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Research Article

NETWORK PHARMACOLOGY AND MOLECULAR DOCKING-BASED INVESTIGATION OF *CALOTROPIS GIGANTEA* LINN. LEAF EXTRACT AGAINST VULVOVAGINAL CANDIDIASIS

VASUNDHARA B BHOSALE[1], AKSHADA AMIT KOPARDE[2]*, VANDANA M THORAT[3], MAYURI V BHOSALE[1]

[1]Department of Pharmaceutical Sciences, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad, Maharashtra, India. [2]Department of Pharmaceutical Chemistry, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad, Maharashtra, India. [3]Department of Pharmacology, Krishna Institute of Medical Science, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad, Maharashtra, India.

***Corresponding author: Akshada Amit Koparde; Email: akshadakakade@gmail.com**

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ABSTRACT

Objectives: This study aimed to elucidate the multitarget therapeutic mechanisms of *Calotropis* gigantea leaf extract against vulvovaginal candidiasis (VVC) using a network pharmacology (NP) approach integrated with molecular docking.

Methods: Gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract of *C. gigantea* leaves identified 38 phytochemicals. Drug-likeness was assessed through SwissADME, and target prediction was performed using Super-PRED and UniProt. VVC-related genes from GeneCards were compared using Venny, and common targets were analyzed through STRING for protein–protein interaction. Pathway enrichment and network construction were done through Kyoto Encyclopedia of Genes and Genomes (KEGG) and Cytoscape. Key compounds were docked with hub protein toll-like receptor 4 (TLR4) using Molsoft ICM.

Results: GC-MS identified key bioactives like 3,6-Octadienal, 3,7-dimethyl- and 1,3,8-p-Menthatriene. Ten overlapping targets with VVC genes were found, with TLR4 as a major hub. KEGG analysis highlighted four pathways, including Toll-like receptor signaling. Docking revealed strong binding of 3,6-Octadienal, 3,7-dimethyl- to TLR4 (−24.9 kcal/mol), exceeding clotrimazole (-18.82 kcal/mol).

Conclusion: *C. gigantea* Linn. shows promise as a multi-target antifungal agent against VVC by modulating immune pathways. NP and docking highlight 3,6-Octadienal, 3,7-dimethyl, as a key bioactive. Among the identified compounds, 3,6-Octadienal, 3,7-dimethyl- exhibited the strongest binding affinity toward TLR4; however, its low concentration (0.11% relative peak area) indicates that its biological impact may be limited and potentially synergistic with other more abundant phytoconstituents. Further *in vitro* and *in vivo* studies are needed to validate these findings and develop clinical formulations.

Keywords: Network pharmacology, Vulvovaginal candidiasis, *Calotropis gigantea* Linn, Molecular docking, 3,6-Octadienal, 3,7-dimethyl-.

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INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common mucosal infection that affects the lower female reproductive tract. It is primarily caused by *Candida albicans*, an opportunistic fungus with polymorphic characteristics. *C. albicans* is a natural part of the human microbiota and can exist in the vaginal environment without triggering any symptoms under normal conditions [1]. VVC remains a prevalent fungal infection, with nearly three-quarters of women experiencing it at least once in their lifetime. *C. albicans* is the predominant causative agent, responsible for 85–95% of reported cases [2]. Although *C. albicans* is the principal etiological agent of VCC, non-*albicans* species such as *Candida glabrata, Candida tropicalis, Candida krusei,* and *Candida parapsilosis* are also capable of colonizing the vaginal niche and contributing to infection. Typically part of the normal vaginal microbiota, *Candida* species can shift from a commensal to a pathogenic state under certain yet unidentified conditions, leading to disease manifestation [3]. VVC is characterized by clinical symptoms such as thick, white, curd-like vaginal discharge, vulvovaginal pruritus, erythema, burning sensations, and dyspareunia. The development of VVC is often associated with a range of predisposing factors, including recent antibiotic use, elevated estrogen levels due to pregnancy, hormonal contraceptives, or hormone replacement therapy, poorly controlled diabetes mellitus, increased sexual activity, and the frequent use of tight-fitting clothing [4].

A subset of women develops recurrent VVC (RVVC), which is characterized by multiple symptomatic episodes separated by symptom-free intervals. RVVC is commonly defined as the occurrence of three or more clinically confirmed episodes within a 12-month period, though some authors suggest a stricter criterion of four episodes annually for diagnosis [5].

Although first-line antifungal therapies, including fluconazole and imidazole agents, result in improved quality of life for approximately 96% of women with VVC, recurrence remains a significant issue, with nearly 63% experiencing repeated infections despite completion of maintenance therapy [6]. Persistent vaginal colonization by *Candida* species often leads to significant discomfort and psychological distress among affected women, particularly in cases where standard antifungal therapies prove ineffective, thereby highlighting critical challenges in the development of future treatment strategies [7-9]. VVC continues to pose a significant public health concern owing to its high global prevalence, frequent persistence of vaginal *Candida* colonization following standard antifungal therapy, and the adverse effects associated with prolonged use of topical azole agents. Recently, two novel antifungal agents – oteseconazole and ibrexafungerp – have emerged as promising therapeutic alternatives, demonstrating enhanced efficacy and a reduced incidence of adverse effects. Azole antifungals exert their action by inhibiting the cytochrome P450 enzyme sterol 14α-demethylase (CYP51), also known as lanosterol 14α-demethylase, which is essential for

converting lanosterol to ergosterol, a fundamental component of the fungal cell membrane [10].

Calotropis gigantea Linn. (Family Asclepiadaceae) is commonly found in dry, barren areas. Its leaves are obovate with a cordate base and measure about 6–20 cm long. Conventionally, the plant is used to manage conditions such as sprains, fatigue, epilepsy, and pain and is also known for its role in treating pregnancy-related issues, toothaches, and earaches [11,12]. Dried leaves of *C. gigantea* are known for their expectorant and anti-inflammatory effects and are used to treat conditions such as paralysis and rheumatic pain. Its leaf extract contains bioactive antifungal compounds, effective against *C. albicans*, and can support the synthesis of silver nanoparticles (AgNP) [13,14].

Network pharmacology (NP) is an interdisciplinary approach grounded in systems biology, integrating multiple pharmacological pathways to understand drug actions and disease mechanisms [34]. Unlike traditional single-target approaches, NP explores how drugs act on multiple targets. It merges computational tools with molecular biology and pharmacology to map disease–target–drug interactions using available data on genes, proteins, and diseases [15].

This study employs NP to identify key bioactive compounds from *C. gigantea* Linn. and explore their potential mechanisms against VVC. Gas chromatography-mass spectrometry (GC-MS) was used to identify plant compounds, which were then screened for drug-likeness. VVC-related genes and compound-associated targets were retrieved from public databases, and their overlap was analyzed through Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. A key signaling pathway and hub gene were identified, followed by virtual screening of selected compounds against the hub gene [16].

Similar methodological approaches of integrating network pharmacology with molecular docking have been successfully used in other plant-based investigations as well [27].

Molecular docking is a computational technique that predicts how ligands bind to receptor proteins, aiding in the design of new therapeutic agents. It is a key tool in structure-based drug discovery and plays a vital role in modern drug development [17,18]. Network pharmacology–based analyses on other medicinal plants have integrated molecular docking to identify bioactive compounds, their signaling pathways, and their key target interactions, supporting the scientific approach used in the present study [28, 32].

NP and molecular docking together provide a comprehensive *in silico* framework ideally suited for the study of complex herbal systems. Herbal extracts such as *C. gigantea* Linn. contain numerous phytoconstituents that can act on multiple molecular targets simultaneously, producing therapeutic effects through synergistic and multi-pathway interactions. Traditional single-target drug discovery approaches often fail to capture this complexity, as they focus on isolated compounds and individual targets. In contrast, NP systematically integrates these multi-component interactions at the molecular and systems levels, while molecular docking provides atomic-level insight into the binding affinity and interaction mode of the active compounds with specific protein targets. Hence, combining these two approaches allows for a mechanistic understanding of the overall pharmacological potential of *C. gigantea* against VVC, laying the foundation for subsequent experimental validation. Thus, this study aimed to identify a bioactive phytoconstituent through NP and molecular docking approaches.

METHODS

Plant collection

The leaves of *C. gigantea* Linn were collected from the rural areas of Shirasi and Bahadurwadi, Sangli, Maharashtra, India. The plant specimen was authenticated by the Department of Botany, Sadguru Gadage Maharaj College, Karad, ensuring accurate identification and classification.

Drying and size reduction

Leaves of *C. gigantea* Linn. were washed, shade-dried at room temperature, and ground into coarse powder using a mechanical grinder [19].

Extraction of *C. gigantea* Linn leaves

To prepare the methanolic extract, 100 g of dried leaf powder was extracted with 1,000 mL of methanol using a Soxhlet apparatus at 50°C for 24 h. The filtrate was collected, concentrated using a rotary evaporator, and stored at 4°C in airtight containers for further use [11].

Phytochemical screening

Preliminary phytochemical screening was performed using standard methods to identify bioactive compounds such as tannins, saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, carbohydrates, proteins, anthraquinones, polyphenols, and glycosides [20,21,33].

GC-MS analysis

GC-MS analysis of the methanolic leaf extract of *C. gigantea* was conducted using a Thermo Scientific Triple Quadrupole system (Trace 1,300 GC with TSQ 8,000 MS) and a TG 5MS column (30 m × 0.25 mm, 0.25 μm). Helium served as the carrier gas at 1 mL/min flow rate with a 1.0 μL injection volume. The injector and ion source temperatures were set at 250°C and 230°C, respectively. The oven temperature was initially 50°C and programmed to reach 280°C [13,45].

Compound database construction and drug-likeness filtering

The information on chemical compounds from *C. gigantea* Linn. was identified using GC-MS analysis. The 38 compounds detected in GC-MS were filtered to select chemical compounds of above 1% relative peak area. PubChem (https://pubchem.ncbi.nlm.nih.gov/) was utilized for the identification of simplified molecular input line entry system (SMILES). The SwissADME (http://www.swissadme.ch/) based SMILES was used to identify the "Drug-likeness" property [16].

Compounds were evaluated for drug-likeness based on Lipinski's Rule of Five, wherein drug-like compounds are expected to have no more than one violation of the five criteria.

a. It must have <5 hydrogen bond donors
b. It must have <10 hydrogen bond acceptors
c. The molecular weight should not be more than 500 daltons
d. The log p-value should be <5 [41].

Identification of target proteins associated with bioactives and VVC

In the NP workflow, potential targets of the identified phytoconstituents were predicted using the Super-PRED database (https://prediction.charite.de) with a probability threshold of ≥0.7 to ensure high-confidence associations where *Homo sapiens* was selected as the species. VVC-related targets were retrieved from the GeneCards database (https://www.genecards.org/) using a relevance score ≥50 as the selection criterion to obtain well-supported gene–disease associations. Overlapping targets between disease and phytochemicals were visualized using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) [22].

Protein–protein interaction (PPI)

A PPI network was generated using the STRING database (https://cn.string-db.org/), focusing on overlapping targets between *C. gigantea* and VVC, with the species restricted to Homo sapiens. A confidence score of 0.150 was set to ensure the highest reliability from the STRING database [45]. These shared genes were further analyzed for gene–gene interactions and KEGG pathway enrichment through the STRING platform [23].

Construction of a component-disease-pathway-target network

Targets related to both *C. gigantea* Linn. compounds and VVC pathways were organized in Excel. Initially, disease-related genes (Node 1) were imported into Cytoscape 3.7.2 [44] to construct a disease network. Then, targets of the plant's active compounds (Node 2) were added.

The two networks were merged using Cytoscape's merge function to identify potential therapeutic targets of *C. gigantea* in VVC. The resulting network highlighted interactions between plant compounds and disease-related genes. Network visualization and analysis were performed using the Network Analyzer plugin, while hub genes were identified using the CytoHubba module [24-26].

Molecular docking

Molecular docking facilitates the study of ligand–protein interactions, helping identify potential binding partners. The 3D structure of the hub protein Toll-like receptor 4 (TLR4) (protein data bank [PDB] ID: 5TZ1) was obtained from the Research Collaboratory for Structural Bioinformatics PDB database (https://www.rcsb.org/) for docking analysis [29,30]. The 2D structures of key phytocompounds and the reference drug clotrimazole were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [31]. Before docking, water molecules were removed, hydrogen atoms were added, and the protein structure was energy minimized using ICM Molsoft.

The binding site was defined based on the coordinates of the ligand in the 5TZ1 structure, encompassing the known active pocket of TLR4. Grid box was generated to cover the binding cavity. Molecular docking was performed using ICM Molsoft software. For each ligand, 10 independent docking runs (confirmations) were performed to ensure convergence of binding poses, and best-scoring conformation based on the ICM docking score (in kcal/mol) was selected for interaction analysis.

The software enabled visualization of molecular interactions in both 2D and 3D formats, highlighting hydrogen bonds, hydrophobic interactions, and binding scores of the docked complexes [29,30].

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary analysis revealed that the leaf extract contains proteins as primary metabolites and various bioactive secondary metabolites, including tannins, steroids, terpenoids, triterpenoids, flavonoids, alkaloids, carbohydrates, polyphenols, and glycosides (Table 1). Their presence suggests the potential medicinal value of the plant.

GC-MS analysis

The GC-MS analysis of the methanolic extract of *C. gigantea* Linn. revealed the presence of 38 different phytochemical constituents, identified based on their retention time (RT) and peak area. These compounds include terpenes, aldehydes, alcohols, fatty acids, and esters, suggesting a diverse phytochemical profile. The major compounds identified were 3, 7, 7-Trimethylbicyclo[4.1.0]hept-3-ene-2,5-dione (RT: 17.448 min, 22.05%), 1, 3, 8-p-Menthatriene (RT: 11.216 min, 19.74%), Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methyl) (RT: 8.104 min, 9.83%), Longifolene (RT: 20.962 min, 4.75%), 9-Undecenal, 2,10-dimethyl- (RT: 37.918 min, 4.71%), p-Cymene-2,5-diol (RT: 26.004 min, 4.26%), and (1R, 4R, 5S)-1-Isopropyl-4-methoxy-4-methylbicyclohexane (RT: 13.736 min, 4.11%).

Table 1: Qualitative analysis of *Calotropis gigantea* Linn leaf extract

Phytochemicals	Results
Tannins	+
Saponins	–
Steroids	+
Terpenoids	+
Triterpenoids	+
Flavonoids	+
Alkaloids	+
Carbohydrate	+
Proteins	+
Anthraquinones	–
Polyphenols	+
Glycosides	+

Indications: "+" means positive activity, "–" means negative activity

A complete list of all compounds with their RT and peak area is presented in Table 2. These compounds are known for various biological activities such as antimicrobial, antioxidant, and anti-inflammatory effects, which could support the therapeutic potential of the extract. The total ion chromatogram (TIC) of the GC-MS analysis is shown in Fig. 1, illustrating the retention peaks of the identified phytochemicals.

Compound database construction and drug-likeness filtering

Out of the 38 identified phytoconstituents, 35 satisfied the applied drug-likeness criteria and were retained for subsequent target prediction and NP analysis.

Identification of gene protein associated with bioactive and VVC

Target prediction was performed using the Super-PRED database, and protein targets were converted to official gene symbols using

Table 2: GC-MS analysis of methanolic extracts of *Calotropis gigantea* Linn. leaves

Sr. No.	Compound name	Retention time	Peak area (%)
01	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methy	8.104	9.83
02	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methyl	9.638	1.87
03	1,3,8-p-Menthatriene	11.216	19.74
04	gamma.-Terpinene	12.045	0.82
05	cis-4-methoxy thujane	13.071	0.69
06	(1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylb	13.736	4.11
07	3,6-Octadienal, 3,7-dimethyl-	14.902	0.11
08	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl	15.464	0.33
09	(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	15.872	0.13
10	Octanoic acid	16.349	0.60
11	3,7,7-Trimethylbicyclo [4.1.0]hept-3-ene-2,5-d	17.448	22.05
12	2,4-Decadienal, (E, E)-	18.268	1.04
13	Benzene, 2-methoxy-1,3,5-trimethyl-	18.760	5.37
14	Tricyclo[5.4.0.0 (2,8)]undec-9-ene, 2,6,6,9-tetr	19.558	1.47
15	2-Undecenal	19.842	1.35
16	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trime	20.347	0.32
17	Longifolene	20.962	4.75
18	2-Tridecanone	22.933	0.22
19	alpha.-Bisabolol	23.368	0.31
20	Epizonarene	23.921	0.08
21	p-Cymene-2,5-diol	26.004	4.26
22	Tetradecanal	26.794	0.17
23	1,8,11-Heptadecatriene, (Z, Z)-	28.164	0.16
24	0s, 11s-Himachala-3 (12),4-diene	28.641	0.14
25	2-Pentadecanone	29.166	0.38
26	Pentadecanal-	29.592	0.31
27	5-Caranol, trans, trans-(+)-	30.115	0.42
28	Tetradecanoic acid	31.420	3.83
29	Hexadecanoic acid, methyl ester	34.228	1.45
30	Phenanthrene, 7-ethenyl-1,2,3,4,4a, 4b, 5,6,7,9,	34.883	1.47
31	9-Tetradecen-1-ol, acetate, (Z)-	35.067	0.58
32	2,3-Dihydrofarnesyl decanoate	35.309	2.34
33	Butyric acid, hexadecyl ester	35.524	0.63
34	Methyl 10-trans, 12-cis-octadecadienoate	37.682	1.66
35	9-Undecenal, 2,10-dimethyl-	37.918	4.71
36	6-Octadecenoic acid	41.324	0.01
37	9,12-Octadecadienoic acid (Z, Z)-	41.541	0.12
38	Oxacycloheptadec-8-en-2-one, (8Z)-	42.673	2.76

GC-MS: Gas chromatography-mass spectrometry

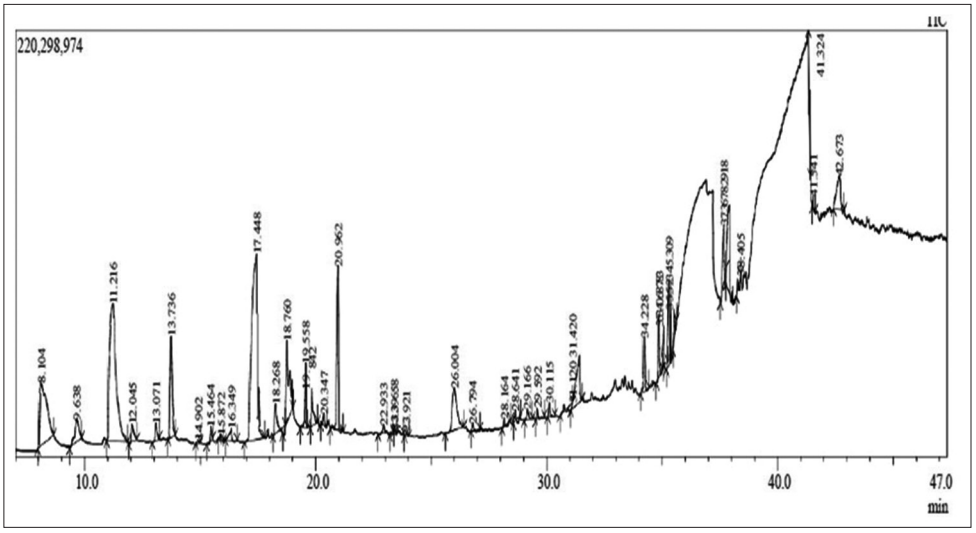


Fig. 1: Gas chromatography-mass spectrometry chromatogram of methanolic extract of *Calotropis gigantea* Linn. leaf

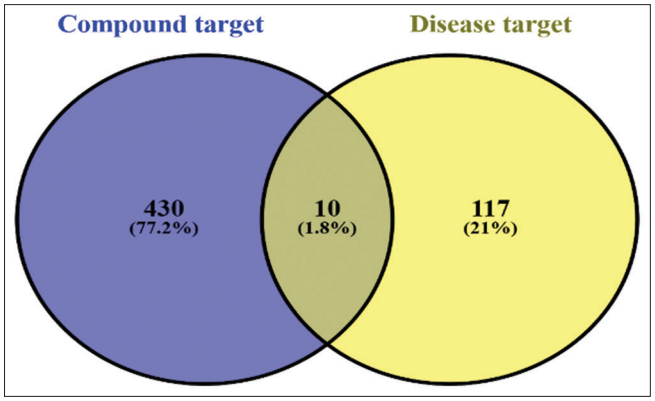


Fig. 2: Venn diagram analysis of the intersection targets between *Calotropis gigantea* Linn. leaves and vulvovaginal candidiasis

UniProt IDs. Targets with a probability score above 0 were considered. From GeneCards, 127 genes related to VVC were identified, while 440 phytoconstituent-related genes were obtained from the STRING database. The intersection revealed 10 common targets between *C. gigantea* bioactives and VVC-related genes. A Venn diagram illustrated these overlapping targets (Fig. 2).

PPI

The PPI network generated through the STRING database (Fig. 3) revealed strong interaction among 10 key targets, including TLR4, STAT1, AR, ESR1, KIT, BRAF, IDO1, SLC5A1, SLC5A2, and ABCB1. TLR4 and STAT1 were identified as central hub proteins with the highest interaction degrees, suggesting their major involvement in immune and inflammatory pathways. These results highlight the functional relevance of the identified targets in the potential mechanism of action. The top 10 hub genes identified based on degree centrality values are summarized in Table 4.

Construction of a component-disease-pathway-target network

The KEGG pathway enrichment analysis showed that 9 target proteins were associated with 4 signaling pathways (False Discovery Rate <0.05). The 4 signaling pathways were directly related to VVC. The description of 4 signaling pathways is presented in Table 3. The top 4 KEGG pathways are as follows: Breast cancer (hsa05224), inflammatory bowel disease (IBD) (hsa0521), Endocrine resistance (hsa01522), and Toll-like receptor signaling pathway (hsa05200). The resulting PPI network consisted of 10 nodes and 33 edges, as illustrated in Fig. 3. The top GO terms for biological process (BPs), molecular function (MFs), and CCs are depicted in Figs. 4-6, respectively.

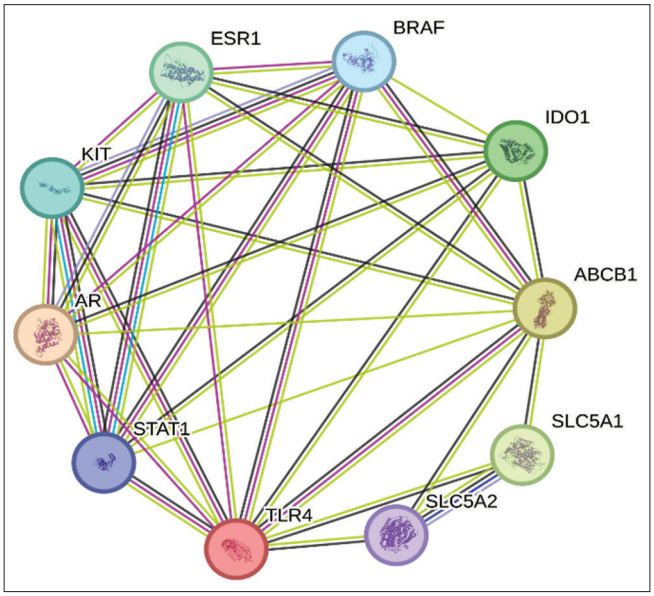


Fig. 3: Overlapping 10 gene-gene networking nodes and edges (false discovery rate <0.05)

A S-T-B network of *C. gigantea* Linn. leaves is displayed in Fig. 3. There were 35 bioactives, 11 target proteins, and 4 pathways. TLR4 emerged as the major target, indicating its central role in the mechanism of action. The identified targets were associated with relevant pathways such as Toll-like receptor signaling, endocrine resistance, and immune response, suggesting that *C. gigantea* exerts its antifungal and anti-inflammatory effects through a multi-target, synergistic approach.

In PPI, the TLR4 target exhibited the highest degree (27) and it is considered as a hub target protein (Fig. 7).

Molecular docking

Based on the PPI network, we employed molecular docking to determine the possibility of binding between VVC-related core targets and phytoconstituents of *C. gigantea* Linn. leaves methanolic extract. TLR4 was selected as a protein receptor because it had connected or interacted with 35 bioactive compounds in the component-disease-pathway target network. The compound with the most negative binding energies is 3,6-Octadienal, 3,7-dimethyl-(-24.9 kcal/mol), indicating very strong interactions with the target proteins. This compound exhibits

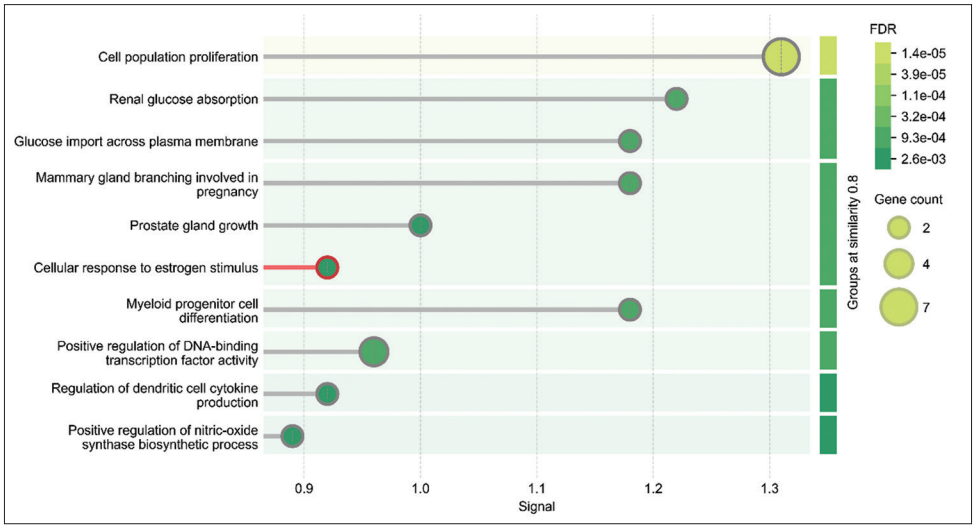


Fig. 4: Biological process (gene ontology) enrichment

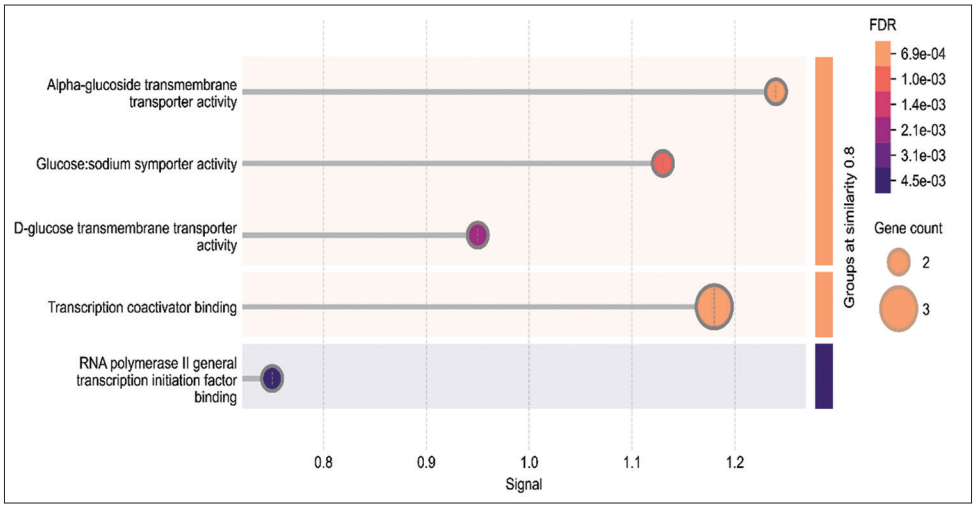


Fig. 5: Molecular function (gene ontology) enrichment

Table 3: The description of 4 signaling pathways

KEGG ID	Pathways	Target protein
hsa05224	Breast cancer	KIT, ESR1, BRAF
Hsa0521	Inflammatory bowel disease	STAT1, TLR4
hsa01522	Endocrine resistance	ESR1, BRAF
hsa04620	Toll-like receptor signaling pathway	STAT1, TLR4

TLR4: Toll-like receptor 4, KEGG: Kyoto encyclopedia of genes and genomes

Table 4: Top 10 hub genes identified from the PPI network based on degree centrality (score) values calculated using CytoHubba in Cytoscape

Rank	Name	Score
1	TLR4	9
2	ABCB1	9
3	IDO1	7
4	BRAF	7
5	ESR1	7
6	AR	7
7	STAT1	7
8	KIT	7
9	SLC5A2	3
10	SLC5A1	3

TLR4: Toll-like receptor 4, PPI: Protein–protein interaction

robust binding, which suggests its potential for high biological activity. The binding energy values of all docked phytoconstituents, including the standard drug clotrimazole as a standard drug (18.82 kcal/mol), are summarized in Table 5. The detailed interaction profile of the standard drug clotrimazole with the 5TZ1 receptor is presented in Table 7. The interaction of the major phytoconstituent 3,6-Octadienal, 3,7-dimethyl- with the 5TZ1 receptor demonstrated stable binding, as shown in the 3D and 2D docking visualizations (Fig. 9). For comparison, the standard antifungal drug clotrimazole exhibited strong interactions with the 5TZ1 receptor, as depicted in the corresponding 3D & 2D docking model (Fig. 10).

DISCUSSION

This study employed a multidisciplinary approach combining phytochemical screening, GC-MS analysis, NP, and molecular docking to investigate the antifungal potential of *C. gigantea* Linn. leaf extract against VVC. The findings confirm that *C. gigantea* is a valuable source of bioactive compounds with significant therapeutic relevance.

Preliminary phytochemical screening revealed the presence of flavonoids, tannins, terpenoids, alkaloids, polyphenols, and glycosides – classes of compounds previously reported to possess antifungal and anti-inflammatory activities [11]. These compounds are known to interfere with fungal cell wall integrity, inhibit virulence factors, and suppress inflammatory pathways, all of which are central to

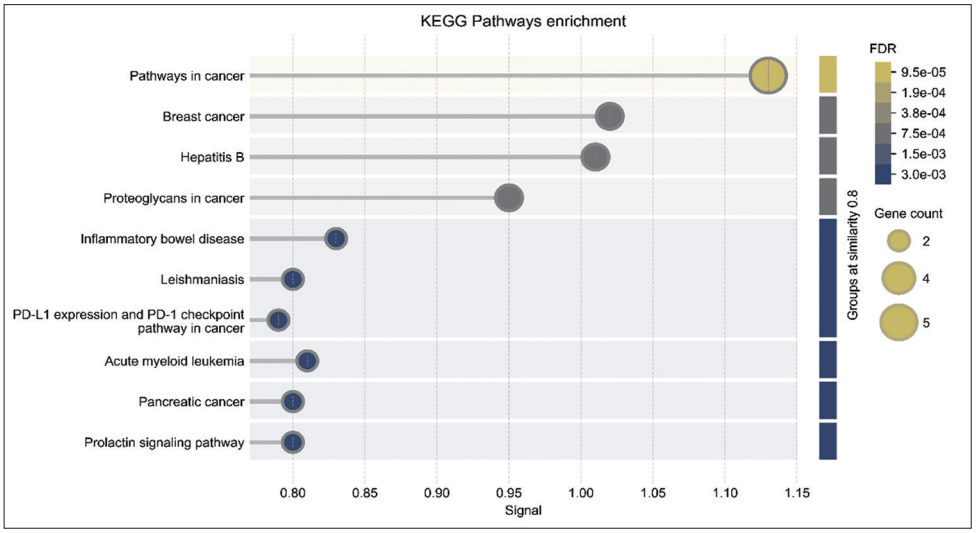


Fig. 6: KEGG pathways enrichment

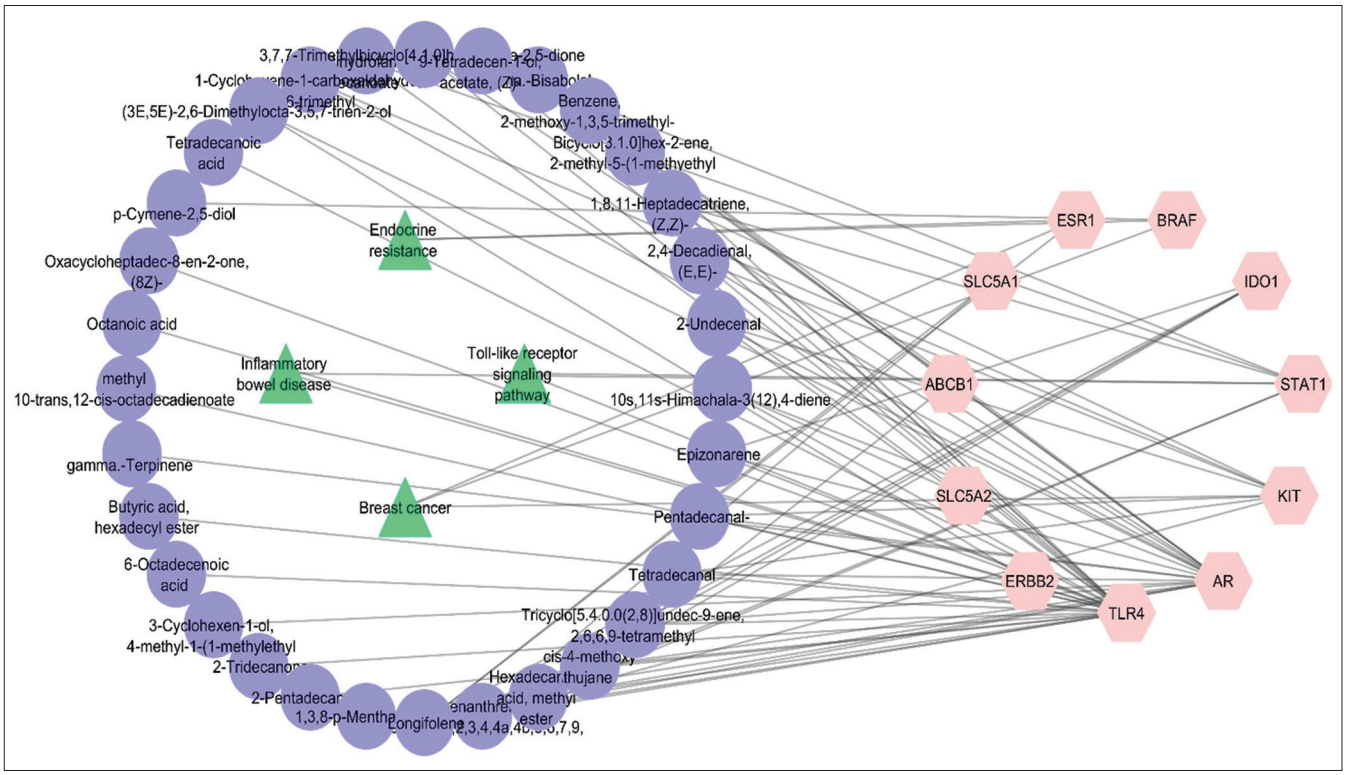


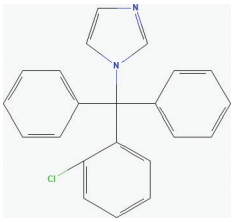
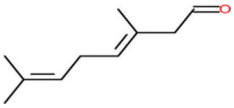
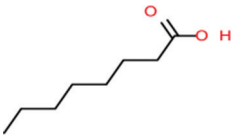
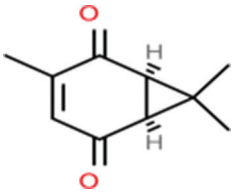
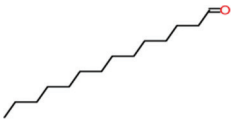
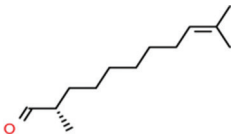
Fig. 7: Component-disease-pathway target network. Green triangles represent pathways, pink hexagons indicate genes involved in disease, blue circles represent phytoconstituents in *Calotropis gigantea* Linn, and the g-ray edges indicate the interactions between the targets and the active ingredients

the treatment of VVC [14]. Similar phytochemical profiles were also reported by Sharma *et al*. 2016 [13], confirming the consistent composition of *C. gigantea* leaves across studies.

GC-MS analysis further validated the presence of 38 compounds, with major constituents such as 3,7,7-trimethylbicyclo[4.1.0]hept-3-ene-2,5-dione, 1,3,8-p-Menthatriene, and Longifolene – compounds that have demonstrated antimicrobial or immunomodulatory activities in earlier reports. A comparable GC-MS profile was reported by Madhavan *et al*. 2020 [19], who also found terpenes and fatty acid derivatives contributing to antifungal action. The detection of p-Cymene-2,5-diol and methyl esters of long-chain fatty acids further supports the extract's membrane-targeting activity against fungal pathogens, potent anti-*Candida* effects of AgNP synthesized using *C. gigantea* extract [12].

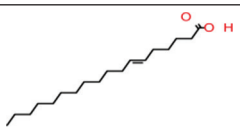
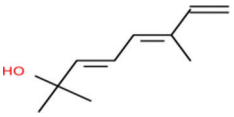
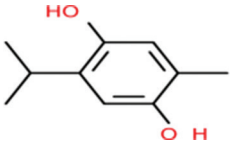
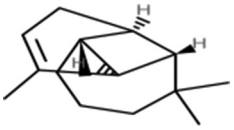
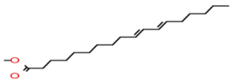
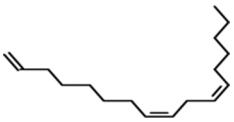
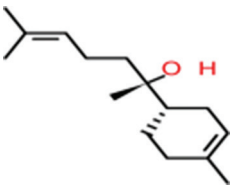
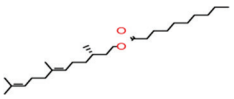
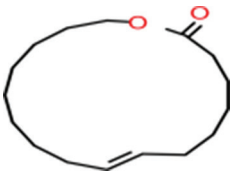
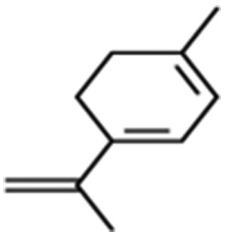
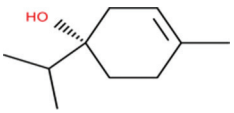
Through NP, 10 overlapping targets between bioactive compounds and VVC-related genes were identified. Notably, key targets such as TLR4, STAT1, ESR1, and BRAF have been implicated in immune modulation and epithelial defense mechanisms. The TLR4 receptor, which showed the highest interaction degree, is a known pattern recognition receptor for fungal pathogens like *C. albicans*. Prior studies have established that modulation of TLR4 signaling can suppress inflammation while enhancing pathogen clearance in mucosal infections [7]. Fig. 8 shows connectivity pattern of the top 10 hub targets was visualized using a protein–protein interaction network constructed through the

Table 5: Virtual docking of TLR4 targets with corresponding active phytoconstituents from *Calotropis gigantea* Linn. leaves extract and standard compound

Sr. No.	Structure	Phytochemical	Binding energy (BE)
1.		Clotrimazole	−18.82
2.		3,6-Octadienal, 3,7-dimethyl-	−24.9
3.		2-Pentadecanone	−22.7
4.		Octanoic acid	−22.28
5.		3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene-2,5-dione	−20.78
6.		Tetradecanal	−20.28
7.		Pentadecanal	−19.6
8.		9-Undecenal, 2,10-dimethyl-	−18.72
9.		2,4-Decadienal, (E, E)-	−18.62
10.		2-Tridecanone	−18.33
11.		9-Tetradecen-1-ol, acetate, (Z)-	−17.91
12.		Tetradecanoic acid	−17.46

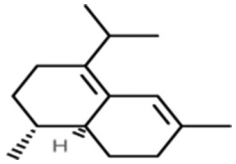
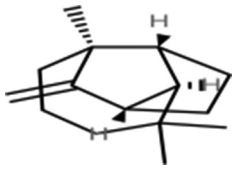
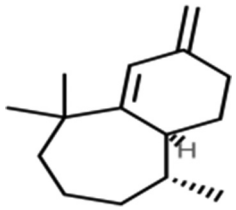
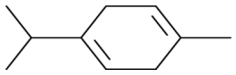
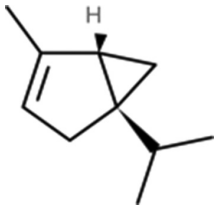
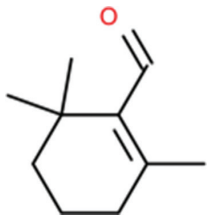
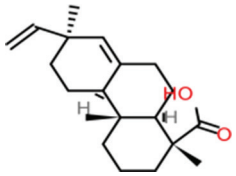
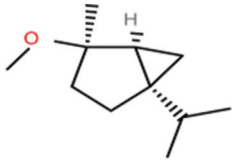
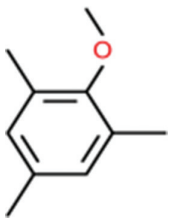
(*Contd...*)

Table 5: (*Continued*)

Sr. No.	Structure	Phytochemical	Binding energy (BE)
13.		6-Octadecenoic acid	−17.35
14.		(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	−17.1
15.		Butanoic acid, hexadecyl ester	−16.19
16.		p-Cymene-2,5-diol	−15.9
17.		Tricyclo[5.4.0.0 (2,8)]undec-9-ene, 2,6,6,9-tetramethyl	−15.44
18.		Methyl 10-trans, 12-cis-octadecadienoate	−14.4
19.		1,8,11-Heptadecatriene, (Z, Z)-	−14.4
20.		alpha.-Bisabolol	−14.25
21.		Hexadecanoic acid, methyl ester	−13.63
22.		2,3-Dihydrofarnesyl decanoate	−13.09
23.		Oxacycloheptadec-8-en-2-one, (8Z)-	−12.98
24.		1,3,8-p-menthatriene	−11.94
25.		3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl	−11.85

(*Contd...*)

Table 5: (*Continued*)

Sr. No.	Structure	Phytochemical	Binding energy (BE)
26.		Epizonarene	−11.85
27.		2-Undecenal	−11.78
28.		Longifolene	−11.67
29.		10s, 11s-Himachala-3 (12),4-diene	−11.12
30.		gamma.-terpinene	−10
31.		Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methyethyl	−9.222
32.		1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl	−8.895
33.		Phenanthrene, 7-ethenyl-1,2,3,4,4a, 4b, 5,6,7,9,	−7.413
34.		cis-4-methoxy thujane	−5.756
35.		Benzene, 2-methoxy-1,3,5-trimethyl-	−4.475

TLR4: Toll-like receptor 4

CytoHubba plugin. Thus, our finding of TLR4 as a hub gene strengthens the hypothesis that *C. gigantea* may exert its effect by restoring innate immune balance.

KEGG pathway enrichment analysis revealed Toll-like receptor signaling, endocrine resistance, breast cancer, and IBD as significantly associated with the compound–target network. These pathways are relevant to VVC pathogenesis, particularly under estrogen-dominant conditions and in women with recurrent infections, highlighting the interplay between hormonal regulation, immune responses, and mucosal integrity.

The enriched GO terms were classified into BP and MF categories. The top GO biological processes were inflammatory response (GO: 0006954), immune response (GO: 0006955), defense response (GO: 0006952), and cell surface receptor signaling pathway (GO: 0007166), suggesting that the target genes are mainly involved in immune and inflammatory regulation. Enrichment in MF included protein binding (GO: 0005515) and cytokine receptor activity (GO: 0004896), while Cellular Component terms such as plasma membrane (GO: 0005886) and cytoplasm (GO: 0005737) indicated membrane-associated signaling roles.

Gene set enrichment across all conditions demonstrated strong statistical significance, with FDR values ranging from 0.0000014 to 0.0045, well below the accepted threshold of 0.05. Enriched pathways were supported by multiple genes (counts of 2–7) and showed high gene ratios, underscoring their biological relevance. Together, these metrics confirm a robust enrichment of immune, cell cycle, and morphogenetic processes – highlighting a coordinated molecular response to the experimental intervention.

TLR4 plays a central role in host defense against *C. albicans* in the vaginal mucosa. It is expressed on epithelial cells, macrophages, and dendritic cells, where it recognizes O-linked mannosyl residues of *Candida* mannoproteins, triggering downstream signaling and cytokine release. This response promotes a Th1-type protective immunity through interleukin-12, interferon gamma, and tumor necrosis factor-α, inhibiting the transition of *Candida* from its commensal yeast form to the invasive hyphal form. Impaired or suppressed TLR4 signaling compromises this defense, allowing fungal overgrowth and symptomatic infection. Modulation of TLR4 activity, therefore, represents a promising therapeutic target to restore mucosal immunity and prevent recurrence [34-36].

Estrogen further contributes to *Candida* virulence by inducing Gpd2-mediated recruitment of Factor H, facilitating evasion of complement-mediated opsonophagocytosis. This hormone-specific adaptation impairs innate immune clearance and supports fungal survival, suggesting that targeting Gpd2 or its regulatory pathways may provide novel strategies for managing estrogen-associated RVVC [37].

Comorbid conditions such as active IBD and breast cancer can also influence VVC susceptibility. IBD is associated with mucosal inflammation and barrier disruption, which may exacerbate vulvovaginal discomfort, while *C. albicans* can worsen epithelial damage through *candida*lysin and IL-17-mediated immune responses [38,39]. Breast cancer treatments, particularly aromatase inhibitors, lower estrogen levels, weakening vaginal immunity and microbiome balance, thereby promoting *Candida* overgrowth. These findings underscore the clinical significance of managing both fungal burden and host immune responses in susceptible populations [41,42].

In the molecular docking analysis, 3,6-Octadienal, 3,7-dimethyl- exhibited the highest binding affinity to TLR4, despite its very low abundance (0.11%) in the *C. gigantea* leaf extract. This highlights a well-recognized phenomenon in phytomedicine, where minor constituents can contribute to the overall biological effect through synergistic interactions with more abundant compounds. Several major phytochemicals, including

Table 6: Detailed view of interaction of 3,6-Octadienal, 3,7-dimethyl- in the binding of 5TZ1 receptor

Amino acids	Distance Å	Interaction type
H377	2.87	Hydrogen bond
Y118	3.78	Hydrophobic bond
L121	3.74	Hydrophobic bond
T122	3.63	Hydrophobic bond
Y132	4.20	Hydrophobic bond
F233	4.01	Hydrophobic bond
L376	3.87	Hydrophobic bond
S378	4.32	Hydrophobic bond
F380	3.47	Hydrophobic bond
M508	4.27	Hydrophobic bond

Table 7: Detailed view of interaction of standard drug clotrimazole in the binding of 5TZ1 receptor

Amino acids	Distance Å	Interaction type
R381	2.90	Hydrogen bond
F105	4.14	Hydrophobic bond
Y132	4.21	Hydrophobic bond
T311	4.40	Hydrophobic bond
L376	3.13	Hydrophobic bond
I379	4.40	Hydrophobic bond
G464	4.13	Hydrophobic bond
H468	3.79	Hydrophobic bond
R469	4.25	Hydrophobic bond
C470	3.63	Hydrophobic bond

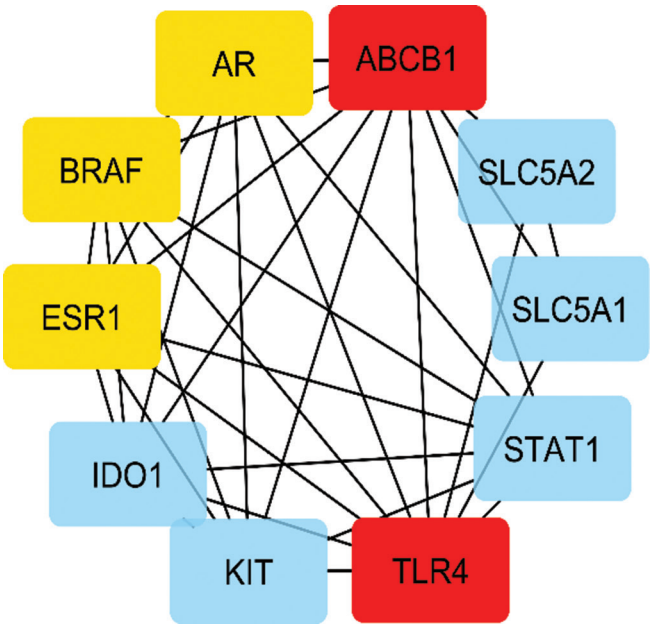


Fig. 8: The PPI network of 10 hub targets of *Calotropis gigantea* Linn. Leaves in the treatment of vulvovaginal candidiasis constructed using the CytoHubba plugin in Cytoscape 3.7.2. The node color ranges from yellow to red, indicating increasing degree values, where red nodes represent proteins with the highest degree of connectivity

2-Pentadecanone, Octanoic acid, 3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene-2,5-dione, also demonstrated favorable binding energies and, given their higher abundance, may play a more prominent role in the therapeutic potential of the extract. Although the absolute docking scores appear unusually low, the relative comparison to the standard antifungal drug clotrimazole indicates that these compounds could effectively

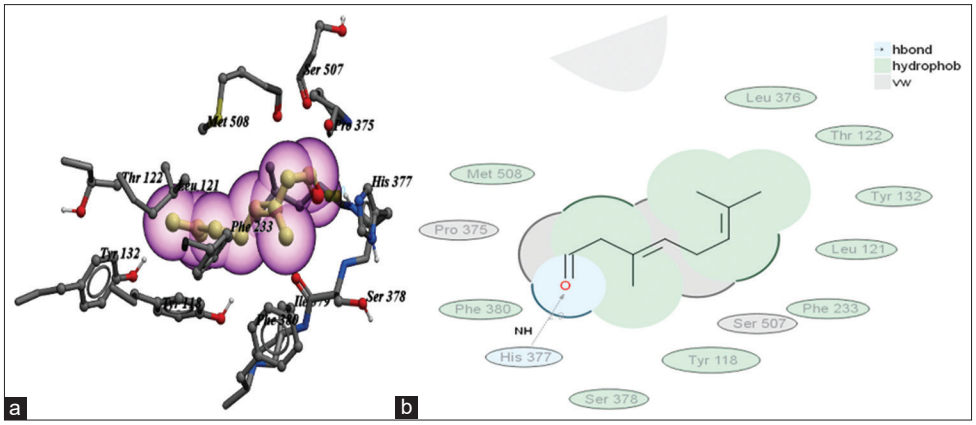


Fig. 9: (a) Binding of 3,6-Octadienal, 3,7-dimethyl- with 5TZ1 receptor (3D diagram). (b) Binding of 3,6-Octadienal, 3,7-dimethyl- with 5TZ1 receptor (2D diagram)

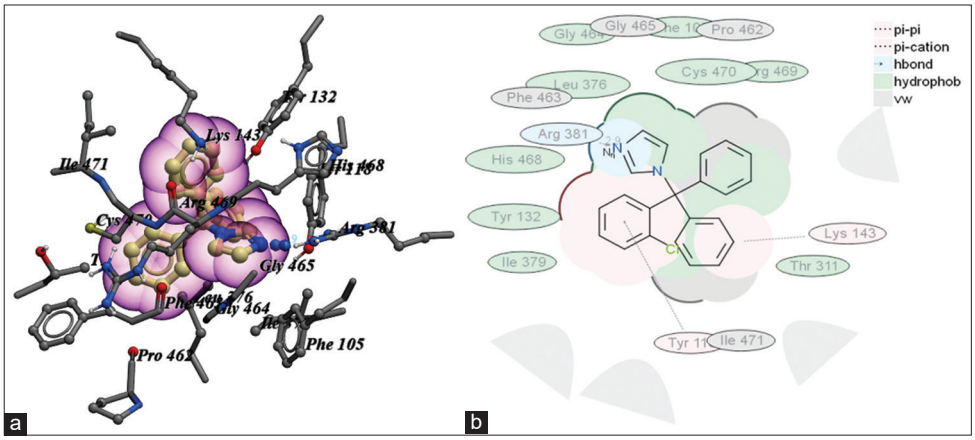


Fig. 10: (a) Binding of clotrimazole with 5TZ1 receptor (3D diagram). (b) Binding of clotrimazole with 5TZ1 receptor (3D diagram)

interact with TLR4. Collectively, these findings suggest that both major and minor constituents may synergistically contribute to the modulation of TLR4-mediated pathways, potentially enhancing the antifungal activity of the extract. Previous molecular docking studies on plant-based antifungals also highlighted similar interactions between terpenoids and immune receptors, validating the approach and supporting the relevance of our docking predictions [40].

The molecular docking analysis of the ligand with the target protein (Table 6) revealed that it forms a hydrogen bond with H377 at a distance of 2.87 Å, which likely stabilizes its position within the binding pocket. In addition, multiple hydrophobic interactions were observed with residues Y118, L121, T122, Y132, F233, L376, S378, F380, and M508, with distances ranging from 3.47 to 4.32 Å. These hydrophobic contacts contribute to the overall stability of the ligand within the active site and enhance binding specificity. Collectively, these interactions suggest that the ligand can effectively occupy the protein's binding pocket, potentially modulating its activity. The pattern of interactions supports the predicted therapeutic relevance of the ligand and provides mechanistic insight into how it may work in synergy with other phytoconstituents, even if present at low abundance, to exert biological effects.

Taken together, the integration of NP and docking results suggests that *C. gigantea* leaf extract acts through a multi-target, synergistic mechanism, modulating host immune responses while directly inhibiting fungal virulence. These findings are consistent with previous bioinformatics-based investigations on medicinal plants such as *Lespedeza bicolor* [16] and *Fumaria indica* [15], which demonstrated similar multi-target antifungal mechanisms through Toll-like receptor and MAPK signaling pathways. The outcomes of the present analysis are in agreement with recent network pharmacology research conducted on medicinal plants for cancer applications [43].

However, this study is limited by its *in silico* nature. Future work should include *in vitro* antifungal assays and *in vivo* validation using animal models of VVC to confirm pharmacological activity and optimize delivery systems. Nonetheless, this study offers a strong scientific foundation and supports further investigation of *C. gigantea* as a novel plant-based *candida*te for treating VVC.

CONCLUSION

The present study demonstrates that *C. gigantea* Linn. leaf extract possesses significant multi-target antifungal potential against VVC. Integration of NP and molecular docking approaches revealed that bioactive compounds, particularly 3,6-Octadienal, 3,7-dimethyl-, interact strongly with key immune-regulatory targets such as TLR4, suggesting modulation of host innate immune responses as a potential mechanism of action. Despite its low abundance, this compound, along with other major phytoconstituents, may act synergistically to enhance antifungal efficacy. Enrichment analyses highlighted relevant pathways, including Toll-like receptor signaling, immune response, and inflammatory regulation, supporting the therapeutic relevance of the extract. While these findings are hypothesis-generating and provide mechanistic insights, experimental validation through *in vitro* and *in vivo* studies is necessary to confirm efficacy and guide the development of optimized delivery systems for vaginal application. Overall, this work lays a foundation for future studies evaluating the therapeutic potential of *C. gigantea* in VVC.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for studies that were the basis of this research.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available upon reasonable request.

ACKNOWLEDGMENT

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AUTHOR'S CONTRIBUTION

Ms. Vasundhara Bhosale and Dr. Akshada Koparde conceptualized and designed the study. Ms. Vasundhara Bhosale handled data collection and prepared the initial draft of the article. Ms. Mayuri Bhosale performed the molecular docking analysis. Dr. Akshada Koparde and and Dr. Vandana Thorat supervised the study, contributed to data analysis and interpretation, and provided essential revisions. All authors have reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING

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REFERENCES

1. Willems HM, Ahmed SS, Liu J, Xu Z, Peters BM. Vulvovaginal candidiasis: A current understanding and burning questions. J Fungi (Basel). 2020;6(1):27. doi: 10.3390/jof6010027, PMID 32106438
2. Martens MG, Maximos B, Degenhardt T, Person K, Curelop S, Ghannoum M, *et al*. Phase 3 study evaluating the safety and efficacy of oteseconazole in the treatment of recurrent vulvovaginal candidiasis and acute vulvovaginal candidiasis infections. Am J Obstet Gynecol. 2022;227(6):880.e1-880.e11. doi: 10.1016/j.ajog.2022.07.023, PMID 35863457
3. Satora M, Grunwald A, Zaremba B, Frankowska K, Żak K, Tarkowski R, *et al*. Treatment of vulvovaginal candidiasis-an overview of guidelines and the latest treatment methods. J Clin Med. 2023;12(16):5376. doi: 10.3390/jcm12165376, PMID 37629418
4. Yano J, Sobel JD, Nyirjesy P, Sobel R, Williams VL, Yu Q, *et al*. Current patient perspectives of vulvovaginal candidiasis: Incidence, symptoms, management and post-treatment outcomes. BMC Womens Health. 2019;19(1):48. doi: 10.1186/s12905-019-0748-8, PMID 30925872
5. Sobel JD. Recurrent vulvovaginal candidiasis. Am J Obstet Gynecol. 2016;214(1):15-21. doi: 10.1016/j.ajog.2015.06.067, PMID 26164695
6. Crousse T, Sobel JD, Smith K, Nyirjesy P. Long-term outcomes of women with recurrent vulvovaginal candidiasis after a course of maintenance antifungal therapy. J Low Genit Tract Dis. 2018;22(4):382-6. doi: 10.1097/LGT.0000000000000413, PMID 29975334
7. Denning DW, Kneale M, Sobel JD, Rautemaa-Richardson R. Global burden of recurrent vulvovaginal candidiasis: A systematic review. Lancet Infect Dis. 2018;18(11):e339-47. doi: 10.1016/S1473-3099(18)30103-8, PMID 30078662
8. Gaziano R, Sabbatini S, Roselletti E, Perito S, Monari C. *Saccharomyces cerevisiae*-based probiotics as novel antimicrobial agents to prevent and treat vaginal infections. Front Microbiol. 2020;11:718. doi: 10.3389/fmicb.2020.00718, PMID 32373104
9. Sustr V, Foessleitner P, Kiss H, Farr A. Vulvovaginal candidosis: Current concepts, challenges and perspectives. J Fungi (Basel). 2020;6(4):267. doi: 10.3390/jof6040267, PMID 33171784
10. Monk BC, Keniya MV. Roles for structural biology in the discovery of drugs and agrochemicals targeting sterol 14α-Demethylases. J Fungi (Basel). 2021;7(2):67. doi: 10.3390/jof7020067, PMID 33498194
11. Usman MR, Usman MA, Patil S. A. Isolation of preliminary phytoconstituents and anti-inflammatory and antipyretic activity of *Calotropis gigantea* Linn. Leaves extracts. Int J Pharm Sci Res. 2012;3(4):1208-14. doi: 10.13040/IJPSR.0975-8232.3(4).1208-14
12. Alafnan A, Sridharagatta S, Saleem H, Khurshid U, Alamri A, Ansari SY, *et al*. Evaluation of the phytochemical, antioxidant, enzyme inhibition, and wound healing potential of *Calotropis gigantea* (L.) Dryand: A source of a bioactive medicinal product. Front Pharmacol. 2021;12:701369. doi: 10.3389/fphar.2021.701369, PMID 34483902
13. Sharma S, Kumari A, Sharma M. Comparative GC-MS analysis of bioactive compounds in methanolic extract of *Calotropis gigantea* (L.) W.T. Aiton Leaf and latex. Int J Pharmacogn Phytochem Res. 2016;8(11):1823-7.
14. Ali EM, Abdallah BM. Effective inhibition of candidiasis using an eco-friendly leaf extract of *Calotropis*-gigantean-mediated silver nanoparticles. Nanomaterials (Basel). 2020;10(3):422. doi: 10.3390/nano10030422, PMID 32121137
15. Batool S, Javed MR, Aslam S, Noor F, Javed HM, Seemab R, *et al*. Network pharmacology and bioinformatics approach reveals the multi-target pharmacological mechanism of *Fumaria indica* in the treatment of liver cancer. Pharmaceuticals (Basel). 2022;15(6):654. doi: 10.3390/ph15060654, PMID 35745580
16. Oh KK, Adnan MD, Cho DH. Network pharmacology approach to bioactive chemical compounds identified from *Lespedeza bicolor* lignum methanol extract by GC-MS for amelioration of hepatitis. Gene Rep. 2020;21:100851. doi: 10.1016/j.genrep.2020.100851
17. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, *et al*. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023;13(1):13398. doi: 10.1038/s41598-023-40160-2, PMID 37592012
18. Sethi A, Joshi K, Sasikala K, Alvala M. Molecular docking in modern drug discovery: Principles and recent applications. In: Gaitonde V, Karmakar P, Trivedi A, editors. Drug Discovery and Development - New Advances. London: IntechOpen; 2020. doi: 10.5772/intechopen.85991
19. Madhavan SA, Vinotha P, Uma V. Phytochemical screening and comparative GC-MS analysis of bioactive compounds present in methanolic leaf and latex extract *Calotropis gigantea* (L.). Asian J Adv Med Sci. 2020;2(2):1-13.
20. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. New Delhi: Nirali Prakashan; 2002. p. 15-163.
21. Kokate CK. Practical Pharmacognosy. 1st ed. New Delhi: Vallabh Prakashan; 2005. p. 111.
22. Sun T, Quan W, Peng S, Yang D, Liu J, He C, *et al*. Network pharmacology-based strategy combined with molecular docking and *in vitro* validation study to explore the underlying mechanism of Huo Luo Xiao Ling dan in treating atherosclerosis. Drug Des Dev Ther. 2022;16:1621-45. doi: 10.2147/DDDT.S357483, PMID 35669282
23. Zhao J, Pan B, Zhou X, Wu C, Hao F, Zhang J, *et al*. *Polygonum cuspidatum* inhibits the growth of osteosarcoma cells via impeding Akt/ERK/EGFR signaling pathways. Bioengineered. 2022;13(2):2992-3006. doi: 10.1080/21655979.2021.2017679, PMID 35129428
24. Gogoi B, Saikia SP. Virtual screening and network pharmacology-based study to explore the pharmacological mechanism of *Clerodendrum* species for anticancer treatment. Evid Based Complement Alternat Med. 2022;2022:3106363. doi: 10.1155/2022/3106363, PMID 36387366
25. Xu H, Wu J, Wang S, Xu L, Liu P, Shi Y, *et al*. Network pharmacology and *in vivo* experiments reveal the pharmacological effects and molecular mechanisms of Simiao Powder in prevention and treatment for gout. BMC Complement Med Ther. 2022;22(1):152. doi: 10.1186/s12906-022-03622-0, PMID 35672755
26. Vinothkanna A, Prathiviraj R, Sivakumar TR, Ma Y, Sekar S. GC-MS and network pharmacology analysis of the ayurvedic fermented medicine, *Chandanasava*, against chronic kidney and cardiovascular diseases. Appl Biochem Biotechnol. 2023;195(5):2803-28. doi: 10.1007/s12010-022-04242-7, PMID 36418713
27. Hu M, Yan H, Li H, Feng Y, Sun W, Ren Y, *et al*. Use of network pharmacology and molecular docking to explore the mechanism of action of curcuma in the treatment of osteosarcoma. Sci Rep. 2023;13(1):9569. doi: 10.1038/s41598-023-36687-z, PMID 37311820
28. Jin J, Chen B, Zhan X, Zhou Z, Liu H, Dong Y. Network pharmacology and molecular docking study on the mechanism of colorectal cancer treatment using Xiao-Chai-Hu-Tang. PLOS One. 2021;16(6):e0252508. doi: 10.1371/journal.pone.0252508, PMID 34125845
29. Shahzadi Z, Yousaf Z, Anjum I, Bilal M, Yasin H, Aftab A,

et al. Network pharmacology and molecular docking: Combined computational approaches to explore the antihypertensive potential of Fabaceae species. Bioresour Bioprocess. 2024;11(1):53. doi: 10.1186/s40643-024-00764-6, PMID 38767701

30. Jain NK, Tailang M, Chandrasekaran B, Khazaleh N, Thangavel N, Makeen HA, *et al*. Integrating network pharmacology with molecular docking to rationalize the ethnomedicinal use of *Alchornea laxiflora* (Benth.) Pax & K. Hoffm. for efficient treatment of depression. Front Pharmacol. 2024;15:1290398. doi: 10.3389/fphar.2024.1290398, PMID 38505421
31. Ma G, Dong Q, Li F, Jin Z, Pi J, Wu W, *et al*. Network pharmacology and *in vivo* evidence of the pharmacological mechanism of geniposide in the treatment of atherosclerosis. BMC Complement Med Ther. 2024;24(1):53. doi: 10.1186/s12906-024-04356-x, PMID 38267978
32. Oh KK, Adnan MD, Cho DH. Network pharmacology study on *Morus alba* L. Leaves: Pivotal functions of bioactives on RAS signaling pathway and its associated target proteins against gout. Int J Mol Sci. 2021;22(17):9372. doi: 10.3390/ijms22179372, PMID 34502281
33. Singh S, Singh S, Mishra RM, Shrivastava MP. Preliminary phytochemical screening of *Calotropis gigantea* Leaf. Int J Sci Res Publ. 2014;4(2):2250-3153.
34. Roeder A, Kirschning CJ, Rupec RA, Schaller M, Korting HC. Toll-like receptors and innate antifungal responses. Trends Microbiol. 2004;12(1):44-9. doi: 10.1016/j.tim.2003.11.003, PMID 14700551
35. Demirezen S, Yucel A, Beksac MS. *Candida* and toll-like receptors. Gynecol Obstet Reprod Med. 2012;18:176-8.
36. Falsetta ML, Foster DC, Woeller CF, Pollock SJ, Bonham AD, Piekna-Przybylska DP, *et al*. Toll-like receptor signaling contributes to proinflammatory mediator production in localized provoked vulvodynia. J Low Genit Tract Dis. 2018;22(1):52-7. doi: 10.1097/LGT.0000000000000364, PMID 29271858
37. Kumwenda P, Cottier F, Hendry AC, Kneafsey D, Keevan B, Gallagher H, *et al*. Estrogen promotes innate immune evasion of *Candida albicans* through inactivation of the alternative compliment system. Cell Rep. 2022;38(1):110183. doi: 10.1016/j.celrep.2021.110183, PMID 34986357
38. Ona S, James K, Ananthakrishnan AN, Long MD, Martin C, Chen W, *et al*. Association between vulvovaginal discomfort and activity of inflammatory bowel diseases. Clin Gastroenterol Hepatol. 2020;18(3):604-11.e1. doi: 10.1016/j.cgh.2019.05.018, PMID 31108226
39. Ho J, Camilli G, Griffiths JS, Richardson JP, Kichik N, Naglik JR. *Candida albicans* and *candida*lysin in inflammatory disorders and cancer. Immunology. 2020;162(1):11-6. doi: 10.1111/imm.13255, PMID 32880925
40. Sherin Rebecca F, Pon Nivedha R, Sharmila R. Molecular docking analysis of bioactive compounds from *Mollugo cerviana* (L.) ser with DHFR for antifungal activity. Bioinformation. 2021;17(11):944-8. doi: 10.6026/97320630017944, PMID 35655907
41. Ashrafi S, Amini AA, Karimi P, Bagherian M, Adibzadeh Sereshgi MM, Asgarhalvaei F, *et al*. Candidiasis in breast cancer: Tumor progression or not? Iran J Basic Med Sci. 2024;27(11):1346-56. doi: 10.22038/ijbms.2024.75408.16379, PMID 39386227
42. Prasanchit P, Pongchaikul P, Lertsittichai P, Tantitham C, Manonai J. Vaginal microbiomes of breast cancer survivors treated with aromatase inhibitors with and without vulvovaginal symptoms. Sci Rep. 2024;14(1):7417. doi: 10.1038/s41598-024-58118-3, PMID 38548910
43. Tipugade O, Sawale JA, Jadhav N. Network pharmacology and molecular docking-based exploration of rubiaceous plants for breast cancer: Phytochemicals, preclinical studies, and regulatory perspectives. Asian J Pharm Clin Res. 2025;18(7):52-71. doi: 10.22159/ajpcr.2025v18i7.54934
44. Jain NK, Agrawal A, Kulkarni GT, Tailang M. Molecular docking study on phytoconstituents of traditional ayurvedic drug Tulsi (*Ocimum sanctum* Linn.) against Covid-19 MPRO enzyme: An *in-silico* study. Int J Pharm Pharm Sci. 2022 Apr 1;14:44-50. doi: 10.22159/ijpps.2022v14i4.43181
45. Sudha D, Malarkodi R, Gokulakrishnan A, Liyakath Ali AR. LC-MS/MS and GC-MS profiling and the antioxidant activity of *Carissa carandas* linn. Fruit extracts. Int J Pharm Pharm Sci. 2024;16(6):39-45. doi: 10.22159/ijpps.2024v16i6.50818