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NETWORK PHARMACOLOGY-BASED EXPLORATION OF THE MOLECULAR MECHANISMS OF *CLITORIA TERNATEA* LINN. ROOT (ŚVETA APARĀJITĀ) IN HYPOTHYROIDISM

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ABSTRACT

Introduction: *Clitoria ternatea* Linn. (Aparajita) is a medicinal plant traditionally recognized in Ayurveda for diverse therapeutic applications. The root of *Shweta Aparajita* has been described in classical texts for *Galganda*, providing a traditional basis for exploring its relevance to thyroid-related disorders. However, the molecular mechanisms underlying its potential thyrogenic activity remain insufficiently characterized. The present study aimed to explore the potential molecular mechanisms of *Clitoria ternatea* root in hypothyroidism using an exploratory network pharmacology approach. **Methods:** Phytoconstituents of *Clitoria ternatea* root were retrieved from IMPPAT 2.0 and published literature, supplemented by compounds identified through LC–MS analysis. The identified compounds were evaluated for drug-likeness and ADME properties using SwissADME and MolSoft. Potential molecular targets were predicted using Swiss Target Prediction. Hypothyroidism-associated genes were retrieved from GeneCards and OMIM, followed by merging, deduplication, and filtering. Common targets were identified using Venn analysis. KEGG pathway enrichment was performed using DAVID v6.8, while compound–target

networks were constructed using Cytoscape 3.10.3. Hub genes were identified using CytoHubba employing Degree, MCC, EPC, and Closeness algorithms. **Results:** A total of 32 phytoconstituents were identified, comprising 8 compounds from IMPPAT 2.0 and 24 compounds from LC–MS analysis; 26 compounds were retained following drug-likeness and ADME screening. Target prediction identified 705 compound–target interactions involving 307 unique targets. GeneCards and OMIM yielded 7,194 and 288 disease-associated entries, respectively, with 7,209 unique hypothyroidism-associated genes retained after processing. Venn analysis identified 158 common targets. KEGG analysis revealed 135 significantly enriched pathways, including 47 hypothyroidism-associated pathways, involving thyroid hormone, PI3K–Akt, MAPK, cAMP, JAK–STAT, PPAR, insulin, NF-κB, and TNF signaling. Hub analysis identified targets including ESR1, ESR2, PTPN1, CA1, CA2, DPP4, XDH, CYP1B1, HDAC6, and PPARA. **Conclusion:** The findings suggest that *Clitoria ternatea* root may exert potential thyrogenic effects through a multi-component, multi-target, and multi-pathway molecular network involving endocrine, metabolic, inflammatory, and cellular signaling processes. These computational findings provide mechanistic insights into its traditional relevance to *Galganda* and warrant validation through experimental molecular and pharmacological studies.

KEYWORDS: *Clitoria ternatea*; Aparajita; Hypothyroidism; *Galganda*; Network pharmacology; Thyrogenic activity; Phytoconstituents; Hub genes.

INTRODUCTION

Hypothyroidism, specifically, is the most common of thyroid disorders in India, affecting one in ten adults. The prevalence of hypothyroidism in India is 11%, compared with only 2% in the UK and 4·6% in the USA. Compared with coastal cities (example, Mumbai, Goa, and Chennai), cities located inland (example, Kolkata, Delhi, Ahmedabad, Bangalore, and Hyderabad) have a higher prevalence (11·7% vs 9·5%).[1]

Hypothyroidism is a common endocrine disorder characterized by inadequate production of thyroid hormones, primarily thyroxine (T4) and triiodothyronine (T3), resulting in disturbances of metabolic and physiological functions.[2] Clinical manifestations may include fatigue, weight gain, cold intolerance, constipation, cognitive changes, and alterations in lipid metabolism. Although thyroid hormone replacement remains the standard therapeutic approach, the complex pathophysiology of hypothyroidism and the persistence of symptoms in some patients emphasize the need to explore additional therapeutic approaches.[3]

In Ayurveda, thyroid-related manifestations considered in the context of *Agni dushti* and *Galganda*. Classical texts describe *Galganda* as a disorder involving swelling of the neck region, with *Vata*, *Kapha*, and *Meda* described among its pathological factors. Although *Galganda* cannot be regarded as synonymous with hypothyroidism, its classical description provides an Ayurvedic basis for investigating medicinal plants traditionally indicated in thyroid-related disorders.

Aparajita, botanically identified as *Clitoria ternatea* Linn. belonging to the family Fabaceae, is a medicinal plant traditionally used for various therapeutic purposes.[4] Classical Ayurvedic literature describes two varieties, *Shweta Aparajita* and *Neela Aparajita*, and attributes several pharmacological properties to the drug.[5] Of particular relevance, the root of *Shweta Aparajita* has been described for use in *Galganda* in classical texts, including *Vangasena Samhita*, *Chakradatta*, and *Bhaishajya Ratnavali*.[6,7] Experimental studies have also reported pharmacological activities of *Aparajita* root, including antioxidant, antihistaminic, and antiasthmatic properties.[8]

Despite its traditional indication in *Galganda* and reported pharmacological activities, the molecular basis underlying the potential thyrogenic activity of *Clitoria ternatea* remains insufficiently explored.[9,10] The presence of multiple phytoconstituents suggests that its biological effects may involve interactions with several molecular targets and signaling pathways rather than a single target. Conventional reductionist approaches may not adequately capture such complex phytochemical–target relationships.[11]

Network pharmacology provides a systems-level approach for investigating the interactions among bioactive phytoconstituents, molecular targets, disease-associated genes, and biological pathways. Therefore, it can provide mechanistic insights into the potential multi-target actions of medicinal plants and help identify key molecular targets and pathways associated with their pharmacological effects.[12]

Accordingly, the present study employed an exploratory network pharmacology approach to investigate the potential molecular mechanisms underlying the thyrogenic activity of *Clitoria ternatea* Linn. in hypothyroidism. The analysis focused on identification of its phytoconstituents, prediction of potential molecular targets, identification of common

hypothyroidism-associated targets, pathway enrichment, and hub gene analysis to provide molecular-level insights into its potential role in thyroid-related disorders.

METHODS

Exploratory Network Pharmacology Analysis

Network pharmacology analysis was performed as an exploratory supplementary component to investigate the potential molecular mechanisms underlying the thyrogenic activity of *Clitoria ternatea* Linn. The analysis involved phytochemical identification, drug-likeness and ADME screening, target prediction, identification of hypothyroidism-associated targets, pathway enrichment, and network analysis.[13]

Selection and Retrieval of Phytochemicals

Phytoconstituents of *Clitoria ternatea* Linn. were identified from the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT 2.0) database and published scientific literature.[14] Constituents identified by LC–MS analysis of the root extract were also included. The PubChem database was used to retrieve the Compound ID (CID) and canonical SMILES notation of the identified phytochemicals, which were subsequently used for computational analyses.[15]

Drug-Likeness and ADME Screening

The identified phytochemicals were evaluated for drug-likeness using SwissADME and MolSoft. Lipinski's Rule of Five was used as the primary criterion, and compounds with not more than one violation were considered for further analysis.[16,17] The shortlisted compounds were subsequently assessed using SwissADME for gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein substrate properties, and interactions with major cytochrome P450 enzymes.[18,19]

Compound Target Prediction

Potential molecular targets of the shortlisted phytochemicals were predicted using SwissTargetPrediction with *Homo sapiens* selected as the target organism.[20] Predicted targets with a probability score of ≥0.4 were retained and compiled to generate the compound–target dataset for subsequent network analysis.[21]

Retrieval of Hypothyroidism-Associated Targets

Hypothyroidism-associated genes were retrieved from the GeneCards and Online Mendelian Inheritance in Man (OMIM) databases using the keyword "Hypothyroidism." The retrieved gene sets were merged, duplicate entries were removed, and relevant targets were retained based on their database-associated relevance scores.[22,23]

Identification of Common Targets

The predicted phytochemical targets were compared with the hypothyroidism-associated genes using a Venn diagram approach.[24] The overlapping genes were considered potential common targets linking *Clitoria ternatea* phytoconstituents with hypothyroidism and were subjected to subsequent network and functional analyses.[25]

KEGG Pathway Enrichment Analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the common targets was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID v6.8).[26] Pathways with $P \leq 0.05$ were considered statistically significant and were selected for further biological interpretation and network construction.[27]

Network Construction and Hub Gene Identification

Compound–target and compound–target–pathway networks were constructed and visualized using Cytoscape [28]. The CytoHubba plugin was used to evaluate network topology and identify highly connected hub genes.[29] The top-ranked genes were considered potential key molecular targets associated with the pharmacological activity of *Clitoria ternatea* in hypothyroidism.[30]

OBSERVATION AND RESULTS

Network Pharmacology of *Clitoria ternatea* Linn.

Based on the phytochemical profiling and in silico analysis, *Clitoria ternatea* Linn. was subjected to network pharmacology analysis to explore its potential molecular mechanisms related to thyrogenic activity. Phytoconstituents identified through the IMPPAT 2.0 database, published literature, and LC–MS analysis were evaluated for drug-likeness and ADME properties, followed by target prediction, disease-gene mapping, pathway enrichment, and network analysis.

Phytochemical Retrieval and Drug-Likeness Screening

A total of **8 phytochemicals** were retrieved from the IMPPAT 2.0 database for the root, while **24 phytochemicals** were identified through LC–MS analysis, resulting in **32 phytochemicals** for preliminary in silico evaluation. The identified compounds belonged to diverse chemical classes, including flavonoids, alkaloids, terpenoids, fatty acid derivatives, and other secondary metabolites.

Table 1: A List of final selected compound after Absorption, distribution, metabolism and excretion screening of *Clitoria ternatea* Linn.

Sr. No	Pubchem Id	Phytochemical Name	Bioavailability	Lipinski	Molecular Weight
1	5280863	Kaempferol	0.55	Yes; 0 violation	286.24 g/mol
2	92785	Taraxerone	0.55	Yes; 1 violation: MLOGP>4.15	424.70 g/mol
3	92097	Taraxerol	0.55	Yes; 1 violation: MLOGP>4.15	426.72 g/mol
4	5490007	(8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,7,8,9,11, 12,14,15,16,17-decahydro-1H -cyclopenta[a] phenanthrene-3,6-dione	0.55	Yes; 1 violation: MLOGP>4.15	426.67 g/mol
5	5280961	Genistein	55	Yes; 0 violation	270.24 g/mol
6	10147	Chelidonine (+)	0.55	Yes; 0 violation	353.37 g/mol
7	6708732	Retusin dimethyl ether	0.55	Yes; 0 violation	312.32 g/mol
8	442206	Elephantopin	0.55	Yes; 0 violation	360.36 g/mol
9	5192	Sebacic acid	0.85	Yes; 0 violation	202.25 g/mol
10	446541	Mycophenolic acid	0.56	Yes; 0 violation	320.34 g/mol
11	6708755	Deoxysappanone B 7,3'-dimethyl ether acetate	0.55	Yes; 0 violation	356.37 g/mol
12	5311390	7-[2 trifluoromethyl-4-(2-hydroxyphenyl) -1,3-dioxancis-5-yl] -hept-5Z-enoic acid	0.55	Yes; 0 violation	374.35 g/mol
13	97143	3-hydroxy-3',4'-dimethoxyflavone 8	0.55	Yes; 0 violation	298.29 g/mol
14	6708795	Isotectorigenin, 7-Methyl ether	0.55	Yes; 0 violation	328.32 g/mol
15	6443968	Tuberonic acid	0.85	Yes; 0 violation	226.27 g/mol
16	14605548	Gibberellin A29	0.56	Yes; 0 violation	348.39 g/mol
17	6918612	Anisodamine	0.85	Yes; 0 violation	305.37 g/mol
18	14146249	8-(1-Hydroxyethyl)etodolac	0.56	Yes; 0 violation	303.35 g/mol
19	65411	Triptonide	0.56	Yes; 0 violation	358.39 g/mol
20	44230574	Naloxol	0.55	Yes; 0 violation	329.39 g/mol
21	5361092	Butorphanol	0.55	Yes; 0 violation	327.46 g/mol
22	54679299	2-Isoprenyl-3-hydroxy-5-methyl-a-pyrone	0.55	Yes; 0 violation	194.23 g/mol
23	5281969	Anandamide	0.55	Yes; 0 violation	347.53 g/mol
24	5283452	(Z)-N-(2-hydroxyethyl)icos-11-Enamide	0.55	Yes; 1 violation: MLOGP>4.15	353.58 g/mol
25	76468	Docosanamide	0.55	Yes; 1 violation: MLOGP>4.15	339.60 g/mol
26	5283127	LTB4 ethanol amide	0.55	Yes; 0 violation	379.53 g/mol

Target Prediction

Target prediction of the shortlisted phytochemicals was performed using SwissTargetPrediction with *Homo sapiens* selected as the target organism. A total of 705 compound–target interactions were initially identified. After removal of duplicate entries, 307 unique protein targets were obtained for further analysis. The predicted targets were

associated with diverse biological functions, including inflammatory response, oxidative stress, signal transduction, immune modulation, metabolic regulation, and endocrine-related processes. Representative targets included ABCG2, TRPC5, IP6K2, HDAC6, NOX4, CYP1B1, ALOX12, ALOX15, PKM, SIRT6, GSK3B, TLR4, MAPK3, NLRP3, NFKB1, HMGCR, FFAR1, and PRKCQ, along with several cytochrome P450 enzymes and receptor-mediated signaling proteins. The identified targets demonstrated the multi-target nature of the phytochemical profile and were subsequently used for hypothyroidism-associated target mapping, identification of overlapping genes, PPI network construction, and pathway enrichment analysis.

Disease Gene Retrieval

Hypothyroidism-associated genes were retrieved from the GeneCards and Online Mendelian Inheritance in Man (OMIM) databases using the keyword "Hypothyroidism." GeneCards yielded 7,194 gene symbols, while 288 disease-associated genes were obtained from OMIM. After merging the datasets and removing duplicate entries, a total of 7,209 unique hypothyroidism-associated genes were identified. The final disease-gene dataset was subsequently used for identification of common targets with the predicted *Clitoria ternatea* targets, PPI network construction, functional enrichment analysis, and compound–target–pathway network development.

Common Target Genes

Venn diagram analysis identified 158 common target genes shared between the predicted targets of *Clitoria ternatea* phytochemicals and hypothyroidism-associated genes. These overlapping targets included genes involved in endocrine regulation, inflammation, oxidative stress, metabolism, and cellular signaling. Representative targets included CA2, TLR4, CYP3A4, XDH, ACE, CFTR, MMP9, HDAC1, HDAC6, NOX4, AKT1, EGFR, STAT3, GSK3B, PPARG, PIK3CA, NFKB1, NLRP3, ESR1, INSR, BRAF, PARP1, VDR etc. The 158 common targets were subsequently subjected to KEGG pathway enrichment, PPI network analysis, and hub gene identification.

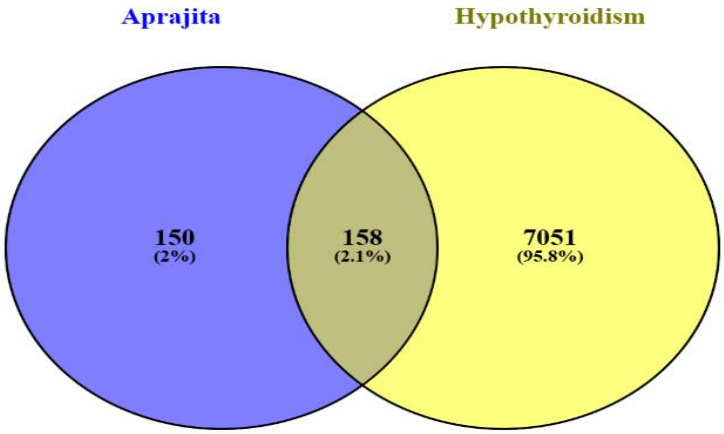
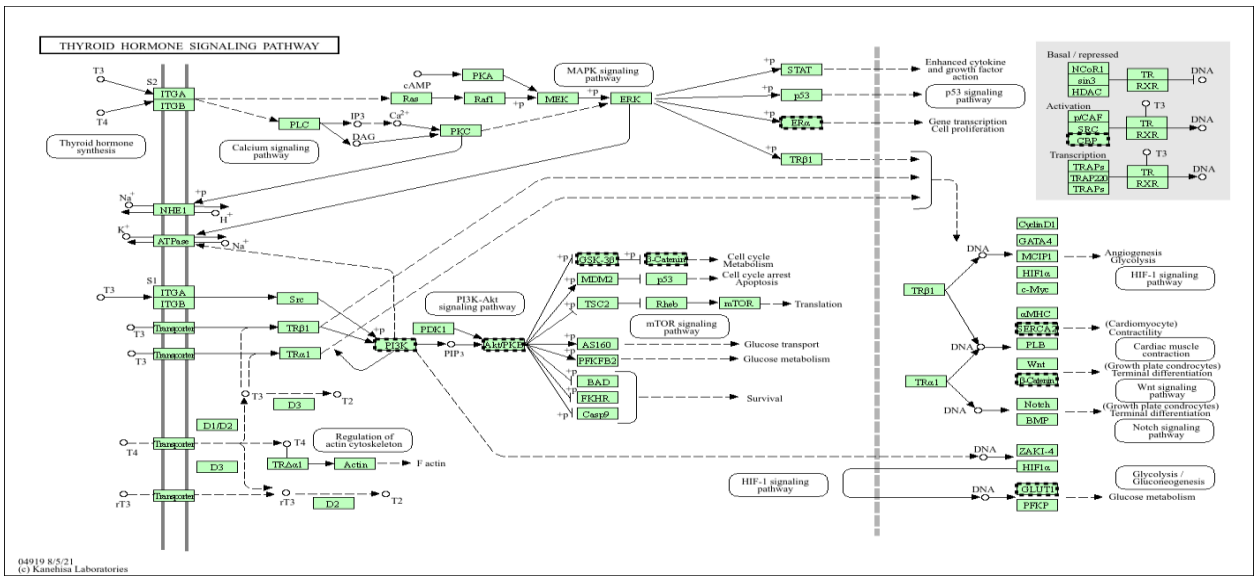
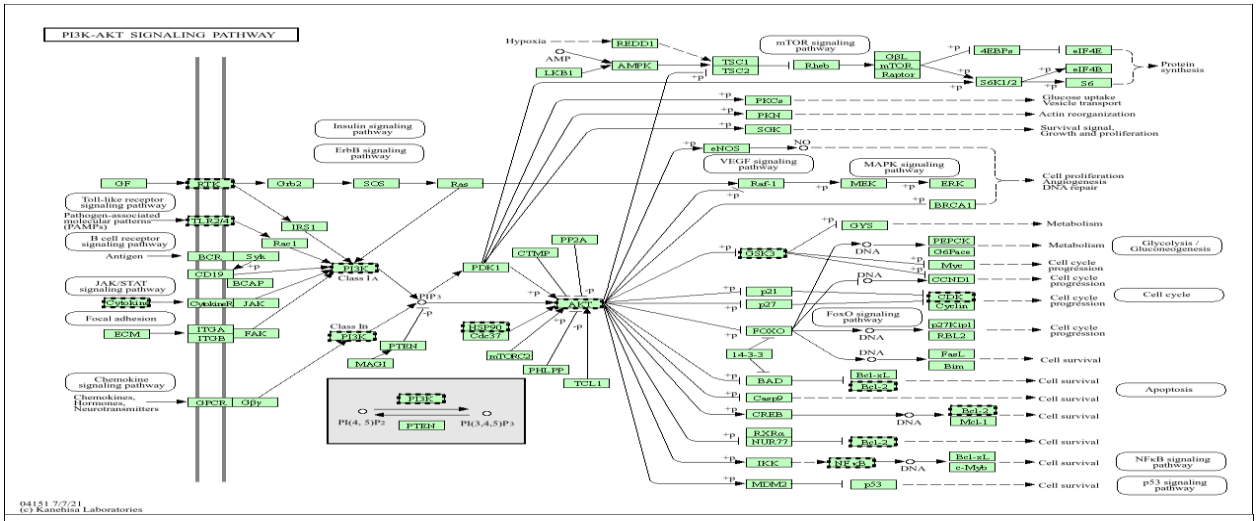


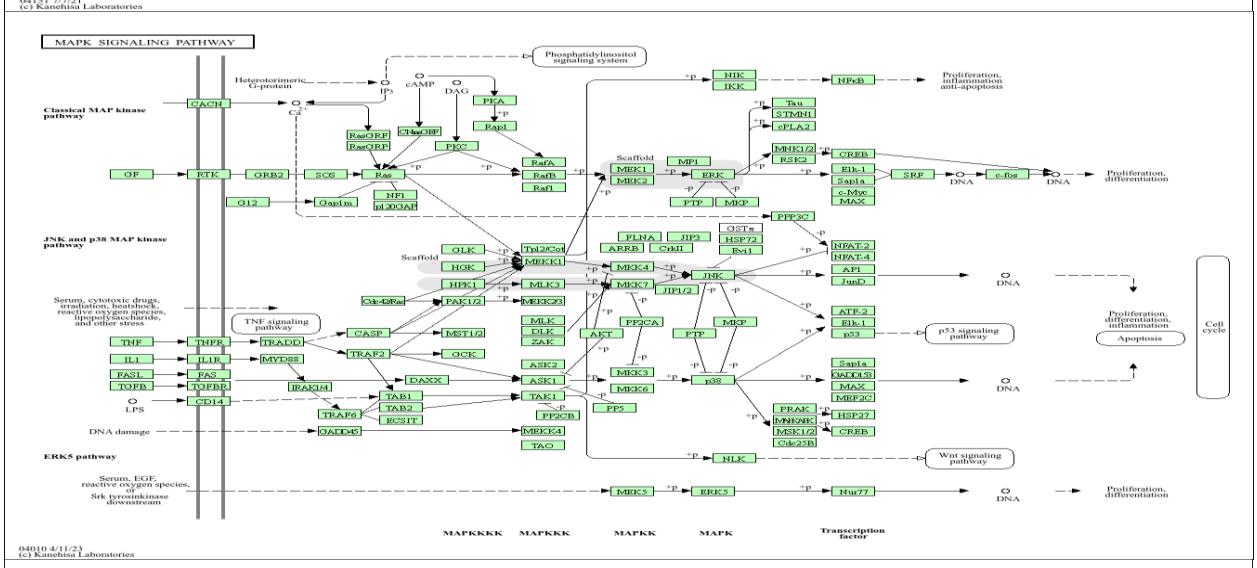
Figure 1 Venn diagram of *Clitoria ternatea* Linn And Hypothyroidism.

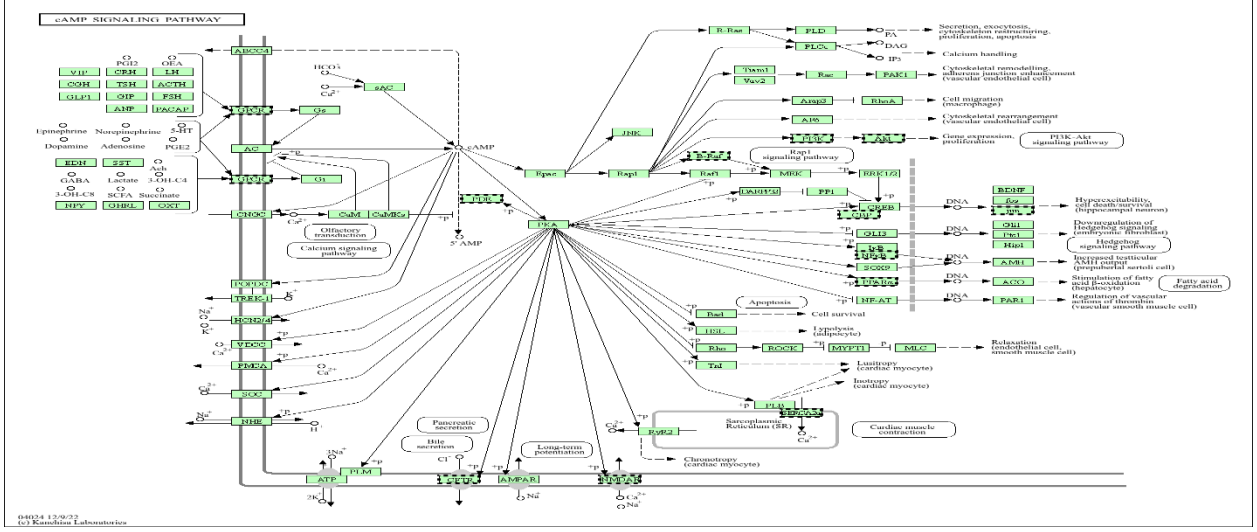
KEGG Pathway Enrichment Analysis

KEGG pathway enrichment analysis of the 158 common target genes was performed using DAVID Bioinformatics Resources. A total of 135 significantly enriched pathways were identified, of which 47 pathways were directly associated with hypothyroidism. The major enriched pathways included the thyroid hormone signaling, PI3K-Akt, MAPK, cAMP, FoxO, JAK-STAT, estrogen, PPAR, insulin, HIF-1, NF-κB, TNF, calcium, and steroid hormone signaling pathways. These pathways are associated with endocrine regulation, metabolic homeostasis, inflammatory responses, and cellular signaling relevant to hypothyroidism. The identified pathways were subsequently considered for further network analysis and biological interpretation.




PI3K-AKT SIGNALING PATHWAY
Hypoxia
REDD1
AMPK
LKB1
mTOR signaling pathway
TSC1
TSC2
Rheb
mTOR
Raptor
4EBPs
eIF4E
eIF4B
S6K1/2
S6
Protein synthesis
PKCs
PDK
SGK
Glucose uptake
Vesicle transport
Actin reorganization
Survival signal, Growth and proliferation
eNOS
NO
VEGF signaling pathway
MAPK signaling pathway
Raf-1
MEK
ERK
BRCA1
Cell proliferation
Angiogenesis
DNA repair
Insulin signaling pathway
ErbB signaling pathway
GF
RTK
Grb2
SOS
Ras
Toll-like receptor signaling pathway
Pathogen-associated molecular patterns (PAMPs)
TLR2/4
IRS1
B cell receptor signaling pathway
Antigen
BCR
Syk
Rac1
CD19
BCAP
JAK/STAT signaling pathway
Cytokine
CytokineR
JAK
Focal adhesion
ECM
ITGA
ITGB
FAK
Chemokine signaling pathway
Chemokines, Hormones, Neurotransmitters
GPCR
Gβγ
Class IA
Class Ia
PIP3
PDK1
PP2A
CTMP
AKT
PI3K
PTEN
MAGI
Class Ib
PIP2
Cdc37
mTORC2
PHLPP
TCL1
PI(4, 5)P2
PI(3,4,5)P3
GYS
Metabolism
PEPCK
G6Pase
Glycolysis / Gluconeogenesis
Myc
CCND1
Cell cycle progression
p21
p27
FoxO signaling pathway
FOXO
p27Kip1
RBL2
14-3-3
FasL
Bim
Cell survival
Bcl-xL
Bad
Casp9
CREB
Bcl-2
Mcl-1
RXRα
NUR77
IKK
NF-κB
c-Myb
MDM2
p53
Apoptosis
NFκB signaling pathway
p53 signaling pathway
04151 7/7/21
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MAPK SIGNALING PATHWAY
Classical MAP kinase pathway
JNK and p38 MAP kinase pathway
ERK5 pathway
Phosphatidylinositol signaling system
Heterotrimeric G-protein
GF
RTK
GRB2
SOS
Ras
G12
Gαq/11α
NF1
p120GAP
RasGRF
RasGEF
CalmBP
PKC
Rap1
RafA
RafB
Raf1
Scaffold
MP1
MEK1
MEK2
ERK
PTP
MKP
MKK1/2
RSK2
CREB
Elk-1
Sap1a
c-Myc
MAX
SRF
c-fos
Proliferation, differentiation
PPP3C
NFAT-2
NFAT-4
AP1
JunD
ATF-2
p53
p53 signaling pathway
Proliferation, differentiation, inflammation
Apoptosis
Cell cycle
Sap1a
DAXX
MAX
MEF2C
HSP27
CREB
Wnt signaling pathway
Proliferation, differentiation
Nur77
NIK
IKK
NFκB
Proliferation, inflammation anti-apoptosis
Tau
STMN1
cPLA2
FLNA
JIP3
ARRB
CrkII
GSTπ
HSP72
Evi1
Grb2/Col
MEKK1
GLK
HGK
HPK1
Cdc42/Rac
MLK3
MLK
DLK
ZAK
ASK2
ASK1
TAK1
TAB1
TAB2
ECSIT
TRAF6
MEKK4
TAO
MKK4
MKK7
MKK3
MKK6
JNK
p38
JIP1/2
PP2CA
PP5
PP2CB
AKT
PTP
MCP
PRAK
MNK1/2
MSK1/2
Cdc25B
MST1/2
GCK
CASP
TRADD
TRAF2
TNF signaling pathway
TNF
TNFR
IL1
IL1R
MYD88
FASL
FAS
TGFB
TGFBR
IRAK1/4
CD14
LPS
DNA damage
GADD45
Ca2+
CACH
CaMKIIF
PKA
Rap1
RapGEF
Gαs
Gαi
Gβγ
PLCβ
PIP2
IP3
DAG
cAMP
Ca2+
Gap1m
Ras
Raf1
MEK1/2
ERK1/2
Elk-1
Sap1a
c-Myc
MAX
SRF
c-fos
Proliferation, differentiation
Serum, cytotoxic drugs, irradiation, heatshock, reactive oxygen species, lipopolysaccharide, and other stress
Serum, EGF, reactive oxygen species, or Src tyrosinkinase downstream
MAPKKKK
MAPKKK
MAPKK
MAPK
Transcription factor
04010 4/11/23
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cAMP SIGNALING PATHWAY
ABCC4
VIP
PGD2
OEA
CRH
LH
CGH
TSH
ACTH
GLP1
OIP
FSH
AMP
PACAP
Epinephrine
Norepinephrine
5-HT
Dopamine
Adenosine
PGE2
EDN
2ST
Ach
GABA
Lactate
3-OH-C4
3-OH-C8
SCFA
Succinate
NPY
GHRL
OXT
HCO3-
sAC
Ca2+
Gs
AC
Gi
cAMP
5'AMP
PKA
Rpac
Rap1
JNK
R-Ras
PLD
PLCε
PA
DAG
IP3
Secretion, exocytosis, cytoskeleton restructuring, proliferation, apoptosis
Calcium handling
Tiam1
Vav2
Rac
PAK1
Cytoskeletal remodelling, adherens junction enhancement (vascular endothelial cell)
Arap3
RhoA
Cell migration (macrophage)
Cytoskeletal rearrangement (vascular endothelial cell)
Gene expression, proliferation
PI3K-Akt signaling pathway
Rap1 signaling pathway
MEK
ERK1/2
PPI
CREB
BDNF
Hyperexcitability, epileptogenesis (hippocampal neuron)
Ghh
Ptc1
Hip1
Hedgehog signaling pathway
Downregulation of Hedgehog signaling (cerebellar fibroblast)
AMH
Increased testicular sertoli cell (prepubertal sertoli cell)
AQ2
Stimulation of fatty acid degradation (hepatocyte)
Fatty acid degradation
PAR1
Regulation of vascular actions of thrombin (vascular smooth muscle cell)
CNGC
CaM
CaMKs
Olfactory transduction
Calcium signaling pathway
HCN1/2/4
TREK
VDCC
PMCA
NCX
NHE
Na+
K+
H+
Cl-
Pancreatic secretion
Bile secretion
Long-term potentiation
PLM
ATP
2K
AMPAR
NMDAR
Ca2+
Na+
Apoptosis
Bad
Cell survival
LIPE
Lipolysis (adipocyte)
Tnt
MYPT1
MLC
Relaxation (endothelial cell, smooth muscle cell)
Luzitropy (cardiac myocyte)
Inotropy (cardiac myocyte)
PLB
SERCA
Sarcoplasmic Reticulum (SR)
RYR2
Cardiac muscle contraction
Chronotropy (cardiac myocyte)
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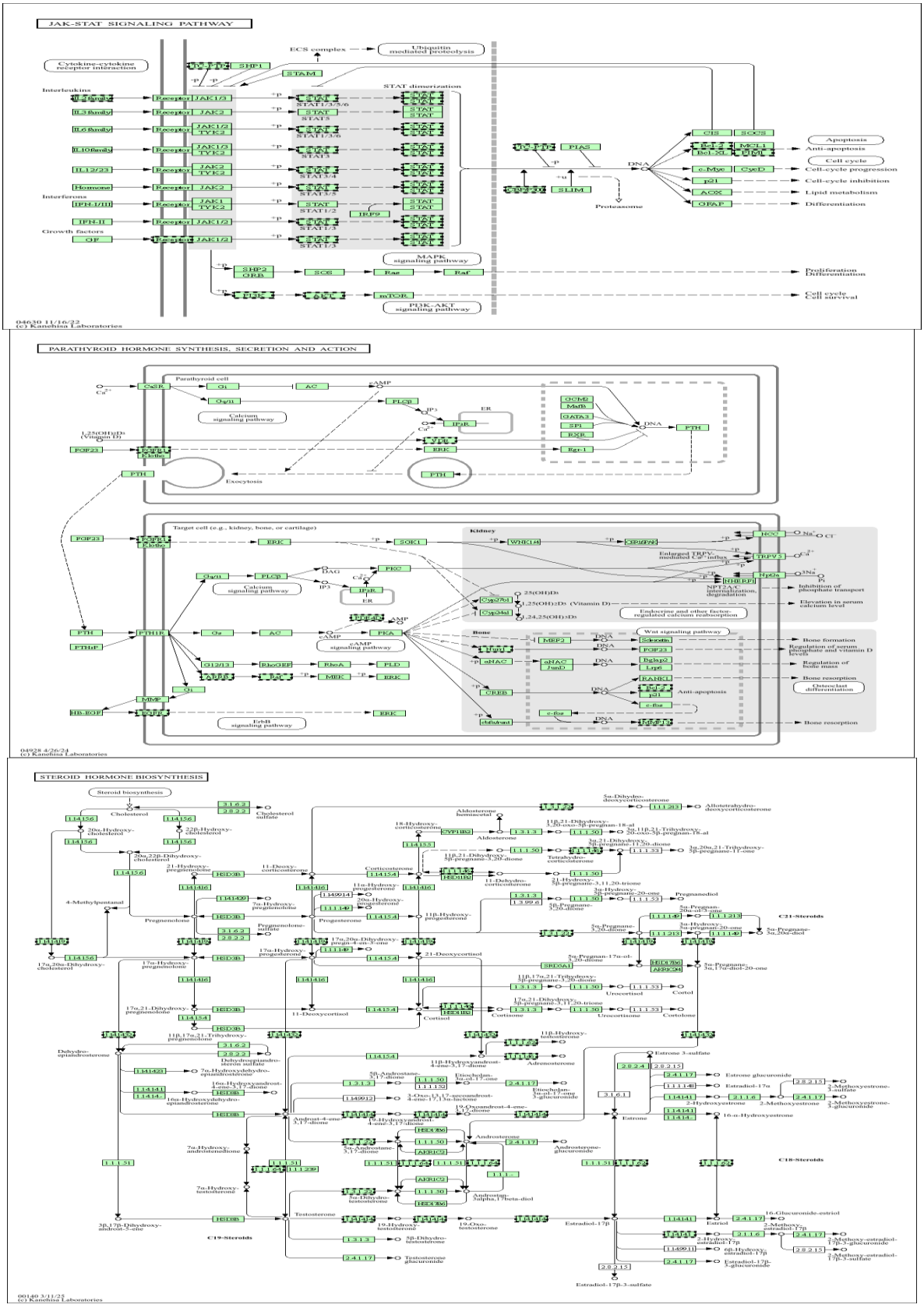


Figure 2: Database for Annotation, Visualization, and Integrated Discovery pathway analysis. (a) Thyroid Hormone Signaling Pathway (b) PI3K-Akt signaling Pathway. (c) MAPK signaling Pathway (d) cAMP signaling pathway. (e) JAK-STAT signaling

pathway. (f) Parathyroid hormone synthesis, secretion_and action (g) Steroid hormone biosynthesis.

Network Analysis

The compound–target interaction network was constructed using Cytoscape version 3.10.3 to visualize the relationships among *Clitoria ternatea* phytoconstituents and hypothyroidism-associated target genes. The network demonstrated a complex multi-component and multi-target interaction pattern, with several phytochemicals interacting with multiple target proteins and several targets being regulated by multiple phytoconstituents.

The observed network architecture highlighted the polypharmacological nature of *Clitoria ternatea* and its potential to modulate multiple molecular targets and signaling pathways associated with hypothyroidism. The identified interactions were further considered for hub gene analysis and mechanistic interpretation.

Multi-Algorithm Hub Gene Ranking

Hub gene analysis was performed using the CytoHubba plugin in Cytoscape version 3.10.3 to identify highly connected and topologically important genes within the interaction network. A multi-algorithm approach was employed using Degree, Maximal Clique Centrality (MCC), Edge Percolated Component (EPC), and Closeness centrality to obtain a robust identification of key network nodes.

The Degree algorithm evaluated direct connectivity, while MCC, EPC, and Closeness assessed network centrality and topological importance from different perspectives. The integrated analysis enabled the identification of consensus hub genes that demonstrated high network connectivity and potential relevance to the molecular mechanisms associated with the activity of *Clitoria ternatea* in hypothyroidism.

Multi-Algorithm Hub Gene Ranking of *Clitoria ternatea*

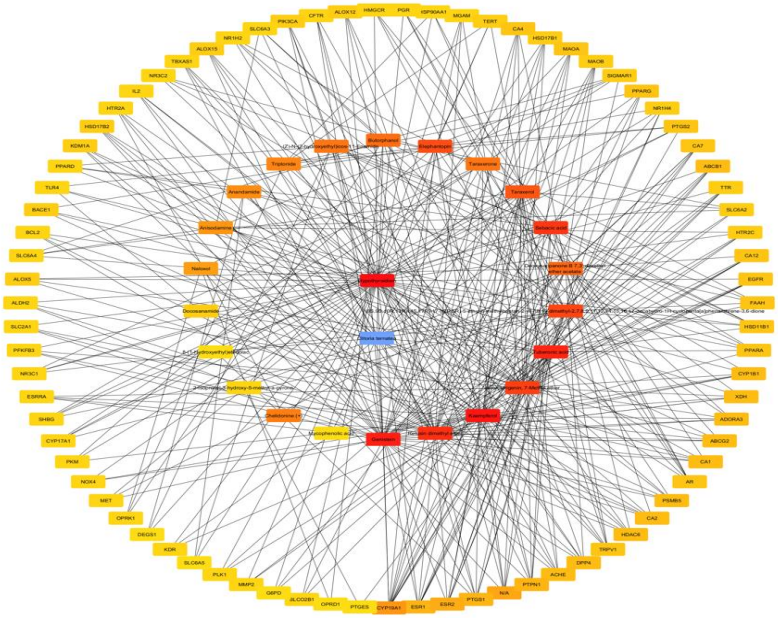


Figure 3: Top 100 Hub Genes of *Clitoria ternatea* Linn.

Multi-Algorithm 100 Hub Gene Ranking

CytoHubba analysis using Degree, MCC, EPC, and Closeness algorithms identified the top 100 hub genes within the interaction network. The prominent hub genes included ESR1, ESR2, PTGS1, PTGS2, PTPN1, CA1, CA2, ADORA3, PSMB5, ACHE, ABCB1, DPP4, XDH, CYP1B1, HDAC6, ABCG2, PPARA, and HSD11B1. These genes showed high topological importance and were considered key candidate targets associated with the potential molecular effects of *Clitoria ternatea* in hypothyroidism.

Multi-Algorithm Hub Gene Ranking of *Clitoria ternatea*

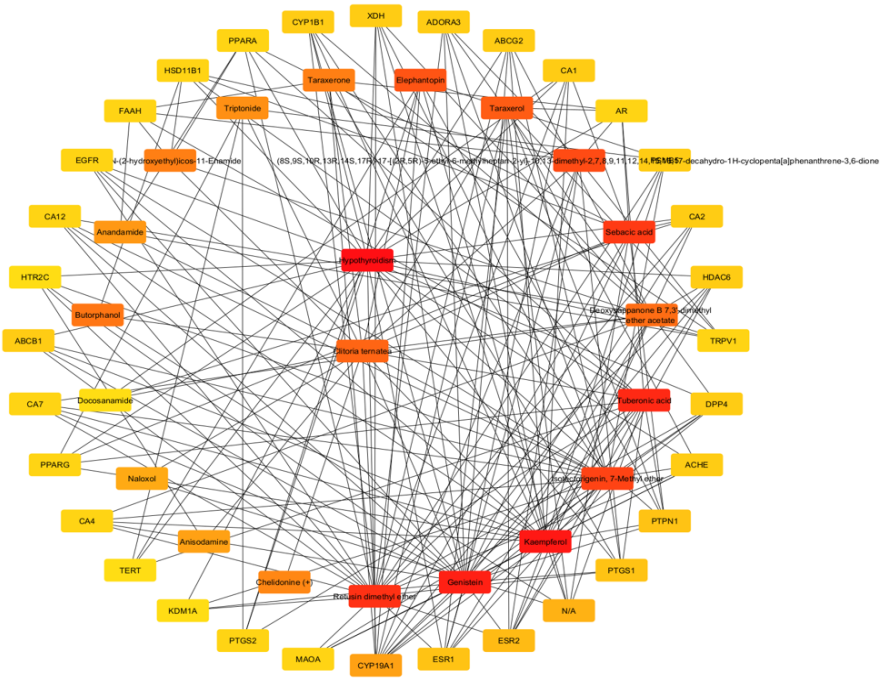


Figure 4: Top 50 Hub Genes of *Clitoria ternatea.*

Multi-Algorithm 50 Hub Gene Ranking

Topological analysis of the PPI network was performed using CytoHubba in Cytoscape 3.10.3, employing Degree, MCC, EPC, and Closeness algorithms for consensus hub identification. The analysis identified the top 50 hub genes, with prominent targets including PTPN1, CA1, ADORA3, PSMB5, ACHE, CA2, ABCB1, DPP4, XDH, CYP1B1, HDAC6, and ABCG2. Several *Clitoria ternatea* phytoconstituents showed high network connectivity with these targets, particularly Kaempferol, Genistein, Tuberonic acid, Retusin dimethyl ether, Sebacic acid, Isotectorigenin-7-methyl ether, Elephantopin, and Taraxerol. These findings identified the major hub targets and phytochemical–target interactions within the network associated with hypothyroidism.

Conclusion of Supplementary Network Analysis

The supplementary network pharmacology analysis indicated that *Clitoria ternatea* Linn. interacts with multiple molecular targets and signaling pathways associated with hypothyroidism. Consensus hub analysis identified important targets including ESR1, ESR2, PTPN1, CA1, CA2, ADORA3, DPP4, XDH, CYP1B1, HDAC6, PPARA, EGFR, PPARG, and TRPV1, along with several other functionally relevant genes.

Phytoconstituents such as Kaempferol, Genistein, Tuberonic acid, Retusin dimethyl ether, Sebacic acid, Isotectorigenin-7-methyl ether, Elephantopin, and Taraxerol showed interactions with multiple targets. Overall, the findings support a multi-target and multi-pathway pharmacological profile of *Clitoria ternatea* and provide molecular-level insights that complement the observed in vivo findings related to its potential thyrogenic activity.

DISCUSSION$

The present exploratory network pharmacology study provides a systems-level perspective on the potential molecular basis of the thyrogenic activity of *Clitoria ternatea* Linn. root in hypothyroidism. The selection of the root is supported by its classical indication in *Galganda*, while the network-based approach enabled investigation of the complex interactions between its phytoconstituents, disease-associated genes, and signaling pathways.

An integrated phytochemical approach combining IMPPAT 2.0, published literature, and LC–MS profiling identified 32 phytoconstituents, of which 26 compounds fulfilled the predefined drug-likeness and ADME criteria. The retention of a substantial proportion of the identified compounds indicates considerable chemical diversity with potentially favorable pharmacokinetic characteristics. This finding provides a rational basis for further target-based investigation and is consistent with the multi-component nature of medicinal plants.

Target prediction further demonstrated the broad molecular interaction profile of the selected phytochemicals. A total of 705 compound–target interactions corresponding to 307 unique protein targets were identified. These targets were associated with endocrine regulation, metabolism, inflammatory responses, oxidative stress, immune modulation, and intracellular signaling. Such a broad target distribution supports the possibility that the biological effects of *Clitoria ternatea* may arise through modulation of multiple interconnected molecular processes rather than through a single target.

Integration with hypothyroidism-associated genes identified 158 common targets, providing a focused set of molecular links between the phytochemical profile and the disease network. Important overlapping targets included AKT1, EGFR, PIK3CA, PTPN1, PPARG, ESR1, TLR4, NFKB1, NLRP3, HDAC6, NOX4, XDH, and CA2, among others. These targets participate in endocrine signaling, metabolic regulation, inflammatory responses, oxidative stress, and cellular signaling, all of which are relevant to the systemic disturbances associated with hypothyroidism.

KEGG enrichment of the common targets identified 135 significantly enriched pathways, including 47 pathways associated with hypothyroidism. Of particular relevance were the thyroid hormone, cAMP, PI3K–Akt, MAPK, JAK–STAT, PPAR, insulin, HIF-1, NF-κB, TNF, calcium, estrogen, and steroid hormone signaling pathways. The enrichment of thyroid hormone signaling is especially relevant because thyroid hormones regulate metabolic activity and energy homeostasis, while the associated cAMP and intracellular kinase pathways participate in hormonal signal transduction and cellular responses.

The PI3K–Akt and MAPK pathways provide an important mechanistic link between endocrine signaling and metabolic or cellular responses. Targets such as AKT1, PIK3CA, INSR, EGFR, PTPN1, BRAF, and MAPK3 were associated with these networks. Their involvement suggests that the phytochemical constituents may potentially influence pathways related to cellular survival, metabolic regulation, receptor signaling, and stress responses. Similarly, enrichment of the cAMP pathway is noteworthy because cyclic AMP participates in hormonal signal transduction, including pathways involved in thyroid regulation.

The enrichment of HIF-1, NF-κB, and TNF signaling further indicates the involvement of cellular stress and inflammatory processes. Hypothyroidism is associated with metabolic alterations and may involve oxidative and inflammatory disturbances. In the present network, genes such as NOX4, NFKB1, TLR4, AKT1, and MAPK-associated targets linked these pathways with the phytochemical–disease interaction network. Thus, the computational findings suggest that modulation of oxidative stress and inflammatory signaling may form part of the broader molecular profile of *Clitoria ternatea*.

The enrichment of PPAR, insulin, adipocytokine, calcium, VEGF, estrogen, and steroid hormone-related pathways further reflects the interconnected nature of endocrine and metabolic regulation. Targets including PPARA, PPARG, ESR1, AKT1, and MAPK-associated proteins were represented within these networks. Such pathway associations are relevant because thyroid dysfunction can influence energy metabolism, lipid handling, hormonal signaling, and cellular homeostasis. However, these pathway associations should be interpreted as predicted molecular relationships rather than evidence of direct pathway activation by the plant.

Hub analysis using multiple CytoHubba algorithms provided additional insight into the central nodes of the network. The identification of genes such as ESR1, ESR2, PTPN1, CA1,

CA2, DPP4, XDH, CYP1B1, HDAC6, ABCG2, PPARA, EGFR, and PTGS1/PTGS2 among the highly ranked targets indicates their topological importance within the constructed network. These genes are associated with diverse processes including endocrine regulation, metabolism, inflammatory signaling, oxidative stress, and cellular regulation. Their high connectivity suggests that they may represent candidate molecular nodes through which multiple phytoconstituents could influence the hypothyroidism-associated network.

Several phytoconstituents, particularly kaempferol, genistein, tuberonic acid, retusin dimethyl ether, sebacic acid, isotectorigenin-7-methyl ether, elephantopin, and taraxerol, showed substantial connectivity within the compound–target network. The prominent representation of kaempferol and genistein across several signaling networks is noteworthy because the computational findings associate these compounds with pathways involved in metabolic, inflammatory, oxidative, and endocrine regulation. Nevertheless, the present network analysis identifies these compounds as candidate contributors based on predicted interactions and does not establish their individual efficacy or synergistic action.

Overall, the findings indicate that the potential activity of *Clitoria ternatea* Linn. root in hypothyroidism involve a multi-component, multi-target, and multi-pathway molecular profile. The convergence of phytochemical targets with hypothyroidism-associated genes and enrichment of thyroid, endocrine, metabolic, inflammatory, and stress-related pathways provides mechanistic support for its traditional use in *Galganda*. Importantly, these findings are computational predictions and should be validated through targeted molecular, biochemical, and pharmacological studies. Thus, the present network analysis serves as a supplementary mechanistic framework for understanding the potential thyrogenic activity of *Clitoria ternatea* root.

REFERENCES

1. Zamwar UM, Muneshwar KN. Epidemiology, Types, Causes, Clinical Presentation, Diagnosis, and Treatment of Hypothyroidism. Cureus, 2023 Sep 30; 15(9): e46241. doi:10.7759/cureus.46241. PMID: 37908940; PMCID: PMC10613832.
2. Bagcchi S. Hypothyroidism in India: more to be done. Lancet Diabetes Endocrinol, 2014 Oct; 2(10): 778. doi:10.1016/S2213-8587(14)70208-6. PMID: 25282085.
3. Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. Indian journal of endocrinology and metabolism, 2011 Jul 1; 15(Suppl2): S78-81.

4. Chunekar KC, Pandey GS., Bhavprakash nighantu. Chaukhambha Bharati Academy, Varansi, 2002; p327 Guduchiadivargya/111.
5. Chunekar KC, Pandey GS., Bhavprakash nighantu. Chaukhambha Bharati Academy, Varansi, 2002; p327., Guduchiadivargya/112.
6. Kumar Roy. Rajeev, Editor- Dr. Ram Kumar Roy., Vangasena Samhita, Varanasi: Prachya Prakashan, First Edition, 1983; p548.
7. Sastri. Ravidutta, Chakradutta of Chakrapani dutta with Padarthabodhini" hindi Commentary Published by Chaukhamba Orientalia, Varanasi, P221.
8. Taur DJ, Patil RY. Evaluation of antiasthmatic activity of Clitoria ternatea L. roots. Journal of ethnopharmacology, 2011 Jun 22; 136(2): 374-6.
9. Tripathi. BN, Madavnidanam, Madhukoshavyakha, vol 2 edition by Chaukhamba surbharti prakashan, Varanasi chaper 38th.
10. Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J. Harrison's Principles of Internal Medicine 14th edition Vol.2 p2020.
11. Luo T.T., Lu Y., Yan S.K., Xiao X., Rong X.L., Guo J. Network Pharmacology in Research of Chinese Medicine Formula: Methodology, Application and Prospective. Chin. J. Integr. Med, 2020; 26: 72–80. doi:10.1007/s11655-019-3064-0
12. Chandran U, Mehendale N, Tillu G, Patwardhan B. Network Pharmacology of Ayurveda Formulation Triphala with Special Reference to Anti-Cancer Property. Comb Chem High Throughput Screen, 2015; 18(9): 846-54. doi:10.2174/1386207318666151019093606. PMID: 26477351.
13. Zhang Z, Zhou J, Guo R, Zhou Q, Wang L, Xiang X, Ge S, Cui Z. Network pharmacology to explore the molecular mechanisms of Prunella vulgaris for treating thyroid cancer. Medicine (Baltimore), 2023 Nov 10; 102(45): e34871. doi:10.1097/MD.0000000000034871. PMID: 37960775; PMCID: PMC10637567.
14. Institute of Mathematical Sciences. IMPPAT: Indian Medicinal Plants, Phytochemistry and Therapeutics. Chennai: The Institute of Mathematical Sciences. Available from: https: //cb.imsc.res.in/ imppat/./. [Last accessed on 2026 July 17].
15. National Center for Biotechnology Information. PubChem Database. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available from: https: // pubchem.ncbi.nlm.nih.gov/./. [Last accessed on 2026 July 17].
16. MolSoft LLC. Drug-Likeness and Molecular Property Explorer. San Diego (CA): MolSoft LLC. Available from: https: //www. molsoft.com/mprop/. [Last accessed on 2026 July 17].

17. Shen M, Tian S, Li Y, Li Q, Xu X, Wang J, et al. Drug-likeness analysis of traditional Chinese medicines: 1. property distributions of drug-like compounds, non-drug-like compounds and natural compounds from traditional Chinese medicines. J Cheminform, 2012; 4: 31.
18. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep, 2017; 7: 42717.
19. Swiss ADME. Available from: https: //www.swissadme.ch/. [Last accessed on 2026 July 17].
20. SwissTargetPrediction. Available from: https: //www. swisstargetprediction.ch/. [Last accessed on, 2026 July 17].
21. Daina A, Michielin O, Zoete V. SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res, 2019; 47: W357-64.
22. GeneCards – The Human Gene Database. Available from: https: // www.genecards.org [Last accessed on 2026 July 20, 15: 58 IST]
23. Online Mendelian Inheritance in Man (OMIM). Baltimore (MD): Johns Hopkins University. Available from: https: //www.omim. org/. [Last accessed on 2025 July 20].
24. Lam F, Lalansingh CM, Babaran HE, Wang Z, Prokopec SD, Fox NS, Boutros PC. VennDiagramWeb: a web application for the generation of highly customizable Venn and Euler diagrams. BMC Bioinformatics, 2016 Oct 3; 17(1): 401. doi:10.1186/s12859-016-1281-5. PMID: 27716034; PMCID: PMC5048655.
25. Bioinformatics and Evolutionary Genomics. Calculate and Draw Custom Venn Diagrams. Universiteit Gent. Available from: https: //bioinformatics.psb.ugent.be/webtools/Venn/. [Last retrieved on 2025 July 22].
26. Huang Da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc, 2009; 4: 44-57.
27. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res, 2000 Jan 1; 28(1): 27-30. doi:10.1093/nar/28.1.27.PMID:10592173;PMCID: PMC102409.
28. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res, 2003 Nov; 13(11): 2498-504. doi:10.1101/gr.1239303. PMID: 14597658; PMCID: PMC403769.

29. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol, 2014; 8 Suppl 4(Suppl 4): S11. doi:10.1186/1752-0509-8-S4-S11. Epub 2014 Dec 8. PMID:25521941; PMCID: PMC4290687.
30. Santri IN, Irham LM, Djalilah GN, Perwitasari DA, Wardani Y, Phiri YV, Adikusuma W. Identification of hub genes and potential biomarkers for childhood asthma by utilizing an established bioinformatic analysis approach. Biomedicines, 2022 Sep 16; 10(9): 2311.