

Multitarget Antimicrobial Potential of *Tridax procumbens*: A Network Pharmacology and Experimental Approach

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ABSTRACT

Background: *Tridax procumbens* L. is a medicinal herb traditionally used to treat microbial infections. However, its molecular mechanisms of antimicrobial action remain poorly understood. This study investigated the multitarget antimicrobial potential of *T. procumbens* using an integrated network pharmacology and experimental approach. **Methods:** Bioactive phytoconstituents were screened using pharmacokinetic and drug-likeness criteria. Potential targets were identified through network pharmacology analysis, followed by protein–protein interaction (PPI), Gene Ontology (GO), and KEGG pathway enrichment analyses. Antimicrobial activity of different solvent extracts was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using the broth microdilution method. Preliminary phytochemical screening was also performed. **Results:** Twenty-one common targets were identified between *T. procumbens* phytoconstituents and antimicrobial-related genes. PPI analysis revealed HIF1A, NFκB1, SRC, TNF, KDR, AXL, ABCG2, XDH, ALOX5, and PTGS1 as major hub genes. Enrichment analyses highlighted pathways associated with EGFR tyrosine kinase inhibitor resistance, VEGF signaling, arachidonic acid metabolism, and immune-related signaling. Phytochemical screening confirmed the presence of alkaloids and cardiac glycosides. Among the extracts tested, the methanolic extract exhibited the strongest antimicrobial activity with MIC values of 6.25 µg/mL against *S. aureus* and 12.5 µg/mL against both *E. coli* and *C. albicans*. **Conclusion:** *T. procumbens* possesses significant multitarget antimicrobial activity mediated through multiple genes and pathways. These findings support its traditional use and highlight its potential as a source of novel antimicrobial agents.

Keywords: *Tridax procumbens*, network pharmacology, antimicrobial activity, hub genes, minimum inhibitory concentration.

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INTRODUCTION

Tridax procumbens L. (Asteraceae) is a widely prevalent medicinal plant that is popularly known by the name coat buttons. It is a medicinal herb that is commonly found in various parts of the world. The plant has been used in folklore remedies for curing various conditions such as wounds, skin infections, stomach problems, inflammation, lung diseases, and microbial infections. As a result of its

wide-ranging uses in ethnomedicine, the plant has been studied for its possible role as a source of bioactive phytochemicals with medicinal importance. Various studies have documented the presence of flavonoids, alkaloids, glycosides, tannins, terpenoids, steroids, and phenolics in this plant [1,2].

The problem of antimicrobial resistance (AMR) is becoming a pressing concern on a global scale [3], making

current antimicrobials ineffective and worsening the situation with infectious diseases. Due to the fast propagation of resistant bacteria and fungi, there is a need to develop new drugs which can act through different biological mechanisms. One of the most promising sources of novel antimicrobials is natural products and medicinal plants due to their structural complexity and capability to influence several targets at once. This means that searching for novel antimicrobials among known medicinal plants could be considered an efficient approach. These phytoconstituents exhibit a broad spectrum of biological activities including antioxidant, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, antidiabetic, and immunomodulatory effects [4-6].

Among all the medicinal plants, *T. procumbens* exhibits considerable antimicrobial properties against a range of pathogens. Some researchers have reported that the bioactivity in the plant is due to its chemical constituents, such as polyphenols, flavonoids [7], alkaloids, and glycosides, which inhibit microbial activities via various modes of action, ranging from interfering with membrane integrity and inhibiting vital enzymes to suppressing virulence factors and oxidative stress pathways [8-10]. Nevertheless, even though there have been several reports regarding the antimicrobial activity of *T. procumbens*, the molecular mechanisms behind them are still unknown [11]. Recently, network pharmacology has emerged as a powerful systems biology approach for investigating the complex interactions among phytochemicals, genes, proteins, and disease-associated pathways [12]. Unlike conventional pharmacology, which primarily follows the “one drug–one target” paradigm, network pharmacology adopts a holistic perspective by considering the synergistic interactions of multiple compounds acting on multiple targets simultaneously [13]. This characteristic makes network pharmacology particularly suitable for studying medicinal plants, which typically contain diverse bioactive constituents with multitarget therapeutic effects. By integrating bioinformatics databases, protein–protein interaction (PPI) networks, Gene Ontology (GO), and pathway enrichment analyses, network pharmacology facilitates the identification of key molecular targets, biological processes, and signaling pathways involved in disease modulation [14].

To elucidate the molecular mechanisms underlying the antimicrobial activity of *T. procumbens*, an integrated network pharmacology approach was employed in the present study [15-17]. The bioactivity of phytoconstituents of *T. procumbens* was predicted using pharmacokinetics and drug-likeness models, followed by predicting the targets and overlapping targets of antimicrobial-associated genes [18]. In total, twenty-one common targets were observed for phytoconstituents of *T. procumbens* and antimicrobial-related genes. Protein-protein interaction network showed that several vital hub genes may play an essential role in the antimicrobial action of the plants, such as HIF1A, NFKB1, SRC, TNF, KDR, AXL, ABCG2, XDH, ALOX5, and PTGS1. Enrichment analysis showed the involvement of EGFR tyrosine kinase inhibitor

resistance pathway, VEGF signaling pathway, arachidonic acid metabolism, Fc epsilon RI signaling pathway, and RIG-I-like receptor signaling pathway in the action of antimicrobials mediated by *T. procumbens* [19].

For experimental validation of the theoretical analysis, leaf extracts from *T. procumbens* were extracted in different polarity solvents and studied in terms of preliminary phytochemical screening and in vitro antimicrobial testing. The phytochemical studies proved the occurrence of biologically effective secondary plant metabolites, namely alkaloids and cardiac glycosides. In addition, for antimicrobial study, the selected microbial strains were subjected to the broth microdilution method, which showed that the methanol extract possessed the highest antimicrobial potential against *S. aureus* (MIC 6.25 µg/mL), *E. coli* (MIC 12.5 µg/mL), and *C. albicans* (MIC 12.5 µg/mL).

Consequently, the current study employs network pharmacology coupled with experimentation to investigate the multitarget antimicrobial efficacy of *T. procumbens*. These results offer scientific explanations of the molecular mechanism behind the antimicrobial action of the drug and justify the use of the medicinal herb as a valuable natural antimicrobial. Moreover, this strategy may help to identify phytoconstituents that have the potential to become leads for antimicrobial drug discovery.

METHODOLOGY:

Software, Chemicals, Drugs and methods

The IMPPAT [20], SwissADME [21], ADMETlab 2.0 [22] used to find plant targets, physicochemical parameters, to assess toxicity and safety profiles of targets of our interest. Venny 2.1, Search Tool for the Retrieval of Interacting Genes (STRING) [23], Cytoscape app [24], DAVID [25], ShinyGO 0.85 databases [26] were utilized for the protein - protein interactions (PPI) and also hub genes identification, pathways involved in the antimicrobial activity. For the phytochemical screening, rotary evaporator (Aditya Scientific) used for solvent separation, to collect the crude extract. To explore antimicrobial activity, we used BHI broth and minimum inhibitory concentration (MIC) method [27]. All other solvents and reagents used in the extraction procedure and phytochemical screening were analytical reagent grades obtained from Merck.

Plant material collection and authentication

The plant fresh material of *T. procumbens* belonging to the family Asteraceae, were collected from in and around the campus of Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Chiyyedu village, Anantapur District, Andhra Pradesh, India. The plant was authenticated by the Department of Botany, College of Sciences, Arts college, Anantapur, and a voucher specimen ID. 2025/001.

Identification of plant targets

At first, the phytoconstituents of *TP* were obtained from the online database of the Dr. Duke (or) IMPPAT (<https://cb.imsc.res.in/impapat/>) we, selected 6 compounds in all. In order to limit the dataset, the compounds were filtered by pharmacokinetic properties and those with an oral bioavailability of ≥ 0.3 (using SwissADME) and a drug-

likeness score of ≥ 0.18 (predicted using Molsoft database, <https://molsoft.com/mprop/>) were selected. The filtered compounds standard SMILES of these phytochemicals were retrieved from PubChem and again filtered through SwissADME to predict their pharmacokinetics and physicochemical properties. Shortlisted ligands were further tested for toxicity using ADMETlab 2.0 to assess their safety profiles.

The compounds those having marked bioavailability and drug likeness, they are subjected to Swiss target prediction (<https://www.swisstargetprediction.ch/>) to identify target Uniport IDs. To convert Uniport IDs to gene name we used retrieve / ID mapping tool [28] (<https://www.uniprot.org/id-mapping>).

Common Targets of *TP* and antimicrobial activity

The *TP* targets and antimicrobial therapies exhibit overlapping genetic targets. These common targets were found and called plant targets which shows antimicrobial activity. Further common genes were identified and the data retrieved from Venny 2.1 online web tool (<https://bioinfogp.cnb.csic.es/tools/venny/>).

Building the PPI network and hub gene identification

Identified common genes from Venny were subjected into the database called STRING (<http://string-db.org/>) to identify PPI data. By selecting multiple proteins and organism as homo sapiens the program is started. To get significant results we selected a cumulative score of more than 0.7 in the database to download the PPI cluster. Further, Cytoscape software (Version 3.10.3) used to visualize the PPI network. The proteins with greater degrees of connectivity were important to ensure the stability of the network. CytoHubba plugin in the Cytoscape software used to get node degrees and top ten hub genes.

Pathway & GO Enrichment-KEGG enrichment analysis

Gene Ontology (GO) and KEGG Pathway used in investigation of significant gene expression data at the biological level. In this study, GO annotations explored and represented as biological processes (BP), molecular functions (MF), and cellular components (CC). GO enrichment results were filtered based on significance level of $p > 0.05$ and a minimum gene number of ≥ 10 . KEGG pathway explored various metabolic and signaling pathways, disease mechanisms, location of targeted hub genes. DAVID database (online free web tool) (<https://davidbioinformatics.nih.gov/>) used to get complete collection of genomic data and functional enrichment analysis.

Preparation of Plant extract and its Procedure

The collected leaves were dried under shaded conditions at ambient temperature ($25 \pm 2^\circ\text{C}$) for 10 to 15 days until achieving a constant weight. The dried leaves were then ground to a coarse powder using a mechanical grinder and sieved through a 60-mesh sieve to form a fine, homogeneous powder. The fine powder was first extracted with diethyl ether to remove the lipophilic components like alkaloids, waxes, fats, and fibers.

The above process involved shaking the powder for 15 minutes with diethyl ether. Then, the defatted plant residue

was separated and air-dried and used for further extractions. The powdered defatted plant material was weighed and distributed into five different samples each weighing 200 grams. All the five samples were put in RBFs and one sample was separately treated by n-hexane and methanol. Also, another sample was treated with ethanol, another sample with n-butanol, and finally the last sample with acetone as described above. Solvent of choice was added to the powder in RBFs in a volume-to-weight (v/w) ratio of 1:5. Then, the mixtures were left to incubate for 7 days at an average temperature of $25 \pm 2^\circ\text{C}$. After maceration, the filtrates were concentrated under vacuum pressure at 40°C to obtain crude extracts, respectively. The crude extracts obtained from the above procedure were kept in tight vials at 4°C . Preliminary screening was done on both extracts using qualitative tests to determine the presence of bioactive secondary metabolites such as alkaloids, flavonoids, tannins, saponins, compounds, and others [29-31].

Phytochemical screening of secondary metabolites

Chemical test for phytochemical screening is a basic procedure carried out for the detection and analysis of various types of secondary metabolites present in plants. Secondary metabolites are organic molecules which do not take part in the processes of plant growth, development, and reproduction but rather function as defensive agents and mediators of interactions between plants and their surroundings. They contain complex chemical composition and high biological activity and making them crucial for drug discovery and pharmacological research [32].

Tests for Alkaloids:

Dragendorff's Test:

To a 1 ml of solution in a test tube, add 1-2 ml of Dragendorff's reagent. Shake the test tube for 1 minute and observe the sample. If a solution is formed a reddish-brown precipitate indicates the presence of alkaloids.

Mayer's test:

Test 1 ml of filtrate in a test tube with 1-2 drops of Mayer's reagent by the sides of the test tube, the formation of a creamy white or yellow precipitate indicates the presence of alkaloids.

Wagner's test:

Treat 1 ml of filtrate in a test tube with 1-2 drops of Wagner's reagent, if it forms a brownish or reddish precipitate indicating the presence of alkaloids.

Hager's test:

Treat 1 ml of solution in a test tube with 1-2 ml of Hager's reagent and shake well for 1 min. If a solution is formed a creamy white precipitate indicates the presence of alkaloids.

Tests for Flavonoids

Shinoda's test:

A little amount of the extract was treated with alcohol and filtered. To the alcohol solution, a few magnesium turnings and a few drops of concentrated hydrochloric acid were added and boiled for 5 minutes. If a solution shows a pink to crimson colour indicates the presence of flavonoids.

Alkaline Reagent Test (NaOH Test):

Approach

Take a test tube and add 1 mL of plant extract. Add sodium hydroxide (NaOH) solution. Formation of a yellow color indicates the presence of flavonoids.

Tests for Tannins**Ferric Chloride Test:**

Take a test tube and add 1 mL of water. Add 2 drops of FeCl₃ solution, then add 1 mL of plant extract. Formation of a blue-black or greenish-black color confirms tannins.

Gelatin Test:

Take a test tube and add 1 mL of plant extract. Add 1 mL of gelatin solution (1%) and NaCl solution. Formation of a white precipitate indicates tannins.

Tests for Saponins**Foam test:**

Take 0.5 gm plant extract, add 2 ml water, and make vigorous shaking. If a solution persists in foam for 10 min, then the sample contains saponins.

Tests for Glycosides**Keller Killiani test:**

Treat 5 ml of the sample solution in a test tube by adding 2 ml of glacial acetic acid, 5% ferric chloride reagent, and conc. H₂SO₄. The lower reddish-brown layer and upper acetic acid layer which turns bluish green colour indicate a positive test for glycosides.

Baljet Test:

Take a test tube and add 1 mL of plant extract. Add 2 mL of sodium picrate solution. Formation of an orange color indicates the presence of cardiac glycosides.

Legal's test:

Dissolve 50 mg of plant extract in pyridine and then add sodium nitroprusside and 10% sodium hydroxide solution, if the solution is given a pink colour indicate the sample contains glycosides.

In Vitro antimicrobial activity of *Tridax procumbens* extracts

The antimicrobial activity of various solvent extracts of *Tridax procumbens* (ethanolic, methanolic, acetone, ethyl acetate, and n-butanol extracts) were evaluated for determining their Minimum Inhibitory Concentration (MIC) against *Staphylococcus aureus* (Gram-positive bacterium), *Escherichia coli* (Gram-negative bacterium), and *Candida albicans* (fungal species) using the broth microdilution method.

Preparation of Culture Medium

Brain Heart Infusion (BHI) broth was used as the growth medium for all test microorganisms. The medium contained infusion from calf brain, beef heart, proteose peptone, dextrose, sodium chloride, and disodium phosphate. The medium was sterilized by autoclaving at 121°C for 15 minutes.

Preparation of Microbial Inoculum

Fresh cultures of *S. aureus*, *E. coli*, and *C. albicans* were obtained from stock cultures. Approximately 5 µL of each microbial suspension was inoculated into 2 mL of sterile BHI broth and incubated to obtain actively growing cultures [33, 34].

Preparation of Extract Solutions

Each extract of *T. procumbens* was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution. Serial two-

fold dilutions were prepared in sterile BHI broth to achieve final concentrations of: 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0.20 µg/mL.

MIC Determination

For each extract, ten sterile test tubes were arranged. The first tube contained 380 µL of BHI broth and 20 µL of extract stock solution. The remaining nine tubes contained 200 µL of sterile BHI broth. From the first tube, 200 µL was transferred to the second tube and mixed thoroughly to obtain a two-fold dilution. This serial dilution procedure was continued up to the tenth tube to obtain the required concentration range.

Subsequently, 200 µL of the prepared microbial inoculum was added to each tube. Positive control tubes containing inoculated broth without extract and negative control tubes containing broth only were included in the experiment. The tubes were incubated at 37°C for 24 h for *S. aureus* and *E. coli*, 28°C for 48 h for *C. albicans*. Following incubation, the tubes were examined visually for turbidity. The MIC was defined as the lowest concentration of extract that completely inhibited visible microbial growth. All experiments were performed in triplicate, and the results were expressed as mean MIC values.

RESULTS & DISCUSSION**Common Targets for antimicrobial activity Vs *T. procumbens***

Venn diagram demonstrated in **Fig. 1** explains the overlying and unique gene targets associated with *Tridax Procumbens* phytoconstituents and antimicrobial activity (data collected from Venny 2.1.0; database). The image reveals unique genes corresponding to the antimicrobial activity, while *Tridax Procumbens* shows targets. A total of 21 genes were common between *Tridax Procumbens* plant and antimicrobial activity respectively.

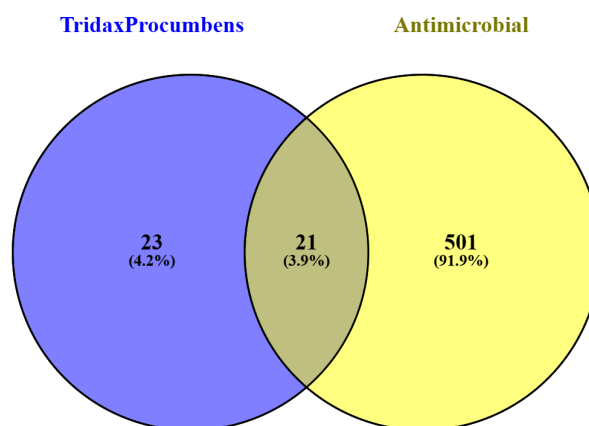


Fig. 1 Venn diagram showing the overlapping and gene targets associated with *Tridax Procumbens* phytoconstituents Vs antimicrobial activity.

PPI Network Construction and Hub Genes Identification

The PPI network construction involved by importing 21 targets of genes into STRING (as shown in **Fig. 2a**). This led to the formation of 21 nodes and 51 edges, average node degree as 4.86 in the network (as depicted in **Fig. 2** obtained by keeping minimum interaction score as medium

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confidence level of 0.4 in settings tab. Further downloaded string interaction file opened in Cytoscape software to visualize the PPI network. In CytoHubba application, the top ten nodes were selected from the network by selecting MCC method. These nodes were HIF1A, MET, NFKB1, SRC, TNF, KDR, AXL, ABCG2, XDH, ALOX5 and PTGS1 were depicted in **Fig. 2b**.

Table 1. Top 10 hub genes, scores Vs corresponding ranks from cytoscape

S. No	Name of Hub gene	Rank	Score
1	HIF1A	1	165
2	NFKB1	2	164
3	SRC	3	156
4	TNF	4	152
5	KDR	5	146
6	AXL	6	120
7	ABCG2	7	31
8	XDH	8	14
9	ALOX5	9	12
10	PTGS1	10	12

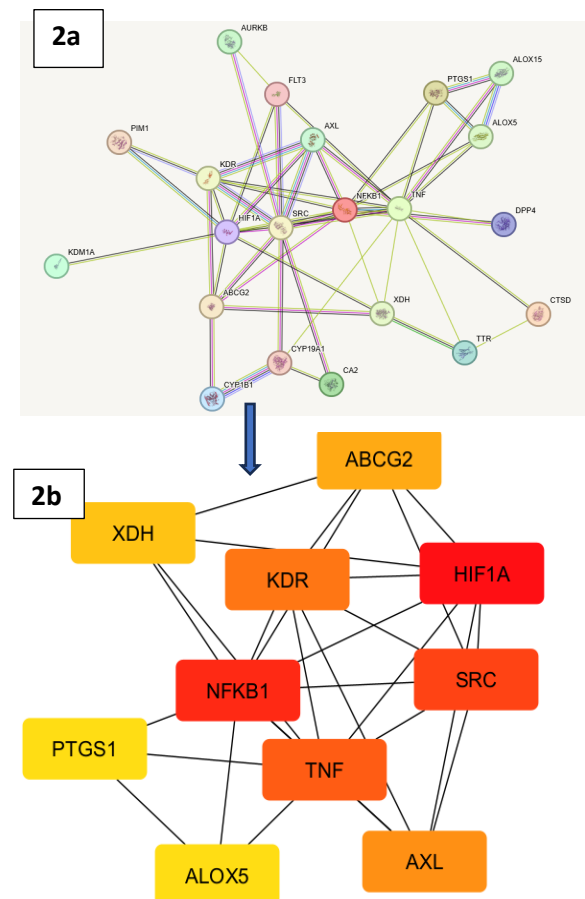


Fig. 2 Protein-protein interactions in a cluster. (a) PPI interactions of target genes. Each node represents, common proteins between *Tridax Procumbens* Vs antimicrobial activity, degrees of nodes represented by colour and size. (b) Representation of top ten hub genes and their interactions by using cytoHubba plugin.

GO and KEGG Pathway Analysis

GO investigation grouped the enriched terms like EGFR Tyrosine Kinase Inhibitor Resistance as shown in **Fig. 3** in categories.

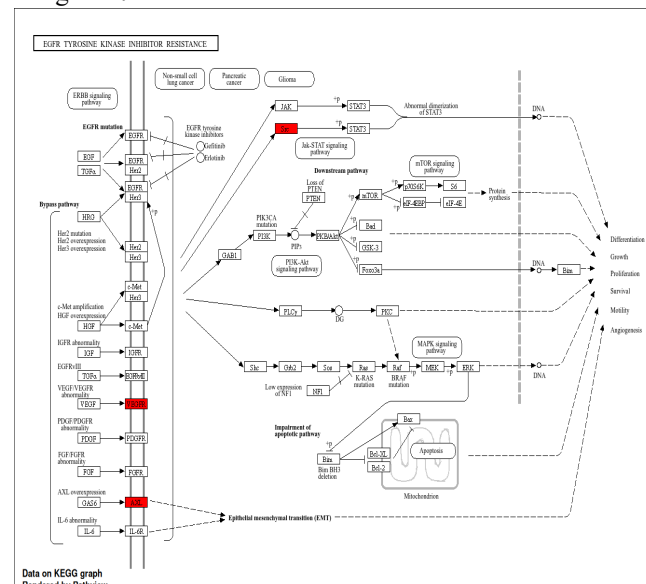


Fig. 3 GO enrichment analysis for the top targets associated with *T. procumbens* exhibiting antimicrobial activity, above diagram demonstrating the EGFR Tyrosine Kinase Inhibitor Resistance term of the top ten hub genes identified by CytoHubba.

KEGG Pathway Enrichment Analysis of Key Target Genes

The KEGG pathway enrichment analysis was performed for the top ten *Tridax Procumbens* associated antimicrobial targets by using Shiny GO 0.85 database by keeping FDR cutoff (p-value) 0.05. The lollipop plot displays the top ten enriched antimicrobial related pathways ranked by fold enrichment, where colour intensity represents the significance level ($-\log_{10}FDR$) and size of bubble represents to the number of genes involved in each pathway as shown in **Fig. 4** and **Fig. 5** represents the microtubule - cytoskeleton representation of top 10 hub genes related pathways.

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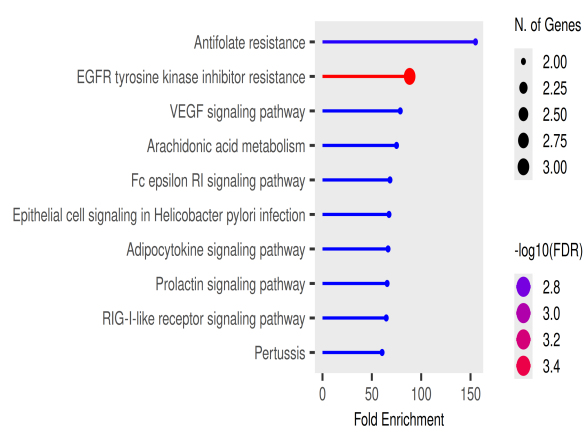


Fig. 4 Enrichment analysis of KEGG pathway for top ten target genes associated with the antimicrobial activity of *Tridax procumbens* derived target genes.

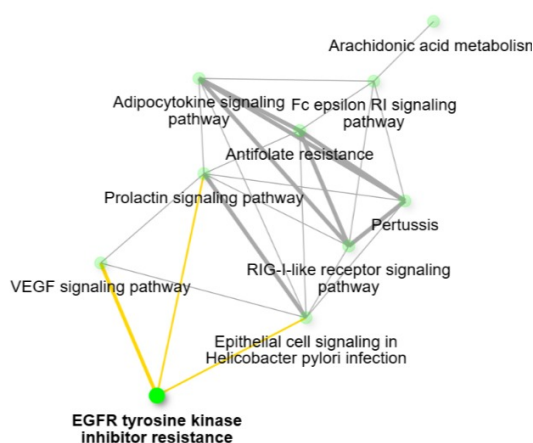


Fig. 5 Microtubule- cytoskeleton representation of top 10 hub genes related pathways

Preliminary Phytochemical Screening

The crude extracts of *T. procumbens* leaves were prepared by solvent extraction using ethanol, methanol, ethyl acetate, n-butanol, and n-hexane. A preliminary phytochemical screening was carried out to determine the presence of important phytochemical components in the maceration extract of *T. procumbens* by following standardized and well-established methods.

The phytochemical screening results of the maceration extract of *T. procumbens* were presented in **Table 2**. The analyses revealed the presence of important biologically active constituents such as alkaloids, Cardiac Glycosides, Tannins, Saponins and Flavonoids compounds in maceration extract represented in **Fig. 6**.

Thin Layer Chromatography (TLC) was executed for *Tridax procumbens* extract on the TLC plate, using the mobile phase (n-hexane: ethyl acetate) in the ratio of 1:4, in the TLC chamber. After the optimized development, the TLC plate was taken out and dried in the air for 5 minutes. Detection of the compounds was conducted by visual inspection, using UV light, and by employing the iodine

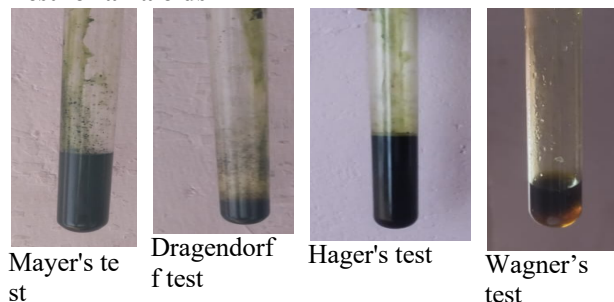
chamber. The identification of the chemical compounds in the mixture was conducted by the appearance of the colors in the iodine vapor and the UV light, and the calculation of the retardation factor (Rf) value.

Table 2. The preliminary phytochemical analysis

S.no	Phytochemical constituents	Test	Positive / Negative
1	Cardiac Glycosides	keller kiliani test Baljet test Legal test	- - +
2	Alkaloids	Mayer's test Dragendorff test Hager's test Wagners's test	- + - +
3	Tannins	Gelatin test Ferric chloride test	- -
4	Saponins	Foam test	-
5	Flavonoids	Shinoda's test Sodium hydroxide test	- -

Table 3 represents the MIC data for *T. procumbens* extracts. Among the tested extracts, the methanolic extract exhibited the highest antimicrobial activity, showing the lowest MIC values against all tested microorganisms. This may be attributed to the higher extraction efficiency of phenolic compounds, flavonoids, tannins, and other bioactive constituents present in *Tridax procumbens*. Ethyl acetate and ethanolic extracts also demonstrated considerable antimicrobial activity, whereas acetone and n-butanol extracts exhibited comparatively lower potency.

Test for alkaloids



Test for flavonoids

Test for tannins

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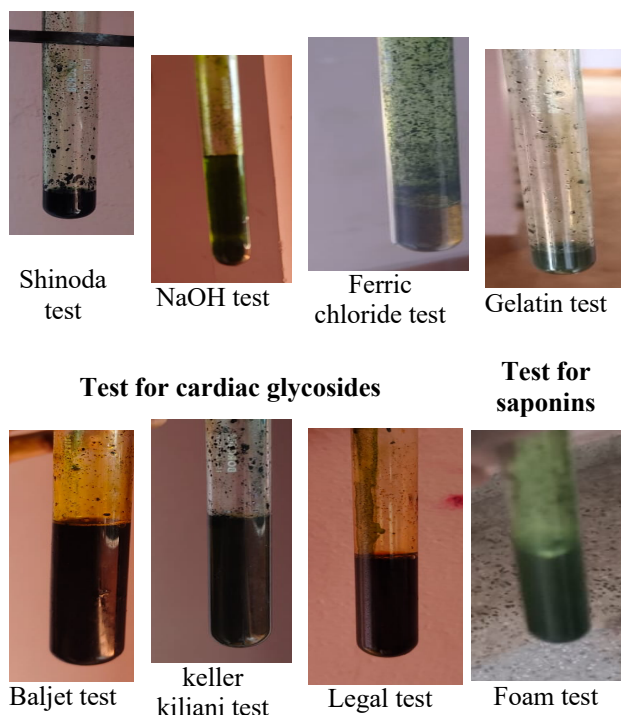


Fig. 6 Images of various phytochemical tests

Table 3. MIC data for *Tridax procumbens* extracts

Plant extract	<i>S. aureus</i> MIC ($\mu\text{g/mL}$)	<i>E. coli</i> MIC ($\mu\text{g/mL}$)	<i>C. albicans</i> MIC ($\mu\text{g/mL}$)
Ethanollic extract	12.5	25	25
Methanolic extract	6.25	12.5	12.5
Acetone extract	25	50	25
Ethyl acetate extract	12.5	25	12.5
n-butanol extract	25	25	50
Ciprofloxacin (Standard)	0.78	0.98	-
Fluconazole (Standard)	-	-	0.85

CONCLUSION

The present study successfully integrated network pharmacology and experimental validation to investigate the multitarget antimicrobial potential of *T. procumbens*. Network pharmacology analysis identified 21 common targets associated with *T. procumbens* phytoconstituents and antimicrobial activity. Protein-protein interaction analysis revealed key hub genes, including HIF1A, NFKB1, SRC, TNF, KDR, AXL, ABCG2, XDH, ALOX5, and PTGS1, suggesting their crucial roles in mediating the biological effects of the plant. Functional enrichment and KEGG pathway analyses further demonstrated the involvement of several signaling pathways related to host defense, inflammation, cellular response, and microbial

pathogenesis, highlighting the multitarget mode of action of *T. procumbens*.

Preliminary phytochemical screening confirmed the presence of biologically active secondary metabolites, particularly alkaloids and cardiac glycosides, which may contribute to the observed antimicrobial activity. In vitro antimicrobial evaluation demonstrated that all solvent extracts exhibited varying degrees of inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Among the tested extracts, the methanolic extract showed the highest antimicrobial efficacy with MIC values of 6.25 $\mu\text{g/mL}$ against *S. aureus* and 12.5 $\mu\text{g/mL}$ against both *E. coli* and *C. albicans*, indicating that polar phytoconstituents are likely responsible for the enhanced antimicrobial effects.

Overall, the findings provide scientific evidence supporting the traditional use of *Tridax procumbens* in the treatment of microbial infections and demonstrate that its antimicrobial activity is mediated through multiple targets and biological pathways. The integration of computational and experimental approaches offers valuable mechanistic insights into the therapeutic potential of *T. procumbens*. Further studies involving bioassay-guided isolation, molecular docking, mechanistic investigations, and in vivo validation are warranted to identify the active compounds and facilitate the development of novel plant-derived antimicrobial agents for combating antimicrobial resistance.

AUTHOR CONTRIBUTIONS

Chilamakuru Naresh Babu: Conceptualization, Methodology, Investigation, Dasari Sravani: Visualization, Writing original draft. Gujjala Vishnuvardhan: Methodology. Validation, Conceptualization. Bhople Supriya: Methodology, Investigation, Formal analysis. Yellamagari Varshini: Data curation, Conceptualization. Kuruva Sheshadri: Methodology, Validation. Singirisetty Triveni: Writing original draft, Methodology, Visualization, Validation and supervision.

DECLARATION OF CONFLICTS OF INTEREST

The authors declare no conflicts of interest, financial or otherwise, that may have influenced the research described in this article.

DATA AVAILABILITY

The data that has been used is confidential.

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