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. This study investigated the phytochemical contents, antiproliferative, and apoptosis-inducing properties of *P. strigosa*. The phytocompounds were identified by the Gas Chromatography Mass Spectrometry (GC-MS) technique. Antiproliferative and apoptosis-inducing properties were conducted in Dalton's lymphoma cells. *In-silico* molecular docking, drug-likeness, and absorption, distribution, metabolism, excretion, and toxicity (ADMET) were carried out to study the binding affinity and drug-likeness of the compounds. The study revealed a dose-dependent antiproliferative activity of the plant. GC-MS study identified 12 compounds from the ethyl acetate extract. Phytompounds **C2**, **C5**, **C6**, **C7**, and **C12** showed the best binding affinity with the anti-apoptotic proteins. The phytocompounds were predicted to possess drug-likeness properties and a good ADMET profile. The findings suggest that *P. strigosa* could be a potential source of anticancer agents.

INTRODUCTION

Cancer is a terrible disease that can start in any body organ from an abnormal genome function leading to the proliferation of cells at inappropriate times and locations. Cells acquire mutations that abolish the regulation of cell division and multiply continuously, forming tumors and invading new body parts (Weinberg, 2015). According to WHO, about 18.1 million cancer patients worldwide and about 10 million deaths in 2020 are due to cancer. Today, one of the six deaths is due to cancer, the second-largest killer in the world (https://www.who.int/news-room/fact-sheets/detail/cancer). Despite significant improvements 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 significant challenges to successful cancer treatment. 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, searching 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 phytocompounds, such as paclitaxel, etoposide, camptothecin, vinblastine, vincristine, uvaribonin, 22-epicalamistrin, etc. are plant-derived natural products with considerable anticancer activity (Cragg *et al.*, 2009; Pettit *et al.*, 2008). *Persicaria strigosa* (R.Br.) Nakai belonging to the family Polygonaceae is a creeping herb that grows well in marshy places. Stems are greenish-brown

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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 bioactivities of *P. strigosa*. From a survey report, Deka and Devi (2015) opined that the leaves of *P. strigosa* can be 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, 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, this study explores the phytochemicals, antioxidants, and antiproliferative properties of *P. strigosa*.

MATERIALS AND METHODS

Identification of plant and preparation of solvent extracts

The sample plant was collected from the Tinali area of Kokrajhar town with the help of local people. The plant specimen was submitted to the Department of Botany, Bodoland University, and identified as *P. strigosa* Nakai. (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* methanolic extract (PSME) was kept at −20°C for further use. The PSME was processed for liquid–liquid solvent fractionation, and three solvent fractions were prepared in n-hexane (PSHE), diethyl ether (PSDE), and ethyl acetate Persicaria strigosa ethyl acetate (PSEE). The solid material obtained after solvent drying was kept at −20°C for further study.

Cytotoxicity study

Cell line and dose preparation

The antiproliferative activity of different solvent extracts was studied for 24, 48, and 72 hours 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 CO_2 incubator at 37°C with 5% CO_2; 80% confluent of exponentially growing cells were sub-cultured and used in the experiments. The different dosage (25, 50, 100, and 200 mg/ml) of plant extracts was freshly prepared in phosphate-buffered saline (PBS).

Cell proliferation and apoptosis assay

After the cells were treated with different concentrations of solvent extracts, cell proliferation was measured by 3-(4,5-Dimethylthiazol-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, 48, and 72 hours, 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 hours under 5% CO_2 and 95% air at 37°C after adding 100 µl of Dimethyl sulfoxide (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 minutes in a dark/cold room. The cells were observed under Fluorescence Microscope and photographed

(Medlab Lx400 FLR Fluorescence Microscope). About 1,000 cells were counted, and the percentages of apoptotic cells were determined based on differential coloring patterns (red/green) of the nucleus.

GC-MS analysis of phytocompounds

The phytocompounds present in the best bioactive fraction, PSEE were further investigated by GC-MS system (Perkin Elmer (USA), make GC-MS instrument, Model: Clarus 680 GC & amp; 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 of 60 m, ID- 0.25 mm, and film thickness of 0.25 µm, and the stationary phase was 5% diphenyl 95% dimethylpolysiloxane. Helium (99.99%) was used as carrier gas at a flow rate of 1 ml/minute. An injection volume of 2 µl 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 minute) with an increasing rate of 7°C/minute up to 200°C (hold for 3 minutes), and then again increased at the rate of 10°C/minute up to 300°C (hold for 5 minutes). The total run time was ~39 minutes. The solvent delay was kept for 8 minutes. MS Protocol Mass Spectra were taken in Electron Impact positive (EI+) mode at 70 eV. A solvent delay of 8 minutes was there for MS scan. Mass range, i.e., m/z range is 50–600 amu.

Identification of peaks

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

Molecular docking

To further elucidate the antiproliferative activity of the plant, molecular docking was carried out with phytocompounds of *P. strigosa* with six 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 performed in AutoDock vina (Trott and Olson, 2010). The grid parameters were set as *x*, *y*, and *z* size-coordinate and grid box center-coordinates: 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.

Analysis of drug-likeness and absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile

The phytocompounds of *P. strigosa* were studied for their drug-likeness properties using SwissADME (Daina *et al.* 2017) and PubChem database. The drug-likeness property of compounds was evaluated based on Lipinski's rule (Lipinski, 2004). Similarly, *in-silico* ADMET properties of identified compounds were studied using the ADMETlab server (Dong *et al.*, 2018).

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 ± SD, $n = 3$ at $p \leq 0.05$ probability level.

RESULTS

Antiproliferative and apoptotic-inducing study

The 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. PSEE showed the most potent cytotoxicity, while PSHE showed the weakest activity. At 25−200 mg/ml, the percentage (%) mortality of cells ranged from 9 ± 19% to 38 ± 4%, 15 ± 2% to 46 ± 4%, and 21 ± 2% to 59 ± 6% at 24, 48, and 72 hours treatment, respectively. The mortality of cells at 72 hours treatment was found to be highest in PSEE, followed by PSDE, PSME, and PSHE. The LD_{50} of plant extracts for cell death at 72 hours treatment was 74.99, 140.93, 308.06, and 396.87 mg/ml, respectively, for PSEE, PSDE, PSME, and PSHE. Statistical analysis showed a significant difference ($p \leq 0.05$) in the mortality of cells at high treatment doses of all the solvent extracts. However, at a lower dose (25 mg/ml), PSDE and PSEE showed no significant difference. Similarly, PSHE and PSME did not show significant differences in terms of mortality of the cells. 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 hours of treatment. Of the four plant extracts, PSEE 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.

GC-MS study

The presence of volatile phytocompounds present in PSEE 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 **[C3]** 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 **[C2]**.

Molecular docking study

Table 2 shows the binding affinities of six anti-apoptotic proteins with the phytocompounds. 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
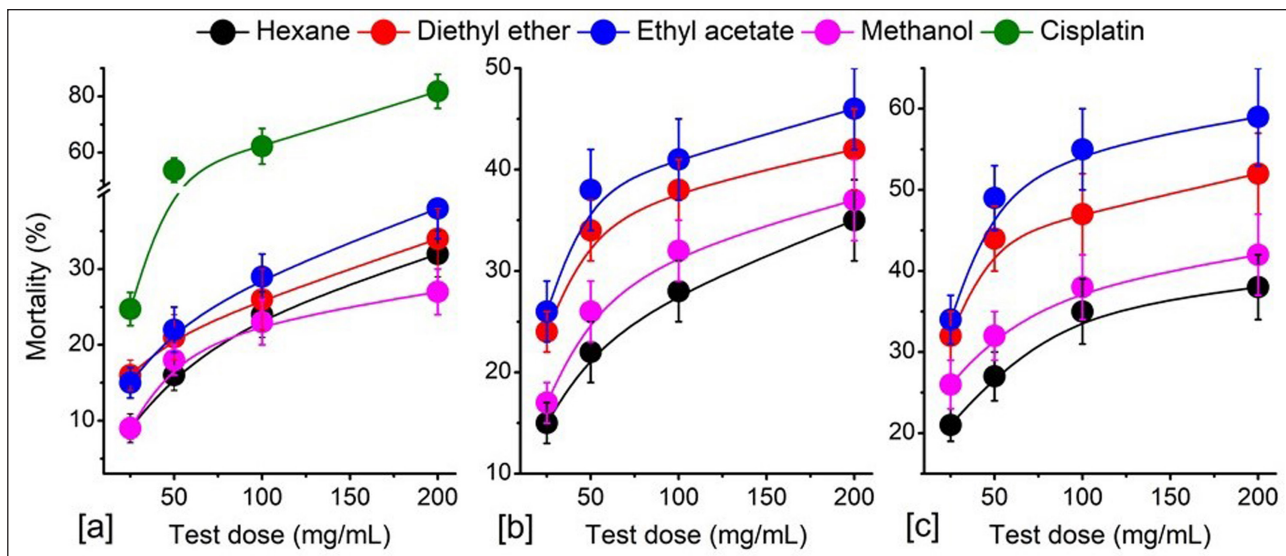

Figure 1. Cytotoxicity study of the solvent fractions of *P. strigosa* against DL cells. (a) 24 hours, (b) 48 hours, and (c) 72 hours treatment. Values are expressed as mean ± SD, $n = 3$.
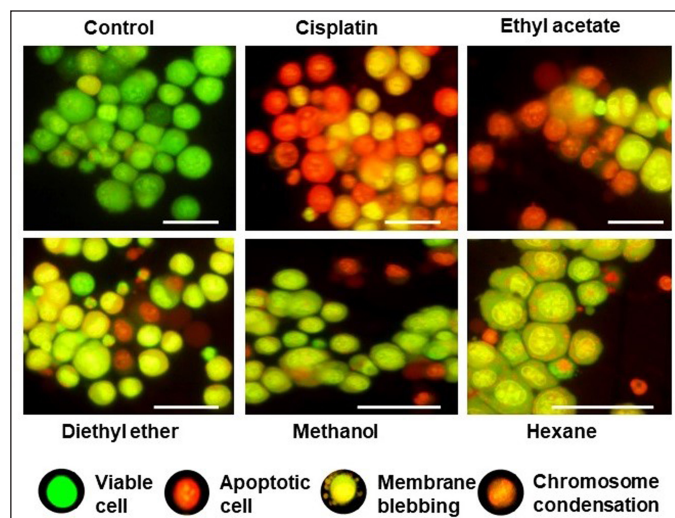
Figure 2. Morphological features of apoptotic and viable cells observed under a fluorescence microscope after 72 hours treatment with different solvent fractions of *P. strigosa*. The ethyl acetate extract shows higher apoptotic cells compared to other plant extracts. Scale bar 10 µm.

BCL-A1/Bfl-1 & **C6** showed one hydrogen bond between proteins and phytocompounds.

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 with <500 Da molecular weight, <5 lipophilicity, <5 H-bond donors, and <10 H-bond acceptors fulfill the drug-likeness property. A compound is found to have drug-likeness properties if the compound does not violate two or more rules. The *in-silico* study revealed that all five compounds possess drug-likeness properties. A major drug-likeness property is the compounds' molecular weight and surface area. All the top five compounds were smaller than 500 Da, and the topological polar surface area (TPSA) values were less than 100 °C². Similarly, the LD_{50} values showed a 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 bioavailability, while the other four compounds have high bioavailability. All the phytocompounds showed high blood-brain barrier properties, indicating less seepage of the compounds from the blood circulation. In addition, all five compounds showed low metabolism profiles suggesting their suitable drug-likeness property. There is a very low probability that the phytocompounds would act as substrates or inhibitors to the cytochromes-P450 enzyme superfamily. In addition to other ADMET properties, the compounds also had low toxicity and carcinogenic effect.

DISCUSSION

Growth and development are essential characteristics of life. An organism grows and becomes an adult because of the new cells' continuous multiplication and replacement of the dead cells. All living cells possess a sophisticated and controlled mechanism of cell division. Aged or damaged cells are eliminated from the body by a standard 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 body parts 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 the 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). In this study, the ethyl acetate fraction of *P. strigosa* showed the most potent antiproliferative and apoptosis-inducing activities. The antiproliferative property of the plant may be due to the phytochemicals present in the plant. Cisplatin, the reference chemical, showed a significant difference ($p \leq 0.05$) compared to plant extracts. GC-MS study identified 12 probable phytocompounds from the ethyl acetate extract of *P. strigosa*. Again, molecular docking was carried out with *P. strigosa* phytocompounds and six anti-apoptotic proteins. The study showed a strong binding affinity of **C2**, **C6**, **C7**, and **C12** phytocompounds with the anti-apoptotic proteins.

Several studies have reported the antiproliferative and inhibitory properties of anti-apoptotic proteins of several medicinal plants and phytocompounds (Erdogan *et al.*, 2020; Kamaruddin *et al.*, 2019; Swargiary *et al.*, 2021). Phytocompounds such as betulinic acid (*Betula alba*), dentatin (*Clausena excavata*), etc. were found to downregulate the expression of the anti-apoptotic BCL-2 gene family (Arbab *et al.*, 2012; Rzeski *et al.*, 2006). 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* (Gabrehiwot *et al.*, 2018). Tricosanoic acid, an important fatty acid, has been reported from plants with rich bioactivities, such as *Cuminum cyminum* and *Coffea robusta* (Dong *et al.*, 2015; Shukla *et al.*, 2018). The bioactive compound Trans-2-Methyl-4-n-butylthiane-S,S-dioxide isolated from the ethyl acetate extract from *Streptomyces parvulus* VITJS11 have been reported in treating microbial infections and indicated their broad spectrum of activity with beneficial virtues for therapeutic use (Naine *et al.*, 2014). 3,5-Bis(1,1-dimethylethyl) phenol, a bioactive compound isolated from the leaves of *Ageratum houstonianum* showed strong binding affinity with human polo-like kinase-1 enzyme suggesting the anticancer potential of the compound (Rizvi *et al.*, 2014). Again, another study found that the bioactive compound 3,5-bis (1,1-dimethylethyl) -phenol extracted from *Moringa oleifera* had the potential as a new anthelmintic drug compound (Novian, 2019). Similarly, the presence of hexacosyl acetate and many other phytocompounds 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–protein interactions that can help design therapeutic protein inhibitors. Van der Waals interaction was the major interaction between the phytocompounds and proteins. Similarly, anonaine, a benzylisoquinoline alkaloid isolated from *Annona*
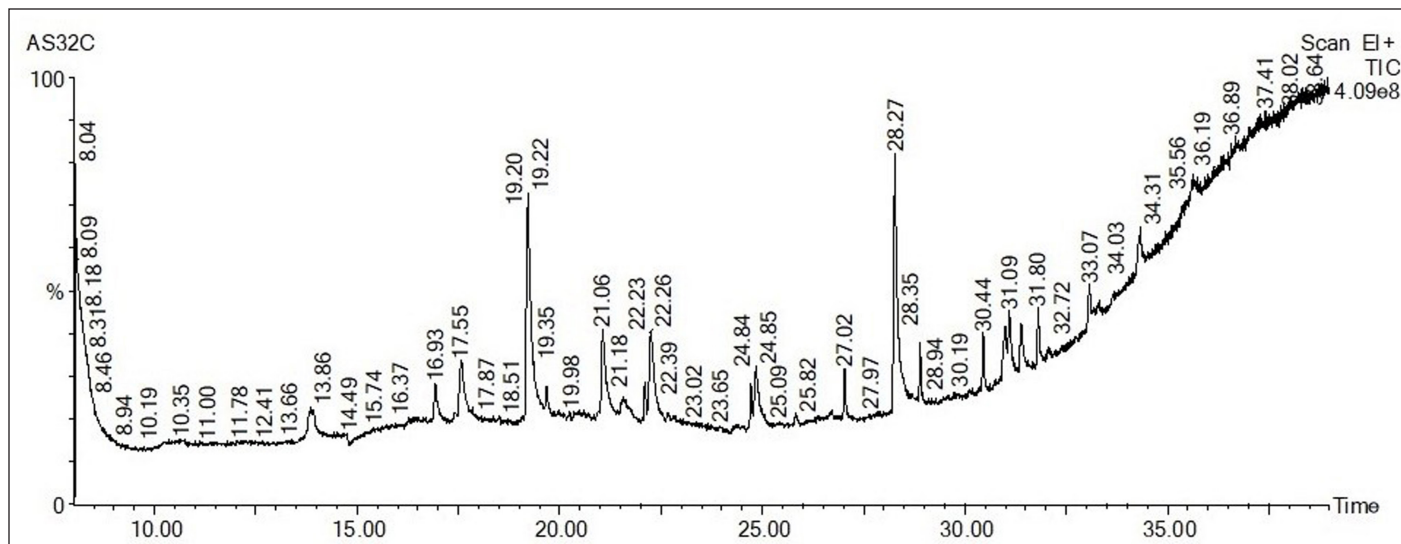
Figure 3. GC-MS chromatogram of ethyl acetate extracts of *P. strigosa* leaves.

Table 1. GC-MS properties of phytocompounds identified from ethyl acetate extract of *P. 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	23,701	2.402	$C_{15}H_{32}O$
2.	Trans-2-Methyl-4-n-butylthiane-S,S-dioxide **[C2]**	17.552	204	543,892	3.057	$C_{10}H_{20}O_2S$
3.	Carbonic acid, tridecyl vinyl ester **[C3]**	19.218	188	9,169,3103	8.200	$C_{16}H_{30}O_3$
4.	Tricosanoic acid, isobutyl ester **[C4]**	19.673	458	91,693,029	0.806	$C_{27}H_{54}O_2$
5.	3N-Hexylthiane-S,S-dioxide **[C5]**	21.064	218	543,815	3.094	$C_{11}H_{22}O_2S$
6.	3,5-Bis(1,1-Dimethylethyl) phenol **[C6]**	22.259	206	70,825	1.382	$C_{14}H_{22}O$
7.	2,5-Bis(1,1-Dimethylethyl) phenol **[C7]**	22.264	206	79,983	2.706	$C_{14}H_{22}O$
8.	Hexacosyl acetate **[C8]**	24.845	424	308,3648	2.122	$C_{28}H_{56}O_2$
9.	Tetradecanoic Acid-10,13-Dimethyl-, Methyl Ester **[C9]**	28.272	270	554,145	6.789	$C_{17}H_{34}O_2$
10.	Methyl-6,10-octadecadienoate **[C10]**	30.442	504	12,255,549	0.752	$C_{19}H_{34}O_2$
11.	1,9-Nonanediol, Dimethanesulfonate **[C11]**	31.008	316	20,244	1.438	$C_{11}H_{24}O_6S_2$
12.	Undec-10-ynoic Acid, Tridec-2-Yn-1-yl Ester **[C12]**	31.388	360	91,697,642	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 phytocompounds from *P. strigose*.

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 *P. strigosa*.

Compounds	MW (<500 Da)	LogP (<5)	HBD (<5)	HBA (<10)	TPSA (Å²)	LD₅₀, mg/kg (acute toxicity)
C2	204.33	2.9	0	2	42.5	1,470.08
C5	218.36	3.4	0	2	42.5	1,816.25
C6	206.32	4.9	1	1	20.2	1,855.92
C7	206.32	4.9	1	1	20.2	1,805.34
C12	360.60	9.1	0	2	26.3	9,099.23

MW – molecular weight, LogP – lipophilicity, HBD – H-bond donor, HBA – H-bond acceptor, TPSA – topological polar surface area.
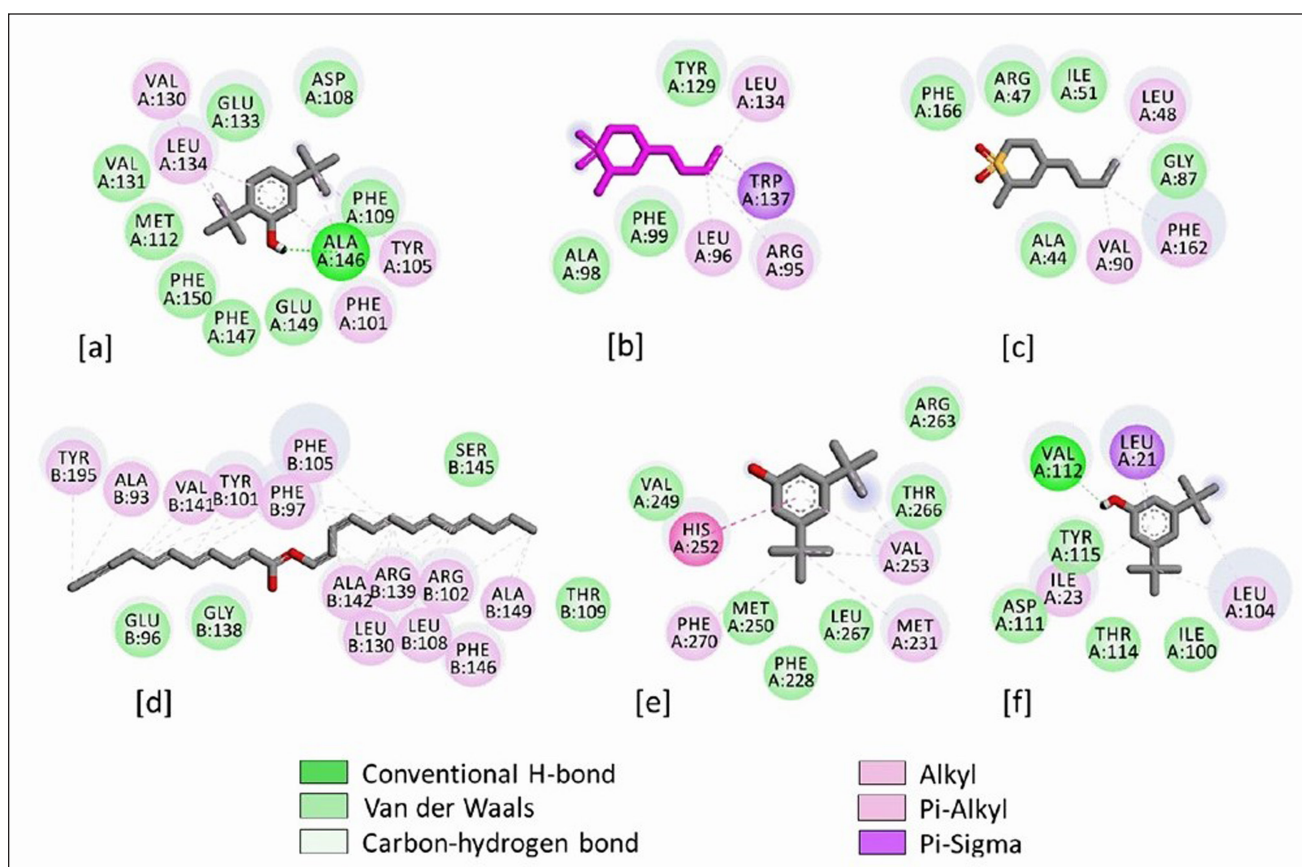
Figure 4. 3D structure of ligand binding site and sphere view of anti-apoptotic proteins. (a) BCL-2 and **C7**, (b) BCL-W and **C2**, (c) BCL-B and **C2**, (d) BCL-XL and **C12**, (e) MCL-1 and **C6**, and (f) BCL-A1/Bfl-1 and **C6**.

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 phytocompounds from *Morinda lucida,* of which two triterpenes (ursolic 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 numerous 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, BCL-2 and BCL-X$_L$ showed the strongest binding

interaction with fisetin (Abd Ghani *et al.*, 2020). In this study, Undec-10-ynoic acid, Tridec-2-yn-1-yl ester showed the most promising inhibitory activity among the phytocompounds. 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 showed potential apoptosis-inducing property.

Studying the drug-likeness and ADMET properties is essential in the present-day drug discovery pipeline. Lipinski's rule predicts that an oral drug is 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 cell membrane permeability, and easy excretion from the body (DiMasi *et al.*, 2003). Such *in-*
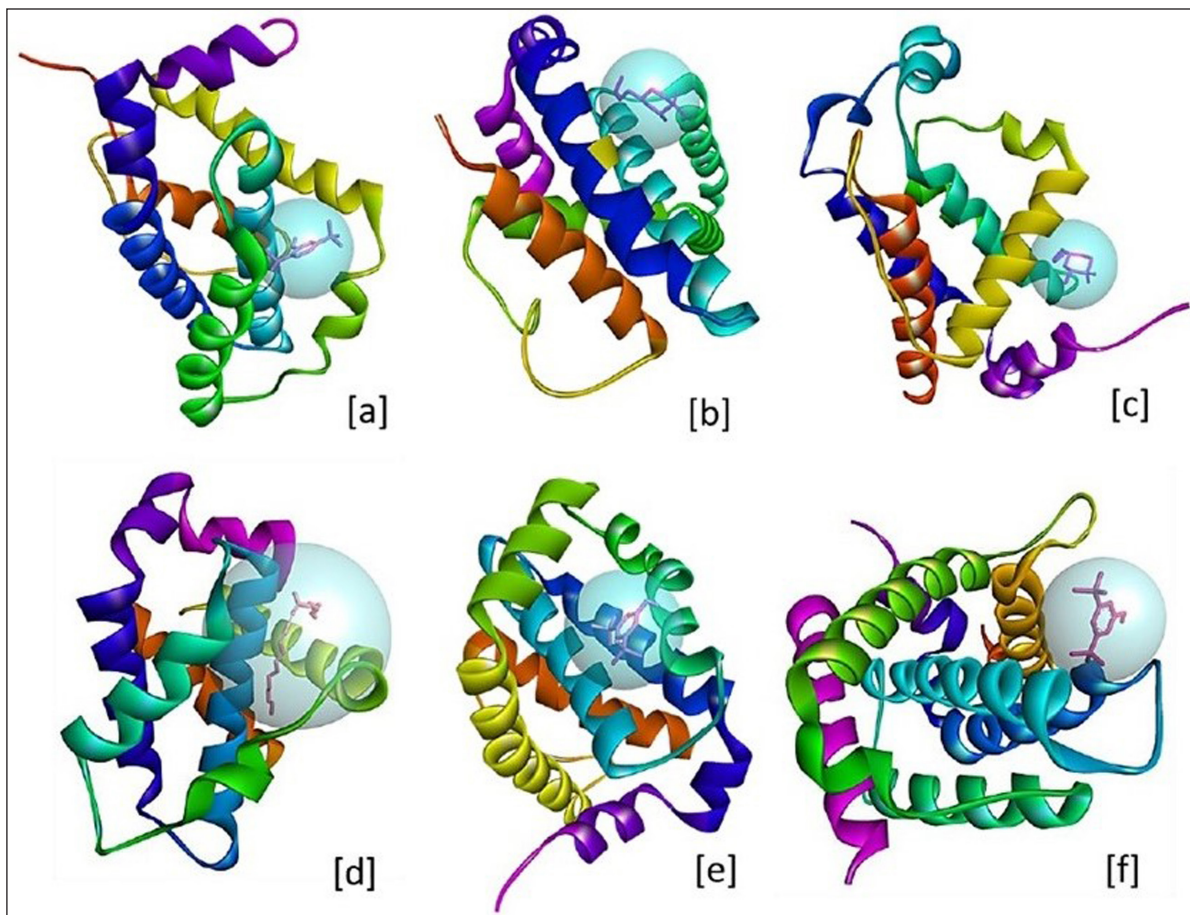
Figure 5. 2D binding interactions of Persicaria strigosa phytocompounds with the anti-apoptotic proteins. (a) BCL-2 and **C7**, (b) BCL-W and **C2**, (c) BCL-B and **C2**, (d) BCL-XL and **C12**, (e) MCL-1 and **C6**, and (f) BCL-A1/Bfl-1 and **C6**.
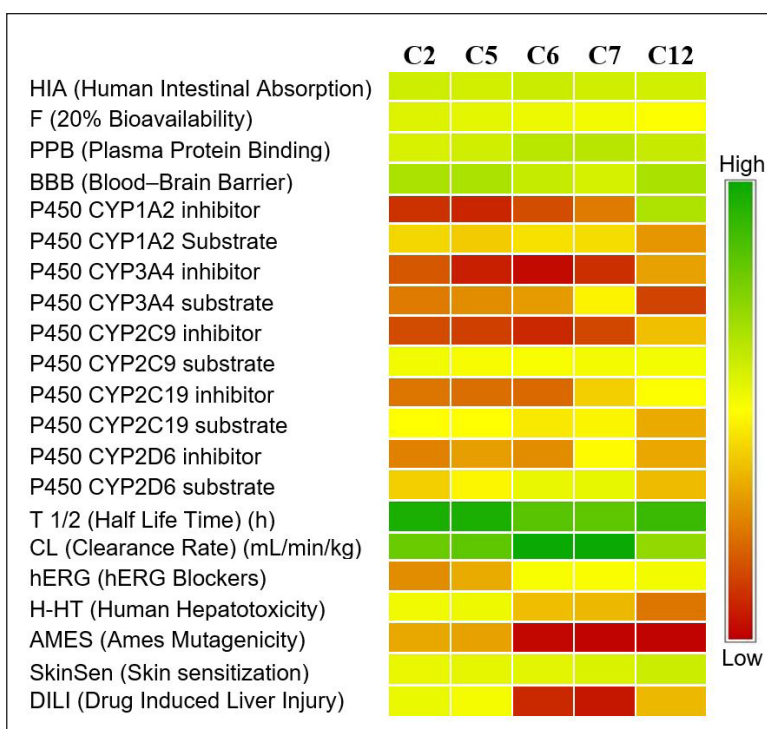

Figure 6. Heat map of ADMET properties of *P. strigosa* phytocompounds (best five).

silico 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 results in significant economic losses (Sertkaya *et al.*, 2016). Undesirable pharmacokinetic 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 helpful guideline for further optimization and drug discovery (Jia *et al.*, 2020). In this study, all five best phytocompounds showed suitable drug-likeness properties with considerable ADMET properties. The high lipophilicity of phytocompounds 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 *P. strigosa* compounds showed low toxicity properties, suggesting the potentiality of a lead therapeutic compound. Compounds having LD_{50} values in the range of 1−50, 51−500, and 501−5,000 mg/kg body weight are considered high, moderate, and low toxicity properties (Lei *et al.*, 2016). The LD_{50} of all five compounds showed more than 600 mg/kg suggesting a low risk of toxicity in the host body.

CONCLUSION

This study investigated the phytochemistry, antipro-liferative, and apoptosis-inducing activity of different solvent extracts of *P. 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 the BCL-2 family gene or inhibition of anti-apoptotic proteins by the phytocompounds. However, further study must be conducted to establish the exact molecular mode of action.

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AUTHOR CONTRIBUTIONS

A.S. designed the study, performed statistical calculations, and wrote the manuscript. A.K.V. conducted the antiproliferative and apoptosis study. M.K.R. is involved in literature collection, manuscript drafting, and docking studies. H.B. was involved in the antioxidant study and statistical calculations. M.D. was involved in data acquisition, and J.K.D. was involved in statistical calculation and manuscript drafting. All the authors read the final manuscript and approved it for publication.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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