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Research Article

Evaluation of Anti-gout Activity of Eugenol and Quercetin from Herbal Sources Using Computational and Analytical Approaches

Samiksha Kondhare, Kumudini Pawar*, Sneha Pawar, Madhuri Nalawade, Hrutuja Wagh, Meera Deshmukh, Pranati Tilak

Department of PharmacyTMV's LokmanyaTilak Institute of Pharmaceutical Sciences, Pune, India

Abstract

Gout is a metabolic disorder characterized by hyperuricemia, which results in the deposition of monosodium urate crystals in joints, leading to recurrent episodes of inflammation, pain, and progressive joint damage. Xanthine oxidase (XO), the key enzyme involved in the final steps of uric acid biosynthesis, is an important therapeutic target for gout management. Although conventional XO inhibitors such as allopurinol are effective, their long-term use may be associated with adverse effects, highlighting the need for safer, plant-derived alternatives. The present study aimed to investigate the antigout potential of the phytochemicals eugenol and quercetin through phytochemical characterization, molecular docking, and in silico pharmacokinetic analysis.Methanolic extracts of clove (Syzygiumaromaticum), bitter melon (Momordicacharantia), and betel leaf (Piper betle) were subjected to preliminary phytochemical screening to identify the presence of bioactive secondary metabolites. Thin Layer Chromatography (TLC) was performed to separate and identify the major phytochemical constituents, while Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) spectroscopy was employed to characterize functional groups and confirm the presence of eugenol and quercetin. Molecular docking studies were carried out using the SwissDock platform to evaluate the binding affinity and interaction patterns of these compounds with the xanthine oxidase enzyme. Furthermore, ADMETlab 2.0 was utilized to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles of the selected phytochemicals, thereby assessing their drug-likeness and safety.The molecular docking analysis demonstrated favorable binding interactions of both eugenol and quercetin with the active site of xanthine oxidase, indicating their potential inhibitory activity. ADMET predictions suggested acceptable pharmacokinetic properties and low toxicity, supporting their suitability as potential therapeutic candidates. Overall, the findings suggest that eugenol and quercetin possess promising antigout activity and may serve as natural xanthine oxidase inhibitors. This integrated experimental and computational approach provides a scientific basis for further in vitro and in vivo investigations to validate their efficacy and facilitate the development of safer, plant-based therapies for gout management.

Keywords

Gout, Clove, Bitter Melon, Betel Leaf, ADMET, Eugenol, Quercetin

*Correspondence: Kumudini Pawar (kumudiniphd30@gmail.com)

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1. Introduction

Gout is a common, painful form of inflammatory arthritis caused by the deposition of monosodium urate crystals in joints and surrounding tissues. It is one of the oldest diseases recorded in medical history and is strongly linked to hyperuricemia, or elevated blood uric acid. Clinically, gout is important not only because of sudden attacks of severe pain, swelling, redness, and reduce joint movement, but also because uncontrolled disease over time can lead to chronic joint damage, tophi, and kidney stones[1, 2].

The main biochemical problem in gout is abnormal purine metabolism. Purines, found in nucleic acids, adenosine triphosphate, and several coenzymes, are continuously synthesized and degraded in the body. During breakdown, adenine and guanine are converted to hypoxanthine, then to xanthine and finally to uric acid [1, 3].

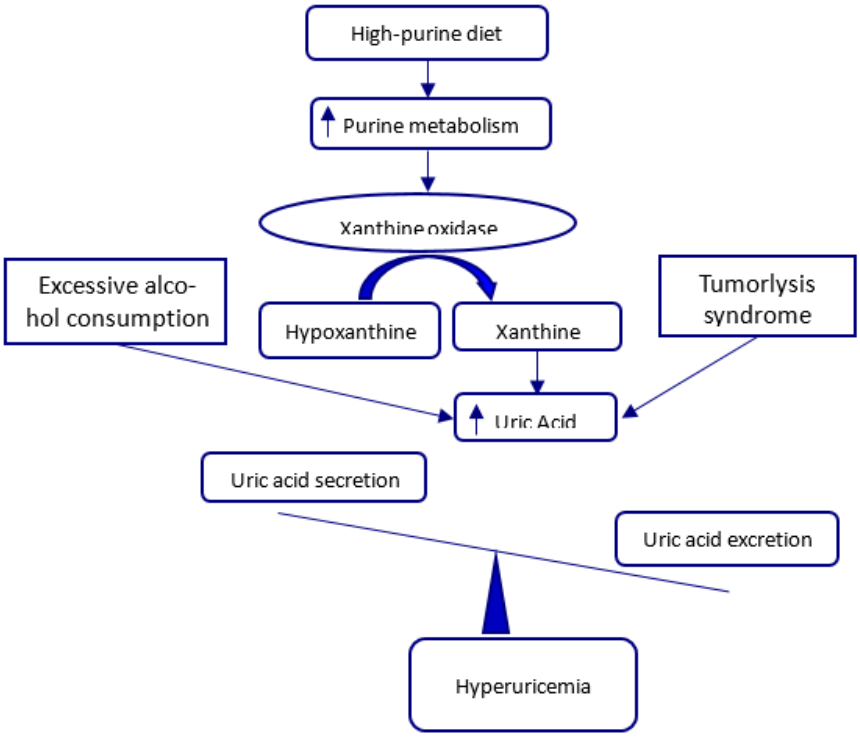

Figure 1. *Uric acid Cycle.*

The final steps of purine breakdown are controlled by xanthine oxidoreductase, which exists as xanthine dehydrogenase and xanthine oxidase. The xanthine oxidase form is especially relevant in gout because it increases uric acid production and also generates reactive oxygen species during the reaction. Higher xanthine oxidase activity therefore contributes to both hyperuricemia and oxidative stress.

Serum uric acid depends on the balance between production and excretion. When this balance is disturbed, uric acid exceeds its solubility and monosodium urate crystals precipitate in tissues. Crystals form more easily in cooler peripheral joint which is why the first metatarsophalangeal joint of the great toe is often affected first [1, 4].

Hyperuricemia may result from increased production due to high-purine diets, alcohol, fructose intake, obesity or rapid cell turnover, or from reduced renal excretion due to impaired kidney function or altered activity of urate transporters such as URAT1 (uratetranspoter 1 protein). Gout is therefore a multifactorial disease involving metabolism, kidney function, diet, genetics, and inflammation[4-6].

When urate crystals are deposited, innate immune cells like macrophages and neutrophils recognize them and activate the NLRP3 inflammasome (NLR family pyrin domain containing 3). This triggers release of IL-1β (interleukin-1 beta) and other mediators, producing the classic signs of an acute gout attack. Repeated episodes can lead to chronic gouty arthritis, joint destruction, and soft tissue tophi[7].

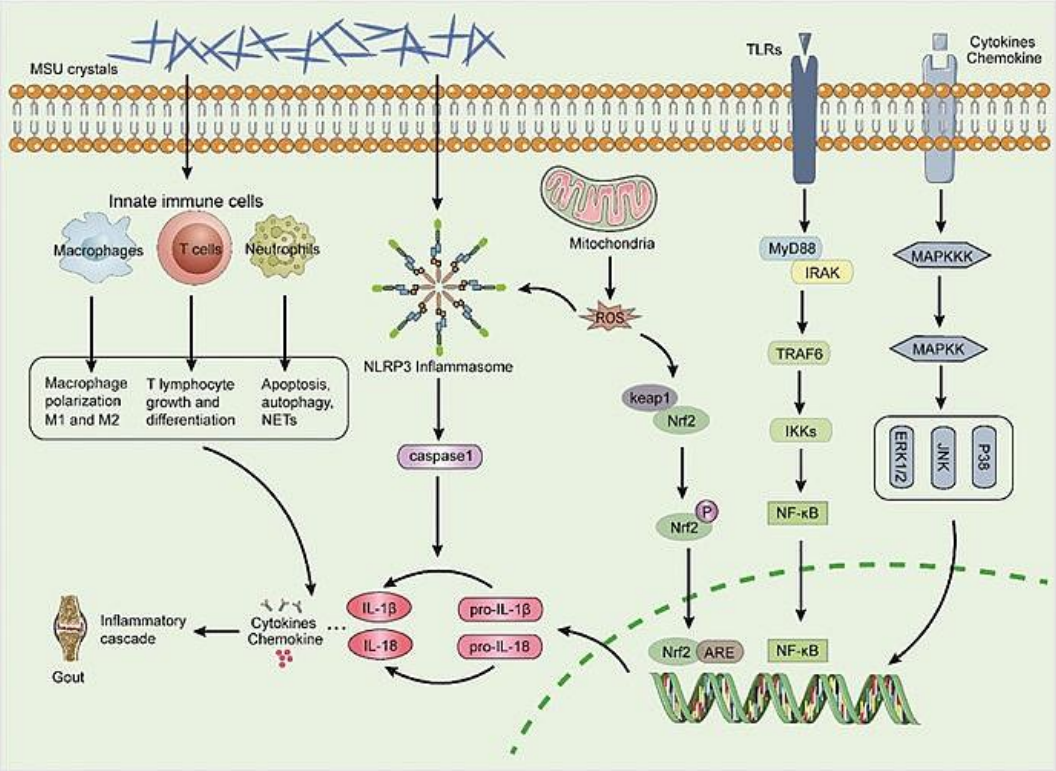

Figure 2.*Molecular Pathogenesis of Gout and NLRP3 InflammasomeSignaling[8].*

Herbal medicines treat gout through multiple mechanisms that target the underlying causes of the disease. They work by inhibiting xanthine oxidase, the enzyme responsible for producing uric acid in the body, which helps lower serum uric acid levels. Herbal compounds also act as uricosurics, increasing the excretion of uric acid through the kidneys. Additionally, they suppress inflammatory mediators like IL-6, TNF-α, and IL-1β that are release when monosodium urate crystals accumulate in joints, thereby reducing pain, swelling, and joint inflammation. The antioxidant properties of herbal medicines also helps neutralize free radicles and reduce oxidative stress associated with gout attacks [3, 9, 10].

Herbal medications have clear advantages over synthetic drugs for treating gout, mainly because they are safer and cause fewer side effects. Studies show that herbal treatments lower uric acid levels and reduce inflammation just as well as standard drugs like allopurinol, and NSAIDs but with far fewer adverse reactions [11, 12].

This better safety profile makes herbal drugs a good option for elderly patients and those with other health problems who cannot tolerate the toxicity of conventional medicines. Synthetic treatments can damage the stomach, trigger allergic reactions, and are not ideal for long-term use. Herbal medicines work well with fewer risks because their natural compounds act together in the body [11, 13].

Common herbal drugs include clove, bitter melon, and betel leaf, along with their active components eugenol and quercetin. Clove lowers uric acid and helps keep body functions normal in people with high uric acid. Bitter melon helps lower inflammation and pain, supports the kidneys in removing excess uric acid, and provides nutrients like iron, magnesium, potassium, and vitamin C. Betel leaf reduces uric acid and boosts antioxidant enzymes more effectively [14-17].

Because synthetic drugs cause unwanted side effects, natural products are becoming important sources of new gout treatment. Herbal medicines are cost-effective, easier to access, and better tolerated, making them strong alternative or addition for patients who need long-term gout management [9, 18].

Many herbs are used for gout, like celery seeds, turmeric, ginger, cinnamon, nettle, hibiscus, guggul, neem, and cherry. They help by blocking xanthine oxidase, lowering inflammation, or increasing uric acid removal. But most of the herbs are backed only by lab tests or animal studies, with very little human data. Also, many of them work in just one way, either by inhibiting xanthine oxidase or by reducing inflammation. Clove, bitter melon, and betel leaf look more promising for gout because they have several benefits together, which most other herbs do not [10, 13, 19, 20].

Clove has eugenol, which directly binds to the active site of xanthine oxidase, making it a more specific and stronger inhibitor than many other herbs. Bitter melon has quercetin that helps to lowers uric acid and inflammation. Betel leaf contains quercetin and high levels of phenols (eugenol), so it can block xanthine oxidase and also boost antioxidant enzymes. These there herbs also appear safe to use and do not carry the toxicity risks seen with some tradition gout remedies. Clove, bitter melon, and betel leaf work through multiple pathways, have

stronger scientific backing, show good potency, making them more reliable options for treating gout compared to other herbs [14, 15, 21, 22].

2. Materials and Method

2.1. Materials

2.1.1. Herbal Plants

Clove, Bitter melon, Betel leaf are commonly considered useful herbal materials for the management of gout because they may help reduce uric acid production and also decrease the inflammation associated with gout attacks [2].

2.1.2. Clove

Clove (Syzygiumaromaticum) is particularly important because it contains eugenol and several phenolic compounds known for their antioxidant and anti-inflammatory properties. In gout management, clove is believed to act mainly through inhibition of xanthine oxidase, the enzyme involved in converting purines into uric acid. By reducing the activity of this enzyme, clove may help decrease uric acid production and lower serum urate levels. Along with this, its anti-inflammatory effects may help reduce joint swelling and pain during acute gout attacks, while its antioxidant activity may protect tissues from oxidative stress and inflammatory damage [3, 14, 23].

2.1.3. Bitter Melon

Bitter melon (Momordicacharantia) has also been reported to possess potential antigout activity, although most evidence is still experimental and supportive in nature. The plant contains several phytochemicals (such as flavonoids) that are associated with antioxidant, anti-inflammatory, and uric acid lowering effects. Bitter melon helps manage gout in two ways, by reducing oxidative stress and inflammation, which can help relieve the severity of gout symptoms and by contributing to maintain uric acid balance by influencing metabolic processes and uric acid elimination [16, 17].

2.1.4. Betel Leaf

Betel leaf (Piper betel) has also shown promising antigout activity. It contains bioactive compounds such as phenolics and other secondary metabolites that inhibit xanthine oxidase activity and thereby reduce uric acid synthesis. Betel leaf possesses anti-inflammatory and antioxidant properties that help to reduce tissue damage and control inflammation in affected joints. Therefore, its beneficial effects are not only to lowering uric acid levels, but also help to reduce the pain, swelling, and inflammation caused by monosodium urate crystals deposition in gout [15].

Phytoconstituents that helps to treat Gout:

2.1.5. Eugenol

Eugenol helps with the swelling and stress to treat gout. Eugenol can lower the levels of things that cause inflammation. These things are called TNF-α, IL-1β, IL-6 and reactive oxygen species. Eugenol also stops some pathways that make inflammation worse. These pathways are called NF-κB, JNK and the NLRP3 inflammasome. They get triggered by urate crystals in the joints. Eugenol reduces the number of cells that gather in the joints. It lessens the swelling and prevents tissue damage in the joints [7, 21].

2.1.6. Quercetin

Quercetin is a plant flavonoid with anti-gout activity mainly through inhibition of xanthine oxidase, which reduce uric acid formation. It also shows anti-inflammatory and antioxidant actions in gout by reducing MSU-crystal induced pain, TNF-α, IL-1β, and inflammasome activation. Quercetin can lower plasma uric acid and can be useful in hyperuricemia [24].

2.1.7. Computational Tools

The molecular docking study was performed using the SwissDock web server, which applies an AutoDockVina-based algorithm for flexible ligand docking with protein targets. The protein structure was downloaded in PDB format prepared using PyMOL Molecular Viewer for visualization and structural modification.

The ligand molecule was obtained from PubChem in the form of SMILES (Simplified Molecular Input Line Entry System) notation and directly uploaded to SwissDock for ligand preparation. For the docking analysis, the required PDBQT files were generated using AutoDock Tools. This step involved assigning atomic charges, defining rotatable bonds, and setting the appropriate atom types necessary for the docking calculation [25].

2.1.8. ADMET Prediction

The ADMET properties, including absorption, distribution, metabolism, excretion, and toxicity of the selected drug compounds, were evaluated using the ADMETlab 2.0 web platform. ADMETlab 2.0 is a freely available online tool widely used for in-silico prediction of physicochemical, pharmacokinetics, and toxicological properties of small molecules. It provides comprehensive ADMET profiling that supports early-stage drug discovery and development studies [26].

For the analysis, the SMILES notation of each compound was obtained from PubChem and used as the input for prediction of ADMET parameters.

2.2. Methods

2.2.1. Extraction of Clove

For the extraction of clove, the maceration method was used. About 10g dried clove powder was taken in a clear glass

beaker, and 35ml of ethanol was added as the solvent. The beaker was covered properly with aluminium foil to minimize solvent loss through evaporation and kept undisturbed at room temperature for round 3-5 days. After the soaking period, the mixture was filtered to remove the solid plant material, and the filtrate was collected separately. The obtained filtrate was then placed on a war water bath maintained at 30-40 ℃ until the solvent evaporated completely; leaving behind the crude clove extract.

2.2.2. Extraction of Bitter Melon

The bitter melon extract was prepared using a conventional maceration technique. Fresh bitter melon fruits were cut into small pieces, dried (sun dried) completely, and ground into a uniform powder. About 10g of powdered material was placed into a 250ml flask, followed by addition of 100ml of ethanol. The mixture was mixed thoroughly and kept at room temperature for 24 hours to all extraction of the bioactive compounds.

Once the maceration method was completed, the mixture was filtered and the liquid filtrate was collected carefully. The filtrate was then heated on a water bath at 40-50 ℃ to evaporate the solvent. After the completion of evaporation, a concentrated crude extract of bitter melon was obtained [16, 17, 27].

2.2.3. Extraction of Betel Leaf

Fresh betel leaves were first washed thoroughly and dried (sun dried) until they became crisp. The dried leaves were then grind into a fine powder. 10g of this powder was transferred into a 250ml flask, and 100ml of ethanol was added as the extraction solvent. The mixture was shaken well and allowed to stand at room temperature for 24 hours for proper maceration.

After completion of the extraction period, the mixture was filtered using filter paper, and the filtrate was collected. The solvent present in the filtrate was evaporated gently on a water bath maintained at 40-50 ℃ to evaporate the solvent. This process resulted crude extract of betel leaf [17].

2.2.4. Phytochemical Screening

1) Alkaloids (Mayer's Test):

Mayer's test was performed for clove by adding 2ml of Mayer's reagent (mercuric iodide-potassium iodide solution) to 2ml of the extract. The formation of a creamy white precipitate immediately after addition of reagent is taken as a positive indication for the presence of alkaloids [28, 29].

2) Alkaloids (Wagner's Test):

Wagner's test was performed for bitter melon and betel leaf and involved mixing 2ml of extract with 2ml of Wagner's reagent (iodine-potassium iodide). A positive result is indicated by the appearance of a brownish to reddish-brown precipitate, produced by the formation of insoluble iodide complexes with alkaloids.

3) Phenolic (Ferric Chloride Test):

The ferric chloride test was carried out for clove, bitter melon, and betel leaf by adding three drops of 5% ferric chloride solution to 2ml of extract. Phenolic compounds give characteristics colour changes blue-black, green, or violet upon reaction with ferric ions. Such immediate colour development is used as a qualitative indicators of phenolic constitutes, including simple phenols and phenolic ethers [28, 29].

4) Flavonoids (Alkaline Test):

Fir the alkaline test 5% sodium hydroxide solution was added drop wise to 2ml of extract until a yellow colour developed; subsequent acidification with dilute acid (Dil. HCl) should decolorize the solution. This test was performed for clove and betel leaf. The appearance of an intense yellow colour that is reversible on acidification is considered positive for flavonoids.

5) Flavonoids (Shinoda Test):

The Shinoda test was performed for bitter melon by adding a small piece of magnesium followed by 5 drops of 5% hydrochloric acid to 2ml extract. Development of pink to red coloration indicates presence of flavonoids [28, 29].

6) Terpenoids/Steroids (Salkowski Test):

The Salkowski test was performed for clove, bitter melon, and betel leaf by mixing 2ml of extract with 2ml chloroform and carefully layering 3 drops of concentrated sulphuric acid. A reddish-brown colouration or a distinct reddish-brown layer at the interface is considered a positive reaction for terpenoids or steroids [29].

7) Tannins:

Tannins testing was done for clove, bitter melon and betel leaf by adding 3-5 drops of 5% ferric chloride to 2ml of extract. The appearance of a white precipitate with gentle or a dark blue/green to blue-black coloration or precipitate is taken positive indication for tannins.

2.2.5. Thin Layer Chromatography (TLC)

TLC analysis of clove, bitter melon, and betel leaf extracts was performed using pre-coated silica gel 60 plates to identify the possible presence of eugenol-related phenolic and quercetin-like flavonoids compounds. The same experimental procedure was applied to all three plant extracts to maintain consistency and allow comparison of the obtained results.

For the detection of eugenol, the primary mobile phase consisted of toluene: ethyl acetate: glacial acetic acid (8: 2: 0.1) was used. Approximately 7.9ml of toluene, 2ml of ethyl acetate, and 0.1ml of glacial acetic acid were mixed to prepare 10ml of solvent system.

For identification of quercetin, a different mobile phase composed of toluene: ethyl acetate: formic acid in the ratio of 5: 4: 1. To prepare 10ml of the solvent system, 5ml of toluene, 4ml of ethyl acetate, and 1ml of formic acid were mixed thoroughly [30, 31].

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR):

ATR-FTIR analysis of the three plant extracts (clove, bitter melon, and betel leaf) was carried out.

2.3. Molecular Docking

2.3.1. Protein Preparation

The three-dimensional structure of the target protein was obtained from the Protein Data Bank (PDB). The protein structure was opened in PyMOL Molecular Viewer to visualize the biomolecule and remove unwanted entities such as water molecules, cofactors not involved in binding, and alternate conformations, while retaining the chain(s) relevant for docking.

Hydrogen atoms were added to the protein structure to ensure correct protonation states at the working pH. The cleaned protein structure was saved in PDB format and used as the target for molecular docking in SwissDock.

2.3.2. Generation of PDBQT Files (AutoDock)

The protein PDB file prepared in PyMOL was imported into AutoDock Tools. Non-standard residues or missing atoms were checked and corrected, and Gasteiger partial charges were assigned to all atoms in the protein.

Rotatable bonds in the protein were defined, and the structure was saved in PDBQT format for the protein, preserving the correct atom types and charge models required.

2.3.3. Ligand Preparation

The structure of the small-molecule ligand was identified in PubChem by its compound name, and its SMILES (Simplified Molecular-Input Line-Entry System) notation was copied from PubChem.

The SMILES were submitted as the ligand input to the SwissDock server under the "Submit Ligand" option, where the platform automatically interprets the SMILES and generates 3D conformation of the ligand.

2.3.4. Docking on SwissDock

The SwissDock web server was accessed, and the AutoDockVina docking algorithm was selected for the study. The target protein was submitted by uploading the PDBQT file generated in previous steps.

The ligand was defined using the SMILES obtained from PubChem, and the "Prepare Ligand" option was selected to generate the ligand model. A search space was defined around the putative binding site. The centre and size of the grid box were set by clicking on a representative atom in the 3D viewer of the protein-ligand complex.

Docking parameters were selected for AutoDockVina, the search level was set to the default and the number of docking poses to generate was specified. The "Check parameters" button was used to validate the docking in out and to estimate the computation time; the job was launched only when the validation passed successfully.

2.3.5. Execution of Docking and Retrieval of Result

After validation, the Start Docking button was clicked to initiate the docking run on the SwissDock. An email address was provided to receive a notification upon completion of the calculation. Upon completion, the result was downloaded from the SwissDock result page. This contained the predicated docking poses, binding-site clustering, and estimated binding free energies for each pose.

2.4. ADMET Prediction

The ADMET profiles of the selected compounds were predicted using ADMETlab 2.0. Initially, the SMILES notation for each drug molecule was retrieved from PubChem by searching either the compound name. The obtained SMILES were then entered into the ADMET 2.0 input section. After submission, the platform generated results for several important ADMET-related parameters, including physicochemical characteristics, drug-likeness, absorption, distribution, and metabolism, excretion, and toxicity properties. The predicted ADMET data were downloaded and analysed to understand the pharmacokinetic behaviour and safety profile [32].

3. Result

3.1. Phytochemical Screening

3.1.1. Phytochemical Screening of Clove

Table 1. *Result of Phytochemical screening of Clove.*

TEST	EXPECTED RESULT	RESULT	EUGENOL	QUERCETIN
Alkaloids (Mayer's Test)	Creamy white precipitate	Pale Yellow colour	Negative	Negative
Phenols (Ferric Chloride Test)	Blue/green/violet or blue-black colour	Blue-black colour	Positive	Positive

TEST	EXPECTED RESULT	RESULT	EUGENOL	QUERCETIN
Flavonoids (Alkaline Test)	Intense yellow colour, decolorizes with acid	Colourless	Negative	Positive
Terpenoids/Steroid (Salkowski Test)	Reddish-brown precipitate or layer	Pale yellow	Negative	Negative
Tannins	Blue-black or greenish	Blue-Black	Positive (Weak)	Positive (Weak)

3.1.2. Phytochemical Screening of Bitter Melon

Table 2. Result of Phytochemical screening of Bitter Melon.

TEST	EXPECTED RESULT	RESULT	EUGENOL	QUERCETIN
Alkaloids (Wagner's Test)	Reddish-brown precipitate	Yellow colour	Negative	Negative
Flavonoids (Shinoda Test)	Pink/red colour	Pink colour	Negative	Positive
Phenols (Ferric Chloride Test)	Blue/green/violet or blue-black colour	Violet colour	Positive	Positive
Terpenoids/Steroid (Salkowski Test)	Reddish-brown precipitate or layer	Pale yellow	Negative	Negative
Tannins	Blue-black or greenish	Blue-Black	Positive (Weak)	Positive (Weak)

3.1.3. Phytochemical Screening of Betel Leaf

Table 3. Result of Phytochemical screening of Betel leaf.

TEST	EXPECTED RESULT	RESULT	EUGENOL	QUERCETIN
Alkaloids (Wagner's Test)	Reddish-brown precipitate	Yellow colour	Negative	Negative
Phenols (Ferric Chloride Test)	Blue/green/violet or blue-black colour	Violet colour	Positive	Positive
Tannins	Blue-black or greenish	Blue-Black	Positive (Weak)	Positive (Weak)
Flavonoids (Alkaline Test)	Intense yellow colour, decolorizes with acid	Colourless	Negative	Positive
Terpenoids/Steroid (Salkowski Test)	Reddish-brown precipitate or layer	Pale yellow	Negative	Negative

3.2. Thin Layer Chromatography (TLC)

Table 4. Result of TLC.

SAMPLES	COMPONENTS	Rf VALUE
(A) Clove	Eugenol	0.7
	Quercetin	0.7
(B) Bitter Melon	Eugenol	0.8
	Quercetin	0.7

SAMPLES	COMPONENTS	Rf VALUE
(C) Betel Leaf	Eugenol	0.7
	Quercetin	0.3

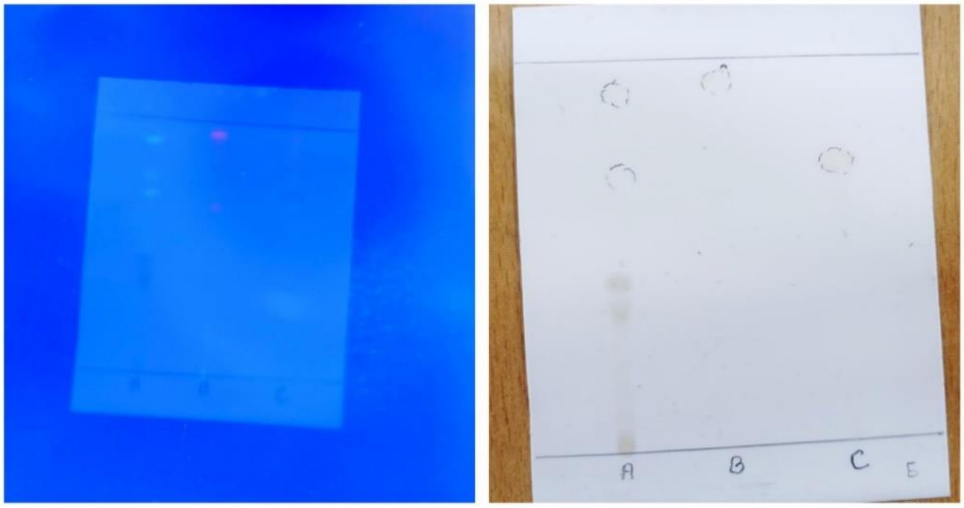

Figure 3. *TLC of Eugenol.*

Figure 4. *TLC of Quercetin.*

The TLC profile of the selected plant extracts indicate efficient separation and suggest the presence of eugenol and quercetin. According to literature survey, Rf values used as reference for comparison. For Eugenol, Rf value was approximately 0.6-0.7 has been reported. For QuercetinRf value was reported in the range of 0.5-0.7. Since the observed Rf values are very close to the literature values, it supports the tentative identification of Eugenol and Quercetin in the tested extracts.

3.3. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

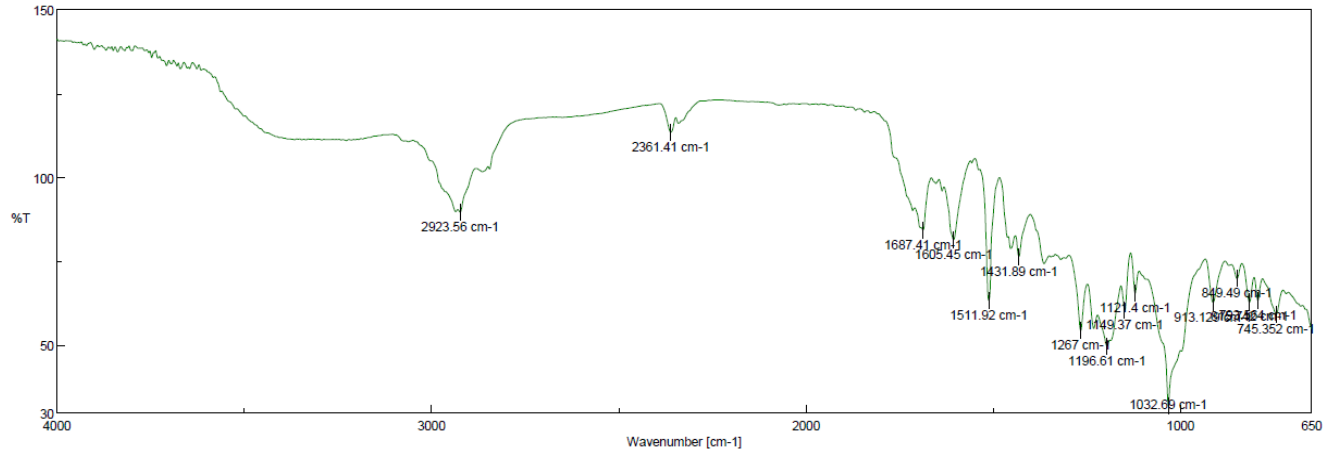

Figure 5. *ATR-FTIR Result of CLOVE.*

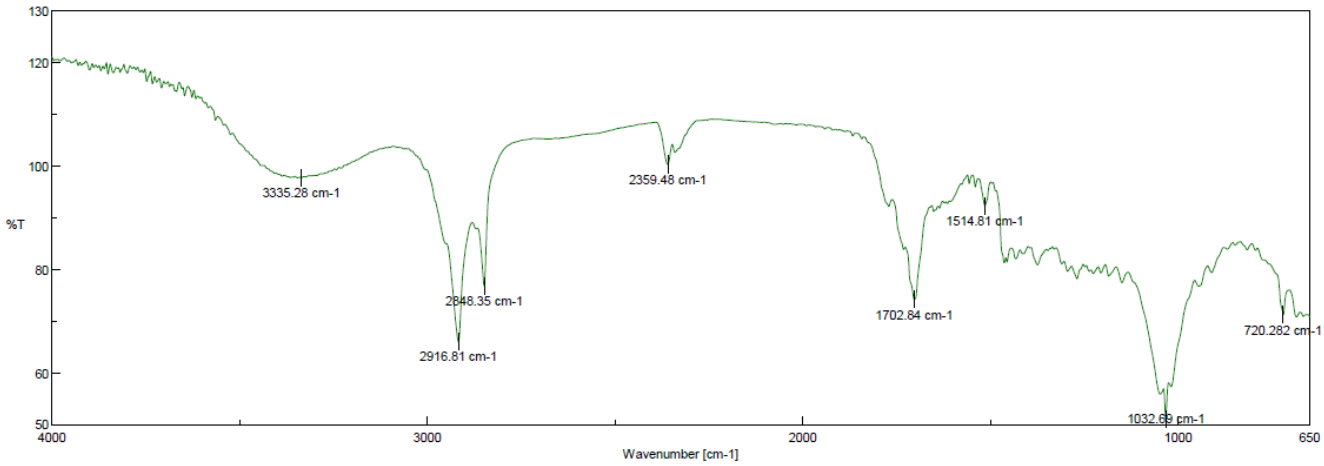

Figure 6. *ATR-FRTIR Result of Bitter Melon.*

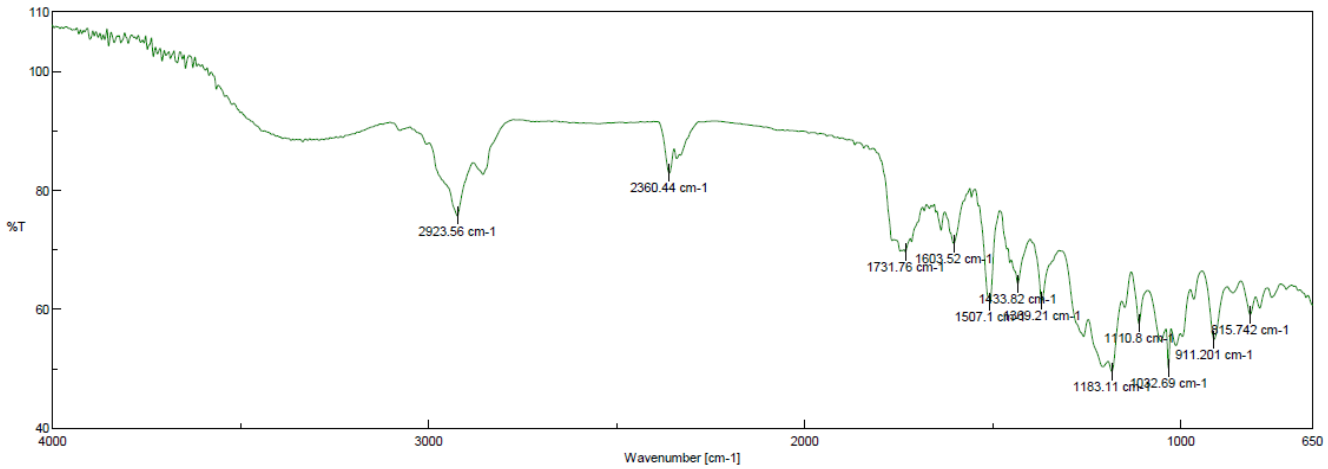

Figure 7. *ATR-FTIR Result of Betel Leaf.*

Table 5. *Peaks and Functional Group of Clove, bitter melon and betel leaf.*

Plant Extract	Characteristics Peaks	Characteristics Functional Groups
Clove	2923.56	C-H Stretching
	1687.41	C=O Stretching
	1605.45, 1511.92	Aromatic C=C Stretching
	1267	C-O Stretching
	1196.61-1032.69	C-O and C-N Stretching
Bitter Melon	3335.28	O-H Stretching
	2916.81, 2848.35	Aliphatic C-H Stretching
	1702.84	C=O Stretching
	1514.81	Aromatic C=C Stretching
	1032.69	C-O Stretching
	720.28	C-H Bending
Betel Leaf	2923.56	Aliphatic C-H Stretching
	1731.76	C=O Stretching
	1603.52, 1507.10	Aromatic C=C Stretching
	1433.82	CH_2 Bending
	1369.21	C-H Bending
	911.20, 815.74	Aromatic C-H Bending

3.4. Molecular Docking

3.4.1. Molecular Docking of Eugenol

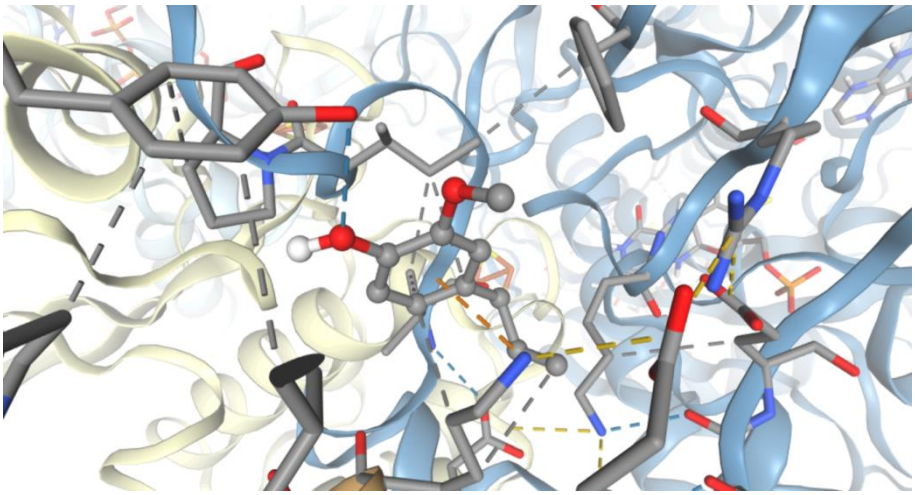

Figure 8. *Molecular Docking of Eugenol (without protein surface).*

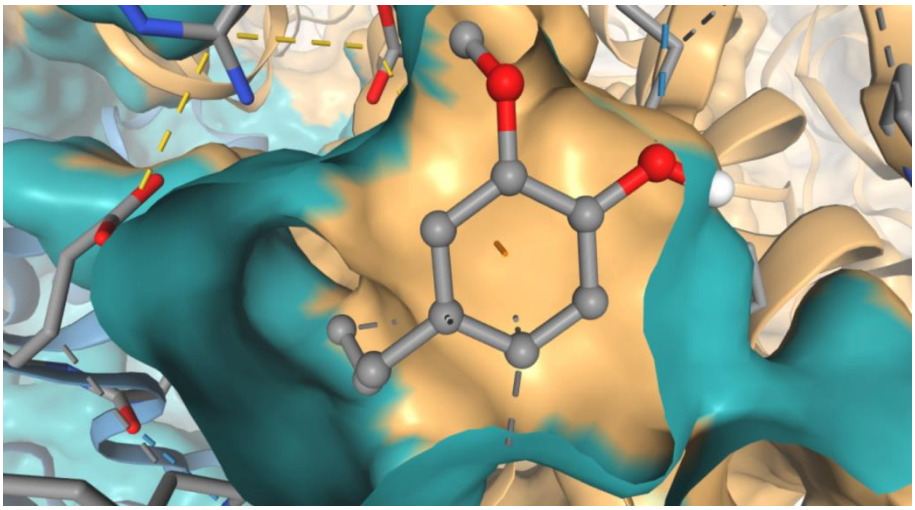

Figure 9. *Molecular Docking of Eugenol (with protein surface).*

Table 6. *Calculated Affinity of Eugenol.*

MODEL	CALCULATED AFFINITY (kcal/mol)
1	-6.018
2	-5.957
3	-5.861
4	-5.668
5	-5.554
6	-5.497
7	-5.449
8	-5.445
9	-5.440
10	-5.306
11	-5.297
12	-5.227
13	-5.160
14	-5.061
15	-4.801
16	-4.639
17	-4.565
18	-4.451
19	-4.376

A total of 19 docking poses (models) were generated. The calculated binding affinities ranged from −6.018 to −4.376 kcal/mol. The best docking pose was Model 1, with the lowest binding energy of −6.018 kcal/mol, indicating the strongest predicted binding among all generated conformations.

3.4.2. Molecular Docking of Quercetin

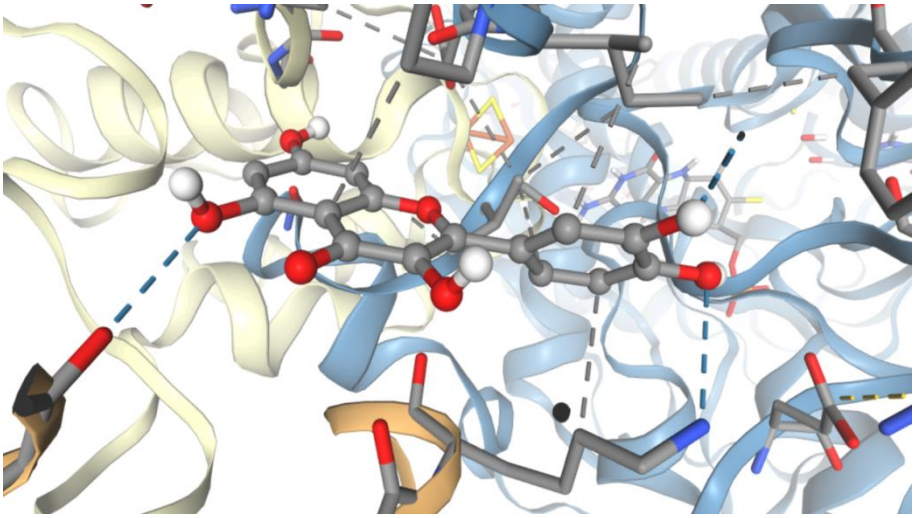

Figure 10. *Molecular Docking of Quercetin (without protein surface).*

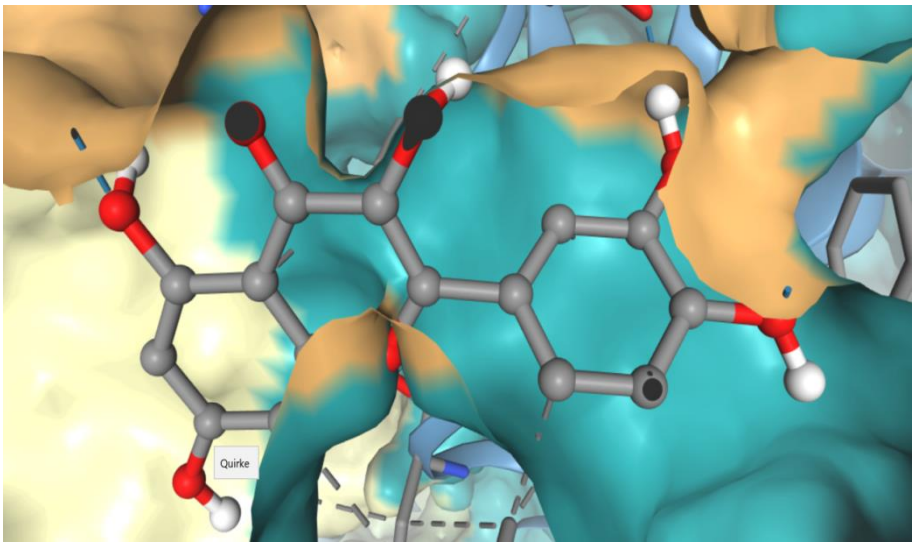

Figure 11. *Molecular Docking of Quercetin (with protein surface).*

Table 7. *Calculated Affinity of Quercetin.*

MODEL	CALCULATED AFFINITY (kcal/mol)
1	-7.992
2	-7.439
3	-6.779
4	-6.710
5	-5.802
6	-5.689
7	-5.534
8	-5.004

A total of 8 docking poses (models) were generated for Quercetin. The calculated binding affinities ranged from −7.992 to −5.004 kcal/mol. The most favourable binding pose was Model 1, which exhibited the lowest binding energy of −7.992 kcal/mol, indicating the strongest predicted interaction between quercetin and the target protein.

The remaining docking poses showed binding affinities of −5.689, −5.534, and −5.004 kcal/mol, indicating comparatively weaker but still favourable interactions. Overall, the docking results demonstrate that quercetin exhibits good predicted binding affinity toward the target protein, with Model 1 representing the most stable and energetically favourable binding conformation. This model is therefore recommended for further analyses, including protein-ligand interaction mapping, hydrogen bond analysis, and binding site characterization.

It can be stated that Quercetin demonstrates good predicted binding affinity, with Model 1 selected as the optimal docking pose for subsequent structural and interaction analyses.

3.5. ADMET Prediction

3.5.1. Physiological Property

Table 8. Physiological Property of Eugenol and Quercetin.

PROPEERTY	EUGENOL	QUERCETIN
Molecular Weight	164.08	302.04
nHA	2	7
nHD	1	5
nROT	3	1
TPSA	29.46	131.36
logS	-2.286	-3.671
logP	2.291	2.155
logD	2.418	1.767

Both Eugenol and Quercetin have good drug-like properties. Eugenol has a low molecular weight, while quercetin is a bit heavier, both with an acceptable size range. The hydrogen bonds are formed well and have the right level of polarity, which helps them interact with biological targets. Their logP and logD values has good balance between fat and water solubility, likely to pass through cell membranes.

3.5.2. Medicinal Chemistry

Table 9. Medicinal Chemistry of Eugenol and Quercetin.

PROPERTY	EUGENOL	QUERCETIN
QED	0.693	0.434
SA score	1.961	2.545
NP score	1.053	1.701
Lipinski Rule	Accepted	Accepted

Eugenol and Quercetin pass the Lipinski rule which means they are good for working as oral drugs. Both have reasonable synthetic accessibility. Eugenol has higher QED scale and is more drug-like compared to Quercetin.

3.5.3. Absorption

Table 10. Absorption of Eugenol and Quercetin.

PROPERTY	EUGENOL	QUERCERTIN
Caco-2 Permeability	-4.373	-5.204
HIA	0.007	0.014
F20%	0.732	0.93
F30%	0.967	0.997

Eugenol showed good permeability in Caco-2 and Quercetin was predicted to have even higher absorption in the human intestine. Both have strong bioavailability scores for F20% and F30%, which means they are likely to get into bloodstream.

3.5.4. Distribution

Table 11. Distribution of Eugenol and Quercetin.

PROPERTY	EUGENOL	QUERCETIN
PPB	92.11%	95.49%
VD	0.833	0.579
BBB Penetration	0.188	0.008
Fu	3.220%	7.423%

Both the compounds (Eugenol and Quercetin) bind well to plasma protein. The predicted volume tells that the compounds are able to spread into tissues. The compounds also have low predicted penetration of the blood-brain barrier.

3.5.5. Metabolism

Table 12. Metabolism of Eugenol and Quercetin.

PROPERTY	EUGENOL	QUERCETIN
CYP1A2 inhibitor	0.901	0.943
CYP2C19 inhibitor	0.716	0.053
CYP2C9 inhibitor	0.313	0.598
CYP2D6 inhibitor	0.85	0.411
CYP3A4 inhibitor	0.288	0.348

The metabolism prediction indicated that the Eugenol and Quercetin exhibit favourable metabolic characteristics. Both compounds shows acceptable interaction with metabolic parameters.

3.5.6. Excretion

Table 13. Excretion of Eugenol and Quercetin.

PROPERTY	EUGENOL	QUERCETIN
CL	14.042	8.284
T1/2	0.887	0.929

Both the compounds clear at acceptable rates and have reasonable half-lives. Eugenol was predicted to clear as compared to Quercetin. The half-life estimation for both compounds indicates sufficient systemic exposure for therapeutic effect.

3.5.7. Toxicity

Table 14. Toxicity of Eugenol and Quercetin.

PROPERTY	EUGENOL	QUERCETIN
hERG Blockers	0.017	0.099
H-HT	0.036	0.1
DILI	0.046	0.98
AMES Toxicity	0.066	0.657
Skin Sensitization	0.792	0.919
Carcinogencity	0.814	0.05
Respiratory Toxicity	0.51	0.072

Eugenol and Quercetin have clean safety profiles and low toxicity risk. Eugenol was predicted to have lower risk of hERG inhibition, drug-induced live injury (DILI), and AMES toxicity, while Quercetin showed a lower risk of carcinogenicity and respiratory toxicity.

4. Conclusion

The findings of this study indicate that eugenol and quercetin, two bioactive phytochemicals derived from medicinal plants, possess significant potential as natural anti-gout agents. Phytochemical screening confirmed the presence of phenolic compounds and flavonoids in the extracts of clove, bitter melon, and betel leaf, highlighting their rich phytochemical composition. Further characterization by Thin Layer Chromatography (TLC) and Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy verified the presence of eugenol and quercetin through their characteristic chromatographic profiles and functional groups.

Molecular docking analysis demonstrated that both compounds interact favourably with the selected gout-related target protein, suggesting their potential inhibitory activity. Among the two, quercetin exhibited a stronger binding affinity (−7.992 kcal/mol) than eugenol (−6.018 kcal/mol), indicating a more stable protein-ligand complex and greater therapeutic potential. These results support the ability of both phytochemicals to bind effectively to the target protein involved in gout pathogenesis.

In addition, ADMET analysis predicted favourable pharmacokinetic properties for both compounds, including good absorption, satisfactory drug-likeness, and an acceptable safety profile with low toxicity risks. These findings suggest that eugenol and quercetin possess characteristics desirable for the development of plant-based therapeutic agents.

Overall, the combined experimental and computational findings demonstrate that eugenol and quercetin are promising natural compounds for gout management. While both compounds showed encouraging anti-gout potential, quercetin exhibited superior binding affinity, making it a particularly promising lead candidate for further investigation. Future studies involving in vitro enzyme inhibition assays, in vivo

efficacy studies, and clinical evaluations are necessary to confirm their therapeutic effectiveness and establish their potential for use in the treatment of gout.

Abbreviations

NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
ADMET	Adsorption, Distribution, Metabolism, Excretion and Toxicity

Author Contributions

Samiksha Kondhare: Conceptualization,Investigation, Resources, Writing – original draft

Kumudini Pawar: Data curation,Formal Analysis, Methodology, Writing – review & editing

Sneha Pawar: Software, Validation

Madhuri Nalawade: FormalAnalysis, Methodology

Hrutuja Wagh: Software, Validation

Meera Deshmukh: Project administration

Pranati Tilak: Project administration

Conflicts of Interest

The authors declare no conflicts of interest.

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