

Phytochemical analysis, antiproliferative and apoptosis-inducing properties of *Persicaria strigosa* Nakai

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Abstract

Persicaria strigosa is an important medicinal plant having several ethnomedicinal values. The present study investigates the phytochemical contents, antiproliferative, and apoptosis-inducing properties of *P. strigosa*. The phytochemicals were identified by the GC-MS technique. Antiproliferative and apoptosis-inducing properties were conducted in DL cells. *In-silico* molecular docking, drug-likeness, and ADMET was carried out to study the binding affinity and drug-likeness of the compounds. The study revealed a dose-dependent antiproliferative activity of *P. strigosa*. Ethyl acetate extract showed the strongest activity compared to other extracts. Plant extract-treated cells showed morphological features of apoptotic cells. GC-MS analysis identified 12 compounds from the ethyl acetate extract. Compound **C2**, **C5**, **C6**, **C7**, and **C12** showed the best binding affinity with the anti-apoptotic proteins. The phytochemicals were predicted to possess drug-likeness property and a good ADMET profile. The findings, thus, suggest that the *Persicaria strigosa* extract could be a potential source of anticancer agents.

Introduction

Cancer is a terrible disease that can start at any organ of the body from an abnormal genome function leading proliferation of cells at inappropriate times and locations. Cells acquire mutations that abolish the regulation of cell division, and cells multiply continuously, forming tumors and then invading new parts of the body (Weinberg 2015). According to WHO, there are about 18.1 million cancer patients worldwide, and about 10 million deaths in 2020 are due to cancer. Today, one out of the six deaths is due to cancer and is the second-largest killer in the world (<https://www.who.int/news-room/fact-sheets/detail/cancer>). Despite significant improvement in healthcare facilities, the cancer burden grows to cause tremendous financial strain to the human populace. At the same time, poor accessibility of quality medicines, high cost, off-the-target drug resistance, and the severity of the side effects remain a big challenge to successful cancer treatment. In this regard, exploring new anticancer compounds from plants and other biological sources could be a good choice. Plant-derived compounds are readily available, possess lesser side effects, and are sometimes more efficacious than the existing drugs (Atanasov et al. 2015).

Plant and plant-derived compounds serve as a promising source of medicine for several diseases (Basu et al. 2020; Fahad et al. 2021; Swargiary and Daimari 2021). Since ancient times, the search for natural medicines from plants and organisms has remained an essential aspect of drug discovery. Today, about 60% of the approved anticancer drugs are directly or indirectly derived from plant sources (Fridlender et al. 2015). Several phytochemicals, such as paclitaxel, etoposide, camptothecin, vinblastine, vincristine, uvaribonin, 22-epicalamistrin, etc., are plant-derived natural products with considerable anticancer activity (Pettit et al. 2008; Cragg et al. 2009). *Persicaria strigosa* (R.Br.) Nakai belonging to the family Polygonaceae (local name *alari gwja*) is a creeping herb that grows well in marshy places. Stems are greenish-brown simple or branched, and leaf blades are green above and prickly on nerves beneath. Geographically, *P. strigosa* is distributed mainly in Southeast Asian countries, including India. There is very little literature regarding the phytochemistry and biological activities of *P. strigosa*. In a survey report,

Deka and Devi (2015) opined that the leaves of *P. strigosa* are used to control dysentery in cattle. The Bodo community of Assam uses the aerial part (mainly leaves) of *P. strigosa* as deworming medicine (Swargiary et al. 2019; Swargiary et al. 2020). The plant is also used to treat jaundice, typhoid, and common sickness. However, no scientific work has been carried out to investigate the bioactivity of the plant. Because of the rich ethnomedicinal values, the present study explores the phytochemicals, antioxidants, and antiproliferative properties of *Persicaria strigosa*.

Materials And Methods

2.1. Identification and preparation of solvent extracts

Persicaria strigosa was collected from Tinali area of Kokrajhar town with the help of local people. The sample specimen was submitted to the Department of Botany, Bodoland University for taxonomic identification (Specimen no. BUBH2018021). Preparation of methanolic crude extract of *P. strigosa* leaves was carried out following the method described in our earlier publication (Swargiary et al. 2016). Dry, semi-solid *P. strigosa* extract obtained kept at -20°C for further use. After the liquid-liquid partitioning method, successful solvent extractions of crude plant extract were carried out with different solvents (increasing solvent polarity) – n-hexane, diethyl ether, and ethyl acetate. The solid material obtained after solvent drying was kept at -20°C for further study.

2.2. Cytotoxicity study

2.2.1. Cell line and dose preparation

The antiproliferative activity of different solvent extracts was studied for 24 h, 48, and 72 h treatment using Dalton's lymphoma (DL) cell line. DL cells were cultured in RPMI-1640 medium supplemented with 10% FBS, gentamycin (20 mg/mL), streptomycin (100 mg/mL), and penicillin (100 IU) in a CO₂ incubator at 37°C with 5% CO₂; 80% confluent of exponentially growing cells were sub-cultured and used in the experiments. The different dosage of plant extracts was freshly prepared in phosphate-buffered saline (PBS).

2.2.2. Cell proliferation and apoptosis assay

After the cells were treated with different concentrations of solvent extracts, cell proliferation was measured by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay in DL (Mosmann 1983; Verma and Prasad 2013). In brief, the cells were treated with different doses (25, 50, 100, and 200 mg/mL) of plant extracts for 24 h, 48 h, and 72 h, then 10 µL of the MTT reagent (5 mg/mL in PBS) was added into each well. Next, the treated plates were incubated for 4 h under 5% CO₂ and 95% air at 37°C following the addition of 100 µL of DMSO into each well and gently shaken. The plates were checked for complete solubilization of crystals, and then absorbance was recorded at 570 nm using Microplate Reader (Rapid Diagnostics; SKU, LISA-R, India). Furthermore, control and treated cells were stained with acridine orange and ethidium bromide (AO/Eb) for 5 min in a dark/cold room. The cells were observed

under Fluorescence Microscope and photographed (Medlab Lx400 FLR Fluorescence Microscope). About 1000 cells were counted, and the percentages of apoptotic cells were determined based on differential colouring patterns (red/green) of the nucleus.

2.3. GC-MS analysis of phytocompounds

The phytocompounds present in the best bioactive fraction (ethyl acetate) of the plant was further investigated by GC-MS system (Perkin Elmer (USA), make GC-MS instrument, Model: Clarus 680 GC & Clarus 600C MS comprising a liquid auto-sampler). The software used in the system was TurboMass Ver. 5.4.2. The capillary column used was 'Elite-5MS', having dimensions, length 60 m, ID-0.25 mm, and film thickness 0.25 μ m, and the stationary phase was 5% diphenyl 95% dimethylpolysiloxane. Helium (99.99%) was used as carrier gas at a flow rate of 1 mL/min. An injection volume of 2 μ L was employed in split-less mode. Injector and ion-source temperatures were kept at 280°C and 180°C, respectively. The oven temperature was programmed at 60°C (1 min) with an increasing rate of 7°C/min up to 200°C (hold for 3 min), and then again increased at the rate of 10°C/min up to 300°C (hold for 5 min). The total run time was ~39 min. The solvent delay was kept for 8 min. MS Protocol Mass Spectra was taken in Electron Impact positive (EI+) mode at 70 eV. A solvent delay of 8 min was there for MS scan. Mass range i.e., m/z range is 50–600 amu.

2.3.1. Identification of peaks

Interpretation of the peaks of GC-MS chromatogram was carried out by library search of the mass spectrum using the National Institute Standard and Technology-2008 database.

2.4. Molecular docking

To further elucidate the antiproliferative property of the plant, molecular docking was carried out with the GC-MS identified compounds of *P. strigosa* with six major anti-apoptotic proteins, BCL-2 (PDB: 1YSW), BCL-X_L (4QVX), MCL-1 (5KU9), BCL-W (2Y6W), BCL-B (4B4S), and BCL-A1/Bfl-1 (5UUK). The 3D structures of proteins and compounds were retrieved from PDB (<https://www.rcsb.org/>) and PubChem databases (<https://pubchem.ncbi.nlm.nih.gov/>). Docking was carried out in AutoDock vina software (Trott and Olson 2010). The grid parameters for docking were set as x, y, z size-coordinate and grid box center-coordinates i.e., 14.496, -3.140, 1.690 and 46, 82, 58 for BCL-2, -15.653, 19.048, 0.789 and 30, 48, 40 for BCL-W, -6.783, 9.665, 2.169 and 38, 38, 30 for BCL-B, 0.308, -21.586, 11.404 and 74, 54, 52 for BCL-X_L, -2.209, -10.161, 24.412 and 54, 42, 52 for MCL-1, and -2.219, 20.505, -4.937, and 36, 44, 44 for BCL-A1/Bfl-1 proteins, respectively. Docking was performed in triplicate (n = 3), and the best docking output was visualized in Discovery studio software.

2.5. Analysis of drug-likeness and ADMET profile

The phytocompounds of *P. strigosa* identified by GC-MS analysis was verified for the drug-likeness properties using SwissADME (Dina et al. 2017) and PubChem database. The drug-likeness property of

compounds was evaluated based on Lipinski's rule (Lipinski 2004). Similarly, *in-silico* absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of identified compounds were predicted using ADMETlab server (Dong et al. 2018).

2.6. Statistical analysis

All the statistical calculations were carried out in Microsoft excel. A one-way ANOVA correlation study was carried out using OriginPro software. Data were expressed as mean $\pm$ standard deviation (SD), $n = 3$ at $P \leq 0.05$ probability level.

Results

3.1. Antiproliferative and apoptotic-inducing study

The mortality of cells and antiproliferative property of different solvent extracts of *P. strigosa* is shown in Figure 1. *In vitro* bioassay showed dose-dependent mortality of DL cells in all the solvent fractions. In all the test doses and treatment times, ethyl acetate extract showed the most potent cytotoxicity while hexane fraction showed the weakest activity. At 25–200 mg/mL concentrations of plant extracts, the percentage (%) mortality of cells ranged from $9 \pm 19\%$ to $38 \pm 4\%$, $15 \pm 2\%$ to $46 \pm 4\%$, and $21 \pm 2\%$ to $59 \pm 6\%$ at 24 h, 48 h, and 72 h treatment, respectively. The mortality of cells at 72 h treatment was found to be highest in ethyl acetate extract ($34 \pm 3\%$ to $59 \pm 6\%$), followed by diethyl ether ($32 \pm 3\%$ to $52 \pm 5\%$), methanol ($26 \pm 3\%$ to $42 \pm 5\%$), and hexane extracts ($21 \pm 2\%$ to $38 \pm 4\%$). The LD_{50} of plant extracts for cell death at 72 h treatment was 74.99 mg/mL, 140.93 mg/mL, 308.06 mg/mL, and 396.87 mg/mL, respectively, for ethyl acetate, diethyl ether, methanol, and hexane extracts of *P. strigosa*. Statistical analysis showed a significant difference ($P \leq 0.05$ level) in the mortality of cells at high treatment doses of all the solvent extracts. However, at a lower dose (25 mg/mL), diethyl ether and ethyl acetate extracts showed no significant difference. Similarly, hexane and methanol extract showed insignificant differences in terms of mortality of the cells. Meanwhile, the percentage of cell death at 24 h treatment is significantly different from the 48 h and 72 h treatment. Like antiproliferative assay, the fluorescence microscopy study also revealed a similar pattern of apoptosis-inducing potential in medicinal plants. Figure 2 shows the fluorescent microscopy images of cells treated with 200 mg/mL plant extracts after 72 h of treatment. Of the four plant extracts, ethyl acetate extract of *P. strigosa* shows the best apoptosis-inducing property. The study observed membrane blebbing, chromatin condensation, and apoptotic bodies in plant extracts treated DL cells. In contrast, the control DL cells showed green nuclei with intact cell and nuclear membrane structures.

3.2. GC-MS study

The presence of volatile phytocompounds present in ethyl acetate extract of *P. strigosa* was analyzed by GC-MS. Figure 3 shows the GC-MS chromatogram of the identified compounds. Peak structure with retention time (RT) 19.218 and RT-28.272 showed the highest percentage height, followed by RT-21.064, RT-17.552, and RT-22.264 compared to other structures. The names of the probable phytocompounds

with the RT and m/z data have been provided in Table 1. The most abundant phytocompounds were identified to be Carbonic acid, tridecyl vinyl ester [3] and Tetradecanoic acid, 10,13-Dimethyl-, Methyl Ester [C9] followed by 3N-Hexylthiane-S,S-dioxide [C5] and Trans-2-Methyl-4-n-butylthiane-S,S-dioxide [2].

3.3. Molecular docking study

Table 2 shows the binding affinities of six anti-apoptotic proteins with the phytocompounds identified by GC-MS. Among the six proteins, BCL-X_L showed the best binding affinity with the phytocompounds (average, -5.40 kcal/mol) followed by BCL-2 (average, -5.21 kcal/mol), BCL-A1/Bfl-1 (average, -4.93 kcal/mol), and BCL-B (average, -4.47 kcal/mol). BCL-W showed the weakest binding affinity (average, -2.14 kcal/mol) with phytocompounds. Of the 12 compounds, **C12** showed the strongest binding affinity of -8.57 kcal/mol with BCL-X_L. Similarly, **C7**, **C5**, **C5**, **C6**, and **C6** showed the best affinities with BCL-2, BCL-W, BCL-B, MCL-1, and BCL-A1/Bfl-1, respectively (Table 3). Overall, **C12**, **C6**, **C7**, and **C2** showed the best binding affinity with all the proteins compared to other compounds, while **C10** showed the weakest affinity.

The binding interactions of phytocompounds with the anti-apoptotic proteins are shown in Figures 4 and 5. The 2D display of the binding interactions revealed 13 amino acid residues of BCL-2 interacting with **C7**. A maximum of 17 residues was observed between BCL-X_L and **C12**. Similarly, **C2** interacted with 7 and 8 residues of BCL-W and BCL-B while **C6** involved 10 and 8 amino acid residues with MCL-1, and BCL-A1/Bfl-1. Van der Waal's interactions and alkyl bonds were the most prominent binding energies between the proteins and phytocompounds. However, in the case of the best docking complex, BCL-XL & **C12**, alkyl bonds were the most predominant interactions (Figure 4d). Alternatively, two docking complexes, BCL-2 & **C7** and BCL-A1/Bfl-1 & **C6** showed one hydrogen bond between proteins and phytocompounds.

3.5. Drug-likeness and ADMET study

The drug-likeness properties of the top five compounds identified from *P. strigosa* are presented in Table 3. All the compounds showed drug-likeness properties as per the Lipinski rule. According to the rule, compounds that possess <500 Da molecular weight, <5 lipophilicity, <5 H-bond donors, and <10 H-bond acceptors fulfil the drug-likeness property. A compound is found to have drug-likeness properties if the compound does not violate two or more rules. *In-silico* study revealed that all the five compounds possess drug-likeness properties. One of the major drug-likeness properties is the molecular weight and surface area of the compounds. All the top five compounds were smaller than 500 Da, and the TPSA values were less than 100 Å². Similarly, the LD50 values showed low toxicity profile suggesting good drug-likeness profiles.

Figure 6 shows the ADMET profiles of the top five best binding phytocompounds. Physiochemically, all the compounds showed poor aqueous solubility but high lipophilicity. **C12** showed the best lipid permeability property. Similarly, the phytocompounds were predicted to have high absorption through intestinal cells. **C12** showed moderate values in terms of bioavailability, while the other four compounds

have high bioavailability. All the phytochemicals showed high blood-brain barrier properties, indicating less seepage of the compounds from the blood circulation. In addition, all the five compounds showed low metabolism profiles suggesting its suitable drug-likeness property. There is a very low probability that the phytochemicals would act either as substrates or inhibitors to the Cytochromes-P450 enzyme superfamily. In addition to other ADMET properties, the compounds were also seen to have low toxicity and carcinogenic effect.

Discussion

Growth and development are essential characteristics of life. An organism grows in size and becomes an adult because of the continuous multiplication and replacement of the dead cells by the newer cells. All living cells possess a sophisticated and controlled mechanism of cell division. Aged or damaged cells are eliminated from the body by a normal cellular mechanism called apoptosis, also known as programmed cell death. Avoidance of normal cellular death leading to the unregulated growth and division of cells and invading other parts of the body is the hallmark of cancer disease (Wong 2011). Dysregulation of apoptosis-related genes and their product is a recurring event in cancer that inhibits cell death induced by many cellular stimuli (Ashkenazi et al. 2017). Anti-apoptotic proteins belonging to BCL-2 family protein (such as BCL-2, BCL-X_L, MCL-1, BCL-W, BCL-B, and BCL-A1/Bfl-1) suppress normal apoptosis. Therefore, it represents an exciting chemotherapeutic target in cancer treatment (Yip and Reed 2008). Several studies have reported the antiproliferative and inhibitory properties of anti-apoptotic proteins of several medicinal plants and phytochemicals (Kamaruddin et al. 2019; Erdogan et al. 2020; Swargiary et al. 2021). Phytochemicals such as betulinic acid (*Betula alba*), dentatin (*Clausena excavata*), etc., were found to downregulate the expression of the anti-apoptotic BCL-2 gene family (Rzeski et al. 2006; Arbab et al. 2012). In the present study, the ethyl acetate fraction of *P. strigosa* showed the strongest antiproliferative and apoptosis-inducing activity. The high antiproliferative property of the plant may be attributed to the phytochemicals present in the extract. Cisplatin, the reference chemical, showed a significant difference (at $P \leq 0.05$ level) compared to plant extracts. GC-MS study identified 12 probable phytochemicals from the ethyl acetate extract of *P. strigosa*. The identified compounds have also been reported from many other plants. The essential oil Trans-2-Methyl-4-n-butylthiane-S,S-dioxide has also been reported in the ethyl acetate fraction of *Croton macrostachyus* (Gebrehiwot et al. 2018). Tricosanoic acid, an important fatty acid, has been reported from several plants having rich bioactivities such as *Cuminum cyminum* and *Coffea robusta* (Dong et al. 2015; Shukla et al. 2018). Rizvi et al. (2014) observed strong binding of 3,5-Bis(1,1-dimethylethyl) phenol with human polo-like kinase-1 enzyme suggesting the anticancer potential of the compound isolated from the leaves of *Ageratum houstonianum*. Similarly, the presence of hexacosyl acetate and many other phytochemicals has been attributed to the α -amylase inhibitory activity of *Croton bonplandianum* (Keerthana et al. 2013).

Molecular docking is an important *in-silico* tool to study drug interactions and target proteins. The study of ligand binding to the active protein site is an effective drug discovery pipeline strategy in designing therapeutic protein inhibitors. Phytochemicals identified from *P. strigosa*, and molecular docking with

six anti-apoptotic proteins revealed a strong binding affinity with **C2**, **C6**, **C7**, and **C12** phytochemicals. Van der Waals interaction was the major interaction between the phytochemicals and proteins. Similarly, anonaine, a benzylisoquinoline alkaloid isolated from *Annona muricata*, showed a high binding affinity with BCL-2, BCL-W, and MCL-1 proteins (Rosdi et al. 2018). Similarly, ginsenosides from *Panax ginseng* showed a strong binding affinity with BCL-2, BCL-XL, and MCL-1 (Sathishkumar et al. 2012). Adewole and Ishola (2019) screened 47 phytochemicals from *Morinda lucida* and two triterpenes (ursolic acid and oleanolic acid), and four phytosterols (cycloartenol, campesterol, stigmasterol, and β -sitosterol) were found to have a strong binding affinity to BCL-2 proteins. Phenolics and flavonoids are bioactive compounds having several biological properties. Our study also revealed high phenolic and flavonoid content in the ethyl acetate fraction of *P. strigosa* compared to other extracts (data not published). Of the five flavonoids, biochanin-A, myricetin, apigenin, galangin, and fisetin, anti-apoptotic proteins BCL-2 and BCL-X_L showed strong binding interaction with fisetin compared to other flavonoids (Abd Ghani et al. 2020). In the present study, Undec-10-ynoic acid, Tridec-2-Yn-1-yl ester shows the most promising inhibitory activity of anti-apoptotic proteins among the phytochemicals. 3,5-Bis(1,1-dimethylethyl)phenol and 2,5-Bis(1,1-Dimethylethyl)phenol as well as Trans-2-methyl-4-n-butylthiane-S,S-dioxide also showed potential apoptosis-inducing property.

The study of drug-likeness of compounds and their ADMET analysis is an important prerequisite in the present-day drug discovery pipeline leading to the optimization of lead compounds. According to Lipinski's rule, an oral drug is predicted to be the most effective if it follows all four rules. According to the rule, a molecule is not considered orally active if it violates two or more of the four rules (Guan et al. 2019). In addition, a candidate drug must also satisfy other properties such as less toxicity, high permeability through the cell membrane, and easy excretion from the body without further deposition (DiMasi et al. 2003). The use of such web-based studies may be helpful because pharmaceutical companies spend millions of dollars to advance a new drug through clinical trials and, failure in the later stages of drug development results in significant economic losses (Sertkaya et al. 2016). Undesirable pharmacokinetics properties and high toxicity are the leading causes of such failure at the clinical trial stage. Therefore, a good balance of potency and ADMET profile offers a useful guideline for further optimization and drug discovery (Jia et al. 2020). In the present study, all the best five phytochemicals showed suitable drug-likeness properties with considerable ADMET properties. High lipophilicity of phytochemicals suggests higher permeability through cell membranes. Similarly, small molecular mass and smaller surface area allow the compounds to pass through the membrane. ADMET study of the *Persicaria strigosa* compounds showed low toxicity properties of the compounds suggesting the potentiality of a lead therapeutic compound. According to Lei et al. (2016), compounds having LD₅₀ values in the range of 1–50 mg/kg, 51–500 mg/kg, and 501–5000 mg/kg body weight are considered high, moderate, and low toxicity properties, respectively. All five compounds showed more than 600 mg/kg suggesting a low risk of toxicity in the host body.

Conclusions

The present study investigated the phytochemistry, antiproliferative, and apoptosis-inducing activity of different solvent extracts of *Persicaria strigosa*. The plant extracts revealed promising apoptosis-inducing properties, which may be attributed to the secondary metabolites present in the ethyl acetate extract of the plant. The bioactivity could be due to the downregulation of BCL-2 family gene or inhibition of anti-apoptotic proteins by the phytocompounds. However, further study needs to be carried out to establish the exact molecular mode of action.

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Tables

Table 1 GC-MS properties of phytochemicals identified from ethyl acetate extract of *Persicaria strigosa* leaves

Sl. no	Compound name & Code	RT	m/z	PC id	PA (%)	MF
1.	3,7,11-Trimethyldodecan-3-ol [C1]	13.861	444	23701	2.402	C ₁₅ H ₃₂ O
2.	Trans-2-Methyl-4-n-butylthiane-S,S-dioxide [2]	17.552	204	543892	3.057	C ₁₀ H ₂₀ O ₂ S
3.	Carbonic acid, tridecyl vinyl ester [3]	19.218	188	91693103	8.200	C ₁₆ H ₃₀ O ₃
4.	Tricosanoic acid, isobutyl ester [4]	19.673	458	91693029	0.806	C ₂₇ H ₅₄ O ₂
5.	3N-Hexylthiane-S,S-dioxide [C5]	21.064	218	543815	3.094	C ₁₁ H ₂₂ O ₂ S
6.	3,5-Bis(1,1-Dimethylethyl) phenol [C6]	22.259	206	70825	1.382	C ₁₄ H ₂₂ O
7.	2,5-Bis(1,1-Dimethylethyl) phenol [C7]	22.264	206	79983	2.706	C ₁₄ H ₂₂ O
8.	Hexacosyl acetate [C8]	24.845	424	3083648	2.122	C ₂₈ H ₅₆ O ₂
9.	Tetradecanoic Acid-10,13-Dimethyl-, Methyl Ester [C9]	28.272	270	554145	6.789	C ₁₇ H ₃₄ O ₂
10.	Methyl-6,10-octadecadienoate [C10]	30.442	504	12255549	0.752	C ₁₉ H ₃₄ O ₂
11.	1,9-Nonanediol, Dimethanesulfonate [C11]	31.008	316	20244	1.438	C ₁₁ H ₂₄ O ₆ S ₂
12.	Undec-10-ynoic Acid, Tridec-2-Yn-1-yl Ester [C12]	31.388	360	91697642	0.927	C ₂₄ H ₄₀ O ₂

RT - retention time, PA - peak area, PC - PubChem id, MW - molecular weight (g/mole), MF - molecular formula

Table 2 Binding affinity (-kcal/mol) of anti-apoptotic proteins with 12 phytochemicals from *Persicaria strigosa*

Compounds	BCL-2	BCL-W	BCL-B	BCL-X _L	MCL-1	BCL-A1/Bfl-1
C1	-4.93	-4.63	-4.56	-4.36	-3.43	-4.3
C2	-5.46	-5.16	-5.2	-5.16	-3.53	-5.56
C3	-4.5	-4.1	-4.36	-3.23	-2.86	-4.51
C4	-3.8	-4.23	-4.5	-3.1	-2.3	-4.22
C5	-5.70	-2.0	-4.07	-6.23	-4.9	-4.97
C6	-6.27	-3.0	-4.93	-6.6	-5.47	-6.13
C7	-6.37	-3.03	-4.77	-6.33	-5.4	-5.9
C8	-5.33	-1.47	-3.57	-7.17	-3.4	-4.8
C9	-5.27	-1.83	-4.0	-6.1	-3.93	-5.2
C10	-3.9	-3.96	-4.66	-2.66	-2.73	-3.3
C11	-4.83	-2.03	-4.07	-5.37	-4.23	-4.67
C12	-6.17	-2.7	-4.97	-8.57	-5.43	-5.57

Values are expressed as the mean of three docking experiments (n = 3); C1-C12 - phytochemicals as listed in table 1

Table 3 In silico drug-likeness properties of top five phytocompounds of *Persicaria strigosa*

Compounds	MW (<500 Da)	LogP (<5)	HBD (<5)	HBA (<10)	TPSA (Å ²)	LD ₅₀ , mg/kg (acute toxicity)
C2	204.33	2.9	0	2	42.5	1470.08
C5	218.36	3.4	0	2	42.5	1816.25
C6	206.32	4.9	1	1	20.2	1855.92
C7	206.32	4.9	1	1	20.2	1805.34
C12	360.60	9.1	0	2	26.3	9099.23

MW - molecular weight, LogP - lipophilicity, HBD - H-bond donor, HBA - H-bond acceptor, TPSA - topological polar surface area

Declarations

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Conflicts of interest/Competing interests: Authors do not have any conflict of interest.

Availability of data and material: The authors confirm that the data supporting the findings of the study are available within the manuscript.

Author Contributions: AS designed the study, carried out statistical calculations, and wrote the manuscript. AKV conducted the antiproliferative and apoptosis study. MKR involved in literature collection, manuscript drafting and docking studies. HB was involved in the antioxidant study and statistical calculations. All the authors read the final manuscript and approved it for publication.

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Consent to participate: Not applicable

Consent for publication: All the authors gave their consent for publication.

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Figures

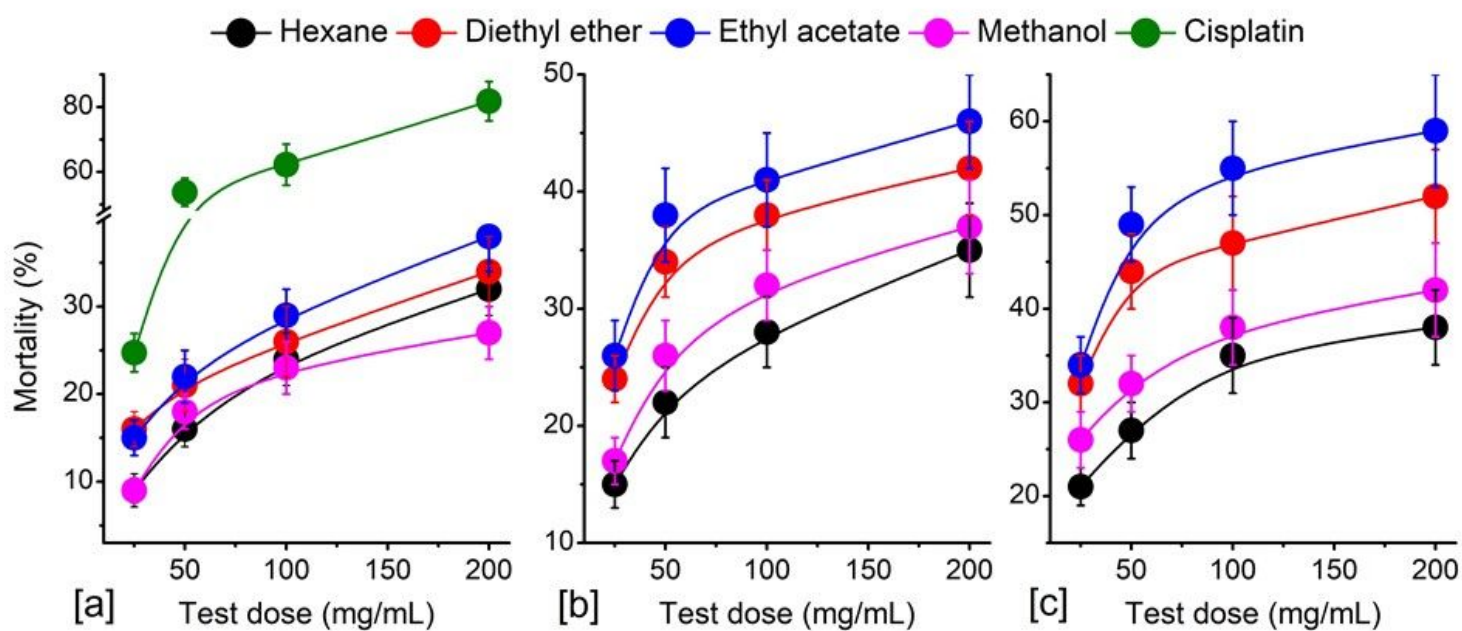


Figure 1

Cytotoxicity study of the solvent fractions of *Persicaria strigosa* against DL cells. (a) 24 h, (b) 48 h, and (c) 72 h treatment. Values are expressed as mean $\pm$ SD, n = 3

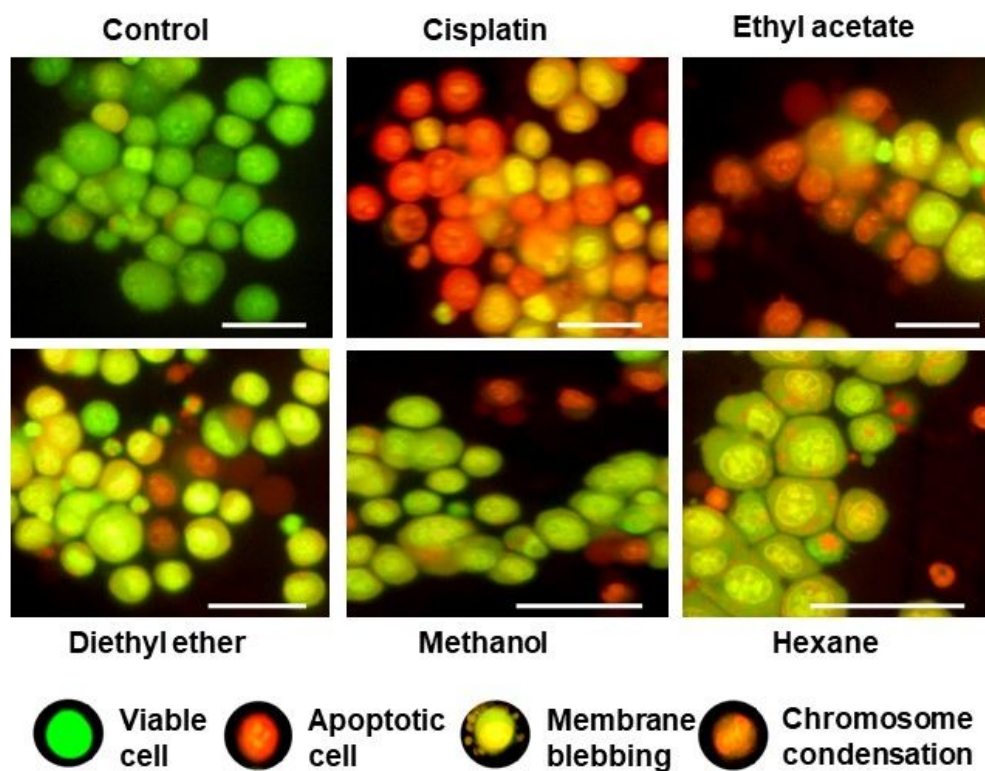


Figure 2

Morphological features of apoptotic and viable cells observed under a fluorescence microscope after 72 h treatment with different solvent fractions of *Persicaria strigosa*. The ethyl acetate extract shows higher apoptotic cells compared to other plant extracts. Scale bar 10 μm

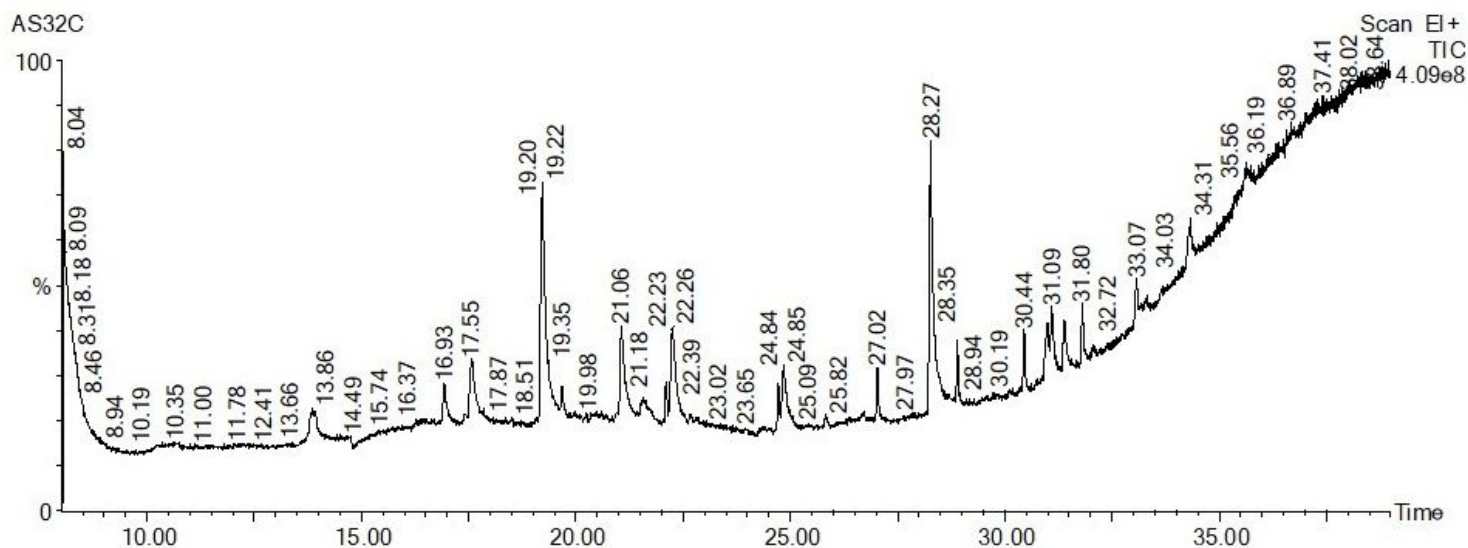


Figure 3

GC-MS chromatogram of ethyl acetate extracts of *Persicaria strigosa* leaves

Figure 4

2D binding interactions of *Persicaria strigosa* phytochemicals with the anti-apoptotic proteins. (a) BCL-2 and **C17**, (b) BCL-W and **C8**, (c) BCL-B and **C17**, (d) BCL-X_L and **C15**, (e) MCL-1 and **C17**, and (f) BCL-A1/Bfl-1 and **C7**

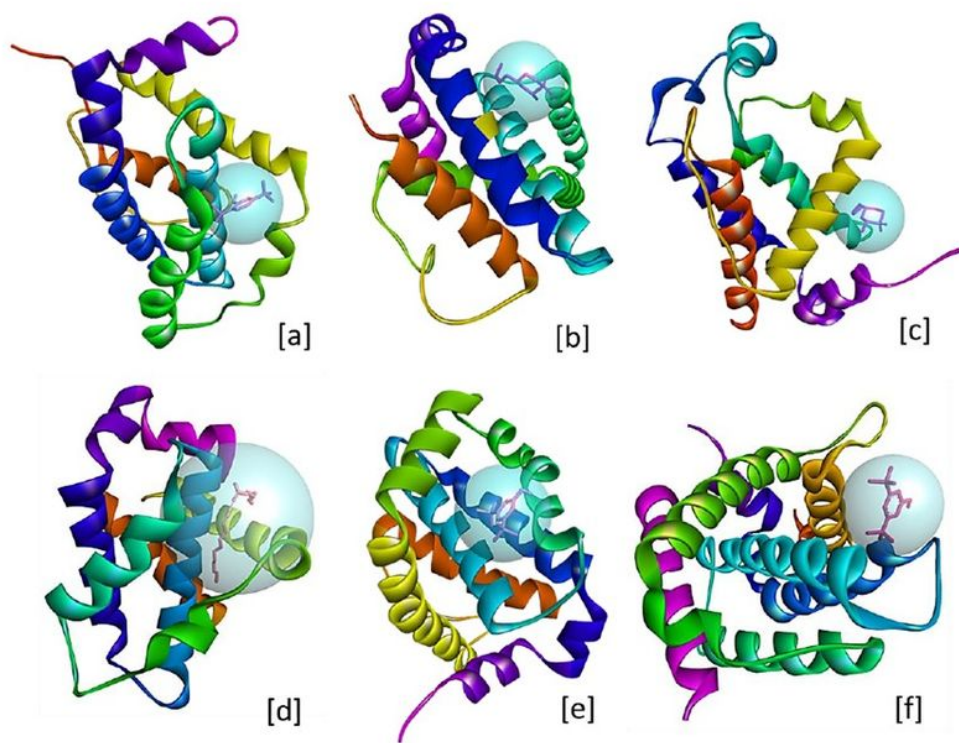


Figure 5

Heat map of ADMET properties of screened phytocompounds of *Persicaria strigosa*

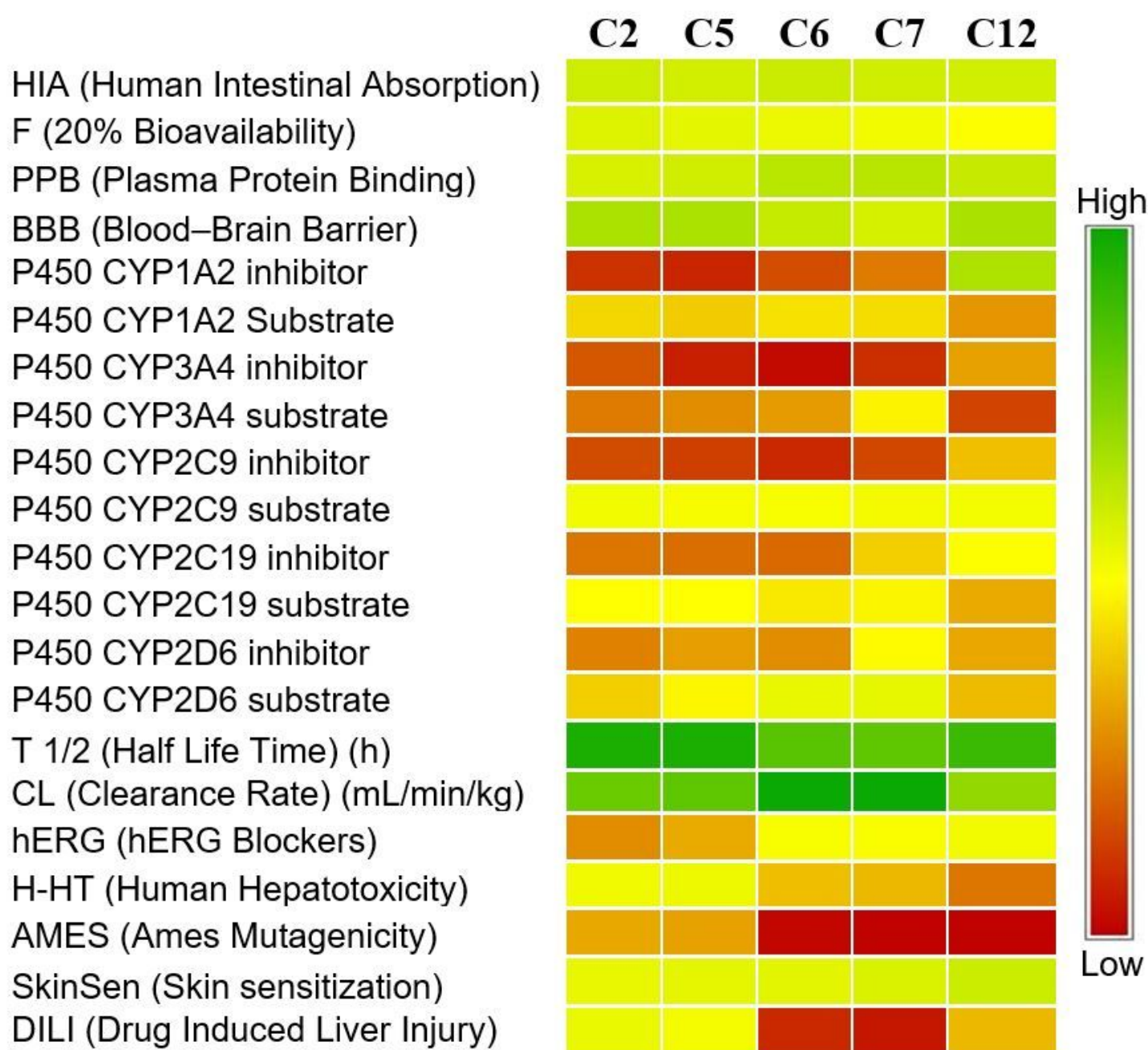


Figure 6

3D structure of ligand binding site and sphere view of anti-apoptotic proteins. (a) BCL-2 and **C17**, (b) BCL-W and **C8**, (c) BCL-B and **C17**, (d) BCL-X_L and **C15**, (e) MCL-1 and **C17**, and (f) BCL-A1/Bfl-1 and **C7**

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