An Efficient Weighted Network Centrality Approach for Exploring Mechanisms of Action of the RP-RB Herbal Formula for Treating Rheumatoid Arthritis

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RESEARCH

An Efficient Weighted Network Centrality Approach for Exploring Mechanisms of Action of the RP-RB Herbal Formula for Treating Rheumatoid Arthritis

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Abstract

Aim : This study outlines an efficient weighted network centrality measure approach and its application in network pharmacology for exploring mechanisms of action of the *Ruellia prostrata (RP)* and *Ruellia bignoniiflora (RB)* herbal formula for treating rheumatoid arthritis.

Method: We first calculated interconnectivity scores all the network targets. We then computed weighted centrality measure using the calculated interconnectivity score to identify the major network targets. To apply our technology to network pharmacology, a tripartite drug target network, was first built to identify the potential RP-RB active compounds. An imbalance network was then built from a union merge of a multi-level network of drug target network and disease target network to explore the mechanism of action of RP-RB compositive compounds in the treatment of rheumatoid arthritis. The major identified network targets were then validated by the enrichment analysis and a molecular docking simulation.

Result : The experimental results indicated that out of 196 compounds, 22 actively interacted with the putative target genes associated with rheumatoid arthritis. An imbalance network simulation identified a total of 33 major network targets. These includes 8 RP-RB compositive compounds, 10 therapeutic targets and 15 putative target genes. The the enrichment analysis based on the Gene Ontology and a KEGG pathway demonstrated that majority of candidate putative target genes were frequently involved in TNF, CCR5, IL-17 and G-protein coupled receptors signaling pathways which are critical in the progression of rheumatoid arthritis . The molecular docking simulation indicated that Glyceryl diacetate-2-Oleate, Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester, Glycidyl oleate, and Oleoyl chloride and candidate known rheumatoid arthritis therapeutic targets had high binding affinity. Molecular descriptor analysis also confirm these compounds were within the Lipinski limits hence indicating their potential drug likeliness for the treatment of rheumatoid arthritis.

Conclusion : This study developed a new technology that integrate interconnectivity-based weighted network centrality measure approach and network pharmacology techniques to explore mechanisms of action of the *Ruellia prostrata (RP)* and *Ruellia bignoniiflora (RB)* herbal formula that could lead us to design and discover alternative drug compounds for the management and treatment of rheumatoid arthritis.

Keywords: Network Centrality; Rheumatoid Arthritis; *Ruellia*

Introduction

Rheumatoid arthritis is an autoimmune disease characterized by pain and synovial inflammation that may result in the destruction of cartilage and bone. Recently, a study has reported a rheumatoid arthritis prevalence of 1% and it affects three times more women than men[1], [2], [3]. Understanding the etiology of rheumatoid arthritis remains an active area in medical research. Studies have shown that rheumatoid arthritis is a multi-factorial disease probably caused by genetic predispositions or environmental factors [4], [5]. Developments of alternative drugs to treat and cure rheumatoid arthritis are still out of our reach, and the development of new therapeutic anti-rheumatic drugs with minimal or no side-effects is a great challenge [6], [7], [8]. Although remedies such as nonsteroidal anti-inflammatory drugs (NSAIDs)[9], corticosteroids [10],[11], disease-modifying anti-rheumatic drugs (DMARDS) [12], and biologic response modifiers ('biologicals') [13] have been used in the management and treatment of rheumatoid arthritis, some of these remedies have adverse side effects [14], [8]. Therefore, it is necessary to develop alternative drugs with minimal or no side effects for the treatment of rheumatoid arthritis. Recently the use of ayurvedic drugs has gained popularity in clinical research. For instance, a study has shown that *Ruellia prostrata* (RP) and *Ruellia bignoniiflora*(RB) herbal medicine and its constituents could have anti-inflammatory and anti-analgesic properties [15] [15], [16]. However, the identification and elucidation of *Rueilla* active constituent compositive compounds and their molecular mechanism of action in treating of rheumatoid arthritis remain unknown [2],[17],[18]. To understand the relationship of interacting components such as drugs, genes, proteins, and disease targets computational methods like network biology, system biology, polypharmacology, and network pharmacology have been recently proposed [19],[20],[21]. These methods apply omics and system biology strategies to decipher the underlying mechanism of action and synergetic effect of multiple targets and multiple components via a network analysis [22],[23]. In general, network pharmacology is widely based on network construction techniques, a network analysis and database quarry techniques which provides a preliminary understanding of the mechanism of action at the system level [24], [25]. Recently, modeling the drug-target interaction in complex biological systems has attracted the attention of medical researchers [26],[21],[23]. In network pharmacology these complex biological system are often presented as complex networks with heterogeneous components that are quantified by a network centrality measure approach [27]. Though several studies in network pharmacology applied traditional centrality measures approaches to investigate the important network components these methods often depend on the network structure and this makes them less efficient when handling with complex dynamic network systems and the area of application [28],[29],[30]. Thus,in this study, we developed an efficient weighted network centrality measure approach for exploring mechanisms of action of the *Ruellia prostrata* and *Ruellia bignoniiflora* herbal formula for treating rheumatoid arthritis. The rest of this paper is organized as follows. In Section 2, we describe our new approach for calculating the interconnectivity score and weighted network centrality and its application in network pharmacology. In Section 3, we present and interpret our results. In Section 4, we discuss our findings and then we draw some pertinent conclusions.

Materials and Methods

Now, we will describe the our new proposed approach for the calculation of the interconnectivity score and weighted centrality measures for the identification of major network components and its application in network pharmacology.

Calculation of the Network-based Interconnectivity Score

We propose a general approach for the calculation of the interconnectivity score among network components. This approach is a local network-based approach that prioritizes network nodes based on their overall connectivity to other nodes in the network. In our calculations we made the following assumptions: (1) There is a direct interaction between a pair of nodes; (2) There are indirect interactions with a path length of two network nodes which we call the shared neighborhood of the two nodes; (3) The network is a tripartite network consisting of nodes i, j and k. Base on these three assumptions, we can calculate interconnectivity using the following:

$$I(i,k) \quad = \quad e(i,k)\left(\frac{2+|N(i)\cap N(k)|}{\sqrt{deg(i)\,deg(k)}}\right), \tag{1}$$

where $I\,(i,k)$ is the interconnectivity between nodes i and k, $deg(i)$ and $deg(k)$ is the degrees of node i and k respectively and N is the size of the shared neighborhood connectivity. Here,

$$e(i,k) \quad = \quad \begin{cases} 1 & \text{if an edge exists between } i \text{ and } k \\ 0 & \text{otherwise} \end{cases} \tag{2}$$

$e(i,k)$ is the edge between node i and k. Therefore, using Equation (1) the interconnectivity score of node j directly interacting with node i and k in their shared neighborhood can be calculated using

$$s\,(j) \quad = \quad \frac{1}{|DEG|}\sum_{k\in DEG(j)} e(i,k)\left(\frac{2+|N(i)\cap N(k)|}{\sqrt{deg(i)\,deg(k)}}\right), \tag{3}$$

where $s\,(j)$ is the interconnectivity score of node j with respect to node k and DEG represents the degree of all set of node k.

Calculation of Weighted Network Centrality

Using the interconnectivity score $s\,(j)$ calculated above, we can introduce a new approach for computing the weighted centrality measure based on the traditional centrality measure such as degree centrality, closeness centrality, and betweenness centrality. Again, in this step we apply Assumption 3, i.e. the network is a tripartite network consisting of nodes i, j and k used to describe our new formulations. Figure 1 shows the three main network centrality measures examined in this study.

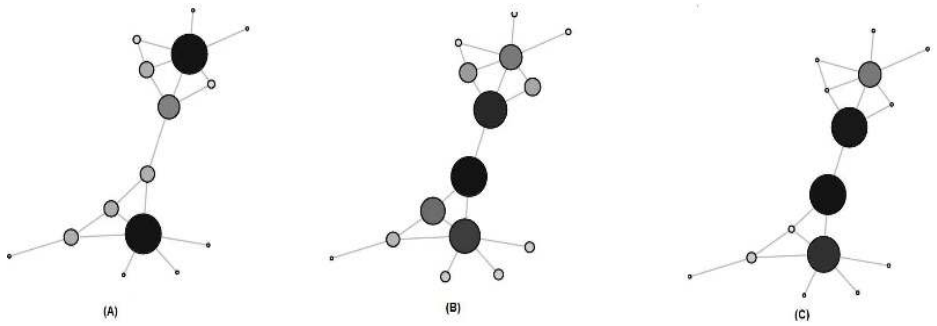

Figure 1 Network centrality measures.(A) degree centrality, (B) closeness centrality, (C) betweenness centrality. The darkness and the size of each node is proportional to its centrality measure.

Weighted Degree Centrality

Generally, degree centrality is defined as the total number of connections or edges attached to the node with respect to other nodes in the network [31] and it usually calculated by

$$C^{D}(i) = \sum_{\substack{j=1 \\ j \neq i}}^{|N|} e\,(i,j)\ , \tag{4}$$

where $C_D\,(i)$is the degree centrality of node i, $e\,(i,j)$is the edge connecting nodes i and j is 1 if it exists otherwise it is 0, and N is the total number of nodes in the network. Therefore using equations (2) and (3), the degree weight of node j is given by

$$w^{DC}\,(j) = \frac{s\,(j)\sum_{k=1}^{n_1} e\,(j,k)}{\ln\left(\sum_{k=1}^{n_1} e\,(j,k)\right)}\ , \tag{5}$$

where $w^{DC}\,(j)$ is the degree weight of node j, $e\,(j,k)$ represents the edge between nodes j and k whose value is 1 if it exists otherwise it is 0, and n_1 is total number of nodes k in the network. Therefore, using Equation (4) the weighted degree centrality score of the network node i can be calculated using

$$\phi^{DC}\,(i) = \sum_{j=1}^{n_2} w^{DC}\,(j)\,e\,(i,j) \tag{6}$$

Here, $\phi^{DC}\,(i)$ is the weighted degree centrality measure of node i with respect to node j, $e\,(i,j)$ represents the edge between nodes j and k whose value is 1 if it exists otherwise it is 0 and n_2 is the total number of nodes i.

Weighted Closeness Centrality

Closeness centrality is often defined as the shortest distance or path or inverse of the sum of the distances between a given node with respect to other nodes in the network [32]. Essentially, closeness centrality is based on the shortest distance and the inverse of sum of the distances between nodes i and j is calculated by

$$C^{C}(i) = \frac{n-1}{\sum\limits_{\forall j \& j \neq i} d(i,j)} = \frac{1}{n(n-1)} \sum_{\forall j \& j \neq i} d(i,j) \ , \tag{7}$$

where $C^{C}(i)$ is the closeness centrality, $d(i,j)$ represents the shortest path or distance between node i and j, while n is the total number of nodes in the network. Here we recalculate the distance by first expressing the sum of the shortest distance between nodes i and j with $\delta = \sum\limits_{\forall j \& j \neq i}^{n} d(i,j)$. Therefore, our new closeness centrality measure is calculated using the equality

$$\frac{n-1}{\delta} \quad = \quad \frac{1}{n(n-1)}\delta \tag{8}$$

Rearranging Equation (8) and taking the positive square root

$$\delta \quad = \quad (n-1)\sqrt{n}\,, \tag{9}$$

where δ is the shortest distance, $\frac{n-1}{\delta}$ represents closeness centrality based on the shortest distance and $\frac{1}{n(n-1)}\delta$ represents closeness centrality based on the inverse of the sum of the distances and n is total number of nodes in the network. Hence the shortest distance between nodes i and j is $\delta(i,j)$. If we take equations (2) and (9), we can calculate a new closeness weight of node j by

$$w^{CC}(j) = \frac{s(j)\sum\limits_{k=1}^{n_1}\delta(j,k)}{\ln\left(\sum\limits_{k=1}^{n_1}\delta(j,k)\right)}\,, \tag{10}$$

where $w^{CC}(j)$ is the closeness centrality weight of node j, $\delta(j,k)$ is the shortest distance or path between nodes j and k and n_1 is the total number of nodes k in the network. Of course,

$$\delta(j,k) \quad = \quad \begin{cases} 1 & \text{if the shortest path exists between } i \text{ and } k \\ 0 & \text{otherwise} \end{cases} \tag{11}$$

Therefore, using Equation (8) we can calculate the weighted closeness centrality score of node i by

$$\phi^{CC}(i) = \sum_{j=1}^{n_2} w^{CC}(j)\,\delta(i,j)\ , \tag{12}$$

where $\phi^{CC}(i)$ is the weighted closeness centrality measure of node i with respect to node j, $\delta(i,j)$ is the shortest distance or path between nodes j and k whose value is 1 if it exists otherwise it is 0 and n_2 is the total number of nodes j.

Weighted Betweenness Centrality

Betweenness centrality is essentially defined as the number of shortest paths passing through the node which acts as a bridge for the shortest path between a pair of nodes [32] and it can be calculated using

$$C^{B}(j) = \sum_{\substack{i=1\\ i\neq j}}\sum_{\substack{k=1\\ k\neq j}} \left(\frac{\sigma^{*}_{ik}(j)}{\sigma_{ik}}\right)\ , \tag{13}$$

where σ_{ik} is the total number of shortest paths from node i to node k and $\sigma^{*}_{ik}(j)$ is the number of shortest paths between nodes i and k with node j acting as an intermediate/bridge node along the shortest path. If we use the betweenness value i.e number of shortest paths between node i and k in Equation (13), then the betweenness weight of node j can be found via

$$w^{BC}(j) \quad = \quad \frac{s(j)\sum\limits_{k=1}^{n_1}\sigma^{*}_{ik}(j)}{\ln\left(\sum\limits_{k=1}^{n_1}\sigma^{*}_{ik}(j)\right)}\ , \tag{14}$$

where $w^{BC}(j)$ is the betweenness centrality weight of node j, $\sigma^{*}_{ik}(j)$ represents the intermediary node between nodes i and j, and n_1 is the total number of node k in the network. Also,

$$\sigma^{*}_{ik}(j) \quad = \quad \begin{cases} 1 & \text{if node } j \text{ exists between } i \text{ and } k \\ 0 & \text{otherwise} \end{cases} \tag{15}$$

Thus, with Equation (15) we can calculate the weighted betweenness centrality score of node i by

$$\phi^{BC}(i) = \sum_{j=1}^{n_2} w^{BC}(j)\,\sigma^{*}_{jk}(i)\ , \tag{16}$$

where $\phi^{BC}(i)$ is the weighted betweenness centrality measure of node i, $\sigma^{*}_{jk}(i)$ represents the intermediary node between nodes j and k whose value is 1 if it exists otherwise it is 0 and n_2 is the total number of nodes j.

Application on Network Pharmacology

We used our proposed approach to explore mechanisms of action of the *Ruellia prostrata* and *Ruellia bignoniiflora* herbal formula for treating rheumatoid arthritis. In the simulation we denoted the chemical compounds, known rheumatoid arthritis therapeutic targets and putative target genes in the network by k, i and j respectively.

Data preparation

The data of compositive compounds of *Ruellia prostrata* (RP) and *Ruellia bignoniiflora*(RB) were obtained from GC-MS peak detention profiles. Their chemical information was then retrieved from PubChem, (http://pubchem.ncbi.nlm.nih.gov/)[33], Chemistry database(http://www.organchem.csdb.cn/scdb/main/slogin.asp,updatedon2014-05-05)[34] and ChemSpider (http://www.chemspider.com/, updated on 2011-12-23)[35]. Rheumatoid arthritis related genes were downloaded from OMIM Morbid Map [36]. Known therapeutic targets of rheumatoid arthritis data were downloaded from DrugBank (http://www.drugbank.ca/,version:3.0)[37] and Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/). All the therapeutic targets were then merged to form a set of rheumatoid arthritis targets. Protein-Protein interaction (PPI) data were obtained from the following existing databases: Human Annotated and Predicted Protein Interaction Database (HAPPI)[38], Reactome [39]; Online Predicted Human Interaction Database (OPHID)[40]; Human Protein Reference Database (HPRD)[41]; Molecular Interaction Database (MINT)[42] and Database of Interacting Proteins (DIP)[43].

Prediction of RP-RB Active Compounds

The active compounds of RP-RB herbs acting on the rheumatoid arthritis therapeutic targets and target genes were identified by first constructing a tripartite drug network of the two herb-compositive compounds-putative target genes. The network was then partitioned by a Markov Clustering algorithm based on [44], [45]. The interconnectivity score described above was then used to prioritizes candidates RP-RB active compounds based on their overall interconnectivity score in the shared neighbourhood connectivity with known rheumatoid arthritis therapeutic targets and putative target genes.

The Identification of Potential Targets of RP-RB Active Compounds

To identify the potential targets of RP-RB active compounds we first built a tripartite drug target network consisting of the two herbs, their active compositive compounds and putative target genes. Then, built a tripartite disease target network consisting of RP-RB compositive compounds-putative target genes-known rheumatoid arthritis therapeutic targets. An imbalance multi level drug-disease network was built by a union merge of drug target network and disease target network. The corresponding RP-RB compositive compounds and candidate putative target genes and known known rheumatoid arthritis therapeutic targets were were identified from the network using the proposed weighted centrality measures approach described that is 'Weighted degree centrality', 'Weighted closeness centrality' and

'Weighted betweenness centrality'. All the potential network targets with high centrality measures values were thought to play an important role the progression of the rheumatoid arthritis disease in the drug-disease network [20], [21].

A Network Major Targets Validation

The major identified putative target genes, known rheumatoid arthritis therapeutic targets and the candidate RP-RB compositive compounds from the network were validated by enrichment analysis based on Gene Ontology annotation system and the KEGG pathway and a molecular docking simulations.

Gene Ontology and a Pathway Enrichment Analysis

The major putative targets of the RP-RB herbs identified from the pharmacological network were validated by Gene Ontology (GO) [46] and a pathway enrichment analysis [47],[48] using Annotation Visualization and Integrated Discovery (DAVID,http://david.abcc.ncifcrf.gov/home.jsp, version 6.7) [49] database and the Kyoto Encyclopedia of Genes and Genomes (KEGG,http://www.genome.jp/Kegg/, last updated: Oct 16, 2012) [50] database, respectively.

A Molecular Docking Analysis

The binding affinity of eight candidate RP-RB compositive compounds towards 10 known RA therapeutic targets identified from the pharmacological network was validated by a molecular docking simulation using Autodock Vina software [51]. The crystal structures of identified known RA therapeutic targets (receptors) were obtained from the RCSB protein database (http://www.pdb.org/, updated on 2014-3-11) [42], [52], [53]. Here, the chemical structures of the RP-RB compositive compounds were downloaded from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/)[33]. For best docking results we set the optimal x, y and z coordinates values of the target receptors and set the limit of exhaustive searches[54]. All the ligands with high binding energy and a low root mean square deviation (RMSD) value were considered to have good molecular interaction with the target receptors [55], [56], [57]. All the files for ligand-receptor structures were saved in .pdbqt according to [54] for molecular interaction visualization using the UCSF Chimera program [58].

Results

The Network Identification of active compounds in the RP-RB herbal formula

We identified the active RP-RB compositive compounds acting on the known rheumatoid arthritis therapeutic targets by first constructing an drug network described above. The active compositive compound in RP-RB herbs were the predicted by the MCL algorithm and interconnectivity approach. The network analysis results indicated that 634 nodes (2 herbs, 196 compositive compounds, and 436 putative target genes) activity interacted (5212 edges) in the network (see Figure 2 (A)). A total of 196 compositive compounds were identified from the network, 103 compounds came from RP herb and 93 came from the RB herb. All the RP-RB compositive compounds actively interacted with with 436 putative target genes, indicating a relationship between the herbs and putative target genes. In addition, out of the

196 RP-RB compositive compounds 22 were common in both herbs (see Figure 2 (B), green nodes with red borders). All these compositive compounds had high a network interconnectivity score indicating their importance in the drug network (see more results given in Additional file 2, Drug-Target-Network analysis).

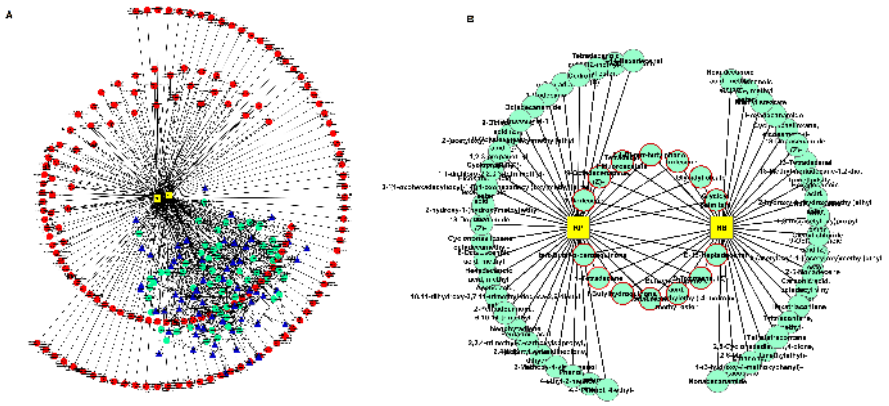

Figure 2 Network-based prediction of active compounds contained in the RP-RB herbal formula acting on rheumatoid arthritis (A) Markov Clustering (MCL) of herb-compositive compound-putative target genes network for the identification of *Ruellia prostrata* (RP) and *Ruellia bignoniiflora* (RB) herbs. (B) The major shared active compounds identified based on the network interconnectivity prediction. Edges: interactions between RP-RB compositive compounds and putative target genes; yellow square nodes: *Ruellia prostrata* (RP) and *Ruellia bignoniiflora* (RB) herbs; green round nodes: RP-RB compositive compounds; red round nodes: known therapeutic targets; blue triangles: putative target genes; green nodes with red border are compounds shared by the two herbs.

A Network Elucidation of Mechanism of Action of the RP-RB Herbal Formula for Treating RA

To ascertain the mechanism of action of the RP-RB herbs for treating Rheumatoid Arthritis an imbalance multi-level network described in the materials and methods section was used. The candidates RP-RB-compositive compound, putative target genes, and known rheumatoid arthritis targets were then identified based on the weighted centrality score values. According to the network analysis the extracted network of Herb-putative target genes identified a total of 438 nodes (Figure 3 (A) showing 2 herbs and 436 putative target genes) actively interacted (3432 edges) in the network. A tripartite drug network of RM-RB herb-compositive compounds-putative target genes identified a total of 634 nodes (containing 2 herbs, 196 compositive compounds and 436 putative targets) and 5123 edges/interactions (see Figure 3 (B)). The analysis also revealed the imbalance network built from a union merge of the drug network and disease network (see Figure 2 (C)) had a total of 1044 nodes (containing 2 herbs, 196 compositive compounds, 436 putative target genes and 410 known RA therapeutic targets) were actively interacting (6964 edges). From our analysis of the imbalance network a total of 33 major network components were identified based on the weighted centrality scores. These includes 8 RP-RB compositive compounds, 15 putative targets and 10 known therapeutic targets for the treatment of rheumatoid arthritis (see Table 1). Among the eight compounds,

three came from RP, which includes Glyceryl diacetate-2-Oleate, Glyceryl-2 Palmitate, and Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester while five came from RB which includes Methyl stearate, Glycidyl oleate, Oleoyl chloride, Hexadecanoic acid, methyl ester and 9-Octadecenoic acid (Z)-, methyl ester. All the major components identified from the network had high 'degree centrality', 'closeness centrality' and 'betweenness centrality' measures. RP-RB compositive compounds with high centrality values indicate the chance of the interaction with majority of putative target genes and therapeutic targets in the network (see more results given in Additional file 2, Drug-Disease Network analysis).

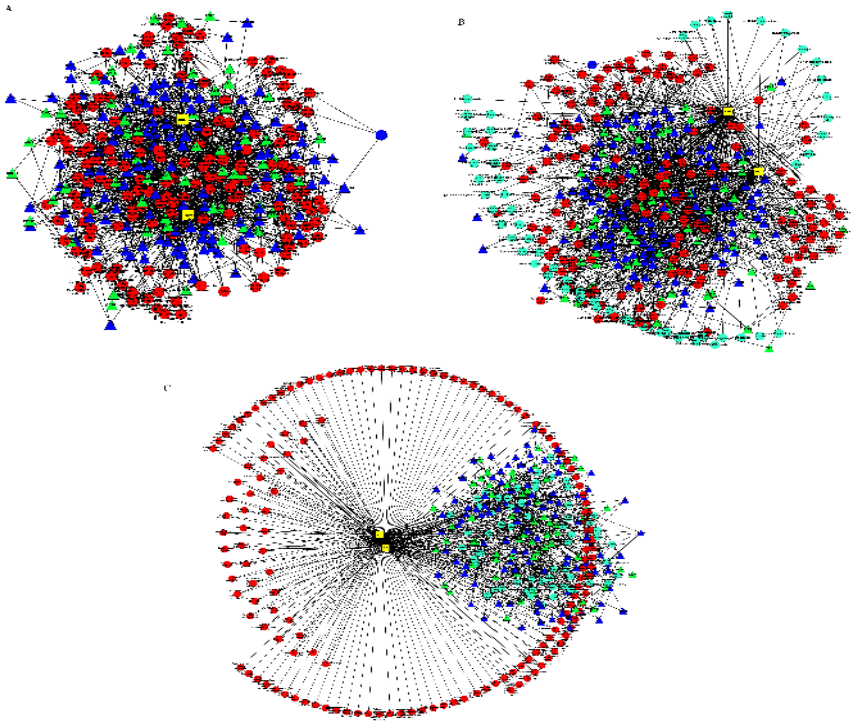

Figure 3 A network elucidation of mechanism of action of RP-RB on rheumatoid arthritis.. (A) An RP-RB herb- putative targets-known rheumatoid arthritis targets network. (B) An RP-RB herb- compositive compound -putative target-known rheumatoid arthritis target network. (C) A major compositive compounds-major putative targets- known rheumatoid arthritis targets network. Edges: interactions among the two herbs, compositive compounds, putative targets and known RA therapeutic targets. Yellow square nodes: two herbs *Ruellia prostrata* (RP) and *Ruellia bignoniiflora* (RB); green round nodes: RP-RB compositive compounds; red round nodes: known RA therapeutic targets; green and blue triangle nodes: putative targets of RP-RB herbs. Nodes with yellow borders are major components with corresponding high 'Degree centrality', 'betweenness centrality', and 'Closeness centrality' measures.

Gene Ontology and Pathway Enrichment Analysis

We validated the major identified putative targets using an enrichment analysis based on the GO annotation system and the KEGG pathway. Figure 4 shows a summary of the major biological processes, molecular function and top-10 KEGG pathways associated with the treatment of rheumatoid arthritis including Interleukin-10 signaling, the IL-17 signaling pathway, TNF signaling pathway, Interleukin-4 and

Table 1 Major RP-RB compositive compounds, therapeutic targets and putative target genes

Network Components	ϕ^{DC}	ϕ^{BC}	ϕ^{CC}
RP-RB Compositive Compounds			
Glycidyl oleate	0.53421	0.04851	0.55812
Glyceryl-2 Palmitate	0.51089	0.05167	0.58919
Oleoyl chloride	0.45887	0.05282	0.47059
9-Octadecenoic acid (Z)-, methyl ester	0.43162	0.05252	0.47516
Methyl stearate	0.44457	0.04894	0.44680
Hexadecanoic acid, methyl ester	0.44716	0.05316	0.46557
Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	0.49120	0.14129	0.48424
Glyceryl diacetate-2-Oleate	0.45089	0.05167	0.48919
RA Therapeutic Targets			
Nitric oxide synthase	0.49822	0.05650	0.56811
Interleukin-1 beta	0.41060	0.04933	0.48550
Delta-type opioid receptor	0.54171	0.11993	0.49435
Tumor necrosis factor	0.46255	0.16964	0.48963
Prostaglandin G/H synthase 1	0.49331	0.39671	0.54107
Prostaglandin G/H synthase 2	0.45508	0.50404	0.47936
Growth-regulated alpha protein	0.41264	0.08876	0.52486
C-C chemokine receptor type 5	0.40256	0.05885	0.46090
Interleukin-6	0.42441	0.06710	0.51582
Cannabinoid receptor 2	0.47004	0.04008	0.43522
Putative Target Genes			
TNF	0.27050	0.09210	0.05880
CXCL10	0.17280	0.01160	0.56402
IL10	0.17280	0.01250	0.54325
IL6	0.30040	0.09297	0.54020
CXCL1	0.10440	0.01510	0.54324
CXCL8	0.12730	0.01600	0.54385
FOXP3	0.41060	0.01490	0.37476
CCR2	0.30040	0.01610	0.47620
CCR5	0.30040	0.02790	0.49220
IL10	0.12730	0.01450	0.53020
CCL2	0.24950	0.01900	0.45480
IL1B	0.14050	0.02530	0.58308
IL17	0.24950	0.01950	0.53740
IL13	0.22120	0.02520	0.53470
CSF2	0.29051	0.09962	0.51014

Interleukin-13 signaling, Toll-like receptor signaling pathway, NF-kappa B signaling pathway, Cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, Chemokine signaling pathway and GPCR ligand binding. Our analysis indicated that the major putative targets were involved in the IL-17 signaling pathway. This pathway plays an important role in neutrophil mobility through T cell activation, and this provides immunity against inflammatory diseases such as psoriasis and rheumatoid arthritis[59]. The TNF signaling pathway was also identified as a significant pathway, Tumor necrosis factor (TNF) is multi-functional cytokine which has an important role in pro-inflammatory cytokine. A study showed that TNF is secreted by inflammatory cells which are involved in inflammation-associated carcinogenesis[60]. Our own analysis showed that the major identified putative targets were also involved in the NF-kappa B signaling pathway which plays a key role in inflammatory and immune responses as well as regulation genes involved in cell death differentiation and proliferation [61]. In addition, cytokines have been implicated in many cases of rheumatoid arthritis by the regulation of chronic inflammatory synovitis[62]. Studies have shown the IL-1 and TNF are the main pro-inflammatory cytokines in RA and their blockage may play a significant role in the management of the RA disease[63]. Our putative targets were also involved in the NOD-like receptor signaling pathway which is involved during tissue injury and

infection by initiating the production of proinflammatory cytokine receptors[16]. It has been reported that the major class of receptors associated with inflammatory joint disease are Toll-like receptors (TLRs) and NOD-like receptors (NLRs)[64]. The major putative targets were also involved in the Cytokine-cytokine receptor interaction biological activity. Chemokines have been reported to be involved in many neurological diseases by primarily inducing inflammation through the localization of leukocytes with their chemotactic activity[62].

A study has shown that Chemokine activities are mediated through G-protein coupled receptors which play a role in inflammatory responses[65]. We also noticed that the major putative targets were involved in the Interleukin-10 signaling pathways which regulate the anti-inflammatory cytokine, and they play a crucial role in preventing the inflammatory and autoimmune diseases[66]. In addition, a study has shown that Interleukin-4 and Interleukin-13 signaling are both associated with the regulation of the type II inflammatory response triggered either by a parasite or allergen[67].

Molecular docking simulation

We performed molecular docking to validate the interaction of the RPM-RBK major compositive compounds and the known rheumatoid arthritis therapeutic targets predicted by network centrality measures. The major identified RPM-RBK compositive compounds (ligands) were docked on the corresponding potential therapeutic targets (receptors).We assigned ligands initials Cp1, Cp2, Cp3, Cp4, Cp5, Cp6, Cp7, and Cp8 to represent Glyceryl diacetate-2-Oleate, Glyceryl-2 Palmitate, Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester, Methyl stearate, Glycidyl oleate, Oleoyl chloride, Hexadecanoic acid, methyl ester and 9-Octadecenoic acid (Z)-, methyl ester, respectively and corresponding receptors TNF, NOSs, IL-1, GRO-alpha, DOP, PTGS1, PTGS2, IL-6, CCR5 and CB2. In this analysis the best ligands (RPM-RBK compositive compounds) were selected based on the binding affinity score and root mean square deviation (RMSD) value. In our analysis the ligands with binding affinity scores in the range of -8.0 kcal/mol and 11.0 kcal/mol with RMSD $\leq$ 2.5 Å were considered to have a good interaction with the target receptors (RA known therapeutic targets).

Based on the results given in Table 2, Cp1 (-10.33 kcal/mol), Cp3(-10.32 kcal/mol), Cp5(-9.96 kcal/mol) and Cp6 (-9.31 kcal/mol) had the highest binding affinity on the TNF target receptor. Another docking analysis revealed that Cp1 (-10.52 kcal/mol), Cp3(-10.32 kcal/mol), Cp5(-9.52 kcal/mol) and Cp6 (-10.02 kcal/mol) bind well on CCR5 target receptor.

Interestingly, Cp1 binds well on both PTGS1 and PTGS2, that is -9.16 kcal/mol and -9.98 kcal/mol, respectively. A similar observation was noted with Cp2, which had a binding affinity of -9.25 kcal/mol and -9.11 kcal/mol for PTGS1 and PTGS2 respectively.

Another nice finding concerning the compositive compound Cp1 and Cp2, is that both bind well on IL-6 target receptor with a binding affinity of -9.82 kcal/mol and 9.02 kcal/mol, respectively. We also observed that Cp3 and CP5 bind well on DOP with binding affinity of -9.16 kcal/mol and 9.43 kcal/mol, respectively. We also noticed that Cp1 and Cp6 binds well on the NOSs target receptor with a binding

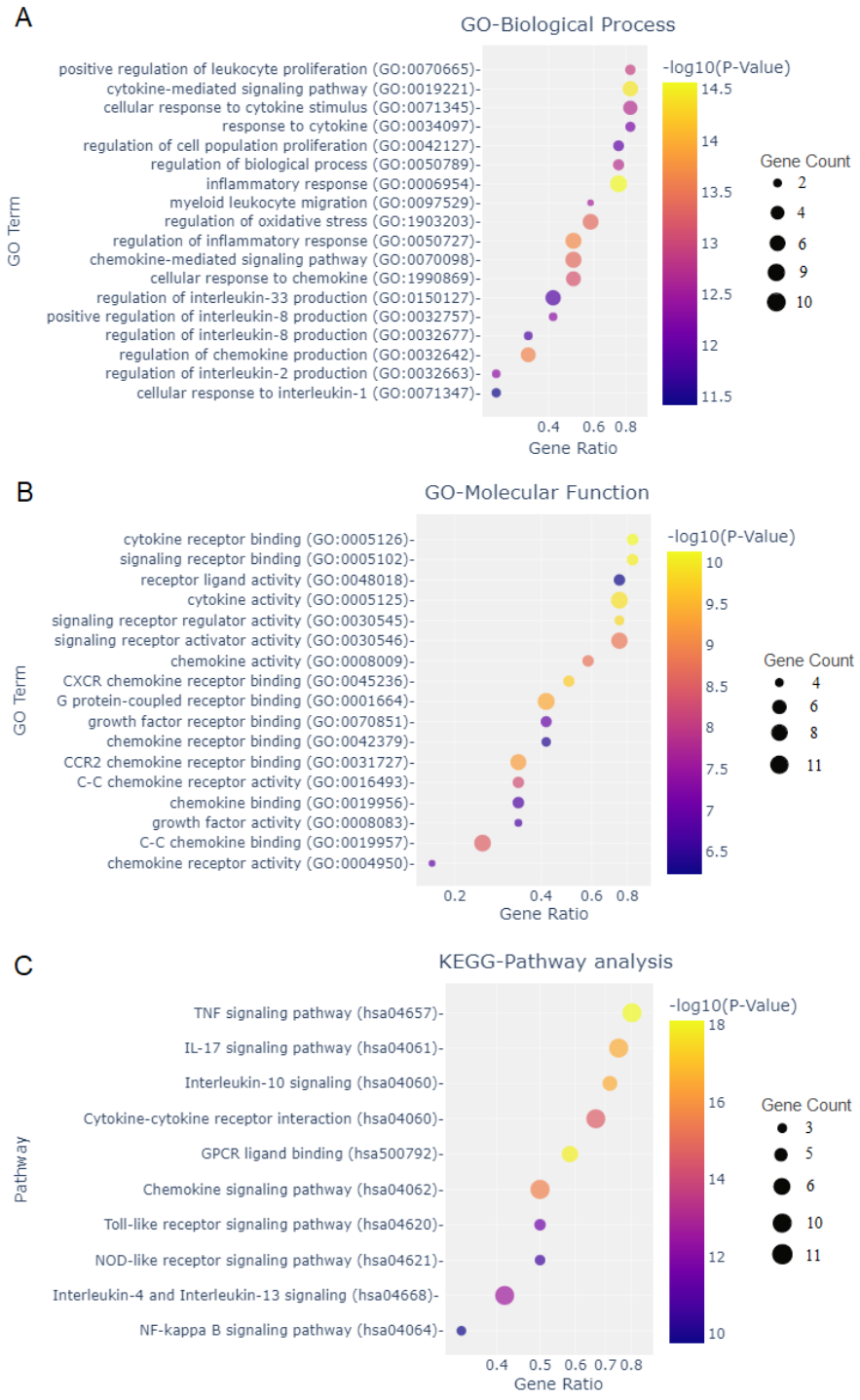

Figure 4 Gene Ontology and the KEGG pathway analysis of major identified putative target genes of the RP-RB herbal formula. Biological process (A), molecular function (B), and top-10 KEGG pathway (C). KEGG, Kyoto Encyclopedia of Genes and Genomes.

affinity of 8.91 kcal/mol and 8.25 kcal/mol, respectively. In addition, Cp1 (-8.82

kcal/mol), Cp4(-8.73 kcal/mol), and Cp6 (-8.62 kcal/mol) bound well on the IL-1β. Similarly, Cp1 (-8.21 kcal/mol), Cp4(-8.87 kcal/mol), and Cp6 (-8.31 kcal/mol) bound well on the GRO-alpha. We also noticed that, Cp1 (-9.06 kcal/mol), Cp4(-8.91 kcal/mol), and Cp5 (-8.96 kcal/mol) bound well to the CB2 target receptor. Based on our analysis, all the selected ligands recorded a binding energy/affinity in the range -8.0 kcal/mol and -10.52 kcal/mol with RMSD in the range 1-to-2.5Å. This means that these ligands are likely to be the best inhibitors of the corresponding target receptors, hence they are good drug candidates due to hydrogen bonds and hydrophobic interactions between these compounds and target receptors. We also evaluated the molecular descriptors of the candidate RP-RB drug compounds. Here, the drug likeliness was evaluated using the Lipinski rule of five which states that "drug-like" molecules have XlogP$\leq$ 5, molecular weight$\leq$ 500, number of hydrogen bond acceptors $\leq$ 10, and number of hydrogen bond donors$\leq$ 5 [68],[69]. We also predicted the compositive compound transport properties using the TPSA, and our results indicated that all the compounds had the TPSA in the range 17.07- to -78.92 Å^2. A good TPSA should be $\leq$ 140Å^2; and this descriptor plays a critical role in characterizing drug absorption properties such as bioavailability, intestinal absorption, Caco-2 permeability and blood-brain barrier penetration[70], [71]. In addition, all the compositive compounds had a rotatable bond in the range 15 to 23 rotatable bonds. According to [72],the mean value of the number of rotatable bond per drug molecule should lie in the range 6 to 170 rotatable bonds. A high number of rotatable bonds indicates high molecular flexibility, which acts as an indicator of a good oral bioavailability of drugs[73]. Our overall analysis shows that Cp1(see Figure 5), Cp3(see Figure 6), Cp5 (see Figure 7) and Cp6 (see Figure 8) are the likely drug candidates in the treatment of rheumatoid arthritis based on their binding affinity to their corresponding targets with the molecular descriptor and Lipinski value within the set limits.

Table 2 Molecular Docking and Molecular Descriptor Analysis of the Major RPM-RBK Compositive Compounds

	RPM-RBK Compositive Compounds															
	Cp1		Cp2		Cp3		Cp4		Cp5		Cp6		Cp7		Cp8	
R.A.T.T.	BE(kcal/mol)	RMSD	BE(kcal/mol)	RMSD	BE(kcal/mol)	RMSD	BE(kcal/mol)	RMSD	BE(kcal/mol)	RMSD	BE(kcal/mol)	RMSD	BE(kcal/mol)	RMSD	BE(kcal/mol)	RMSD
TNF	-10.33	1.212	-8.51	2.311	-9.98	1.921	-8.11	2.473	-9.96	1.774	-9.31	1.605	-8.01	2.398	-8.15	2.431
NOSs	-8.91	2.163	-7.51	3.063	-8.72	2.269	-7.49	3.064	-8.31	2.123	-9.25	1.664	-6.49	3.261	-7.45	3.134
IL-1	-8.82	2.311	-8.39	2.341	-8.73	2.129	-8.53	2.311	-8.71	2.391	-8.45	2.111	-8.32	2.211	-8.19	2.362
GRO-alpha	-8.21	2.146	-7.43	3.046	-8.11	2.379	-8.87	2.228	-7.96	3.008	-8.31	2.217	-7.19	3.148	-7.29	3.066
DOP	-8.45	2.121	-8.23	2.211	-9.163	1.218	-7.59	2.91	-9.43	1.627	-8.37	2.011	-7.81	3.083	-7.81	3.018
PTGS2	-9.87	1.411	-9.27	1.511	-9.65	1.632	-6.85	3.914	-9.71	1.613	-9.38	1.494	-6.89	3.231	-7.65	3.041
PTGS1	-9.98	1.721	-9.11	1.371	-9.83	1.575	-7.39	3.053	-8.38	2.176	-8.09	2.278	-7.38	3.131	-6.97	3.171
IL-6	-9.82	1.263	-9.02	1.671	-8.81	2.163	-7.52	3.016	-8.61	2.037	-8.42	2.126	-7.29	3.213	-7.52	3.264
CCR5	-10.52	1.019	-8.02	2.228	-10.32	1.135	-8.52	2.366	-9.52	1.147	-10.02	1.201	-7.19	3.214	-7.62	3.111
CB 2	-9.06	1.847	-8.13	2.143	-8.35	2.378	-8.91	2.119	-8.96	2.041	-8.11	2.263	-7.91	2.911	-8.09	2.482
M.D.A.																
Xlog P	8.02		6.09		7.5		8.32		7.72		8.6		7.37		7.89	
H-bond Donors	0		2		0		0		0		0		0		0	
H-bond Acceptos	6		4		6		2		3		1		2		2	
nrotb	23		18		22		17		18		15		15		16	
TPSA	78.92		66.76		78.92		26.3		38.83		17.07		26.3		26.3	
MW	440.62		330.5		414.58		298.51		338.53		300.91		270.46		296.5	

[1] **Cp1**: Glyceryl diacetate-2-Oleate; **Cp2**: Glyceryl-2 Palmitate; **Cp3**: Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester; **Cp4**: Methyl stearate; **Cp5**: Glycidyl oleate;**Cp6**: Oleoyl chloride; **Cp7**: Hexadecanoic acid, methyl ester; **Cp8**: 9-Octadecenoic acid (Z)-, methyl ester.[2] **TNF**: Tumor necrosis factor; **NOSs**: Nitric oxide synthase; **IL-1β**:Interleukin-1 beta;**GRO-alpha**: Growth regulated alpha protein; **DOP**: Delta-type opioid receptor; **PTGS1**: Prostaglandin G/H synthase 2; **PTGS2**: Prostaglandin G/H synthase 1; **IL-6**: Interleukin-6; **CCR5**: C-C chemokine receptor type 5; **CB2**: Cannabinoid receptor 2. [3] **XlogP**: Molecular Lipophilicity Potential; **nrotb**:Number of rotatable bond; **TPSA**: Topological Polar Surface Area; **MW**: Molecular Weight;**R.A.T.T**: Rheumatoid Arthritis Therapeutic Target;**M.D.A**: Molecular Descriptor Analysis

Discussion

Recently, network science has found new applications in medical and drug discovery research[74]. Network pharmacology along with the system biology paradigm

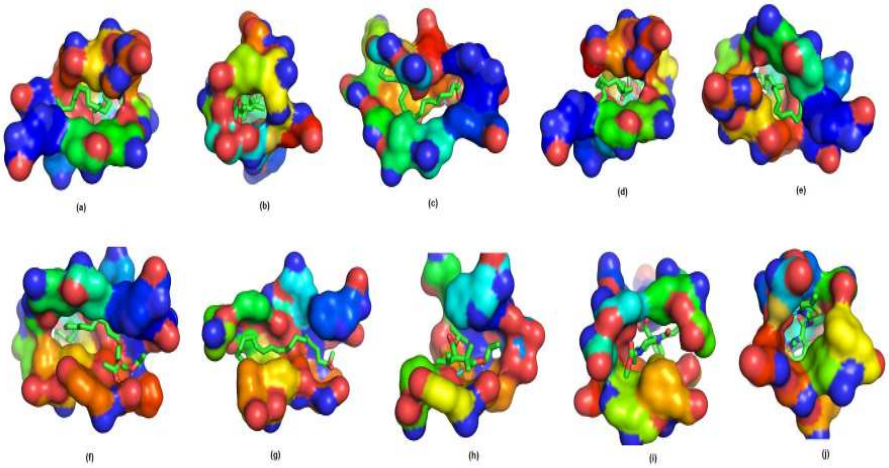

Figure 5 Molecular docking visualization of the interaction of Glyceryl diacetate-2-Oleate (Cp1) and major targets. Molecular docking visualization of the interaction of Glyceryl diacetate-2-Oleate (Cp1) and major targets: **(a)** Cp1-TNF, **(b)** Cp1-NOSs, **(c)** Cp1-IL-1β, **(d)** Cp1-GRO-alpha, **(e)** Cp1-DOP, **(f)** Cp1-PTGS1, **(g)** Cp1-PTGS2, **(h)** Cp1-IL-6, **(i)** Cp1-CCR5 and **(j)** Cp1- CB2. The green color represents the hydrogen bond interaction with main amino acids, blue indicate mild polar i.e. hydrogen bond interactions with side chain amino acid and pink represents the hydrophobic interactions.

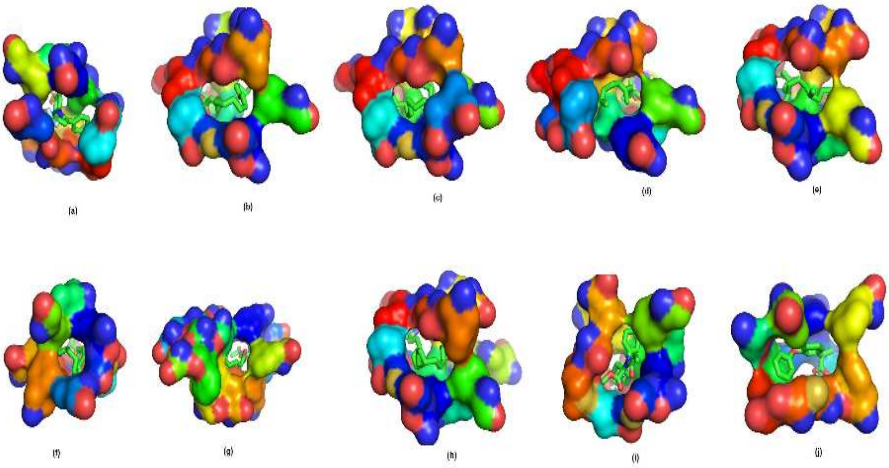

Figure 6 Molecular docking visualization of the interaction of Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester (Cp3) and major targets. (a) Cp3-TNF, **(b)** Cp3-NOSs, **(c)** Cp3-IL-1β, **(d)** Cp3-GRO-alpha, **(e)** Cp3-DOP, **(f)** Cp3-PTGS1, **(g)** Cp3-PTGS2, **(h)** Cp3-IL-6, **(i)** Cp3-CCR5 and **(j)** Cp3- CB2. The green color represents the hydrogen bond interaction with main amino acids, blue indicate mild polar i.e. hydrogen bond interactions with side chain amino acid and pink represents the hydrophobic interactions.

have both provided a breakthrough in drug discovery research by establishing the relationship between drug molecules and disease, and the potential therapeutic targets[75]. The development of multi-target therapeutics drug compounds is likely to be a promising paradigm for discovering drugs and offer a greater efficacy level with minimal or no adverse effects and toxicity compared to monotheraputic drug compounds [76]. A study has reported herbal medicine to be characterized

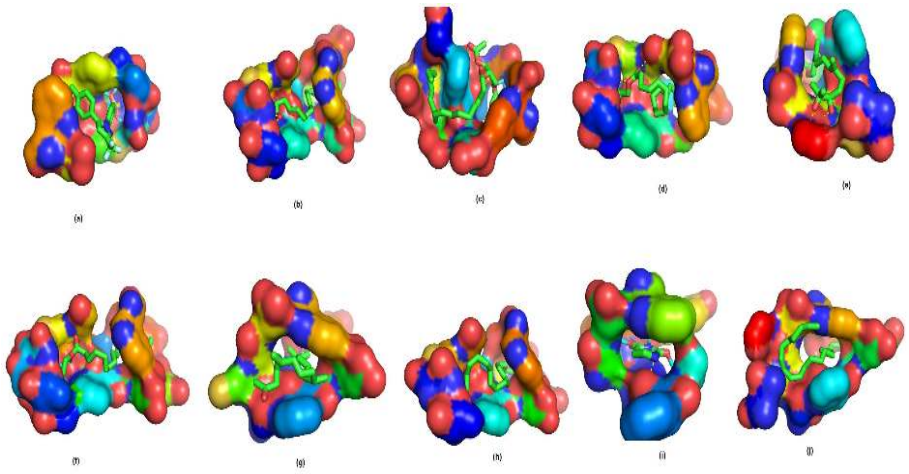

Figure 7 Molecular docking visualization of the interaction of Glycidyl oleate (Cp5) and major targets. (a) Cp5-TNF, **(b)** Cp5-NOSs, **(c)** Cp5- IL-1β, **(d)** Cp5-GRO-alpha, **(e)** Cp5-DOP, **(f)** Cp5-PTGS1, **(g)** Cp5-PTGS2, **(h)** Cp5-IL-6, **(i)** Cp5-CCR5 and **(j)** Cp5- CB2. The green color represents the hydrogen bond interaction with main amino acids, blue indicate mild polar i.e. hydrogen bond interactions with side chain amino acid and pink represents the hydrophobic interactions.

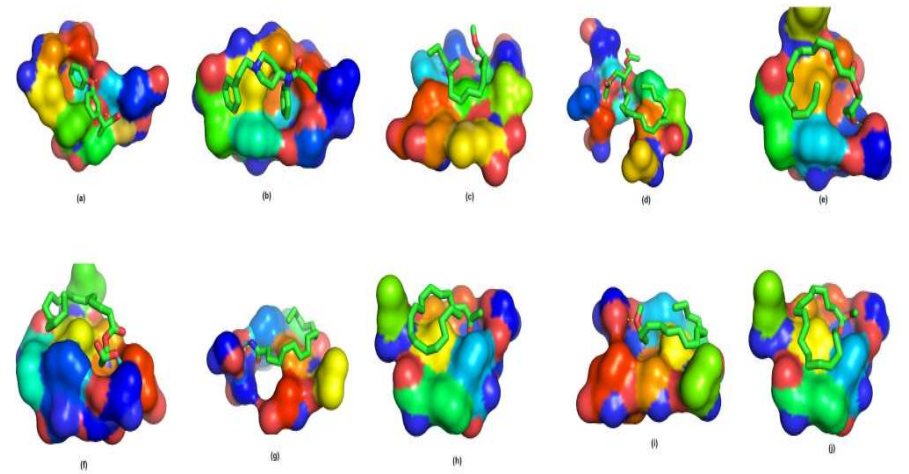

Figure 8 Molecular docking visualization of the interaction of Oleoyl chloride (Cp6) and major targets. (a) Cp6-TNF, **(b)** Cp6-NOSs, **(c)** Cp6- IL-1β, **(d)** Cp6-GRO-alpha, **(e)** Cp6-DOP, **(f)** Cp6-PTGS1, **(g)** Cp6-PTGS2, **(h)** Cp6-IL-6, **(i)** Cp6-CCR5 and **(j)** Cp6- CB2. The green color represents the hydrogen bond interaction with main amino acids, blue indicate mild polar i.e. hydrogen bond interactions with side chain amino acid and pink represents the hydrophobic interactions.

by multi-components/compounds and multi-targets with promising prospects for the discovery of new lead drug compounds with multi-therapeutic use[68]. This can be achieved by developing an efficient computational approach to discover and elucidated multi-agents/components and their mechanisms of action.

In this study we developed an efficient weighted network centrality measure approach that can explore relationships among the active RP-RB compositive com-

pounds and putative target genes and known therapeutic targets of rheumatoid arthritis for the first time. This approach allows us to understand the mechanism of action of the *Ruellia prostrata* and *Ruellia bignoniiflora* herbal formula in treating rheumatoid arthritis. Our network pharmacology findings revealed the following things:

First, the drug target network built from RP-RB herbs-compositive compounds-putative target genes partitioned via MCL and interconnectivity score enabled us identify the potential active compounds contained in *Ruellia prostrata* and *Ruellia bignoniiflora* herbal formula acting on rheumatoid arthritis. Calculation interconnectivity score by MCL allowed us to prioritize significant active compounds and RA associated putative target genes in the network [77], [78] (see more results given in the supplementary files). Secondly, the imbalance network built by a union merge of the multi-level drug target network and disease target network provided a clue to the mechanism of action of *Ruellia prostrata* and *Ruellia bignoniiflora* herbal formula used in the treatment of rheumatoid arthritis (see more results given in the supplementary files). Third, a network target validation by Gene ontology and KEGG pathway analysis provided a clue to help understand the molecular function of the putative target genes interacting with RP-RB compositive compounds. The majority of putative targets were involved in the TNF signaling pathway, Interleukin-10 signaling, IL-17 signaling pathway, Interleukin-4 and Interleukin-13 signaling, Toll-like receptor signaling pathway, NF-kappa B signaling pathway, Cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, Chemokine signaling pathway and GPCR ligand binding which play a role in the progression of rheumatoid arthritis disease (see more results given in the supplementary files). Finally, a molecular docking simulation and molecular descriptor analysis allowed us to understand the inhibitory properties and drug likeliness of the major identified corresponding RP-RB compositive compounds in the treatment of rheumatoid arthritis. A molecular docking simulation indicated that Glyceryl diacetate-2-Oleate (Cp1), Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester (Cp3), Glycidyl oleate (Cp5) and Oleoyl chloride (Cp6) had a high binding affinity to all major identified known rheumatoid arthritis therapeutic targets. The molecular descriptor analysis also confirmed that these compounds were within the Lipinski set limits. This allow us to understand the molecular mechanism of action of RP-RB herbal formula in the treatment of rheumatoid arthritis.(see more results given in the supplementary files).

Conclusions

In this study we developed a technology that integrate an efficient weighted network centrality measure approach and network pharmacology techniques to explore the relationship among the compositive compounds of *Ruellia prostrata* and *Ruellia bignoniiflora* and their putative target genes and known therapeutic targets of rheumatoid arthritis. The results of drug target network using an interconnectivity network-based approach by MCL revealed that among 196 compositive compounds of the RM-RK herbal formula, 22 were actively interacted with putative target genes associated with rheumatoid arthritis. The imbalance multi-level network built by a union merge of drug target network and disease target network indicated that 8

RP-RB compositive compounds, 10 known RA therapeutic targets and 15 putative target genes plays a significant role in the treatment of the rheumatoid arthritis disease. Gene ontology and KEGG pathway analysis indicated that the majority of the identified putative target genes were frequently involved in the IL-17 signaling pathway, TNF signaling pathway, Interleukin-4 and Interleukin-13 signaling, Toll-like receptor signaling pathway, NF-kappa B signaling pathway which play a role in the treatment of rheumatoid arthritis. A molecular docking simulation and molecular descriptor analysis indicated that Glyceryl diacetate-2-Oleate (Cp1), Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester (Cp3), Glycidyl oleate (Cp5) and Oleoyl chloride (Cp6) had a high binding affinity with all the major target receptors with molecular descriptor values showing their drug likeliness properties, hence indicating their potential as candidate drug compounds for treating rheumatoid arthritis. The findings of this study provide promising results that could lead us to design and discover of alternative drug compounds for the management and treatment of rheumatoid arthritis. Though our approach is a purely *in silico* method, clinical experiments are required to test and validate the hypotheses of our computational methods.

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Author's contributions
JD and PJO conceived the idea and formulated the new weighted network centrality measures. AH contributed to the data collection and curation. PJO analysed the data, implemented the model, computational framework and methodology, and performed the experiments. All authors discussed the results and contributed to the writing of the original draft manuscript. All three authors read and approved the final manuscript.

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Additional Files

Additional file 1 —Data
This file contains the data for for the *Ruellia prostrata* and *Ruellia bignoniiflora* chemical compounds, putative target genes and know rheumatoid arthritis targets used for network construction.

Additional file 2 –Supplementary results
This file contains additional results for network analysis and Gene Ontology and Pathway analysis and molecular docking simulation.

Additional file 3 –Figures
This file contains list of figure used presented in this article.

Supplementary Files

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