RESEARCH PAPER

Gyrocarpus asiaticus Exerts Analgesic Activity by Inhibiting COX-1 and NET: An Integrated In Silico Network Pharmacology Approach and In Vivo Studies

Madduri Padmavathamma [1*], Shanmugapandian P [1] and A.V.V.Sowjanya [2]

[1]*Department of Pharmacy, Prist Deemed University, Thanjavur ,Tamil Nadu, India*
[2]*Division of Pharmacology, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila, Vishvavidyalayam , Tirupati, India*
[1*]*padmavathammam@gmail.com*

Received: 1st Mar, 2026; Revised: 7th Mar 2026; Accepted: 28th March, 2026; Available Online: 30th March, 2026

ABSTRACT
Gyrocarpus asiaticus has long history in traditional medicine for reducing inflammation and pain, its molecular mechanisms are still unclear. Network pharmacology helped to identify pain- related targets, molecular docking showed a strong interaction between T-muurolol and PTGS1 (COX-1) as well as SLC6A2 (norepinephrine transporter). Which confers both central and peripheral processes. To confirm these findings, ethanolic extract was evaluated in acetic acid-induced writhing and tail immersion animal models. At a dose of 400 mg/kg, the extract produced a significant reduction in the writhing model, similar to the pain relief effects of aspirin. In the tail immersion test also the reaction time was prolonged. In summary, the results support the traditional medicinal use of *G. asiaticus* by showing its pain-reducing effects through targets that involve monoaminergic and inflammatory pathways.
Keywords: *Molecular docking, network pharmacology, analgesic activity, and Gyrocarpus asiaticus.*
How to cite this article: Padmavathamma M, Shanmugapandian P, Sowjanya AVV. Gyrocarpus asiaticus Exerts Analgesic Activity by Inhibiting COX-1 and NET: An Integrated In Silico Network Pharmacology Approach and In Vivo Studies. Int J Drug Deliv Technol. 2026;16(25s): 538-546. DOI: 10.25258/ijddt.16.25s.66
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

World Health Organization estimates that more than 80% of the world's population relies on traditional plant-based drugs for basic healthcare. This shows how important plant-based products are in global healthcare.[1] So far, at least 119 bioactive compounds from 90 plant species have been included in standard pharmacopeia. [2]

The deciduous tree *Gyrocarpus asiaticus* mainly grows in the Tirumala hills of India. Locally, it is called Taniki or Nalla Poliki. Historically, it has been used to treat inflammation, cancer, and gastrointestinal issues, although its exact pain-relieving mechanisms are still unknown. [3,4] Early studies indicate that the plant has various pharmacological properties, including heart-strengthening, antioxidant, and liver-protective effects. [5]

Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are the main stays to treat pain. However, these drugs often have serious side effects, including damage to the gastrointestinal lining, breathing issues, kidney damage and the risk of addiction. [6-8] Thus, there is a need for safer pain relievers. Network pharmacology is based on systems biology, is a relatively new field. It provides a broad "multi-component, multi-target, multi-pathway" approach to uncovering the complex relationships between plant compounds and the human body. [9] Unlike the traditional "one drug, one target" method, which often does not fully explain how herbal medicines work, network pharmacology supports the combined effects of traditional medicine. It allows for a deeper understanding of how drugs interact with diseases by predicting potential targets, biological mechanisms, and signalling pathways involved in their healing effects, thus providing valuable insights into drug-disease interactions.[10]

These computer-based predictions are validated by simulating the atomic-level binding processes and affinities between small-molecule ligands and target macromolecules using molecular docking.[11] Binding energies and types of interactions, such as hydrogen bonds and π–π stacking, structure-based approach yields significant insights into mechanisms. GO/KEGG enrichment analysis and Protein-Protein Interaction (PPI) networks predict pain related targets of phytoconstituents of *Gyrocarpus asiaticus*. Two *in-vivo* animal models were used to confirm the analgesic activity.

MATERIALS AND METHODS

Chemicals and reagents:

Analytical-grade chemicals and reagents were used in this experiment.

**Author for Correspondence: padmavathammam@gmail.com*

Plant Collection and Authentication:
The whole species of *Gyrocarpus asiaticus* was collected from the Tirumala Hills in Andhra Pradesh, India. Prof. Madhava Chetty, Sri Venkateshwara University, Botany Department, Tirupati, Andhra Pradesh verified the authenticity of the plant sample. A voucher specimen (No. 0711) was added to the Institutional herbarium for further use.

Preparation of Extract
The material was mechanically ground into a coarse powder (sieve size 10/44). Soxhlet device was used to extract 500 grams of the powder. The ethanolic extract (GAE) was obtained by first defatting with petroleum ether and then extracting with 95% ethanol, a rotary flash evaporator was used to concentrate the extract. [12]

Phytoconstituents Identification and Target Prediction
Reported phytoconstituents were identified through literature review, and their structures were retrieved from PubChem (http://pubmed.ncbi.nlm.nih.gov/). Potential human protein targets were predicted using SwissTargetPrediction (https://www.swisstargetprediction.ch/). Pain- related genes were collected from DisGeNET (https://www.disgenet.org/) and GeneCards (https://www.genecards.org). A single set of pain-related targets were produced by merging the results and eliminating duplicates. Overlapping targets between phytoconstituents and pain- related genes were identified and visualized using a Venn diagram (https://bioinfogp.cnb. csic.es/tools/venny/).

Building PPI Networks and Determining Important Targets for Analgesic Phytoconstituents
Using the STRING 12.0 database (https:// string-db.org/), a Protein–Protein Interaction (PPI) network was built with Homo sapiens as the organism and a medium confidence score (>0.4) as the minimum interaction threshold for analysing interactions between the common targets of pain and the chosen phytoconstituents.

For analysis and visualization, the network was imported into Cytoscape 3.10.4 (https://cytoscape.org/). The Network Analyzer and CytoHubba plug-ins were utilized for determining topological properties. The most important possible analgesic targets of the phytoconstituents were identified as hub targets based on high degree centrality, betweenness centrality, and closeness centrality.

GO and KEGG Pathway Enrichment Analysis
For enrichment analysis, the common targets were uploaded to the ShinyGO database (https:// bioinformatics.sdstate.edu/go/) with Homo sapiens selected as the species and a false discovery rate (FDR) threshold of less than 0.05. Using enrichment bar plots, lollipop charts, and network diagrams based on the Kyoto, the most important biological processes, molecular activities, cellular components, and pathways were shown using the Gene Ontology (GO) and the Encyclopedia of Genes and Genomes (KEGG) enrichment pathways. The top ten hub targets by degree value were highlighted.

A Phytoconstituent–Target–Pain–Pathway network was constructed and investigated in Cytoscape 3.10.4 to elucidate the multi-target, multi-pathway processes underlying the analgesic activity of the selected phytoconstituents.

Molecular Docking and Visualization
To evaluate binding affinities and interaction modes between the chosen phytoconstituents and important pain-related target proteins (such as COX-1/2, TRPV1, and opioid receptors), molecular docking was carried out using the SwissDock web server (http://www.swissdock.ch). The RCSB PDB database was used to obtain protein structures, and PubChem was used to prepare ligands. Binding energy, interaction types, and pose reliability were employed to evaluate the docking results. Key interactions, such as hydrophobic contacts, hydrogen bonds, vander Waals forces π–π stacking with active-site residues, were identified by visualizing and analyzing the docked complexes using BIOVIA Discovery Studio. These findings provide information on the molecular processes underlying the chosen phytoconstituents' analgesic therapeutic potential.

Pharmacological studies
The National Institute of Nutrition, Hyderabad, India, supplied Swiss albino female mice weighing 20–25 grams, 8–10 weeks old. A clean 12:12-hour light and dark cycle was maintained for the duration of the study. The mice were given an unrestricted supply of water and the normal laboratory pellet diet (Hindustan lever). The study protocol (IAEC no:1995/PO/Re/S/2017/CCSEA) was approved by the Institutional Animal Ethics Committee, and the studies were conducted in compliance with the CCSEA Guidelines.

ANALGESIC ACTIVITY
The analgesic potential of the ethanolic extract of *Gyrocarpus asiaticus* (GAE) was evaluated in Swiss albino mice using two established nociception models. These models were selected to validate the *in silico* finding inhibition of PTGS1 (COX-1) and the modulation of the SLC6A2 (Norepinephrine transporter).

1. Acetic Acid-Induced Writhing Test
The acetic acid-induced writhing method was used to evaluate the extract's peripheral analgesic activity.[13] Acetic acid was injected into the abdominal cavity of the animals as algesic. Prior to the experiment, Swiss albino mice were fasted over and allocated into five groups at random (n = 6 per group). Group I received 2% v/v aqueous Tween 80 as the negative control. Aspirin (20 mg/kg, p.o.) to Group II, which was regarded as the positive control. *Gyrocarpus asiaticus* ethanolic extract (GAE) was administered orally to the remaining three groups (Groups III, IV, and V) at doses of 100, 200, and 400 mg/kg suspended in 2% Tween 80, respectively. An intraperitoneal injection of 1% aqueous acetic acid at a dose of 0.1 mL per 10 g body weight was used to induce pain 30 minutes after the vehicle, standard medication or test extract. Each mouse

was held in a transparent glass observation chamber after receiving an injection of acetic acid, and the number of typical abdominal constrictions (writhes) was counted for 15 minutes.

2. Tail Immersion Method

The tail immersion method is a dependable way to examine the role of spinal and supraspinal pain pathways. It was used to evaluate centrally activity analgesic activity. Mouse tail was immersed in water preheated to 51 ± 0.5 °C, tail flick latency was measured.[14] Tramadol (10 mg/kg, p.o.) or *Gyrocarpus asiaticus* ethanolic extract (GAE) was administered at doses of 100, 200, and 400 mg/kg/PO. Reaction times were measured at 0, 30, 60, and 90 mins, a cut-off time of 15 seconds was maintained to prevent thermal damage to the tail tissue. However, values at 60 mins are presented in results. Centrally acting analgesics typically improve descending inhibitory pain pathways by affecting monoaminergic neurotransmission. This model is particularly relevant for linking the *in silico* docking results with SLC6A2.

Statistical analysis

Data was **represented** as mean ± SEM (n = 6). One-way analysis of variance (ANOVA) followed by dunnets test was used. The significance level p value < 0.05 was considered significant.

RESULTS

Identification of Phytoconstituents

Gyrocarpus asiaticus phytoconstituents were collected from literature (PubMed, Google Scholar, IMPPAT), yielding 46 compounds from seeds, leaves, and bark. PubChem provided the SMILES/3D structures.

Prediction of Potential Biological Targets of Selected Phytoconstituents

The SwissTargetPrediction web server was utilized to anticipate possible biological targets of the chosen phytoconstituents. Only targets with probability > 0 were kept in the SMILES format, and predictions have been restricted to Homo sapiens. Among the phytoconstituents that were screened, 49 protein targets were found.

Database Mining of Targets Associated with Analgesic Activity / Pain

The DisGeNET and GeneCards databases were used for pain/analgesic-related targets using the keywords "pain," "analgesic," "nociception," "antinociceptive," "inflammatory pain," "neuropathic pain," and "chronic pain." DisGeNET identified 986 genes linked to pain, while GeneCards determined 1323 targets with a relevance score of a minimum 5.0. In order to identify key therapeutic targets involved in analgesic activity and pain modulation, duplicate entries were removed and the combined unique gene set was used for subsequent overlap analysis and network construction.

Identification of Potential Therapeutic Targets for Analgesic Activity

The 218 phytoconstituent-predicted targets and the 1323 pain-related targets from GeneCards (plus 986 from DisGeNET) showed 45 overlapping targets in a Venn diagram (Venny 2.1.0) (Figure 1). PPI network construction and functional enrichment analysis done on common targets, which are thought to be significant mediators of *Gyrocarpus asiaticus's* analgesic action.

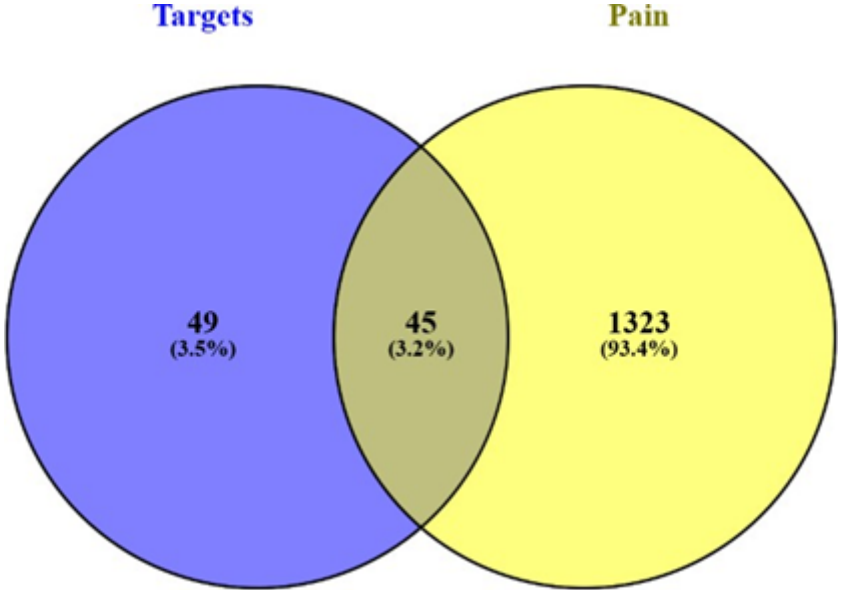

Fig 1: Venn diagram of target phytoconstituents compared with genes of analgesia

Network Analysis of Protein-Protein Interactions (PPI)

The 45 overlapping targets (confidence score > 0.7, Homo sapiens) were imported into the STRING 12.0 database in order to create the PPI network. The network consisted of 20 edges and 93 nodes. After removing unconnected nodes, the network was visualized and examined using Cytoscape 3.10.3's CytoHubba plugin. Topological analysis using cytohubba identified the top hub genes based on degree, betweenness centrality, and closeness centrality, including HIF1A, ESR1, PIK3CA, KDR, PARP1, HMGCR, APP, KIT, CREBBP and JAK2.These top 10 Hub genes and they were chosen for further

molecular docking confirmation.

KEGG Pathway Enrichment Analysis with GO

ShinyGO database provided 45 potential targets for KEGG pathway and Gene Ontology (GO) enrichment studies. GO annotation was performed with a significance threshold of $P < 0.01$ for each of the three main categories: cellular component (CC), molecular function (MF), and biological process (BP). The findings were displayed using dual-axis bar and line graphs, with the names of GO terms on the X-axis, the proportion of input genes on the primary Y-axis (bar graph), and −log10 (P-value) on the secondary Y-axis (line graph) (Figure 2A–C). The same significance cut off ($P < 0.01$) was used for the KEGG pathway enrichment analysis. The results are displayed as a bar chart (Figure 3).

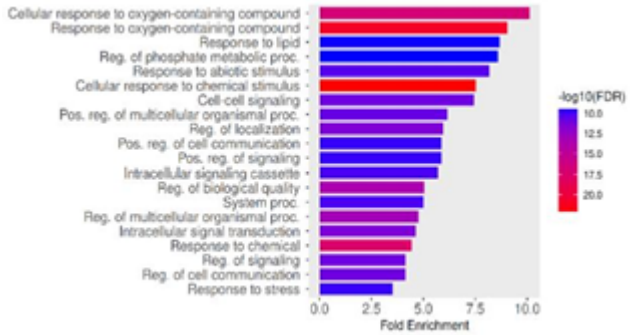

Fig A: GO biological process

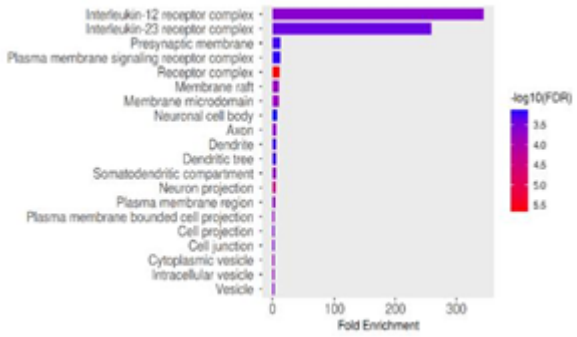

Fig 2B: GO cellular components

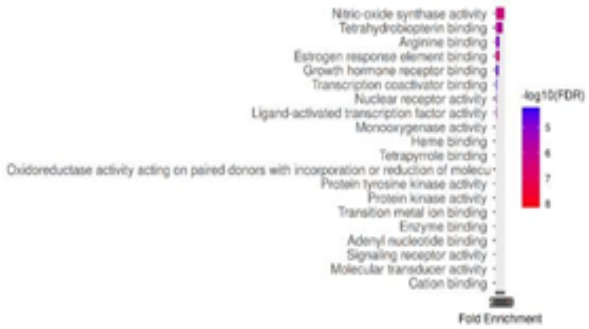

Fig 2C: GO Molecular function

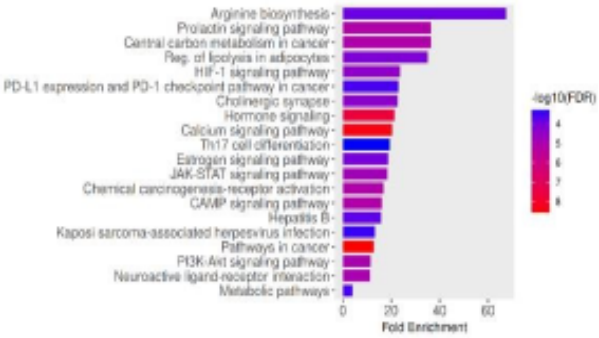

Fig 3: KEGG pathway

Building the Compound-Target-Pathway System

The Compound–Target–Pathway network, which illustrates the interactions between phytoconstituents, therapeutic targets, and related biological pathways linked to analgesic efficacy, was constructed using Cytoscape (v3.10.3) (Figure 4). Nodes represent compounds, targets, and routes, while edges show how they relate to one another functionally.

Based on degree of rank, the top ten hub targets were determined by network topology analysis using the CytoHubba plugin (Table 1). In order determine their role in the observed analgesic effects, these hub targets were then selected for molecular docking analyses.

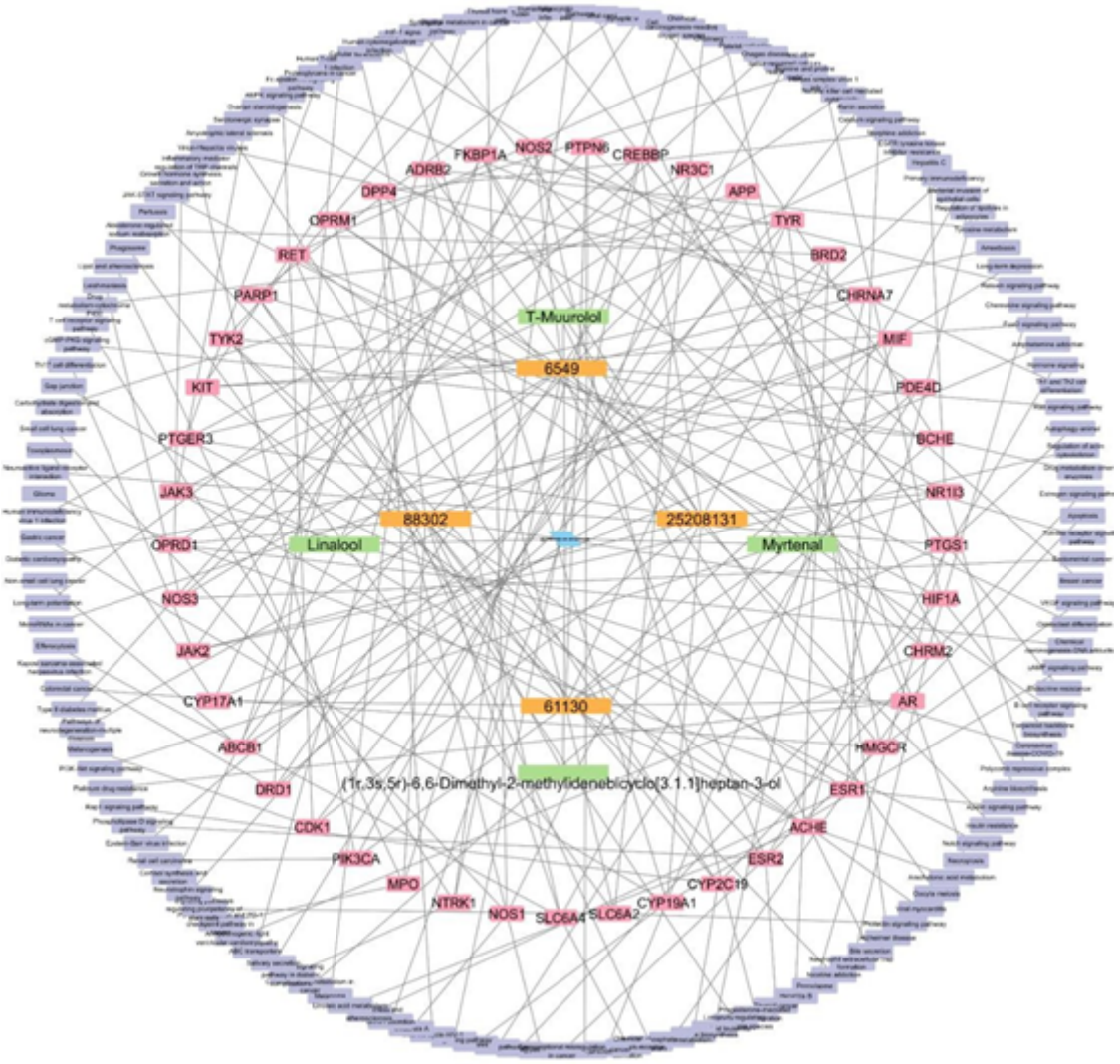

Fig 4: Cytoscape network of phytoconstituents with associated genes of analgesics **Green.** phytoconstituents **Orange** PC ID's of selected phytoconstituents **Light pink** Target attributes **Light blue** gene pathway attribute

Table 1: PDB ID's of hub protein targets of selected phytoconstituents against analgesic activity

Rank	Gene	PDB ID'S	Protein name
1	HIF1A	4ZPR	Hypoxia-inducible factor 1-alpha
2	ACHE	4EY7	Acetylcholinesterase
3	NR1I3	7YXD	Apoptosis regulator Nr-13
4	PTGS1	5KIT	Prostaglandin-endoperoxide synthase 1 (COX-1)
5	SLC6A2	4KN0	solute carrier family 6 member 2 protein
6	CHRM2	4MQT	Cholinergic receptor muscarinic 2
7	CYP19A1	4J24	Cytochrome P450 family 19 subfamily A member 1.
8	BCHE	1P0P	Butyrylcholinesterase
9	SLC6A4	9D71	solute carrier family 6 member 4
10	CYP2C19	3APV	cytochrome P450 family 2 subfamily C member 19

Molecular Docking Analysis

The ten hub target proteins were computationally docked with selected phytoconstituents. The complete docking scores are presented in Table 2, while the top five protein-ligand complexes with the highest binding affinities have been selected based on the docking scores (Table 3). Key molecular interactions between the ligands and target proteins are illustrated in two-dimensional interaction diagrams and representative three-dimensional docking poses (Figure 5).Among all interactions T-Muurolol exhibited the highest binding affinity towards SLC6A2 (-

8.613 kcal/mol). Strong binding was also observed with BCHE (-7.557 kcal/mol) and PTGS1 (-7.257 kcal/mol).

Table 2: Docking Scores of phytoconstituents against hub proteins

Protein (Gene)	PDB ID	Ligand Name	Docking Score (kcal/mol)
ACHE	4EY7	T-Muurolol	-5.878
ACHE	4EY7	Myrtenal	-5.461
ACHE	4EY7	Bicyclic monoterpenoid	-5.391
NR1I3	7YXD	Myrtenal	-3.822
NR1I3	7YXD	Linalool	-3.746
NR1I3	7YXD	T-Muurolol	-3.645
PTGS1	5IKT	T-Muurolol	-7.257
PTGS1	5IKT	Bicyclic monoterpenoid	-5.673
PTGS1	5IKT	Linalool	-5.092
SLC6A2	4KN0	T-Muurolol	-8.613
SLC6A2	4KN0	Bicyclic monoterpenoid	-7.157
SLC6A2	4KN0	Myrtenal	-7.112
CHRM2	5ZKC	Linalool	-4.866
CHRM2	5ZKC	T-Muurolol	-4.841
CHRM2	5ZKC	Bicyclic monoterpenoid	-4.411
CYP19A1	4J24	T-Muurolol	-6.729
CYP19A1	4J24	Myrtenal	-5.503
CYP19A1	4J24	Bicyclic monoterpenoid	-5.503
BCHE	1P0P	T-Muurolol	-7.557
BCHE	1P0P	Bicyclic monoterpenoid	-6.031
BCHE	1P0P	Myrtenal	-5.645
SLC6A4	9D71	T-Muurolol	-6.993
SLC6A4	9D71	Bicyclic monoterpenoid	-5.546
SLC6A4	9D71	Myrtenal	-5.337
CYP2C19	3APV	T-Muurolol	-6.306
CYP2C19	3APV	Linalool	-5.176
CYP2C19	3APV	Bicyclic monoterpenoid	-4.864
HIF1A	4ZPR	T-Muurolol	-3.855
HIF1A	4ZPR	Bicyclic monoterpenoid	-3.618
HIF1A	4ZPR	Linalool	-3.616

Table 3: Top 5 Phytoconstituents Protein interaction Docking Scores

S.no	Target gene (PDB ID)	Ligand	Docking score (k/cal)	Interacting residues	No. of interactions	Standard Drugs
1	SLC6A2 (4KN0)	T-Muurolol	-8.613	TYR76 TRP118 TRP150 TRP187 THR98 HIS151 PHE78 TYR101	8	Duloxetine (-9.370)
2	BCHE (1P0P)	T-Muurolol	-7.557	HIS438 PHE329 TRP82 THR120	4	Rivastigmine -6.960
3	PTGS1 (5IKT)	T-Muurolol	-7.257	ASN375 HIS226 GLY225 GLN374 TRP139 PHE142 ARG376 LEU145	8	Indomethacin-8.384
4	SLC6A2	(1r,3s,5r)-	-7.157	TYR101 TYR76	5	Duloxetine

	(4KN0)	6,6-Dimethyl-2-methylidene bicyclo[3.1.1] heptan-3-ol		TRP118 TRP187 THR98 PHE78		-9.370
5	SLC6A2 (4KN0)	Myrental	-7.112	TYR76 TRP118 TRP187 TYR101	4	Duloxetine -9.370

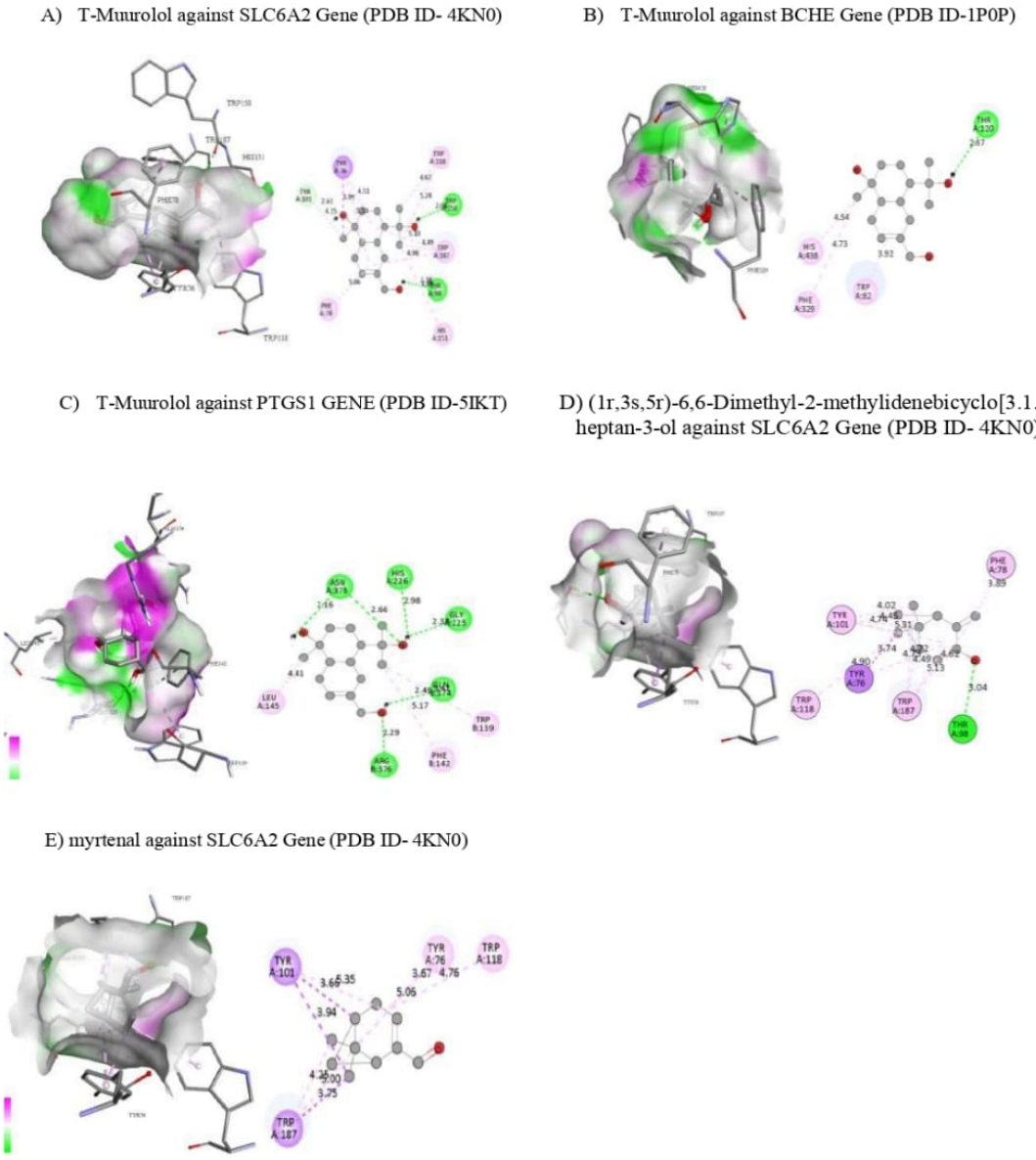

Fig 5: 2D and 3D Interactions and top scoring phytoconstituents against targets

***Gyrocarpus asiaticus* (GAE) ethanolic extract's analgesic activity**

The analgesic effect of the ethanolic extract of *Gyrocarpus asiaticus* (GAE) at oral doses of 100, 200, and 400 mg/kg was evaluated in two nociception models employing Swiss albino mice (n = 6 per group). The standard drugs for the acetic acid-induced writhing was aspirin (20 mg/kg) and tramadol (10 mg/kg) for tail immersion. While GAE produced a dose-dependent increase similar to the standard, tramadol significantly increased latency. Aspirin and GAE both significantly decreased writhing responses in the writhing model, with GAE exhibiting dose-dependent inhibition. Peripheral analgesic activity increased gradually and in a dose-dependent manner (Table 4).

Table 4: *Gyrocarpus asiaticus* ethanolic extract's analgesic effects in Swiss albino mice

Group	Treatment	Tail Immersion	Acetic acid induced Writhings
		Reaction time in seconds	No of Writhes
I	Control	2.2 ± 0.1	53.3 ± 3.5
II	Standard Drug (Aspirin/Tramadol)	6.1 ± 0.31*	13.12 ± 3.46***
III	GAE100mg/kg	3.8 ± 0.21	23.5 ± 2.54***
IV	GAE200mg/kg	4.3 ± 0.18	19.9 ± 3.9***
V	GAE400mg/kg	4.9 ± 0.42*	13.7 ± 2.9***

*p<0.5, **p<0.0, p<0.001 vs control group

DISCUSSION

The current study used network pharmacology, molecular docking, and *in vivo* validation of analgesic potential of *Gyrocarpus asiaticus* (GAE). In the present study molecular docking studies indicated that T- muurolol binds strongly to PTGS1, with a value of −7.257 kcal/mol. This suggests it can effectively inhibit the enzyme within its active site. Important interactions with ASN375, HIS226, and ARG376 indicate stable binding between the ligand and the protein. GAE significantly lowered pain in both peripheral and central systems, by influencing cholinergic signaling pathways, monoaminergic transporters, and inflammatory mediators. Multi-target action supports the concept of systems pharmacology, which states that effective treatments for complex conditions often need regulation at a network level rather than concentrating on a single target.

These computational predictions were tested using the acetic acid-induced writhing model, intraperitoneal acetic acid releases inflammatory agents like PGE_2 and $PGF_2\alpha$, leading to abdominal contractions. A key gene PTGS1 or COX-1 synthesizes prostaglandins from arachidonic acid, irritates peripheral nociceptors and causes inflammatory hyperalgesia, aspirin-like drugs reduce prostaglandin production. Inhibition of abdominal constriction is correlated with peripheral analgesic activity[15]. Inhibiting cyclooxygenase can relieve pain, aspirin-like drugs reduce prostaglandin production. [16,17] In this study, GAE dose dependently reduced Writhings on par with aspirin. This supports basis for blocking peripheral prostaglandin production provides effective pain relief mediated by PTGS1.

SLC6A2, also known as the norepinephrine transporter (NET), was the main target with the highest docking affinity for T-muurolol at −8.613 kcal/mol. Brainstem controls spinal pain transmissions. NET controls the reuptake of norepinephrine in the central nervous system. Norepinephrine levels increase as NET is blocked, which raises the activation of α2-adrenergic receptors in the descending inhibitory pathways, inhibition of the descending noradrenergic system reduces pain.[18] In the tail immersion model GAE prolonged tail flick latency suggest central pain relief, involving both supraspinal and spinal pathways. The longer reaction time supports monoaminergic regulation linked to SLC6A2 inhibition.

Further, T-muurolol displayed a strong affinity of −7.557 kcal/mol for BCHE. Inhibition of Butyryl cholinesterase improves cholinergic neurotransmission, activating the cholinergic system can relieve pain through muscarinic receptor pathways. [19-20]

The KEGG pathway analysis identified neuroactive ligand-receptor interaction, PI3K-Akt signaling, which plays a key role in inflammatory hyperalgesia and nerve sensitization[21] and HIF-1 signaling pathways controls responses to low oxygen, which can increase pain.[22] Phytochemical constituents such as linalool, myrtenal, and T-muurolol exhibited affinities for multiple targets. Hermann Wagner's (2011) hypothesis on phytochemical synergy suggests that interactions among several components can improve therapeutic effectiveness while lowering side effects.[23]

The broad-spectrum pain-relieving effects of GAE observed in both chemical and thermal pain models can be explained by its actions at multiple targets. It inhibits PTGS1 for peripheral anti- inflammatory pain relief, modulates SLC6A2 for central monoaminergic pain inhibition, and enhances cholinergic activity through BCHE.

These findings suggest that GAE reduces prostaglandin production and regulates signaling pathways related to pain sensitivity. A strong point of this study is the agreement between *in-vivo* and computational predictions. The predicted inhibition of PTGS1 from docking was confirmed by a significant decrease in writhing caused by acetic acid. Additionally, the proposed role of SLC6A2 in central monoamines was supported by increased thermal latency.

CONCLUSION

In silico docking of key components like myrtenal and T-muurolol to targets such as PTGS1 and SLC6A2 supports its analgesic activity in writing and thermal models and explains it's traditional use in analgesia.

REFERENCES

1. Rates SMK. Plants as source of drugs. Toxicon. 2001;39(5):603–613. DOI: 10.1016/S0041-0101(00)00154-9.
2. Fabricant DS, Farnsworth NR. The value of medicinal plants used in traditional medicine for drug discovery. Environmental Health Perspectives. 2001;109(Suppl 1):69–75. DOI: 10.1289/ehp.01109s169.
3. Cragg GM, Newman DJ. Natural Products: A continuing source of novel drug leads. Biochimica et

Biophysica Acta. 2013;1830(6):3670–3695. DOI: 10.1016/j.bbagen.2013.02.008.

4. Wojdyło A, Oszmiański J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry. 2007;105(3):940–949. DOI: 10.1016/j.foodchem.2007.04.038.
5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. Journal of Natural Products. 2007;70(3):461–477. DOI: 10.1021/np068054v.
6. Vane JR, Botting RM. Mechanism of action of nonsteroidal anti-inflammatory drugs. The American Journal of Medicine. 1998;104(3):2S–8S. DOI: 10.1016/S0002-9343(97)00203-9.
7. Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001;413(6852):203–210. DOI: 10.1038/35093019.
8. Woolf CJ. Central sensitization: Implications for the diagnosis and treatment of pain. Pain. 2011;152(3):S2–S15. DOI: 10.1016/j.pain.2010.09.030.
9. Hopkins AL. Network pharmacology: The next paradigm in drug discovery. Nature Chemical Biology. 2008;4(11):682–690. DOI: 10.1038/nchembio.118.
10. Li S, Zhang B. Traditional Chinese medicine network pharmacology: Theory, methodology and application. Chinese Journal of Natural Medicines. 2013;11(2):110–120. DOI: 10.1016/S1875-5364(13)60037-0.
11. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: A powerful approach for structure-based drug discovery. Current Computer-Aided Drug Design. 2011;7(2):146–157. DOI: 10.2174/157340911795677602.
12. Sander LC. Soxhlet extractions. Journal of Research of the National Institute of Standards and Technology. 2017;122. DOI: 10.6028/jres.122.004.
13. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacological Reviews. 2001;53(4):597–652. DOI: 10.1124/pr.53.4.597.
14. Sewell RD, Spencer PS. Antinociceptive activity of narcotic agonists and partial agonists measured by the tail immersion and tail-flick procedures. British Journal of Pharmacology. 1976;58(2):275–278. DOI: 10.1111/j.1476-5381.1976.tb07514.x.
15. Collier HOJ, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. British Journal of Pharmacology and Chemotherapy. 1968;32(2):295–310. DOI: 10.1111/j.1476-5381.1968.tb00973.x.
16. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biology. 1971;231:232–235. DOI: 10.1038/newbio231232a0.
17. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: Structural, cellular and molecular biology. Annual Review of Biochemistry. 2000;69:145–182. DOI: 10.1146/annurev.biochem.69.1.145.
18. Fields HL, Basbaum AI. Brainstem control of spinal pain-transmission neurons. Annual Review of Physiology. 1978;40:217–248. DOI: 10.1146/annurev.ph.40.030178.001245.
19. Soreq H, Seidman S. Acetylcholinesterase: New roles for an old actor. Nature Reviews Neuroscience. 2001;2(4):294–302. DOI: 10.1038/35067589.
20. Ikeda H, Stark J, Fischer H, Wagner M, Drdla R, Jäger T, Sandkühler J. Synaptic amplifier of inflammatory pain in the spinal dorsal horn. Science. 2006;312(5780):1659–1662. DOI: 10.1126/science.1127233.
21. Xu Q, Fitzsimmons B, Steinauer J, O'Neill A, Newton AC, Hua XY, Yaksh TL. Spinal phosphoinositide 3-kinase–Akt–mTOR signaling pathway in inflammatory hyperalgesia. Journal of Neuroscience. 2011;31(6):2113–2124. DOI: 10.1523/JNEUROSCI.2139-10.2011.
22. Semenza GL. Targeting HIF-1 for cancer therapy. Nature Reviews Cancer. 2003;3(10):721–732. DOI: 10.1038/nrc1187.
23. Wagner H. Synergy research: Approaching a new generation of phytopharmaceuticals. Fitoterapia. 2011;82(1):34–37. DOI: 10.1016/j.fitote.2010.08.018.