Int. J. Pharm. Investigation, 2024; 14(2):399-408.
https://www.jpionline.org

Original Article

Molecular Docking Studies on Phytoconstituent Isolated from *Nyctanthes arbortristis* Linn.

Devyani Rajput, Dharmendra Jain, Sushil Kumar Kashaw, Umesh Kumar Patil*

Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar, Madhya Pradesh, INDIA.

ABSTRACT

Background: *Nyctanthes arbortristis* Linn., popularly referred to as Parijat or Night-flowering Jasmine, has garnered significant attention in traditional medicinal practices owing to its wide-ranging array of phytochemical constituents. The focus of this study lies in the molecular docking investigations conducted on a specific phytoconstituent isolated from this plant. **Materials and Methods:** The phytoconstituent was extracted from *Nyctanthes arbortristis* Linn. through rigorous isolation and purification techniques. Its chemical structure and purity were characterized using state-of-the-art spectroscopic and analytical methods, including UV, ^{1}H NMR, IR, and HPLC. The molecular docking analysis of phytoconstituent with (target) proteins was carried out using Schrodinger (V5.0). **Results and Discussion:** Crocetin, a carotenoid compound, was derived from the ethanolic extract of *Nyctanthes arbortristis* Linn. seeds. In the molecular docking analysis, the binding scores of the compound with the binding sites of PDB ID: 2FGI, 1ERE, 3K23 and 2GHM were -7.956, -8.246, -7.530, and -5.625 kcal/mol, respectively. **Conclusion:** The isolated chemical, crocetin, has the potential to be an effective anticancer agent, according to computational analyses.

Keywords: *Nyctanthes arbortristis* L., Parijat, Crocetin, Docking, HPLC.

Correspondence:
Umesh Kumar Patil
Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar-470003, Madhya Pradesh, INDIA
Email: umeshpatil29@gmail.com

Received: 18-10-2023;
Revised: 27-11-2023;
Accepted: 19-12-2023.

INTRODUCTION

Nyctanthes arbortristis, also known as coral jasmine or night jasmine, is a plant that has gained attention for its therapeutic properties and medicinal applications. In traditional medicine, it has found application in the management of diverse conditions, including but not limited to sciatica, arthritis, intermittent fevers, inflammation, malaria, viral infections, and leishmaniasis.[1] The plant harbors phytoconstituents possessing pharmacological properties, encompassing iridoid glucosides and other compounds that have demonstrated anticancer, anti-inflammatory, anti-allergic, immunomodulatory, and antiviral characteristics. Additionally, *Nyctanthes arbortristis* has been found to have anti-bacterial and anti-fungal characteristics. Research has also explored the use of *Nyctanthes arbortristis* as a natural pigment for dyeing textile fibers, showing promising results in terms of color yield, fastness properties, and functional properties of the dyed fabric. Overall, *Nyctanthes arbortristis* is a plant with diverse therapeutic potential and further research is needed to explore its full range of benefits.[2]

Crocetin, a natural carotenoid compound found in various plant sources, has garnered significant attention due to its potential therapeutic properties. From these potential sources, the seeds of *Nyctanthes arbortristis*, a plant celebrated for its cultural and medicinal importance in South Asia, emerge as a particularly auspicious storehouse of crocetin.[3] Numerous pharmacological effects of crocetin have been demonstrated, including antioxidant, anti-inflammatory, and anticancer characteristics. Its molecular structure, characterized by a conjugated polyene system and functional groups, makes it an intriguing candidate for drug development.[4]

The isolation of crocetin from *Nyctanthes arbortristis* seeds presents an exciting avenue for harnessing the bioactive potential of this compound. This endeavor aligns with the broader context of exploring natural compounds as potential therapeutic agents, considering the increasing demand for safer and more effective medicines.[5]

In this research paper, we embark on a comprehensive study that combines the isolation of crocetin from *Nyctanthes arbortristis* seeds along with computational molecular docking study. This dual approach aims to shed light on the Efficiency of isolation process and to explore the molecular interactions of crocetin with specific biological targets. This research holds significant promise for both pharmaceutical development and the understanding of the pharmacological mechanisms of crocetin. The elucidation of

DOI: 10.5530/ijpi.14.2.50

crocetin's molecular interactions and its isolation from *Nyctanthes arbortristis* seeds may open new avenues for drug discovery and contribute to the broader field of natural product-based therapeutics.

MATERIALS AND METHODS

Plant material

Seeds of *Nyctanthes arbortristis* Linn. were procured from the local region of Deori, located in Sagar, Madhya Pradesh, India, and subjected to authentication at the Department of Botany, Dr. Harisingh Gour Vishwavidyalaya, Sagar, M.P. A voucher specimen (No. BOT/H/02/45/01) of the plant seeds has been meticulously preserved in the Herbal Drug Project laboratory within the Department of Pharmaceutical Sciences at Dr. Harisingh Gour Vishwavidyalaya, Sagar M.P. This specimen serves as a valuable point of reference for future research endeavors.

Extraction and isolation of Crocetin

The seeds of *N. arbortristis* were initially cleansed by rinsing them under a continuous flow of tap water and subsequently air-dried. These dried seeds were then subjected to maceration with ethanol for approximately 24 hr at room temperature. The resulting extract, displaying a dark brown color, was obtained by filtering the solid residue (marc). The extract was further processed by evaporating it using a rotary flash evaporator and subsequently drying it under vacuum conditions, ensuring that the temperature did not exceed 50°C.[6]

To isolate crocetin from the dried NAT extract, 10 g of the extract were suspended in a solution comprising 50 mL of distilled water and 10 mL of concentrated hydrochloric acid. The resulting suspension was subjected to reflux for duration of three hr over a water bath and subsequently allowed to cool. Following this, the mixture was partitioned into approximately 50 mL of diethyl ether. The diethyl ether layer was carefully separated and then treated with a saturated aqueous solution of sodium bicarbonate to saturation.[7] Following this process, the sodium bicarbonate layer was separated, and concentrated hydrochloric acid was incrementally introduced until the pH exhibited acidity, as confirmed by litmus paper. The addition of hydrochloric acid resulted in the formation of an orange-colored precipitate, which was subsequently filtered and then dissolved in diethyl ether. The solvent (ether) was allowed to evaporate at room temperature, yielding solid crocetin. For additional purification, crocetin was subjected to a column chromatography technique, utilizing a 1:1:0.1 hexane-ethyl acetate system with the inclusion of methanol.[8]

Purification and characterization of active compound

The structural confirmation of the extracted crocetin was accomplished by scrutinizing its UV, IR, and ^{1}H-NMR spectral data, while its purity was quantified employing High-Performance Liquid Chromatography (HPLC). HPLC analysis was carried out using an Agilent 1260 system, which was coupled with mass spectrometry (API-4000, AB SCIEX). Separation was executed using an Atlantis dC18 column (2.1×150 mm, 3 µm, Agilent), and the column oven was consistently maintained at 40°C. The mobile phase used in the analysis comprised a mixture of water (A) and acetonitrile (B).[9]

To prepare stock solutions of crocetin, a concentration of 1 mg/mL was meticulously dissolved in methanol and preserved at an ultra-low temperature of -80°C. Subsequently, a linear calibration curve was meticulously constructed, covering a concentration range from 10 to 10,000 ng/mL for crocetin.[10]

The gradient conditions for the analysis of crocetin were as follows: initially, mobile phase B was set at 10% for 0.5 min, after which it was ramped up to 65% over 1 min and held at that level for an additional minute. Subsequently, there was a progressive increase to 75% of mobile phase B over the next 1.5 min, followed by a swift elevation to 99% within 0.5 min. The mobile phase was maintained at 99% for an additional 3.5 min before reverting to the initial 10% within 1 min. To ensure column pressure equilibration, a min period at 10% was observed at the end of the analysis. Throughout this process, the flow rate remained constant at 0.25 mL/min, and each injection for analysis utilized a fixed volume of 7.5 µL.[11]

Docking Study using Glide Module of Schrödinger Software

Glide, which stands for Grid-based Ligand Docking with Energetics, is a computational approach designed to identify favorable interactions between a receptor molecule, often a protein, and one or more ligand molecules. It's important to clarify that each ligand under consideration should be a single, distinct molecule, while the receptor can encompass various components, including a protein and a cofactor. Glide offers two distinct docking options: rigid and flexible. In flexible docking, the algorithm takes care of generating a range of conformations for each input ligand. This flexibility allows for the exploration of different positions, orientations, and conformations of the ligand concerning the receptor, collectively referred to as a ligand pose. The algorithm employs a series of hierarchical filters to evaluate interactions between the ligand and the receptor. These filters employ a grid-based methodology and the empirical ChemScore function to assess the spatial compatibility of the ligand with the designated active site and to analyze the complementarity of ligand-receptor interactions. These techniques enable the exploration and assessment of potential binding interactions, contributing to our understanding of molecular docking.[12] Poses that pass the initial screening undergo the final phase of the algorithm. In this stage, the algorithm utilizes an OPLS 3e model-based grid approximation to calculate and minimize the

nonbonded interaction energy between the ligand and receptor. Positions with the lowest energy levels are prioritized, aiming for energetically favorable configurations. By default, the scoring of poses is carried out using the GlideScore multi-ligand scoring method developed by Schrödinger. This final phase in the Glide docking process plays a crucial role in evaluating and ranking the potential binding interactions, contributing to our understanding of ligand-receptor interactions based on energetics and structural fit.[13] The poses of each ligand are subsequently organized based on a composite model score. This score encompasses not only the nonbonded contact energy but also, in flexible docking scenarios, the internal energy of the generated ligand conformation, along with the GlideScore. In summary, Glide employs a sophisticated methodology to assess and order ligand poses based on energetics, interaction compatibility, and spatial congruence. The approach amalgamates energy minimization, empirical scoring functions, and grid-based filters to identify and prioritize the most favorable ligand-receptor interactions.[14]

Protein preparation

The precision of Glide results is directly linked to the quality of the initial protein structures. Schrödinger provides the Protein Preparation Wizard, a comprehensive protein preparation tool designed to ensure chemical accuracy and enhance protein structures for effective interaction with Glide and related products. LigPrep, also developed by Schrödinger, functions as a complementary ligand preparation tool, offering analogous capabilities. It is highly advisable to employ these tools for the preparation of both protein and ligand structures to achieve the best possible outcomes. For incorporating a ligand/protein co-crystallized structure into Maestro, the import can be facilitated from the Protein Data Bank (PDB).[15] To enhance computational efficiency in Glide, particularly for multimeric complexes, it is advisable to retain a single ligand-receptor subunit. Nonetheless, if the active site requires the presence of two identical chains, it is imperative to retain both of them. Decisions regarding the retention or removal of water molecules are crucial. Waters are typically eliminated, with the exception of those correlated to metals.[16] Waters connecting the ligand and protein may be retained based on specific considerations. Adjustments to cofactors, metal ions, and the protein structure are necessary. Repairs are warranted for structures lacking residues in proximity to the active site. Notably, the establishment of bonds between the ligand or a cofactor and a metal within complex protein structures demands meticulous handling of formal charges and the ordering of ligand bonds. It's essential to exercise caution during the process of protein structure minimization. This step, regulated by a user-specified RMSD (Root-Mean-Square Deviation) tolerance, guarantees constrained minimization in relation to the initial protein coordinates. Ultimately, a comprehensive evaluation of the resultant structures is of paramount importance. This assessment should encompass verifying the accurate orientation of water molecules, resolving any steric conflicts, and addressing potential hydrogen-bonding issues to uphold the structural integrity and credibility of the prepared systems.[17]

Ligand preparation

The fidelity of docked structures is pivotal for producing accurate results that mirror authentic ligand configurations within protein-ligand complexes. Schrödinger's LigPrep is adept at generating top-notch, all-atom 3D structures for a diverse array of drug-like compounds, provided with initial 2D or 3D structures in SDF formats. The LigPrep protocol consists of a sequence of operations aimed at data transformation, structural correction, incorporation of structural variations, elimination of superfluous structures, and fine-tuning molecular conformations. Many of these steps are optional and can be tailored through command-line arguments or preferences set in the LigPrep panel.[18] The sequential steps include: 1. Convert Structure Format: Convert input structures into a compatible format. 2. Select Structures: Choose relevant structures for processing. 3. Add Hydrogen Atoms: Introduce hydrogen atoms to achieve appropriate protonation states. 4. Remove Unwanted Molecules: Eliminate undesired molecular entities. 5. Neutralize Charged Groups: Neutralize charged functional groups. 6. Generate Ionization States: Derive ionization states for the molecules. 7. Generate Tautomer: Generate tautomeric forms for flexibility. 8. Filter Structures: Apply filters to refine the selection of structures. 9. Generate Alternative Chirality: Introduce alternative chirality where applicable. 10. Generate Low-Energy Ring Conformations: Generate energetically favourable ring conformations. 11. Remove Problematic Structures: Eliminate structures causing computational issues. 12. Optimize Geometries: Conduct geometric optimization for structural refinement. 13. Convert Output File: Convert the final output file into the desired format.[19]

It is important to note that the flexibility of LigPrep allows users to tailor these steps based on specific requirements, ensuring the creation of accurate and realistic ligand structures for subsequent docking simulations.

Receptor Grid Generation

The docking process involves the utilization of several sets of fields to represent the shape and characteristics of the receptor within a grid, facilitating the accurate scoring of ligand poses. The creation and configuration of this receptor grid are pivotal to the entire procedure, and this is effectively achieved through the receptor grid generation panel. Before initiating the actual ligand docking, it is imperative to create receptor grids, and this process mandates the presence of a "prepared" structure. This structure must be an all-atom representation, with correct bond ordering and formal charges. The grid generation relies on the OPLS 2005 force field, which is well-equipped with a wide variety of predefined atom types and is especially adept at managing the precise treatment of metal components within the receptor.

The receptor grid creation panel is a comprehensive tool with five distinct tabs, each serving a unique function in specifying options for the generation of the receptor grid. These tabs are aptly named: receptor, site, constraints, rotatable groups, and excluded volumes. They collectively allow for the fine-tuning of parameters essential for creating an accurate and effective receptor grid for the subsequent ligand docking process.[20]

Receptor Tab

This tab is the starting point where the user defines the precise section within the Workspace system where receptor grids should be calculated. It offers parameters for customization, including adjusting van der Waals radii for receptor atoms and the choice of using partial charges from either the force field or the input structure. This step is crucial in ensuring that the grid is accurately aligned with the receptor.

Site Tab

Moving on, the Site Tab is pivotal in configuring and preparing scoring grids based on the structure found in the workspace. It plays a fundamental role in defining the regions where ligand binding will be assessed, setting the stage for the subsequent docking simulations.

Constraints Tab

This tab serves as a tool for defining Glide constraints during the receptor grid generation. These constraints are based on structural or pharmacological insights, specifying which receptor-ligand interactions are essential for the binding mode. The inclusion of constraints streamlines the docking process by excluding ligands, conformations, or poses that do not meet the predefined criteria for successful docking.

Rotatable Groups Tab

Within this tab, the flexibility of certain functional groups in residues such as Ser, Thr, Tyr, and Cys is addressed. These groups can adopt various orientations in the presence of different ligands. Glide accommodates this variability, allowing them to optimize their orientation during ligand docking, ultimately leading to more favorable interaction outcomes.

Excluded Volumes Tab

The Excluded Volumes Tab provides a means of specifying conditions that restrict ligands from occupying specific spatial regions during docking. For example, if it is known that ligands do not bind in a particular pocket near the active site, this tab can be employed to prevent ligands from entering that region. It grants the ability to exclude ligands from designated spatial areas throughout the docking process, further refining the accuracy of the simulations.

Ligand docking

For the successful execution of a Glide ligand docking task, a well-defined combination of receptor grids and one or more ligand structures forms the foundation. It's essential that the correct Lewis structure for each ligand is determinable, as ligands failing this criterion are automatically excluded from the docking process. Moreover, Glide possesses an in-built mechanism to filter out ligands that contain unparametrized components, such as tin, or those consisting of atom types incompatible with the OPLS force field, including explicit lone pair "atoms." The Ligand Docking panel encompasses a series of tabs, each serving distinct functions: "ligands," "settings," "core," "constraints," "torsional constraints," and "output." These tabs collectively provide a structured interface to configure, fine-tune, and monitor the ligand docking simulation.[21] It's worth emphasizing that in cases where a ligand cannot produce a valid Lewis structure or contains elements incompatible with the force fields, Glide will systematically exclude it from the docking process. Molecular modeling studies using the Glide module from Schrödinger were performed to investigate potential interactions between the most potent derivative and the target protein.[22]

Docking study

The molecular docking investigations featuring Crocetin were carried out using the receptor proteins of four distinct targets, namely the Fibroblast growth factor receptor, Oestrogen receptor, Glucocorticoids receptor, and p38 MAP Kinase. The receptor structures for these proteins were referenced using the respective PDB IDs, which were 2FGI, 1ERE, 3K23, and 2GHM. For conducting these docking studies, the Glide module software integrated within Schrödinger Maestro version 13.5 was utilized. To initiate this process, the protein structures were retrieved from the Protein Data Bank. Subsequently, these protein structures underwent a refinement phase through the "protein preparation workflow" within Maestro wizard version 13.5, ensuring their suitability for the docking investigations. This comprehensive approach allowed for an in-depth exploration of the potential interactions between Crocetin and the selected protein targets, shedding light on the compound's therapeutic prospects across this different receptors.[23] The molecular docking study followed a structured workflow designed to enhance the protein structure. Several optimization steps were included in this process, such as generating different states, performing refinement procedures, and implementing constrained minimization. The optimization focused on factors like hydrogen-bonded groups and dehydration, utilizing the default force field OPLS 3e to ensure the accuracy of the protein structure. The refined protein structure was then utilized to create a grid surrounding the ligand molecule, which facilitated the docking simulations. These simulations unveiled a variety of ligand conformations, each accompanied by distinct binding energy scores. To assess their potential, the conformations were ranked according to these scores, with higher rankings

indicative of more favorable binding affinities. This systematic ranking approach aided in the identification and prioritization of ligand poses based on their binding strengths.[24-27]

RESULTS

Structure elucidation of compound

Crocetin was obtained in the form of an orange-red powdered substance. Analysis of its UV-Vis spectral data revealed distinctive absorption bands (Figure 2A) within the range of 261 to 432 nm, which corresponds to ester bonds and all-trans carotenoids, respectively. The FT-IR analysis displayed characteristic bands at specific wavelengths: 2921, 1463, and 1376 cm^{-1} were indicative of C-H bonds, while 2923, 2853 cm^{-1}, and 1704 cm^{-1} were associated with carbonyl groups (C=O). Furthermore, bands at 1660, 1277, and 1578 cm^{-1} indicated the presence of C=C groups, and a band at 1168 cm^{-1} denoted Trans C=C groups (Figure 2B).

The ^1H NMR spectrum of the compound, observed in a DMSO solution, revealed distinct signals confirming the presence of terminal methyl groups, methylene groups, and methyl groups attached to double bonds. These signals included 2.045=d, J=5.5 Hz (12H) (representing 4 CH_3 groups located at positions 6 and 6' in Figures 1 and 2C), 2.309-S (6H) (indicative of 2*CH_3 groups) (located at positions 2 and 2' in Figures 1 and 2C), 7.210-S (2H) (representing 2 CH_3 groups associated with the OCH_3 group in Figures 1 and 2C), and 7.554-7.540=d, J=7.0 Hz (6H) (representing 6*CH groups located at positions 4, 4', and 6 in Figures 1 and 2C). Additionally, a signal at 7.898-S (2H) (indicating 2*H groups) was observed (located at positions 3 and 3' in Figures 1 and 2C).

Based on this comprehensive analysis, the molecule (as depicted in Figure 1) is confirmed to be crocetin, specifically dimethyl (2E, 4E, 6Z, 8E, 10E, 12E, 14Z)-2, 6, 11, 15-tetramethylhexadeca-2, 4, 6, 8, 10, 12, 14-heptaenedioate.

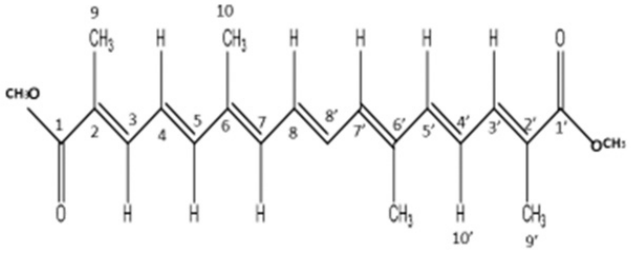


Figure 1: Structure of crocetin.

To calculate the percentage purity of crocetin by HPLC (High-Performance Liquid Chromatography). This is the area under the crocetin peak in the HPLC chromatogram. It represents the quantity of crocetin in the sample. This is the total area under all the peaks in the HPLC chromatogram. 80 % of purity was calculated for the isolated crocetin as compared to the marketed crocetin by an HPLC analysis. The results were shown in Table 1 and Figure 3.

Molecular docking

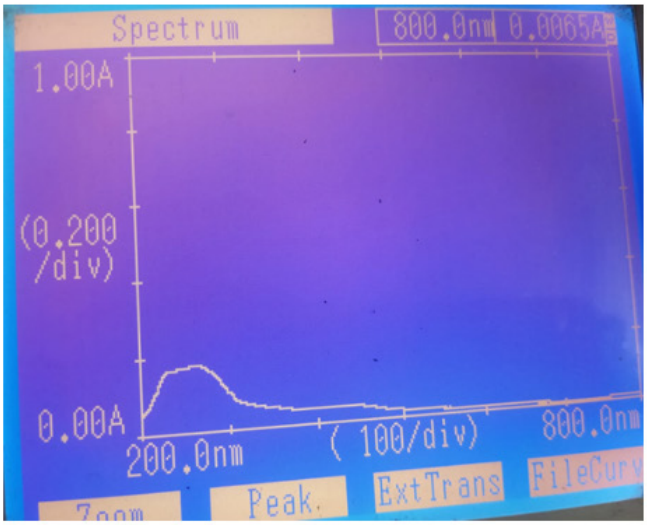
The Glide module of the Schrödinger software was used to conduct a molecular docking research to clarify potential interactions between a number of strong ligands and a target protein. The potential for interactions between inhibitors and specific amino acid residues within the target protein plays a crucial role in determining the inhibition of enzyme activity. In this study, Crocetin was chosen for docking investigations to probe the binding sites within the Fibroblast growth factor receptor, Oestrogen receptor, Glucocorticoids receptor, and p38 MAP Kinase receptors, with reference to their respective PDB IDs: 2FGI, 1ERE, 3K23, and 2GHM. These docking studies aimed to elucidate the binding interactions between Crocetin and the active sites of these receptors. A grid of dimensions five by five was generated for each Receptors (PDB ID: 2FGI, 1ERE, 3K23 and 2GHM), and subsequent XP docking was performed on each generated five grid. The docking results revealed with an XP docking score value of -7.956, -8.246, -7.530, and -5.625 Kcal/mol respectively as shown in Table 3. These interactions, as evidenced by the docking scores and the involved amino acid residues, were identified as crucial contributors to the inhibitory activity of Crocetin against Fibroblast growth factor receptor, Oestrogen receptor, Glucocorticoids receptor, and p38 MAP Kinase receptors as shown in Table 2.

DISCUSSION

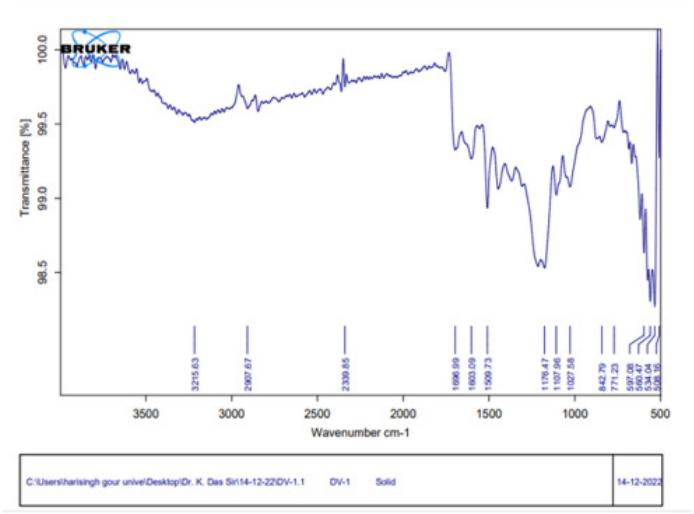
The phytoconstituent isolated from *Nyctanthes arbortristis* Linn. represents an intriguing compound with potential pharmacological significance. This discussion aims to explore key aspects of this phytoconstituent, including its traditional uses, chemical properties, and potential applications in modern medicine.[28-30]

Table 1: Peak area percentages.

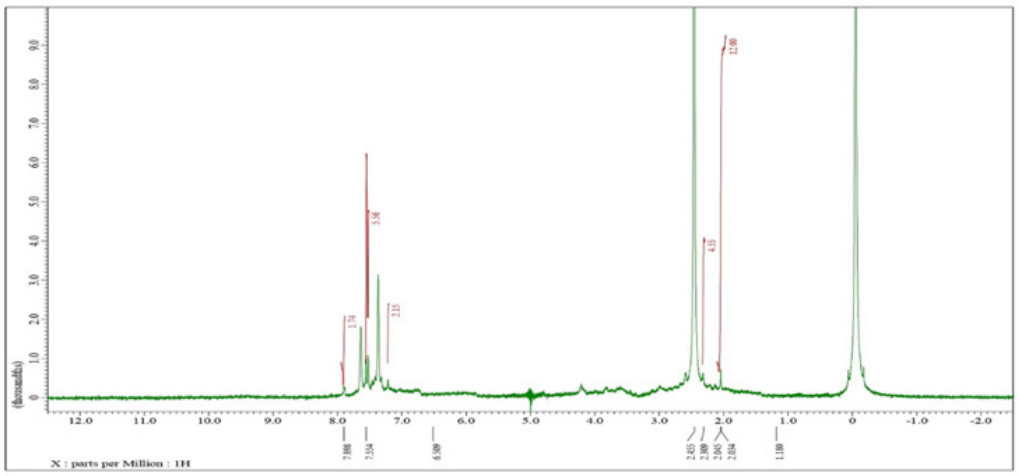
Name of compound	Retention time	Area	Area%	Height
Marketed Crocetin	3.28	8003368	100.00	1082470.88
Isolated Crocetin	2.47	150743.02	13.02	924791
	2.71	49661.36	4.29	476805
	2.88	30142.16	2.60	444726
	3.34	927643.63	80.09	7430335



A



B



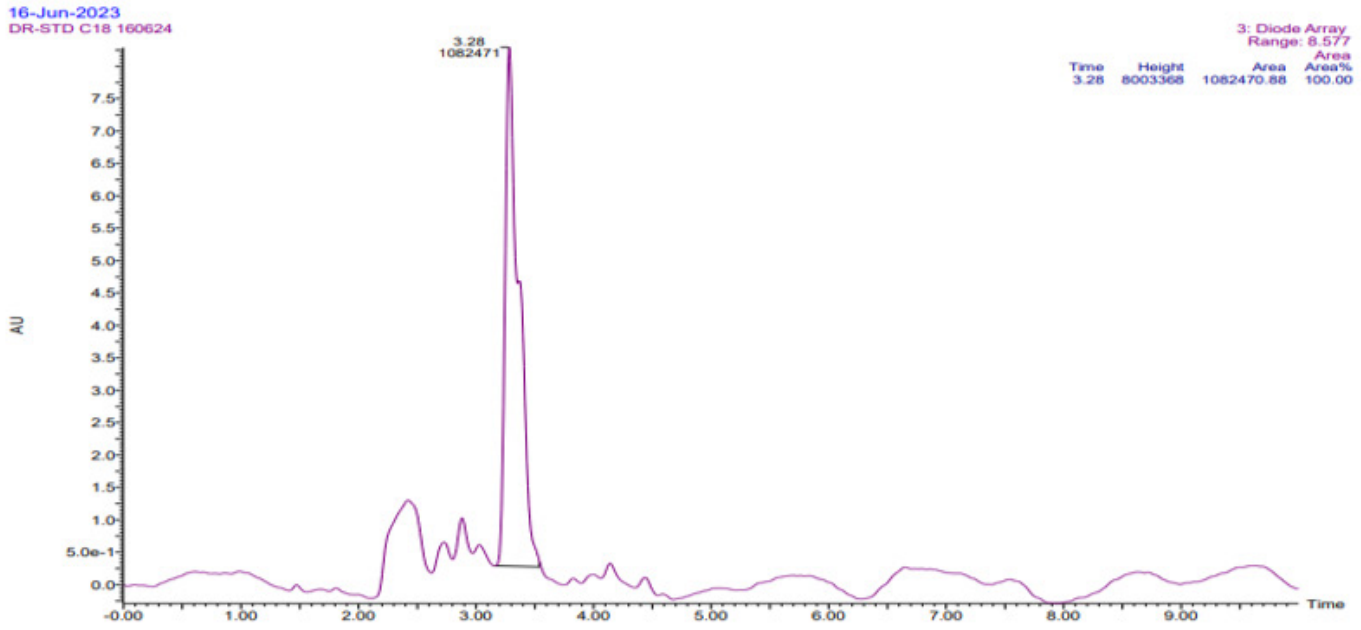
C

Figure 2: Spectrums of crocetin on hyphenated spectroscopic techniques (A) UV-Vis (B) FT-IR (C) ^{1}H NMR.

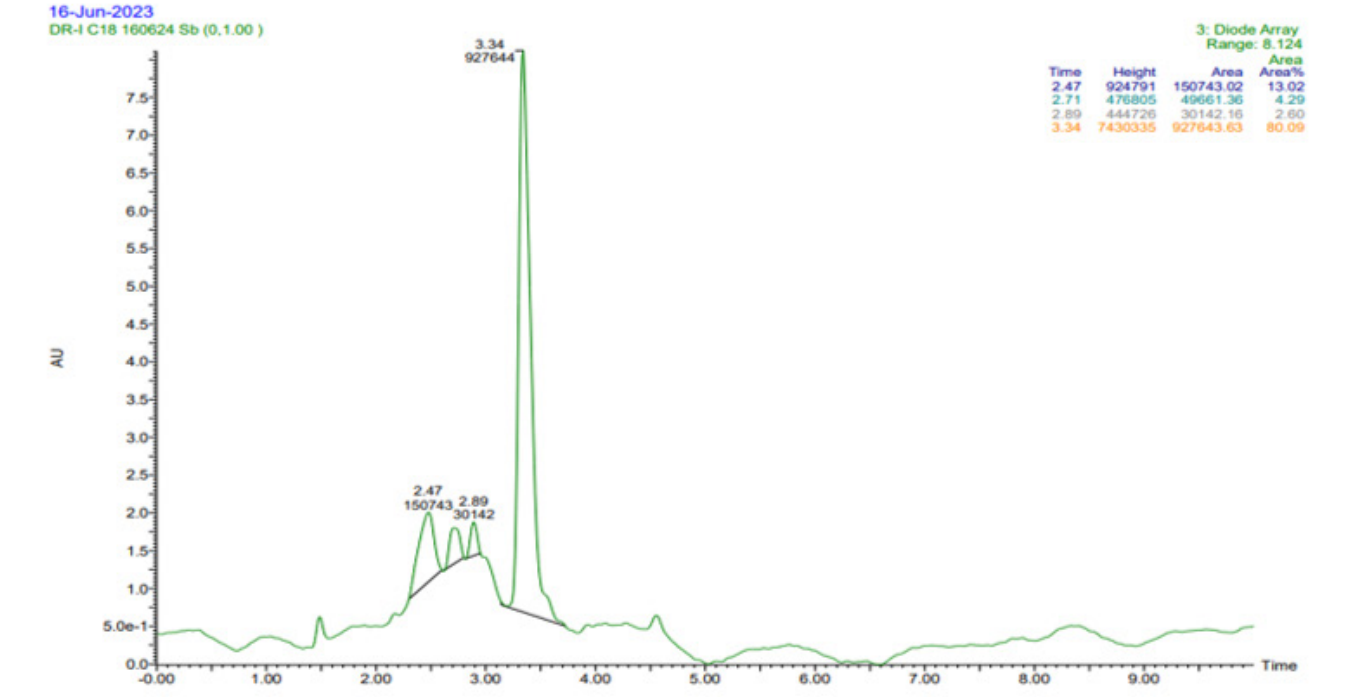
Research on the phytoconstituent's chemical composition is a key area of interest. Understanding the compound's structure, purity, and physicochemical properties is crucial. To confirm the identity and purity of the isolated compound, characterization techniques such as Nuclear Magnetic Resonance (NMR), Infrared (IR) spectroscopy, and High-Performance Liquid Chromatography (HPLC) are employed. These techniques provide valuable insights into the compound's structural and chemical characteristics.

The molecular docking studies conducted on the phytoconstituent isolated from *Nyctanthes arbortristis* Linn. provide a platform for in-depth exploration and understanding of its potential therapeutic applications. This discussion delves into the key findings, implications, and limitations of the research, offering insights into the compound's interactions with biomolecular targets.

The docking simulations revealed significant binding affinities between the Crocetin and Fibroblast growth factor receptor, Oestrogen receptor, Glucocorticoids receptor, and p38 MAP Kinase receptors. The favorable binding energies observed suggest a high likelihood of interaction, emphasizing the compound's potential as a biologically active agent. Additionally, the specificity of binding to particular targets underscores the compound's selectivity, a critical aspect in drug design.



A



B

Figure 3: HPLC chromatogram showing %Purity of A: Marketed Crocetin B: Isolated Crocetin.

Table 2: Docking scores of Crocetin (active compounds) with different proteins

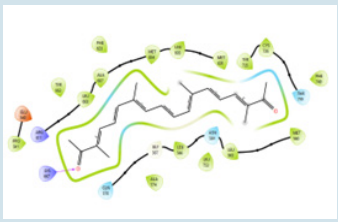
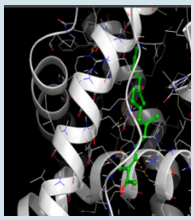
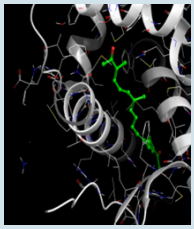
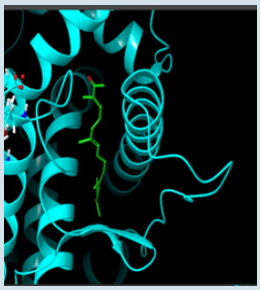
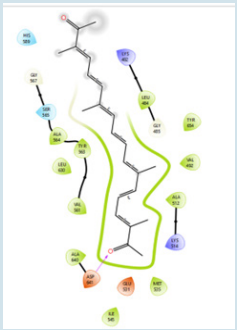
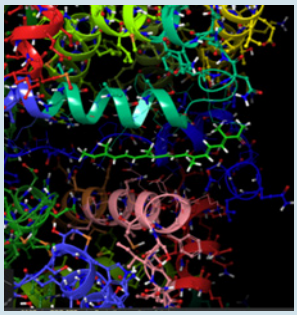
PDB ID	Docking Score	2D Interaction	3D Interaction
2FGI	-7.956		
1ERE	-8.246		
3K23	-7.530		
2GHM	-5.625		

Table 3: Validation of Docking Procedure: The verification of the correctness of the conducted docking procedure was carried out using AutoDock Vina software. Subsequently, ligands was redocked into the identical binding site of the protein, serving as a benchmark for assessing the accuracy and reliability of the docking methodology.

PDB ID	Docking result
2FGI	-9.1
1ERE	-8.9
3K23	-7.2
2GHM	-6.5

The choice of molecular targets in the docking studies is pivotal. The research focused on targets associated with diseases and biological processes relevant to the compound's traditional medicinal use. The promising interactions with these targets suggest potential therapeutic applications, including anti-inflammatory, antioxidant, or anti-cancer effects. These findings align with the historical use of *Nyctanthes arbortristis* in traditional medicine.

This study sets the stage for further investigations. Future research should focus on validating the predicted interactions

through experiments. Both *in vitro* and *in vivo* studies play a critical role in evaluating the compound's effectiveness, safety, and pharmacokinetics. These studies provide valuable data on how the phytoconstituent interacts with biological systems and its potential for drug development. Additionally, structure-activity relationship (SAR) studies offer insights into how the compound's structure relates to its bioactivity, guiding efforts to optimize it for therapeutic use.

CONCLUSION

The research presented in this paper aimed to elucidate the therapeutic potential of a phytoconstituent isolated from *Nyctanthes arbortristis* Linn. a plant with a rich history in traditional medicine. Through comprehensive molecular docking studies, we have probed the interactions between Crocetin and biologically relevant molecular targets, shedding light on its potential pharmacological potential as an anticancer drug. In conclusion, our research bridges traditional medicinal knowledge with modern computational biology, emphasizing the importance of phytoconstituents as potential sources of novel therapeutic agents. The knowledge obtained through this research opens new avenues for future explorations and holds the promise of inspiring the development of novel drugs and therapeutic approaches. This takes us one step further in unlocking the therapeutic potential found within the diverse world of natural compounds.

ACKNOWLEDGEMENT

The authors extend their gratitude to the Department of Pharmaceutical Sciences at Dr. Harisingh Gour Vishwavidyalaya, Sagar, (M.P.). Special recognition is also given to Priyanshu Nema for his invaluable contributions to the Docking Studies.

CONFLICT OF INTEREST

The authors affirm that there is no conflict of interest to disclose.

ABBREVIATIONS

UV: Ultra-Violet; **IR:** Infrared Radiation; **NMR:** Nuclear Magnetic Resonance; **HPLC:** High performance liquid chromatography; **PDB:** Protien data bank; **NAT:** *Nyctanthes arbortristis;* **LIGPREP:** Ligand preparation; **SER:** Serine; **THR:** Threonine; **TYR:** Tyrosinase; **CYS:** Cystine.

REFERENCES

1. Agrawal J, Pal A. *Nyctanthes arbortristis* Linn-A critical ethnopharmacological review. J Ethnopharmacol. 2013; 146(3): 645-58. doi: 10.1016/j.jep.2013.01.024, PMID 23376280.
2. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbortristis* L. J Ethnopharmacol. 1994; 42(1): 31-7. doi: 10.1016 /0378-8741(94)90020-5, PMID 8046941.
3. Giaccio M. Crocetin from saffron: an active component of an ancient spice. Crit Rev Food Sci Nutr. 2004; 44(3): 155-72. doi: 10.1080/10408690490441433, PMID 15239370.
4. Guo ZL, Li MX, Li XL, Wang P, Wang WG, Du WZ, *et al.* Crocetin: a systematic review. Front Pharmacol. 2021; 12: 745683. doi: 10.3389/fphar.2021.745683, PMID 35095483.
5. Sah AK, Verma VK. Phytochemicals and pharmacological potential of *Nyctanthes arbortristis*: A comprehensive review. Int J Res Pharm Biomed Sci. 2012; 3(1): 420-7.
6. Nirmal SA, Pal SC, Mandal SC, Patil AN. Analgesic and anti-inflammatory activity of β-sitosterol isolated from *Nyctanthes arbortristis* leaves. Inflammopharmacology. 2012; 20(4): 219-24. doi: 10.1007/s10787-011-0110-8, PMID 22207496.
7. Khatune NA, Islam ME, Rahman MAA, Mosaddik MA, Haque ME. *In vivo* cytotoxic evaluation of new benzofuran derivative isolated from *Nyctanthes arbortristis* L. on Ehrlich Ascite Carcinoma cells (EAC) in mice. J Med Sci. 2003; 3(2): 169-73.
8. Gadgoli C, Shelke S. Crocetin from the tubular calyx of *Nyctanthes arbortristis*. Nat Prod Res. 2010; 24(17): 1610-5. doi: 10.1080/14786411003754363, PMID 20954088.
9. Kannan M, Singh AR. An immunopharmacological investigation of Indian medicinal plant *Nyctanthes arbortristis* Linn. World Appl Sci J. 2010; 11(5): 495-503.
10. Mishra AK, Upadhyay P, Dixit J, Kumar P, Tiwari KN, Mishra SK, *et al.* Ameliorative activity of ethanolic flower extract of *Nyctanthes arbortristis* (L.) against scopolamine-induced amnestic effect and profiling of active compounds using gas chromatography-mass spectrometry and ultraperformance liquid chromatography-quadrup. Pharmacogn Mag. 2018; 14:(59s).
11. BAWARI S, Tewari D, Sah AN. Ameliorative Effects of Raphanus sativus L., *Nyctanthes arbortristis* L. and *Ficus palmata* Forssk. on Calcium Oxalate Crystallization Events of Stone Formation *in vitro*. Indian J Pharm Sci. 2022; 84(2).
12. El-Azab AS, Al-Omar MA, Abdel-Aziz AA, Abdel-Aziz NI, el-Sayed MA, Aleisa AM, *et al.* Design, synthesis and biological evaluation of novel quinazoline derivatives as potential antitumor agents: molecular docking study. Eur J Med Chem. 2010; 45(9): 4188-98. doi: 10.1016/j.ejmech.2010.06.013, PMID 20599299.
13. Chaudhary S, Gupta RK, Kumar A, Tarazi H. Hepatoprotective and antioxidant potential of *Nyctanthes arbortristis* L. leaves against antitubercular drugs induced hepatotoxicity. J Pharm Pharmacogn Res. 2018; 6(3): 205-15.
14. Seal A, Aykkal R, Babu RO, Ghosh M. Docking study of HIV-1 reverse transcriptase with phytochemicals. Bioinformation. 2011; 5(10): 430-9. doi: 10.6026/97320630005430, PMID 21423889.
15. Islam MT, Khalipha ABR, Bagchi R, Mondal M, Smrity SZ, Uddin SJ, *et al.* Anticancer activity of thymol: A literature-based review and docking study with Emphasis on its anticancer mechanisms. IUBMB Life. 2019; 71(1): 9-19. doi: 10.1002/iub.1935, PMID 30308112.
16. Vishwakarma RK, Negi DS, Negi A. Abortitristoside A and desrhamnosylverbanscoside: the potential COX-2 inhibitor from the leaves of *Nyctanthes arbortristis* as anti-inflammatory agents based on the *in vitro* assay, molecular docking and ADMET prediction. Chem Pap. 2023; 77(6): 3035-49. doi: 10.1007/s11696-023-02686-7.
17. Abo-Zeid Y, Ismail NSM, McLean GR, Hamdy NM. A molecular docking study repurposes FDA approved iron oxide nanoparticles to treat and control COVID-19 infection. Eur J Pharm Sci. 2020; 153: 105465. doi: 10.1016/j.ejps.2020.105465, PMID 32668312.
18. Pierce AC, Jacobs M, Stuver-Moody C. Docking study yields four novel inhibitors of the protooncogene Pim-1 kinase. J Med Chem. 2008; 51(6): 1972-5. doi: 10.1021/jm701248t, PMID 18290603.
19. Nath A, Kumer A, Khan MW. Synthesis, computational and molecular docking study of some 2, 3-dihydrobenzofuran and its derivatives. J Mol Struct. 2021; 1224: 129225. doi: 10.1016/j.molstruc.2020.129225.
20. Scotti L, Bezerra Mendonca FJ, Ribeiro FF, Tavares JF, da Silva MS, Barbosa Filho JM, *et al.* Natural product inhibitors of topoisomerases: review and docking study. Curr Protein Pept Sci. 2018; 19(3): 275-91. doi: 10.2174/1389203718666170111114442, PMID 28079013.
21. Shaji D, Suzuki R, Yamamoto S, Orihashi D, Kurita N. Natural inhibitors for severe acute respiratory syndrome coronavirus 2 main protease from *Moringa oleifera, Aloe vera*, and *Nyctanthes arbortristis*: molecular docking and ab initio fragment molecular orbital calculations. Struct Chem. 2022; 33(5): 1771-88. doi: 10.1007/s11224-022-02021-y, PMID 35938063.
22. Mhatre S, Naik S, Patravale V. A molecular docking study of EGCG and theaflavin digallate with the druggable targets of SARS-CoV-2. Comput Biol Med. 2021; 129: 104137. doi: 10.1016/j.compbiomed.2020.104137, PMID 33302163.
23. Patil NS, Rohane SH. Organization of Swiss Dock: in study of computational and molecular docking study; 2021.
24. Najafi Z, Mahdavi M, Saeedi M, Karimpour-Razkenari E, Asatouri R, Vafadarnejad F, *et al.* Novel tacrine-1, 2, 3-triazole hybrids: *in vitro, in vivo* biological evaluation and docking study of cholinesterase inhibitors. Eur J Med Chem. 2017; 125: 1200-12. doi: 10.1016/j.ejmech.2016.11.008, PMID 27863370.
25. Jain N, Jain P, Rajput D, Patil UK. Green synthesized plant-based silver nanoparticles: therapeutic prospective for anticancer and antiviral activity. Micro Nano Syst Lett. 2021; 9(1): 5. doi: 10.1186/s40486-021-00131-6.
26. Banik A, Ghosh K, Patil UK, Gayen S. Identification of molecular fingerprints of natural products for the inhibition of breast cancer resistance protein (BCRP). Phytomedicine. 2021; 85: 153523. doi: 10.1016/j.phymed.2021.153523, PMID 33662771.
27. Singhai A, Ahmad Y, Patil UK. Phyto-therapeutic potential of *Aconitum ferox* Roots in CFA-induced arthritis in rat model. Indian J Pharm Educ Res. 2022; 56:s725-35.

28. Rahman M. Chemical Constituent of *Nyctanthes arbortristis* Linn Leaf. Nat Prod J. 2013; 3(1): 71-6. doi: 10.2174/2210315511303010013.
29. Awasthi M, Amin SA, Shukla V, Jain S, Patil UK, Gayen S. Structural requirements of some derivatives based on natural alkaloid lycorine for their dengue inhibitory activity to accelerate dengue drug discovery efforts. Indian J Nat Prod Resour (IJNPR) [Formerly Natural Product Radiance (NPR)]. 2016; 7(3): 221-8.
30. Singhai A, Patil UK. Amelioration of oxidative and inflammatory changes by *Peganum harmala* seeds in experimental arthritis. Clin Phytosci. 2021; 7: 1.

Cite this article: Rajput D, Jain D, Kashaw SK, Patil UK. Molecular Docking Studies on Phytoconstituent Isolated from *Nyctanthes arbortristis* Linn. Int. J. Pharm. Investigation. 2024;14(2):399-408.