phytoconstituents had a binding potency between -3 and -7 kcal/mol., while the previously reported phytoconstituents had a binding affinity between -4 and -10 kcal/mol., which was quite higher. Generally, a higher negative docking score indicates a higher binding affinity or potency against the respective target, according to the AutoDock software (Swain and Hussain, 2022). From a close observation, *P. grantum* derive phytochemicals showed comparatively higher potency than the other two plant-derived candidates and displayed higher potency against PA_PqsA and SA_PyK, the other two target enzymes (Table 3). Taking the average docking score: P5 (cyclohexanone, 4-methyl-) from *P. guajava* with an average docking score of -5.27 kcal/mol.; P18 (1,4-dioxaspiro [5.5] undecan-2-one) from *S. cumini* with an average docking score of -6.57 kcal/mol.; and P21 (1,3,5-tris(cyclohexyl)pent-1-ene) from *P. grantum* with an average docking score of -7.87 kcal/mol., respectively. However, among the three sets of literature-based phytoconstituents from the aforementioned three plants, P39: ursolic acid (-9.5 kcal/mol.) was more potent against KP_FabG, P43: epifriedelanol (-9.3 kcal/mol) against KP_FabG, and P56: punicacortein A (-9.8 kcal/mol) against PA_PqsA and MRSA_PyK. When compared to the standard drug, amikacin was better at binding to all target enzymes except PA_PqsA (Table 3).

In addition, we have also elucidated the protein-ligand interactions of selected potential docking complexes, where we found a higher number of non-covalent interactions like pi-pi, van der Wall, hydrogen bond, etc., in reported and GC-MS-derived candidates as per their potency in form of binding efficacy (Figure 1). Despite the reported compounds exhibiting higher therapeutic efficacy, the overall results suggest that the presence of these bioactive compounds synergistically enhanced the crude extracts' antibacterial activity. However, the exact percentage of these bioactive compounds in the crude extracts remains unknown. Sometimes highly abundant candidates may not be active against bacteria, whereas low percentile candidates may be active. Therefore, validating each candidate with a specific biological activity through various experimental models before mainstream application is crucial.

Toxicity and drug-ability profile prediction

The toxicity profiles of all phytoconstituents, along with standard amikacin were recorded in Table 4. According to predicted profiles with a higher green color indication, most candidates are non-toxic. Narratively, both sets of candidates demonstrate high safety from HT and CT, while the CG profiles of most candidates showed

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Table 4. Recorded toxicity and drug-ability profiles of selected GM-MS-derived phytoconstituents and selected existing phytoconstituents including standard amikacin.

Sl. No.	GC-MS derived phytoconstituents					Sl. No.	Existing phytoconstituents from literature				
	HT	CG	CT	TC	LD_{50}		HT	CG	CT	TC	LD_{50}
P1	IA(0.87)	A(0.64)	IA(0.82)	V	5000	P31	IA(0.68)	IA(0.62)	IA(0.87)	V	2500
P2	IA(0.94)	IA(0.53)	IA(0.76)	III	300	P32	IA(0.72)	IA(0.51)	IA(0.84)	VI	10000
P3	IA(0.85)	IA(0.73)	IA(0.81)	VI	7200	P33	IA(0.83)	IA(0.59)	IA(0.90)	IV	2991
P4	IA(0.89)	IA(0.78)	IA(0.83)	VI	7800	P34	IA(0.61)	IA(0.56)	IA(0.91)	IV	2000
P5	IA(0.72)	IA(0.78)	IA(0.72)	IV	500	P35	IA(0.80)	IA(0.75)	IA(0.69)	V	5000
P6	IA(0.76)	IA(0.51)	IA(0.80)	V	5000	P36	IA(0.68)	IA(0.72)	IA(0.98)	V	3919
P7	IA(0.82)	IA(0.65)	IA(0.72)	V	3700	P37	IA(0.69)	IA(0.68)	IA(0.99)	V	3919
P8	IA(0.79)	IA(0.55)	IA(0.84)	IV	1000	P38	IA(0.69)	IA(0.68)	IA(0.99)	III	159
P9	IA(0.58)	IA(0.59)	IA(0.62)	V	3000	P39	IA(0.52)	IA(0.57)	IA(0.99)	IV	2000
P10	IA(0.79)	IA(0.55)	IA(0.84)	IV	1000	P40	IA(0.55)	IA(0.64)	IA(0.93)	IV	2000
P11	IA(0.84)	IA(0.72)	IA(0.81)	VI	15380	P41	IA(0.87)	IA(0.60)	IA(0.94)	IV	890
P12	IA(0.81)	IA(0.54)	IA(0.88)	V	3200	P42	IA(0.83)	IA(0.59)	IA(0.90)	IV	2991
P13	IA(0.77)	IA(0.56)	IA(0.78)	V	5000	P43	IA(0.78)	IA(0.79)	IA(0.93)	IV	940
P14	IA(0.79)	IA(0.54)	IA(0.80)	VI	15380	P44	IA(0.61)	IA(0.56)	IA(0.91)	IV	2000
P15	IA(0.81)	IA(0.54)	IA(0.79)	III	880	P45	IA(0.68)	IA(0.91)	IA(0.98)	V	3919
P16	IA(0.59)	IA(0.65)	IA(0.70)	II	48	P46	IA(0.69)	IA(0.68)	IA(0.99)	III	159
P17	IA(0.81)	IA(0.65)	IA(0.68)	V	5000	P47	IA(0.73)	IA(0.50)	IA(0.93)	V	5000
P18	IA(0.82)	IA(0.56)	IA(0.74)	V	5000	P48	IA(0.52)	IA(0.63)	IA(0.74)	IV	900
P19	IA(0.78)	IA(0.54)	IA(0.81)	VI	15380	P49	IA(0.84)	IA(0.86)	IA(0.52)	V	5000
P20	IA(0.67)	IA(0.64)	IA(0.69)	II	11800	P50	IA(0.69)	IA(0.68)	IA(0.99)	III	159
P21	IA(0.80)	IA(0.52)	IA(0.86)	V	5000	P51	IA(0.52)	IA(0.60)	IA(0.83)	IV	820
P22	IA(0.75)	IA(0.51)	IA(0.82)	VI	15380	P52	IA(0.72)	IA(0.51)	IA(0.84)	VI	10000
P23	IA(0.76)	IA(0.69)	IA(0.67)	V	2400	P53	IA(0.54)	IA(0.82)	IA(0.83)	V	2500
P24	IA(0.71)	IA(0.60)	IA(0.74)	II	34	P54	IA(0.83)	IA(0.59)	IA(0.90)	IV	2991
P25	IA(0.82)	IA(0.58)	IA(0.88)	IV	2000	P55	IA(0.59)	IA(0.65)	IA(0.70)	V	3200
P26	IA(0.84)	IA(0.63)	IA(0.88)	IV	940	P56	IA(0.87)	IA(0.78)	IA(0.58)	V	2170
P27	IA(0.77)	IA(0.58)	IA(0.70)	VI	11730	P57	IA(0.82)	IA(0.70)	IA(0.79)	V	3000
P28	IA(0.77)	IA(0.66)	IA(0.72)	IV	2000	P58	IA(0.69)	IA(0.68)	IA(0.99)	III	159
P29	IA(0.69)	IA(0.62)	IA(0.93)	IV	1190	P59	IA(0.86)	IA(0.70)	IA(0.78)	V	2260
P30	IA(0.80)	IA(0.52)	IA(0.76)	V	4100	P60	IA(0.55)	IA(0.64)	IA(0.93)	IV	2000
Std.	IA(0.97)	IA(0.71)	IA(0.77)	V	4000	Std.	IA(0.97)	IA(0.71)	IA(0.77)	V	4000

HT, hepatotoxicity; CG, carcinogenicity; CT, cytotoxicity; TC, toxicity class; LD_{50}, fifty percent lethal dose (mg/kg).

moderate to high risk. The GC-MS produces potential candidates such as P5, which is highly safe in all three toxicity classes, while P198 and P21 pose a CG risk. Similarly, P39 is comparatively safe from both HT and CG, while P43 and P56 are highly secure from all the toxicity classes. All of the toxicity classes showed that amikacin had a higher safety rating. The majority of the candidates were classified as class IV and V by the toxicity class (TC), indicating that their non-toxicity ranged from 300 to 5000 mg/kg. On the other hand, the ProTox tool (Table 4) classified candidates P2, P15, P20, P24, P38, P46, P50, and P58 as class II and III, indicating their non-toxicity within 5 to 50 mg/mg. According to the toxicity class (TC), all candidates' predicted LD50 (mg/kg) also showed similar trends, ranging from a minimum of 50 mg/kg to a maximum of 10000 mg/kg. As an approved antibiotic, standard amikacin showed a non-toxic profile with TC=IV and LD_{50}=4000 mg/kg. The results suggest that the derived candidates from three medicinal plants are non-toxic, even at higher concentrations, and could potentially serve as an ideal antibacterial lead candidate. At the same time, the predicted overall drug-likeness (DL) score (Table 3) showed that all three plants' GC-MS-derived phytoconstituents had a negative score, while most of the other candidates had a positive DL score. Mainly among

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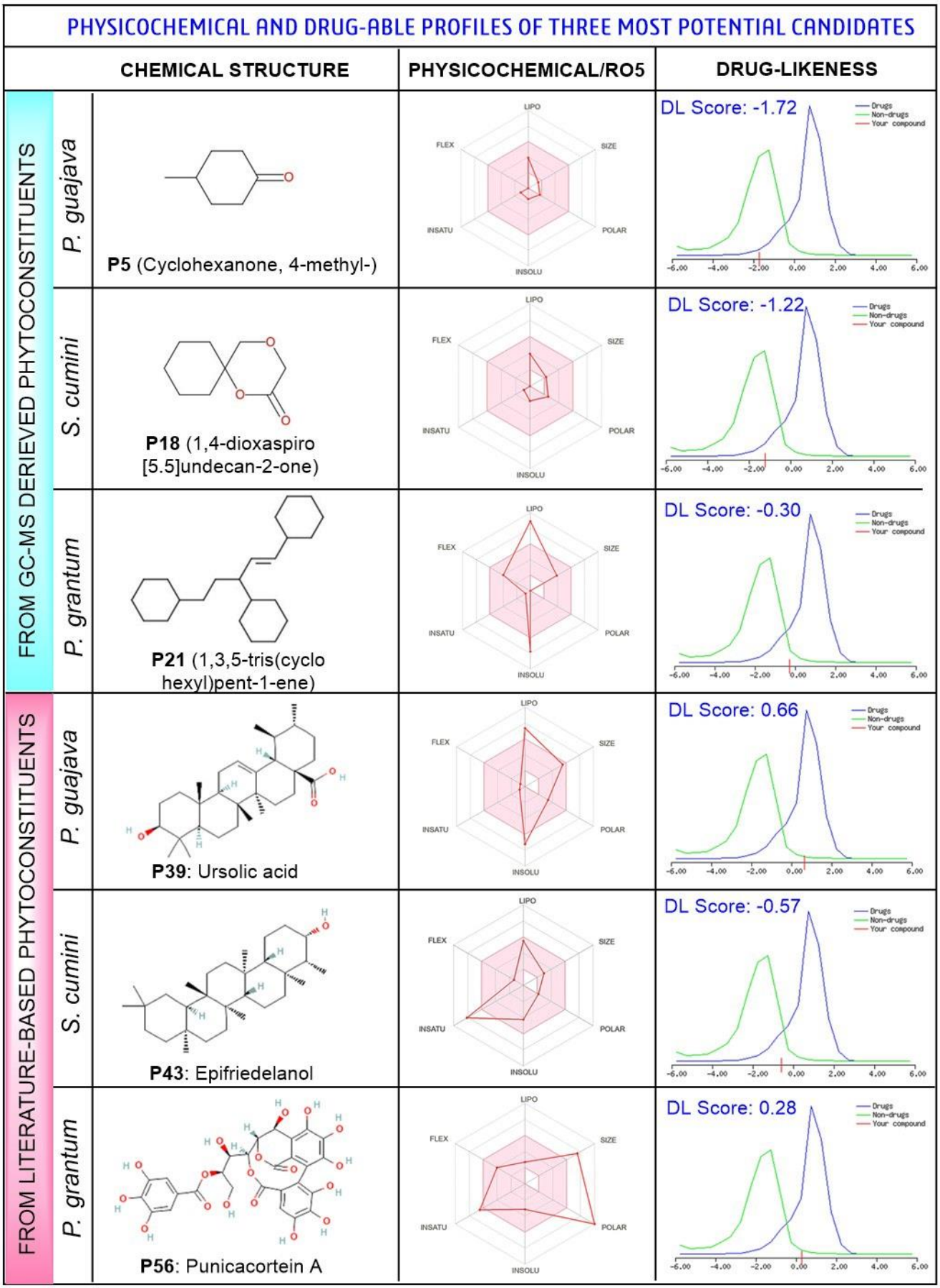

Figure 2. The physicochemical or RO5 profiles and overall drug-likeness scores of the three most promising compounds from both sets (GC-MS and literature)-based phytoconstituents are graphically presented based on their anticipated outcomes.

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P39, P43, and P56, both P39 (0.66) and P56 (0.28) showed positive drug likeness scores, while P43 (-0.57) showed a negative DL score (Figure 2). Technically, the DL-score shows how well a candidate expressed their suitability as a drug by comparing it to the chemical structure of FDA-approved drugs. It could help choose bioactive molecules in the early stages of the drug discovery process, with higher chances of experimental success. Therefore, an ideal candidate should have potential, nontoxicity and suitable RO5 profiles. We didn't find any drug-able components in our GC-MS sets, but existing phytoconstituents showed that the three medicinal plant leaf extracts above have a higher number of active drug-able phytochemicals. This makes us want to find more bioactive constituents for Indian medicinal plants for mainstream antibacterial therapy.

Exploring biological activity through experimental study is crucial for unlocking the therapeutic potential of traditional remedies used for centuries by various cultures. This research bridges the gap between traditional knowledge and modern medicine, offering a pathway to discovering new, effective drugs that can address unmet medical needs (Swain et al., 2022a; Sahoo et al., 2022b and 2022c). It also ensures that traditional practices are scientifically validated, which can lead to safer and more standardized treatments. Additionally, this exploration helps preserve cultural heritage, promotes biodiversity, and provides affordable healthcare solutions, particularly in underserved regions, while fostering ethical practices in the sharing and use of indigenous knowledge. Following the same path, the study of ethnomedicines against bacterial infections and antibiotic-resistant strains is critical in addressing the growing global health crisis of antimicrobial resistance. Traditional remedies, often derived from plants and other natural sources, offer a rich reservoir of bioactive compounds that may possess novel mechanisms of action against resistant bacteria (Swain et al., 2022a; Sahoo et al., 2022b and 2022c). By scientifically validating these ethnomedicines, we can discover new antibacterial agents that are effective where conventional antibiotics fail, potentially slowing down the spread of resistance. This research not only diversifies the arsenal of available treatments but also emphasizes the importance of sustainable and culturally respectful approaches to drug discovery, providing affordable and accessible solutions to combat bacterial infections worldwide.

The quantitative phytochemical investigation, antibacterial potency evaluation, and drug-ability assessment of leaf extracts from three Indian medicinal plants provide valuable insights into their therapeutic potential. The study revealed that these extracts are rich in bioactive compounds, such as flavonoids and phenolics, known for their antimicrobial properties (Swain and Padhy, 2015). The antibacterial assays demonstrated significant activity against a range of bacterial strains, including some antibiotic-resistant ones, highlighting the potential of these plant extracts as alternative treatments (Swain et al., 2022a; Sahoo et al., 2022b and 2022c). Additionally, bioinformatics tools were used to predict the toxicity and drug-ability of the identified compounds along with some existing compounds showing favorable profiles, which support the antibacterial potency of crude extracts. Additionally, antibacterial agents derived from various ethnomedicinal crude extracts are non-toxic and possess additive anti-inflammatory and antioxidant potencies, which could aid in improving treatment outcomes and preventing antibiotic resistance (Swain and Padhy, 2015).

Researchers use several advanced techniques and approaches to isolate and locate potential bioactive candidates, where bioinformatics tools play a crucial role in accelerating the drug selection process at the early stage of drug discovery. Generally, crude extracts are composed of various classes of phytoconstituents, but each constituent has specific biological activity and identification of such biologically active, drug-able natural phytochemicals is essential to modern drug discovery (Swain et al., 2021a and 2021b). This comprehensive approach not only validates the traditional use of these plants in Indian medicine but also emphasizes the importance of integrating phytochemical analysis with modern bioinformatics for the development of new antibiotic agents. In the present study, bioinformatics tools played a crucial role in enhancing the understanding of these natural remedies' therapeutic potential. By leveraging bioinformatics, the study was able to assess the compounds' drug-likeness, target affinity, and potential off-target effects, providing a more comprehensive evaluation of their suitability as drug candidates (Swain et al., 2022a; Sahoo et al., 2022b and 2022c). Using both traditional phytochemical analysis and advanced computational methods together made it easier to find promising antimicrobial agents and gave researchers more information about how they work and how safe they are, which made a big difference in the drug discovery process as a whole (Swain et al., 2021a and 2021b). Nevertheless, bioinformatics tools are coding and programming-dependent, and they require proper hypotheses and expertise to produce more reliable results. Furthermore, while the results may not be able to recommend a candidate for human consumption, they

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provide a cost-effective platform for exploring potency, predicting toxicity, and determining drug-ability profiles. This platform aids in guiding a systematic approach to select potential leads, thereby increasing experimental success within limited resources (Swain et al., 2022a). In summary, the approach is a more ideal and effective strategy at the early phase of drug discovery to explore the antibacterial properties of individual phytochemicals, paving the way for the development of new and effective natural antimicrobial agents.

Conclusion

The traditional ethnomedicinal practice plays a crucial role in modern drug discovery, where plant-derived metabolites or phytochemicals are well-known conservative sources of alternative and complementary medicines with less toxicity. Primarily, the majority of research focuses on exploring the multi-potential biological activities and utilizing these unique natural scaffolds in mainstream medicine through scientific investigations. The current study aimed to identify potential antibacterial candidates by utilizing crude extracts that exhibited potential antibacterial activity against various pathogens. We chose methanol-derived crude extracts for quantitative phytochemical analysis based on an in vitro antibacterial study. The results showed a rich bioactive compound profile, indicating that these traditional Indian plants have natural medicinal value. The bioinformatics study revealed that existing candidates and GC-MS derivative candidates showed that the low molecular weight GC-MS derivative candidates had lower drug potential and ability profiles than literature-based candidates such as ursolic acid and punicacortein A. All three crude extracts synergistically showed potential antibacterial activity due to being composed of a large number of phytoconstituents (both GC-MS and existing candidates) in the crude form. The demonstrated antibacterial potency against various pathogenic strains further validates their use in traditional medicine and suggests promising applications in modern antimicrobial treatments, where advanced computational tools speed up the selection process of leads candidates from a bunch at the early stages towards accelerating the phytochemical-based antibacterial therapy.

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Declaration of Competing Interest

The authors declare that they do not have any conflict of interest.

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