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Original Article

Elucidating the role of *Shatapushpa* (*Anethum graveolens* L.) in Rheumatoid Arthritis through *in vitro* and *in silico* Analysis

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ABSTRACT

Introduction: Day by day the prevalence of the diseases are increasing throughout the globe. One such disease that is having increase in incidence is Rheumatoid Arthritis (RA). With the rise in the occurrence there is need of newer drugs with safety and efficacy. *Shatapushpa* (*Anethum graveolens* L.) in Ayurveda is used in majority of the inflammatory conditions in various mode of administrations not only in oral form but also through parenteral route in the form of *basti* (enema). It is one of the major ingredients in *Erandamooladi niruha basti* (Enema prepared with decoction based on *Ricinus communis* L. roots). The pharmacological action of the drug has proven its effect on the disease but the exact mechanism of it is still not clear. **Materials and Methods:** On performing macroscopic, physico-chemical, basic phytochemical analysis, Thin Layer Chromoatography, Total flavanoid quantification and network pharmacology the plant phytochemicals and disease targets were obtained from the databases. Followed by that overlapping of drug and disease target was done to find out the common targets. The Protein interaction, pathway analysis and topological analysis was made with overlapping targets. Molecular docking was done with the top 3 phytochemical and 3 targets with highest degree in topological analysis. **Results and Discussion:** The basic physicochemical and Phytochemical analysis result revealed that Shatapushpa churna (*Anethum graveolens* L.) was in the standard API limits. The total flavonoid content result gave a quantification of 4.54±0.47 mg QE/ gram per extract. A total of 200 common targets, 10 pathways and 60 phytochemicals were obtained at last. Scoplotein had shown highest binding affinity towards PIK3CA target of the disease. The phytochemical has docked in the places where the control drug Methotrexate has shown its binding. These, evidence gave us the probable mechanism of Shatapushpa (*Anethum graveolens* L.) in RA. **Conclusion:** Safety and efficacy tests on animals and human subjects are required to validate the results.

Keywords: *In silico* Analysis, *In vitro* Analysis, Rheumatoid Arthritis, *Shatapushpa*.

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INTRODUCTION

Plants are used as medicine since centuries in various traditional medicinal system. They have helped us in curing and managing various illness. *Panchakarma* (five therapeutics) in *Ayurveda* is majorly used in managing almost 70-80% of the disease, one among the *panchakarma* (five therapeutics) is *basti* (enema). This *basti* (enema) helps in normalizing the vitiated *vata dosha* (body constituency according to *Ayurveda*). The *basti* is classified into 2 types: 1. *Niruha basti* (Enema based on mixture of 5 products) and 2. *Anuvasana basti* (Enema based on Oil).

The *Niruha basti* is a mixture of 5 components namely, 1. *Madhu* (Honey) 2. *Saindhava* (Salt) 3. *Kalka* (Paste) 4. *Kwatha* (Decoction) 5. *Awapa* (Adjuvant). The *Niruha basti* is prepared by adding *dravyas* into the *kalwa yantra* (Mortar and pestle) and triturating them for 45 - 60 min. In this the *Kalka Dravya* (paste like substance) is very important because of its action in increasing the bioavailability, boosting potency, promoting colonic action and guaranteeing therapeutic efficiency. To get the best results, the right quantity and mixing order must be maintained (Mantha *et al.*, 2023). *Erandamoola niruha basti* (Enema prepared with decoction based on *Ricinus communis* L. roots) is one of the commonly used enema therapies in the clinical practice, in various diseases such as Rheumatoid arthritis,

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Osteo-arthritis and Sciatica. In this *Kalka Dravya* (paste like substance), *Shatapushpa* (*Anethum graveolens* L.) is used. Its role in increasing the bio-availability, boosting the potency and increasing the effect of the enema is mentioned in various classical texts.

As of 2020, rheumatoid arthritis affects 17.6 million individuals worldwide, and its prevalence has increased by 14.1% since 1990. Despite a 23.8% decrease in mortality, disability is still high. Smoking is a risk factor, and the effects are greater in women. Globally, the condition is expected to affect 31.7 million people by 2050 (Deane *et al*., 2021). NSAIDs, corticosteroids, DMARDs, TNF inhibitors, Janus Kinase (JAK) inhibitors, and interleukin 6 inhibitors are examples of current therapy methods that have demonstrated efficacy in treating rheumatoid arthritis. Though there are so many medications they also give possible side-effects such as Renal failure, heart failure, Gastro-intestinal ulceration, weight gain, muscle weakness, diabetes, etc., (Radu *et al*., 2021).

Shatapushpa (*Anethum graveolens* L.) which is having various properties such as Anti-inflammatory, Analgesic, Anti-microbial, Anti-cancer, Hypolipidemic, Anti-diabetic and Anti-ulcer activity (Talreja *et al*., 2025). With these pharmacological actions it is used in the management of Rheumatoid arthritis. Though its usage is mentioned in various classical literatures, the real mechanism of action behind it is still unknown due to presence of numerous phytochemicals. Thus, by assessing the basic phytochemical activity, thin layer chromatography, flavonoid quantification, computational pharmacology and assessing its molecular activity on the selected target will give us a clear picture why it is used in treatment of Rheumatoid arthritis in both single drug preparation and in compound formulation i.e. *Erandamooladi niruha basti.*

MATERIALS AND METHODS

First, raw ingredients were obtained, then an *in vitro* and *in silico* evaluation of the Shatapushpa churna was performed, and the process layout is displayed in Figure 1.

Ethical statement

Since the preparation, *in vitro* and *in silico* investigation doesn't involve any human or animal participants the permission from Institutional Ethical Committee and Institutional Animal Ethical Committee was not required.

Statistical analysis

As the research doesn't require any statistical analysis as it doesn't involve any comparison of the groups, the statistical analysis wasn't performed. For the *in silico* analysis the *p*-value in the KEGG pathway was performed in the database itself and it didn't require any Statistical software.

Procuring *Shatapushpa,* Physico-chemical, Phytochemical, TLC and Total Flavonoid analysis

The drug *Shatapushpa* was procured from KLE Ayurveda GMP certified pharmacy and followed by that phytochemical activity, thin layer chromatography, flavonoid quantification and physico-chemical activity was performed as per API volume 3.

Gathering Phyto-chemicals

Two databases were searched for phytochemicals using the term "*Apium graveolens*": Dr. Duke's Phytochemical and Ethnobotanical Databases (Lans *et al*., 2020) and IMPPAT 2.0 (Mohanraj *et al*., 2018) (Indian Medicinal Plants, Phytochemistry and Therapeutics). The phytochemicals that were discovered were screened mostly based on oral bioavailability and pharmacological similarity. We first gathered information on the canonical smiles of each phytochemical using the PubChem database (Kim *et al*., 2016). Afterwards, the SwissADME database (Diana *et al*., 2017) was used to forecast a number of ADME variables. The web tool BindingDB (Liu *et al*., 2007), which is based on similarities, revealed that phytochemicals have a target with a similarity score of ≥0.85. Using the UniProtKB database, (Apweiler *et al*., 2004), the target names were standardized after the disease targets linked to each phytochemical were identified.

Gathering disease target and Overlapping targets

Human Protein Atlas (HPA) and Gene cards were the two databases used in collecting the disease targets related to Rheumatoid arthritis (Thul *et al*., 2018; Sterlzer *et al*., 2016). The term "Rheumatoid arthritis" was used in both the databases and the targets were collected. Overlapping targets related to Rheumatoid arthritis from the two databases and *Shatapushpa* (*Anethum graveolens* L.) related targets from Uniprot KB was put into Venny 2.1 to find out the common targets related to both disease and the drug (Collazos JCO).

Protein Interaction

The common targets were uploaded in STRING database and the construction of Protein-Protein Interaction (PPI) of the Phytochemicals of *Shatapushpa* (*Anethum graveolens* L.) and Rheumatoid arthritis targets was made (Szklarczky *et al*., 2023). We chose "*Homo sapiens*," and a medium degree of confidence was set at 0.50 FDR to confirm reliability.

Pathway analysis

After the PPI, the KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathway analysis of the targets was analysed. The selection of "*Homo sapiens*" as the species that could serve as the pathway of *Shatapushpa* (*Anethum graveolens* L.) and Rheumatoid arthritis.

Network creation

Network of the Phytochemicals, targets and the pathway was constructed using Cytoscape 3.7.2 (Shannon *et al.*, 2023). The network analyser was used to do the topological evaluation. To ascertain the function and connection between the active phytochemicals and the overlapping targets of rheumatoid arthritis, the network was analysed in terms of degree strength.

Molecular docking

The Phytochemicals and targets which had higher degree in the topological evaluation was chosen for its molecular mechanism. The binding affinity of the particular chemical to the particular target was assessed. For this the chemical 3-dimensional structure was retrieved from Pub Chem database and the target compound was retrieved from RCS PDB (Berman *et al.*, 2000). The targets were analysed and the charges were added using Auto dock 1.5.7 (Morris *et al.*, 2009). After this the targets and the Phytochemicals were binding was done using PyRx. (Dallakyan *et al.*, 2015). Later these was assessed on binding affinity and visualisation was done using Biovia tool (Dassault systems, 2021).

RESULTS

Physico-chemical, Phytochemical, Thin Layer Chromatography and Flavonoids

The *Shatapushpa* that was procured from GMP certified pharmacy was subjected for physico-chemical, phytochemical, TLC and flavonoid quantification. *Shatapushpa* Churna's organoleptic characteristics, which indicate identification, purity, and lack of adulteration, were found to be consistent with standard descriptions. Low microbiological risk and a good shelf life are indicated by moisture content within certain bounds. Low siliceous and inorganic contamination is confirmed by ash tests.

Table 1: Macroscopic and Physico-chemical results of *Shatapushpa churna*.

Parameter	Standard Limit	Result
Form	Churna	Churna
Colour	Greenish yellow	Greenish yellow
Taste	Pungent	Pungent
Odour	Aromatic	Aromatic
Parameter	Permissible Limit	Result
Moisture content	≤ 5%	4.389%
Total ash	≤ 10%	8.913%
Acid insoluble ash	≤ 2.5%	1.812%
Water soluble ash	NA	7.384%
Water soluble extractive	≥ 25%	30.035%
Alcohol soluble extractive	≥ 8%	20.999%

High extractive values support therapeutic potency by indicating a rich abundance of active components that are soluble in water and alcohol. The antioxidant, anti-inflammatory, and digestive properties can be understood by the presence of flavonoids and tannins. The presence of cardiac glycosides indicates possible impacts on metabolism and circulation. Alkaloids in alcohol extract show that some bio actives are more soluble in organic solvents. A complex phytochemical profile is shown by the several unique Rf values under UV and daylight, which provide a trustworthy fingerprint for *Shatapushpa Churna* authentication and quality control. At a total flavonoid content of 4.54±0.47 mg QE/g of extract, the alcoholic extract of *Shatapushpa Churna* exhibited a moderate to noticeable flavonoid concentration. *Shatapushpa Churna's* medicinal relevance is strengthened by the fact that flavonoids are recognized to play a major role in protecting against oxidative stress, enhancing pharmacological efficacy, and scavenging free radicals. Table 1 gives the macroscopic, physicochemical results of *Shatapushpa chiurna*, while the Table 2 reveals the phytochemical values and flavonoid quantification. Tables 1 and 2 gives a detailed result of the *Shatapushpa* (*Anethum graveolens*) *chruna in vitro* study.

Analysis of the obtained Phytochemicals

A total of 815 phytochemicals related to *Shatapushpa* (*Anethum graveolens* L.) was obtained from the plant databases. After selecting them on the basis of part used "seed" and removing the duplicated from the phytochemicals we got 174 Phytochemicals. The Pub Chem ID related to these Phytochemicals were collected. These were uploaded and on the basis of "drug likeliness" and "absorption", a total of 102 phytochemicals were obtained from Swiss ADME. After this the canonical similes obtained were uploaded into Binding DB followed by that retrieving the Gene

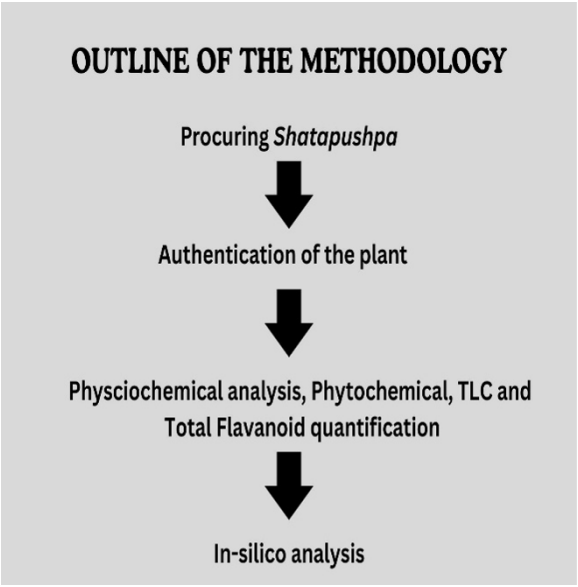

Figure 1: Outline methodology of *in vitro* and *in silico* analysis of *Shatapushpa* (*Anethum graveolens* L.) *churna*.

ID of the target, we found 333 Phyto-targets related to 102 Phytochemicals.

Analysis of the disease targets and Overlapping targets

There were 6629 targets related to Rheumatoid arthritis in both the databases. These disease targets and the Phyto-targets were put into Venny 2.1.0 and the overlapping targets were recovered. There were a total of 200 common targets for both disease and the plants Figure 2 depicts the common targets between the plant and the disease.

Protein Interaction Network

A PPI network of common targets in both *Shatapushpa* (*Anethum graveolens* L.) related Phyto-targets and Rheumatoid arthritis targets were uploaded in STRING database. Figure 3 illustrates the PPI Network.

KEGG Pathway enrichment

The top 10 KEGG pathways through which the common targets help in management of Rheumatoid arthritis is depicted in Figure 4. These pathways play a potential role in the disease.

Table 2: Phytochemical values, TLC and flavonoid quantification.

Phytochemical Test	Water Extract		Alcohol Extract	
Carbohydrates	Positive		Positive	
Reducing sugars	Positive		Positive	
Monosaccharides	Positive		Positive	
Pentose sugar	Negative		Negative	
Non-reducing sugar	Negative		Negative	
Hexose sugar	Negative		Negative	
Proteins	Negative		Negative	
Amino acids	Positive		Negative	
Steroids	Negative		Negative	
Flavonoids	Positive		Positive	
Alkaloids	Negative		Positive	
Tannins	Positive		Positive	
Cardiac glycosides	Positive		Positive	
Anthraquinone glycosides	Negative		Negative	
Saponin glycosides	Negative		Negative	
Observation Condition	R_f Values			
Short UV (254 nm)	0.08, 0.17, 0.31, 0.72, 0.87			
Long UV (366 nm)	0.05, 0.08, 0.11, 0.16, 0.25, 0.32, 0.72, 0.80, 0.87			
Day light	0.05, 0.14			
Total flavonoid content				
Extract	Reading I (mg)	Reading II (mg)	Reading III (mg)	Mean±SD (mg QE/g of extract)
Alcoholic extract	4.27	5.08	4.27	4.54±0.47

Table 3: Molecular docking analysis of 5 chemical compounds and 4 targets.

Phytochemicals	Binding Affinity			
	HRAS (8be6)	PIK3CA (7r9y)	NFKB1 (1svc)	PIK3CB (3ihy)
Methotrexate	-7.7	-9.3	-6.7	-8.2
Scoplotein	-6.2	-7	-5.4	-6.7
4-vinylguaiacol	-5.3	-6.9	-4.9	-5.9
Geranyl acetate	-5.5	-5.7	-5.1	-5.8
Apiole	-5.6	-6.3	-4.9	-6

Network creation

The top 10 pathways, 200 common targets and 60 Phytochemical that are obtained by following these processes were used construction of the target-pathway-compound network. The network that is created is portrayed in Figure 5. The targets are in the big circle out-side with red circular shape, inside those targets are the phytochemicals with blue color circular layout and the pathways are in downward triangular shape in the right side.

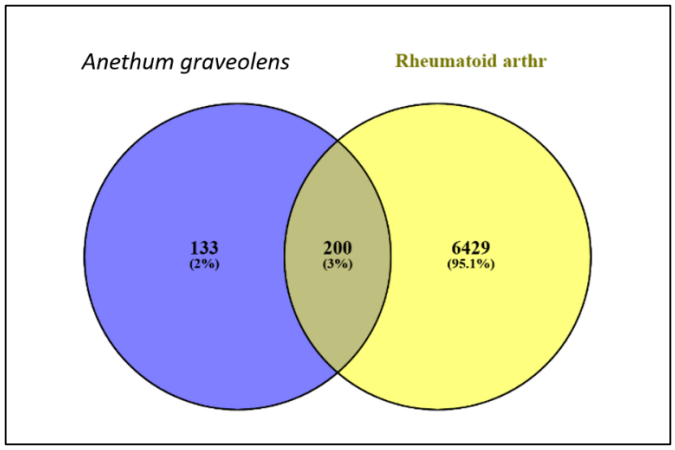

Figure 2: Overlapping targets of *Shatapushpa* (*Antheum graveolens* L.) and Rheumatoid arthritis.

Analysis of the Molecular docking

With the network constructed the top 4 phytochemicals (Apiole, Geranyl acetate, Scoplotein and 4-vinylguaiacol) and targets were downloaded and with this the modern medication that is Methotrexate was taken as control drug and with that the docking was done using the tools. Followed by this the binding affinity of the Phytochemicals, targets and the control drug was assessed. Scoplotein has shown the same binding affinity like the control drug methotrexate. Scoplotein has shown highest binding affinity towards PIK3CA and even methotrexate has shown good binding affinity towards that target. The Figure 6. shows the docking visualization of the control drug methotrexate and scoplotein against the selected targets. Table 3 depicts the binding affinity of the chemical constituents and the targets.

DISCUSSION

The *Shatapuspha kalka* is used in the preparation of *Erandamoola niruha basti*, which gives potency to the whole combination. *Kalka Dravya* may aid in the formation of a homogeneous colloid of *Sneha Dravya* and *Kashaya Dravya* by binding the hydrophilic and lipophilic ends of *Basti Dravya*. *Shatapushpa* possesses unique *Shoolaghna* and *Vatanuloman* properties. Additionally, because of its antibacterial, anti-inflammatory, analgesic, antiemetic, anti-convulsive, gastric, and mucosal protecting

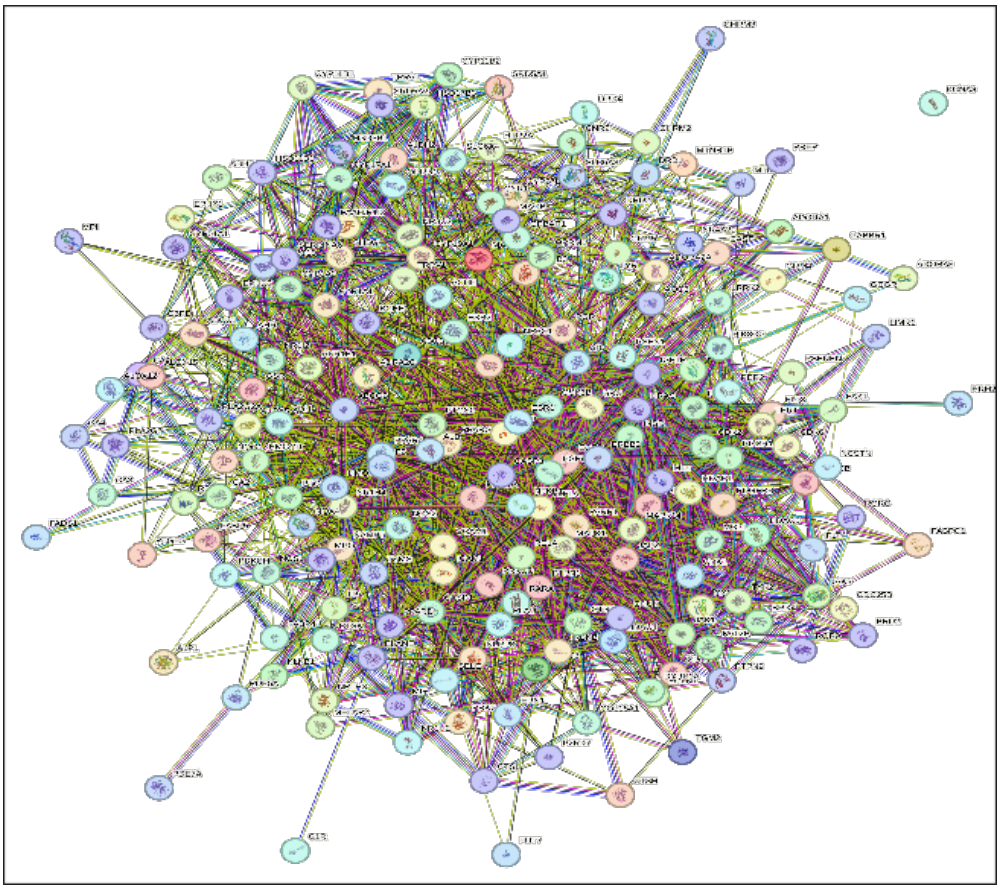

Figure 3: PPI Network of the common targets.

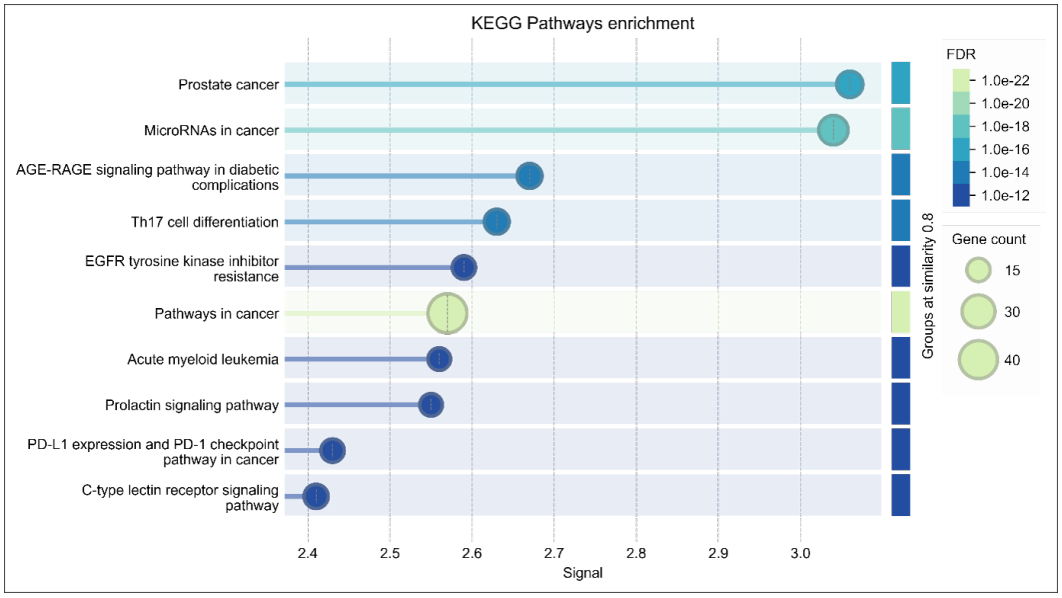

Figure 4: KEGG pathway analysis of the targets.

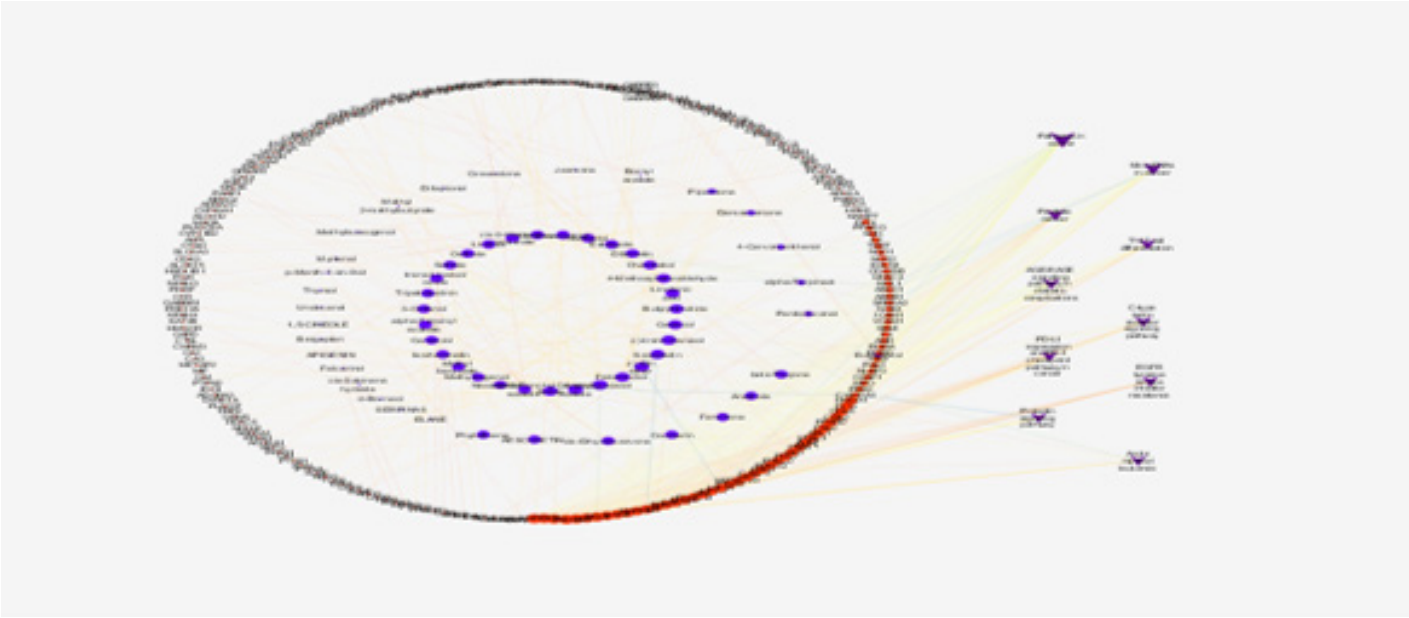

Figure 5: Topological analysis of *Anethum graveolens*.

qualities, it soothes painful ulcers, abdominal pain, eye disorders, and uterine pain. Since the 1980s, Methotrexate has been used to treat Rheumatoid Arthritis (RA), and it is still frequently the first drug prescribed for RA. (Friedman B *et al.*,2019) The results of the binding energy indicate that the phytochemical binds with the protein's active site with a comparable affinity to the conventional medication.

The *churna* authenticity and acceptability were confirmed by its organoleptic characteristics, which included form, color, taste, and odor, all of which met conventional requirements. Because the moisture content (4.389%) was within allowable bounds, there was less chance of microbiological growth and acceptable stability. The values of acid-insoluble ash (1.812%) and total ash (8.913%) were within acceptable bounds, indicating low levels of siliceous and inorganic contaminants. Higher alcohol-soluble (20.999%) and water-soluble (30.035%) extractive values show that the active ingredients are easily extracted, demonstrating the formulation's adequate quality and purity. In a study conducted on seeds of *Anethum graveolens* L. It revealed that the ash value was more than 10%. When compared to the current study the values are more and further more in the same study the flavonoid

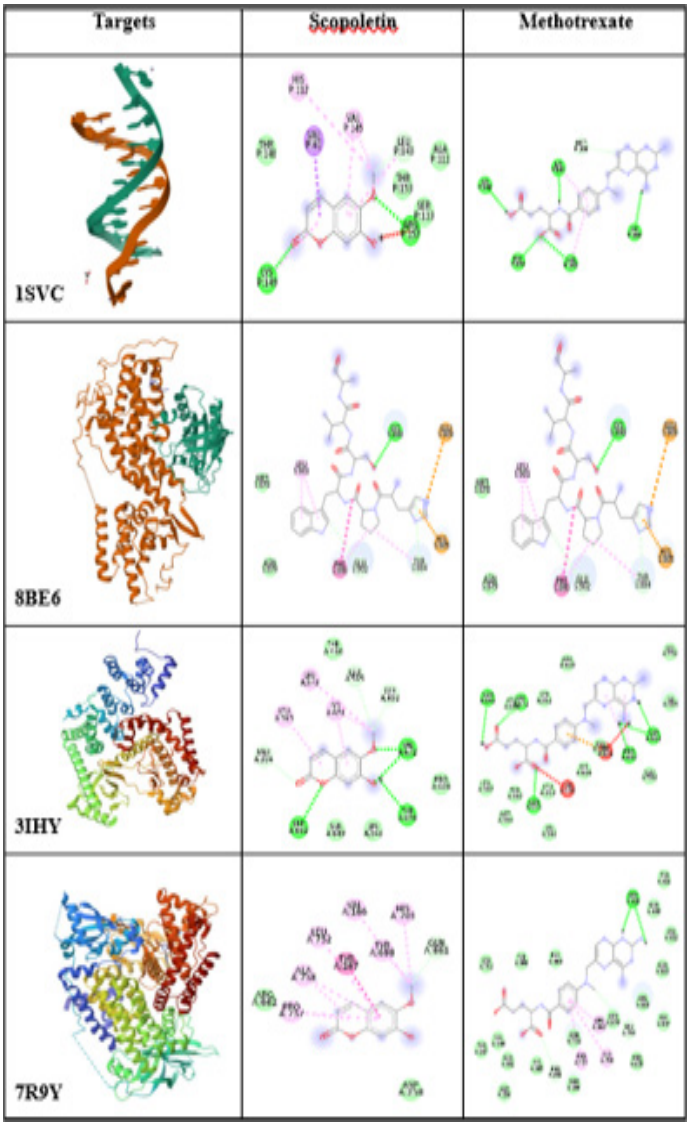

Figure 6: Docking Analysis of Phytochemicals, Control drug and Targets.

content is more when compared to this with a value of 13.79 ± 0.69 QE/ g of extract while the current study has given only 4.54±0.47 mg QE/g of extract this maybe because the study we have performed with hydro-alcoholic extract while in the study done in the past it was with aqueous and ethanolic extract (Hadi *et al*., 2024). TLC profiling exhibited multiple well-resolved spots under UV and daylight conditions, reflecting chemical complexity and providing a reproducible fingerprint for standardization. Collectively, these findings validate the formulation's quality and support its suitability for further pharmacological and clinical investigations.

Scopoletin's anti-arthritic properties in rats with adjuvant-induced arthritis were investigated. When administered intraperitoneally at doses of 50 and 100 mg/kg, scopoletin decreased articular index scores and paw swelling in both infected and non-inoculated animals. By lowering the number of new blood vessels in the synovium and the synthesis of significant endogenous angiogenic inducers, scopoletin can reduce the clinical symptoms of rat adjuvant-induced arthritis (Pan 2010). Scopoletin can prevent RAW 264.7 cells from producing proinflammatory cytokines, particularly IL-6, when exposed to lipopolysaccharide *in vitro*, most likely by preventing MAPK pathway activation (Yao *et al*., 2012). Scopolettin may have an anti-RA effect by inhibiting the synthesis of pro-inflammatory cytokines. Molecular docking investigations in this study showed that the chosen phytochemical component Scopoletion and the control medication methotrexate had similar binding affinities -9.3kcal/mol towards target PIK3CA.

Geranyl acetate has been demonstrated that geranyl acetate inhibits pro-inflammatory cytokines, such as TNF-α and IL-1β, which are important mediators in the pathophysiology of RA. In a study using a Complete Freund's Adjuvant (CFA) mouse model, an extract from Zanthoxylum (andaliman), which is rich in monoterpenoids such geranyl acetate and geraniol, shown anti-arthritic benefits. The extract relieved joint inflammation and cartilage damage, lowered COX-2, IL-1β, iNOS, and MMP-1 expression, and considerably decreased arthritic assessment scores (Setiadi *et al*., 2022).

HRAS by acting upstream of important inflammatory pathways such PI3K-AKT, NF-κB, and MAPK/ERK, which are frequently activated in RA synovial Fibroblasts (FLS), HRAS promotes angiogenesis, cytokine secretion, cell proliferation, and MMP expression (Sadeghi Shaker *et al*., 2023). According to this study, RA synovial tissues, particularly the intimal lining, have markedly elevated levels of H-Ras mRNA and protein. The increase is associated with synovial hyperplasia, indicating a function in tissue invasion and chronic inflammation (de Launay *et al*., 2010).

PIK3CB the catalytic subunit of class I PI3K enzymes, which are crucial for inflammation, immune cell signalling, and survival, is encoded by PIK3CB. Through Fcγ receptors, PI3Kβ plays a crucial role in neutrophil activation, which results in Reactive Oxygen Species (ROS) production, Chemotaxis, Degranulation (Qu *et al*., 2016). In RA, Fibroblast-Like Synoviocytes (FLS) are hyperproliferating and resistant to apoptosis.

MicroRNAs are short, non-coding RNAs that post-transcriptionally control gene expression. They have been linked to the aetiology of autoimmune illnesses like Rheumatoid Arthritis (RA) and cancer (Kim *et al*., 2002). Dysregulated miRNA expression is a contributing factor to joint degeneration, synovial hyperplasia, and persistent inflammation in RA. For instance, the immune response and inflammatory signalling pathways are regulated by miR-155 and miR-146a, which are elevated in RA (Peng *et al*., 2015). The disease process in RA is also significantly influenced by the miRNA-mediated interactions between various cell lines. Interestingly, the intricate relationships and integration of multilayer signalling between miRNAs and other epigenetic variables are essential for the pathophysiology of RA but are still mostly unclear (Stanczyk *et al*., 2008). Still, there is not enough

in vivo experimental data to discuss the pleiotropic function of miRNAs in RA clinical conditions.

With these above phytochemicals present in *Shatapushpa* (*Anethum graveolens* L.) we came to know the mechanism of the drug in *Amavata* (Rheumatoid arthritis). The phytochemicals present in the plant has major role on the disease and the targets. Especially molecular docking visualisation has shown that where the control drug Methotrexate has docked, in the same binding site Scoplotein has also shown its docking. Additionally, methotrexate and scopoletein have a close binding affinity. All this result conclusively suggests the efficacy and mechanism of *Shatapushpa* (*Anethum graveolens* L.) in RA.

Although the study has given satisfying results, still there are some limitations for this study. The whole process is solely depended on computational method. Especially the phytochemicals which the main mode of action is substantiated is majorly procured from the database. Thus, this may bring bias for this study due to the continuous update in the databases. Nevertheless, further study on *in vivo* and followed by that clinical research on human samples are needed to evaluate the safety and efficacy of the drug.

CONCLUSION

The current research suggests that the paste made from *Anethum graveolens* L. Known as *Shatapushpa*, has therapeutic potential for the treatment of RA. The main research question the mechanism of action of *Shatapushpa* (*Anethum graveolens* L.) is clear by the network pharmacology and followed by the docking. In order to optimize treatment and meet unmet clinical requirements, a deeper understanding of the underlying mechanisms of RA is necessary (Peng *et al*., 2023).

Bioinformatics research support this, showing that *Shatapushpa's* active phytoconstituents such as scopoletin, geranyl acetate, apiole. HRAS and PIK3CB targets and important molecular pathways linked to RA, including as micro RNAs in cancer and pathway in cancer. *Shatapushpa* has demonstrated anti-inflammatory and immunomodulatory effects similar to those of methotrexate, suggesting its potential as a natural alternative or complementary agent in the management of rheumatoid arthritis. Strong computational evidence for its anti-inflammatory and immunomodulatory actions is provided by the combination of gene interaction network research, pathway enrichment, and molecular docking. As a result, *Shatapushpa Kalka* shows promise as an adjuvant or alternative treatment for RA, deserving of additional experimental support and clinical studies.

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ABBREVIATIONS

RA: Rheumatoid Arthritis; **IMPPAT:** Indian Medicinal Plant, Phytochemistry and Therapeutics; **KEGG:** Kyoto Encyclopaedia of Genes and Genomes; **NSAIDs:** Non- Steroidal Anti-inflammatory Drugs; **DMARDs:** Disease Modifying Anti-Rheumatic Drugs; **ADME:** Absorption, Distribution, Metabolism and Excretion; **CFA:** Complete Freund's Adjuvant; **PPI:** Protein - Protein Interaction; **TLC:** Thin Layer Chromatography; **SD:** Standard Deviation; **ROS:** Reactive Oxygen Species; **GMP:** Good Manufacturing Practices; **PIK3CA:** Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; **PIK3CB:** Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Beta; **HRAS:** Harvey Rat sarcoma viral oncogene homolog; **QE:** Quercetin Equivalent; **API:** Ayurvedic Pharmacopoeia of India; **HPA:** Human Protein Atlas; **RCS PDB:** Research Collaboratory for Structural Bioinformatics Protein Data Bank; **R_f:** Retention factor; **UV:** Ultraviolet; **FDR:** False Discovery Rate; **iNOS:** inducible Nitric Oxide Synthase; **COX-2:** Cyclooxygenase-2; **MMP-1:** Matrix metalloproteinase-1; **FLS:** Fibroblast-like synoviocytes; **TNF-α:** Tumor Necrosis Factor-alpha; **IL-1β:** Interleukin-1 beta; **IL-6:** Interleukin-6; **MAPK:** Mitogen-activated protein kinase.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

AK: Research concept and design, Critical revision of the article; **RM:** Research concept and design, Data analysis and interpretation, Writing the article; **KM:** Collection and/or assembly of data; **VSVK:** Research concept and design, Data analysis and interpretation, Writing the article.

REFERENCES

Apweiler, R., Bairoch, A., Wu, C. H., Barker, W. C., Boeckmann, B., *et al.* (2004). UniProt: The Universal Protein knowledgebase. Nucleic Acids Research, 32, D115-D119. https://doi.org/10.1093/nar/gkh131

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., *et al.* (2000). The Protein Data Bank. Nucleic Acids Research, 28(1), 235-242. https://doi.org/10.1093/nar/28.1.235

Collazos, J. C. O. Venny 2.1.0 [Internet]. Agencia Estatal Consejo Superior de Investigaciones Científicas. https://bioinfogp.cnb.csic.es/tools/venny/

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7(1), 42717. https://doi.org/10.1038/srep42717

Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. Methods in Molecular Biology, 1263, 243-250. https://doi.org/10.1007/978-1-4939-2269-7_19

Dassault systèmes [BIOVIA Discovery Studio visualizer] (version 21.1.0) [Software]. (2021). Dassault Systèmes.

de Launay, D., Vreijling, J., Hartkamp, L. M., Karpus, O. N., Abreu, J. R. F., *et al.* (2010). Silencing the expression of Ras family GTPase homologues decreases inflammation and joint destruction in experimental arthritis. The American Journal of Pathology, 177(6), 3010-3024. https://doi.org/10.2353/ajpath.2010.091053

Deane, K. D., & Holers, V. M. (2021). Rheumatoid arthritis pathogenesis, prediction, and prevention: An emerging paradigm shift. Arthritis and Rheumatology, 73(2), 181-193. https://doi.org/10.1002/art.41417

Friedman, B., & Cronstein, B. (2019). Methotrexate mechanism in treatment of rheumatoid arthritis. Joint Bone Spine, 86(3), 301-307. https://doi.org/10.1016/j.jbspin.2018.07.004

Hadi, N., Drioiche, A., Bouchra, E. M., Baammi, S., Abdelaziz Shahat, A., *et al.* (2024). Phytochemical analysis and evaluation of antioxidant and antimicrobial properties of essential oils and seed extracts of *Anethum graveolens* from Southern Morocco: *In vitro* and *in silico* approach for a natural alternative to synthetic preservatives. Pharmaceuticals, 17(7), 862. https://doi.org/10.3390/ph17070862

Kim, G., Jun, J.-B., & Elkon, K. B. (2002). Necessary role of phosphatidylinositol 3-kinase in transforming growth factor beta-mediated activation of Akt in normal and rheumatoid arthritis synovial fibroblasts. Arthritis and Rheumatism, 46(6), 1504-1511. https://doi.org/10.1002/art.10314

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., *et al.* (2016). PubChem substance and compound databases. Nucleic Acids Research, 44(D1), D1202-D1213. https://doi.org/10.1093/nar/gkv951

Lans, C., & Van Asseldonk, T. Dr. (2020). Duke's phytochemical and ethnobotanical databases, a cornerstone in the validation of ethnoveterinary medicinal plants. In Medicinal and aromatic plants of the world (1st ed.) (pp. 219-246). Springer Cham.

Liu, T., Lin, Y., Wen, X., Jorissen, R. N., & Gilson, M. K. (2007). BindingDB: A web-accessible database of experimentally determined protein-ligand binding affinities. Nucleic Acids Research, 35(suppl_1), D198-D201. https://doi.org/10.1093/nar/gkl999

Mantha, U., Madikonda, P. K., & Johar, B. [In preparation] of Niruha Basti. AYUSHDHARA. (2023). A conceptual analysis and importance of Kalka Dravya, 10(5), 42-48.

Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. P. B., Aparna, S. R., *et al.* (2018). IMPPAT: A curated database of Indian medicinal plants, phytochemistry and therapeutics. Scientific Reports, 8(1), 4329. https://doi.org/10.1038/s41598-018-22631-z

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., *et al.* (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry, 30(16), 2785-2791. https://doi.org/10.1002/jcc.21256

Pan, R., Gao, X.-H., Li, Y., Xia, Y.-F., & Dai, Y. (2010). Anti-arthritic effect of scopoletin, a coumarin compound occurring in *Erycibe obtusifolia Benth*. stems, is associated with decreased angiogenesis in synovium. Fundamental and Clinical Pharmacology, 24(4), 477-490. https://doi.org/10.1111/j.1472-8206.2009.00784.x

Peng, X., Wang, Q., Li, W., Ge, G., Peng, J., *et al.* (2023). Comprehensive overview of microRNA function in rheumatoid arthritis. Bone Research, 11(1), 8. https://doi.org/10.1038/s41413-023-00244-1

Peng, Y., & Croce, C. M. (2016). The role of microRNAs in human cancer. Signal Transduction and Targeted Therapy, 1(1), 15004. https://doi.org/10.1038/sigtrans.2015.4

Qu, Y., Wu, J., Deng, J.-X., Zhang, Y.-P., Liang, W.-Y., *et al.* (2016). MicroRNA-126 affects rheumatoid arthritis synovial fibroblast proliferation and apoptosis by targeting PIK3R2 and regulating PI3K-AKT signal pathway. Oncotarget, 7(45), 74217-74226. https://doi.org/10.18632/oncotarget.12487

Radu, A.-F., & Bungau, S. G. (2021). Management of rheumatoid arthritis: An overview. Cells, 10(11), 2857. https://doi.org/10.3390/cells10112857

Sadeghi Shaker, M., Rokni, M., Mahmoudi, M., & Farhadi, E. (2023). Ras family signaling pathway in immunopathogenesis of inflammatory rheumatic diseases. Frontiers in Immunology, 14, 1151246. https://doi.org/10.3389/fimmu.2023.1151246

Setiadi, A. Y. L. A., Karmawan, L. U., & Yanti. (2022). Anti-arthritic and anti-inflammatory effects of Andaliman extract and Nanoandaliman in inflammatory arthritic mice. Foods, 11(22), 3576. https://doi.org/10.3390/foods11223576

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., *et al.* (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Research, 13(11), 2498-2504. https://doi.org/10.1101/gr.1239303

Stanczyk, J., Pedrioli, D. M. L., Brentano, F., Sanchez-Pernaute, O., Kolling, C., *et al.* (2008). Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis and Rheumatism, 58(4), 1001-1009. https://doi.org/10.1002/art.23386

Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., *et al.* (2016). The GeneCards suite: From gene data mining to disease genome sequence analyses. Current Protocols in Bioinformatics, 54(1), 1.30.1-1.30.33. https://doi.org/10.1002/cpbi.5

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., *et al.* (2023). The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Research, 51(D1), D638-D646. https://doi.org/10.1093/nar/gkac1000

Talreja, S. B., Shrirao, A. V., Mohale, D. S., Kochar, N. I., & Chandewar, A. V. (2025). The pharmacological potential of *Anethum graveolens*. Int. J. Adv. Res. Sci. Commun Technol., 5(5), 366-372.

Thul, P. J., & Lindskog, C. (2018). The human protein atlas: A spatial map of the human proteome. Protein Science, 27(1), 233-244. https://doi.org/10.1002/pro.3307

Yao, X., Ding, Z., Xia, Y., Wei, Z., Luo, Y., *et al.* (2012). Inhibition of monosodium urate crystal-induced inflammation by scopoletin and underlying mechanisms. International Immunopharmacology, 14(4), 454-462. https://doi.org/10.1016/j.intimp.2012.07.024

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