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Original article

Density functional theory (DFT), molecular docking, and xanthine oxidase inhibitory studies of dinaphthodiospyrol S from *Diospyros kaki* L

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ABSTRACT

In this work, we investigated *Diospyros kaki* extract and an isolated compound for their potential as xanthine oxidase (XO) inhibitors, a target enzyme involved in inflammatory disorders. The prepared extract was subjected to column chromatography, and dinaphthodiospyrol S was isolated. Then XO inhibitory properties were assessed using a spectrophotometry microplate reader. DMSO was taken as a negative control, and allopurinol was used as a standard drug. The molecular docking study of the isolated compound to the XO active site was performed, followed by visualization and protein–ligand interaction. The defatted chloroform extract showed the highest inhibitory effect, followed by the chloroform extract and the isolated compound. The isolated compound exhibited significant inhibitory activity against XO with an IC_{50} value of 1.09 μM. Molecular docking studies showed that the compound strongly interacts with XO, forming hydrogen bond interactions with Arg149 and Cys113 and H-pi interactions with Cys116 and Leu147. The binding score of −7.678 kcal/mol further supported the potential of the isolated compound as an XO inhibitor. The quantum chemical procedures were used to study the electronic behavior of dinaphthodiospyrol S isolated from D. *kaki*. Frontier molecular orbital (FMO) analysis was performed to understand the distribution of electronic density, highest occupied molecular orbital HOMO, lowest unoccupied molecular orbital LUMO, and energy gaps. The values of HOMO, LUMO, and energy gap were found to be −6.39, −3.51 and 2.88 eV respectively. The FMO results indicated the intramolecular charge transfer. Moreover, reactivity descriptors were also determined to confirm the stability of the compound. The molecular electrostatic potential (MEP) investigation was done to analyze the electrophilic and nucleophilic sites within a molecule. The oxygen atoms in the compound exhibited negative potential, indicating that they are favorable sites for electrophilic attacks. The results indicate its potential as a therapeutic agent for related disorders. Further studies are needed to investigate this compound's in vivo efficacy and safety as a potential drug candidate.

1. Introduction

The most selective and accurate production of various bioactive substances occurs in medicinal plants' factories of phytochemicals (Uddin et al., 2011). Throughout the Pakistan, there are more than 6000 distinct species of wild plants, and 400 to 600 of them are regarded to

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have medicinal properties. Over the past ten years, there has been a huge global growth in the value of conventional medical systems. Although modern medicine may be available in many developing countries, current estimates show that a sizeable fraction of the population in these countries still predominantly relies on traditional healers. Because of historical and cultural influences, herbal remedies typically continue to be popular (Bibi et al., 2015). Many secondary metabolites have been studied and are essential components of contemporary therapy. Because plant-based medications are safer and more efficient, several plants are being researched for their potential as new medicines. Diospyros kaki is a member of the Ebenaceae family, sometimes known as Japanese or Oriental persimmon (Xie et al., 2015). The 500-species strong Ebenaceae family is extensively distributed in the tropics and subtropics. The genus Diospyros (Syn: persimmon, ebony), which has more than 249 species, is the most significant both numerically and economically. The Chinese-born persimmon (*Diospyros kaki*), one of them, has a long and storied history of usage in traditional remedies. It was also claimed to be the species that produces the most fruit. (Mallavadhani et al., 1998). In Korea, China, Japan, and India, persimmon was commonly available (Funayama and Hikino, 1979). Persimmon leaves have diverse biological and pharmacological qualities, making them useful as a medication, health drink, and cosmetic. Ancient Chinese medical texts mention the utilization of persimmon leaves for traditional medicine. The use of persimmon leaves as a home medicine for chronic lower thigh ulcers dates back to Lan Mao's 1556 Ming period work Diannan Bencao. Bencao Zaixin, a book of Chinese materia medica, was published in 1841 (Qing dynasty) (Xie et al., 2015) and stated that cough, hematemesis, promoting salivation, and quenching thirst could all be treated with persimmon leaves. Consequently, persimmon leaves might be used to cure lung distension (also known as "Fei zhang" in traditional Chinese theory), according to Fen Lei Cao Yao Xing, a medical treatise written in the late Qing Dynasty (Xie et al., 2015). Persimmon leaves have historically been used to treat heart illness, cerebral arteriosclerosis, hypertension, cough, and internal bleeding in Chinese herbal remedies, according to the Guangxi standard of the 1990s (Han et al., 2002). The Naoxinqing pill, a patented and authorized Traditional Chinese Medicine (TCM) medication used to treat cerebral arteriosclerosis, has been listed in the Chinese Pharmacopeia 2010. It is manufactured from an extract of persimmon leaves (Pei et al., 2013).

Due to the high vitamin C content of persimmon leaves, it is well known as an anti-aging and nutrient-rich tea in Japan, which boosts sales (Tsurunaga et al., 2011, Martínez-Las Heras et al., 2016). Another discovery regarding persimmon leaves is that when consumed as a tea, the flavonoids are digested by intestinal bacteria and subsequently absorbed into the blood from the gastrointestinal system (Bei et al., 2009, Zhang et al., 2015). The vitamin C content of persimmon tea has also been shown to be helpful, at least partially replacing the pricey citrus fruits. This tea has a taste that is pleasant and uplifts individuals. While tea prepared from green leaves is quite good, tea made from dried leaves is superior and has a flavor that is somewhat similar to sassafras (Gibbons and McPhee, 1962). According to Diannan Bencao, consuming persimmon leaves during the day and washing your face with persimmon leaf decoction would significantly lighten your complexion. As a result, it is known as "beautiful leaves" in Taiwan and may one day be used in a skin carefulness product. In East Asia, persimmon leaves were also utilized as a healthy dietary addition. Japanese persimmon leaf extract and jasmine use in creating anti-tobacco sweets in Japan is noteworthy (Mallavadhani et al., 1998). *Diospyros kaki*, also known as the Japanese persimmon, is a widely cultivated fruit tree traditionally used in Chinese and Korean medicine to treat various diseases, including hyperuricemia. It possesses multiple secondary metabolites, including vitamins, tannins, flavonoids, sugars, lipids, and terpenoids (Mallavadhani et al., 1998). The leaves of Diospyros kaki have also been found to contain carotenes, kryptoxanthin, lignin, resins, chlorophyll, hemicelluloses, polysaccharides, and amino acids. Previous studies have reported the XO inhibitory activity of various parts of *Diospyros kaki*, including leaves, fruits, and stems. Dinaphthodiospyrol S is a natural compound isolated from the stem bark of *Diospyros kaki*, which has been reported to exhibit various pharmacological activities, including antioxidant, anti-inflammatory, and antitumor activities (Rauf et al., 2020). However, the XO inhibitory activity of dinaphthodiospyrol S and its underlying mechanism of action has not been fully elucidated (Singh and Joshi, 2011).

In this study, we aimed to evaluate the XO inhibitory activity of the extract from *Diospyros kaki* and dinaphthodiospyrol S and to investigate their binding modes and interactions with XO using molecular docking simulations. Our findings could provide valuable insights into the potential use of *Diospyros kaki* and its natural compounds as XO inhibitors for hyperuricemia treatment. The scheme of the conducted study is shown in Fig. 1.

2. Materials and methods

2.1. Plant collection

Diospyros kaki roots used in this study were collected from the village Razagram, Khall Dir (Lower), in July 2021. The plant specimen was identified by a plant taxonomist in the Department of Botany, University of Swabi, Khyber Pakhtunkhwa, Pakistan. The voucher specimen NO. UOS/ Bot-43 was stored in the herbarium of the Department mentioned above.

2.2. Extraction and isolation of compound

The plant's roots having a total weight of 8.09 kg were collected. The collected plant material was dried for two weeks at room temperature under shade and ground with the help of a grinder machine. The powder plant material (8.00 kg) was subjected to extraction using chloroform which afforded chloroform extract. The obtained extract was concentrated at low pressure and temperature, yielding dark red crude extract (176.34 g). The crude extract was defatted with hexane through a soxhlet apparatus to remove the fixed oil and dyes, which afforded defatted chloroform extract (110.16 g). From the defatted extract, 20 g was subjected to column chromatography (250 mm long by 4 to 5 mm in internal diameter). The column was eluted with hexane and ethyl acetate, which yielded 120 subfractions (AFF1-AFF120). Based on the TLC profile the sub-fraction AFF12-AFF20 was combined which afforded subfraction SM1 the SM1 was subjected to repeated column chromatography which yielded dinaphthodiospyrol S (80.12 mg). The chemical structure of dinaphthodiospyrol S was reported previously by our research group (Rauf et al., 2020) as shown in Fig. 2.

2.3. Enzyme inhibition activities

2.3.1. Xanthine oxidase inhibitory properties

The A spectrophotometry (SpectraMax340) microplate reader tested the enzyme inhibitor effecte XO (EC1.1.3.22) was used as a milk source, and the legend used was xanthine (Alam et al., 2021). The reaction mixture consisted of 10 μL of one mmol/L^{-1} of the pure sample, which was dissolved in DMSO, 125 μL of phosphate buffer (0.1 mol/L) with pH of 7.4, the XO (0.003 units) was mixed in buffer (25 μL) and the same volume of xanthine with mixed. This mixture was incubated for 10 min and pre-read in the UV region. After adding Xanthine nine readings for 30 min with equal intervals were taken. The DMSO was taken as a negative control, and allopurinol was used as the standard drug. The IC_{50} was calculated using EZ-FIT software, while the percent effect was calculated using the following formula.

$$Percent\ effect = 100 - \frac{\text{OD test sample}}{\text{OD Control}} X100$$

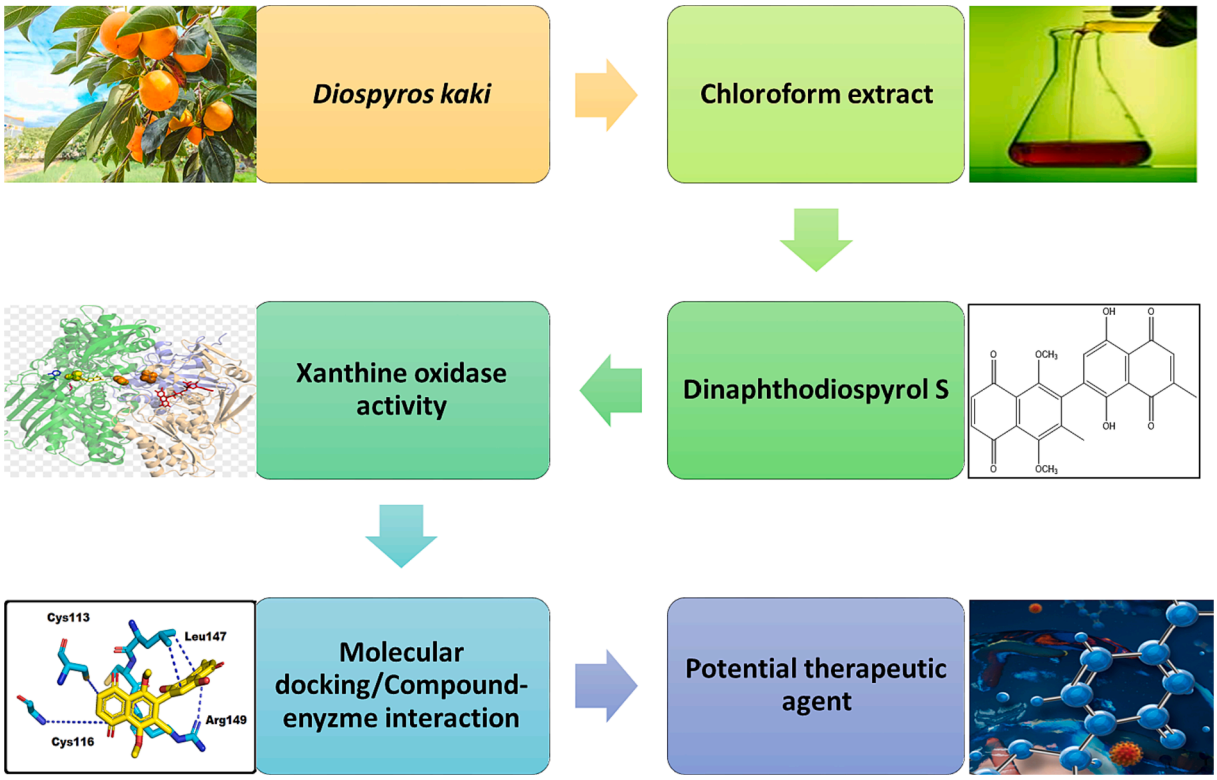

Fig. 1. The scheme of study of this work.

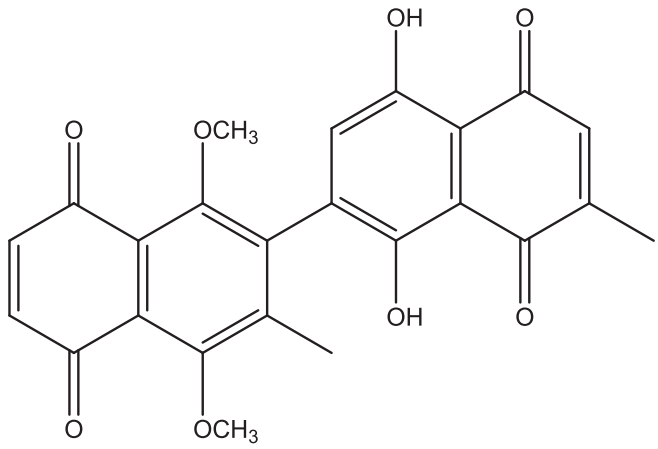

Fig. 2. Structure of dinaphthodiospyrol S isolated from *D. kaki.*

2.4. Molecular docking

The molecular docking study of the isolated compound into the active site of xanthine oxidase was performed to determine the best binding pose of the isolated compound by utilizing Molecularthe Operating Environment (MOE) (Inc, 2016). The three-dimensional structure of xanthine oxidase with PDB ID (3NVY) was extracted from the protein data bank (https://www.rcsb.org) (Santi et al., 2018). The water molecules were removed adequately before the three-dimensional protonation and minimization steps. In addition, the ligand atoms were set to flexible for determining low-energy protein–ligand complexes during molecular docking (Shamim et al., 2020). Finally, protein–ligand visualization and interaction were carried out using Pymol v.1.7 (Peele et al., 2020).

2.5. Computational procedure

All the calculations were performed using the Gaussian 09 program, and for visualizing the results, GaussView 5.0 software was employed. Additionally, GaussView 5.0 was used to generate input files for the research. First of all, the optimization of the structure was done through density functional theory (DFT) calculations, employing the 6-311G(d, p) basis set and the ωB97XD functional. Frequency analysis is conducted using the same level of theory to confirm the validity of the structure as the true minima on the potential energy surface (PES). The frontier molecular orbital FMO and the molecular electrostatic potential (MEP) calculations were performed at B3LYP/6311G(d,p) level of theory to investigate the electronic structure and chemical and physical properties respectively. Furthermore, the global chemical reactivity descriptors were also calculated to understand the stability of the compound.

3. Results

3.1. Xanthine oxidase inhibitory effect

Various extracts of *D. kaki* and one of the isolated compounds (Dinaphthodiospyrol S) demonstrated variable degrees of xanthine oxidase inhibitory effect, as shown in Table 1. The results from the table suggest that the extracts of *D. kaki* and the isolated compound Dinaphthodiospyrol S possess significant inhibitory potential against xanthine oxidase, an enzyme responsible for the production of uric acid. Among

Table 1
XO inhibitory activity of extracts and dinaphthodiospyrol S from *D. kaki.*

Samples	Concentration	% Effect	IC_{50}
Chloroform extract	50 μg/mL	78.32	64.09 ± 2.88 μg/mL
Defatted chloroform extract	50 μg/mL	85.98	25.98 ± 2.09 μg/mL
Dinaphthodiospyrol S	0.5 mM	86.43	1.09 ± 1.08 μM
Allopurinol	0.5 mM	98.01	0.890 ± 0.02 μM

the extracts tested, the defatted chloroform extract showed the highest percent effect (85.98 %) with an IC_{50} value of 25.98 μg/mL, indicating its potential as a xanthine oxidase inhibitor. Similarly, the isolated compound Dinaphthodiospyrol S demonstrated a significant inhibitory effect (86.43 %) with an IC_{50} value of 1.09 μM, indicating its potential as a lead molecule for further drug development.

Moreover, the positive control drug allopurinol exhibited potent inhibitory activity against xanthine oxidase with a high percent effect (98.01 %) and low IC_{50} value (0.890 ± 0.02 μM), confirming the reliability of the assay used in this study.

A lower IC_{50} value indicates a higher potency, suggesting that a relatively lower concentration of the compound is required to achieve a substantial inhibitory effect.

These findings support the traditional use of *D. kaki* as a medicinal plant for treating hyperuricemia and related diseases.

3.2. Molecular docking analysis

Molecular docking analysis is a computational method that predicts the binding orientation of a ligand (small molecule) within the binding site of a protein. In this study, the isolated compound from *Diospyros kaki* was subjected to molecular docking analysis to investigate its binding interactions with xanthine oxidase. The results of the molecular docking analysis revealed that the isolated compound had a significant interaction with xanthine oxidase. The compound formed one hydrogen bond acceptor interaction with Arg149, which is an essential residue for xanthine oxidase activity. Additionally, the compound formed three H-pi interactions with Cys116 and Leu147, important hydrophobic residues in the active site of xanthine oxidase. Furthermore, Cys113 formed one hydrogen bond donor interaction with the isolated compound.

The binding score obtained from the molecular docking analysis was −7.678 kcal/mol, which suggests that the isolated compound has significant inhibitory potential against xanthine oxidase. The binding pattern and interaction of the protein–ligand complex are listed in Table 2.

The binding orientation of the isolated compound in the active site of xanthine oxidase is depicted in Fig. 3.

3.3. Geometry optimization

Geometry optimization was carried out at ωB97XD/6-311G(d,p) level of DFT. ωB97XD functional is regarded as the optimal choice for non-covalent interactions (Alkhalifah et al., 2022). The bond lengths between C–C, C-,O and C = C are 1.48, 1.35, and 1.21 Å respectively. The optimized geometries are true minima, as evidenced by the absence of any negative frequencies (Ullah et al., 2022). The optimized geometry is shown in Fig. 4.

3.4. Frontier molecular orbital (FMO) analysis

Frontier molecular orbitals (FMOs), i-e the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), and their energy gap, are crucial in quantum chemistry, providing vital insights into molecular behavior, properties, and reactivity (Ali et al., 2020). The electronic structure of the compound was

Table 2
Docking score and protein–ligand interaction of isolate compound with XO.

Carbon atom	Interacting residues	interaction type	Distance	Energy (kcal/mol)	Docking score
C2	Cys113	H-donor	4.40	−0.8	−7.678
027	Arg149	H-aaceptor	3.35	−0.5	
6-ring	Cys116	pi-H	4.64	−0.4	
6-ring	Leu147	pi-H	4.35	−0.5	
6-ring	Leu147	pi-H	3.70	−0.6	

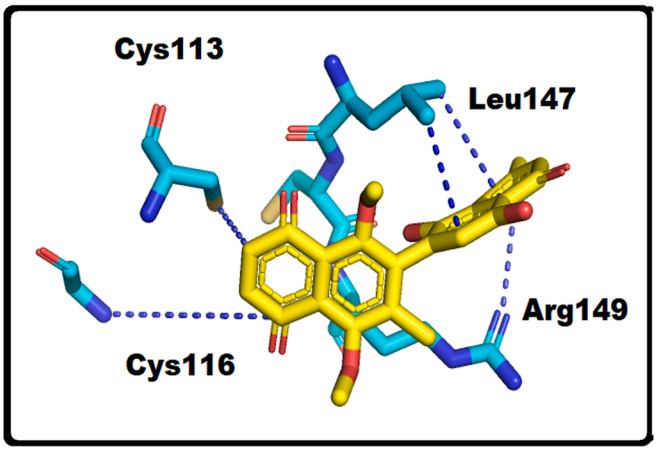

Fig. 3. The isolated compound interacted strongly and significantly with the active site of XO with a docking score (-7.678 kcal/mol).

investigated using B3LYP the functional of DFT with a 6-311G(d,p) basis set. The energy of HOMO, LUMO and energy gap (E_{gap}) between the HOMO and LUMO are displayed in Fig. 5 and summarized in **Table-3**. The negative energy values of HOMO and LUMO confirmed the stability of the compound (Rasool et al., 2022).

3.5. Global chemical reactivity descriptors (GCRD)

The energy levels of the HOMO and LUMO are frequently linked to the ionization potentials and electron affinities of the systems. These energy differences are used to determine various significant properties, including softness, hardness, ionization energy, electron affinity, electronegativity as well as electrophilicity index. The HOMO-LUMO gap of a substance serves as an indicator of its hardness, with a harder molecule having a more substantial energy gap (Muhammad et al., 2019). The hardness (η) is calculated as

$$\eta = \frac{I - A}{2} \tag{1}$$

Where $I = -E_{HOMO}$ and $A = -E_{LUMO}$ respectively. The softness (S) of a molecule is determined as

$$S = \frac{1}{2\eta} \tag{2}$$

Equations (3) and (4) can be used to compute the electron affinity (A) and ionization energy (I) values respectively

$$A = -E_{LUMO} \tag{3}$$

$$I = -E_{HOMO} \tag{4}$$

The values of electronegativity (χ), chemical potential (μ) and electrophilicity index (ω) were calculated using Eqs. (5), 6 and 7

$$\chi = \frac{I + A}{2} \tag{5}$$

$$\mu = -\left(\frac{I + A}{2}\right) \tag{6}$$

$$\omega = \frac{\mu^2}{2\eta} \tag{7}$$

The ionization energy (I) of the compound is found to be 6.39 eVwhicht is indicative of its thermal stability. The electron affinity value is equal to 3.51 eV which is less than ionization energy, indicating a reasonable attraction for electrons. The value of hardness is 1.44 eV which is greater

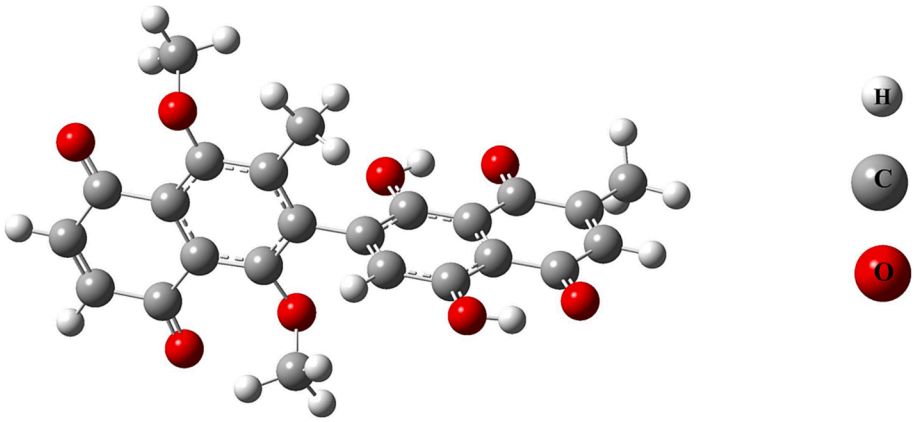

Fig. 4. Stable optimized geometry of Dinaphthodiospyrol S at ωB97XD/6-311G(d,p) level of DFT.

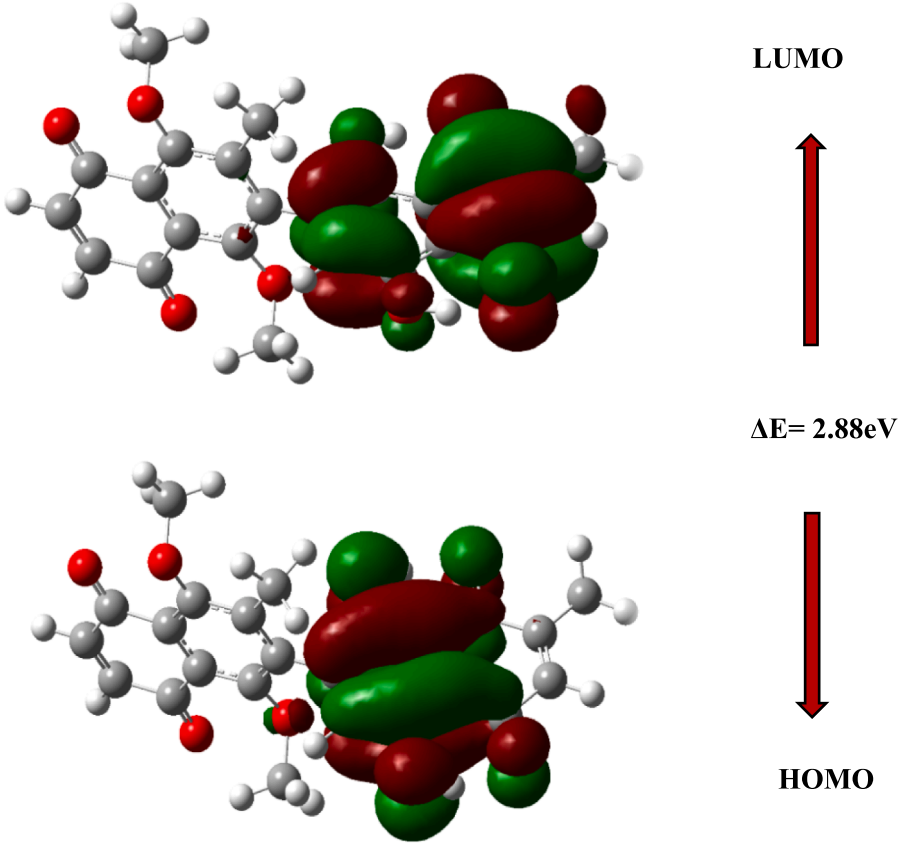

Fig. 5. Frontier Molecular Orbitals of dinaphthodiospyrol S.

than the value of softness which is calculated to be 0.35 $e^{-1}V^{-1}$. The energy gap of 2.88 eV is associated with greater hardness, lower reactivity, and higher stability. Stability is also directly related to the chemical potential. The values of chemical potential and electronegativity index are calculated to be −4.95 and 4.95 eV, respectively. A high value of electrophilicity index i-e 8.50 eV indicates that the molecule has a more electrophilic character. The values of chemical reactivity descriptors are shown below in Table 3.

3.6. Molecular electrostatic potential (MEP)

The molecular electrostatic potential (MEP) plot is a valuable tool for investigating the chemical and physical properties of a chemical system. It helps to identify potential electrophilic and nucleophilic sites on molecular structures, with colors indicating the magnitude of electrostatic potential. The decreasing order of potential magnitude is blue > green > yellow > orange > red (Alarfaji et al., 2022, Rasool et al., 2023).

$$V(r) = \sum \left(\frac{Z_A}{R_A} - r \right) - \int p \frac{(\mathrm{r}')}{(\mathrm{r}' - \mathrm{r}) d\mathrm{r}'} \tag{8}$$

The red regions represent negative potential, proposing susceptibility to electrophilic attacks, while blue regions indicate highly positive potential, signifying nucleophilic sites. In the current study, oxygen atoms in the compound exhibited negative potential, making them favorable for electrophilic attacks as shown in the Fig. 6.

Table 3
The calculated global chemical reactivity descriptors (GCRD) at ωB97XD/6-311G level of theory.

Parameters	eV
E_{HOMO}	−6.39
E_{LUMO}	−3.51
Band Gap	2.88
Ionization Energy (I)	6.39
Electron Affinity (A)	3.51
Chemical Hardness (η)	1.44
Chemical potential (**μ**)	−4.95
Electronegativity index (***X***)	4.95
Electrophilicity index (ω)	8.50
Softness (S) $e^{-1}V^{-1}$	0.35

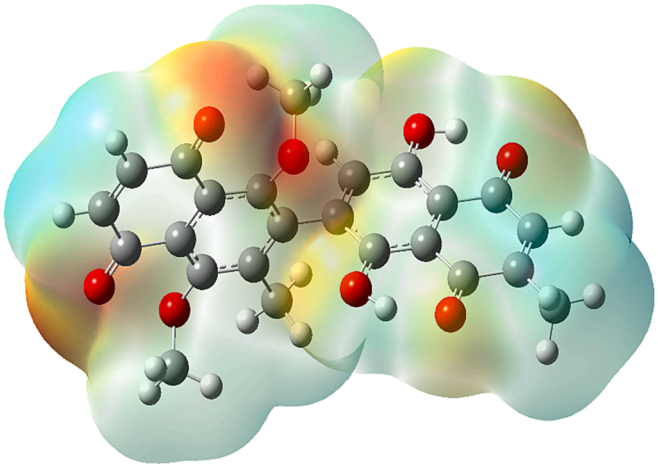

Fig. 6. MEP analysis of compound (Dinaphthodiospyrol S).

4. Discussion

Natural products are the primary source of therapy for various ailments. These natural products are considered safe and effective by most of the world's population (Tariq et al., 2021, Rahman et al., 2023). Due to this perception, the demand, as well as usage of plant-based therapeutic agents, is increasing with time in developing and developed countries, and a lot of natural products are developed as new drugs for the treatment of various disorders such as skin (Marcarino et al., 2022, Saldívar-González et al., 2022). *Diospyros kaki*, also known as the persimmon tree, is a plant that has been traditionally used in oriental medicine to treat various ailments. This plant contains a variety of bioactive secondary metabolites, including naphthoquinone, which has been identified as having potential therapeutic properties. Naphthoquinone is a class of natural compounds found in many plants. It has been found to possess a range of therapeutic properties, including antioxidant, antimicrobial, and anticancer properties, making it a potential candidate for treating a variety of diseases. One promising therapeutic use of naphthoquinone is as an inhibitor of xanthine oxidase, an enzyme involved in inflammatory disorders such as gout and cardiovascular diseases. While traditional drugs such as allopurinol or febuxostat are effective in treating gout, they may lead to poor patient compliance due to their prolonged use. *Diospyros kaki* extract has been reported to significantly lower uric acid levels, making it a good indication for gout treatment. Moreover, research is ongoing to explore the potential of XO inhibitors as a treatment for hypoxic-ischemic encephalopathy (HIE), a neurological disorder that affects the brain. *Diospyros kaki* has varieties of bioactive secondary metabolites, including naphthoquinone. Naphthoquinones are a class of natural compounds found in many plants and have been identified as having a range of therapeutic properties. One such compound is naphthoquinone isolated from the plant *Diospyros kaki*, also known as the persimmon tree. Studies have shown that this compound has potential therapeutic uses, including as an inhibitor of xanthine oxidase, an enzyme involved in inflammatory disorders. XO inhibitors have been used to treat gout and cardiovascular diseases. Additionally, naphthoquinones have been found to have antioxidant, antimicrobial, and anticancer properties, making them potential candidates for the treatment of a variety of diseases. *Diospyros kaki* extract has been traditionally used in oriental medicine to treat various ailments, and the isolation of naphthoquinone from the plant adds to its potential as a therapeutic agent. In the current research, *Diospyros kaki* was subjected to an XO inhibitory effect. Various extract and one of the isolated compounds were tested for anti-XO effect. The most common human health issue with XO is gout. This painful disorder is treated with allopurinol or febuxostat (O'Dell et al., 2022). Although both are safe and effective drugs, they fail to eradicate hyperuricemia. So, the prolog use of these medications affects the human lifestyle and ultimately leads to poor patient compliance. The search for safe, effective, and economical natural products is essential to resolve these issues. *Diospyros kaki* has been reported with significant ($p < 0.001$) lowering of uric acid (Shahat et al., 2022), which is a good indication for the curing of gout. This metabolic disorder is attributed to the elevated level of uric acid (Osada et al., 1993). Research is also in progress that XO inhibitors might be a potential drug candidate for curing hypoxic-ischemic encephalopathy (HIE) (Chaudhari and McGuire, 2012). In the current research, our tested samples significantly antagonized the XO, indicating that these extracts and isolated compounds might prove a good XO antagonist in the treatment of hyperuricemia and HIE. However, a detailed study is recommended of these tested samples agonist the aforementioned human disorders. Molecular docking analysis was applied to understand the binding affinity and interaction between a protein and a ligand molecule. The compound "Dinaphthodiospyrol S" was thoroughly analyzed through an optimization process using the DFT method, employing the ωB97XD functional and a 6-311G(d, p) basis set. The electronic properties of the studied compound were investigated at the B3LYP/6-311G(d, p) level of theory. The energy values of HOMO and LUMO provide insights into charge transfer within the molecule. A molecule with a HOMO that has higher energy tends to exhibit a more reactive nature. Likewise, a lower energy LUMO is favorable for nucleophilic reactions. The higher HOMO energy values of Dinaphthodiospyrol S suggest that the compound is inclined to effectively donate electrons to acceptor molecules possessing low-energy molecular orbitals. Additionally, lower LUMO energies signify that Dinaphthodiospyrol S is also prone to receiving electrons. The visual representation of FMO demonstrates that the electronic density of HOMO is distributed widely over dihydroxy benzene moiety and methyl-p-benzoquinone, while LUMO density is shifted slightly towards the methyl group of methyl substituted para benzoquinone that is an indication of intramolecular charge transfer. In the calculation of chemical reactivity descriptors, the results revealed high value of ionization energy hence the stability of the compound. There is an inverse relationship between chemical reactivity and the global softness of compounds with their energy gap (E_{gap}) (Muhammad et al., 2021). Conversely, global hardness and stability are positively correlated with the energy gap in the studied molecule. The results demonstrated that the value of hardness is greater than that of softness indicating stability of the system. Chemical potential reflects the inclination of electrons to escape, while electronegativity signifies the ability of the compound to accept electrons. The negative values of chemical potential demonstrate the stability of the compound while the high values of electronegativity are representative of the ability to attract electrons. The global electrophilicity index (ω) measures the tendency of a system to accept electrons (Vektariene et al., 2009). The higher value in this case specifies the electrophilic character of the studied compound Furthermore, the protein–ligand interaction pattern obtained from the docking analysis showed that the isolated compound formed one hydrogen bond acceptor interaction with Arg149, three H-pi interactions with Cys116 and

Leu147, while Cys113 formed one hydrogen bond donor. These interactions play a crucial role in determining the stability and strength of the complex formed between the protein and ligand. Overall, the molecular docking analysis results support the inhibitory potential of the isolated compound against xanthine oxidase, which can have implications for the development of new drugs for related disorders.

5. Conclusions

The present study reveals the potential of *Diospyros kaki* extract and an isolated compound as inhibitors of xanthine oxidase, with the isolated compound demonstrating significant inhibitory activity against the enzyme. We employed quantum chemical techniques to investigate the electronic properties of dinaphthodiospyrol S, a compound isolated from D. *kaki*. Our study involves frontier molecular orbital (FMO) analysis, which helps us to comprehend the distribution of electronic density, to identify the highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), and to determine the energy gap. Specifically, we obtain the values for HOMO, LUMO, and the energy gap to be −6.39 eV, −3.51 eV, and 2.88 eV, respectively with intramolecular charge transfer. Additionally, we calculated chemical reactivity descriptors to confirm the compound's stability. Furthermore, our investigation of molecular electrostatic potential (MEP) reveals electrophilic and nucleophilic sites within the molecule. Notably, the oxygen atoms in the compound display a negative potential, signifying their susceptibility to electrophilic attacks. The molecular docking results suggest the compound forms strong interactions with the enzyme, indicating its potential as a therapeutic agent for inflammatory disorders. Further studies are needed to explore this compound's in vivo efficacy and safety and to study the toxicity to use it as a potential drug candidate for treating inflammatory diseases.

Disclosure statement

The authors report there are no competing interests to declare.

Author's contribution

Tareq Abu-Izneid contributions to the conception, and study design, Abdur Rauf, Zubair Ahmad, acquisition, analysis, isolation, interpretation of the obtained data, Abdul Wadood, docking analysis Naveed Muhammad, experimental of biological screening, Yahya S. Al-Awthan, Omar S. Bahattab, Characterization and analysis, Hassan A. Hemeg, Saima Naz, Dorota Formanowicz Analysis, formatting, editing. All authors took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the journal; gave final approval of the version to be published; and agreed to take responsibility and be accountable for the contents of the work.

Additional information

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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