RESEARCH PAPER

Phytochemical Profiling and Antioxidant Activity of Pergularia Daemia (L.) Extracts: a Preliminary Study Toward Antivenom Drug Discovery

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ABSTRACT
Snakebite envenomation is a neglected public health issue in tropical and subtropical countries. Existing antivenom treatments do not effectively combat the venom-induced local tissue damage. Bioactive plant derivatives provide on option of novel supplementary therapy approaches. Therefore, the aim of this investigation is to characterize the phytochemical composition, antioxidant activity, and perform silico docking against β-neurotoxic phospholipase A2 proteins. A methanolic extract of Pergularia daemia (L.) was screened for the presence of flavonoids, steroids, glycosides, saponins, tannins, coumarins, proteins, amino acids, and reducing sugars. GC–MS analysis identified several components in which methyl oleate (27.11%), methyl linoleate (20.90%), and methyl palmitate (11.95%) as the predominant constituents. The compounds exhibit potent free radical scavenging capacity in DPPH and ABTS radical assays respectively with IC_{50} values of 42 and 31 µg/mL, respectively. Molecular docking against three β-neurotoxic phospholipase A_2 proteins (β-bungarotoxin, 1BUN; phospholipase A_2, 1A3D; and taipoxin, 3VBZ) revealed favourable binding interactions for several identified compounds. Folic acid binding -7.077 kcal /mol for 1A3D and-7.619 kcal/mol for 3VBZ. While phorbol with -7.248 kcal/mol. for 1BUN. Collectively, the data produced in the present study indicates the richness of P. daemia contains chemically diverse metabolites with potent antioxidant properties. Most compounds had interactions with venom-associated phospholipase A_2 proteins, which are important for development of naturally derived candidates for snakebite therapeutics.
Keywords: Snakebite, envenomation, Pergularia daemia, Antioxidant, Docking, Phytochemicals
How to cite this article Sivarami Reddy Bhavanam, Prashantha CN, Monika Devireddy , Ramachandra. R. Phytochemical Profiling and Antioxidant Activity of Pergularia Daemia (L.) Extracts: a Preliminary Study Toward Antivenom Drug Discovery Int J Drug Deliv Technol. 2026;16(89s): 1571-1582; DOI: 10.25258/ijddt.16.89s.162
Source of support: Nil.
Conflict of interest: None

1.INTRODUCTION

Snakebite envenomation remains a critically neglected health issue around the world particularly in tropical and subtropical regions where populations are predominantly rural and agriculture-based and are exposed to the hazard of venomous snakes [1, 2, 3]. World Health organization (WHO) as an A grade Neglected Tropical disease because of its significant implications in human mortality, lifelong impairment and socio-economic burden [4, 5]. Globally there occur about 4.5–5.4 million snake bites resulting in 2.7 million envenomed and 81 000 to 138 000 death cases per year [6, 7]. Many individuals suffer lifelong physical impairment by resulting tissue damage including necrosis, hemorrhages and amputation of the extremities [8,9].

Snakebite is as unique emergency as it presents in almost every part of the globe. It is unique emergency based on the symptoms and complications vary with type of snake, dose, site of bite, species, age and general condition of patients [10, 11]. The management of venom-induced symptoms through anti snakebite may be effective in counteracting general toxemia but it is often ineffective in dealing with local symptoms like massive tissue swelling, tissue necrosis, hemorrhage and localized ischemic injury to limbs [12, 13, 14]. Accessibility of antivenom dose is a major problem in rural areas, high cost of production, need for cold chain, Batch-to batch variations and serious adverse reactions of anti-snakebite like serum sickness and anaphylaxis are limitations of this therapy, hence efforts are being devoted to search adjuncts in addition to anti-snakebite to reduce local as well as systemic toxicities [15, 16].

Natural products have continuously played an undeniable and influential role in drug discovery and development processes [17]. The secondary metabolites such as (flavonoids, phenolics, alkaloids, terpenoids, tannins, saponins, glycosides and others) displayed variety of biological activities including antioxidants, anti-inflammatory, antimicrobial and enzyme modulation activities etc. [18, 19, 20]. Amongst them, antioxidant activity may be considered as prime importance in snakebite pathology since oxidative stress caused by venom mediated to increase Lipid peroxidation (LPx), Protein oxidation (POx), Mitochondria dysfunction and inflammation [21, 22]. Hence, many antioxidants active medicinal plants have been explored in snake bite associated problems and used in combination with anti-

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venom drug for overcoming severe consequences related to venom and oxidative stress damage [23]. Thus, phytochemical characterization followed by biological investigation is one the best strategy to identify active component from plants for developing such drugs. *Pergularia daemia (L.)* is a profusely branched perennial woody medicinal vine commonly known in South Asia, tropical Africa and commonly utilized by different indigenous systems like Ayurveda, Siddha, and local folk medicine for its beneficial effect in various diseases such as inflammation, wounds, diabetes, respiratory disorder, snake bites etc. [24, 25]. Recent studies revealed its antioxidant, anti-inflammatory, antimicrobial, anti-hepatotoxic, anti-diabetic and wound healing property according to diverse of its Phytochemicals. Although various phytochemicals have been identified from various extracts of the plant species but the integrative study on phytochemical profile, antioxidant potency, and interaction with molecular targets associated with venom intoxication is still lagging [26, 27].

In the era of rapidly evolving analytical tools such as gas chromatography-mass spectrometry (GC-MS), and computational techniques such as molecular docking, a well-established protocol along with phytochemical screening and in vitro assays can make significant improvements in identifying and prioritizing bioactive natural products. In the present study, the phytochemical profile of the methanolic extract of *P daemia (L)* was scrutinized by qualitative phytochemical screening and GC-MS; its potential as antioxidants in DPPH and ABTS scavenging assay were analyzed; moreover, the binding affinity of some important phytochemicals targeting β-neurotoxic phospholipase A proteins by molecular docking; by putting together the information, thus, *P. daemia* might be presented as an interesting source to leading potential for snakebite envenomation complementary therapy.

MATERIALS AND METHODS

1. Plant material

Fresh aerial parts of *Pergularia daemia (L.)* were collected during the flowering season from University of Agricultural Sciences (UAS), GKVK, Bangalore, Karnataka, India. The plant was taxonomically identified and confirmed by a trained botanist at the University of Agricultural Sciences, Bengaluru (Specimen Ref No: 4858) was deposited in the institutional herbarium. The plant material was washed with distilled water, shade-dried at ambient temperature (25–30 °C) for approximately two weeks, pulverized into a fine powder using a laboratory grinder, and stored in airtight containers until extraction [28, 29].

2. Preparation of methanolic extract

Approximately 100 g of powdered plant material was extracted with methanol using a Soxhlet apparatus for 8–10 h. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at temperatures below 40 °C. The resulting semi-solid extract was dried to constant weight, the extraction yield was calculated, and the extract was stored at 4 °C in amber-colored bottles until further analysis [30].

3. Preliminary phytochemical screening

Qualitative phytochemical screening of the methanolic extract was performed following standard protocols to detect major classes of secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids, glycosides, carbohydrates, proteins, and reducing sugars. Dragendorff's and Mayer's tests were used for alkaloids, ferric chloride for phenolics and tannins, Shinoda test for flavonoids, frothing test for saponins, Liebermann–Burchard reaction for steroids and terpenoids, Keller–Killiani test for cardiac glycosides, Molisch's test for carbohydrates, Biuret test for proteins, and Benedict's test for reducing sugars. The intensity of each reaction was qualitatively scored as absent (−), weak (+), moderate (++), or strong (+++) [31,32,33].

4. Gas chromatography–mass spectrometry (GC–MS) analysis

Chemical profiling of the methanolic extract was performed using a GC–MS analysis was performed using an Agilent 8890 GC coupled with a 5977B MSD equipped with an HP-5MS column (30 m × 0.25 mm × 0.25µm). One microliter of filtered extract was injected in split mode. Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The injector temperature was maintained at 250 °C, while the oven temperature was programmed from 60 to 280 °C. Mass spectra were acquired under electron ionization (70 eV) over a mass range of m/z 50–600. Phytochemical constituents were identified by comparing the acquired mass spectra with those in the National Institute of Standards and Technology (NIST) Mass Spectral Library (2023). Compounds with high spectral similarity were considered positively identified, and their retention time, molecular formula, molecular weight, and relative peak area were recorded [34, 35].

5. Antioxidant assays

5.1 DPPH radical scavenging assay

Antioxidant activity was evaluated using the DPPH free radical scavenging assay. Briefly, 1 mL of freshly prepared 0.1 mM DPPH solution was mixed with plant extract at concentrations ranging from 5 to 100 µg mL^{-1} and incubated for 30 min in the dark at room temperature. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid served as the positive control. Radical scavenging activity (%) was calculated using:

$$\text{Scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where *A* control and *A* sample represent the absorbance of the control and sample, respectively. IC_{50} values were determined from dose–response curves [36, 37].

5.2 ABTS radical scavenging assay

The ABTS radical cation was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating

the mixture in the dark for 12–16 h. Before use, the solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Plant extracts (5–100 µg mL^{-1}) were mixed with the ABTS solution, incubated for 6 min, and absorbance was measured at 734 nm. Gallic acid was used as the reference standard. Radical scavenging activity and IC_{50} values were calculated as described for the DPPH assay [38, 39].

6. Molecular docking

The crystal structures of β-bungarotoxin (PDB ID: 1BUN), phospholipase A_2 (PDB ID: 1A3D), and taipoxin (PDB ID: 3VBZ) were retrieved from the Protein Data Bank (https://www.rcsb.org). Protein structures were prepared using AutoDock Tools (v1.5.7) by removing water molecules and co-crystallized ligands, followed by the addition of polar hydrogen atoms and Kollman charges. Phytochemicals identified by GC–MS analysis were selected as ligands. Their three-dimensional structures were obtained from the PubChem database, converted using Open Babel, energy-minimized with the MMFF94 force field, and saved in PDBQT format. Docking simulations were performed using AutoDock Vina (v1.2.5) with the grid box centered on the catalytic site of each protein. Docking was conducted using an exhaustiveness value of 8, and the pose with the lowest binding free energy was selected for analysis. Protein–ligand interactions, including hydrogen bonds, hydrophobic contacts, π–π interactions, π–alkyl interactions, and van der Waals interactions, were visualized using BIOVIA Discovery Studio Visualizer (2021) [40, 41, 42].

7. Statistical analysis

All experiments were performed independently in triplicate (n = 3), and results are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Preliminary phytochemical screening

The quantitative study was performed to detect the presence of different classes of secondary metabolites. (Table 1). Positive results were found with the presence of flavonoids, alkaline-reacting substances, steroids, glycosides, saponins, tannins, reducing sugars (Benedict's test), proteins/amino acids (ninhydrin test), and coumarins. Whereas anthocyanins, terpenoids, and quinones were found to be absent. Flavonoids, tannins, saponins, steroids, and glycosides are the compounds which have been founded, and they show the complex nature on the phytochemical screening of methanolic extract of *P. Daemia*. Phenol group of compounds, non-phenolic class was also included in the Phytochemical screening as illustrated in Table 1.

Table 1. Preliminary phytochemical screening of methanolic extract of Pergularia daemia

Phytochemical constituent	Intensity
Flavonoids	+++
Tannins	++
Saponins	++
Steroids	++
Glycosides	++
Coumarins	+
Proteins/Amino acids	+
Reducing sugars	+
Anthocyanins	–
Terpenoids	–
Quinones	–

GC–MS profiling of the methanolic extract of Pergularia daemia

The methanolic extract of P. daemia leaf samples showed the presence of twelve components with chemical class including fatty acid methyl esters, diterpenoids, ketones, sterols, alcohols, and sesquiterpenoids using GC-MS analysis (Figure 1). The retention time ranged from 10.55 to 19.13 min of which methoxy acetate; 2-undecanamethoxy was the highest peak percentage as 44.09%. Followed by methyl hexadecanoate; methyl palmitate included 9-octadecenoic acid methyl ester (methyl oleate; 27.11%), 9,12-octadecadienoic acid methyl ester (methyl linoleate; 20.90%), 2-pentadecanone, 6,10,14-trimethyl- (9.62%), and neophytadiene (8.14%). Additional constituents detected at lower relative abundances comprised methyl hexadec-9-enoate (4.92%), methyl stearate (4.12%), ursodeoxycholic acid (0.83%), phytol (0.59%), trans-β-ionone (0.40%), ethyl iso-allocholate (0.17%), and corymbolone (0.04%). Many other components have been reported to exert protective and therapeutic effect like antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and anticancer activities according to published literature. A comprehensive summary of the retention time, molecular formula, relative

peak area, and reported biological activities of the identified phytochemicals is presented in Table 2

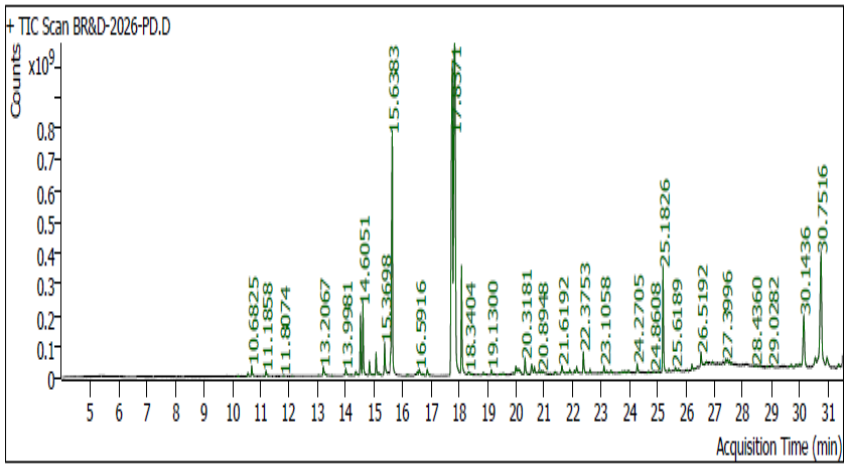


Figure 1. GC-MS analysis of methanolic extract of P. daemia

Table 2. Phytochemical constituents identified by GC–MS analysis of the methanolic extract of P. daemia.

RT (min)	Compound	Molecular formula	Peak area (%)
10.5503	trans-β-Ionone	$C_{13}H_{20}O$	0.40
12.1193	Corymbolone	$C_{15}H_{24}O_2$	0.04
14.5152	Neophytadiene	$C_{20}H_{38}$	8.14
14.6051	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	9.62
15.3698	Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$	4.92
15.6383	Hexadecanoic acid, methyl ester (Methyl palmitate)	$C_{17}H_{34}O_2$	44.09
16.7139	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	0.17
17.7542	9,12-Octadecadienoic acid methyl ester (Methyl linoleate)	$C_{19}H_{34}O_2$	20.90
17.8371	9-Octadecenoic acid methyl ester (Methyl oleate)	$C_{19}H_{36}O_2$	27.11
18.0818	Methyl stearate	$C_{19}H_{38}O_2$	4.12
18.3404	Ursodeoxycholic acid	$C_{24}H_{40}O_4$	0.83
19.1300	Phytol	$C_{20}H_{40}O$	0.59

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of the methanolic extract of *P. daemia* was evaluated using the DPPH radical scavenging assay over a concentration range of 5–100 µg mL^{-1} (Figure 2). The extract exhibited a concentration-dependent increase in radical scavenging activity, with absorbance values increasing from 0.346 at 5 µg mL^{-1} to 0.627 at 100 µg mL^{-1} (Table 3). Under identical experimental conditions, the reference standard ascorbic acid showed absorbance values ranging from 0.134 to 0.283 across the same concentration range. The calculated IC_{50} value for the methanolic extract was 42 µg mL^{-1}, whereas the IC_{50} value for ascorbic acid was 94 µg mL^{-1}. These results demonstrate a concentration-dependent response of the extract in the DPPH assay. Statistical analysis indicated significant differences among the tested concentrations ($P < 0.05$).

Table 3. DPPH radical scavenging assay of the methanolic extract of Pergularia daemia.

Concentration (µg mL^{-1})	Ascorbic acid	*Pergularia daemia* extract

Control	0.134	0.346
5	0.134	0.346
10	0.159	0.367
20	0.164	0.389
50	0.201	0.492
100	0.283	0.627

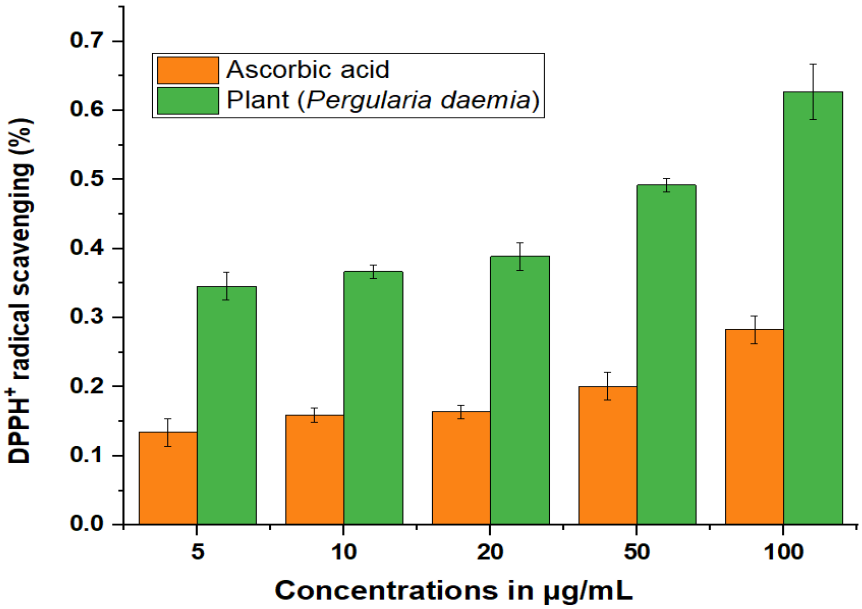


Figure 2. DPPH radical scavenging activity of the methanolic extract of P. daemia and the reference antioxidant ascorbic acid at concentrations ranging from 5 to 100 µg mL⁻¹. Data are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.05

ABTS radical scavenging activity

The antioxidant capacity of the methanolic extract was further evaluated using the ABTS radical cation decolorization assay (Figure 3). Similar to the DPPH assay, the extract exhibited a concentration-dependent response over the concentration range of 5–100 µg mL⁻¹. The measured absorbance increased from 0.682 at 5 µg mL⁻¹ to 1.269 at 100 µg mL⁻¹ (Table 4). In comparison, the absorbance values for the reference standard gallic acid ranged from 0.239 to 0.562 under identical assay conditions. The IC_{50} value of the methanolic extract was 31 µg mL⁻¹, whereas gallic acid exhibited an IC_{50} value of 78 µg mL⁻¹. Statistical analysis demonstrated significant differences among the tested concentrations ($P < 0.05$).

Table 4. ABTS radical scavenging assay of the methanolic extract of *Pergularia daemia*.

Concentration (µg mL⁻¹)	Gallic acid	*Pergularia daemia* extract
Control	–	–
5	0.239	0.682
10	0.256	0.741
20	0.281	0.789
50	0.349	0.923
100	0.562	1.269

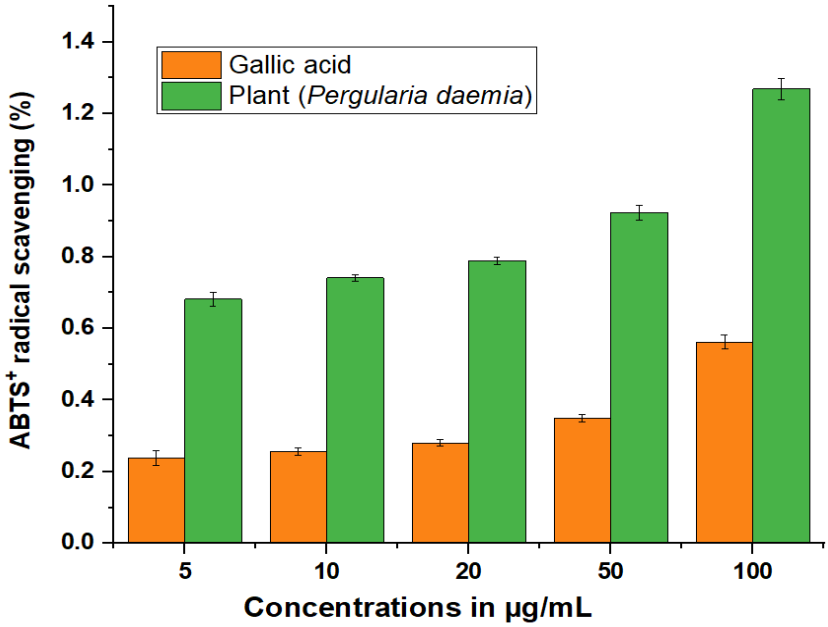


Figure 3. ABTS radical scavenging activity of the methanolic extract of Pergularia daemia and the reference antioxidant gallic acid at concentrations ranging from 5 to 100 µg mL⁻¹. Data are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.05).

Molecular docking analysis against β-neurotoxic phospholipase A₂ proteins

To evaluate the potential interactions of the GC–MS-identified phytochemicals with snake venom phospholipase A₂ (PLA₂) proteins, molecular docking was performed against three experimentally validated β-neurotoxic PLA₂ targets: β-bungarotoxin (1BUN), phospholipase A₂ (1A3D), and taipoxin (3VBZ). Ten representative phytochemicals were docked using AutoDock Vina, and the resulting protein–ligand complexes were analysed using BIOVIA Discovery Studio Visualizer. Binding affinities and hydrogen-bond interactions for all docked compounds are summarized in Table 6.

3.4.1 Docking against β-bungarotoxin (1BUN)

Docking analysis identified phorbol as the highest-affinity ligand for β-bungarotoxin, with a binding energy of −7.248 kcal mol⁻¹, followed by salvinorin A (−7.207 kcal mol⁻¹), ursodeoxycholic acid (−6.920 kcal mol⁻¹), dihydroxanthin (−6.770 kcal mol⁻¹), and folic acid (−6.380 kcal mol⁻¹) (Table 6). Among the evaluated ligands, folic acid formed the greatest number of hydrogen bonds (eight), whereas dihydroxanthin, trans-β-ionone, and methyl oleate each formed two hydrogen bonds. Benzofuranone, salvinorin A, and ursodeoxycholic acid each formed one hydrogen bond, while phorbol, corymbolone, and ethyl iso-allocholate interacted predominantly through non-hydrogen-bond interactions.

3.4.2 Docking against phospholipase A₂ (1A3D)

For phospholipase A₂ (1A3D), folic acid exhibited the most favorable binding affinity (−7.077 kcal mol⁻¹), followed by methyl oleate (−6.014 kcal mol⁻¹), dihydroxanthin (−5.780 kcal mol⁻¹), ursodeoxycholic acid (−5.734 kcal mol⁻¹), and salvinorin A (−5.718 kcal mol⁻¹). Folic acid also showed the highest number of hydrogen bonds (six) among the docked compounds. Salvinorin A established four hydrogen bonds, whereas dihydroxanthin and trans-β-ionone each formed two hydrogen bonds. Benzofuranone, corymbolone, methyl oleate, phorbol, and ursodeoxycholic acid each formed one hydrogen bond, while ethyl iso-allocholate showed no hydrogen-bond interactions (Table 6).

3.4.3 Docking against taipoxin (3VBZ)

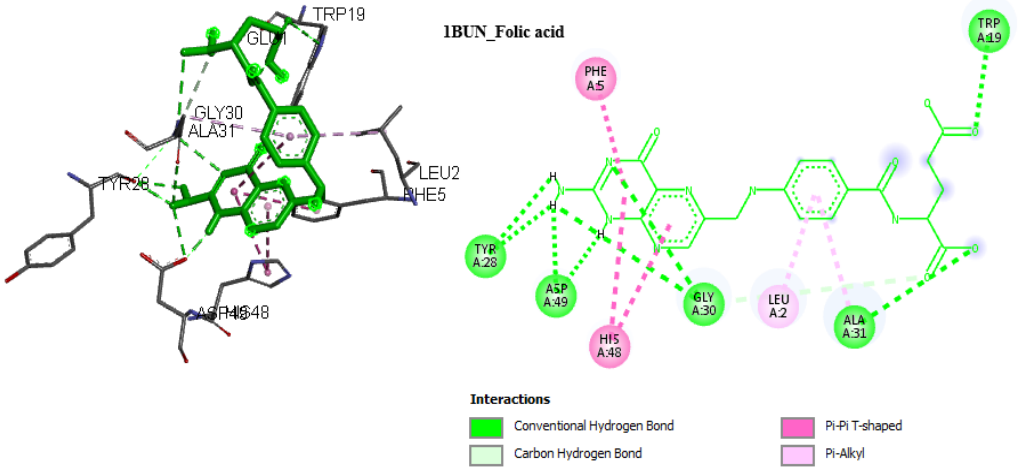
Docking against taipoxin (3VBZ) similarly identified folic acid as the highest-affinity ligand, with a binding energy of −7.619 kcal mol⁻¹. The next highest-ranked compounds were phorbol (−6.695 kcal mol⁻¹), dihydroxanthin (−6.261 kcal mol⁻¹), ursodeoxycholic acid (−6.173 kcal mol⁻¹), ethyl iso-allocholate (−6.116 kcal mol⁻¹), and Salvinorin A (−6.099 kcal mol⁻¹). Folic acid formed four hydrogen bonds with the target protein, whereas Salvinorin A and trans-β-ionone each established three hydrogen bonds. Corymbolone and phorbol formed two hydrogen bonds, while dihydroxanthin and methyl oleate each formed one hydrogen bond. Benzofuranone, ethyl iso-allocholate, and ursodeoxycholic acid did not form hydrogen bonds with the target protein (Table 6).

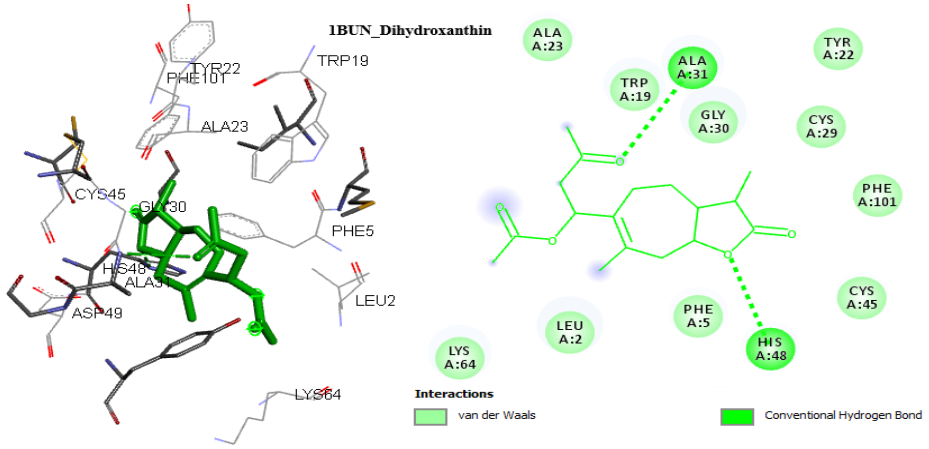
Overall, the docking analysis demonstrated ligand-specific differences in binding affinity and intermolecular interactions across the three β-neurotoxic PLA₂ proteins. Folic acid consistently exhibited the most favorable interaction profile with phospholipase A₂ (1A3D) and taipoxin (3VBZ), whereas phorbol showed the strongest predicted binding toward β-bungarotoxin (1BUN). The complete docking scores and hydrogen-bond interactions

for all evaluated phytochemicals are presented in Table 6

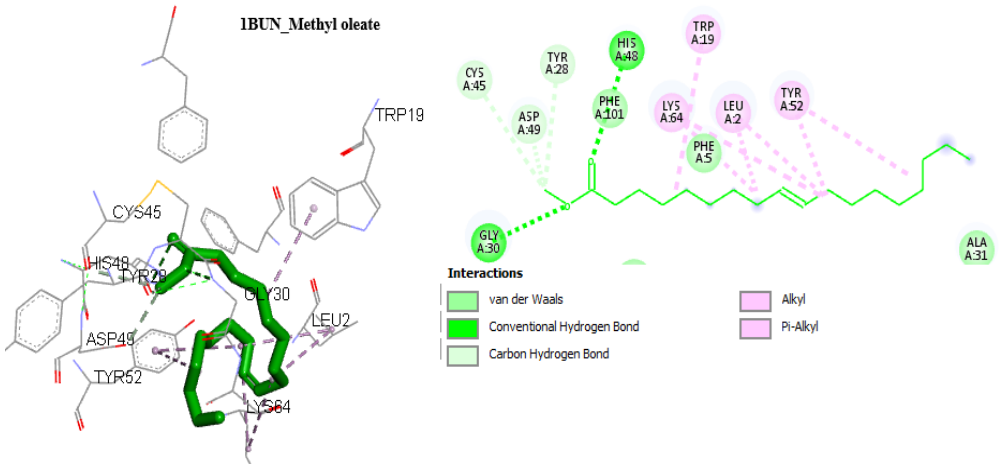
Table 6. Molecular docking results of GC–MS-identified phytochemicals against β-neurotoxic phospholipase A_2 proteins.

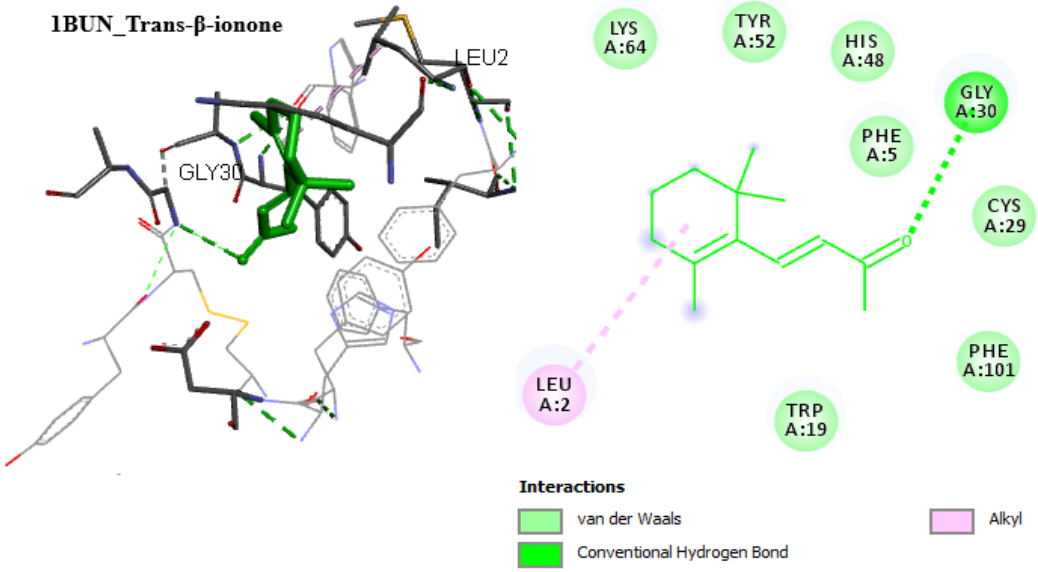
Phytochemical	β-Bungarotoxin (1BUN)		Phospholipase A_2 (1A3D)		Taipoxin (3VBZ)	
	Binding affinity (kcal mol^{-1})	H-bonds	Binding affinity (kcal mol^{-1})	H-bonds	Binding affinity (kcal mol^{-1})	H-bonds
Benzofuranone	−5.431	1	−5.382	1	−5.742	0
Corymbolone	−5.965	0	−5.261	1	−5.984	2
Dihydroxanthin	−6.770	2	−5.780	2	−6.261	1
Ethyl iso-allocholate	−5.884	0	−5.147	0	−6.116	0
Folic acid	−6.380	8	−7.077	6	−7.619	4
Methyl oleate	−5.214	2	−6.014	1	−5.587	1
Phorbol	−7.248	0	−5.437	1	−6.695	2
Salvinorin A	−7.207	1	−5.718	4	−6.099	3
Trans-β-ionone	−5.628	2	−5.546	2	−5.801	3
Ursodeoxycholic acid	−6.920	1	−5.734	1	−6.173	0

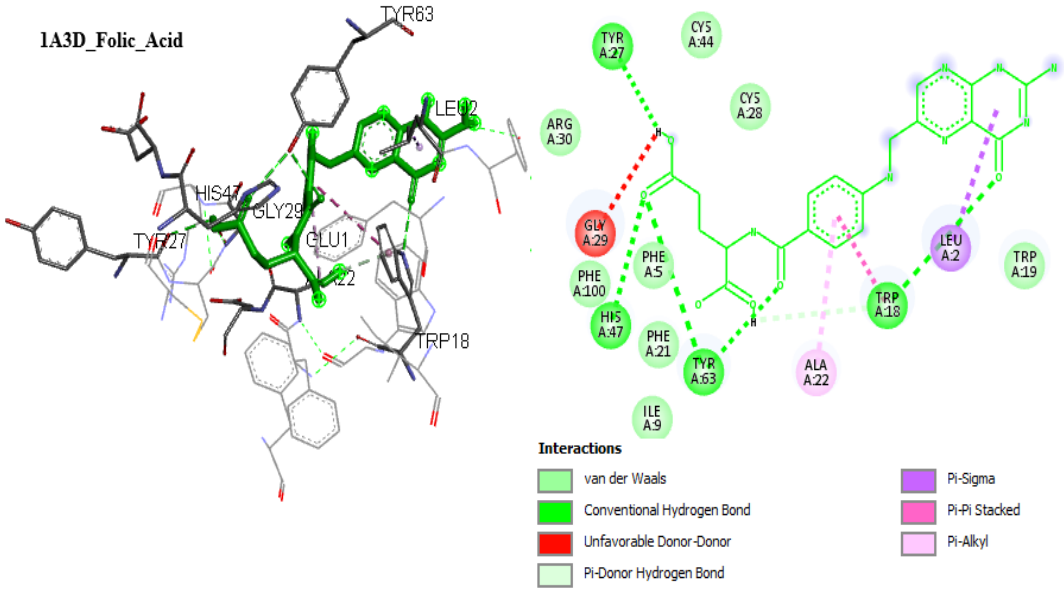


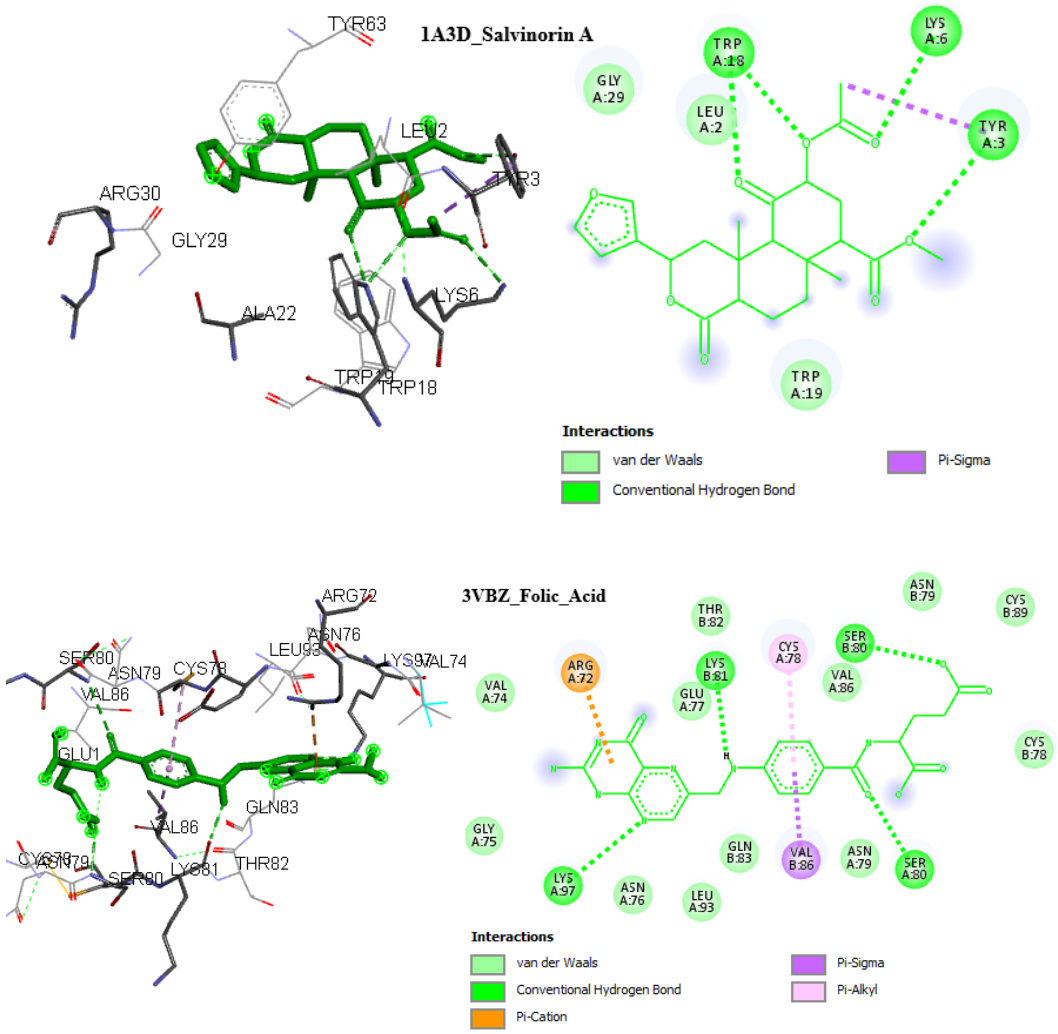


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1BUN_Methyl oleate
TRP19
CYS45
HIS48
TYR28
GLY30
LEU2
ASP49
TYR52
LYS64
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Alkyl
Pi-Alkyl


1BUN_Trans-β-ionone
LEU2
GLY30
LYS A:64
TYR A:52
HIS A:48
GLY A:30
PHE A:5
CYS A:29
PHE A:101
LEU A:2
TRP A:19
Interactions
van der Waals
Conventional Hydrogen Bond
Alkyl


1A3D_Folic_Acid
TYR63
LEU2
HIS47
GLY29
TYR27
GLU1
TRP18
Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Donor-Donor
Pi-Donor Hydrogen Bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl



DISCUSSION

The present study combined phytochemical profiling, antioxidant evaluation, and molecular docking to investigate the pharmacological potential of Pergularia daemia. The integrated findings indicate that the methanolic extract contains structurally diverse phytochemicals with antioxidant activity and predicted interactions with venom-associated phospholipase A_2 (PLA_2) proteins, supporting its potential as a source of bioactive compounds for snakebite research [43].

The phytochemical screening and GC–MS analysis demonstrated that the extract contains multiple classes of secondary metabolites, including flavonoids, tannins, saponins, steroids, and fatty acid methyl esters. Similar phytochemical profiles have previously been reported for P. daemia, suggesting that these metabolites contribute to its reported medicinal properties. The predominance of methyl palmitate, methyl oleate, methyl linoleate, phytol, and neophytadiene is noteworthy, as these compounds have been associated with antioxidant, anti-inflammatory, and antimicrobial activities [44, 45]. The coexistence of chemically distinct metabolites indicates that the biological activity of the extract is likely mediated through multiple compounds acting in concert rather than a single active constituent.

Oxidative stress is a key contributor to venom-induced tissue damage, promoting lipid peroxidation, inflammation, and cellular injury. Consistent with this mechanism, the methanolic extract exhibited concentration-dependent antioxidant activity in both DPPH and ABTS assays [46]. Although these in vitro assays cannot directly predict therapeutic efficacy, the observed free radical scavenging activity suggests that P. daemia contains metabolites capable of reducing oxidative stress associated with snakebite pathology. This activity is likely attributable to the combined contribution of flavonoids, phenolic constituents, diterpenoids, and fatty acid derivatives identified in the extract [47].

Molecular docking further demonstrated that several phytochemicals exhibited favorable binding affinities toward β-neurotoxic PLA_2 proteins. Folic acid and phorbol showed the strongest predicted interactions with the selected protein targets, while methyl oleate, ursodeoxycholic acid, trans-β-ionone, and ethyl iso-allocholate also demonstrated favorable binding profiles [48, 49]. The observed hydrogen-bonding and hydrophobic interactions suggest that these compounds can occupy the predicted ligand-binding regions of PLA_2 proteins. However, docking analysis provides only a structural prediction of ligand–protein interactions and should not be

interpreted as evidence of enzyme inhibition or therapeutic efficacy [50].

A major strength of this study is the integration of phytochemical characterization with functional antioxidant assays and computational target analysis. This workflow enables the prioritization of bioactive compounds for subsequent experimental validation and provides a rational framework for natural product-based antivenom research. Nevertheless, several limitations should be acknowledged. GC–MS primarily detects volatile and semi-volatile metabolites and may not identify non-volatile bioactive constituents. In addition, antioxidant assays and molecular docking represent preliminary screening approaches and do not account for bioavailability, metabolism, or physiological complexity [52, 53]. Therefore, the identified compounds should be further evaluated using molecular dynamics simulations, phospholipase A_2 inhibition assays, cell-based models, pharmacokinetic analyses, and in vivo validation.

Overall, the present findings demonstrate that Pergularia daemia contains chemically diverse phytochemicals with antioxidant activity and favorable predicted interactions with β-neurotoxic PLA_2 proteins. These results provide a scientific basis for further investigation of P. daemia-derived metabolites as potential lead compounds for the development of complementary therapeutic strategies against snakebite envenomation.

CONCLUSION

The present study demonstrates that the methanolic extract of Pergularia daemia contains a diverse array of bioactive phytochemicals with antioxidant activity and favorable predicted interactions with β-neurotoxic phospholipase A_2 proteins associated with snake venom. Qualitative phytochemical screening and GC–MS profiling identified several pharmacologically relevant metabolites, including fatty acid methyl esters, terpenoids, steroidal derivatives, and other secondary metabolites that may collectively contribute to the biological activity of the extract. The concentration-dependent antioxidant activity observed in both DPPH and ABTS assays indicates an effective free radical scavenging capacity, suggesting a potential role in reducing oxidative stress associated with venom-induced tissue injury. Molecular docking further identified several compounds with favorable binding affinities toward β-bungarotoxin, phospholipase A_2, and taipoxin, supporting their potential as candidate molecules for future antivenom research. However, the present findings are based on in vitro antioxidant assays and computational predictions and therefore require further validation through phospholipase A_2 inhibition assays, molecular dynamics simulations, cell-based studies, pharmacokinetic evaluation, and in vivo models. Overall, this study provides a scientific foundation for the further exploration of P. daemia-derived phytochemicals as potential complementary therapeutic agents for snakebite envenomation

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