Characterising the cardioprotective potential of *Sida rhombifolia*, *Polygonum chinense* and *Phyla nodiflora* aqueous extracts: investigating its effect on foam cell formation

Xiao Wei Lee, Wei Sheng Siew, Siau Hui Mah and Wei Hsum Yap*

Faculty Health and Medical Sciences,
School of Biosciences,
Taylor's University,
No. 1, Jalan Taylors, 47500 Subang Jaya,
Selangor, Malaysia
Email: leon20001205@gmail.com
Email: vin.siew@gmail.com
Email: SiauHui.Mah@taylors.edu.my
Email: WeiHsum.Yap@taylors.edu.my
*Corresponding author

Abstract: Cardiovascular diseases represent one of the leading causes of mortality. Studies have shown that medicinal plants with anti-inflammatory and antioxidant activities are potential cardioprotective agents. This study aimed to determine cardioprotective potential of *Sida rhombifolia*, *Polygonum chinense* and *Phyla nodiflora* in inhibiting macrophage foam cells formation and its regulatory mechanisms. The findings showed that *S. rhombifolia* and *P. nodiflora* have minimal cytotoxicity effect on THP-1 macrophages, however *P. chinense* exhibited cytotoxic effect with an IC50 of 11.83 µg/mL. All plant extracts showed significant inhibition of foam cells formation at 10 µg/mL. PPI network and gene enrichment analysis showed that the three plant extracts may regulate the steroid biosynthesis and arachidonic acid metabolism, and MDM2, PTGS2, SIRT1, PARP1, RELA, GSK3B, CYP17A, and CYP19A1 were predicted as the targets. In conclusion, S. rhombifolia and P. nodiflora showed promising cardioprotective effect, which could be potential therapeutic treatment and prevention towards atherosclerosis.

Keywords: atherosclerosis; foam cell; network pharmacology.

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Biographical notes: Xiao Wei Lee is an accomplished biotechnology graduate from Taylor's University, having completed a BSc in Biotechnology with a commendable CGPA of 3.71. His academic journey was enriched by a diverse range of coursework spanning cell biology, microbiology, biochemistry, genetics, and biotechnology applications. Notably, he undertook an internship at University Malaya, where he honed his research skills in areas such as PCR, ELISA assays, and bacterial studies. Beyond academics, he actively

participated in the Taylor's University School of Biosciences Club and achieved recognition as the Best Poster Presenter at the Intervarsity Biochemistry Seminar 'Skeptiko'.

Wei Sheng Siew is a dedicated doctoral student at Taylor's University, pursuing research in biomedical sciences. With a strong background in biotechnology and biomedical science, he has contributed to several publications, notably on atherosclerosis, macrophage lipid homeostasis, and CRISPR/CAS applications. Actively participating in conferences, he has presented his work both orally and through poster presentations. He has honed his skills in techniques like cell culture, CRISPR, qRT-PCR, and bioinformatics, making him a well-rounded researcher. His passion lies in unravelling intricate biological mechanisms and exploring innovative therapeutic approaches.

Siau Hui Mah is an Associate Professor at the School of Biosciences, Taylor's University, Malaysia. She holds a PhD in Natural Products Chemistry from Universiti Putra Malaysia. Her research interests include natural products chemistry, structure-activity relationship studies, and the design and synthesis of biologically active compounds. She has published extensively in prestigious journals and received numerous awards, including the President's Award for Excellence in Research and Innovation from Taylor's University in 2017. She is actively involved in professional organisations and serves on the committee of the American Chemical Society-Malaysia Chapter.

Wei Hsum Yap is an Associate Professor at the School of Biosciences, Taylor's University, Malaysia. She holds a PhD in Science from Universiti Tunku Abdul Rahman. Her research interests include molecular pharmacology, macrophage foam cells, and skin models. She has published extensively in prestigious journals, including *Frontiers in Pharmacology*, *International Journal of Molecular Sciences*, and *Life Sciences*. He is actively involved in research projects related to natural products, inflammation, and skin diseases. She has supervised numerous graduate students and has presented her work at various national and international conferences.

1 Introduction

Cardiovascular disease (CVD) is a major healthcare challenge globally. It is estimated that 18 million deaths were associated with CVD in 2019, which represent 32% of the global deaths (Rashmi et al., 2014). Atherosclerosis is a chronic inflammatory condition that leads to CVD, and it is characterised by deposition of fatty streak lesions in the arterial intima layer. The underlying pathogenesis is primarily resulted from the cellular uptake of modified lipoproteins (LDL) in macrophages, leading to enhanced inflammation and release of free radicals which increased recruitment of monocytes and macrophages to the lesion site (Nikhlesh and Gadiparthi, 2019). Statins represent one of the most effective therapeutics for the prevention and treatment of CVD and atherosclerosis (Taylor et al., 2013). However, it also poses side effects such as muscle pain, sleeping disorders, digestion complications, liver damage, and neurological side effects (Ramkumar et al., 2016).

Natural products derived from plants, fungi, algae have been proved to have abundant sources of phytochemicals which contribute to potential drug leads. There are many

studies reporting that medicinal plants with anti-inflammatory and anti-oxidant activities are potential cardioprotective agents (Adegbola et al., 2017). Recent studies have shown that *Sida rhombifolia*, *Polygonum chinense* and *Phyla nodiflora* possess anti-inflammatory and antioxidant effects (Mah et al., 2017; Wu et al., 2020; Lin et al., 2014). *S. rhombifolia* also known as arrowleaf S., is a perennial plant derived from the Malvacaea family that is widely distributed around the eastern and western hemisphere. *S. rhombifolia* have been scientifically proven to exhibit antibacterial (Masih et al., 2014), antioxidant (Shyur et al., 2005), anti-anxiety (Sundaraganapathy et al., 2013), anti-obesity, cardioprotective effect (Thounaojam et al., 2013), lipid lowering (Patel et al., 2009), anti-inflammatory, anti-cholinergic and cytotoxicity (Mah et al., 2017) properties.

P. chinense also known as creeping smartweed or Chinese knotweed, is a perennial plant derived from the Polyganaceae family. *P. chinense* is found in countries such as, China, Japan, Indian Subcontinent, Indonesia, Malaysia, and Vietnam. *P. chinense* have been shown to exhibit gastroprotective (Ismail et al., 2012), anti-diarrhoea (Xiao et al., 2013), antiproliferation (Chen et al., 2020), wound healing (Wu et al., 2012), anticomplement (Zheng et al., 2018), anti-inflammatory (Hossen et al., 2015), antibacterial, antifungal (Zeng et al., 2022), antioxidant (Seimandi et al., 2021), antibacterial and cytotoxic (Tran et al., 2017) properties. On the other hand, *P. nodiflora* also known as the frog fruit, sawtooth fogfruit or turkey tangle, is an ornamental plant derived from the Verbenaceae family. *P. nodiflora* are widely distributed and it can be found in South America and USA. There are studies reporting that *P. nodiflora* possess anti-melanogenesis (Yen et al., 2012), anti-inflammatory, analgesic, antipyretic (Forestieri et al., 2012), antioxidant (Lin et al., 2014), antinociceptive (Amir et al., 2011), antibacterial (Priya and Ravindhran, 2015), cytotoxic (Ko et al., 2014), hepaprotective and antioxidant (Arumanayagam and Arunmani, 2015) properties.

Bioinformatics has recently been utilised for network pharmacology analysis of natural products for their multi-target mechanisms. This study evaluates the cardioprotective effects of *S. rhombifolia*, *P. chinense* and *P. nodilfora* in terms of inhibiting uptake of oxidised LDL in THP-1 macrophages, and their potential regulatory mechanisms in regulating foam cell formation using bioinformatics analysis. The bioactive phytochemicals of *S. rhombifolia*, *P. chinense* and *P. nodiflora* were screened and putative targets of bioactive phytochemicals were predicted utilising the Swiss target prediction and verified using Uniprot Database. Protein-protein interaction networks of natural products putative targets were established to identify candidate targets using Cytoscape. GO and KEGG enrichment analyses were performed on the targets.

2 Methods

The experimental design for the study was included in the supplementary Figure 1.

2.1 Preparation of natural products extracts

The aqueous extracts of *S. rhombifolia*, *P. chinense* and *P. nodiflora* were prepared as described (Mah et al., 2017). The dried plant sample ground into fine powder and extracted in a Soxhlet apparatus with water for 5 h. The plant extracts were freeze dried after that. The master stock concentration of 10mg/mL was prepared by dissolving 10 mg

of the extracts in 1 mL of DMSO. The same stocks were used for all experiments in this study.

2.2 Cell culture

Human monocytic cell line THP-1 (ATCC TIB-202) was maintained in RPMI-1640 medium supplemented with 10% FBS. THP-1 monocytes (2 x 10^5 cells/mL) are stimulated with 100 ng/mL PMA and incubated for 72 hours in a 5% CO_2 humidified incubator at 37ºC (Starr et al., 2018; Gažová et al., 2020). PMA-differentiated THP-1 macrophages are used for subsequent cell viability and foam cells analysis.

2.3 Resazurin cell viability assessment

Cell viability assessment was analysed using resazurin assay (Phang et al., 2020). Briefly, THP-1 macrophages were treated with 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL of natural products extracts (*S. rhombifolia*, *P. chinense* and *P. nodiflora*) for 24 hours. After incubation, 20 µL of Resazruin reagent (0.15 mg/mL) were added into each well and further incubated for 3 hours in dark. The percentage cell viability was determined by measuring the fluorescence intensity at 550 nm excitation/ 599 nm emission wavelength using BMG FLUOstar OPTIMA Microplate Reader (BMG Labtech, Germany). The cell viability was calculated by normalising the fluorescence values to non-treated cell samples (100% cell viability). The experiments were performed in three independent experiments in triplicates.

2.4 Copper sulphate ($CuSO_4$)-induced LDL modification

Low-density lipoproteins (LDL) were extracted from blood plasma by gradient density ultracentrifugation as previously reported (Phang et al., 2020). LDL oxidation was performed based on a previous study with minor modifications (Xu et al., 2010). Briefly, LDL was exposed to 50 µM for $CuSO_4$ 24 hours at 37ºC to generate oxidised LDL (oxLDL). Native LDL (nLDL) that was incubated PBS alone at 37ºC was also prepared as a control. The extent of oxidation was determined by thiobarbituric acid-reactive substances (TBARS) assay.

2.5 Oil Red O staining

Differentiated THP-1 macrophages were either non-treated, treated in the presence of nLDL oxLDL, or oxLDL in the presence of 10 µg/mL of natural products extract (*S. rhombifolia*, *P. chinense* and *P. nodiflora*) for 24 hours, and cells were stained with Oil Red O (ORO) as described (Phang et al., 2020). Briefly, the medium in the sample wells was removed and gently washed with 1× PBS twice. Next, 10% formalin solution (neutral buffered, Sigma-Aldrich) was added to each well and incubated for 30 minutes, prior to washing with 1× PBS twice. After washing, sample wells were rinsed with 60% isopropanol for 15 seconds, followed by staining with filtered ORO working solution for 30 minutes, protected from light. After incubation, the ORO working solution was removed and the sample wells were de-stained with 60% isopropanol for 15 seconds. The residue of ORO solution was removed, and sample wells were washed with deionised water twice. Lastly, the sample wells were submerged in deionised water prior to viewing

under inverted microscope (Eclipse T*i*, Nikon). Images were taken using Eclipse-Ti inverted microscope and ImageJ software was used for analysis of lipid uptake.

2.6 Potential target prediction

The chemical compounds of *S. rhombifolia*, *P. chinense* and *P. nodiflora* were identified using PubMed database. Swiss target prediction was used to identify the potential targets of the chemical compounds. All targets were standardised and verified using Uniprot Database. *Homo sapiens* was selected as the target organisms.

2.7 GO and KEGG enrichment analysis

The target proteins identified were submitted to R studio for Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis using the Molecular Signatures Database (MSigDB). *Homo sapiens* was selected as the target organism, and the FDR (False Discovery Rate) adjusted *p-value* was used evaluating the enrichment analysis. *P* value < 0.05 is considered significant.

2.8 PPI network analysis

Protein-protein interaction (PPI) network was constructed using STRING database. *Homo sapiens* was selected as target organism and the high confidence (0.700) was the criterion for evaluation of interaction score. PPI network was imported to Cytoscape (version 3.9.0) for PPI analysis. MCODE – Cytoscape plugin app was used to identify clusters (highly interconnected regions) in the PPI network. Cluster analysis was performed using default settings where network scoring (degree cutoff = 2), and cluster finding (node score cutoff = 0.2; K-Core = 2; Max. Depth = 100) were applied. The identified cluster with a high MCODE score (score > 10) along with its clustered proteins were identified.

2.9 Statistical network

All experimental data were expressed as mean ± SD. All experiments were performed independently with three replicates each. Statistical analysis was performed by SPSS (version 25) using one-way analysis of variance (ANOVA) to assess significant differences between groups. For all tests, $p > 0.05$ is considered significant.

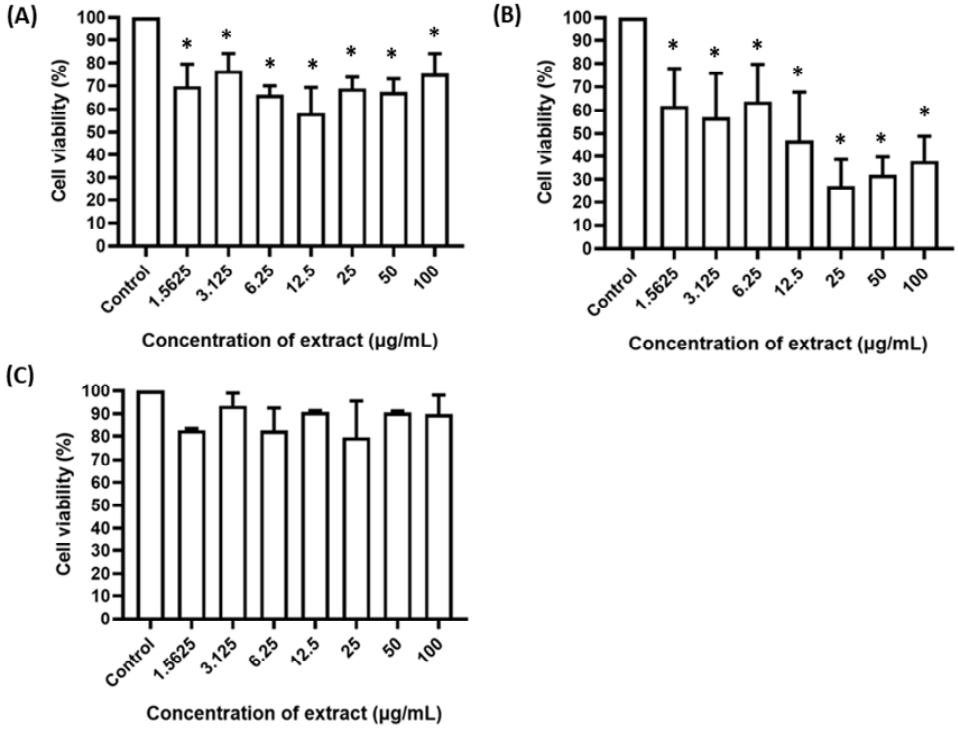
3 Results

3.1 Cytotoxic effects of Sida rhombifolia, Polygonum chinense and Phyla nodiflora in THP-1 macrophages

The cytotoxicity of the three plant natural products extracts was assessed in THP-1 macrophages using Resazurin assay. *S. rhombifolia* extracts showed moderate cytotoxic effects with overall inhibition at 60%–70% cell viability across the concentrations tested (Figure 1). *P. chinense* water extracts on the other hand exerted higher toxicity effects

with 30%–40% cell viability (p<0.05) at 25 µg/mL. Meanwhile, the aqueous extracts of *P. nodiflora* exhibit no toxicity effects in THP-1 macrophages.

Figure 1 *In vitro* cytotoxic activity of (a) *Sida rhombifolia* (b) *Polygonum chinense* (c) *Phyla nodiflora* aqueous extracts at concentrations ranging from 1.563 to 100 µg/mL

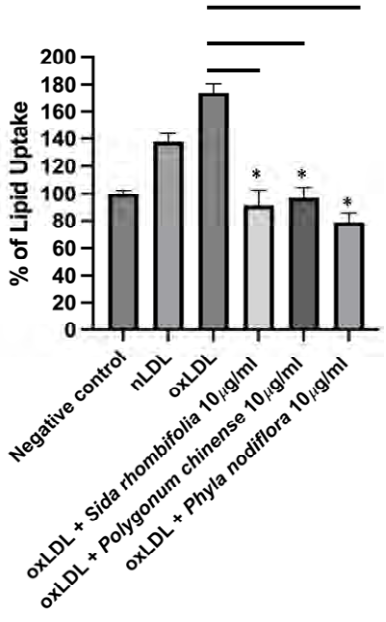


Note: The experiment was performed in triplicates of three independent repeats and the values obtained represent mean ± SD. One-way ANOVA was used to determine the statistical significance of the results. Tukey's Post-hoc test was performed to compare the means to the control group. *Represent $p < 0.05$ when compared to Control (untreated cells).

3.2 Sida rhombifolia, Polygonum chinense and Phyla nodiflora inhibit foam cell formation

Considering that the three plant extracts have minimal or moderate cytotoxicity on THP-1 macrophages at 10 µg/mL, the plant extracts were tested for their effects on macrophage foam cells formation. The results showed that all three plant extracts showed significant reduction of intracellular lipids accumulation in THP-1 macrophages using ORO straining (p<0.05) (Figure 2).

Figure 2 The effects of *Sida rhombifolia*, *Polygonum chinense* and *Phyla nodiflora* on THP-1 macrophage foam cell formation

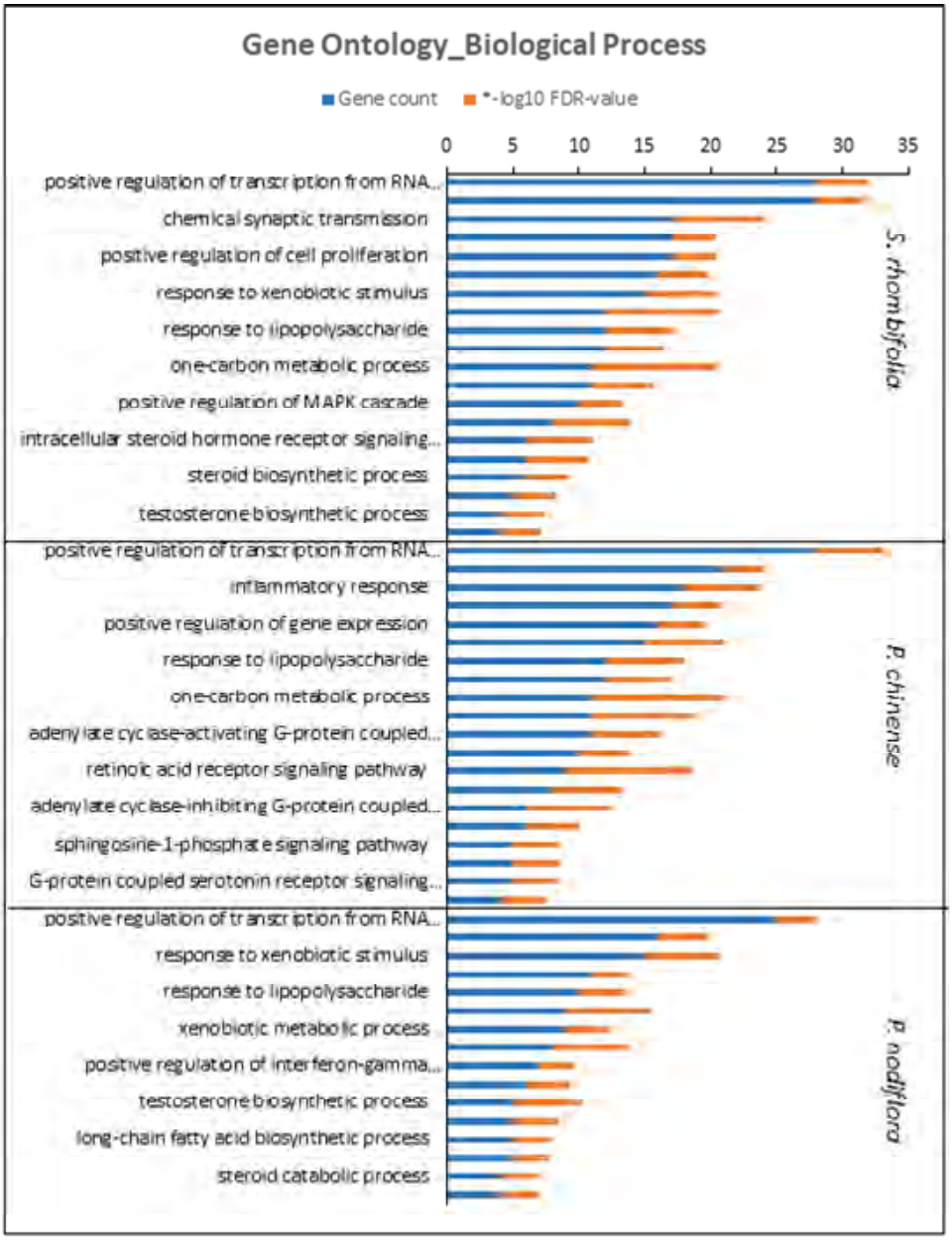


Notes: THP-1 macrophages were either non-treated (negative control), incubated with only nLDL, or oxLDL, as well as incubated with oxLDL in the presence of 10 μg/mL of *S. rhombifolia, P. chinense* and *P. nodiflora*. THP-1 macrophages are stained with ORO and lipids uptake were quantified by ImageJ analysis. Triplicates of three independent tests were performed for this experiment, the values are tabulated in (mean ± SD). One way ANOVA was performed to determine the statistical significance of the results. Subsequently to negative control. *Represent $p<0.05$ compared to oxLDL.

3.3 Bioinformatics analysis on the molecular interactions of Sida rhombifolia, Polygonum chinense and Phyla nodiflora

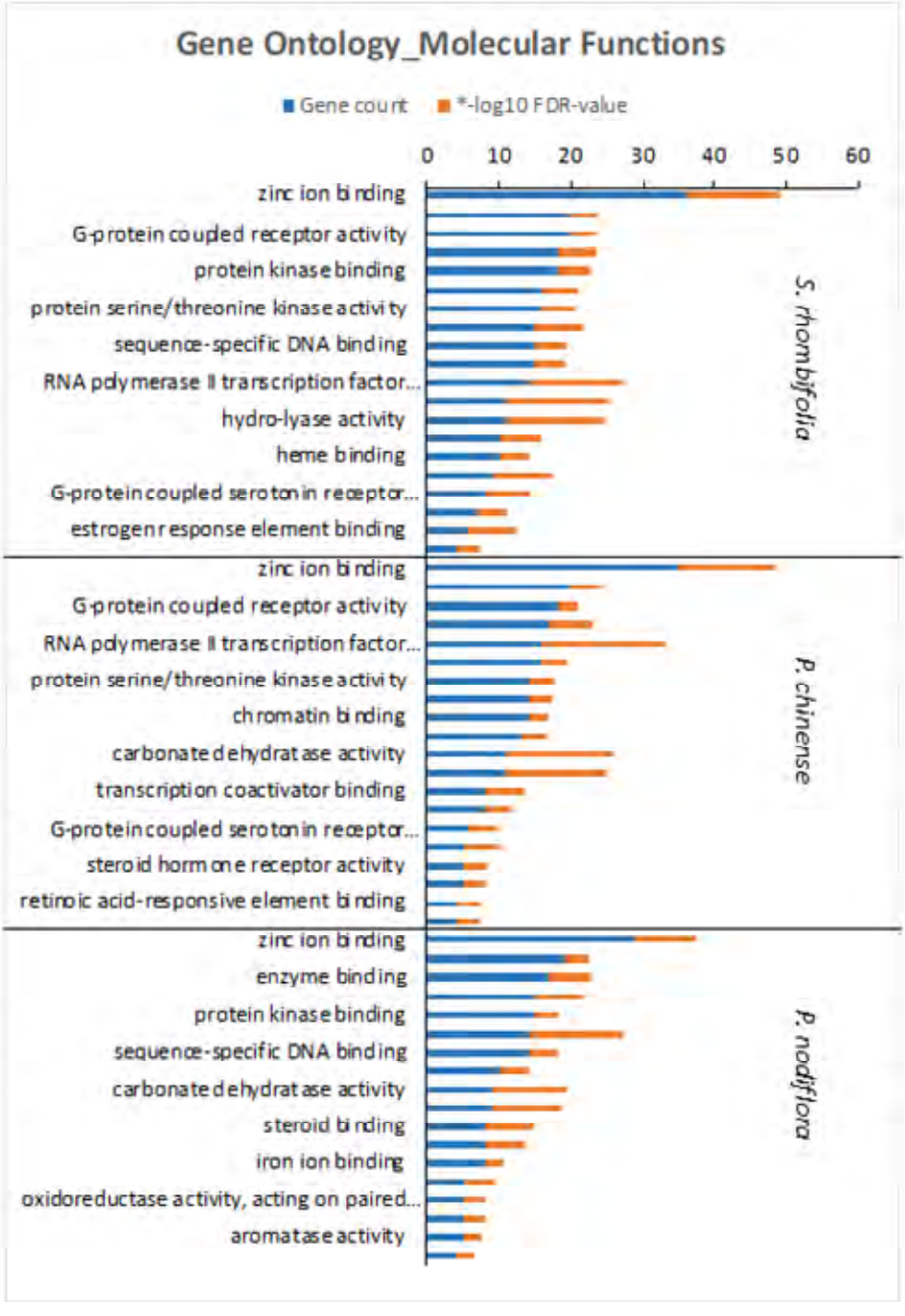
The phytochemical compounds of *S. rhombifolia*, *P. chinense* and *P. nodiflora* were identified from PubMed Database. The natural compounds found in *S. rhombifolia*, *P. chinense* and *P. nodiflora* are submitted to TargetNet (http://targetnet.scbdd.com/). A total hit of 199 protein targets (probability > 80%) which could interact with *S. rhombifolia* compounds were identified. Meanwhile, *P. chinense* showed 179 protein targets, and *P. nodiflora* had a total hit of 186 protein targets. Next, the identified protein targets from all three species were subjected to GO and KEGG enrichment analysis using DAVID tool (https://david.ncifcrf.gov/home.jsp). The results were exported for further analysis. Data that showed FDR-value > 0.05 were considered significant. Common GO terms which include biological processes and molecular functions were presented in Figure 3 and 4, whereas enriched pathways from KEGG database was listed in Figure 5.

Figure 3 GO analysis (biological processes) of *S. rhombifolia*, *P. chinense* and *P. nodiflora* targets (see online version for colours)



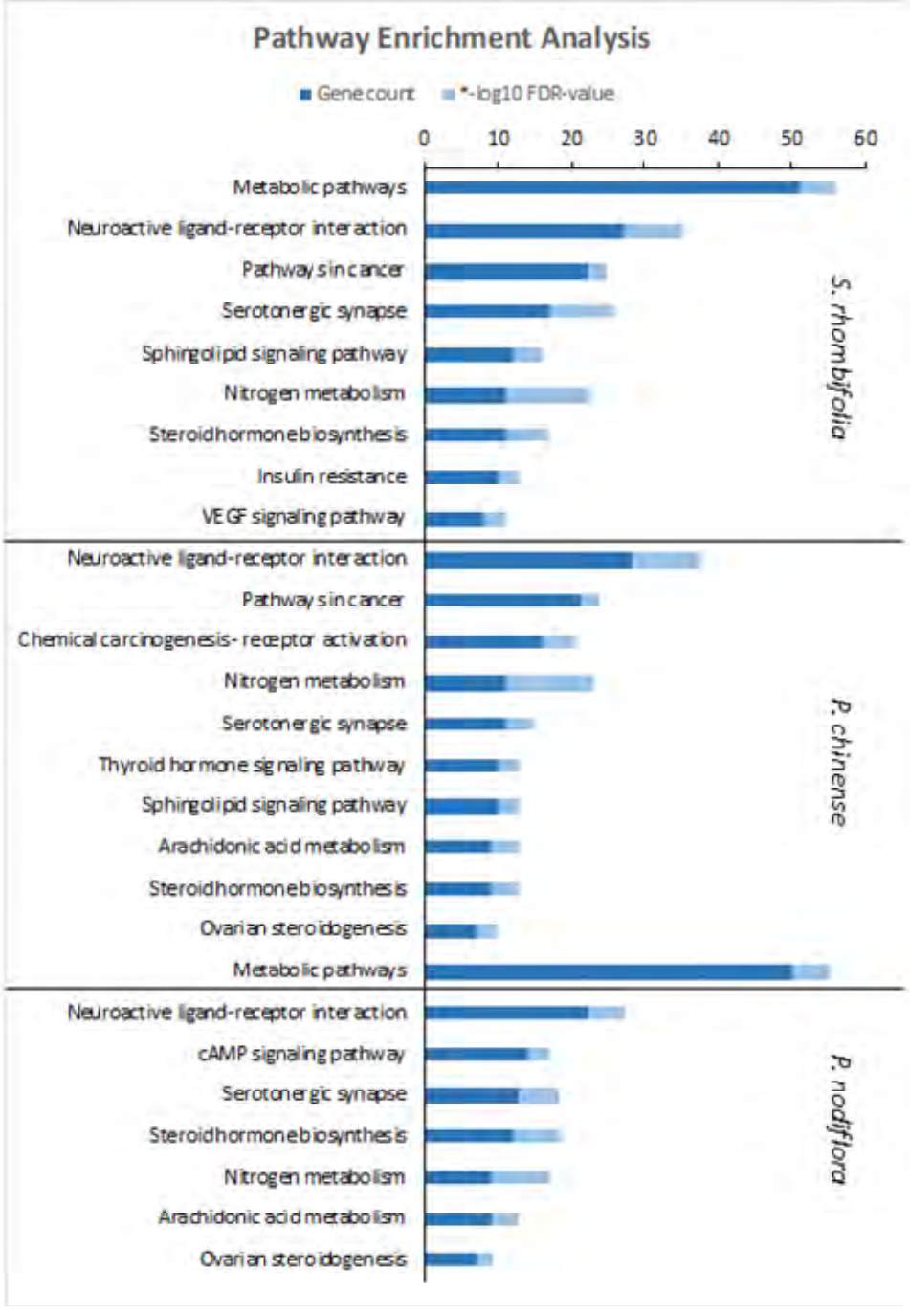
Note: GO biological process were identified for the query targets and their corresponding interactors using Cytoscape software.

Figure 4 GO analysis (molecular functions) of *S. rhombifolia*, *P. chinense* and *P. nodiflora* targets (see online version for colours)



Note: GO molecular functions were performed for the query targets and their corresponding interactors using Cytoscape software.

Figure 5 KEGG pathway enrichment analysis of *S. rhombifolia*, *P. chinense* and *P. nodiflora* targets (see online version for colours)



Note: The graph shows the common pathways enriched from all three different species, which are identified through DAVID database.

Table 1 Hub gene analysis for the clustered proteins from the module of *S. rhombifolia*, *P. chinense* and *P. nodiflora* along with the results of centrality analysis

	Name	*Degree*	*Betweenness*	*Closeness*	*Eccentricity*	*Neighbourhood connectivity*	*Radiality*	*Stress*
S. rhombifolia	ESR1	18	0.256196742	0.90909091	2	9.88888889	0.99444444	330
	AR	15	0.146146617	0.8	2	10.4666667	0.98611111	222
	PARP1	13	0.04696533	0.74074074	2	11.0769231	0.98055556	88
	GSK3B	13	0.015728906	0.68965517	3	11.0769231	0.975	36
	SIRT1	13	0.015728906	0.68965517	3	11.0769231	0.975	36
	MDM2	13	0.015728906	0.68965517	3	11.0769231	0.975	36
	TERT	10	0.00199457	0.625	3	12.3	0.96666667	6
	RELA	10	0.005263158	0.625	3	12.1	0.96666667	12
	DNMT1	10	0.00199457	0.625	3	12.3	0.96666667	6
	HDAC4	9	0.000584795	0.60606061	3	12.6666667	0.96388889	2
	HNF4A	9	0.003508772	0.60606061	3	12.1111111	0.96388889	8
	CYP19A1	9	0.065263158	0.64516129	2	9.55555556	0.96944444	140
	CYP17A1	8	0.033216374	0.625	2	9.25	0.96666667	88
	HSD17B1	8	0.033216374	0.625	2	9.25	0.96666667	88
	CASP9	8	0.00075188	0.58823529	3	12	0.96111111	2
	HSD17B3	8	0.033216374	0.625	2	9.25	0.96666667	88
	MCL1	8	0.00075188	0.58823529	3	12	0.96111111	2
	ABCB1	8	0.002690058	0.58823529	3	11.875	0.96111111	6
	STS	6	0	0.58823529	2	11	0.96111111	0
	HSD11B2	5	0	0.43478261	3	7.6	0.92777778	0
	HSD11B1	5	0	0.43478261	3	7.6	0.92777778	0

Table 1 Hub gene analysis for the clustered proteins from the module of *S. rhombifolia*, *P. chinense* and *P. nodiflora* along with the results of centrality analysis (continued)

	Name	*Degree*	*Betweenness*	*Closeness*	*Eccentricity*	*Neighbourhood connectivity*	*Radiality*	*Stress*
P. chinense	ESR1	23	0.178069515	1	1	11.7826087	1	258
	SIRT1	18	0.030968066	0.82142857	2	13.2777778	0.9905482	102
	HSP90AA1	18	0.030968066	0.82142857	2	13.2777778	0.9905482	102
	GSK3B	18	0.030968066	0.82142857	2	13.2777778	0.9905482	102
	AR	18	0.084015622	0.82142857	2	12.6111111	0.9905482	134
	PARP1	15	0.021602673	0.74193548	2	14	0.98487713	60
	PTGS2	15	0.02497961	0.74193548	2	13.8	0.98487713	68
	ESR2	15	0.055884936	0.74193548	2	12.9333333	0.98487713	90
	RELA	14	0.0098328	0.71875	2	14.7857143	0.98298677	38
	CASP9	13	0.008836815	0.6969697	2	14.1538462	0.98109641	32
	CDK1	12	0.005991593	0.67647059	2	14.9166667	0.97920605	22
	DNMT1	12	0.00517912	0.67647059	2	15.0833333	0.97920605	20
	TERT	11	0.002501725	0.65714286	2	15.1818182	0.97731569	10
	MCL1	10	0.001453981	0.63888889	2	15.5	0.97542533	6
	HDAC4	10	0.001937073	0.63888889	2	15.7	0.97542533	8
	APP	9	0.00154809	0.62162162	2	15.4444444	0.97353497	6
	CYP19A1	9	0.009617918	0.62162162	2	12.6666667	0.97353497	18
	HNF4A	9	0.002446829	0.62162162	2	15.2222222	0.97353497	8
	ABCB1	9	0.002584855	0.62162162	2	15.4444444	0.97353497	8
	NOS3	8	0.000494071	0.60526316	2	16.125	0.97164461	2
	STS	7	0	0.58974359	2	12.2857143	0.96975425	0
	HSD17B3	7	0	0.58974359	2	12.2857143	0.96975425	0
	CYP17A1	7	0	0.58974359	2	12.2857143	0.96975425	0
	HSD17B1	7	0	0.58974359	2	12.2857143	0.96975425	0

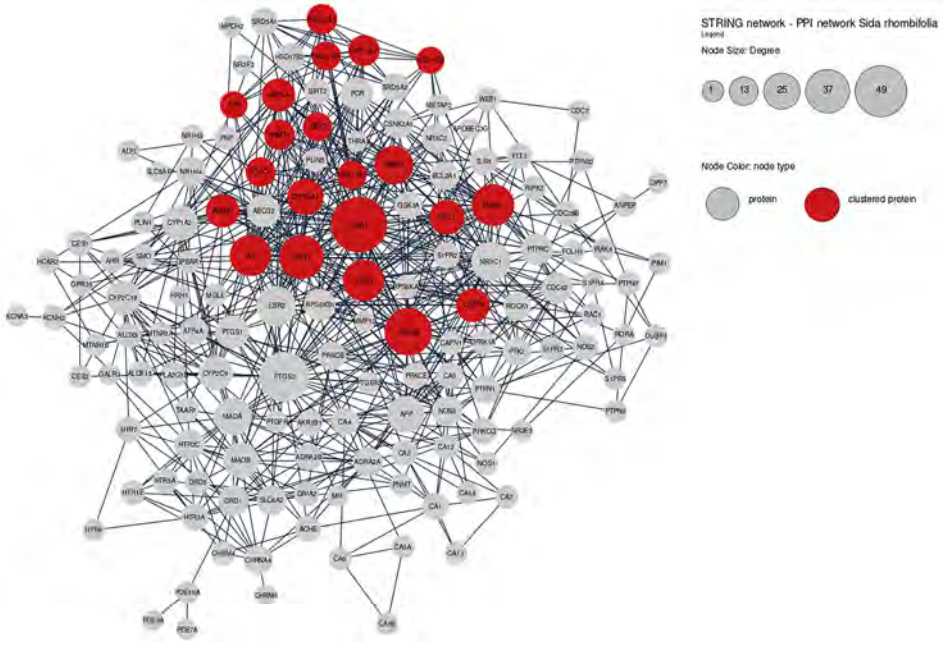
Table 1 Hub gene analysis for the clustered proteins from the module of *S. rhombifolia*, *P. chinense* and *P. nodiflora* along with the results of centrality analysis (continued)

	Name	*Degree*	*Betweenness*	*Closeness*	*Eccentricity*	*Neighbourhood connectivity*	*Radiality*	*Stress*
P. nodiflora	ESR1	14	0.157198969	0.85	2	10.1428571	0.98739496	180
	AR	14	0.163981586	0.85	2	10.1428571	0.98739496	190
	CYP17A1	13	0.023164907	0.80952381	2	11.5384615	0.98319328	64
	HSD17B3	13	0.023164907	0.80952381	2	11.5384615	0.98319328	64
	HSD17B1	13	0.023164907	0.80952381	2	11.5384615	0.98319328	64
	CYP19A1	13	0.023164907	0.80952381	2	11.5384615	0.98319328	64
	SRD5A2	12	0.020153703	0.77272727	2	11.75	0.9789916	58
	AKR1C3	12	0.017956574	0.77272727	2	11.75	0.9789916	52
	SRD5A1	11	0.010815063	0.73913043	2	11.6363636	0.97478992	30
	HSD17B2	11	0.008925654	0.73913043	2	11.8181818	0.97478992	24
	ESR2	10	0.024101307	0.70833333	2	11.9	0.97058824	40
	STS	10	0.003288399	0.70833333	2	12.4	0.97058824	8
	HSD11B1	9	0.000919118	0.5862069	3	11.7777778	0.94957983	2
	HSD11B2	8	0	0.56666667	3	12	0.94537815	0
	DNMT1	6	0.007352941	0.56666667	3	8.83333333	0.94537815	6
	HDAC4	5	0	0.5483871	3	8.8	0.94117647	0
	HNF4A	5	0	0.5483871	3	8.8	0.94117647	0
	TERT	5	0	0.5483871	3	8.8	0.94117647	0

Based on the results in Figure 3, chemical compounds present in all three plants showed that they modulate similar biological processes including inflammatory response, response to lipopolysaccharide, and steroids/fatty acid biosynthetic process. Meanwhile, molecular functions of GO analysis (Figure 4) showed association to enzyme binding, hydro-lyase activity, and steroid binding. Furthermore, *in-silico* KEGG analysis (Figure 5) of chemical compounds found in all 3 different plant species showed regulation of steroid hormone biosynthesis. The cholesterol biogenesis pathway is enriched in *S. rhombifolia* and *P. nodiflora*. The findings highlighted that the compounds identified from the plants might be capable of regulating the biogenesis of cholesterol as well as steroid hormones.

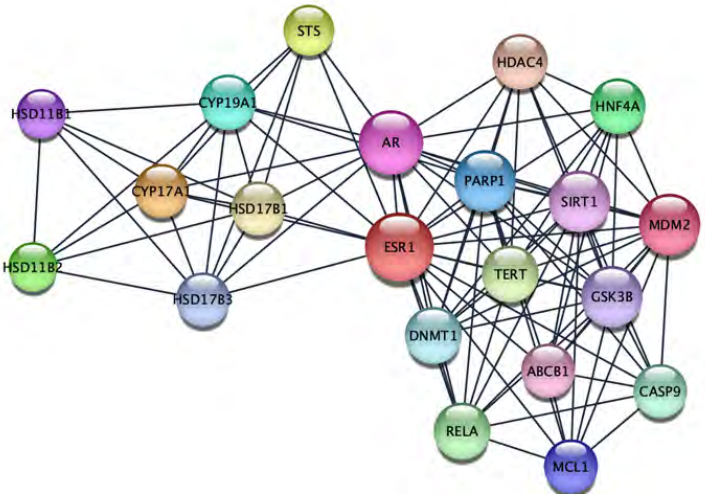
PPI network construction was then performed to identify functional proteins or protein complexes that are associated with the regulation of lipid metabolism. The PPI network of *S. rhombifolia* consists of 145 nodes and 699 edges (Figure 6), while the PPI network of *P. chinense* contains 130 nodes and 625 edges (Figure 8). On the other hand, the *P. nodiflora* PPI network showed 135 nodes and 584 edges (Figure 10). The identified cluster with high MCODE score (> 10), along with its clustered proteins from *S. rhombifolia* (Figure 7), *P. chinense* (Figure 9) and *P. nodiflora* (Figure 11) were tabulated (Table 1). The protein targets were shown to regulate processes associated with lipids biosynthesis, telomerase stability and epigenetics, as well as fatty acids metabolisms.

Figure 6 The overall PPI network of *Sida rhombifolia* generated using STRING database (medium confidence > 0.40) (see online version for colours)



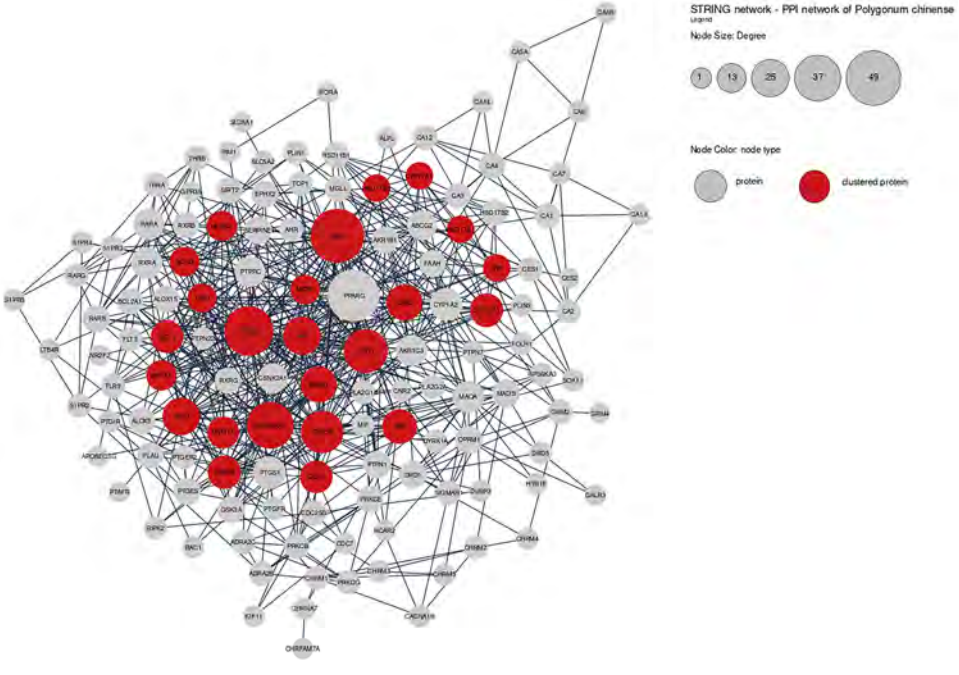
Notes: The PPI network layout was pre-processed using organic layout. Protein clusters within the PPI network were identified using the MCODE plugin of Cytoscape software, and clusters with a high score value (score > 10) were selected for hub genes identification. Clustered proteins for the highest score cluster – module 1 were highlighted in red.

Figure 7 PPI network of the module 1 of *Sida rhombifolia* (see online version for colours)



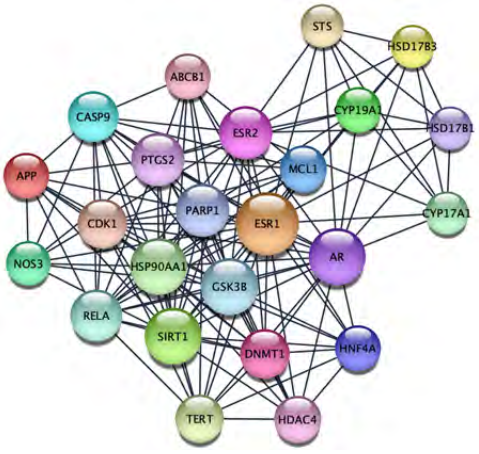
Note: The identified module has a MCODE score of 10.3.

Figure 8 The overall PPI network of Polygonum chinense generated using STRING database (medium confidence > 0.40) (see online version for colours)



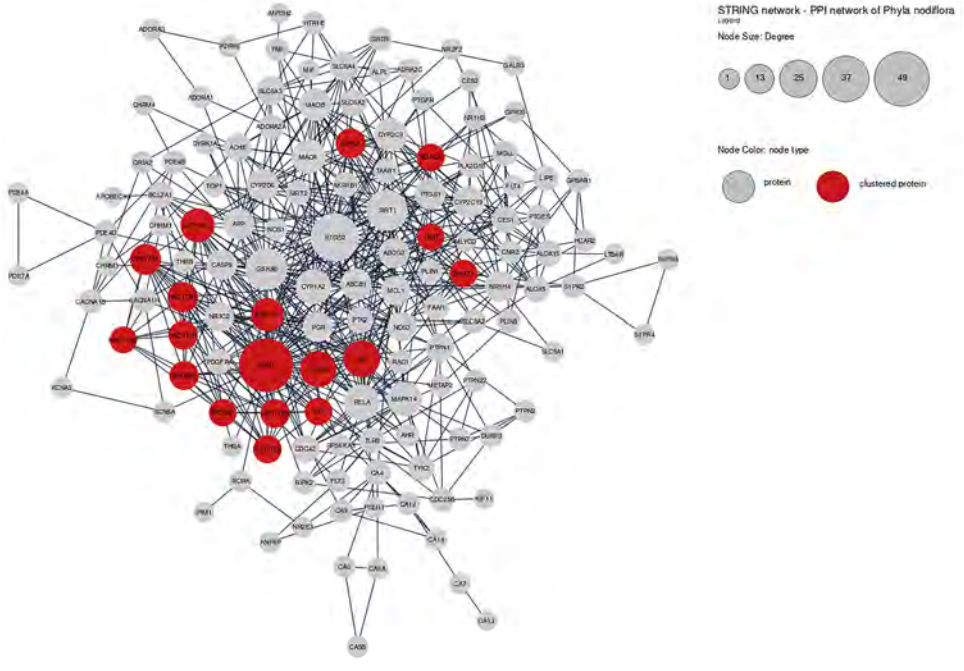
Notes: The PPI network layout was pre-processed using organic layout. Protein clusters within the PPI network were identified using the MCODE plugin of Cytoscape software, and clusters with a high score value (score > 10) were selected for hub genes identification. Clustered proteins for the highest score cluster – module 1 were highlighted in red.

Figure 9 PPI network of the module 1 of *Polygonum chinense* (see online version for colours)



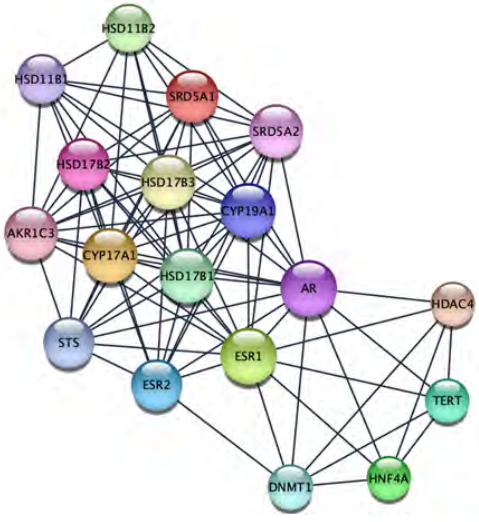
Note: The identified module has a MCODE score of 12.9.

Figure 10 The overall PPI network of Phyla nodiflora generated using STRING database (medium confidence > 0.40) (see online version for colours)



Notes: The PPI network layout was pre-processed using organic layout. Protein clusters within the PPI network were identified using the MCODE plugin of Cytoscape software, and clusters with a high score value (score > 10) were selected for hub genes identification. Clustered proteins for the highest score cluster – module 1 were highlighted in red.

Figure 11 PPI network of the module 1 of *Phyla nodiflora* (see online version for colours)



Note: The identified module has a MCODE score of 10.8.

4 Discussion

The three aqueous plants extracts have shown potential cardioprotective activities in this study. All three plants extracts (10 µg/mL) significantly suppressed uptake of oxLDL in THP-1 macrophages. Both *S. rhombifolia* and *P. nodiflora* showed moderate to low cytotoxicity levels in THP-1 macrophages, while *P. chinense* exhibited significant toxicity with an IC_{50} of 11.83 µg/mL. Network pharmacology analysis of the chemical compounds present in the plants showed that they modulate similar biological processes in metabolic pathways, ligand-receptor interactions, and arachidonic acid metabolism. Specifically, *S. rhombifolia* has been shown to regulate sphingolipids and steroids biosynthesis, while *P. nodiflora* and *P. chinense* modulate the arachidonic acid metabolism pathway. The results suggest that the bioactive phytochemicals within these plant extracts could potentially target key signaling pathways associated with lipids metabolism, which lead to reduced lipids uptake.

Phytochemical analysis of *S. rhombifolia* showed that there are a variety of functional bioactive components including those from the classes of terpenoids, alkaloids, flavonoids, and quinine. *S. rhombifolia* has minimal cytotoxic effects in THP-1 macrophages where the cell viability remained at 70%–80% at all concentrations tested (1.53–100 µg/mL). Other research however indicated that *S. rhombifolia* induced toxic effects in brine shrimp lethality assay (Mah et al., 2017). Meanwhile, there are studies reporting that flavonoids such as kaempferol can inhibit lipids accumulation and promote cholesterol efflux from THP-1 macrophages (Li et al., 2013). Other than that, studies have shown that terpenoids in *S. rhombifolia* such as ursolic acid and oleanolic acid, reduce the reactive oxygen species (ROS) production, and promote anti-inflammation effect towards foam cells (Jakub et al., 2021). The protective potential of *S. rhombifolia* was further supported by network pharmacological analysis in this study where it was shown to regulate the metabolic pathways, ligand receptor interactions, and steroid

biosynthesis. Notably, the PPI analysis demonstrated that *S. rhombifolia* regulates murine double minute 2 (MDM2), an E3 ubiquitin ligase targets various substrates for ubiquitination, thereby regulating their stability, conformation, and localisation (Fåhraeus and Olivares-Illana, 2014). MDM2 is overexpressed in human atherosclerotic lesions (Ihling et al., 1998) and studies also showed that MDM2 mediate ox-LDL-induced inflammation through modulation of mitochondrial damage (Zeng et al., 2020).

S. rhombifolia also modulated multiple targets that control key signaling pathways involved in antioxidative stress including PARP1, SIRT1, RELA and GSK3B. It was shown that SIRT1 inhibition promotes atherosclerosis through impaired autophagy (Yang et al., 2017). Meanwhile, recent studies have also identified the role of GSK3β and PARP in vascular calcification and its impact on atherosclerotic lesions (Cai et al., 2023; Cui et al., 2020). Meanwhile, several targets that are involved in telomerase regulation and epigenetics control, including telomerase reverse transcriptase (TERT), DNA methyltransferase 1 (DMNT1) and Histone deacetylase (HDAC), were identified. Studies have shown that statin therapy and endurance exercise both enhance telomerase activity, and that TERT and telomerase have emerged as a novel target to treat cardiovascular aging and its related inflammatory signalling (Hoffmann et al., 2021). Recent epigenome-wide association study has shown an association between DNA methylation and histone deacetylation with the sizes, lipid compositions, and lipid concentrations (Gomez-Alonso et al., 2021; Burg et al., 2021), inferring a possible role of DMNT1 and HDAC and their involvement of epigenetic mechanisms in the regulation of lipid metabolism pathways.

The PPI network modulated by key compounds in *S. rhombifolia* have shown to regulate the CYP17A1 and CYP19A1 which are involved in the CYP450 pathway. CYP17A1 is involved in the production of steroid hormone synthesis and catalyses the production of dehydroepiandrosterone (DHEA), which act as an essential ligand for PPARα activity (Milona et al., 2019). On the other hand, studies revealed positive correlation between CYP19A1 expression with genes that are involved in fatty acid metabolism (Wang et al., 2022). The anti-inflammatory potential of *S. rhombifolia* was further supported by KEGG analysis where it was shown to regulate the VEGF pathway. VEGF pathway is critical in promoting angiogenesis and formation of new blood vessels which can help to supply oxygen and nutrients to the atherosclerotic plaques (Kieran et al., 2012).

Meanwhile, *P. chinense* exhibited cytotoxic activity against THP-1 macrophages. The cell viability decreased to 30% at all tested concentrations (1.53–100 μg/mL) compared to untreated cells. The phytochemical compounds present in *P. chinense* including terpenoids, flavonoids, quinones, and phenylpropanoids have been linked to regulation of foam cells formation. There are studies reporting that catechins such as procyanidin, epicatechin, and gallocatechin play a role in modulating cell cellular signaling pathways that lead to reduction of inflammation, platelet aggregation and an elevation of vascular reactivity (Velayutham et al., 2009). Meanwhile, quercetin, one of the target phytochemicals found in *P. chinense* had been shown to increase macrophage cholesterol efflux, a process that leads to reduced foam cell formation (Sun et al., 2015). *In-silico* analysis showed regulation of SIRT1, GSK3B, PARP1, and RELA which are linked to cardiovascular inflammation. Interestingly, *P. chinense* also modulate prostaglandin-endoperoxide synthase 2 (PTGS2), a key target that is associated with conversion of arachidonic acid to prostaglandins. There are studies reporting that PTGS2 expression is positively correlated with severity of atherosclerosis (Zhou et al., 2021). The PPI network

is supported by the corresponding regulation of arachidonic acid pathway in the KEGG pathway.

P. nodiflora shows lowest toxicity among all three-plant natural extracts which maintained high cell viability at 80% in all concentrations (1.53–100 µg/mL) tested compared to the untreated cells control. The findings contrasted with the other studies which showed cytotoxicity in lung cancer cells (Vanajothi et al., 2012). The phytochemical analysis of *P. nodiflora* has shown the presence of triterpenoids, flavonoids and hallerone. Notably, *P. nodiflora* contained numerous other flavonoids such as onopordin, cirsiliol, eupafolin, hispidulin, larycitrin, nodifloretin, jaceosidin, nepetin, gonzalitosin I and linalool. It was shown that cornoside found in *P. nodiflora* has anti-inflammatory properties, which can help to reduce inflammation in arteries and prevent the formation of plaque (Jang and Park, 2021). The GO analysis showed that *P. nodiflora* regulates the process in steroid hormones biosynthesis, through target proteins including SRD5A1 and SRD5A2, as well as HSD11B1, HSD11B2, HSD17B3 and HSD17B1, all which are responsible for hormones production and participates in the corticosteroid receptor-mediated anti-inflammatory response, as well as metabolic and homeostatic processes.

5 Conclusions

The current study provides a foundation to support the understanding the potential regulatory effects in foam cells formation and their underlying molecular mechanisms. It is imperative that the three plants extracts should be further tested on atherosclerotic *in vivo* models for in-depth analysis on their cardioprotective effects against foam cells formation and plaque development.

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