

Original Research

Unveiling the antibacterial potential of latex-derived phytocomponents from *Calotropis gigantea* targeting bacterial Penicillin-binding proteins: chemical profiling, *in silico* docking, and in vitro lab validation

Arun Dev Sharma^{1*}, Amrita Chauhan¹

Abstract

Uncontrolled use of currently accessible medicines has caused antibiotic resistance in bacteria worldwide. This work aimed to undertake molecular docking and chemical profiling of bioactive components extracted from *Calotropis gigantea* latex, with wet lab confirmation to follow. Gas chromatography was used to analyze the phytochemical profile. The docking tool, Cb-dock2, was used for in-silico analysis. G⁺ and G⁻ bacterial strains were utilized to confirm the wet lab results. Chemical profiling revealed 18 peaks, and some major bioactive compounds were like phytol, 4-methyl-2-phenylindole and α -tocospiro A. Docking analysis revealed strong interaction against bacterial Penicillin-binding Protein (PBP1a) with docking scores ranging from -5.2 to -7.9 (Vina score). The PBP-Ligand complex has Van der Waals, hydrogen bond, pi-cation, and alkyl bonding interactions, according to the results of the interaction investigations. *Calotropis* latex successfully suppressed the development of bacterial strains (G⁺ and G⁻) with inhibition zones ranging from 1.1 cm to 2.2 cm, as demonstrated by in-vitro tests, indicating their potential as antibacterial medications. The study found that latex-based bioactive compounds have the potential to be used in the pharmaceutical and biotechnology sectors by facilitating the creation of structural alterations that lead to stronger medications. Therefore, this latex-derived compound may open a new pipeline into the development of multi-target antibiotics against a broad-spectrum multidrug-resistant G⁻ bacterium.

Keywords

Penicillin-binding protein, calotropis latex, in-silico studies, anti-bacterial

¹ P.G Dept of Biotechnology, Lyallpur Khalsa College, Mohyal Nagar Jalandhar, Punjab (144008), India

* Corresponding author:

E-mail address: arundevsharma47@gmail.com

Citation: Sharma, A. D., Chauhan, A., (2024). Unveiling the antibacterial potential of latex-derived phytocomponents from *Calotropis gigantea* targeting bacterial Penicillin-binding proteins: chemical profiling, *in silico* docking, and in vitro lab validation. Acta Biologica Slovenica 68 (1)

Received: 12.09.2024 / **Accepted:** 21.10.2024 /

Published: 29.10.2024

<https://doi.org/10.14720/abs.68.0119803>

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY SA) license

Razkritje antibakterijskega potenciala iz lateksa pridobljenih fitokomponent iz *Calotropis gigantea*, usmerjenih proti bakterijskim proteinom, ki vežejo penicilin: kemijsko profiliranje, *in silico* docking in *in vitro* potrditev v laboratoriju

Izvleček

Nenadzorovana uporaba trenutno dostopnih zdravil je povzročila razvoj odpornosti bakterij proti antibiotikom po vsem svetu. Namen tega dela je bil izvesti analizo molekularnega prileganja in kemijsko profiliranje bioaktivnih sestavin, pridobljenih iz lateksa *Calotropis gigantea*, čemur bo sledila potrditev z eksperimenti. Za analizo fitokemičnega profila je bila uporabljena plinska kromatografija. Za *in silico* analizo je bilo uporabljeno orodje Cb-dock2. Za potrditev rezultatov v laboratoriju so bili uporabljeni sevi G+ in G- bakterij. Kemijsko profiliranje je pokazalo 18 vrhov, nekatere glavne bioaktivne spojine pa so bile fitol, 4-metil-2- fenilindol in α -tokospiro A. Analiza prileganja je pokazala močno interakcijo z bakterijskim proteinom, ki veže penicilin (PBP1a), z rezultati dokiranja od -5,2 do -7,9 (ocena Vina). V kompleksu PBP-ligand so glede na rezultate preiskav interakcij prisotne Van der Waalsove vezi, vodikova vez, π -kation in alkilna vez. Lateks kalotropisa je uspešno zaviral razvoj bakterijskih sevov (G+ in G-) z inhibicijskimi conami od 1,1 cm do 2,2 cm, kot so pokazali *in vitro* testi, kar kaže na njihov potencialno uporabnost kot antibakterijska zdravila. Študija je pokazala, da imajo bioaktivne spojine na iz lateksa *Calotropis gigantea* potencial za uporabo v farmacevtskem in biotehnološkem sektorju, saj omogočajo ustvarjanje strukturnih sprememb, ki vodijo k močnejšim zdravilom, zato lahko ta spojina, pridobljena iz lateksa, odpre novo pot za razvoj multitarčnih antibiotikov proti širokemu spektru G- bakterij z multiplo rezistenco.

Ključne besede

penicillin vezajoč protein, mleček kalotropisa, *in silico* študije, protibakterijsko delovanje

Introduction

Since they were developed, antibiotics have significantly benefited human health and the battle against bacterial illnesses. However, in recent years, antibiotic abuse has resulted in medicine-resistance-created antibiotic-resistant microorganisms (ARMs), which present serious health risks to individuals. Therefore, the researchers' current goal is to develop innovative, safe drugs (Mir et al., 2022). The emergence of antibiotic resistance has made microbes one of the greatest risks to human health (Harris et al., 2022). G-bacteria are unique to hospitals and communities; they have developed resistance to all currently available antibiotics.

β -lactam antibiotics have been targeting membrane-bound macromolecules involved in forming cell walls, known as penicillin-binding proteins (PBPs), for over 70 years. Numerous PBPs are found in bacteria, and some are starting to show their special roles in the cell division cycle (Straume et al., 2020). Peptidoglycan is depleted and may cause cell death when β -lactam antibiotics block tran-

septidation or carboxypeptidation activities (Chan et al., 2016). This powerful mechanism, which has made penicillin and its analogues the most extensively used antibiotics globally for all infectious illnesses over the past 70 years, is under pressure due to the emergence of penicillin and its analogue resistance. This highlights the necessity for cutting-edge, all-natural antibiotic remedies. Additionally, it has been observed that the naturally occurring substance moenomycin inhibits the actions of glycosyltransferases, which cause bacterial cells to die by breaking down peptidoglycan (Masters et al., 2020). Glycan chain polymerization (transglycosylation) and glycan chain cross-linking (transpeptidation) are catalyzed by PBPs. Certain PBPs hydrolyze the last D-alanine of the stem pentapeptide (DD-carboxypeptidation) or the peptide link between the two glycan chains (endopeptidation), according to Straume et al. (2020). One of the most promising antibiotic targets is the mechanism involved in bacterial cell wall production. A comparable macromolecular framework is absent from human metabolism, but many proteins, including PBPs, are

involved in the metabolism of bacteria, which is essential to life. These two prerequisites must be met in order to produce antibiotics. Furthermore, it has been noted that the naturally occurring compound moenomycin suppresses the activities of glycosyltransferases, which degrade peptidoglycan and kill bacterial cells (Masters et al., 2020). PBPs are thought to be great candidates for the creation of bacterial inhibitors due to their important role. The function of the PBP protein can be blocked to prevent bacterial replication. No PBP with a similar cleavage specificity has been found in humans; hence, the inhibitors are unlikely to be considered dangerous.

Because they are safer, more ecologically friendly, and have no unfavourable side effects, conventional medical procedures are therefore improving and growing in popularity (Mir et al., 2022). People still favour natural herbal remedies over synthetic ones despite the many advantages that synthetic drugs provide. Because they include multiple essential botanical components in various plant sections, medicinal plants are unique in that they may treat and cure a wide range of human health concerns (Salm et al., 2023; Chaachouay and Zidane, 2024). Natural plant products, like plant-based latex, are rich in bioactive compounds that vary in both biology and molecular structure, making them important tools for the creation of new drugs (Nasim et al., 2022).

Calotropis gigantea is commonly known as the giant milkweed and belongs to the Asclepiadaceae family (Wijeweera et al., 2020). In traditional Ayurvedic medicine, *Calotropis gigantea* is called "Sweta Arka" and "Rakha Arka", respectively, and it is designated an ethnomedical useful plant (Julius et al., 2021). It is a perennial shrub native to diverse regions encompassing tropical Africa and Asia (Wijeweera et al., 2020). This species is widely distributed across roadsides, railway tracks and deserted land pockets and can be found in countries such as Bangladesh, Burma, China, India, Indonesia, Malaysia, Pakistan, the Philippines, Thailand, Nepal, and Sri Lanka, where it holds significant economic importance (Gyawali et al., 2020). *Calotropis gigantea* is commonly known as milkweeds because of the latex they produce (Wijeweera et al., 2020). Plants produce white latex when sliced or broken. Several plant parts, including the flowers, stem, and leaves, can be used to extract latex (Timilsina et al., 2020). This white latex is loaded with several bioactive compounds. However, due to the biologically active substances such as alkaloids, flavonoids, terpenoids, glycosides, phenolics, tannins, and

saponins that it contains (Kumar et al., 2021), plant latex has made a significant contribution to medicine. Moreover, some ethnomedicinal applications of *Calotropis* plant parts include pesticide, anti-inflammatory, anti-fertility, antiulcer, fungicide, antiseptic, insecticide, anti-diabetic, and anti-helminthic activity (Wadhwani et al., 2021). Nevertheless, very little research on antioxidant bioactive compounds has been done on the latex of such a neglected plant species, even though this plant is widely utilized by the local communities as food ethnomedicine and is thought to be very nutritious. Consequently, in order to support *Calotropis gigantea* applications in traditional medicine, research into the latex secondary metabolite compositions, particularly its richness in phytonutrients, is still needed. Through the use of computational approaches to drug discovery, sophisticated methodologies that may be applied to screen drugs derived from phytochemicals found in a variety of medicinal plants have been established. Computational prediction models are critical to guide the technique selection process for drug and technology development. Furthermore, they have been used in the in-silico prediction of pharmacokinetic, pharmacological, and toxicological parameters, per Loza-Mejía et al. (2018). Molecular docking has become a practical and affordable approach for drug discovery and evaluation. As per the findings of Lee et al. (2019), this technique provides a valuable understanding of drug-receptor interactions and may be applied to predict the orientation of a potential medication upon binding to a target protein. However, because of its intricacy, the precise mechanism behind *Calotropis gigantea* latex's antibacterial action is ultimately unclear. We've spoken about how we think *Calotropis gigantea* latex's several bioactive ingredients may help reduce the threat posed by both G⁺ and G⁻ bacteria that are resistant to multiple drugs. Consequently, further research is needed to determine the possible medicinal advantages and workable modes of action of *Calotropis gigantea* latex. The objective of this study was to perform phytochemical screening and in-silico docking of the most common compounds present in *Calotropis gigantea* latex against target proteins involved in the bacterial life cycle. Additionally, it will offer new insights into future projections for figuring out the dose of crucial antibacterial drugs. This will increase our knowledge of the potential therapeutic use of the latex from the *Calotropis gigantea* plant. These techniques seek to offer a thorough comprehension of the characteristics and chemical makeup of *Calotropis gigantea* latex, as well as its potential therapeutic effects.

Material and Methods

Collection of samples

Wildly growing *Calotropis gigantea* (Supplementary Fig. 1) plant samples were collected from roadside locations close to the study area (31.30 latitudes North and 75.60 longitude East). The plant was verified by the botany department, and BT103 was noted on the voucher. This city experiences long, hot summers and chilly winters due to its humid subtropical climate. The study was carried out between January and May of 2024.

Preparation of extracts

The latex (about 30 mL) was collected from the *Calotropis gigantea* plant by breaking the stems of the plant with hands and then squeezing the broken stems to collect the latex from them into the Eppendorf tubes and stored at 4 °C for further use.

GC-FID analysis of latex

Chemical profiling was done using a gas chromatography flame ionization detector (GC-FID) system (Chemtron 2045) for the study of bioactive chemicals (Sharma and Kaur, 2022a). To identify and measure the bioactive chemicals contained in *Calotropis gigantea* latex, it was mixed with double volumes of isopropyl alcohol/water (8:2 v/v). Shaking the mixture by hand, vortexing it, and letting it lie overnight at room temperature were the next steps before centrifuging it for 15 minutes at 4 °C at 10,000 rpm. After that, the supernatant was collected and subjected to GC-FID examination. A 0.2 µl injection volume of extract was used. A 2-meter-long stainless-steel column fitted with a 10% OV-17 load on an 80–100% mesh Chromosorb W (HP) filter was provided by GC. The carrier gas was nitrogen gas at a rate of 30 mL per minute. The temperature of the injector and detector was maintained at 200°C and 250°C, respectively. A 0.2µl sample volume was injected. The ramping temperature settings for the oven were 110°C initially, then 200°C at a pace of 2°C per minute. Bioactive compounds were identified by comparing the relative retention duration with published data or standards (Adam, 2007).

Ligands Preparation

For in-silico docking studies, the predominant phytochemicals in *Calotropis gigantea* latex (Phytol, 4-Methyl-2-phenylindole and α -Tocospiro A) were chosen were ligands for seven bacterial Penicillin-binding protein (PBP1a). The following link was used to retrieve SMILES of ligands: <https://pubchem.ncbi.nlm.nih.gov/>. The 3-D structures of ligands were generated using UCSF-chimera after SMILES were obtained from the NCBI-PubChem database.

Protein preparation

Targets bacterial penicillin-binding protein (PBP1a) was the protein involved in the bacterial life cycle and was hence selected as a key target for docking. The RCSB database (<https://www.rcsb.org/>) was used to retrieve the target macromolecule (PDB IDs: PBP1a:3UDF). The PBP1a structure lacks water molecules and natural inhibitors before docking research. The dock-prep function in the Chimera program (chimera tool 1.14) was used to lower energy. The optimization process of dock preparation takes atomic structure, bond length, and charge anomalies into consideration.

Molecular Docking

A molecular docking study was performed on the selected phytocompounds using the CB-Dock2 application (<https://cadd.labshare.cn/cb-dock2/php/index.php>). The protein receptor (PBP1a) and ligand molecules were then sent to the CB-Dock2 service in pdb file format. A .pdb file with the ideal docked structure was generated and downloaded.

In-vitro Anti-bacterial Activity

The antimicrobial activity of calotropis latex was assessed using the agar disc diffusion method against four test organisms: G+ *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 121), and G- *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424). The microorganisms originate from IMTECH, the Institute of Microbial Technology in Chandigarh. *Calotropis* latex (volume: 5-150µl) was impregnated onto sterilized paper discs (5 mm diameter). New inoculums were created using 12-hour-old cultures for each microbial strain. After swabbing the LB-Agar plates with bacterial suspension, they were allowed to dry for thirty minutes. Next, the calotropis latex-impregnated paper discs

were positioned in the middle of the petri plates. The plates underwent incubation for 24 hours at 37°C after being kept at room temperature for a period of 20 minutes to enable the calotropis latex out of the disc to dissociate into the liquid. Streptomycin (10µg/disc) served as the positive control. After incubation, a zone of inhibition encircling the disc may be observed using a transilluminator; this radius represents the antibacterial activity of calotropis latex.

Statistical Analysis

The average $\pm$ standard deviation is displayed for each study; all investigations were carried out in triplicate using at least three different trials unless otherwise stated. ANOVA (analysis of variance) was utilized with GraphPad Prism v. 6.0 (GraphPad Software, Inc., San Diego, CA, USA) to identify significant differences. Tukey's multiple comparisons test was employed to evaluate mean differences between groups. Variations were considered significant when $p < 0.05$.

Results and Discussion

Gas chromatography analysis

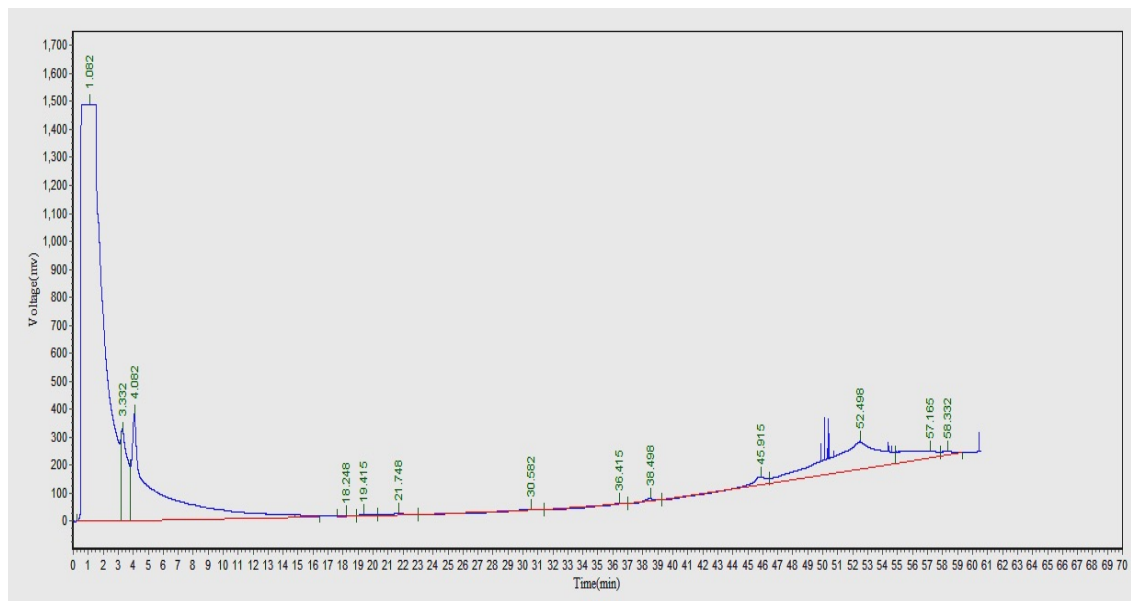
Phytochemical profiling is the qualitative test based on colour formations and/or precipitates of herbal formulations as indicators of the presence of secondary metabolites in plant material (Bouzidi et al., 2023). Many contemporary analytical techniques have been developed, and they are crucial in determining the active ingredients, medicinal properties, pharmacological properties, and other details of a substance's application in the fields of biochemical, pharmaceutical, and clinical research. GC-FID phytochemical analysis is one of the contemporary methods for identifying and isolating phytoconstituents. One modern technique for identifying and isolating phytoconstituents in plants is GC-FID (Bouzidi et al., 2023; Nkwocha et al., 2023; Onyeike et al., 2023).

The typical chromatogram depicting phytochemical compounds present in calotropis latex received by GC-FID is evident in Figure 1. Additionally provided are the noted peaks and the duration of their retention. GC-FID analysis of latex extracted from *Calotropis gigantea* led to the identification of 13 compounds. Oxygenated diterpenes, i.e. Phytol, with other minor components, were found to

be the most dominant phytochemicals at 64%. Acyclic hydrogenated diterpene alcohol, or phytol, is the main component and precursor to synthetic forms of vitamins K and E. It is also directed as phytosol or floral alcohol (Gutbrod et al., 2021). Phytol is used in the production of fragrances. According to Islam et al. (2018), it is also used commercially in shampoos, toilet soaps, detergents, and cosmetics. Additionally, some cannabis distillates use it as a flavouring or dilution. The microscopic peaks could be caused by the bioactive compounds present in trace amounts. The latex composition turned out to be different from previous investigations. Prior research has likewise confirmed the existence of both main and minor elements, albeit with different compositions. For example, in our investigation, phytol accounted for 24% of the total component; in contrast, Al-Rowaily et al. (2020) found that the phytol content was 8.73%. The current findings were entirely consistent with the *C. procera* EO chemical profile that was published and obtained from the Egyptian delta, where oxygenated diterpene phytol was found in amounts of 38% (Wahba and Khalid, 2018). These findings were consistent with those published for other Egyptian *C. procera* organs, where the primary chemicals were identified as diterpenes, specifically E-phytol. However, the profile of Indian *C. gigantea* (Singh and Javed, 2013) showed no diterpenes at all, which was at odds with the current results. Overall, the variance in the metabolites' production, including that of latex, may be the cause of the considerable disparity in chemical components. Environmental and climatic factors such as temperature, altitude, seasonality, plant age and water availability and soil conditions such as weather, extraction technique, soil moisture, harvest season, the distance between plants, harvest time, drying method, storage conditions, conditions, and genotype, all have a direct impact on the biosynthesis of latex components (Borges et al., 2018, Amin et al., 2019).

Molecular Docking studies

In-silico study was conducted by targeting PBP 1 (Penicillin-binding protein), which is essential for the final steps of peptidoglycan (PG) bacterial cell wall biosynthesis (Galinier et al., 2023). The primary structural element of the bacterial cell wall, PG is necessary to preserve the form, integrity, and survival of the cell. Owing to transpeptidase activity, PBP1 is involved in connecting the peptide side chains of developing glycan strands in cell wall synthesis, thus



S. No	Ret. Time (RT)	Bioactive Compound	Conc. (%)
1	1.082	Phytol	64.4103
2	3.332	Butanoic acid, 2[(phenyl methoxy)imino] trimethylsilyl ester	4.4500
3	4.082	4-Methyl-2- phenylindole	16.2854
4	18.248	Cyclohexanol,3-methyl	0.0594
5	19.415	Ergost-5-en-3-ol	0.0841
6	21.748	β -Cyclocitral	0.1554
7	30.582	Heptadecyl acetate	0.1362
8	36.415	α -amryin	0.1346
9	38.498	Lup-20(29)-en-3-ol acetate	0.1547
10	45.915	Guanidine nitrate	0.4865
11	52.498	α -Tocospiro A(alpha-tocopheroids)	10.9942
12	57.165	Ursa-12-en-3 β -yl pentanoate	2.3091
13	58.332	Astilbin	0.3400

Figure 1. Chemical profile and phytocompounds identified calotropis latex. Green font indicates major compounds used for docking analysis.

Slika 1. Kemični profil in identificirane fitokomponente lateksa kalotropisa. Črke v zeleni barvi označujejo glavne spojine, uporabljene za analizo prileganja.

being selected as the ideal target for docking studies. The bioactive compounds with the most potential for inhibition are those with the lowest vina score with their targets. According to Agu et al., 2023, molecular docking is an efficient computational method applied here to anticipate and determine the potential binding mode that emerges

between a chemical and a specific target protein or receptor. Penicillin-binding proteins (PBP1) were used in this study's molecular docking assays, with the most prevalent phytocompounds found in calotropis latex. The docking score of every bioactive molecule against the PBP1 and other pertinent data are displayed in Table 1.

Table 1. Molecular docking of α -amylase with the bioactive component of latex derived as ligand.Tabela 1. Molekularno prileganje α -amilaze z bioaktivno komponento lateksa kot ligandom.

Ligand	Vina score	Cavity volume (Å ³)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
Phytol	-5.2	2357	93, 48, -1	27, 27, 27	Chain A: LEU64 ALA65 GLU67 ASP68 SER69 SER70 PHE71 ASN134 TRP205 ARG209 GLN212 LEU213 Chain B: GLN506 MET509 ASP510 PHE511 GLY512 LEU513 GLN514 GLU515 ASP516 LEU532 ILE534 GLN535 THR538 ARG549 VAL550 GLN551 PRO552
4-Methyl-2- phenylindole	-7.4	2357	93, 48, -1	29, 20, 32	Chain A: ALA66 GLU67 ASP68 SER69 SER70 PHE71 ASN134 LYS185 ARG202 TRP205 ARG209 GLN212 LEU213 Chain B: LYS255 LEU426 GLN427 TRP429 GLN506 MET509 ASP510 GLY512 LEU513 GLN514 GLU515 ASP516 LEU532 ILE534 GLN535 THR538 ARG549 GLN551 PRO552
α -Tocospiro A	-7.9	2357	93, 48, -1	29, 29, 29	Chain A: ALA66 GLU67 ASP68 SER69 SER70 PHE71 ASN134 LEU135 LYS185 ALA186 TYR190 ARG202 TRP205 ARG209 GLN212 LEU213 GLY214 TYR215 Chain B: GLU247 ARG250 SER251 GLU252 VAL254 LYS255 GLU259 LYS421 PHE422 ASN423 LEU426 GLN427 TRP429 MET509 ASP510 GLY512 LEU513 GLN514 GLU515 LEU532 ILE534 GLN535 THR538 ARG549 GLN551 PRO552 GLU572 GLN631 ILE633
Phytol	-5.2	2357	93, 48, -1	27, 27, 27	Chain A: LEU64 ALA65 GLU67 ASP68 SER69 SER70 PHE71 ASN134 TRP205 ARG209 GLN212 LEU213 Chain B: GLN506 MET509 ASP510 PHE511 GLY512 LEU513 GLN514 GLU515 ASP516 LEU532 ILE534 GLN535 THR538 ARG549 VAL550 GLN551 PRO552
4-Methyl-2- phenylindole	-7.4	2357	93, 48, -1	29, 20, 32	Chain A: ALA66 GLU67 ASP68 SER69 SER70 PHE71 ASN134 LYS185 ARG202 TRP205 ARG209 GLN212 LEU213 Chain B: LYS255 LEU426 GLN427 TRP429 GLN506 MET509 ASP510 GLY512 LEU513 GLN514 GLU515 ASP516 LEU532 ILE534 GLN535 THR538 ARG549 GLN551 PRO552
α -Tocospiro A	-7.9	2357	93, 48, -1	29, 29, 29	Chain A: ALA66 GLU67 ASP68 SER69 SER70 PHE71 ASN134 LEU135 LYS185 ALA186 TYR190 ARG202 TRP205 ARG209 GLN212 LEU213 GLY214 TYR215 Chain B: GLU247 ARG250 SER251 GLU252 VAL254 LYS255 GLU259 LYS421 PHE422 ASN423 LEU426 GLN427 TRP429 MET509 ASP510 GLY512 LEU513 GLN514 GLU515 LEU532 ILE534 GLN535 THR538 ARG549 GLN551 PRO552 GLU572 GLN631 ILE633

*Green font indicates common residues among ligands involved in interactions.

Figure 2 shows 3D models of the PBP1 complex with affinities ligands and their interactions. With vina scores ranging from -5.2 to -7.9, the three common phytochemicals found in the latex were demonstrated to have binding potentials. α -Tocospiro-A exhibited the most efficient binding of all the bioactive components, with a docking score of -7.9, followed by 4-Methyl-2- phenylindole (vina core -7.4) and phytol (vina score -5.2). The ligand's affinity for the receptor is determined by its ability to create hydrogen bonds or hydrophobic interactions with the receptor protein's active site residues during docking (Sharma and Kaur, 2022b). It was observed that almost all phytochemicals interacted

with the PBP1a receptor via Van der Waal, alkyl, and Pi-alkyl interactions (Table 1). Notable all phytocompounds involved some common residues located at A and B chains to interact with the PBP1a receptor, as shown in Table 1. Antibacterial agents have a wide range of targets at which they can prevent the formation of cell walls. For many years, these systems have been regarded as significant targets for antimicrobial agents (Bruning et al., 2011). It has been discovered that natural substances prevent PBP from forming cell walls. The transpeptidase action of PBP is attributed to the C-terminal module (Contreras-Martel et al. 2017). Studies have demonstrated that therapies that mimic the structure

of the D-peptide moiety, D-body, such as β -lactam antibiotics, can obstruct the processes of transpeptidation or carboxypeptidation, which depletes peptidoglycan and may even result in cell death (Da-Costa et al. in 2018). This results in decreased cell wall integrity and the inability to transfer peptidoglycan (Moon et al., 2018). The results of Sripathi and Ravi (2017) reported that molecular docking analyses to determine the antibacterial efficiency of the most active components of *Plectranthus hadiensis* essential oil against PBPs are consistent with our findings. Additionally, molecular docking investigations of *Pogostemon cablin* essential oil components against PBPs were published by Yang et al.

(2013). The study's findings suggest that plant-based metabolites might be the primary source of antibacterial chemicals. Based on the aforementioned observations, it can be inferred that phytocompounds exhibited a strong affinity for PBP1a upon ligand binding. Consequently, it was postulated that PBP1a alteration results in a modification of the bacterial cell wall and enzyme conformation. By stopping bacterial viability, all of these occurrences lessen the bacteria's capacity to invade the host cell. Previous research has also shown through in-silico results that multi-pharmacological drugs have antibacterial properties that can regulate the proliferation of microbial strains (Lima et al., 2019).

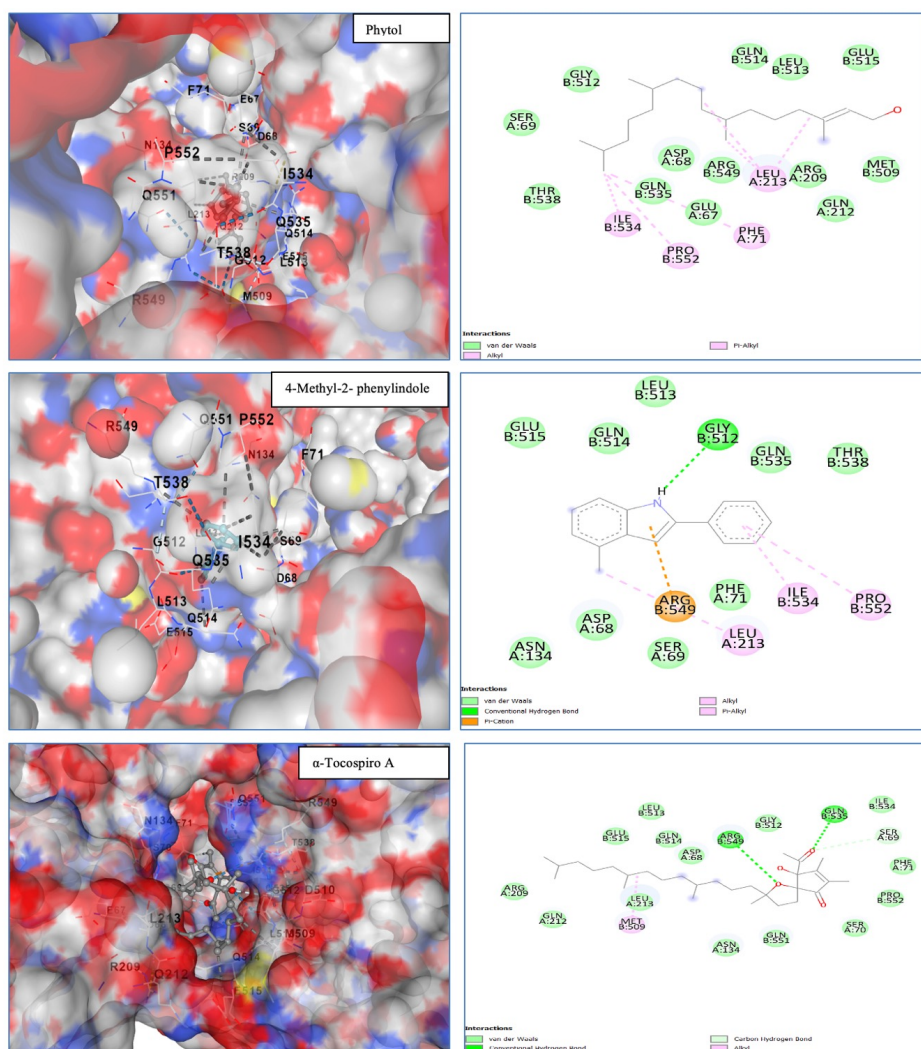


Figure 2. 2D models and 3D interactions of phytocompounds with PBP1a receptor.

Slika 2. 2D-modeli in 3D-interakcije fitokomponente z receptorjem PBP1a.

Antibacterial potential of latex

The wet lab experiment study was designed to assess the anti-bacterial potential of calotropis latex against four bacterial strains to validate the in-silico findings. According to the current analysis, calotropis latex exhibited potent antibacterial activity comparable to synthetic antibacterial drugs. Table 2 and Supplementary Figure 1-5 present the findings.

At 150µl, calotropis latex showed very strong antibacterial activity against *P.aeruginosa* (MTCC 424 G-) and *Staphylococcus aureus* (MTCC 3160 G+) with inhibition zone (IZ) of 2.2cm and 1.6cm, respectively. *E.coli* (MTCC 40 G-) and *B.subtilis* (MTCC 121 G+) exhibited moderate inhibitory activity with IZ values of 1.1 cm and 1.2 cm, respectively. The potent antibacterial properties of calotropis latex could

be attributed to the presence of major and minor bioactive components in toto that suppressed partners such as microbial adhesions, cell wall envelop proteins, and non-specific interactions with carbohydrates, or affected the inhibition of hydrolytic enzymes (proteases) (Saddiq et al. (2022). Calotropis latex may have multiple targets or a single target, contributing to its varying antibacterial activity against different bacterial strains. Additionally, prior research has shown that the synergistic effects of major and minor components, rather than the high concentration of a single chemical compound, are often responsible for anti-microbial activity (Vaou et al., 2022). Previous studies have shown that anti-microbial activity can occasionally be attributed to the combined effects of major and minor chemical components as opposed to a high concentration of a single main chemical molecule (Vaou et al., 2022).

Table 2. Anti-bacterial activity of calotropis latex of different microbial cultures.

Tabela 2. Protibakterijsko delovanje lateksa kalotropisa na različne mikrobne kulture.

Microbial culture	Volume (µl)	Inhibition zone (cm)
<i>Escherichia coli</i> MTCC 40	NC	-
	PC	2±0.02a
	5	NI
	20	0.7±0.03c
	50	0.9±0.03c
	150	1.1±0.04c
<i>Bacillus subtilis</i> MTCC121	NC	-
	PC	1.5±0.01d
	5	NI
	20	0.6±0.03c
	50	0.9±0.02c
	150	1.2±0.02c
<i>Pseudomonas aeruginosa</i> MTCC424	NC	-
	PC	1.6±0.02d
	5	NI
	20	1±0.02c
	50	1.3±0.04d
	150	2.2±0.02e
<i>Staphylococcus aureus</i> MTCC3160	NC	-
	PC	1.7±0.01d
	5	NI
	20	0.5±0.02c
	50	1.0±0.03c
	150	1.6±0.04d

Note: PC: Positive Control, NC: negative control (Streptomycin 10 µg); NI: no inhibition. Results are expressed as Mean ± SD of three determinations (n = 3). Mean values with different letters (a-d) across the columns are considered significantly different at p < 0.05.

Nejhad et al.'s study from 2023 also mentioned the antibacterial activity of an aqueous extract of *Calotropis procera* leaves against *E. coli*, but IZ was only 14.1 mm. One significant finding from our study's CEO was that G-bacteria were highly toxic to antibiotics. This is notable because earlier studies have suggested that G-bacteria's thicker cell walls allow them to express resistance to drugs, and antibiotics in particular, making them more resistant than G+ bacteria (Saxena et al., 2023). The chemical components found in the plant combination are often responsible for the antibacterial action of a plant extract. Rather than coming from the presence of a single major active element, a medicinal plant's optimum effectiveness may come from the combination of multiple diverse ingredients (Nejhad et al., 2023). The primary cause of the variations in antibacterial activity is the plant's varied concentration of secondary metabolites, which may be impacted by elements including species and extraction techniques. Compared to G+ bacteria, which have a single cell wall structure, G- bacteria enclose a multilayered cell wall structure, increasing their resistance to antimicrobial agents (Nooshkam et al., 2019; Shahidi et al., 2020). In recent times, there has been a growing significance placed on the antibacterial properties of plant flavonoids against various microbial species. Plant extracts typically have antimicrobial properties because they contain phenolic compounds with free hydroxyl groups, like flavonoids. These substances can disrupt bacterial membrane permeability, change cellular peptidoglycan, bind to the active site of enzymes, form hydrogen bonds with enzymes, and disrupt soluble and extracellular proteins, glutamate, and phosphate of bacteria, among other mechanisms (Shahidi et al., 2020).

Conclusion

Antibiotic resistance to both G+ and G- bacteria is now present in human populations and might endanger health around the globe. PBP's are currently the primary focus of infection. The goal of this work was to examine the bioactive components of calotropis latex that may be utilized to block bacterial infection pathways. Effective docking of all major phytochemicals with PBP1 and antibacterial activity has been demonstrated by docking and in vitro investigations. Therefore, we suggest that calotropis latex, which is included in herbal medications, might be a viable treatment choice and function as a bacterial PBP inhibitor. To make space for these compounds in drug development, more research utilizing in vitro and in vivo models should be carried out to validate these compounds.

Funding

Funding: Dept of Science and Technology, Govt. of India, DST/SEED/SCSP/STI/2019/253

Author's contributions

Conceptualization, ADS; methodology, AC.; software, AC; validation, ADS.; formal analysis, ADS and AC; investigation, ADS; writing—original draft preparation, ADS; writing—review and editing, ADS; visualization, AC; supervision, ADS; project administration, ADS; funding acquisition, ADS. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

- Agu, P.C., Afiukwa, C.A., Orji, O.U., Ezeh, E.M., Ofoke, I.H., Ogbu, C.O., Ugwuja, E.I., Aja, P.M., 2023. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. *Scientific Reports*, 13, 13398. DOI: 10.1038/s41598-023-40160-2.
- Nejhad, A., Alizadeh Behbahani, B., Hojjati, M., Vasiee, A., Mehrnia, M.A., 2023. Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of *Calotropis procera* leaf aqueous extract. *Scientific Reports*, 13, 14716. DOI: 10.1038/s41598-023-42086-1.
- Al-Rowaily, S.L., Abd-ElGawad, A.M., Assaeed, A.M., Elgamal, A.M., Gendy, A.E.G.E., Mohamed, T.A., Dar, B.A., Mohamed, T.K., Elshamy, A.I., 2020. Essential Oil of *Calotropis procera*: Comparative Chemical Profiles, antimicrobial Activity, and Allelopathic Potential on Weeds. *Molecules*, 25, 5203. DOI: 10.3390/molecules25215203, PubMed: 33182287.
- Amin, S.M., Hassan, H.M., El Gendy, A.E.N.G., El-Beih, A.A., Mohamed, T.A., Elshamy, A.I., Bader, A., Shams, K.A., Mohammed, R., Hegazy, M.E.F., 2019. Comparative chemical study and antimicrobial activity of essential oils of three *Artemisia* species from Egypt and Saudi Arabia. *Flavour and Fragrance Journal*, 34, 450–459. DOI: 10.1002/ffj.3525.
- Borges, R.S., Lima, E.S., Keita, H., Ferreira, I.M., Fernandes, C.P., Cruz, R.A.S., Duarte, J.L., Velázquez-Moyado, J., Ortiz, B.L.S., Castro, A.N., Ferreira, J.V., da Silva Hage-Melim, L.I., Carvalho, J.C.T., 2018. Anti-inflammatory and antialgic actions of a nanoemulsion of *Rosmarinus officinalis* L. essential oil and a molecular docking study of its major chemical constituents. *Inflammopharmacology*, 26, 183–195. DOI: 10.1007/s10787-017-0374-8.
- Bouzidi, A., Azizi, A., Messaoudi, O., Abderrezak, K., Vidari, G., Hellal, A.N., Patel, C.N., 2023. Phytochemical analysis, biological activities of methanolic extracts and an isolated flavonoid from Tunisian *Limoniastrum monopetalum* (L.) Boiss: An in vitro and in silico investigations. *Scientific Reports*, 13, 19144. DOI: 10.1038/s41598-023-46457-6.
- Bruning, J.B., Murillo, A.C., Chacon, O., Barletta, R.G., Sacchetti, J.C., 2011. Structure of the *Mycobacterium tuberculosis* D-alanine: D-alanine ligase, a target of the antituberculosis drug D-cycloserine. *Antimicrobial Agents and Chemotherapy*, 55, 291–301. DOI: 10.1128/AAC.00558-10.
- Chaachouay, N., Zidane, L., 2024. Plant-derived natural products: A source for drug discovery and development. *Drugs and Drug Candidates*, 3, 184–207. DOI: 10.3390/ddc3010011.
- Chan, L.C., Gilbert, A., Basuino, L., da Costa, T.M., Hamilton, S.M., Dos Santos, K.R., Chambers, H.F., Chatterjee, S.S., 2016. PBP 4 mediates high-level resistance to new-generation cephalosporins in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, 60, 3934–3941. DOI: 10.1128/AAC.00358-16.
- Contreras-Martel, C., Martins, A., Ecobichon, C., Trindade, D.M., Mattei, P.J., Hicham, S., Hardouin, P., Ghachi, M.E., Boneca, I.G., Dessen, A., 2017. Molecular architecture of the PBP2-MreC core bacterial cell wall synthesis complex. *Nature Communications*, 8, 776. DOI: 10.1038/s41467-017-00783-2.
- Da Costa, T.M., De Oliveira, C.R., Chambers, H.F., Chatterjee, S.S., 2018. PBP4: A new perspective on *Staphylococcus aureus* β -lactam resistance. *Microorganisms*, 6, 57. DOI: 10.3390/microorganisms6030057.
- Galinier, A., Delan-Forino, C., Foulquier, E., Lakhal, H., Pompeo, F., 2023. Recent advances in peptidoglycan synthesis and regulation in bacteria. *Biomolecules*, 13, 720. DOI: 10.3390/biom13050720.
- Gutbrod, P., Yang, W., Grujicic, G.V., Peisker, H., Gutbrod, K., Du, L.F., Dörmann, P., 2021. Phytol derived from chlorophyll hydrolysis in plants is metabolized via phytenal. *Journal of Biological Chemistry*, 296, 100530. DOI: 10.1016/j.jbc.2021.100530.
- Gyawali, R., Bhattarai, B., Bajracharya, S., Bhandari, S., Bhetwal, P., Bogati, K., Neupane, S., Shrestha, S., Shrestha, A.K., Joshi, R., Paudel, P.N., 2020. α -amylase inhibition, antioxidant activity and phytochemical analysis of *Calotropis gigantea* (L.) Dryand. *Journal of Health and Allied Sciences*, 10, 77–81. DOI: 10.37107/jhas.143.
- Harris, A.D., Samore, M.H., Lipsitch, M., Kaye, K.S., Perencevich, E., Carmeli, Y., 2002. Control-group selection importance in studies of antimicrobial resistance: Examples applied to *Pseudomonas aeruginosa*, *Enterococci*, and *Escherichia coli*. *Clinical Infectious Diseases*, 34, 1558–1563. DOI: 10.1086/340533, PubMed: 12032889.
- Islam, M.T., Ali, E.S., Uddin, S.J., Shaw, S., Islam, M.A., Ahmed, M.I., Chandra Shill, M., Karmakar, U.K., Yarla, N.S., Khan, I.N., Billah, M.M., Pieczynska, M.D., Zengin, G., Malainer, C., Nicoletti, F., Gulei, D., Berindan-Neagoe, I., Apostolov, A., Banach, M., Yeung, A.W.K., El-Demerdash, A., Xiao, J., Dey, P., Yele, S., Jóźwik, A., Strzałkowska, N., Marchewka, J., Rengasamy, K.R.R., Horbańczuk, J., Kamal, M.A., Mubarak, M.S., Mishra, S.K., Shilpi, J.A., Atanasov, A.G., 2018. Phytol: A review of biomedical activities. *Food and Chemical Toxicology*, 121, 82–94. DOI: 10.1016/j.fct.2018.08.032.
- Julius, O.O., Oluwasusi, V.O., Ibiyemi, M.F. and Oluwatobi, F.B., 2021. Antibacterial Activities and Phytochemical Screening of Crude Extracts of *Calotropis gigantea* (Giant Milk Weed). *South Asian Journal of Research in Microbiology*, 9(3), pp.24-31.
- Kumar, H., Sharma, S., Vasudeva, N., 2021. Pharmacological profile of *Calotropis gigantea* in various diseases: A profound look. *Int. J. Creat. Res Thoughts*, 9, 2987–2996.
- Lee, K., Kim, D., 2019. In-silico molecular binding prediction for human drug targets using deep neural multi-task learning. *Genes*, 10, 906. DOI: 10.3390/genes10110906, PubMed: 31703452.
- Lima, S.L., Colombo, A.L., de Almeida Junior, J.N., 2019. Fungal cell wall: Emerging antifungals and drug resistance. *Frontiers in Microbiology*, 10, 2573. DOI: 10.3389/fmicb.2019.02573.
- Loza-Mejía, M.A., Salazar, J.R., Sánchez-Tejeda, J.F., 2018. In silico studies on compounds derived from *Calceolaria*: Phenylethanoid glycosides as potential multitarget inhibitors for the development of pesticides. *Biomolecules*, 8, 121. DOI: 10.3390/biom8040121.
- Masters, E.A., de Mesy Bentley, K.L., Gill, A.L., Hao, S.P., Galloway, C.A., Salminen, A.T., Guy, D.R., McGrath, J.L., Awad, H.A., Gill, S.R., Schwarz, E.M., 2020. Identification of penicillin binding protein 4 (PBP4) as a critical factor for *Staphylococcus aureus* bone invasion during osteomyelitis in mice. *PLOS Pathogens*, 16, e1008988. DOI: 10.1371/journal.ppat.1008988.

- Mir, W.R., Bhat, B.A., Rather, M.A., Muzamil, S., Almilaibary, A., Alkhanani, M., Mir, M.A., 2022. Molecular docking analysis and evaluation of the antimicrobial properties of the constituents of *Geranium wallichianum* D. Don ex Sweet from Kashmir Himalaya. *Scientific Reports*, 12, 12547. DOI: 10.1038/s41598-022-16102-9.
- Moon, T.M., D'Andréa, É.D., Lee, C.W., Soares, A., Jakoncic, J., Desbonnet, C., Garcia-Solache, M., Rice, L.B., Page, R., Peti, W., 2018. The structures of penicillin-binding protein 4 (PBP4) and PBP5 from *Enterococci* provide structural insights into β -lactam resistance. *Journal of Biological Chemistry*, 293, 18574–18584. DOI: 10.1074/jbc.RA118.006052.
- Nasim, N., Sandeep, I.S., Mohanty, S., 2022. Plant-derived natural products for drug discovery: Current approaches and prospects. *Nucleus (Calcutta)*, 65, 399–411. DOI: 10.1007/s13237-022-00405-3.
- Nejhad A., Behbahani AB, Hojjati M, Vasiee A, Mehrnia MA., 2023. Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of *Calotropis procera* leaf aqueous extract. *Sci Rep* 13, 14716.
- Nkwocha, C., Felix, O.J., Idoko, N.R., 2023. GC-FID spectroscopic analysis and antioxidant activities of methanolic fraction of *Cassia tora* leaves. *Pharmacological research. Modern Chinese Medicine*, 9, 100338.
- Nooshkam, M., Falah, F., Zareie, Z., Tabatabaei Yazdi, F., Shahidi, F., Mortazavi, S.A., 2019. Antioxidant potential and antimicrobial activity of chitosan–inulin conjugates obtained through the Maillard reaction. *Food Science and Biotechnology*, 28, 1861–1869. DOI: 10.1007/s10068-019-00635-3.
- Onyeike, E.N., Egbuna, C., Patrick-Iwuanyanwu, K.C., 2023. Phytochemical screening and quantitative analysis of *Annona muricata* Leaf ethanolic extract by gas chromatography-flame ionization detection (GC-FID). *IPS Journal of Drug Discovery Research and Reviews*, 2, 1–4. DOI: 10.54117/ijddrv2i1.15.
- Saddiq, A.A., Tag, H.M., Doleib, N.M., Salman, A.S., Hagagy, N., 2022. Antimicrobial, antigenotoxicity, and Characterization of *Calotropis procera* and Its Rhizosphere-Inhabiting Actinobacteria: In vitro and in vivo Studies. *Molecules*, 27, 3123. DOI: 10.3390/molecules27103123.
- Salm, S., Rutz, J., van den Akker, M., Blaheta, R.A., Bachmeier, B.E., 2023. Current state of research on the clinical benefits of herbal medicines for non-life-threatening ailments. *Frontiers in Pharmacology*, 14, 1234701. DOI: 10.3389/fphar.2023.1234701.
- Saxena, D., Maitra, R., Bormon, R., Czekanska, M., Meiers, J., Titz, A., Verma, S., Chopra, S., 2023. Tackling the outer membrane: Facilitating compound entry into Gram-negative bacterial pathogens. *Npj Antimicrobials and Resistance*, 1, 17. DOI: 10.1038/s44259-023-00016-1.
- Shahidi, F., Tabatabaei Yazdi, F., Nooshkam, M., Zareie, Z., Fallah, F., 2020. Chemical modification of chitosan through non-enzymatic glycosylation reaction to improve its antimicrobial and anti-oxidative properties. *Iran. Food Science and Technology Research J*, 16, 117–129.
- Sharma, A.D., Kaur, I., 2022a. A novel therapeutic eucalyptol from eucalyptus essential oil targeting penicillin binding proteins (PBPs) against gram-positive and gram-negative bacteria: Molecular docking and experimental approach. *Arabian Journal of Medicinal and Aromatic Plants*, 8, 24–42.
- Sharma, A.D., Kaur, I., 2022b. Aroma profile and in-silico molecular docking studies of essential oil against "Aspergillosis" from *Eucalyptus polybrachtea*. *South African Journal of Botany*, 147: 754–763. <https://doi.org/10.1016/j.sajb.2022.03.011>
- Singh, M., Javed, K., 2013. Chemical characterization and antimicrobial activity of *Calotropis gigantea* Linn. Flower essential oil collected from northern plain of India. *International Journal of Advanced Biotechnology and Research*, 4, 533–541.
- Sripathi, R., Ravi, S., 2017. Molecular docking studies of the constituents present in the essential oil of *Plectranthus hadiensis* against bacterial proteins. *International Journal of Chemical Sciences*, 15, 185.
- Straume, D., Piechowiak, K.W., Olsen, S., Stamsås, G.A., Berg, K.H., Kjos, M., Heggenhougen, M.V., Alcorlo, M., Hermoso, J.A., Håvarstein, L.S., 2020. Class A PBPs have a distinct and unique role in the construction of the pneumococcal cell wall. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 6129–6138. DOI: 10.1073/pnas.1917820117.
- Timilsina, H., Modi, B. and Basnyat, R., 2020. Phytochemical, antimicrobial and ethnobotanical study of *Calotropis gigantea*. *Journal of Health and Allied Sciences*, 10(2), pp.23-27.
- Vaou, N., Stavropoulou, E., Voidarou, C.C., Tsakris, Z., Rozos, G., Tsigalou, C., Bezirtzoglou, E., 2022. Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. *Antibiotics*, 11, 1014. DOI: 10.3390/antibiotics11081014.
- Wadhwani, B.D., Mali, D., Vyas, P., Nair, R., Khandelwal, P., 2021. A review on phytochemical constituents and pharmacological potential of *Calotropis procera*. *RSC Adv.* 4:11(57):35854-35878. doi: 10.1039/d1ra06703f
- Wahba, H.E., Khalid, K.A., 2018. Comparative study on essential oil composition in various organs of Sodom apple (*Calotropis procera*) grown wild in Egypt. *Asian Journal of Plant Sciences*, 17, 85–90. DOI: 10.3923/ajps.2018.85.90.
- Wijeweera, W.P.S.N., Senaratne, K.A.D.W., Dhileepan, K., 2021. Studies on the fruit feeding weevil, *Paramecops farinosa* (Coleoptera: Curculionidae). in Sri Lanka as a prospective weed biological control agent of invasive weed, *Calotropis* spp. *Journal of Biological Control*, 34, 241–250.
- Yang, X., Zhang, X., Yang, S.P., Liu, W.Q., 2013. Evaluation of the antibacterial activity of patchouli oil. *Iranian Journal of Pharmaceutical Research*, 12, 307–316. PubMed: 24250637.