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Research Article

Isolation, Characterization, *In-silico* and Enzyme Inhibition Studies of *Bougainvillea spectabilis* against a Hyperoxaluria Initiator Glycolate Oxidase

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ABSTRACT

Glycolate oxidase has long been thought to be a key player in the formation of oxalate accumulation in the human body. Both the endogenous synthesis of oxalate and clinically identified targets for the therapy of primary hyperoxaluria are affected by this disorder. The role of glycolate oxidase has been investigated in order to provide additional insight into the possible molecular pathways involved. The presence of flavonoids in the ethanolic extract led to the conclusion that it was suitable following phytochemical screening. Column chromatography was used to isolate the active component using the proper solvent system. The structure of the active, separated phytoconstituents was ascertained using a variety of spectral techniques, including FTIR, NMR, and GCMS analysis. Following structure elucidation, glycolate oxidase (PDB: 2RDT) and quercetin were used as standards in molecular docking investigations. Studies on *in-vitro* enzyme inhibition were carried out to verify the outcome. The investigation of the isolated compound's spectral data determined that the structure might be 4-hydroxy-3-nitro coumarin. Additional molecular docking studies were carried out using conventional standard flavonoid quercetin to speculate on the compound's potential mechanism of action. The chemical was discovered to be effective during the inhibition of the target enzyme by occupying the majority of the amino acid active sites. *In-vitro* enzyme inhibition tests provided additional confirmation for the computational investigations. The isolated compound's significant IC_{50} value in this study was demonstrated in comparison to standard quercetin's efficacy. These findings supported the isolated compound's potential mechanism of action, which involves inhibiting the enzyme glycolate oxidase in terms of its anti-urolithiasis efficacy. The study also provided justification for the compound's potential therapeutic application in urolithiasis.

Introduction

The renaissance of herbs is a global phenomenon. Herbal products are safe today, unlike synthetics, which are thought to be harmful to both humans and the environment. A greater emphasis is being placed on the use of plant materials as a source of medicines for a wide range of human ailments. These include population growth, an inadequate supply of drugs, the prohibitive cost of treatments, the adverse effects of several allopathic drugs, and the emergence of resistance to currently used drugs for infectious diseases. But the blind dependence on artificial substances has ended, and people are turning back to natural materials in the hope of security and safety.[1-3] Several scientists and researchers have recently focused on the pure active compounds and natural extracts from plant species utilized in herbal and traditional pharmaceuticals.[3-6] Nowadays, the newly discovered phytochemical contents of locally grown medicinal plants that herbalists often utilize to treat a wide range of illnesses need to be isolated, identified, and characterized. Further investigation is required to determine whether locally grown medicinal plants have anti-urolithiasis qualities, as this could indicate their potential as sources of therapeutic chemicals.[7-9]

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Most of the world's traditional healthcare systems have included plant-based medications for thousands of years. Ayurvedic treatments frequently use a variety of medicinal herbs. India is habitat to thousands of officially recognized species of medicinal plants, many of which contain physiologically beneficial compounds that have anti-urolithiasis properties and were used in traditional medicine to treat kidney stone disease. Surgery is the primary method of treating urolithiasis for the majority of people worldwide. Adverse consequences of surgical therapy include renal fibrosis, hemorrhage, hypertension, and tubular necrosis.[10] Even with the drawbacks of medication, surgical stone excision is still thought to be the best method for relieving symptoms, even if it is expensive and has a high recurrence rate. Consequently, physicians have recently turned their attention to plant-based therapies.

The third most common urinary tract ailment is urolithiasis. Nearly 80% of urinary calculi in northern India are either calcium oxalate stones or calcium oxalate mixed with calcium phosphate. Urolithiasis is fairly common throughout the world.[11] The primary cause of idiopathic calcium oxalate kidney disease (CaOx) stone disease in humans is hyperoxaluria. Urine contains oxalate, a powerful crystallization-inducing ingredient whose retention exacerbates cell damage and starts the lithogenesis process.[12] Despite tremendous advancements in medicine. There is no effective treatment for renal calculi. But phytomedicines have given us a different kind of treatment option in addition to increasing our understanding of the pathophysiology of many diseases.

*Bougainvillea f*rom South America is member of the family Nyctaginaceae. Because of its great adaptability to many agro-climatic zones across the globe. It is primarily utilized in arid landscapes for horticulture, pharmaceutical, agricultural, and environmental industries. It comprises roughly 18 species. The Brazilian species *Bougainvillea spectabilis* is also utilized extensively. The height is six feet. Compared to other *Bougainvilleas*, it has darker leaves that are hairy and have noticeable hairs on the underside. It has bright red or purple bracts that open in the summer. The leaves of *B. spectabilis* are reported to have antidiabetic, hepatoprotective, antiviral, antibacterial, antiulcer, antioxidant, antifertility, antiinflammatory, antihyperlipidemic, and insecticidal properties. Reported constituents are flavonoids, betacyanine, alkaloids, and tannins used as a medicine for various disorders.[13]

The bioactive chemicals found in the leaf of the *Bougainvillea* plant were investigated in this study. Pet-ether, chloroform ethyl acetate, ethanol, and water were utilized for plant material extraction. Using different spectrometric techniques the separation and characterization of the leaf's bioactive components were accomplished. So, based on previous literature, the study focuses on identification and possible mechanism of action of particular compounds in the plant responsible for its urolithiatic activity. Srivastava S (1962) reported the presence of oxalic acid oxidase in the leaves of *B. spectabilis*.[14] Sunil Jawla (2013) isolated pinitol and quercetin from the plant *B. spectabilis,* from which quercetin is a potent flavonoid compound commercially used in herbal formulations for many kidney disorders, including kidney stones.[15] Anil K chillar (2013), with many scientists, reported antioxidant activity from the plant, preferably due to the presence of wide variety of flavonoids in the plant.[16] Thushara Bindu (2016) investigated the nephroprotective activity of the ethanolic extract of the leaves of the plant using gentamycin as inducer.[17] Rajeswari P (2018) also indicated from their *in-vitro* study that aqueous extract of another species of *Bougainvillea* i.e., *B. glabra* showed anti-urolithiatic potential.[18] Das PK and Vaghela JS (2021) reported the *in-vitro* anti-urolithiatic activity of the plant *B. spectabiils*.[19] Das PK Vaghela JS (2021) evaluated the *in-vivo* anti-urolithiatic activity of the plant using sodium oxalate as an inducer.[20]

Primary hyperoxaluria is initiated by the conversion of glyoxylate to oxalic acid, which is primarily carried out by the enzyme glycolate oxidase. In this study, we used *in-silico* and *in-vitro* enzyme inhibition investigations in order to inhibit the enzyme using the plant-isolated compound. As far as we are aware, the anti-urolithiasis potential of *B. spectabilis* has not yet been explored through computational studies or enzymatic analysis. Determining and characterizing phytoconstituents in its ethanolic extracts for chemical profiling by IR, NMR, and mass spectrometry technique, as well as evaluating its potential for preventing urolithiasis through computational and enzyme inhibition studies to correlate with the flavonoid content, are therefore worthwhile endeavors when screening *B. spectabilis* for the presence of phytochemicals. Despite the fact that the precise mechanisms by which the plant *B. spectabilis* works are still unknown, in recent studies on urolithiasis against the plant found that *B. spectabilis* can inhibit the action of the enzyme glycolate oxidase, which is thought to be a powerful initiator of primary hyperoxaluria. Examining the isolated plant compound and its interaction with glycolate oxidase was the primary goal of this investigation. Docking analysis was also carried out to identify the residues involved in the binding of isolated compounds and their inhibitory effect on gycolate oxidase.

Materials and Methods

Collection and Authentication of Plant Material

In January, the leaves of *B. spectabilis* Willd. were collected from the local areas in and around Madhya Pradesh and validated the herbarium specimen of the plant that was deposited in the Department of Pharmacognosy, PG Govt College Khargone.

Processing of Plant Material

The freshly collected leaves were thoroughly washed many times to wipe out any type of dirt, debris, or crystals found on the leaves, shade-dried, and then ground using a mechanical pulverizer. After passing it through a sieve, the fine powder was collected.

Chemicals and Reagents

Chemicals i.e., petroleum ether, n-hexane, chloroform, ethyl acetate, ethanol, methanol, and all other chemicals used in the experiment were of analytical grade and were purchased from authorized scientific labs.

Preparation of the Plant Extracts

In 200 g of dried and coarsely chopped leaves were extracted in a soxhlet extractor with petroleum ether, chloroform, ethyl acetate, ethanol, and water using continuous hot percolation for 18 to 24 hours. Finally, the concentrated extracts were evaporated to dryness.

Phytochemical Screening of *B. spectabilis*[21]

Due to the variety of phytochemical constituents present inside the medicinal plants they act as natural healers in addition to this they also cure various human ailments. To review the qualitative chemical composition of crude extracts from *B. spectabilis* the preliminary phytochemical screening was performed by using coloration and precipitation reaction to recognize the chief natural chemical groups. Specific reactions in this study discovered the presence or absence of the phytochemical compounds in the crude extracts tested.

TLC study of plant B. spectabilis[22]

TLC analysis of ethanolic plant extract was performed. TLC plates were examined using a UV light, iodine vapor was applied to create TLC plates, or reagent was sprayed onto the plates to aid visualization. It was common practice to experiment with several solvent systems to find one that provided the best separation for TLC. The result of the solvent system with TLC report is illustrated in Table 1 and Figs. 1 and 2.

Column chromatography[23]

For column packing the wet packing method was used. Activated silica gel (neutral) in slurry form was made in a solvent system and was transferred into the column by a funnel. A suitable solvent was combined with about 20 grams of plant extract in ethanol and 80 grams of silica gel for CC (60–120 mesh). The combinations were triturated with a pestle until they were uniform, dry, and able to flow freely. As above, the mixtures were made and fed gradually into the column with the assistance of a glass funnel devoid of unsettling the silica bed. Afterward, a suitable solvent system was dispensed into the column for the revelation of components. After column chromatography the elutes obtained were processed to preparative TLC as requisite to get pure compounds. (Fig. 3)

Spectral analysis, structure elucidation, identification study of the isolated compound from the ethanolic extract of the plant B. spectabilis

The most common methods for determining the structure of natural materials are fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectroscopy. With the help of these spectral methods, the structures can be elucidated.

- IR Spectroscopy: is used to determine the types of functional groups present in isolated compounds.
- NMR Spectroscopy: It gives an idea about the compound's structural backbone (No. of Hydrogen and Carbon).
  - ^{13}C-NMR: To determine how many different kinds of carbon atoms make up the molecule.
  - ^{1}H-NMR: The goal is to determine how many and what kinds of hydrogen atoms are present in the chemical, as well as the nature of the bonds between them.
- Mass Spectroscopy: To determine the molecular weight of the compound.

IR spectra of the isolated compounds were recorded by using KBr pellets on a Shimadzu 8400S FTIR spectrophotometer at Central Instrumentation Laboratory, Punjab University, Chandigarh, and all the spectra were recorded in the range of 650 to 4000 cm^{-1} and values were expressed in terms of cm^{-1} The spectrum was attached. The IR peak is shown in Fig. 4.

^{1}H-NMR and ^{13}C-NMR spectra of isolated compounds were recorded on Bruker, Advance Neo (500 MHz) NMR

Table 1: Docking result of isolated compound with standard drug

Compound	*Mol Dock score*	*H-Bond Interaction*	*Steric Interaction*	*Interaction with reported amino acid*
Isolated compound	-121.298	His260, Arg263, Arg167, Tyr26	Arg263, His260, Arg167, Tyr26, Trp110	04
Quercetin	-128.818	Arg167, Tyr26, Arg263, His260, Gln130	Val209, Trp110	04

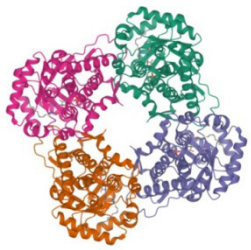


Fig. 1: 3D Structure of 2RDT protein

spectrophotometer at Indian Institute of Science Education and Research Bhopal (IISER Bhopal), Where $CDCl_3$ was used as a solvent and tetra methyl silane (TMS) as internal standard and chemical shift were expressed in δ ppm (Fig. 5). All these GCMS analyses were performed in the sophisticated High-tech laboratory of IISER Bhopal.

Isolated compounds in ethanolic extract of *B. spectabilis* were identified by comparing their mass spectra and peak molecular ions with known compounds through NIST compound libraries provided by IISER Bhopal.

GC-MS analysis chromatogram revealed an active flavonoid phytoconstituent in the isolated compound from ethanolic extract of *B. spectabilis*. Their chromatogram, peak molecular ion, and molecular formulas are illustrated in Fig. 6.

In-silico study of the active compounds[24]

In drug design according to structure-based, the target protein's structure is known, and following the docking procedure, interaction or bio-affinity is calculated for each tested compound to create a new therapeutic molecule that interacts more effectively with the target protein.

This investigation focuses on molecular modeling and docking studies by selecting a standard compound, flavonoid quercetin, popularly used for its chemopreventive and chemotherapeutic properties in many human disorders, especially eradicating kidney stones. The investigation of enzyme–ligand interactions has revealed a possible mechanism of action of leads in the management of calcium oxalate stones. So in the molecular docking study, we use quercetin as a standard molecule against isolated compounds.[25, 26]

Quercetin inhibited the enzyme glycolate oxidase (GOX), preventing stone formation in several models of urolithiasis utilizing sodium oxalate and ethylene glycol.[25] Oxalate production requires several enzymes, but one of the most important is glycolate oxidase, which oxidizes glycolate to glyoxylate and then to oxalate. An alternate substrate reduction approach, by which glyoxylate production

The computational study tries to justify the selective interaction of GOX key amino acids with the standard flavonoid quercetin when compared with the isolated compounds of the plant *B. spectabilis*, which is further confirmed by *in-vitro* enzyme inhibition Thus, discovering active lead molecules from the plant extract with modern analytical methods and advanced *in-silico* techniques revealed better leads for chemotherapy of urolithiasis, mediated possibly through GOX inhibitory effects. We further propose a structure-based drug design to get a molecule with high efficacy. Since urolithiasis is linked to overactivity of the human glycolate oxidase enzyme, small compounds or specially designed ligands that occupy most of the binding sites of the receptor that are already established in co-crystallization ligand could function as a possible mechanism of action of inhibition of the enzyme GOX.

The protein's crystal structure used in this investigation was obtained using coordinates retrieved from the protein databank (PDB) at the research collaborative for structural bioinformatics (http://www.RCSB.org). To explore the binding pattern of ligand molecules into their binding site, a molecular docking study was carried out with isolated compounds by using Molegro Virtual Docker (MVD 6.0) software. To validate the methodology of the docking procedure docking is performed, where a co-crystallized ligand is extracted initially and is docked once again to the active site of the receptor and results are compared with the binding pose of the co-crystallized ligand and natural co-crystallize ligand. The root mean square deviation (RMSD) value between the native co-crystallized ligand and the docked pose is calculated to confirm the accuracy of the docking technique X-ray crystallography structures of human GOX in complex with CDST, was retrieved from RCSB protein data bank [PDB ID: 2RDT].

In-vitro GOX enzyme inhibition assay[26]

The isolated compound, along with the standard compound, which showed significant docking score and interactions, was subjected to re-evaluate using the *in-vitro* enzyme inhibition activity to validate the preliminary docking results. Additionally, this study justifies and explains the enzyme inhibitory activity of the compound in terms of IC_{50} value.

The GOX reaction was coupled with the peroxidase reaction and assayed at 30°C by monitoring H_2O_2 production. The assay conditions were 1 mM, pH 7.8, Tris–HCl containing 5 µM glycolate, 1-µM anti pyrine, 0.5 U horse radish peroxidase, 2 µM phenol, and 0.1 µM FMN in a final volume of 3.0 mL. Assays were initiated by the addition of glycolate and the absorbance increase at 520 nm was then monitored for 2 minutes. One unit of glycolate oxidase activity was defined as the amount of enzyme required to produce 1-µmol H_2O_2 per min. GOX inhibition by isolated flavonoid at variable concentrations, 0.1, 0.3, 0.5, and 1.0 µM was measured and their IC_{50} values were determined. The inhibitory effect of quercetin was studied at inhibitory concentrations of equal to the isolated flavonoid 0.1, 0.3, 0.5, and 1.0 µM against variable concentrations of glycolate (1–5 µM). In a reaction mixture the inhibitor and substrate concentrations were maintained identical to have equal competition for enzymes active site. The results are illustrated in Table 2 and Fig. 8.

RESULTS

The results of the phytochemical study revealed the presence of most phytochemicals in ethanolic extract as compared to any other extract. So, from the findings of this result, we decided to isolate the phytoconstituents flavonoids from ethanolic extract for further justification of its antiurolithiatic potential of the plant *B. spectabilis.*[27] After analyzing the ethanolic extract of the plant using TLC method by applying different solvent systems and,

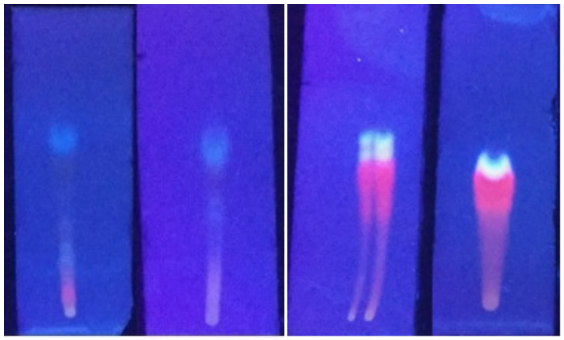


Fig. 2: TLC visuals of ethanolic extract

Fig. 3: Column chromatography of ethanolic extract

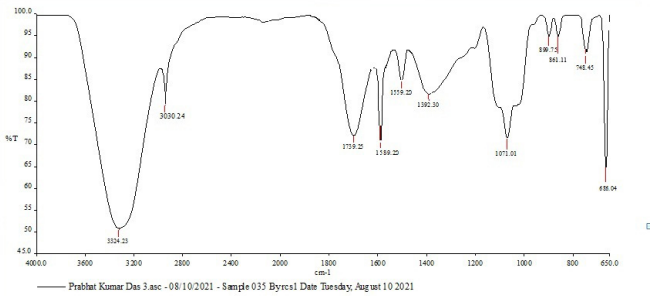


Fig. 4: FTIR analysis of isolated compound

visualizations were done by examining the TLC plates under a UV lamp of 254 nm after spraying the 10% aqueous H_2SO_4, we found that solvent systems $CHCl_3$: Methanol with diversified ratio was established to be the most appropriate solvent system for isolating flavonoid compound present in ethanolic extract of *B. spectabilis.* So, the above solvent system was selected for further column chromatographic studies. (Fig. 2)

The dried ethanolic extract of *B. spectabilis* (15 gm) was mixed with 80 gm silica gel (60–120 mesh) to make the material adsorbed in the silica gel. Different solvent systems according to the polarity were used in the column to isolate the compound.

The column was eluted with solvent increasingly initially from chloroform only, then chloroform with methanol with different ratios in increasing polarity to identify the exact ratio in which compounds were eluted and fractions were collected. One compound was eluted from the solvent system Chloroform: Methanol with ratio 8:2 was eluted from the column. (Fig. 3)

Different spectral analyses like FTIR, NMR, and GCMS studies were performed in various sophisticated labs of IISER Bhopal and Punjab University to identify the isolated compounds.

In FTIR (KBr) υ cm^{-1} interpretation a broad absorption band in the range of 3324 indicated the presence of OH group. A narrow band in the range of 3030 indicates aromatic CH stretching. A broad band in the range of 1739 indicates the Ketone group (C=O). Another broad band of 1589 indicates C=C ring stretching. A narrow band in the range of 1392 indicates a C-OH bending peak. A broad peak of 1071.01 indicates the C-O Stretching mode of ether. A group of broad and narrow bands in the range of 686 to 899 indicating aromatic out-of-plane bending mode (Fig. 4).

In ^{13}C-NMR ($CDCl_3$, 500 MHz, ppm), interpretation peak at the range of 117.5 to 128.7 indicates the presence of aromatic carbon. Another peak at 162.5 indicates the presence of ketone, while a peak at 197.5 indicates the presence of the C-OH group. In ^{1}H-NMR interpretation peak at the range of 15.024 indicates the presence of the hydroxyl group. The simultaneous peak at the range of 7.020 to 7.286 indicates the presence of aromatic hydrogen (Fig. 5).

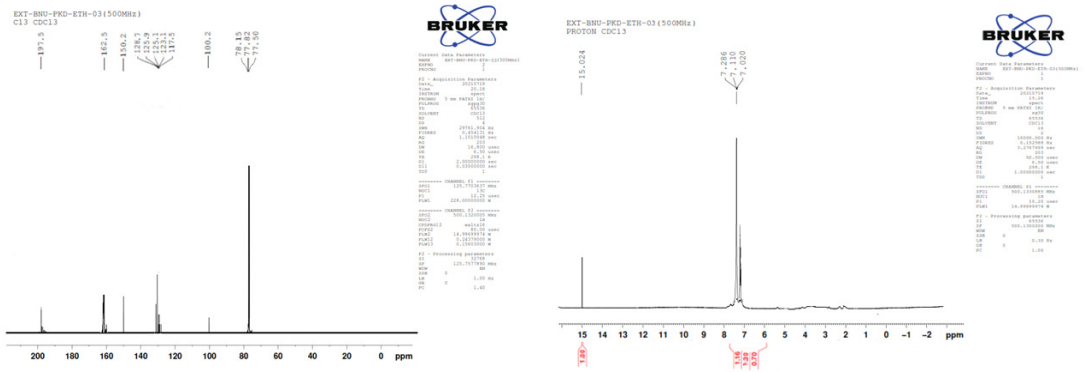


Fig. 5: NMR analysis of isolated compound

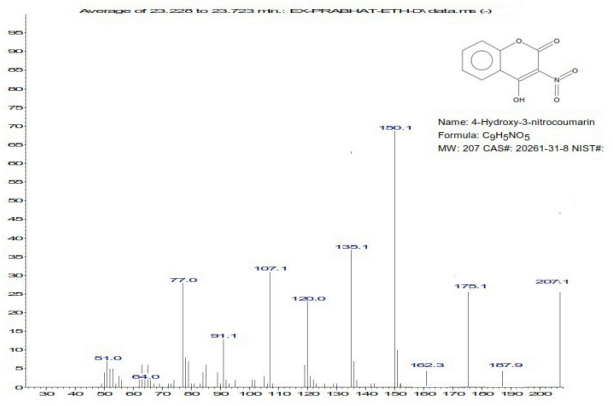
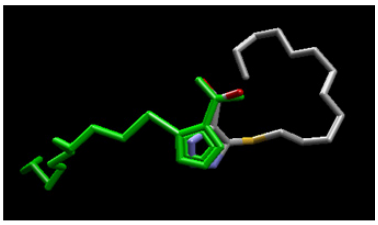
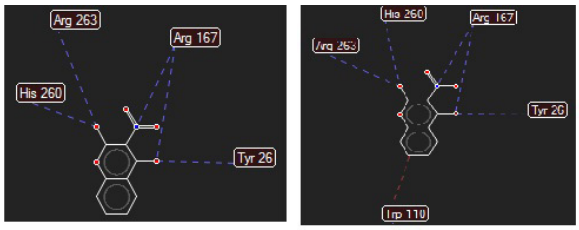


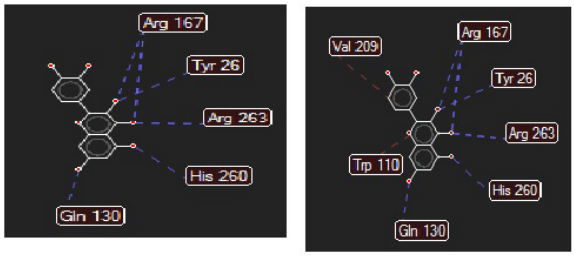
Fig. 6: GCMS analysis of the isolated compound



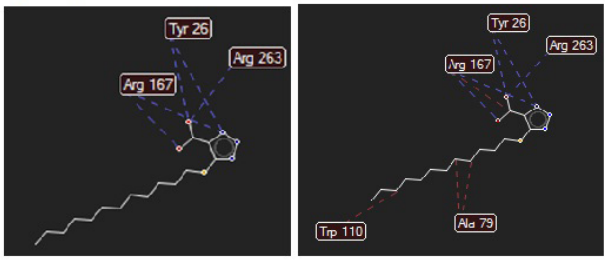
7A **RSMD VALUE**



7B **Hydrogen Bond & Steric Interaction of isolated compound**



7C **Hydrogen Bond & Steric Interaction of Standard**



7D **Hydrogen Bond & Steric Interaction of Co-Crystallised ligand**

Fig. 7: Docking result of compounds

Mass spectroscopy from the molecular ion peak of 207 indicates the confirmation of the proposed structure having a molecular weight of 207. After observing its fragmentation and interpretation of FTIR and NMR by comparing the structure with the NIST compound library, we finally assumed the isolated compound might be 4-hydroxy-3-nitro coumarin.

Molecular docking studies of isolated compound with quercetin as a standard has also been performed to screen the inhibitory activity against protein target glycolate oxidase (PDB: 2RDT). The docking study revealed that the isolated compounds exhibited the most significant affinity score against glycolate oxidase and showed the best significant hydrogen bond interaction at the protein's active site. Reported hydrogen bond interaction with co-crystallized ligand CDST (4-carboxy-5-dodecylsulfanyl-1, 2, 3-triazole) are Arg167, tyr26, Arg263 and steric interactions are Trp110, Ala79, Arg167 (Fig. 7D). The MolDock score of the isolated compounds and quercetin was found to be -121.298 and -128.818, respectively (Table 1).

Standard drug quercetin has occupied the major amino acids with Arg167, Tyr26, Arg263, His260, Gln130, Val209, and Trp110 among which five hydrogen bonds Arg167, Tyr26, Arg263, His260, Gln130 matched with the co-crystallized ligand (Fig. 7C).

After docking isolated compound occupied the amino acids with His260, Arg263, Arg167, Tyr26, and Trp110, among them four hydrogen bond interactions found to match with

Table 2: *In-vitro* enzyme inhibition values of the compound

Concentration	*Eth-03*	*Quercetin*
2.5	50.42	66.47
2	38.65	52.53
1.5	31.53	48.66
1	25.62	40.25
0.5	18.64	30.32
0.3	8.14	15.52
0.1	0.03	0.25
IC_{50} VALUE µM	2.5	1.9

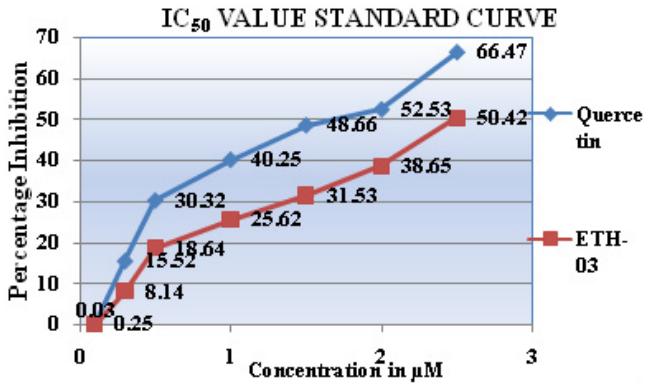


Fig. 8: *In-vitro* enzyme inhibition values of the compound

the co-crystallized ligand (Fig. 7B). The co-crystallized ligand was also validated, showing the RMSD value of 1.67 A^{o} (Fig. 7A).

As indicated in Table 2 & Fig. 8, the isolated compound exhibit good inhibitory activity with IC_{50} value 2.5 µM, compared to the IC_{50} value of quercetin, which is 1.9 µM as a potent non-competitive inhibitor.

Discussion

Human GOX is a flavin mononucleotide (FMN)-dependent a-hydroxy acid-oxidizing protein found in both plants and animals, and it belongs to the class of enzymes called flavoprotein oxidases, which plays a key role in oxalate synthesis leading to deposition of calcium oxalate stones. In humans, GOX is located in liver peroxisomes, catalyzing the FMN-dependent oxidation of glycolate to glyoxylate and oxalate, a key metabolite leading to increased excretion of oxalate. The ability of GOX to produce oxalate, a key metabolite in the formation of kidney stones, leads to liver oxalate overproduction in patients with primary hyperoxaluria type I. Hence, GOX attracts the attention of researchers as a potential target in the management of excess oxalate synthesis to save life of frequent kidney stone formers due to genetic aberrations. Therapeutic intervention of GOX in this consequence thus found potential in the treatment of calcium oxalate urolithiasis. In the present investigation, we explored enzyme GOX inhibition properties of the isolated compounds in search of potential leads from traditional resources.

Since ethanolic extract contains most phytochemical constituents, particularly flavonoids, we chose to focus on isolating the active flavonoid compounds responsible for kidney stone treatment.

After solvent optimization through TLC, we isolated the active constituents from the ethanolic extract by using the solvent system chloroform: Methanol with a ratio of 8:2.

We assumed the structure of the isolated compounds using spectral analysis techniques like FTIR, NMR, and GCMS. From FTIR analysis, we found the presence of characteristic absorption bands of different functional groups like hydroxyl, ether, ketone, aromatic CH group, etc. From ^{13}C-NMR analysis, we found the presence of characteristic signals corresponding to Aromatic Carbon, C=O, and C-OH group. The ^{1}H-NMR interpretation indicates the relative number of protons corresponding to each peak like the aromatic hydrogen and hydroxyl group. We found that the molecular peak ion of 207 from mass spectroscopy indicates the assumption that the proposed structure has a molecular weight of 207. After observing its fragmentation by comparing the structure with the NIST compound library, we assumed the compound might be 4-hydroxy-3-nitro coumarin. The outcomes of spectrum analysis support the underlying structures by exposing patterns, frequencies, and behaviors that point to particular characteristics or components.

Virtual screening experiments are the most convenient way to incorporate protein in the docking process by performing docking using an ensemble of static receptor conformations. Molecular docking is used in modern drug design to help understand the interaction between ligands and target protein. These techniques are supported to the design of novel drug which has specific activity by the mechanism of drug-receptor interaction. Computer-aided drug design (CADD) helps identify small molecules by orienting and scoring them in a protein's active binding site. The docking simulation technique was performed using Molegro Virtual Docker (MVD 6.0) software with enzyme glycolate Oxidase [PDB ID: 2RDT] as the protein target. This program selected the best docked based on criteria such as structure-based ligand binding position. The parameter to identify the best ligand binding position was the RMSD. In computational studies, we predicted the active sites of the enzyme glycolate oxidase after docking with inhibitor CDST. A docking score is a value that reflects the binding energy required to form a bond between the ligand and receptor, which predicts the activity of compounds. It also causes the bond between the ligand and the receptor to be more stable. The binding energy values of the isolated compound as well as the standard showed the approximate docking score -121.298 and -128.818 kcal/mol that value a comparable docking score of the isolated compound than quercetin used as a standard compound. After that, we compared the isolated compound and standard with these reported active sites. After analyzing the isolated compound with CDST and quercetin-occupied amino acids, we found that the isolated compound showed the highest affinity towards occupied amino acids in both hydrogen bonding and steric interactions. In the case of comparison with CDST three amino acids were common in hydrogen bonding and one amino acid was common in steric interaction, which showed its similar affinity and potency as standard quercetin.

After all the observations from the computational study of the isolated compound with the target enzyme glycolate oxidase, we finally came to the conclusion the isolated compound, occupied most of the amino acids in the case of CDST occupied amino acids and occupation of all the amino acids in case of standard quercetin as well, showed its highest potency against inhibition of the target enzyme glycolate oxidase and could be used as a primary ingredient in case of treatment of urolithiasis. Further verification of the result against the target enzyme, *in-vitro* enzyme inhibition assay to be conducted to prove its efficacy.

In-vitro enzyme inhibition study of isolated compounds showed good inhibitory activity when compared to the IC_{50} value of quercetin.

These results demonstrated conclusively that *B. spectabilis* has the potential to prevent and reverse urolithiasis by inhibiting the enzyme glycolate oxidase.

Conclusion

The current study confirmed that the isolated compound might be the compound 4-hydroxy-3-nitrocoumarin, isolated from column chromatographic separation of ethanolic crude extract of the plant *B. spectabilis* exhibited significant anti-urolithiasis potential in comparison to the commercially available drug cystone. In docking studies targeting the well-known urolithiasis-inducing enzyme GOX, we found isolated compound as the most effective inhibitor of this enzyme by comparing the maximum occupation of amino acids with the maximum number of hydrogen bond interactions and steric interactions to the standard and co-crystallization ligands. Additionally, *in-vitro* enzyme inhibition research confirmed our result regarding docking research.

Isolated coumarin compound is active owing to the presence of an electron-releasing group (-OH at the R position and ketone group). As reported in the literature, the hydrophobic nature of the GOX surface suggests that an electron-releasing group may bind to a receptor and exhibit significant inhibitory activity. This suggests that our isolated compound may be a good inhibitor of the GOX enzyme, confirming its action against urolithiasis. Anti-urolithiasis activity would have been aided by the plant's encouraging antioxidant activity as a result of the presence of flavonoids. Additional exhaustive target-based research must be conducted on plant extracts that may lead to a novel chemical unit for eradicating urolithiasis and preventing its recurrence. To assist the human race in eradicating urolithiasis, additional extensive pharmacological research is required to determine the precise mechanism of action of the plant material.

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References

1. Akerele OA, Estrada N, Hawkes JG. Summary of WHO guidelines for the assessment of herbal medicines, HerbalGram. 1991; 22: 13–28.
2. Jarrar N, Abu-Hijleh A, Adwan K. Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin-resistant *Staphylococcus aureus*. Asian Pacific Journal of Tropical Medicine.2010; 22(10): 121–123.
3. Bhattacharjee, Chatterjee SK, Chandra G. Isolation and identification of antibacterial components in seed extracts of *Argemone mexicana* L. (Papaveraceae). Asian Pacific Journal of Tropical Medicine. 2010; 547–551.
4. Bhimba BV, Meenupriya J, Joel EL, Naveena DE, Kumar S, Thangaraj M. Antibacterial activity and characterization of secondary metabolites isolated from mangrove plant *Avicennia officinalis*. Asian Pacific Journal of Tropical Medicine. 2010; 544–546.
5. Jeeshna MV, Manorama S, Paulsamy S, Antimicrobial property of the medicinal shrub, *Glycosmispentaphylla*. Journal Basic Applied Biology. 2009; 5 (7): 25–27.
6. Nadkarni KM, Nadkarni AK. Indian *Materia Medica*. Popular Prakashan, Bombay. 1976; 850–885.
7. Boumba VA, Mitselou A, Vougiouklakis T. Fatal poisoning from ingestion of *Datura stramonium* seeds. Veterinary and Human Toxicology. 2004; 46: 81–82.
8. Darabpour E, Motamedi H, Nejad SMS. Antimicrobial properties of *Teucrium polium* against some clinical pathogens. Asian Pacific Journal of Tropical Medicine. 2010; 23: 124–127.
9. Dorman HJ, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology. 2000; 88: 308–316.
10. Aeckart KSJ, Schroder FH. Effect of extra corporeal shock wave lithotripsy (ESWL) on renal tissue. Urol Res. 1989; 17(1):3-7.
11. Mitra SK, Gopumodhavan S, Venkataranganna MV, Sundaran R. Effect of cystone, a herbal formulation on glycolic acid–induced urolithiasis in rats. Phytother Res. 1998; 12: 372–374.
12. Kumar R, Mukherjee M, Bhandari M, Kumar A, Sidhu H, Mittal RD. Role of Oxalabacter formigenes in calcium oxalate stone disease, A study from north India. Eur Urol 2002; 41: 318–322.
13. Ghogar A, Jiraungkoorskul K, Durg S, Jiraungkoorskul W, Paper *Flower Bougainvillea spectabilis*: Update Properties of Traditional Medicinal Plant. Journal of natural remedies. 2016; 16 (3): 82-87.
14. Srivastava SK, Krishnan PS. An Oxalic Acid Oxidase in the Leaves of *Bougainvillea spectabilis*. Biochem. J, 1962; 85: 33-38.
15. Jawla S, Isolation of Antidiabetic Principle from *Bougainvillea spectabilis* Willd (Nyctaginaceae) Stem Bark. Tropical Journal of Pharmaceutical Research. October 2013; 12(5): 761-765.
16. Chhillar AK, Evaluation of antimicrobial and antioxidant activities of *Bougainvillea spectabilis*. Int J Pharm Pharm Sci. 2013; Vol 5, Suppl 3: 178-182.
17. Thushara B. Nephroprotective Activity of the Ethanolic Extract of *Bougainvillea Spectabilis* Leaves Against Gentamycin Induced Renal Dysfunction in Wistar Rats. Indo American Journal of Pharmaceutical Research.2016; 6(07): 6291-6298.
18. Rajeswari. P, Keziya priya, Bhanusree. *In Vitro* Evaluation of Anti-Urolithiatic Activity of Aqueous Extract of *Bougainvillea glabra* (Leaves). Ijppr. Human Journals, 2018; 12 (3): 409-421.
19. Das P, Vaghela J. Evaluation of *In- Vitro* Litholytic Activity of Plant *Bougainvillea Spectabilis*. J Adv Sci Res. 2021; 12 (4) Suppl 1: 311-315.
20. Das P, Vaghela JS. Evaluation of Anti-urolithiatic Activity of *Bougainvillea spectabilis* Willd. against Sodium Oxalate-induced Nephrolithiasis in Rats. International Journal of Pharmaceutical Sciences and Drug Research 2023;15(1):57-64.
21. Trease and evans. Text book of Pharmacognosy. 16th ed. Saunders edition (Elsevier). 2009; 133-148.
22. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Second edition. Springer Verlag Berlin Heidelberg *New York Tokyo*, 1996; 384.
23. Sharma BK. Instrumental methods of chemical analysis, Goel Publishing House, Meerut, 1979; 623.
24. Imam SS, Gilani SJ. Computer Aided Drug Design: A Novel Loom To Drug Discovery. Org. Med. Chem. 2017; 1(4):1-6.
25. Chhatre S, Nesari T, Somani G, Kanchan D, Sathaye S. Phyto pharmacological overview of *Tribulus terrestris*. Phcog Rev. 2014;8: 45-51.
26. Shirfule. Exploring glycolate oxidase (GOX) as an antiurolithic drug target: Molecular modeling and in vitro inhibitor study. International Journal of Biological Macromolecules. 2011; 49: 62–70.
27. Das P, Vaghela J, Badore N. Pharmacognostical, Phytochemical and Fluorescence analysis of the plant *Bougainvillea spectabilis* (Willd.). Research J. Pharm. and Tech.2021; 14(7): 3733-3738.

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