



INTEGRATED miRNA–TARGET NETWORK ANALYSIS REVEALS ANTI-ATHEROSCLEROTIC MECHANISMS OF SIKKANCHAR MANAPAGU: AN IN SILICO SYSTEMS PHARMACOLOGY STUDY

Seethaladevi A.^{1*}, Varunapriya M.³, Balamurugan A.³

¹Third Year PG Scholar, Department of Noinadal, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu.

²Third Year PG Scholar, Department of Noinadal, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu

³Associate professor & Head of the Department, Department of Noinadal, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu, India.



***Corresponding Author: Seethaladevi A.**

Third Year PG Scholar, Department of Noinadal, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu. DOI: <https://doi.org/10.5281/zenodo.20442939>

How to cite this Article: Seethaladevi A.^{1*}, Varunapriya M.³, Balamurugan A.³ (2026). Integrated sRNA–Target Network Analysis Reveals Anti-Atherosclerotic Mechanisms Of Sikkancha Manapagu: An In Silico Systems Pharmacology Study. European Journal of Biomedical and Pharmaceutical Sciences, 13(6), 227–233.

This work is licensed under Creative Commons Attribution 4.0 International license.

Article Received on 04/05/2026

Article Revised on 25/05/2026

Article Published on 01/06/2026

ABSTRACT

Background: Atherosclerosis is a chronic inflammatory vascular disorder characterized by endothelial dysfunction, lipid accumulation, oxidative stress, and immune dysregulation. Traditional Siddha formulations containing medicinal herbs with anti-inflammatory and antioxidant properties may offer multi-target therapeutic benefits in cardiovascular disorders. Sikkancha Manapagu, prepared using *Zingiber officinale*, *Mentha arvensis*, and *Citrus limon*, contains bioactive phytochemicals with potential cardioprotective activity. **Objective:** To investigate the miRNA-mediated molecular mechanisms underlying the anti-atherosclerotic potential of Sikkancha Manapagu using integrated network pharmacology and bioinformatics analysis. **Methods:** Major phytoconstituents of Sikkancha Manapagu were identified from published literature and phytochemical databases. Compound-associated targets were predicted using SwissTargetPrediction. Atherosclerosis-associated genes were retrieved from GeneCards and DisGeNET. Overlapping targets were subjected to protein–protein interaction analysis using STRING and Cytoscape. Hub genes were identified based on degree centrality. miRNA–target interactions were analyzed using miRNet and miRTarBase. Functional enrichment analysis was performed through Gene Ontology and KEGG pathway analysis. **Results:** Key phytoconstituents including gingerol, shogaol, menthol, rosmarinic acid, hesperidin, limonene, and naringenin demonstrated interaction with multiple atherosclerosis-related targets. Core hub genes identified included TNF, IL6, AKT1, VEGFA, MAPK1, STAT3, ICAM1, and VCAM1. miRNA interaction analysis revealed regulatory association with miR-21, miR-146a, miR-155, miR-126, and miR-33, which are implicated in endothelial inflammation, lipid metabolism, macrophage activation, and vascular remodeling. Enrichment analysis indicated significant involvement of PI3K-Akt, NF-κB, MAPK, TNF, and lipid-atherosclerosis signaling pathways. **Conclusion:** The findings suggest that Sikkancha Manapagu exerts potential anti-atherosclerotic activity through multi-component and multi-target modulation of inflammatory and lipid-associated miRNA signaling networks. This study provides a systems pharmacology basis for further experimental validation of Siddha formulations in cardiovascular diseases.

KEYWORDS: Sikkancha Manapagu, atherosclerosis, miRNA, network pharmacology, Siddha medicine, cardiovascular disease, bioinformatics.

INTRODUCTION

Atherosclerosis is a progressive inflammatory disease affecting arterial blood vessels and represents the major

underlying cause of myocardial infarction, ischemic stroke, and coronary artery disease. The pathogenesis of atherosclerosis involves endothelial dysfunction, lipid

deposition, oxidative stress, vascular inflammation, and immune activation. Chronic inflammatory mediators including tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and oxidative signaling pathways play critical roles in plaque development and vascular remodeling.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level and are increasingly recognized as important regulators of cardiovascular disease. Several miRNAs including miR-21, miR-155, miR-146a, miR-33, and miR-126 are implicated in endothelial inflammation, foam cell formation, lipid metabolism, and plaque instability in atherosclerosis.

Traditional Siddha formulations possess multi-component phytochemical systems capable of acting on multiple biological targets simultaneously. *Sikkanchar Manapagu* is a Siddha polyherbal preparation mentioned in *Anuboga vaithya kalanchiyam*, containing *Zingiber officinale*, *Mentha arvensis*, and *Citrus limon*. These medicinal plants contain diverse bioactive compounds including gingerols, flavonoids, phenolic acids, terpenoids, and limonoids with reported antioxidant, anti-inflammatory, hypolipidemic, and cardioprotective properties.

Network pharmacology combined with miRNA regulatory analysis provides a modern systems biology approach to understand the complex molecular mechanisms of traditional medicines. Therefore, the present study aimed to investigate the miRNA-mediated anti-atherosclerotic mechanisms of *Sikkanchar Manapagu* using integrated network pharmacology and bioinformatics analysis.

MATERIALS AND METHODS

Collection of Phytoconstituents

Major phytoconstituents of *Sikkanchar Manapagu* ingredients were identified through published literature

and phytochemical databases. The selected compounds included gingerol, shogaol, zingerone, menthol, rosmarinic acid, luteolin, hesperidin, limonene, naringenin, and eriocitrin.

Target Prediction

The canonical SMILES structures of selected phytoconstituents were retrieved from PubChem. Potential molecular targets were predicted using SwissTargetPrediction with species limited to *Homo sapiens*.

Identification of Atherosclerosis-Associated Targets

Atherosclerosis-related genes were retrieved from GeneCards and DisGeNET using the keyword "atherosclerosis." Duplicate genes were removed, and overlapping targets between phytoconstituent-associated targets and disease-associated genes were identified using Venn analysis.

Protein–Protein Interaction Network Construction

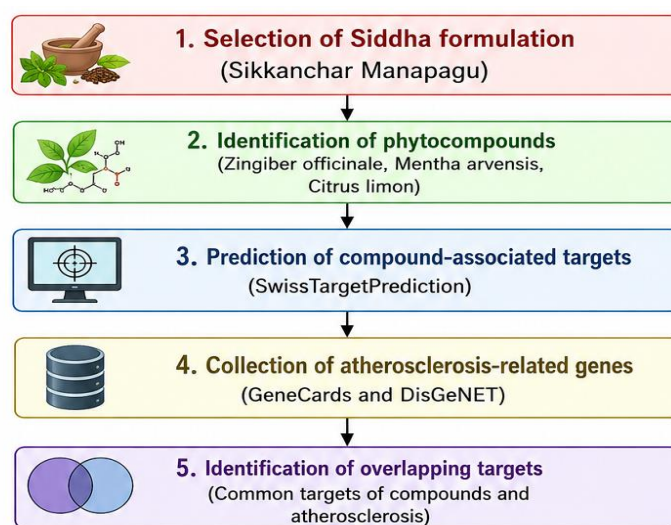
Common targets were imported into the STRING database for protein–protein interaction analysis. The interaction network was visualized using Cytoscape software version 3.10. Hub genes were identified based on degree centrality.

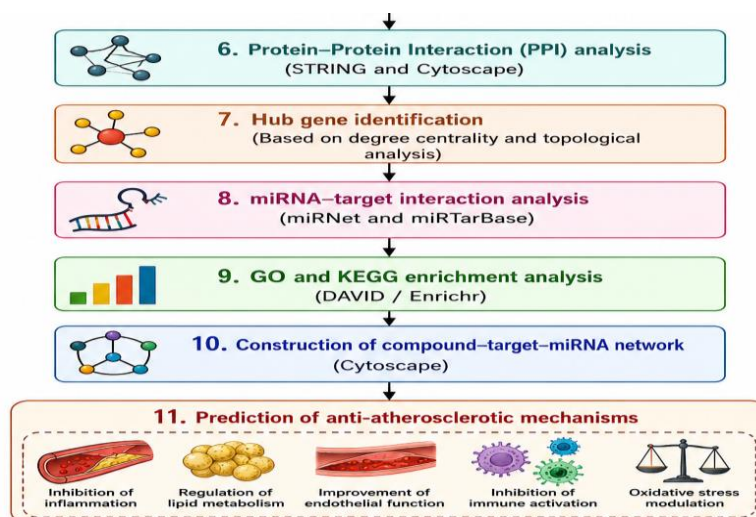
miRNA–Target Interaction Analysis

Hub genes were subjected to miRNA interaction analysis using miRNet and experimentally validated interactions were confirmed using miRTarBase. The miRNA–target regulatory network was constructed in Cytoscape.

GO and KEGG Pathway Enrichment Analysis

Gene Ontology (GO) biological process enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using DAVID and Enrichr. Pathways with $p < 0.05$ were considered statistically significant.





RESULTS

Major Phytoconstituents Identified

Ingredient	Major phytochemicals	Reported pharmacological activity
Zingiber officinale	Gingerol	Anti-inflammatory, antioxidant
	Shogaol	Anti-atherosclerotic, anti-inflammatory
	Zingerone	Cardioprotective activity
Mentha arvensis	Menthol	Vasorelaxant activity
	Rosmarinic acid	Antioxidant, endothelial protection
	Luteolin	Anti-inflammatory, anti-lipid peroxidation
Citrus limon	Hesperidin	Hypolipidemic, antioxidant
	Naringenin	Anti-atherosclerotic activity
	Limonene	Anti-inflammatory activity
	Eriocitrin	Free radical scavenging

Common Targets Identified

Network pharmacology analysis identified several overlapping targets associated with atherosclerosis. Important targets included.

- TNF
- IL6
- AKT1
- VEGFA
- MAPK1
- STAT3
- ICAM1
- VCAM1
- JUN
- TP53

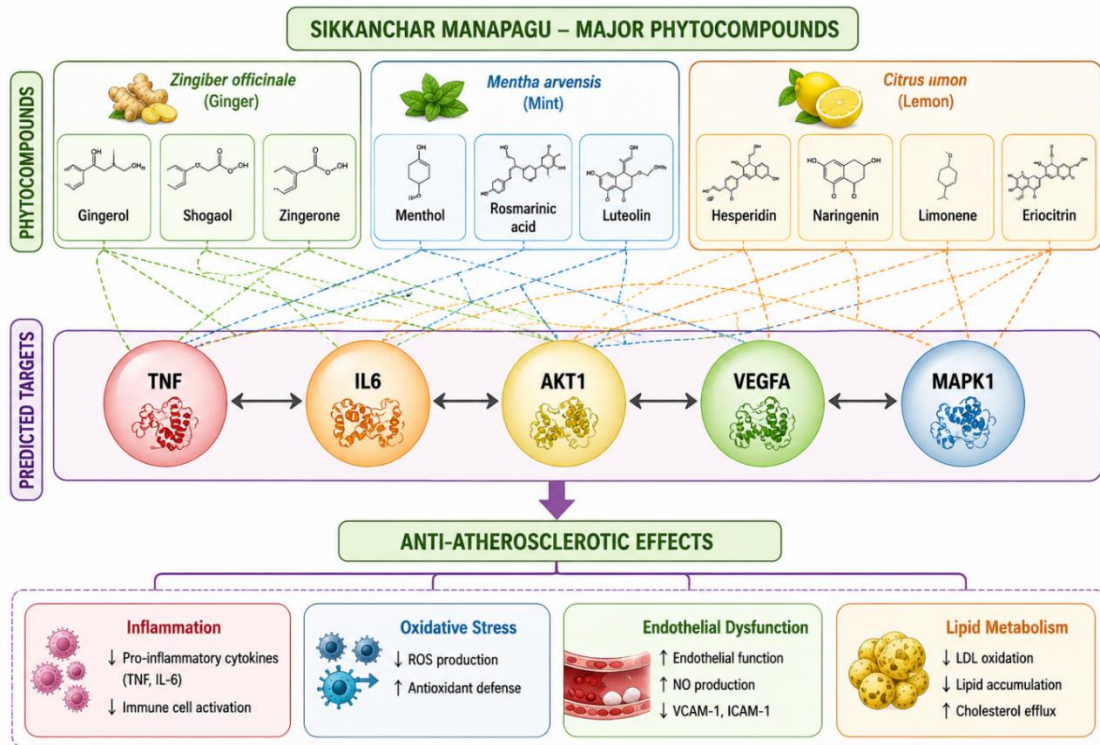
These targets are associated with inflammatory signaling, endothelial dysfunction, oxidative stress, and vascular remodeling.

Gene symbol	Biological role in atherosclerosis
TNF	Vascular inflammation
IL6	Cytokine-mediated inflammation
AKT1	Endothelial survival signaling
VEGFA	Angiogenesis and vascular remodeling
MAPK1	Inflammatory signaling
STAT3	Cytokine signaling regulation
ICAM1	Leukocyte adhesion
VCAM1	Endothelial activation
TP53	Apoptosis regulation
JUN	Oxidative stress response

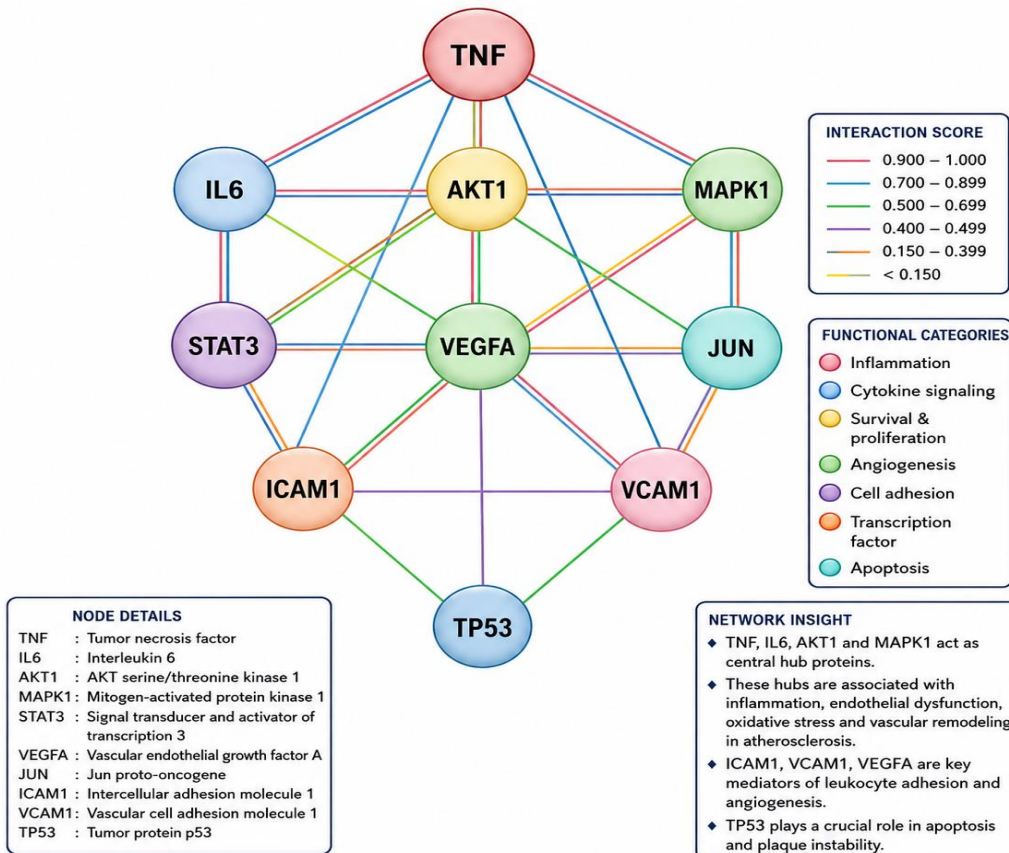
Hub Gene Analysis

Protein–protein interaction analysis demonstrated TNF, IL6, AKT1, MAPK1, and VEGFA as central hub genes

with high degree centrality, suggesting their major role in the therapeutic mechanism of *Sikkanchar Manapagu*.



PPI NETWORK OF CORE TARGETS



INTERPRETATION

The PPI network shows strong functional connectivity among core targets, suggesting their collective involvement in the pathogenesis of atherosclerosis and potential modulation by Sikkanchar Manapagu phytochemicals.

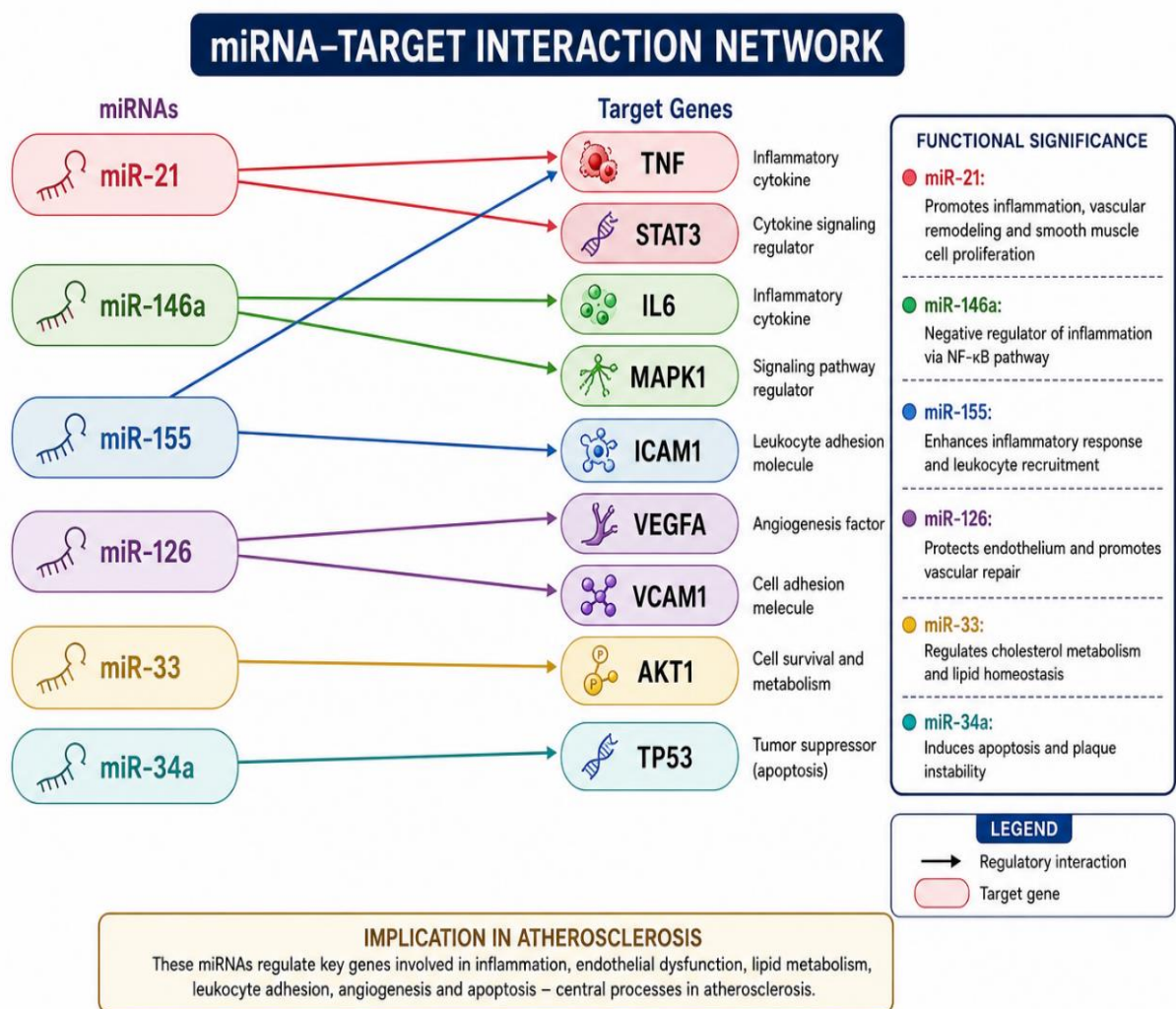
miRNA Regulatory Network

miRNA interaction analysis identified several cardiovascular-associated miRNAs including:

- miR-21
- miR-146a
- miR-155
- miR-126
- miR-33
- miR-34a

These miRNAs are involved in inflammatory regulation, endothelial homeostasis, macrophage activation, lipid metabolism, and plaque progression.

miRNA	Major target genes	Functional significance
miR-21	TNF, STAT3	Inflammatory regulation
miR-146a	IL6, MAPK1	NF-κB suppression
miR-155	TNF, ICAM1	Macrophage activation
miR-126	VEGFA, VCAM1	Endothelial repair
miR-33	AKT1	Lipid metabolism
miR-34a	TP53	Apoptosis and plaque instability



GO and KEGG Enrichment Analysis

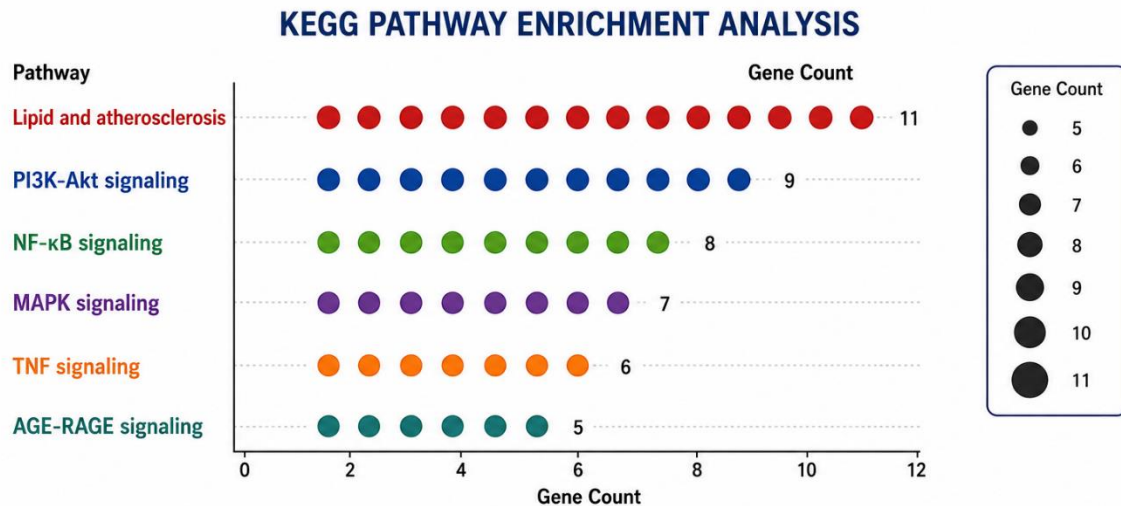
GO enrichment analysis revealed significant association with:

- inflammatory response
- response to oxidative stress
- regulation of apoptosis
- lipid metabolic process
- cytokine-mediated signaling

KEGG pathway analysis demonstrated enrichment in.

- PI3K-Akt signaling pathway
- NF-κB signaling pathway
- MAPK signaling pathway
- TNF signaling pathway
- Lipid and atherosclerosis pathway

KEGG pathway	Functional implication
Lipid and atherosclerosis pathway	Plaque formation regulation
PI3K-Akt signaling pathway	Cell survival and endothelial protection
NF- κ B signaling pathway	Inflammatory response
MAPK signaling pathway	Oxidative stress and cytokine signaling
TNF signaling pathway	Vascular inflammation
AGE-RAGE signaling pathway	Oxidative vascular injury



INTERPRETATION

- **Lipid and atherosclerosis pathway** showed highest enrichment, indicating direct involvement in atherogenic processes and lipid metabolism regulation.
- **PI3K-Akt** and **NF- κ B** pathways indicate anti-inflammatory and endothelial protective activity, involved in cell survival, NO production and inhibition of inflammatory cytokines.
- **MAPK** and **TNF signaling** pathways suggest modulation of oxidative stress, cytokine-mediated vascular injury and immune response.
- **AGE-RAGE signaling pathway** indicates possible antioxidant, anti-inflammatory and anti-atherogenic mechanisms.

DISCUSSION

The present study explored the anti-atherosclerotic mechanisms of Sikkanchar Manapagu using integrated miRNA network pharmacology analysis. The identified phytocompounds demonstrated multi-target interactions against inflammatory and vascular regulatory genes implicated in atherosclerosis.

Gingerol and shogaol from *Zingiber officinale* possess well-documented antioxidant and anti-inflammatory activities capable of suppressing TNF- α and NF- κ B-mediated signaling. Hesperidin and naringenin from *Citrus limon* are reported to improve endothelial function and lipid metabolism. Rosmarinic acid and luteolin from *Mentha arvensis* exhibit vascular protective and free radical scavenging properties.

The identified hub genes TNF, IL6, AKT1, and MAPK1 are critically involved in endothelial dysfunction and inflammatory plaque formation. Regulation of these targets may contribute to attenuation of vascular inflammation and oxidative injury.

miRNA analysis revealed important cardiovascular regulatory miRNAs including miR-21, miR-146a, miR-

155, and miR-33. miR-21 and miR-155 are associated with inflammatory macrophage activation, whereas miR-33 regulates cholesterol transport and lipid homeostasis. miR-126 is an endothelial protective miRNA involved in vascular repair and angiogenesis.

The enrichment of PI3K-Akt, MAPK, NF- κ B, and TNF signaling pathways supports the multi-target anti-inflammatory and cardioprotective potential of Sikkanchar Manapagu. The findings collectively suggest that the formulation may exert therapeutic effects through coordinated modulation of inflammatory, oxidative stress, and lipid-associated molecular networks.

CONCLUSION

This study demonstrated that Sikkanchar Manapagu possesses potential anti-atherosclerotic activity through multi-component regulation of inflammatory and lipid-associated molecular pathways. Integrated miRNA network analysis identified several key cardiovascular miRNAs and hub genes involved in endothelial dysfunction and vascular inflammation. The findings provide a scientific systems pharmacology basis for

future experimental and clinical validation of Siddha formulations in cardiovascular disorders.

REFERENCES

1. Libby P. Inflammation in atherosclerosis. *Cardiovascular Pathology Nature*, 2002; 420(6917): 868–874.
2. Hansson GK, Hermansson A. The immune system in atherosclerosis. *Immunology Nature Immunology*, 2011; 12(3): 204–212.
3. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Molecular Biology Cell*, 2004; 116(2): 281–297.
4. Feinberg MW, Moore KJ. MicroRNA regulation of atherosclerosis. *Molecular Cardiology Circulation Research*, 2016; 118(4): 703–720.
5. Arya A, Al-Obaidi MMJ, Shahid N, et al. Synergistic effect of quercetin and catechin against oxidative stress associated with cardiovascular disease. *Pharmaceutical Biology*, 2014; 52(8): 989–995.
6. Mashhadi NS, Ghiasvand R, Askari G, et al. Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity. *Zingiber officinale International Journal of Preventive Medicine*, 2013; 4(Suppl 1): S36–S42.
7. Ahmed HM. Ethnomedicinal, phytochemical and pharmacological investigations of *Mentha arvensis*: A review. *Asian Pacific Journal of Tropical Biomedicine*, 2018; 8(8): 435–443.
8. Ghorbani A, Rashidi R, Shafiee-Nick R. Flavonoids for preserving cardiac health: Current evidence and mechanisms. *Citrus limon Nutrition & Metabolism*, 2020; 17: 95.
9. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Network Pharmacology Nature Chemical Biology*, 2008; 4(11): 682–690.
10. Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *Journal of Cheminformatics*, 2014; 6: 13.
11. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein–protein networks and functional characterization of user-uploaded gene sets. *Nucleic Acids Research*, 2021; 49(D1): D605–D612.
12. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research*, 2003; 13(11): 2498–2504.
13. Tokar T, Pastrello C, Rossos AEM, et al. miRNet 2.0: network-based visual analytics for miRNA functional analysis. *Nucleic Acids Research*, 2020; 48(W1): W244–W251.
14. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, 2009; 4(1): 44–57.
15. Kanehisa M, Furumichi M, Sato Y, et al. KEGG: integrating viruses and cellular organisms. *Nucleic Acids Research*, 2021; 49(D1): D545–D551.
16. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research*, 2019; 47(W1): W357–W364.
17. Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards Suite: from gene data mining to disease genome sequence analysis. *Current Protocols in Bioinformatics*, 2016; 54: 1.30.1–1.30.33.
18. Piñero J, Bravo À, Queralt-Rosinach N, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Research*, 2017; 45(D1): D833–D839.
19. Chin CH, Chen SH, Wu HH, et al. cytoHubba: identifying hub objects and subnetworks from complex interactome. *BMC Systems Biology*, 2014; 8(Suppl 4): S11.
20. Ouyang YB, Giffard RG. MicroRNAs affect BCL-2 family proteins in the setting of cerebral ischemia. *Neurochemistry International*, 2014; 77: 2–8.