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Mechanistic insight anti-arthritis efficacy of bio-actives of *Moringa oleifera*: *In-silico* molecular docking

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Abstract
Background: Rheumatoid arthritis (RA), an autoimmune, inflammatory, and chronic condition, affects patients differently in terms of how severely it affects their joints. Risk factors include age, gender, genetics, and environmental exposure (tobacco usage, air pollution exposure, and occupational exposure). If Felty syndrome is not treated, it can progress and lead to a number of complications, such as rheumatoid vasculitis, persistent joint deterioration requiring arthroplasty, and Felty syndrome requiring splenectomy. Since there is no known cure for RA, the goals of treatment are to reduce discomfort and stop/slow further damage. Moringa oleifera Lam., popularly known as munga, is one of the most significant plants that is widely cultivated in India. Moringa oleifera Lam is a plant that is widely used as a dietary supplement. It possesses valuable pharmacological qualities, such as anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-cancer, antimicrobial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic action. In every area, this herb has excellent therapeutic qualities. It is a good source of vitamin A, vitamin C, and milk protein. The numerous active phytoconstituents present include alkaloids, proteins, quinine, saponins, flavonoids, tannin, steroids, glycosides, fixed oil, and lipids, to name just a few.
Aim and Objective: The current study's objective was to assess the anti-arthritic efficacy of *M. oleifera* leaves.
Method: *In -silico* molecular modelling studies for assessment of anti-arthritic potential of *M. oleifera* leaf was designed taking quercetin and niazirinin as lead molecule found in the ethanolic leaf extract (as per literature survey) against GLS-1 protein. A grid-based docking strategy was used to determine the binding using the Auto Dock software.
Result: The finding of molecular modelling of lead molecule with GLS-1 protein showed that both the selected molecules have good affinity towards GLS-1 protein. The binding energy of quercetin and niazirinin against GLS-1 protein was found to be -4.8 & -5.94 Kcalmol^{-1} respectively.

Keywords: RA, quercetin, niazirinin and GLS-1 protein

Introduction
Rheumatoid nodules, vasculitis, ocular inflammation, and cardio pulmonary disease are all symptoms of rheumatoid arthritis, a systemic disease. Rheumatoid arthritis does not have a hereditary basis. Researchers claim that certain people are genetically predisposed to the disease [1]. People who carry these genes do not inevitably develop rheumatoid arthritis. A "trigger," such as an illness or environmental factor, frequently "activates" the genes. When the body is exposed to this trigger, the immune system responds inappropriately. Instead of defending the joint, the immune system begins to produce chemicals that assault the joint. This is what might lead to the development of rheumatoid arthritis. Being an autoimmune disease, healthy tissues are mistakenly assaulted by the immune system of the body [2]. While the lining of healthy joints is extremely thin and home to few blood vessels, the lining of rheumatoid arthritis-affected joints is thick and densely populated with white blood cells. Two substances released by white blood cells, interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-alpha), are uncomfortable and contribute to joint degeneration [3]. Recent research has revealed the existence of novel cytokines as IL-17 and IL-18. These cytokines cause synovial fibroblasts and chondrocytes in the surrounding articular cartilage to secrete proteoglycan- and collagen-degrading enzymes, which leads to tissue breakdown and the generation of RANK ligand (RANKL), a role in the genesis of chronic arthritis. The creation of many cytokines and inflammatory mediators causes the synovium to start growing and spreading, a condition

known as pannus. The next stage, fibrosis, is known as ankylosis and causes a lack of joint motion [4-6].

The *Moringa oleifera* plant is also known as the drumstick tree and the horse radish tree. Zeatin, quercetin, beta-sitosterol, kaempferol, and caffeoylguinic acid are all present in significant and uncommon amounts in munga plants. Iron, potassium, calcium, copper, zinc, magnesium, manganese, and other essential elements are found in Moringa oleifera. Deic, palmitic and stearic acid, saponins, glycoside, gum, and protein are the primary components of the moringa plant. Vitamins: B1, B2, B3, C, and A (8855 IU per 100g). Calcium, iron, phosphorus, and magnesium are minerals. Significant amounts of the vitamins A, B, and C, riboflavin, nicotinic acid, folic acid, pyridoxine, beta-carotene, calcium, iron, and alpha-tocopherol can be found in the leaves, flowers, and pods. The high flavonoid concentration of the Moringa genus contributes to its potent antioxidant effect. Most of the flavonoids in the genus are found in their flavanol and glycoside forms. Most frequently occurring flavonoids in the genus are rutin, quercetin, rhamnetin, kaempferol, apigenin, and myricetin. The species' most abundant glucosinolate is 4-O-(-L-rhamnopyranosyloxy)-benzyl glucosinolate. The most prevalent phenolic acid in M. oleifera leaves is gallic acid. Additionally, elagic acid, ferulic acid, caffeic acid, o-coumaric acid, and chlorogenic acid are present in the leaves. The leaves and seeds were used to isolate -sitosterol. Antispasmodic, anti-hypertensive, anti-inflammatory, anti-fertility, anti-hyperglycemic, anti-hyperlipidemic, and hypocholesterolemic, as well as antiviral, anti-leishmanial, anti-convulsant, anti-microbial, and anticancer activities were all demonstrated by the plant [7-9].

Experimental works

Molecular docking studies of Qucertin and Naizirinin against GLS-1 Protein Ligand Preparation

Using Chem Sketch [10], the two-dimensional structures of the produced ligands were transformed into their three-dimensional structures, which were then optimised with three-dimensional geometry. Examples of these ligands include quercetin and niazirinin. For Auto Dock compatibility, the optimised structures were stored in PDB format. The prepared ligands' fundamental structures are listed below:

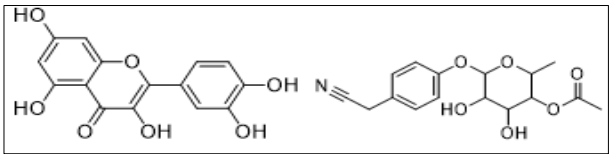


Fig 1: 2D structure of quercetin and niazirinin

Preparation of the grid file

By creating a grid box around the active sites, Autodock's regions of interest were identified by taking grid area into account. Grid boxes are essential to the docking process because they are designed to cover all amino acids other than those found in receptors that are present in active sites and required for binding. Three thumbwheel widgets on the grid box allow us to adjust the number of points in the x, y, and z dimensions. Table 1 provides the grid points and spacing [11-12].

Table 1: Grid parameters used in current docking analysis of GLS1

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	GLS1	42	40	40	0.375	25.53	10.109	44.068

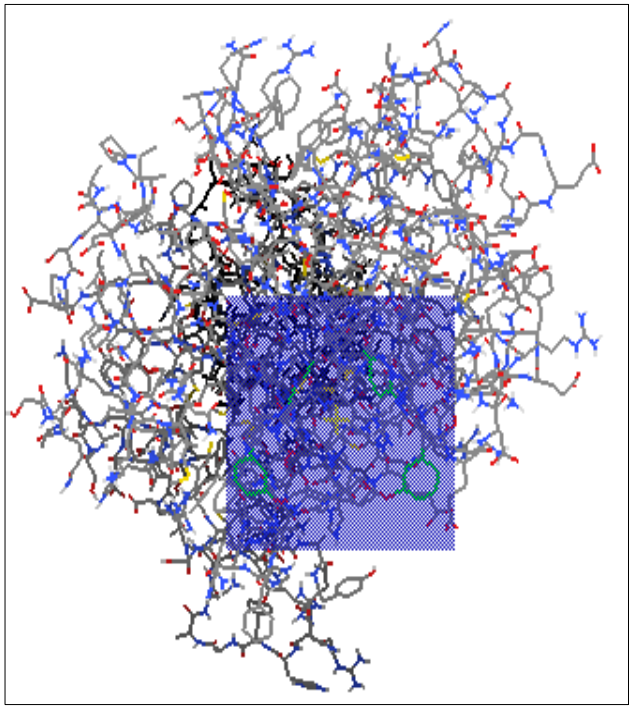


Fig 2: Grid box covering all active sites in GLS1 receptor

Preparation of the docking file

Autodock 4.2 was used as the docking tool for all calculations. Pymol, Chimera, DS visualizer, MMP Plus, and other applications were used to carry out the visualisation and other tasks required for docking research [20, 14].

Docking Study

Crystal structure: The Protein Data Bank portal allows users to obtain the crystal structure of the protein containing the GLS1 receptor. The Protein Data Bank's 1n45.pdb file, which contains all of the major receptor and structural information, was used [15]. Chimera software was used to separate the intricate ligand.

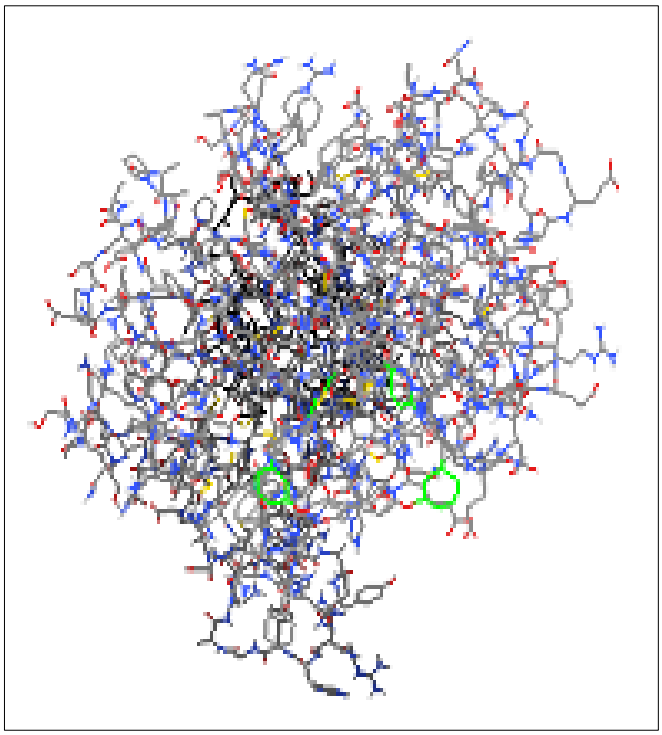


Fig 3: Crystal structure of GLS1 receptor (PDB ID-1n 45)

Processing of Protein

Chains A, B, C, and D make up the four chains of the receptor protein that was downloaded. Out of these two chains, chain A was chosen for the experiment and the other chains were eliminated. Using the Chimera software [16], the binding ligand EN2 was isolated from the macromolecular complex.

Molecular Docking Simulation Studies

Autodock was used to dock ligands like quercetin and niazirinin against the human GLS1 receptor. While no receptor residues were rendered flexible, all of the linkages in each ligand were kept flexible [17].

Toxicity & ADME-T Studies

The online programme OSIRIS looked at the ligand molecules quercetin and niazirinin to anticipate the presence of any toxic groups as well as the presence of any toxic groups and ADME-T characteristics [18].

Result and Discussion

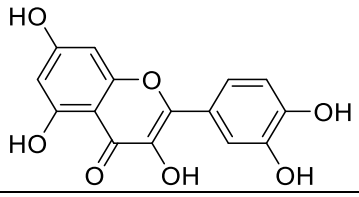
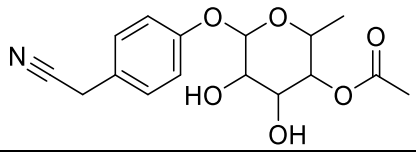
Quercetin and niazirinin were chosen as the lead molecules in the ethanolic leaf extract (according to the literature survey) for their anti-GLS-1 protein activity in in silico molecular modelling studies for the evaluation of the anti-arthritic potential of *M. oleifera* leaf. Molecular docking research using computational methods was used to evaluate anti-arthritic activity. According to a literature review, the ethanolic leaf extract of Moringa oleifera contains the antioxidants quercetin and niazinin; hence, these substances were considered for docking against the GLS-1 protein.

GLS1, the enzyme that turns glutamine into glutamate, inhibits RAFLS growth directly, which lessens the pathological severity of the autoimmune disease, arthritis. The synovial fluid of RA patients contains elevated levels of glutamate. This is directly related to an increase in IL-6 levels. Therefore, as proposed by Flood S *et al*; 2004, blockage of these metabolic enzymes offers cell-specific, unique biological therapeutic targets for the creation of novel drugs against RA [19].

In-silico molecular modelling of quercetin and niazirinin against GLS-1 protein showed that both the lead molecule effective binds with target protein but niazirinin exert high bonding affinity. The binding energy of quercetin and niazirinin against GLS-1 protein was found to be -4.8 & -5.94 $Kcalmol^{-1}$ respectively (Table 2). The binding mode showed in fig. 4-5 The docking interaction result revealed that the niazirinin showed conventional hydrogen binding at LEU A:323, LYS A:398, PHE A: 322, ARG A:387 and Pi-sigma binding at LEU A:321 & TYR A:394 having HIS A: 330, ASN A:324 & LYS A:320 weak vander wall interaction whereas quercetin binds covalently at LEU233 & PHE322 having Pi-sigma interaction at LEU 321, TYR394 & ARG 317 with conventional hydrogen bonding at LYS 320.The 2D binding interaction showed in fig. 6-7 & 3D binding interaction displayed in fig.8-11.

The pharmacokinetic profile of quercetin & naizirnin reveals that it is having good pharmacokinetic profile but with the presence of any major toxic effects including mutagenicity, tumorogenicity and reproductive effects. The pharmacokinetic and toxicity profiling results of ligands like quercetin and niazirinin were shown in Fig 12-13.

Table 2: Results of docking of ligands like quercetin and niazirinin against human GLS1 receptor

Sl. No.	Compound Name	Structure	Binding Energy (Kcal/mole)
1.	Quercetin		-4.8
2.	Niazirinin		-5.94

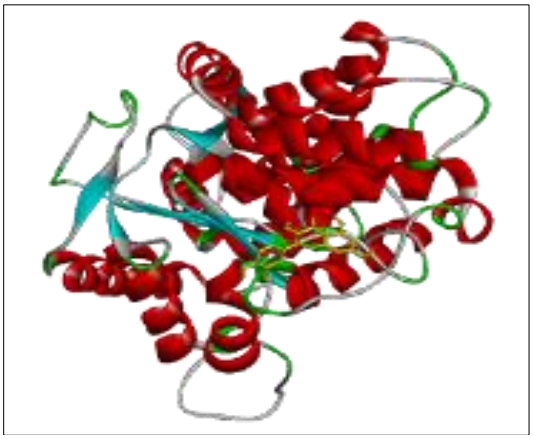


Fig 4: Binding mode of quercetin within the active site of human GLS1 receptor

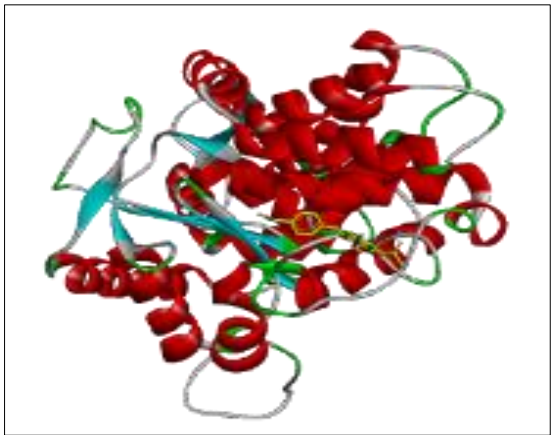


Fig 5: Binding mode of niazirinin within the active site of human GLS1 receptor

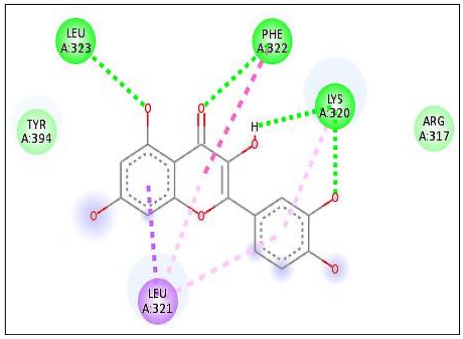


Fig 6: Two-dimensional binding mode of quercetin within the active site of human GLS1 receptor

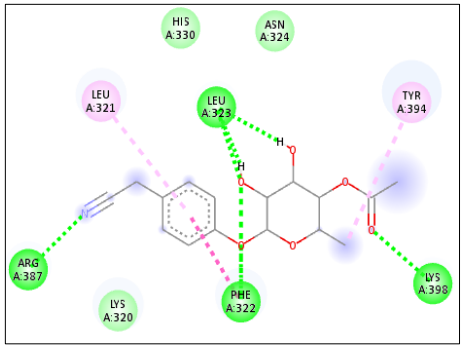


Fig 7: Two-dimensional binding mode of niazirinin within the active site of human GLS1 receptor

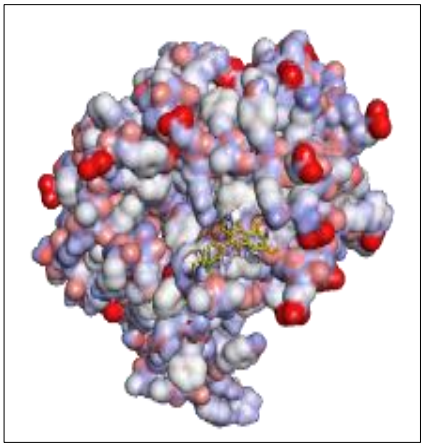


Fig 8: Three-dimensional binding conformation of quercetin within the active site of human GLS1 receptor

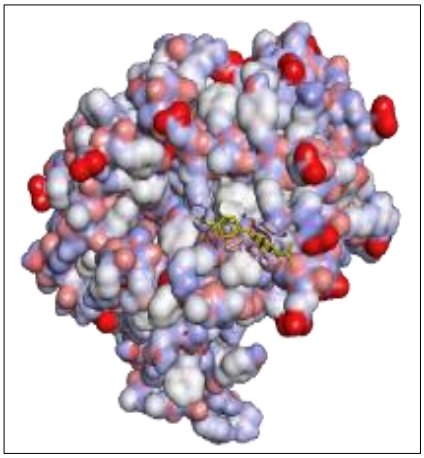


Fig 9: Three-dimensional binding conformation of niazirinin within the active site of human GLS1 receptor

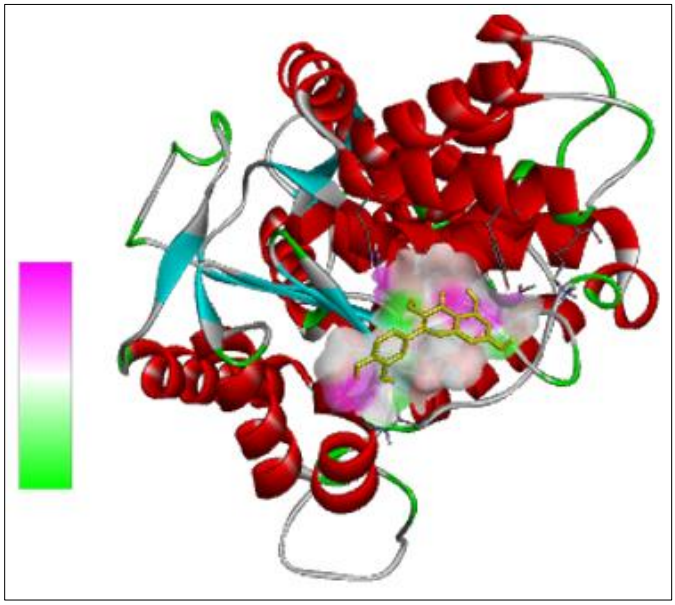


Fig 10: Three-dimensional binding mode of quercetin within the active site of human GLS1 receptor

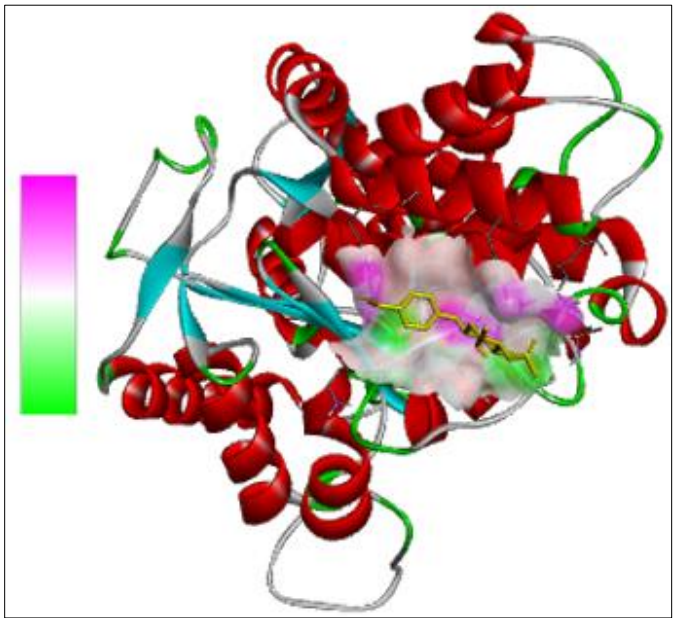


Fig 11: Three-dimensional binding mode of niazirinin within the active site of human GLS1 receptor

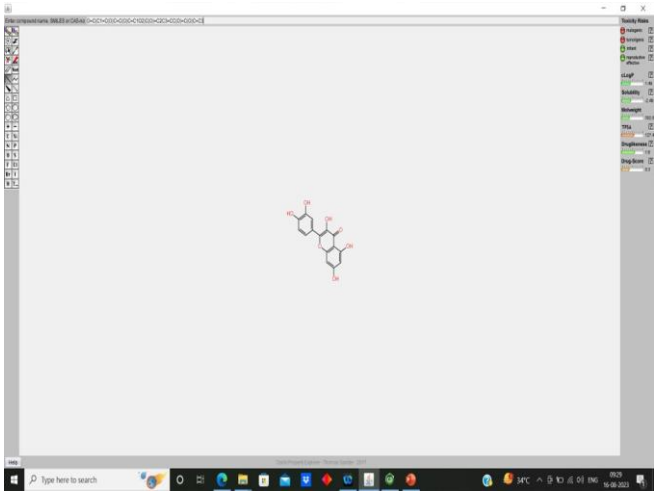


Fig 12: Pharmacokinetic and toxicity profiling of Quercetin

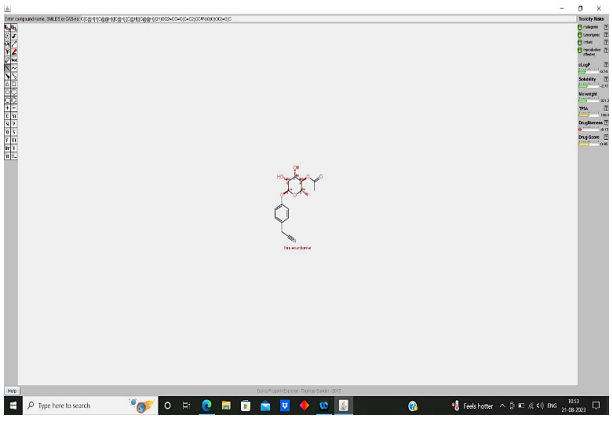
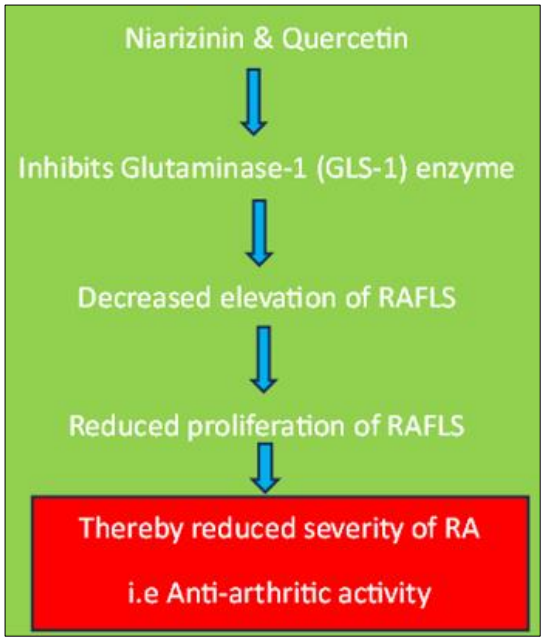


Fig 13: Pharmacokinetic and toxicity profiling of Niazirinin

Divulgence of Investigation

The findings of the present investigation showed that the ethanolic leaf extract of *M. olegifera* includes flavonoids and phenolic glycosides. Quercetin and niarizinin, which were chosen as the main chemicals for a computationally based prediction study to assess their efficacy in treating arthritis, were found in leaves, according to a literature review. According to the current study's findings, quercetin and niarizinin have an inhibitory effect on the GLS-1 protein.



Conclusion

The finding of the current suggested that quercetin and niazirinin has potent inhibitory action on GLS-1 protein and thereby reduced severity of RA.

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