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Original Article

IN SILICO PREDICTION OF ESSENTIALS OIL FROM *CYMBOPOGON NARDUS* AS IMMUNOMODULATOR IN RHEUMATOID ARTHRITIS

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ABSTRACT

Objective: Rheumatoid arthritis (RA) is an autoimmune disease involving the synovial lining of the major joints. Our study involves bioactive compounds of *Cymbopogon Nardus* as possible Rheumatoid arthritis drugs *in silico* targeted TNF-α, JAK1/2, JAK3, PAD4, and DHFR.

Methods: Using computational docking and receptors from the Protein Data Bank (PDB) files 2AZ5, 3EYG, 3LXY, 1DLS, and 1WDA. Molegro Virtual Docker 6.0 was utilized to undertake an *in silico* anti-arthritis drugs study. ChemDraw 3D was utilized to minimize the ligand's energy before docking, and the structures Native Ligand were employed as positive control medications. A pharmacokinetic and toxicological study was performed using SwissADME (ADME) and PK-CMS.

Results: Using the Moldock SE mechanism calculates the binding (atom) energies of each protein (Enzyme) and each ligand at the least energetic conformation state. Docking results of ten tested bioactive compounds have not displayed the Lowest rerank score scores and best fit within the prominent active site residues.

Conclusion: The Essentials oils from *Cymbopogon nardus* have not effectively suppressed the Rheumatoid Arthritis pathway through inhibition of TNF-α, JAK1/2, JAK3, PAD4, and DHFR which can serve as potential lead compounds for the development of new drugs for the treatment of Rheumatoid Arthritis.

Keywords: Rheumatoid arthritis, *Cymbopogon nardus, In silico*, Molecular docking, Essentials oils

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INTRODUCTION

Rheumatoid arthritis (RA) is an immune-dysregulated illness that causes bone and cartilage damage. It causes joint discomfort, swelling, and sometimes deformity and is characterized by persistent inflammation. In extreme circumstances, other numerous organs may be impacted as well. Genetic factors such as human leukocyte antigen (HLA), protein tyrosine phosphatase, non-receptor type 22 (PTPN22), signal transducer and activator of transcription 4 (STAT4) and peptidyl arginine deiminase, type IV (PADI 4), epigenetic, and environmental factors have been linked to the development of the disease [1].

Immunological homeostasis is disrupted in RA, causing immune components to become hostile against the body's own cells, causing persistent inflammation through the production of a variety of cytokines triggered by T and B cells. When peptidyl arginine is converted to peptidyl citrulline by the process of citrullination that occurs from these released B cells, it contributes to the formation of immune complexes and the activation of the complement system in the joints, which results in the emergence of an antibody known as rheumatoid factor and anti-citrullinated proteins. T cells penetrate the synovial membrane, causing macrophages and synovial fibroblasts to become tissue-destructive effector cells. These stimulate the production of various inflammatory cytokines, which contribute to the loss of bone and cartilage, which promotes joint degeneration [2].

Immune cells are penetrated as a result of the immunological complexes formed, leading to swelling and congestion. TNF-α is a pro-inflammatory cytokine that plays a key role in inflammation and tissue death. TNF-α is an inflammatory protein released by macrophages in response to septic shock. Furthermore, because TNF-αoverexpression and synovial cell proliferation are hallmarks of RA, they are possible therapeutic targets for treating the disease. TNF levels were observed to be greater in the blood plasma and synovial fluid of RA patients. Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), JAK 1/2/3, and Granulocyte-macrophage colony-stimulating factors are all autocrine stimulators and paracrine inducers for this cytokine. It also boosts the proliferation of a wide range of cells, including macrophages, activated T-cells, B-cells, synovial cells, and endothelial and fibroblast cells, as well as induces the production of adhesion molecules [3].

Non-steroidal anti-inflammatory medicines (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), and contemporary therapies including b-DMARDs and ts-DMARDs are currently used to treat RA. While DMARDs have been shown to significantly relieve disease symptoms and limit disease progression in RA patients, they also have significant adverse effects. These medications are beneficial in slowing the course of the disease, but they also have adverse effects such as bacterial and viral infections, TB reactivation, cancer, hematologic abnormalities, and cardiovascular ailments [4]. Plants are a valuable source of medicine and are essential to the survival of ethnic and tribal societies. Medicinal plants are used to cure a variety of human and animal ailments all around the world. Essential dietary components such as carbohydrates, protein, and fat can be found in medicinal plants. Many medications are obtained directly or indirectly from plant resources, and plants are frequently a valuable supply of medicines [5].

The pharmacological potential of the Poaceae family's Cymbopogon genus has been examined. Citronella or *Cymbopogon nardus* (L.) Rendle is grass grown in subtropical and tropical parts of Asia, Africa, and America, including Brazil. The EO is a complex mixture of monoterpene and sesquiterpene hydrocarbons (10 and 15 carbon atoms, respectively), and their oxygenated derivatives such as alcohols, aldehydes, ketones, phenylpropanoids, and other minor compounds. EOs are also called volatile oils or ethereal oils, as they have a high degree of evaporation when exposed to air at room temperature. This feature confers a strong odor to plants, both to attract pollinators and to repel insects and herbivores [6].

Compounds derived from aromatic plants are known as essential oils (EOs). Their volatile compounds have long been employed for

bactericidal, virucidal, fungicidal, antiparasitic, insecticidal, anticancer, antioxidant, antidiabetic, cardiovascular, cosmetic, and dietary reasons. Aromatic grasses of the genus Cymbopogon (Poaceae family) are a unique group of plants that generate a wide range of monoterpene Essential oils compositions. Evidence from ethnopharmacology suggests that they have a wide range of qualities that justify their usage in a variety of disciplines, including pest management, cosmetics, and anti-inflammation compounds. These plants might possibly be useful as anti-tumor and chemopreventive agents [7].

Molecular modeling and structure-based drug design have shown to be a viable method for discovering cancer inhibitors and have become a well-known approach in contemporary drug development. Until the advent of computational drug discovery, medications were discovered by accident via try and error. Computer-aided drug design (CADD) is a computational chemistry approach for creating and optimizing pharmacological lead compounds using computer simulations, calculations, and predictions of the drug-receptor connection. This approach may considerably enhance drug screening success rates, minimize research blindness, have cheap cost and short cycle benefits and is an essential tool in drug research and development. Molecular docking is the most often utilized computational chemistry tool in structure-based drug design. It uses computer simulation to place ligands into the binding site of the target, then calculates the physical and chemical parameters to predict the binding force and models of the ligand-receptor complexes, allowing for high-throughput, virtual screening of unknown compounds and greatly improving the speed of new drug design and discovery [8, 9].

MATERIALS AND METHODS

Materials

The 10 essential oil *Cymbopogon nardus* (L.) Rendle that were evaluated for the study came from the literature [11]. Receptors from the Protein Data Bank (PDB) TNF-α (PDB: 2AZ5), JAK 1 (PDB: 3EYG), JAK3 (PDB: 3LXK), PAD4 (PDB: 1DLS), and DHFR (PDB: 1WDA)

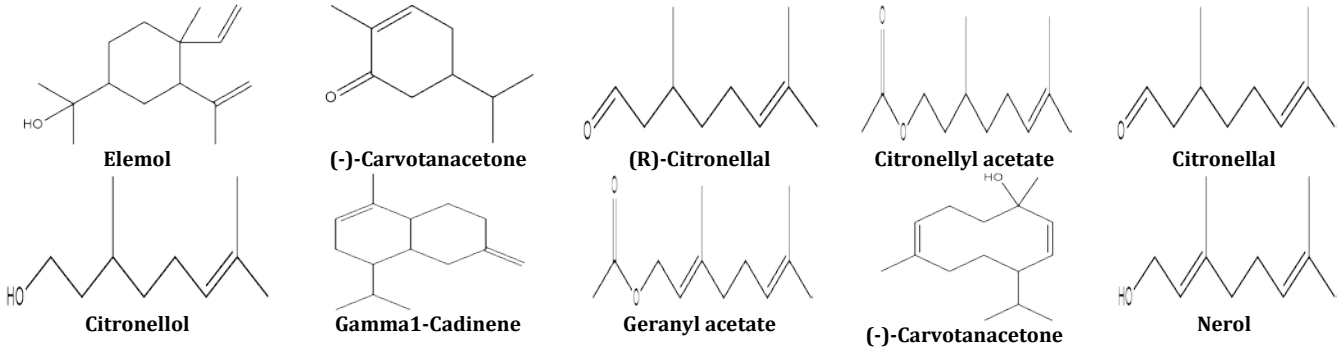

Fig. 1: Structures of 10 essential oils from the *Cymbopogon nardus* (L.) rendle

Preparation of ligand

The structures were created in. mol2 format using Chem3D and energy minimization using the MM2 force field. MVD software was used to assign the structures missing bond orders, charges, bonds, and hybridization states [12].

Preparation of protein

The protein's 3D structure was obtained from the Protein Data Bank, and the protein was created by deleting all water molecules, ligands, cofactors, assigning bonds, bond order, hybridization, and charges using MVD software [10].

Docking parameters

With a grid resolution of 0.3 A, the scoring function is used. The docking radius was then increased to 15 to encompass the voids on the protein structure. The docking algorithm used was the MolDock SE (Simplex Evolution), and the constrain poses to the cavity, energy minimization, and optimize H-bonds boxes were all checked, and the maximum iterations = 1500, maximum population size = 50, energy threshold was set at 100, and the values for Tries were 10, and 30 for a min, quick, and max tries, respectively. Furthermore, in the simplex evolution, the Max step is 300 and the neighbor distance factor is 1.00. Pose clustering was used to ensure a wide range of binding modes [13].

Pharmacokinetics

The term ADME/Tox is used to characterize drugs absorption, distribution, metabolism, excretion, and toxicity. The *in silico* ADME/Tox profile is a valuable technique for predicting the pharmacological and toxicological features of drug candidates, particularly in pre-clinical phases. *In silico* models have been used to enhance ADME/Tox predictions. The application of these models has especially contributed to medication optimization and preventing late-stage failures, which are extremely crucial since such failures result in the significant fruitless investment of time and money. The candidate leads can be predicted using pkCSM [14].

Drug-likeness

Lipinski's rule of five well-used criteria to understand if a compound can be taken orally or not, such as molecular weight (MW) 500, octanol/water partition coefficient (AlogP) 5, and the number of hydrogen bond donors (HBDs) 5 and number of hydrogen bond acceptors (HBAs) 10 were used to check compounds for their adaptability. According to the rule of five, a compound cannot be consumed orally if it does not fulfill at least two of the five requirements [15].

RESULTS

Molecular docking result analysis

The chemicals investigated were put through a molecular docking study to see if they could block TNF-(PDB: 2AZ5), JAK 1 (PDB: 3EYG), JAK3 (PDB: 3LXK), PAD4 (PDB: 1DLS), and DHFR receptors from the Protein Data Bank (PDB) (PDB: 1WDA). Using Molegro Virtual Docker 6.0, a total of 10 essential oils from *Cymbopogon nardus* (L.) Rendle was docked, with the ligands targeting TNF-, JAK1/2, JAK3, PAD4, and DHFR. The top ten compounds were chosen for further investigation. Rank these chemicals according to their affinity for certain receptors (table 1).

When compared to its greatest rerank score, essential oils from *Cymbopogon nardus* (L.) Rendle, the 307 (Native Ligand) complexed with TNF-receptor displayed significant binding affinity (Rerank score: -105.622). (rerank score: -74.8272) Two of the 307 hydrogen bonding contacts with conserved TNF amino acid residues are shown in the crystal structure, one from chain GLY121 and the other from chain GLY122 [16].

Table 1: Rerank scores of docked compounds attenuates rheumatoid arthritis

Ligand	Rerank score				
	TNF-α (PDB: 2AZ5)	JAK 1/2 (PDB: 3EYG)	JAK3 (PDB: 3LXK)	PAD4 (PDB: 1WDA)	DHFR (PDB: 1DLS)
Native	-105.622	-99.6079	-105.569	-107.816	-144.262
Elemol	-74.8272	-64.1568	-74.0575	-69.3855	-76.6046
gamma1-Cadinene	-73.7337	-71.9447	-74.2081	-62.6078	-74.0229
Geranyl acetate	-73.2064	-70.947	-77.5096	-98.1481	-84.291
Citronellyl acetate	-72.9204	-71.6576	-77.6574	-94.1818	-80.5562
Germacren-4-ol	-64.7265	-64.4698	-70.4769	-65.1255	-68.53
(R)-Citronellal	-63.6733	-59.7493	-64.7768	-81.6171	-63.9672
Nerol	-62.4947	-64.1907	-67.0658	-81.3487	-67.9286
Citronellol	-61.6292	-59.6929	-60.8725	-78.6005	-63.6407
Citronellal	-58.9689	-59.9921	-67.5056	-76.7056	-67.6295
(-)-Carvotanacetone	-54.0609	-57.0766	-59.6626	-68.4141	-57.7547

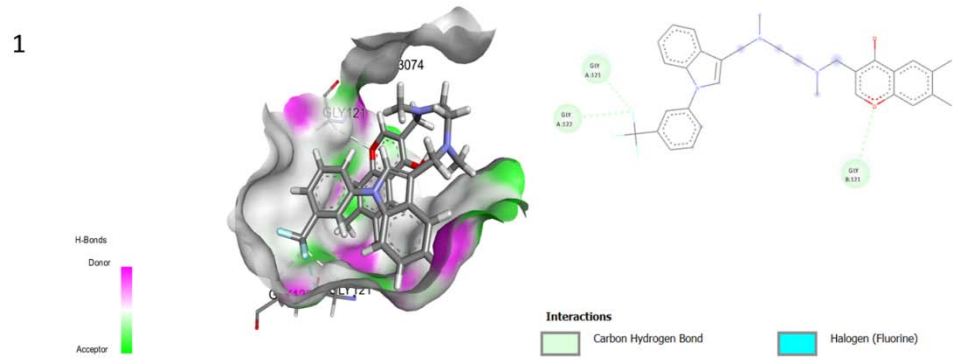

Fig. 2: The 2D/3D-interaction diagram of 307 in the catalytic pocket of TNF-α (PDB ID: 2AZ5) exposing key interactions

The PTR (Native Ligand) complexed with JAK-1 and JAK-2 receptor (fig. 2) exhibited potent binding affinity (Rerank score: -99.6079) when compared with its highest rerank score essential oils from *Cymbopogon nardus* (L.) Rendle (rerank score: -64.1568) The PTR displayed hydrogen bonding interactions with conserved amino acid residues of JAK-1 and JAK-2 crystal structure two are presented two hydrogen bonds between the hinge region of JAK1 and PTR, linking Glu957O with pyridoneN2 and Leu959N with pyridone0O. Therefore, unlike PTR makes numerous contacts with the glycine loop with both the oxygenO21 and the nitrileN24 forming hydrogen bonds with conserved Gly residues from the glycine loop Gly882 and making van der Waals contacts with several residues. A large number of van der Waals interactions with the JAK1 PTK domain also stabilize the inhibitor. Both the pyrolopyrimidine and piperidine rings (chair conformation) are sandwiched between the hydrophobic residues of the N-terminal lobe Leu881, the C-terminal lobe Leu1010, and the hinge Phe958. The methyl group of the piperidine ring points towards the C-terminal lobe, making van der Waals contact with ASN1008 [17].

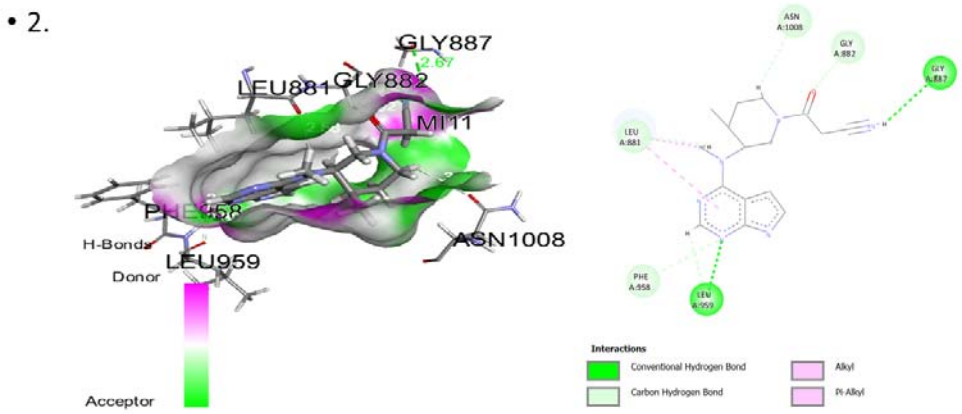

Fig. 3: The 2D/3D-interaction diagram of *PTR* in the catalytic pocket of JAK 1/2 (PDB: 3EYG) exposing key interactions

The MI1 (Native Ligand) complexed with JAK-3 receptor (fig. 3) exhibited potent binding affinity (Rerank score: -105.569) when compared with its highest rerank score essential oils from Cymbopogon nardus (L.) Rendle (rerank score: -77.6574). As observed in JAK-3 crystal structures, the pyrrolopyrimidine ring is situated against the hinge region and forms one hydrogen bond with the main chain atoms of Leu905 (3.1 Å). In the JAK3-MI1 structure, this residue is observed to participate in hydrogen bonding interactions with Asn954 The piperidine ring is situated in the hydrophobic environment of the JAK3 binding cavity, forming

hydrophobic interactions with Val836., the terminal cyano group packs against the glycine-rich loop, and forms polar interactions with main-chain atoms spanning this flexible loop, particularly Gly834 [18].

The BAG (Native Ligand) complexed with PAD4 exhibited potent binding affinity (Rerank score: -107.816) when compared with its highest rerank score essential oils from *Cymbopogon nardus (L.)* Rendle (rerank score: -98.1481). As observed in the Arginine side-chain nitrogen atoms of the substrate are hydrogen-bonded by Asp473 and Asp350. The coordination of Ca1 also stabilizes residues Phe633–His644 as an α-helix (α16), thereby facilitating the formation of a hydrogen bond between Arg639 and the N1 atom of the substrate. Hydrogen bonding also occurs between two main chain peptide oxygen atoms (O1 and O2) of the substrate and Arg374. The aliphatic portion of the substrate arginine side chain is in close hydrophobic contact with Trp347 [19].

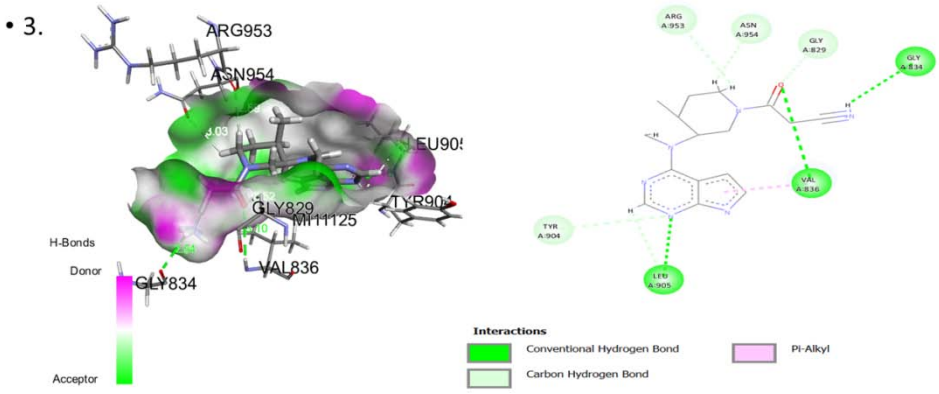

Fig. 4: The 2D/3D-interaction diagram of MI1 in the catalytic pocket of JAK 3 (PDB: 3LXK) exposing key interactions

The MTX (Native Ligand) complexed with DHFR (fig. 4) enzymes exhibited potent binding affinity (Rerank score: -144.262) when compared with its highest rerank score essential oils from *Cymbopogon nardus* (L.) Rendle (rerank score: -84.291). complexed MTX and DHFR enzymes ave five hydrogens bond which is ILE7, ARG28, GLU30, ASN64, VAL115

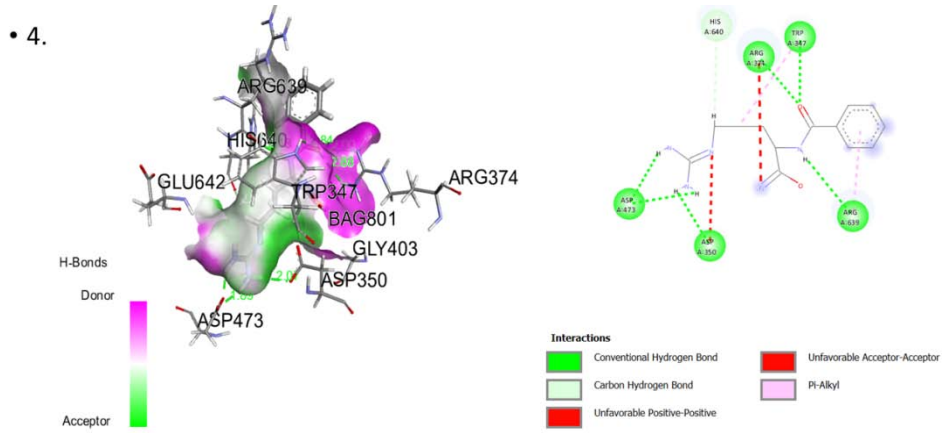

Fig. 5: The 2D/3D-interaction diagram of PTR in the catalytic pocket of PAD4 (PDB: 1LDS) exposing key interactions

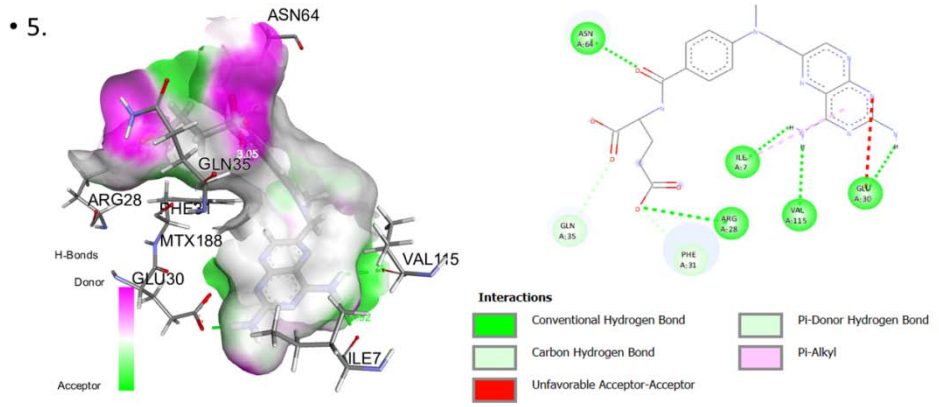

Fig. 6: The 2D/3D-interaction diagram of PTR in the catalytic pocket of DHFR (PDB: 1WDA) exposing key interactions

CONCLUSION

TNF-a, JAK1/2, JAK3, PAD4, and DHFR are cytokines and enzymes that are primarily implicated in tissue degeneration at RA locations and might be a viable target for RA therapy. In this study, we have carried out an *in silico* screening of the phytoconstituents of essential oils from *Cymbopogon nardus* (L.) Rendle This study demonstrated that ten compounds (Elemol, gamma1-Cadinene, Geranyl acetate, Citronellyl acetate, Germacren-4-ol, Nerol, (R)-Citronellal, Citronellol, Citronellal, and (-)-Carvotanacetone) from the selected phytocompounds showed docking rerank scores have no more stable affinity than the complex native and protein. To summarize, phytoconstituents present in *Cymbopogon nardus* (L.) Rendle possesses strong inhibitory effects against TNF-a and could be further evaluated for their anti-arthritic effect, as well as for the development of alternative drugs with fewer side effects for the treatment of RA.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

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