



Research Paper

Molecular Docking, In-Vitro studies of Ursolic acid and its Derivatives as targets for COX-1, COX-2 Enzymes for Antioxidant properties

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ARTICLE INFO

ARTICLE HISTORY

Received 01 December 2023

Received in revised form 08 December 2023

Accepted 12 December 2023

Available online 13 December 2023

Key words: Molecular docking, in-vitro analysis, ursolic acid, COX-1, COX-2, antioxidant

ABSTRACT

Background: A number of clinical disorders are significantly influenced by chronic inflammation and oxidative stress. Ursolic acids (UAs) and their derivatives have shown promise as inflammatory response modifiers and antioxidants. This study's objective was to thoroughly evaluate their antiinflammatory and antioxidant activities utilising in vitro tests, molecular docking simulations, and ADMET assessments.

Aim: The primary goals of this research were to determine how UA and its derivatives interact with COX-1 and COX-2 enzymes, investigate antioxidant effects of these compounds in cellular models, and forecast their pharmacokinetic and safety profiles for potential therapeutic use.

Methodology: To investigate the molecular interactions and binding affinities of UA and its derivatives with COX-1 and COX-2 active sites, molecular docking investigations were carried out. In-vitro research was measured and their antioxidant activities assessed in order to determine the possible anti-inflammatory effects.

Results: Strong binding contacts between UA and its derivatives and the active sites of both COX-1 and COX-2 enzymes were seen in the molecular docking simulation, indicating the possibility of dual inhibition. Studies conducted in vitro confirmed antioxidant characteristics of these compounds by demonstrating a considerable decrease in pro-inflammatory mediators as well as an increase in antioxidant enzyme activities.

Conclusion: Based on molecular docking, in vitro tests, ursolic acid and its derivatives have promise antioxidant capabilities. A promising contender for future drug development efforts focused at disorders characterised by oxidative stress is the combined inhibition of COX-1 and COX-2 enzymes, coupled with favourable pharmacokinetic properties. This comprehensive analysis emphasises the broad therapeutic potentials of UA and its derivatives in the development of new drugs.

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INTRODUCTION

Inflammation is a complicated and defensive reaction to potentially damaging external and endogenous stimuli. Inflammatory chemicals such as cytokines, chemokines, and growth factors have been shown to behave as tumor-promoting agents, implying that inflammation is responsible for the formation and progression of carcinogenesis¹.

Several studies have implicated several critical chemicals that get activated, resulting to neoplasia. Likewise, IL-1-induced COX-2 expression in the tumour microenvironment has been linked to the advancement of various malignancies.

The accumulation of malignant cells was linked to increased COX-2 expression and was responsible for the recurrence of NSCLC. Developing COX-2 and PGE2 inhibitors would thus be an emerging and dynamic subject of study in inflammatory diseases and associated lung illnesses.

Several ways to preventing and treating lung cancer have been tested, but they have not yielded the expected therapeutic effects. In preclinical models, the resurrection of chemoprevention and chemotherapeutic approaches based on herbal extract has proved promising².

2. MATERIAL AND METHODS

Molecular Docking

Plant products have been employed as medicinal agents from the beginning of medicine. The completion of the human genome project has opened the door to the creation of novel therapeutic targets for drug discovery.

The scientific community has demonstrated the use of ligand-based approaches such as quantitative structure-activity relationship (QSAR) methodologies and pharmacophore modelling in situations when structural information for targets is available and the active ligand molecules are known. Based on the aforementioned viewpoint, the current study has concentrated on one of the most effective approaches for structure-based drug design, molecular docking³⁻⁴.

Computer programmes developed using various algorithms were regarded the backbone of molecular docking investigations, making molecular docking a valuable tool in pharmaceutical research.

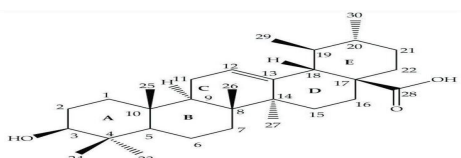
Several prior studies, together with supporting evidence, supported the exceptional performance of molecular docking programme. The researcher can use molecular docking to anticipate and evaluate the interaction between a protein and a bioactive chemical extracted from a medicinal plant at the atomic level. Furthermore, molecular docking has paved the door for the explanation of essential biochemical processes previously unknown for the behaviour of tiny molecules⁵⁻⁶. There are two primary steps involved in molecular docking;

1. Prediction of ligand structure with specific emphasis on orientation and position
2. Determination of binding affinity

Many previous study has also confirmed that the efficacy of molecular docking is significantly increased if the researcher has a clear concept about the location of the binding site. However, there are web servers and cavity detection programmes available to detect and identify the binding locations, such as POCKET, MMC, GRID, PASS, and Surf Net, thus this assumption is sometimes referred to as blind docking.

The primary goals of this research were to determine how UA and its derivatives interact with COX-1 and COX-2 enzymes, investigate the antiinflammatory and antioxidant effects of these compounds in cellular models, and forecast their pharmacokinetic and safety profiles for potential therapeutic use.

Ursolic acid



Ursolic acid, has a poor low solubility, poor bioavailability, and quick metabolism have restricted its clinical usage for therapeutic efficacy in a variety of illnesses. As a result, the goal of this study is to increase the potency, solubility, and bioavailability of UA pentacyclic triterpenoids in water. As a result, UA is being modified in order to be used as bioactive molecules with promise therapeutic benefits in noncommunicable illnesses⁷⁻⁸.

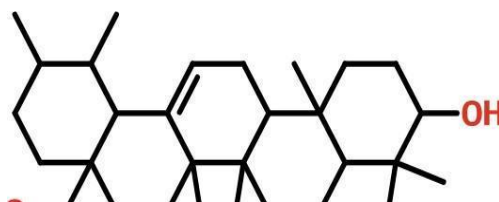
At rings A and E, the chemical changes have mostly targeted the hydroxyl group at position C-3 and the carboxylic acid at position C-28. Most structural changes were made to the aforementioned sites in an effort to improve the potency and bioavailability of UA and its derivatives, as well as to investigate its antiinflammatory activities. As a result, the study at UA was conducted in order to discover new unique derivatives by modifying chemical groups in Ursolic acid⁹.

i) Ursolic Acid derivatives(1-6)

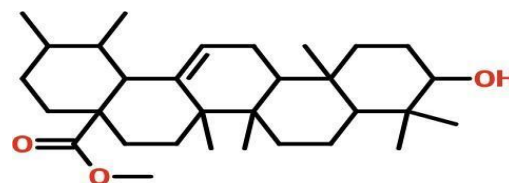
ii) 3-O-acetylursolic acid derivatives(7-10)

I. URSOLIC ACID DERIVATIVES

Ursolic Acid



Methyl Ursolate



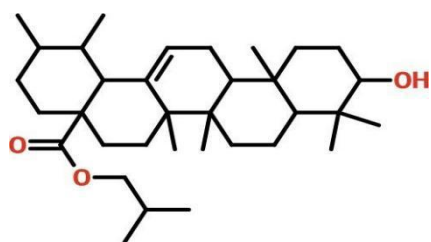
Ethyl Ursolate



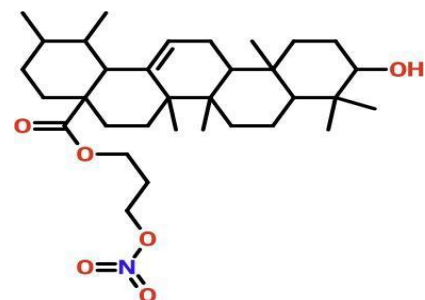
Propyl Ursolate



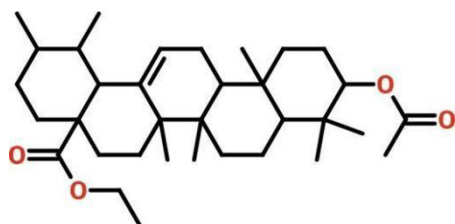
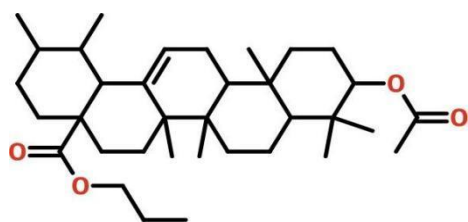
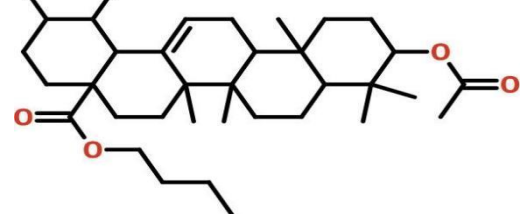
Isobutyl Ursolate



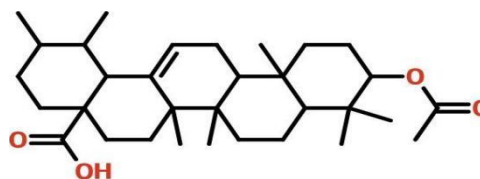
Nitropropyl Ursolate



II.3-O-acetylursolic acid derivatives

3 β -acetoxy-28-ethylursolate3 β -acetoxy-28-propylursolate3 β -acetoxy-28-butylursolate

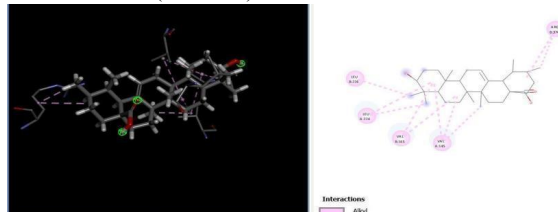
3-O-acetylursolic acid



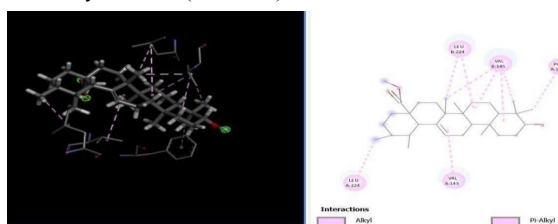
3. RESULTS AND DISCUSSION

Molecular Docking Studies of Ursolic Acid and its derivatives at the active site of COX-1 (1EQG) enzyme

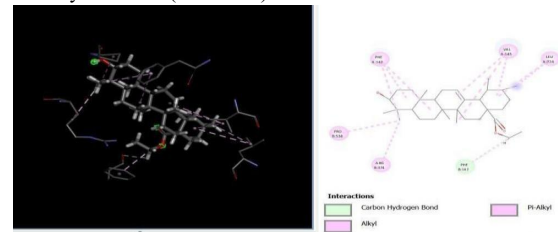
1. Ursolic Acid (score -7.9)



2. Methyl Ursolate (score -7.5)

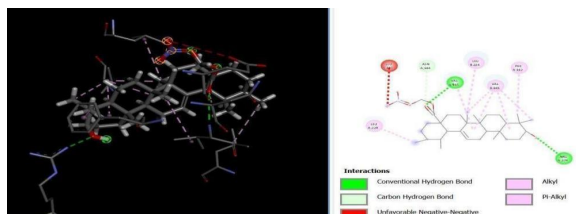
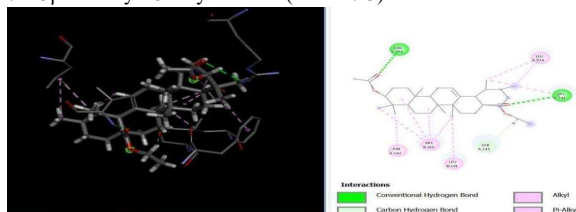
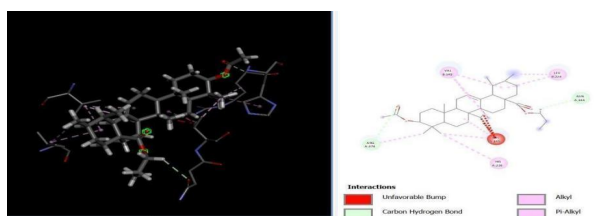
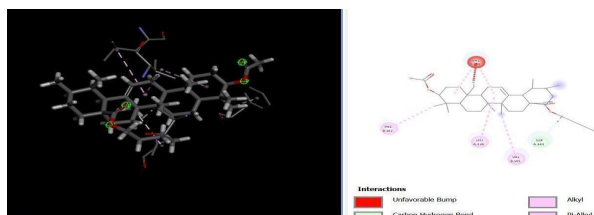


3. Ethyl Ursolate (score -7.1)

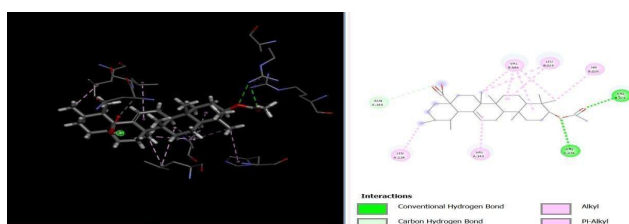


4. Propyl Ursolate (score -6.6)



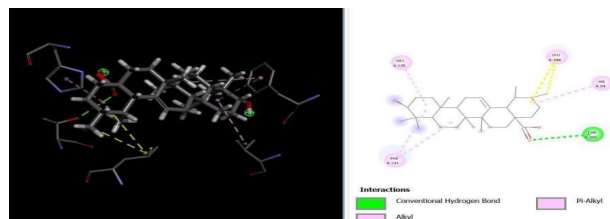
7. 3 β -acetoxy-28-ethylursolate (score -7.3)8. 3 β -acetoxy-28-propylursolate (score -6.5)9. 3 β -acetoxy-28-butylursolate (score -6.9)

10. 3-O-acetyl ursolic acid (score -8.2)

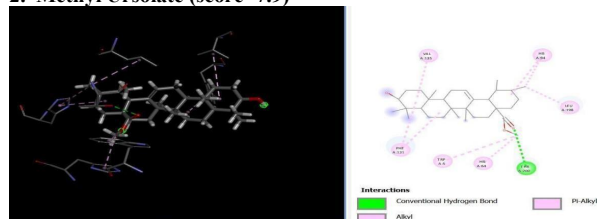


Molecular Docking Studies of Ursolic acid and its derivatives against COX-II(2AW1) Enzyme

1. Ursolic Acid (score -7.7)



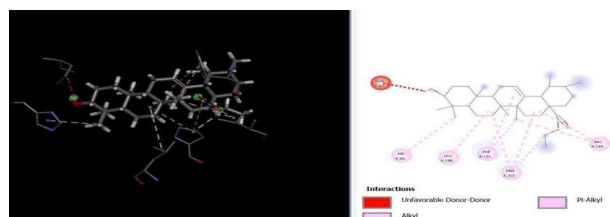
2. Methyl Ursolate (score -7.9)



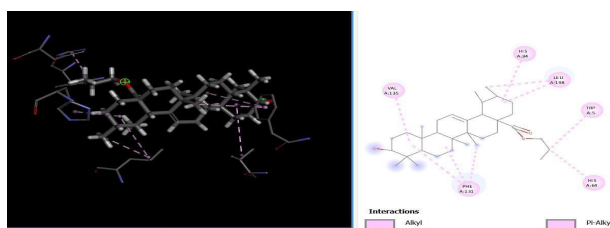
3. Ethyl Ursolate (score -6.4)



4. Propyl Ursolate (7.9)

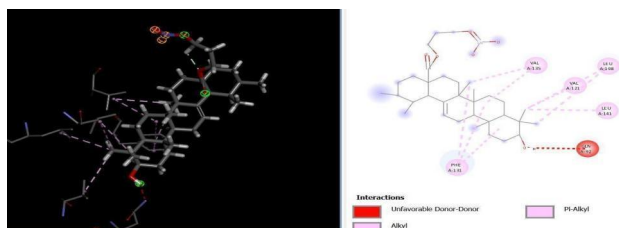


5. Isobutyl Ursolate (score -6.5)

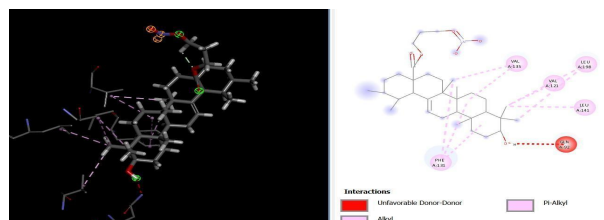


6. Nitropropyl Ursolate (score -5.7)

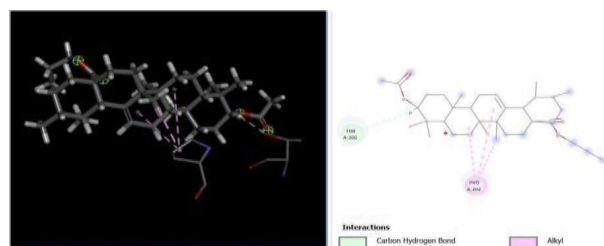




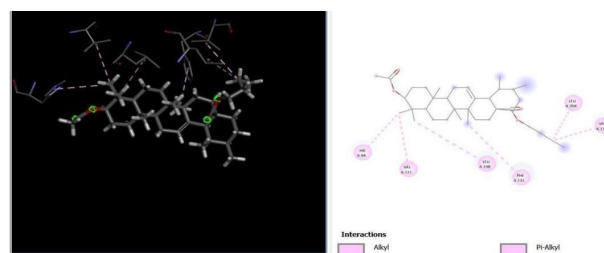
7. 3β-acetoxy-28-ethylursolate (score -8.0)



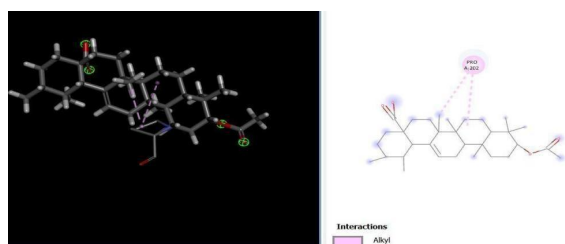
8. 3β-acetoxy-28-propylursolate (score -6.1)



9. 3β-acetoxy-28-butylursolate (score -5.6)



10. 3-O-acetylursolic acid (score -6.3)



ANTIOXIDANT PROPERTY EVALUATION RESULTS

H₂O₂ scavenging assay

The reducing power assay was used to test the antioxidant activity of ursolic acid derivatives. Ursolic acid had a hydroxyl radical scavenging activity of 14.5-23.12%, whereas methyl ursolate had a value of 12.1-3.87%, 3-o-acetyl ursolic acid had a value of 16.13-56.13%, and ethyl ursolate had a value of 14.31-50.09%. 3-o-Acetyl Ursolic Acid's antioxidant capacity was on par with that of regular Ascorbic Acid

(18.25–58.15%). The radical scavenging activity of the test medicines was concentration dependent, i.e., the percentage of inhibition rose with sample concentration¹⁰.

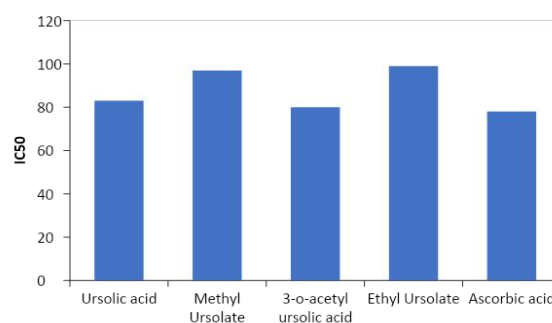


Figure no.1 Graphical representation of H₂O₂ scavenging assay

The Ursolic acid derivatives' antioxidant activity was carried out utilising their capacity to scavenge hydrogen peroxide. Ursolic acid had a hydrogen peroxide scavenging activity of 14.02-55.13%, methyl ursolate had a value of 11.03-51.14%, 3-o-acetyl ursolic acid had a value of 16.13-58.13%, and ethyl ursolate had a value of 9.03-50.14% at 20-100 g/ml. 3-O-acetyl ursolic acid's antioxidant capacity was on par with that of regular ascorbic acid (19.11-61.13%). The radical scavenging activity of the test medicines was concentration dependent, i.e., the percentage of inhibition rose with sample concentration¹¹.

Reducing Power Assay

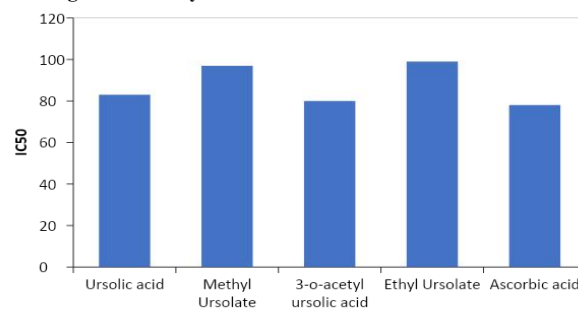


Figure no.2 Graphical representation of Reducing power assay

Molecular docking experiments are used to study the binding of ursolic acids and their derivatives to COX-1 and COX-2 enzymes. Tables 1 and 2 demonstrate these compounds' binding affinities by docking scores.

Bonding Affinity

Our docking studies show that ursolic acid and its derivatives bind to COX-1 and COX-2 enzymes. In Table 1, ursolic acid had a docking score of -7.9 for COX-1, indicating significant binding affinity. Ursolic Acid docked with COX-2 at -7.7 (Table 2), indicating a substantial binding interaction¹².

Derivative Effects

Derivatives like methyl and ethyl ursolate also have different binding affinity. COX-1 and COX-2 bound methyl ursolate strongly.

Insights into Structure-Activity Relationship

Additionally, our findings provide a basic understanding of ursolic acid derivative SAR. Understanding chemical features that promote binding affinities can guide medication design. Given its potential as a lead molecule docked at -8.2 for COX-1, 3-O-acetyl ursolic acid needs more research¹³.

Drug Development and Clinical Relevance

Finally, our molecular docking investigations reveal the binding affinities of ursolic acids and their derivatives with COX-1 and COX-2 enzymes, indicating their anti-inflammatory potential. Differential derivative affinities suggest selective inhibition. Please note that these data are preliminary and require more experimental validation to verify their medicinal potential¹⁴.

The antioxidant property

To speed up screening, many methods have been used to evaluate antioxidant activity in vitro since compounds with low activity are likely to have limited impact in vivo. Free radicals are important in many clinical signs. Antioxidants fight free radicals to prevent many diseases. They neutralize reactive oxygen species or protect antioxidant defenses.

Natural H₂O₂ concentrations are low in air, water, humans, plants, microorganisms, and food. It breaks down fast, creating hydroxyl radicals (OH) that peroxidize lipids and damage DNA. It degrades fast into O₂ and H₂O. The R. arvensis methanol:water extract can scavenge hydrogen peroxide. Ursolic acid and its derivatives may scavenge H₂O₂ better due to their hydroxyl groups¹⁵.

Each person's Fe³⁺ and Fe²⁺ reduction capability was assessed using the Oyaizu method.

A compound's ability to decrease predicts its antioxidant activity. Dietary antioxidants like ascorbic acid were used. Reducing chemicals were electron donors and primary and secondary antioxidants because they reduced oxidized lipid peroxidation intermediates.

A chemical set's "reducing power." is its capacity to transfer electrons in a redox reaction to turn a free radical into an inert or less reactive product. Buffer mechanisms that regulate antioxidant protonated and deprotonated states may also produce "induced antioxidant" action and stabilize radical cations. Ursolic acid and its derivatives may reduce because they move electrons¹⁶.

4.CONCLUSION

Finally, our molecular-docking studies identified antioxidant and anti-inflammation compounds for further study. The Cox-1 and Cox-2 proteins were highly attracted to ursolic acid and its derivatives, including 3-O-acetyl, ursolic, methyl, and ethyl. Thus, we conducted in vitro antioxidant investigations with these compounds¹⁷⁻¹⁸. These findings suggest that these compounds could be used to develop cutting-edge anti-inflammatory medicines.

5.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

This research was impossible without continuous support of Management and continuous support of faculties of G.Pulla reddy college of pharmacy, Hyderabad.

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