Implementation of System Pharmacology and Molecular Docking Approaches to Explore Active Compounds and Mechanism of Ocimum Sanctum against Tuberculosis

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Abstract: Worldwide, Tuberculosis (TB) is caused by Mycobacterium tuberculosis bacteria. Ocimum sanctum, commonly known as holy basil (Tulsi), is an herbaceous perennial that belongs to the family Lamiaceae and is considered one of the most important sources of medicine and drugs for the treatment of various diseases. The presented study aims to discover the potential phenomenon of Ocimum sanctum in the medicament of tuberculosis using a network pharmacology approach. Active ingredients of Ocimum sanctum were fetched through two different databases and from literature review and then targets of these compounds were harvested by SwissTargetPrediction. Potential targets of TB were downloaded from GeneCards and DisGNet databases. After screening of mutual targets, enrichment analysis through DAVID was performed. Protein–protein interaction analysis reveals those ten targets that formed the core PPI network. Furthermore, molecular docking analysis reveals that active compounds have the greater binding ability with the potential target to suppress TB.

Keywords: Ocimum sanctum; tuberculosis; network pharmacology

1. Introduction

Herbal plant species remain essential for humanity since they provide different and modern medicines for the health sector [1]. Herbal ingredients, which have therapeutic potential, have increasingly become a hub for novel medicinal remedies [2]. Because of extensive multi-target therapeutic actions, the vast varieties of phytochemicals have attracted the attention of researchers. The therapeutic advantages of medicinal plants are: they are economically cheap, safe, effective, and readily available [3]. Medicinal plants have now emerged as a viable option since they are high in a variety of secondary metabolites such as tannins, phenolics, alkaloids, and flavonoids, which improve growth, innate immune response, and disease resistance in humans. Ocimum sanctum (holy basil)

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belongs to the Lamiaceae family, is considered one of the major sources of medicines, drugs with secondary metabolites, and essential oils recommended for different diseases [4,5]. Ocimum sanctum also shows anti-microbial, anti-spasmodic, anti-asthmatic, anti-rheumatic, and hepatic-protective activities [6,7]. Because of their unrivalled potential in treating many problems, all parts of the plant are beneficial in therapeutics [8].

With an estimated 1.7 billion persons infected, Mycobacterium tuberculosis (M.tb) is the most common disease in humans. The causal factor of TB is Mycobacterium tuberculosis, which mainly affects the lungs. Tuberculosis is transmitted between humans through aerosol inhalation, usually between people who live in close quarters. Unlike other mycobacterial species, Mycobacterium tuberculosis has no reservoir in the environment, meaning it is mainly transmitted between humans [9]. People living with HIV are more likely to obtain active tuberculosis [10]. Tuberculosis is a disease of poverty occurring in developing countries and is the leading cause of mortality and morbidity in these countries [11]. The World Health Organization (WHO) claims that 87% of new TB cases were reported in 2019 in 30 countries, eight of which accounted for two-thirds of the TB cases, and most of which are Asian countries like Pakistan, India, and Bangladesh. Increased multidrug-resistant TB is an equally important public health issue [12].

The current therapies against TB are the use of antibiotics like rifampicin [13], isoniazid [14], and pyrazinamide [13–15]. However, the problem is the emergence of drug resistance of mycobacteria against these modern drugs. In contrast, herbal medicines used by either extraction or anti-pathogen decoction will not allow the pathogen to develop resistance [16]. Hence, herbal treatment is an efficient and cheap therapy used as an anti-tuberculosis drug. The importance of medicinal plants has been increasing for a few years; herbal plants are the primary source of bioactive molecules and potential drugs that play a vital role in controlling various types of pathogens [17].

Network pharmacology is the new in-silico drug discovery approach developed by Hopkins in 2007 [18]. This drug discovery approach combines data science and clinical therapeutics [19]. Network pharmacology is the method used to study the synergistic action [20] and underlying mechanisms of traditional herbal medicines [21]. Network pharmacology has grown in popularity in recent years and is now a widely used strategy in drug discovery. To identify the processes that affect the simultaneous treatment methods of conventional medicines, network pharmacology has an holistic in-silico strategy for designing a “protein-compound/disease-gene” network [22]. The active components of O. sanctum were investigated using a network pharmacology-based method in this study. This research sheds fresh light on the molecular mechanisms of O. sanctum against tuberculosis and potentially accelerating the drug discovery process. In light of the results of this investigation, it is believed that the network pharmacology technique adopted here could be a new beginning for discovering the active molecule that could be examined in the laboratory and give a foundation for studying the pharmacological mechanism of O. sanctum. The procedural-specific schematic diagram is shown in Figure 1.
Figure 1. Schematic diagram of network pharmacology to explore mechanism of O. sanctum to treat Tuberculosis.
2. Materials and Methods

2.1. Screening of Chemical Constituents of *O. sanctum*

All the information about chemical constituents of *Ocimum sanctum* was collected from published literature [6], KNAPSAcK Core System database (http://www.knapsackfamily.com/ 15 August 2021), and traditional Chinese medicine system pharmacology (TCMSP) (https://old.tcmsp-e.com/ 16 August 2021). The plant name as *Ocimum sanctum* was used to search the active components in the KNAPSAcK database [23], while a literature search was done through Google Scholar, PubMed and the CNKI database. The physicochemical properties and 3D structure of the whole identified compounds were searched in the PubChem (http://www.ncbi.nlm.nih.gov/pubchem/ 21 August 2021) database via the name and CID/SID number. The canonical SMILES of all the active compounds were collected from PubChem database. Then these canonical SMILES were used to find the pharmacokinetics properties of the entire active chemical constitute. The ADMET parameters of each ingredient were analyzed using the admetSAR tool and TCMSP database, e.g., drug-likeness (DL) and oral bioavailability (OB) [24]. OB is the oral bioavailability of pharmacological ingredients, and DL indicates similarity between a drug and a component, which can indicate potential drug. Subsequently, the active compounds that met the Drug Likeness (DL $\geq 0.18$) and Oral Bioavailability (OB $\geq 30$) [25] criteria were chosen as novel compounds and employed for further screening.

2.2. Potential Target Screening of Tuberculosis

Putative targets of selected active ingredients were found through SwissTargetPrediction (http://www.swisstargetprediction.ch/ 22 August 2021) through giving the canonical SMILES strings from PubChem (http://pubchem.ncbi.nlm.nih.gov/ 22 August 2021). For this study, species were chosen as *Homo sapiens*, and the predicted target results were collected. SwissTargetPrediction is the most widely used software to predict the most likely protein targets of bioactive chemicals. Using the keyword of “tuberculosis”, the potential targets were selected from GeneCards (http://www.genecards.org/ 23 August 2021) and DisGeNET (http://www.disgenet.org/ 23 August 2021). The targets of the two databases were combined. [26]. Furthermore, all redundant genes were removed from the study. The standard name of the target was obtained using UniProtKB, with the organism chosen as “*Homo sapiens*”. A Venn diagram was constructed by achieving the mutual targets of disorder and drug. Those common targets of disease and compounds were considered potential targets for further analysis.

2.3. Gene Function Annotation

The functional enrichment database DAVID (http://david.ncifcrf.gov/ 24 August 2021) gives the comprehensive annotation of genes, GO analysis provides the gene function classification, and it comprises biological process (BP), cellular component (CC), and molecular function (MF), with pathway analysis provided by KEGG database. The 72 common targets were pasted into DAVID for GO annotation and pathway enrichment analysis and the species selected as “*Homo sapiens*”. The enriched pathways with a probability score of less than 0.05 were selected. For the visualization of these GO annotation and KEGG pathways, bubble maps were drawn using the ggplot2 package of R-language.

2.4. Network Pharmacology Analysis

2.4.1. Compound-Target Network construction

To create the compound-target network, the active components of *Ocimum sanctum* and the tuberculosis therapeutic target were entered into Cytoscape (version 3.8.2) (http://cytoscape.org/ 25 August 2021). Cytoscape is a free software for viewing and merging complicated networks with any sort of data types. The network nodes symbolize the chemical constituents and targets, while edges show their interaction. The “network analyzer” was used to evaluate the essential properties of the network [27]. The network
was filtered based on “degree”, network node attributes in which the degree represents the number of connected nodes with a particular network node.

2.4.2. Prediction of Hub Genes and PPI Network

The correlation between the therapeutic targets of TB was evaluated using the STRING database (version 11.0, Manufactured by: ELIXIR, Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK) (https://string-db.org/ 25 August 2021). The organism was named “Homo sapiens,” and the combined score was set to 0.4 or above (Figure 2). Cytoscape was used to visualize the PPI network. The CytoHubba plugin was used to find the hub genes and nodes with a higher degree [28] and referred to for further investigation. The highest degree indicates that the targeted genes are highly linked.

Figure 2. PPI Network of 72 intersected key targets (O. sanctum and Tuberculosis).

2.4.3. Target–Compound–Pathway Network Construction

The top 20 pathways were evaluated using DAVID based on KEGG pathway analysis to design the target–compound–pathway network with Cytoscape [29].

2.5. Molecular Docking

Molecular docking facilitates the identification of interactions of ligands to their correlating proteins. For the validation of the results of this study, molecular docking was used. The SDF file formats of the 3D structure of active ingredients were downloaded from the PubChem database and optimized these structures, the PDB format file of hub genes was downloaded from Protein Data Bank (RCSB) PDB, (http://www.rcsb.org/ 28 August 2021). The finest protein crystal structure (smaller resolution value, complete structure, and
human protein) was selected for docking. For the removal of ligands, water molecules and for correcting protein structure, Chimera (version 1.15, Manufacturer is RBVI, Resource for Biocomputing, Visualization, and Informatics, University of California, 600 16th Street, M/S 2240, San Francisco, CA, USA, 94158-2517) was used. The ligand and protein molecules were charged, hydrogenated, and normalized using AutoDockTool, and PDBQT file format was created. Molecular docking was performed between the refined ingredients and protein using AutoDock Vina. To calculate the binding energy, the Lamarckian genetic algorithm was selected, and chimera software was used for the visualization of the docked results. By measuring the strong affinity between chemicals and their related targets, this stage aimed to investigate the binding energy between them; the higher the affinity, the lower the binding energy.

3. Results

3.1. Active Ingredients Screening of *O. sanctum*

After the duplicates have been found, filtered, and removed, about 48 active compounds of *Ocimum sanctum* were collected from a literature survey and two different databases. After performing ADMET analysis on these 48 compounds, 8 active ingredients were selected (Table 1) as effective components using DL and OB (DL ≥ 0.18 & OB ≥ 30). These unique compounds were evaluated through PubChem to determine their chemical structure.

| Chemicals     | Oral Bioavailability | Drug Likeness | Chemical Structures |
|---------------|----------------------|---------------|---------------------|
| Campesterol   | 37.58                | 0.71          | ![Chemical Structure](attachment:image1.png) |
| Cirsimaritin  | 30.35                | 0.3           | ![Chemical Structure](attachment:image2.png) |

Table 1. OB and DL values of active ingredients of *O. sanctum*. 
| Chemicals         | Oral Bioavailability | Drug Likeness | Chemical Structures |
|------------------|----------------------|---------------|---------------------|
| Triterpenoid glycoside | 34.11                | 0.63          | ![Chemical Structure](image) |
| Luteolin         | 36.16                | 0.25          | ![Chemical Structure](image) |
| ß-sitosterol     | 36.91                | 0.75          | ![Chemical Structure](image) |
| ß-carotene       | 37.18                | 0.58          | ![Chemical Structure](image) |
| Stigmasterol     | 43.83                | 0.76          | ![Chemical Structure](image) |
Table 1. Cont.

| Chemicals                  | Oral Bioavailability | Drug Likeness | Chemical Structures |
|----------------------------|----------------------|---------------|---------------------|
| Luteolin-7-O-glucuronide   | 36.16                | 0.25          |                     |

3.2. Analysis of Network Pharmacology
3.2.1. Identifying Potential Targets

The 352 targets were retrieved from 8 chemical ingredients of *Ocimum sanctum* through the SwissTargetPrediction database (supplementary data Table 1). The potential targets of tuberculosis were found in the GeneCard and DisGeNet databases, which yielded 2407 and 1257 results, respectively. After removing the duplicates and merging the disease related targets 632 disease targets were screened out. (supplementary data Table 2). Potential mapping of these 352 targets of active ingredients with 632 targets of TB results in 72 common targets (Figure 3), which were considered potential targets of *Ocimum sanctum* against tuberculosis.

Table 2. Degree of 8 active compounds analyzed in Cytoscape.

| Compounds                  | Class Categories | Degree |
|----------------------------|------------------|--------|
| Campesterol                | Sterol           | 17     |
| Cirsimaritin               | Flavones         | 21     |
| Triterpenoid glycoside     | Terpenoid        | 22     |
| Luteolin                   | Flavones         | 21     |
| ß-sitosterol               | Sterol           | 20     |
| ß-carotene                 | Carotenoid       | 15     |
| Stigmasterol               | Sterol           | 19     |
| Luteolin-7-O-glucuronide   | Flavones         | 29     |

Figure 3. Venn diagram showing common targets.
3.2.2. Compound-Target Network

A network between potential targets and active compounds was constructed using Cytoscape to analyze the interaction between the active compounds and potential targets. A compound-target network was created with 72 potential target genes and eight active ingredients of *O. sanctum*. Analyzing the network with a "network analyzer" shows that the compound-target network comprises 165 edges and 80 nodes (Figure 4).

![Compound-target network between active compounds and common targets of disease and compounds (red color shows active ingredients and purple color shows potential targets).](image)

In the network, red-colored nodes at the center represent the constituents of *O. sanctum*, while the rest of the purple-colored nodes show the potential targets of TB. Furthermore, the 165 edges depict the interaction of chemicals and targets. The degree of eight active compounds was analyzed in a compound-target network (Table 2). In the table the flavones and terpenoids have the comparatively highest degree while carotenoids and sterols were comparatively lower. All eight targets were selected for further molecular docking analysis. Target-Compound network analysis shows that one active ingredient can affect many
targets, while the same target may interact with more than one active compound. This reflects the multi-target and multi-components effects of the *O. sanctum* in the medication for TB.

3.2.3. Analysis of PPI Network

Protein–protein interactions (PPI) are extremely important because of their versatility, adaptability, and selectivity. Protein–protein interaction analysis reveals the correlation between the targets. After visualizing the PPI network in Cytoscape, 72 nodes with 587 edges were found (Figure 5). CytoHubba plugin was utilized to find the Hub genes. In the CytoHubba, there are twelve topological methods of analysis. From these 12 methods, the degree method was used to predict Hub genes. The highest degree shows more connectivity of the targets with other ones, indicating that it could be a key target. The blue nodes in the network show the top 10 hub genes with high degree values, comprising AKT1 (49), TNF (47), EGFR (44), STAT3 (43), PTGS2 (40), MAPK1 (37), CASP3 (37), MMP9 (35), IL2 (34), MAPK14 (28) (Figure 6).

![Figure 5. PPI network analysis of 72 targets. According to topological analysis, ten blue targets are key nodes (central blue nodes are hub genes with higher degree and yellow nodes represent other potential targets).](image-url)
3.3. KEGG Pathway and Go Analysis

To illustrate the molecular mechanism of *O. sanctum* in the medication for TB, GO annotations and KEGG pathway analysis were operated on 72 anti-tuberculosis targets. GO analysis recognized 202 biological processes (BP), including response to the drug, response to hypoxia, signal transduction, and many more; 28 cellular components (CC), which include extracellular region, cytosol, plasma membrane, and organelle membrane; and 53 molecular functions (MF) including serine-type endopeptidase activity, protein kinase activity, heme-binding, histone kinase activity. KEGG analysis predicted 84 pathways regarding the anti-tubercular targets. Applying the cutoff value $p < 0.05$ top 20 GO annotations (BP, CC, and MF) (Figure 7) and 20 KEGG pathways (Figure 8) were selected. GO annotations and KEGG pathway were shown in the figure by drawing a bubble map with R-language, and also the KEGG pathways were shown in the target-compound-pathway network.

3.4. Target–Pathway–Compound Network Analysis

The mechanism of *O. sanctum* in TB was studied using network analysis. For this, the top 20 enriched pathways were selected by DAVID analysis, and the target–pathway–compound network was created with Cytoscape. There were 102 nodes and 311 edges in the network, with eight active components, 72 potential targets, and 20 relevant pathways (Figure 9). The node color and size were set according to their degree values. The targets of *O. sanctum* active components show coordination with diverse paths and are connected to each other, and play a major role in the treatment of TB, which widely embodies traditional Chinese medicine’s multi-target, multi-component, and multi-pathway physiognomies.
3.5. Molecular Docking

The prospective targets of components with the ability to reduce the incidence of TB were screened using molecular docking. The significant binding affinity between components and binding pockets of target proteins was successfully predicted using docking analysis. For the molecular docking, five target genes AKT1, MAPK1, MAPK14, CASP3, and TNF were selected by comparing the hub genes with the results provided by KEGG analysis in the pathway of tuberculosis. All the eight active components from *O. sanctum* were docked with the five potential targets of tuberculosis. The lower (more negative) the binding energy, the stronger the anticipated affinity for binding of the ligand against the target in molecular docking. The range of the binding score between all the compounds and targets was from $-2.35$ to $-10.55$ kcal/mole (Table 3). The docking results show that triterpenoid glycoside and stigmasterol have higher binding energy with key tuberculosis targets. This outcome evaluated the accuracy of the current study. Exploring the connection between these five targets is crucial to comprehend the anti-TB activity of active components fully.
3.4. Target–Pathway–Compound Network Analysis

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Table 3. Docking results show the binding energies of potential targets with compounds. The green color shows the high, yellow color show that moderate while red color show low binding energy.

| Compounds                | AKT1  | MAPK1 | MAPK14 | CASP3 | TNF  |
|--------------------------|-------|-------|--------|-------|------|
| β-carotene               | −5.73 | −7.35 | −2.35  | −10.23| −8.36|
| β-sitosterol             | −3.85 | −5.21 | −6.37  | −7.65 | −9.31|
| Campesterol              | −9.80 | −5.66 | −8.24  | −2.57 | −6.57|
| Leutolin                 | −4.36 | −6.34 | −7.36  | −5.56 | −7.78|
| Luteolin-7-O-glucuronide| −6.31 | −6.88 | −5.67  | −3.69 | −10.31|
| Triterpenoid Glycoside   | −10.55| −9.36 | −9.85  | −4.33 | −7.32|
| Cirismartin              | −3.00 | −5.31 | −7.34  | −4.37 | −5.24|
| Stigmasterol             | −7.64 | −9.19 | −4.37  | −10.27| −9.37|

In this analysis, almost all the active ingredients show the high binding affinities with the core targets, which declared that active ingredients of *O. sanctum* have the better binding ability with the tuberculosis core targets. Docking results of five targets with chemical constituents are displayed (Figure 10).
3.5. Molecular Docking

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**Figure 9.** Target-compound-pathway network between active compounds, targets genes and enrichment pathways, node size and color show the interaction of node.
4. Discussion

In recent years, natural product research has earned more interest [30,31]. The use of network pharmacology techniques promotes a better understanding of the extensive connections and association between drugs, their targets, and their possible mechanisms of action [32–34]. The drug development complexities from natural resources lead to methodological difficulties [35]. The newly developed drug is meager due to the lack of ADME properties, and the high-budget nature of development more difficulties to drug discovery methods [36]. Therefore, pharmaceutical experts pay much attention to ADME-based screening in medication development [37].

TB is a highly chronic, infectious, and contagious disease. Isoniazid and rifampicin are widely utilized as first-line anti-TB drugs, particularly pulmonary TB. However, their efficacy has been found to be reduced due to hepatotoxicity, CNS toxicity, or multi-organ toxicity [38]. Only a few medicines, such as bedaquiline, delamanid, and pretomanid, have been licensed to treat multi-drug resistant-TB in the last 46 years. As a result, the quest for new drugs has become more targeted. An elevated source of phytoconstituents with health benefits could be a suitable TB therapeutic candidate in this scenario.

*Ocimum sanctum* is an herb suitable for all reasons, which is reported to have antioxidant, anti-bacterial, anti-fungal, anti-inflammatory, immunological effect, anti-asthmatic, hepatoprotective, and anti-stress activities [39,40]. *Ocimum sanctum* is a mainly rich source of phytochemicals; more than 60 chemical compounds including phenolics, flavonoids, terpenoids, steroids, eugenol, sitosterol, etc. are reported in *Ocimum sanctum*. This work serves as a benchmark for the initial screening of *O. sanctum*’s bioactive chemicals and a novel therapeutic notion for further research into *O. sanctum*’s mechanisms for TB treatment.

According to GO annotation results, anti-tuberculosis targets of *O. sanctum* were mainly involved in serine-type activity, response to oxidative activity, endopeptidase activity, and protein serine/threonine kinase activity (STPK) [41]. Nine STPKs comprise an extracytoplasmic sensor domain and an intracellular kinase domain, indicating external signal transducers. The regulatory domains of the other two STPKs detect the change in the cell and are found in the cytoplasm [42]. In response to hypoxia, hypoxia increased the antigen-specific up-regulation of granulysin mRNA and protein in human CD4+ and CD8+ T lymphocytes [43]. The anti-microbial effector mechanism is activated by hypoxia [44,45].
Negative regulation of the apoptotic process [46,47] and serine pathway [48] is a significant activity that the key targets of *O. sanctum* would suppress. KEGG pathway exploration revealed that multiple gene targets of the *Ocimum sanctum* served an imperative role in numerous tuberculosis-related pathways. Amongst these, tuberculosis which is the main targeted pathway in this study, genes like MAPK1, AKT1, TNF, VDR, CASP3, and five other target genes of *Ocimum sanctum* which are involved in this pathway. According to KEGG pathway research, targets were found to be enriched in tuberculosis-related pathways’ TNF signaling pathway. Tumor necrosis factor-alpha (TNF) is an important element in the control of *Mycobacterium tuberculosis* [49,50] by enhancing T cell responses and promoting macrophage phagolysosomal fusion (which improves CD4+ T cell immunity by improving antigen presentation) and apoptosis [51,52] (which can lead to CD8+ T cell cross-priming) [53]. In HIF-1 signaling pathway, activation of hypoxia-inducible factor 1(Hif-1) enhanced the autophagy in Tuberculosis infected cells and production of IL-6 and TNF-α [54] that control the *mycobacterium tuberculosis* infection. At the same time, T cell receptor signaling pathway [55], up-regulates the T cell receptor which results to enhances the immunity which controls further infection. By the KEGG pathway analysis, it is revealed that many targeted genes participated in numerous metabolic pathways. The disease mechanism can be prevented by targeting the genes that disrupt metabolic pathways.

By network analysis, five key targets TNF, AKT1, MAPK14, MAPK1, and CASP3 were screened out to check their binding affinity with eight active ingredients of *O. sanctum* as anti-tuberculosis activity. Stigmasterol and Triterpenoid Glycoside were successfully docked with the five potential targets with high binding affinity compared with other ingredients. Triterpenoid Glycoside could bind with MAPK1 and MAPK14 to suppress tuberculosis activity. Stigmasterol shows a high affinity with MAPK1 and TNF to inhibit tuberculosis. Sterols, terpenoids, and flavones were reported to play a crucial role in the anti-tuberculosis process. Further network analysis discloses that *O. sanctum* produces therapeutic effects on tuberculosis, suppressing the key targets of tuberculosis. The current study elaborates the active molecules, their prospective targets, and associated pathways to treat TB in light of network pharmacology, thus giving a conceptual framework for further experimental investigation.

5. Conclusions

This study lays a new scientific validity for establishing the effectiveness of multi-component, multi-target compound regimens and identifying additional TB treatment targets. Network pharmacology combined with a molecular docking approach was used to determine the molecular mechanism of *O. sanctum* for the treatment of TB. Network analysis shows that *O. sanctum* comprises multi-targeting agents that simultaneously operate on multiple tuberculosis pathways. Furthermore, data suggested that the AKT1, MAPK14, TNF, MAPK1, and CASP3 genes are prospective and effective therapeutic targets for preventing and reducing TB, potentially resulting in positive efficacy in TB. Nevertheless, there are some limitations to the present study, as more phytochemical and pharmacological research is needed to confirm our observations.

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