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EVALUATION OF *LASHUNA* (*ALLIUM SATIVUM L* L) BULB IN THE MANAGEMENT OF RHEUMATOID ARTHRITIS WITH NETWORK PHARMACOLOGY AND DOCKING MECHANISM

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ABSTRACT

Rheumatoid Arthritis (RA) is a disease that has caused a serious issue in our society with a whooping increase in its percentage of prevalence from 1990 to 2021. Morning stiffness, rise in temperature and pain during any activity are few symptoms that are prevalent among the RA patients. As the prevalence increases there is a need for drug discovery with more safety and efficacy. *Lashuna* (*Allium sativum* L) is a drug of choice in case of RA as it is mentioned in several classical texts of *Ayurveda*. Though the drug is used and mentioned for treatment, exact mechanism of the drug will give us further more understanding in its usage. For this computational method was used to recover phytochemicals related to the drug was obtained from plant databases and targets related to disease were collected form disease databases. After recovering the overlapping targets and generating PPI network, the KEGG pathway analysis done. With this topological analysis of 'Target – Compound – Pathway' was done. Followed by this molecular docking and visualization of the targets and phytochemicals was done. A total of 53 overlapping targets, 10 pathways and 25 phytochemicals were derived. Among this based on the degree in topological analysis 3 targets and phytochemicals were chosen and docking results showed that Quercetin has highest binding affinity towards FABP4 and PDGFRA targets of the disease. Further in-vivo and Clinical studies are required to find out its dosage and efficacy in huge volunteers.

Keywords: Rheumatoid arthritis, *Lashuna*, In-silico analysis, Molecular docking

INTRODUCTION:

In 2019, Rheumatoid arthritis affected 18 million persons globally **[1]**. Women make up over 70% of those with rheumatoid arthritis, and 55% of those over 55 have the disease. The hands, wrists, feet, ankles, knees, shoulders, and elbows are the joints most frequently impacted by rheumatoid arthritis, despite the fact that it is a systemic autoimmune disease that affects many body systems **[2]**. The disease in common has prevalence of it in 3rd to 5th decade of a person's life.

RA have symptoms such as inflammation of joints, smoking and obesity as causative factor, and the prevalence is more in women in both RA **[3]**. Though the modern system of treatment such as NSAIDs, Corticosteroids, DMARDs, TNF inhibitors, Janus kinase (JAK) Inhibitors, Interleukin 6 Inhibitors have proven its efficacy in RA still the side-effects of these drugs are present such as Osteoporosis, weight gain, elevated risk of infections and in chronic conditions without medication no relief is there in patients **[4]**.

So, with these issues, a potential drug which can have both efficacy and safety is required for management of RA. *Lashuna* (*Allium sativum L*) a plant in *Ayurveda* (Indian Traditional Medicine) is indicated in *Vata-vyadhi* (disease caused due to *vata* imbalance) and *Ama vata* (RA). *Allium sativum L* is having various properties such as Anti-inflammatory, Anti-oxidant, Anti-cancer, Anti-microbial, Anti-diabetic activity and Hepato-protective activity **[5]**. Though there are studies which support its activities on these conditions, the mechanism through which the drug acts on the disease is still a big question mark. Pinpointing the phytochemicals, targets and the pathway of *Lashuna* (*Allium sativum L*) will give us scientific foundation and future practice for clinicians in the management of RA.

MATERIALS AND METHODS:

The analysis of *Lashuna* (*Allium sativum L*) through network pharmacology consists of 6 steps: 1. Collection of the data from different databases 2. Collection of the targets related to the disease from the databases 3. Analysis of the Protein construction 4. Analysis of the enrichment pathway 5. Construction of the network and 6. Docking of the collected target and phytochemical.

Collection of the phytochemical from plant database:

The phrase "*Allium sativum L*" and "part use bulb" were used to search two databases for phytochemicals in *Lashuna*: IMPPAT 2.0 (Indian Medicinal Plants, Phytochemistry, and Therapeutics 2.0) **[6]** and Dr. Duke's Phytochemical and Ethnobotanical Databases **[7]**. Oral bioavailability and drug similarity served as the two primary screening criteria for the phytochemicals that were found. Using the PubChem database **[8]**, data on each phytochemical's canonical smiles was first

obtained. Several ADME variables were then predicted using the SwissADME database **[9]**. Utilizing the similarity-based web tool Binding DB **[10]**, *Lashuna* phytochemicals were shown to have a target with a similarity score of ≥0.85. Once the disease targets associated with each phytochemical related to *Lashuna* bulb was obtained, the target names were standardized using the UniProt KB **[11]** database.

Procuring targets related to & RA from databases:

The disease "Rheumatoid Arthritis" were used in two databases Gene Cards **[12]** and HPA (Human Protein Atlas) **[13]**, targets related to the diseases were collected from both the websites and further overlapping of the disease and Phyto-targets were done from Venny 2.1.0 web tool **[14]**.

Protein interaction:

The overlapping targets were uploaded in STRING version 11.0 **[15]** and the protein network related to disease targets and phytochemicals was constructed. To verify reliability, we selected "Homo sapiens," and a medium level of confidence was established at 0.50 FDR.

KEGG pathway analysis:

KEGG study was conducted after "Homo sapiens" was chosen as the species that could be the pathway of *Lashuna's* impact on RA. The top 10 pathways were chosen and described.

Network construction:

The Target-Compound-Pathway network of *Lashuna* was built using Cytoscape 3.7.2 **[16]** after data pertaining to active phytochemicals, enriched pathways, and intersected targets had been exported and processed. The topological evaluation was carried out using the network analyser. The network was examined in terms of degree strength in order to determine the role and relationship between the active phytochemicals and the overlapping targets of RA.

Molecular docking:

The crystal structures of the top three compounds and the three most well-known target proteins were obtained from the RSCB PDB **[17]** and Pub Chem databases, respectively. Using the Biovia DS program, water molecules were eliminated and polar connections were added to stabilize the protein structures. The docking approach was employed to predict the binding relationship of top target proteins and ligands, while PyRx **[18]** software was utilized to automatically identify binding sites. The interaction with the best docking scores in both 2D and 3D structures has been demonstrated using the Biovia Discovery Studio application.

RESULTS:

Analysis of the collected phytochemicals from plant databases:

Total 538 phytochemicals were collected from both IMPPAT and Dr. Dukes Phytochemical and Ethnobotanical Databases for the term "*Allium sativum L*". After doing the toxicity

and ADME screening with Swiss ADME, the total available phytochemicals were 209 based on the bio-availability, oral absorption and Lipinski violation. With Binding DB, the Smilies were screened and with Uniprot KB the targets were acquired with a total of 181 targets for 34 phytochemicals in total.

Analysis of Overlapping targets between Phyto-targets and Disease targets:

A total of 6629 targets of RA was acquired from Gene Cards and HPA (Human Protein Atlas) databases. After this the disease targets and the Phyto-targets of *Lashuna* (*Allium sativum L*) was uploaded in the Venny 2.1.0. The final intersection of *Lashuna* (*Allium sativum L*) was 53 for RA. The overlapping targets of *Lashuna* (*Allium sativum L*) and RA in **Figure 1**.

Protein Construction:

A PPI network was constructed using the String database. Following the import of 53 *Lashuna*-related common targets into the STRING platform with RA. A medium degree of confidence, set at 0.50, was used to create the PPI network. PPI of RA is shown in **Figure 2**.

KEGG pathway Analysis:

KEGG functional enrichment analysis to investigate the signalling pathways was carried out. The top 10 involved paths of RA are displayed in **Figure 3**.

Merged Network of Target- Phytochemical-Pathway:

The interaction link between 10 KEGG pathways, 53 intersecting targets, and 25 active ingredients for RA was uploaded and the network was constructed using Cytoscape 3.7.2 software. The network of RA is depicted in **Figure 4**.

Docking analysis:

Molecular docking was done to analyse the binding energy of the plant phytochemical and gene targets. 3 phytochemical which had better degree in the network was chosen for both the disease they were Quercetin, Apigenin and Kaempferol. Three targets PGDFRA, FABP4 and FABP5 of RA was chosen as these were most likely shown a substantial interaction with the three main targets that were identified based on binding energies ≥ 5. In this analysis All the Phytochemicals have shown good binding affinity towards the specific targets and the visualisation of the docking is picturised in **Figure 5** for RA. In **Figure 5**, the first row is Apigenin – FABP5, middle row is Quercetin – FABP4 and the last row depicts the binding of Kaempferol – PDGFRA.

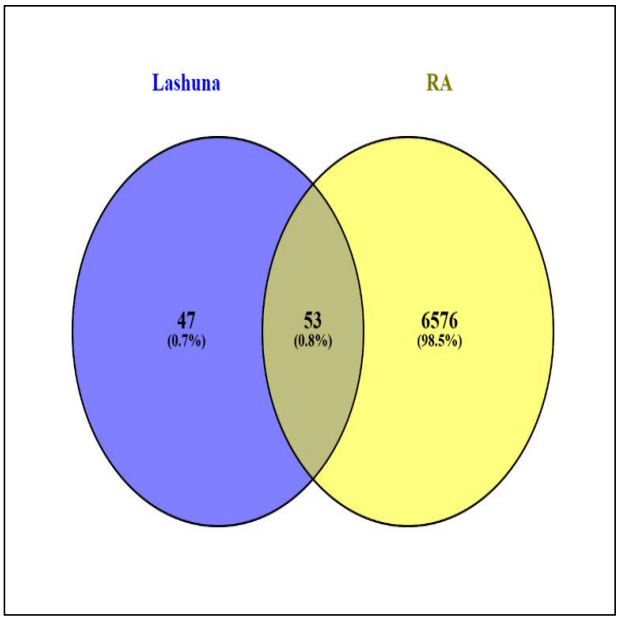

Figure 1: Common targets of *Lashuna* (*Allium sativum* L) and RA

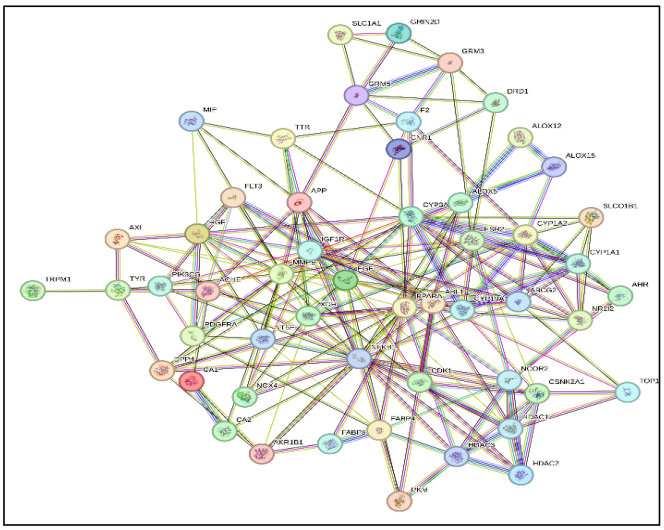

Figure 2: PPI Network of common targets

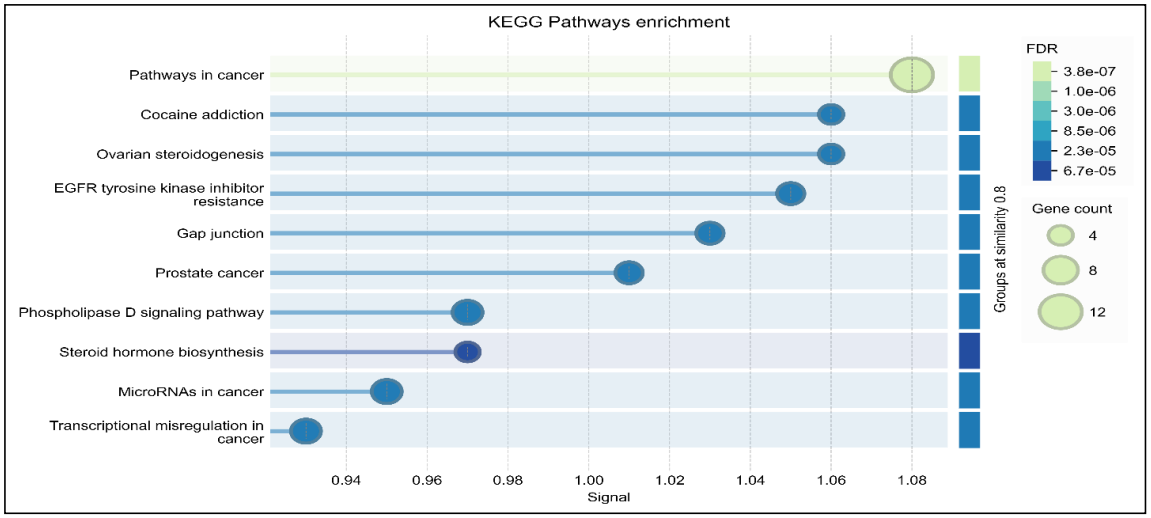

Figure 3: Top 10 pathways of *Lashuna* (*Allium sativum L*)

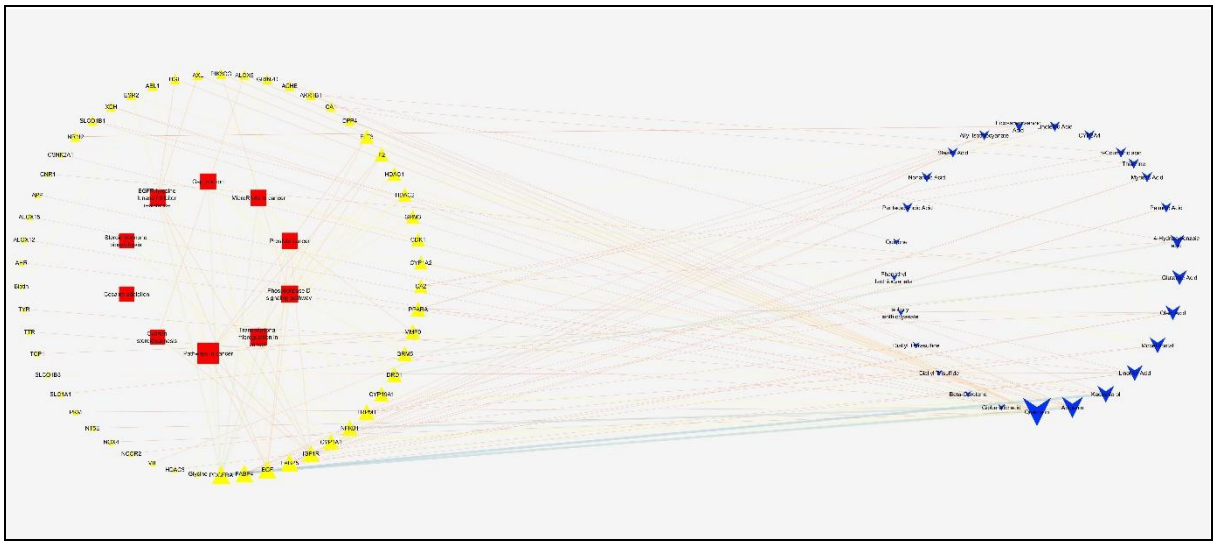

Figure 4: Topological analysis of the 'Target – Compound – Pathway'

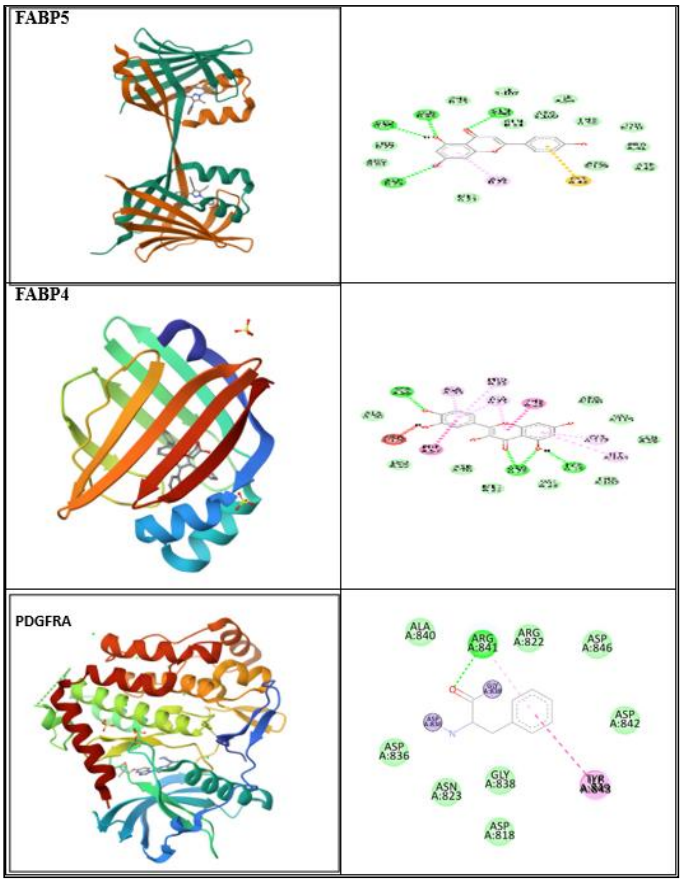

Figure 5: Docking analysis of the Phytochemicals and the targets

Table 1: Docking of *Lashuna* (*Allium sativum* L) in RA

Phytochemicals	Binding Affinity		
	FABP5 (4azm)	FABP4 (2nnq)	PDGFRA (6joj)
Quercetin	-7.3	-8.4	-8.4
Apigenin	-7.6	-8.3	-8
Kaempferol	-7.1	-8.3	-8.3

DISCUSSION:

Plants and herbs are extensively used in *Ayurveda* for management of many diseases. *Lashuna* (*Allium sativum L*) which is used in *Ayurveda* extensively in many forms such as *vati* (tablet), *Kshirapaka* (milk preparation) and as *rasayana* (rejuvenation) purpose has many pharmacological actions such as Anti-inflammatory, Anti-oxidant, Anti-cancer, Anti-microbial, Anti-diabetic activity and Hepato-protective activity **[8]**. In *Amavata* (RA) this plant is given in management of the disease due to its *Katu rasa* (spice taste), *Katu vipaka* (post digestion effect), *Ushna virya* (Hot potency) and *Kapha-vata hara* (pacifies *Kapha* and *vata doshas*) these properties of these plants help in reducing the symptoms of the disease. It has textual proves in *Samhitas* like *Kashyapa Samhita* and *Madhava Nidhana*, where the *rogagnata* (disease indication) of *Lashuna* (*Allium sativum L*) is told as *Amavatahara* (Manages RA) **[19]**.

So, with this classical reference and Clinical data about garlic's ability to treat Rheumatoid arthritis was reviewed. Clinical research of *Allium sativum L* on RA patients revealed that, when compared to a placebo, an 8-week garlic supplement (1,000 mg daily) significantly decreased CRP, TNF-α, pain, fatigue, and joint symptoms in women with RA. ESR did not change significantly. Although longer-term research is advised, garlic may be a useful supplementary therapy **[20]**. These evidences and literature review has given *Lashuna* has role in RA, but to substantiate the mechanism of action to know how *Lashuna* (*Allium sativum L*) works in RA we used Bio-informatics method and we got few Phyto-chemicals, Disease targets and Pathways through which *Lashuna* (*Allium sativum L*) works on RA.

Quercetin in RA:

Important cytokines implicated in the pathophysiology of RA, including TNF-α, IL-1β, IL-6, and IL-17, are downregulated by quercetin **[21]**. The additional factor compared to RA is IL-17, which primarily causes cartilage and joint degeneration as well as inflammation. When these cytokines are combined, the result is increased pain and inflammation. Oxidative stress is linked to RA. Quercetin reduces oxidative damage to joints by neutralizing ROS and boosting the activity of SOD, catalase, and glutathione **[22]**. Quercetin's antioxidant properties lessen oxidative damage to synovial cells and joint cartilage. By inhibiting the release of histamines and other inflammatory mediators, it also contributes to the inhibition of mast cells and neutrophils **[23]**. Additionally, in a clinical research, 70 female RA patients were given 500 mg/kg of quercetin, and the patients' condition significantly improved in terms of blood parameters such serum HS-CRP and TNF-α levels, post-activity discomfort, and early morning stiffness **[24]**.

PDGFRA role in RA:

One receptor tyrosine kinase that binds platelet-derived growth factors (PDGFs) is called PDGFRA (Platelet-Derived Growth Factor Receptor Alpha). Indeed, it does Autophosphorylation of PDGF ligands upon binding to PDGFRA results in the activation of synovial fibroblasts (RA-FLS) and initiates subsequent signalling pathways. This causes cartilage to be destroyed via fibroblast migration, proliferation, and the release of matrix-degrading enzymes (like MMPs). By promoting the activation of pericytes and the recruitment of endothelial cells, PDGFRA aids in angiogenesis **[25]**. In the pannus, this promotes blood vessel development, which keeps the inflammation going. It also encourages the production of inflammatory cytokines such as IL-1β and TNF-α. Synovial tissues in RA patients generally exhibit increased PDGFRA; using inhibitors may assist to lessen inflammation and the development of pannus **[26]**. *Lashuna* (*Allium sativum L*) which contains Apigenin, Quercetin and Kaempferol in the plant may help in reducing the pannus formation and also suppresses the proliferation and invasion of RA-FLS since all had good binding affinity PDGFRA target and it may probably act as PDGF inhibitor which will help in management of RA.

Cancer pathway role in RA:

Synovial fibroblasts (RA-FLS) and immune cells are activated by the PI3K/AKT/mTOR Pathway, one of the "Pathways in Cancer," which supports angiogenesis in pannus tissue, cell survival and proliferation, and resistance to apoptosis **[27]**. The NF-KB Pathway Pro-inflammatory cytokines (IL-1, TNF-α) and anti-apoptotic proteins are induced by chronic activation of immunological and synovial cells in RA. It encourages cartilage degradation and inflammation **[28]**. The characteristics of the cancer pathways are similar to those of RA, including hyperproliferation, apoptosis resistance, and genetic mutations. For example, the symptoms of RA such as epigenetic changes and RA-FLS hyperplasia, are similar to those of cancer including tumour growth, tumour suppression mutation and apoptosis resistance **[29]**. So, with this we can give a justification that *Lashuna* (*Allium sativum L*) does Anti-arthritic activity through cancer pathway and helps in reducing hyper-proliferation, apoptosis resistance and inflammation.

These pathway, compounds and targets have become short-term evidence that *Lashuna* (*Allium sativum L*) has effect on RA. Moreover, the binding affinity of the targets and compounds were also assessed through molecular docking has provided potential evidence of the drug in Anti-arthritic activity. But there are certain limitations in this study such as the study mainly relies on multiple databases, but the reliability of the data from the databases is still a question mark. Because of this reliability there is a question in the KEGG enrichment pathway analysis which

becomes the base for the drug to do its action on the particular disease. Besides, this study assessment of the phytochemicals derived from the plant and its study on the disease may give and add up as potential evidence for the validation of *Lashuna*'s (*Allium sativum L*) activity on RA.

CONCLUSION:

Bheshaja pariksha (Standardisation of medicine) is very important for usage of any *Dravya* (substance) in clinical usage according to *Ayurveda.* According *Acharya Charaka*, any plant or a drug before using it in clinical practice should go through the 10-fold examination and in that *vyadhi* -Various diseases in which drug can be therapeutically used is one factor **[30]**. The importance given for the drug action in various disease should be also given for mode of action of drug, through computational pharmacological evaluation the drug mechanism becomes much easier. Though there are few lacunas in this area, a proper assessment of *Lashuna*'s (*Allium sativum L*) activity on RA through pre-clinical data and further on human subjects may give a conclusive result on our ancient textual preachings.

ACKNOWLEDGEMENT:

Nil

CONFLICT OF INTEREST:

Nil

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