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INVESTIGATIONS ON THE INCLUSION OF BETULINIC ACID INTO CD-MOF-1 PERFORMED BY QUANTUM MECHANICS CALCULATIONS AND MOLECULAR DOCKING SIMULATION

(Kajian Kemasukan Asid Betulinik ke dalam CD-MOF-1 Melalui Pengiraan Mekanik Kuantum dan Simulasi Dok Molekul)

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Abstract

In recent years, CycloDextrin Metal-Organic Frameworks (CD-MOFs) have been investigated to develop potential drug carriers and improve the solubility of some molecules by their encapsulation. Betulinic acid (BA) or 3β-hydroxy-lup-20(29)-en-28-oic acid is a pentacyclic triterpene of the lupane family which has a wide range of biological activities, and it is considered a promising candidate for clinical application but, its high hydrophobicity and limited aqueous solubility contribute to its poor bioavailability. In this work, we show through computational studies that CD-MOF-1 can strongly encapsulate betulinic acid molecules through hydrogen bond interactions. For this purpose, the optimized geometry of BA was implemented by the Density Functional Theory using the function of Becke, Lee, Yang, and Parr (DFT/B3LYP) method with a 6-31+G(d) basis set using the Gaussian09 program and GaussView 5.0 for visualization. We noticed that BA has a lower energy gap (Egap = 6.1615 eV) indicating that it is soft, less stable, more reactive, and polarizable. According to the map of electrostatic potential (MEP), the active site of betulinic acid is the carboxylic group and the latter molecule preferred electrophilic attacks. The molecular docking was performed using AutoDock Vina v1.1.2 program and Discovery Studio Visualizer 16.1. The best binding affinities of BA and CD-MOF-1 had the lowest values of -8.2 KCal/mol and -11.5 KCal/mol for the simple and the packed CDMOF-1 structure respectively. The docking revealed that BA bound in the hydrophobic cavities of CD-MOF-1 through hydrogen bonds interactions which are [CD-MOF-$1_{OH13\cdots O28}$BA (2.95 Å)], [CD-MOF-$1_{O13\cdots H30}$BA (2.54 Å)], and [CD-MOF-$1_{OH12\cdots H30}$BA (2.12 Å)] for the simplest structure and [CD-MOF-$1_{OH12A\cdots O28}$BA (3.00 Å)], [CD-MOF-$1_{H13A\cdots H30}$BA (2.72 Å)] and [CD-MOF-$1_{OH2A\cdots H30}$BA (2.66 Å)] for the packed structure 1×1×1. The packed CD-MOF-1 structure is the best with accurate results, this may be the factor of enhancement of betulinic acid solubility and bioavailability. The present theoretical results indicate the possibility of forming the host-guest inclusion complex between BA and CD-MOF-1 which may enhance the solubility of BA and then its efficiency for drug delivery.

Keywords: CD-MOF, betulinic acid, quantum mechanics, molecular docking, solubility

Abstrak

Kebelakangan ini, pembangunan kerangka kerja logam-organik siklodekstrin (CD-MOF) telah dikaji sebagai pembawa ubat yang berpotensi dan meningkatkan keterlarutan beberapa molekul melalui pengkapsulan mereka. Asid betulinik (BA) atau asid 3β-hidroksi-lup-20(29)-en-28-oik ialah triterpena pentasiklik daripada keluarga lupana yang mempunyai pelbagai aktiviti biologi, dan ia dianggap sebagai calon yang berpotensi untuk aplikasi klinikal, namun hidrofobisitinya yang tinggi dan keterlarutan dalam air yang terhad menyumbang kepada bioavailabilitinya yang lemah. Dalam kajian ini, kajian pengiraan menunjukkan bahawa CD-MOF-1 boleh memerangkap molekul asid betulinik dengan kuat melalui interaksi ikatan hidrogen. Untuk tujuan ini, geometri BA yang optimum telah dilaksanakan melalui kaedah Teori fungsi ketumpatan menggunakan fungsi Becke, Lee, Yang, dan Parr (DFT/B3LYP) dengan set asas 6-31+G(d) menggunakan program Gaussian09 dan GaussView 5.0 untuk visualisasi. Kami mendapati bahawa BA mempunyai jurang tenaga yang lebih rendah (Egap = 6.1615 eV) menunjukkan bahawa ia lembut, kurang stabil, lebih reaktif dan boleh dipolarisasi. Menurut peta potensi elektrostatik (MEP), tapak aktif asid betulinik ialah kumpulan karboksilik dan molekul terakhir lebih cenderung kepada serangan elektrofilik. Pendokkan molekul telah dilakukan menggunakan program AutoDock Vina v1.1.2 dan Discovery Studio Visualizer 16.1. Perkaitan pengikatan terbaik BA dan CD-MOF-1 mempunyai nilai terendah -8.2 KCal/mol dan -11.5 KCal/mol untuk struktur CDMOF-1 yang ringkas dan padat. Pendokkan mendedahkan bahawa BA terikat dalam rongga hidrofobik CD-MOF-1 melalui interaksi ikatan hidrogen iaitu [CD-MOF-$1_{OH13\cdots O28}$BA (2.95 Å)], [CD-MOF-$1_{O13\cdots H30}$BA (2.54 Å)], and [CD-MOF-$1_{OH12\cdots H30}$BA (2.12 Å)] untuk struktur paling ringkas dan [CD-MOF-$1_{OH12A\cdots O28}$BA (3.00 Å)], [CD-MOF-$1_{H13A\cdots H30}$BA (2.72 Å)] and [CD-MOF-$1_{OH2A\cdots H30}$BA (2.66 Å)] untuk struktur tersusun 1×1×1. Struktur CD-MOF-1 tersusun adalah yang terbaik dengan hasil yang tepat, yang berkemungkinan faktor peningkatan keterlarutan asid betulinik dan bioavailabiliti. Keputusan teori semasa menunjukkan kemungkinan pembentukan kompleks kemasukan hos antara BA dan CD-MOF-1 yang boleh meningkatkan keterlarutan BA dan kemudian kecekapannya untuk penghantaran ubat.

Kata kunci: CD-MOF, asid betulinik, mekanik kuantum, dok molekul, keterlarutan

Introduction

For millennia, natural products have been used as a source of natural bioactive compounds for the medication and treatment of various sicknesses [1]. Alkaloids, steroids, quinones, limonoids, flavonoids, as well as another significant component, terpenoids, are all being explored in medicine for their potential to improve health. Based on their chemical skeleton, terpenoids can be classified as monoterpenes, sesquiterpenes, diterpenes, triterpenoids, and steroids [2]. Triterpenes molecules, in particular, are the most abundant terpenoids and their study has increased dramatically during the last decade due to their different kinds of bioactivities [3], including liver protection, antioxidant, anti-cancer, immunomodulatory, anti-inflammatory, and anti-obesity [2, 4]. Betulinic acid is one of this family's most frequently studied compounds [3]. Indeed, betulinic acid (BA) or 3β-hydroxy-lup-20 (29)-en-28-oic acid is a pentacyclic triterpene of the lupane family isolated primally from the bark of white birch plants [5] (Figure 1). BA has a wide range of biological activities such as its cytotoxic, anti-inflammatory, antiviral (especially anti-HIV), antimalarial, anti-tumor, antibacterial, and antioxidant activities [6, 7]. Its first anticancer effect against melanoma was reported by Pisha et al. [8]. A main advantage of BA and its derivatives is that they are cytotoxic to different human tumor cells, while cytotoxicity is much lower in normal cells [9]. Many subsequent investigations have discovered that BA was demonstrated to be a very promising anticancer agent against various tumor cell lines such as breast, colon, lung, and brain [10, 11].

Therefore, betulinic acid is considered a promising candidate for clinical application [12, 13]. Despite its strong cytotoxic effect, BA exhibits low water solubility, characterized by high hydrophobicity leading to poor bioavailability [5, 10], it belongs to the Biopharmaceutics Classification System (BCS) class IV that is characterized by both low solubility and permeability [14]. To overcome these inconveniences, several approaches for improving the physicochemical and pharmacokinetic profile of the hydrophobic small drug molecules have been conducted such as cocrystallization, amorphous solid dispersions, liposomes, solubilization in co-solvents, complexation with β-cyclodextrin and nanoemulsions [6, 15, 16]. Although these approaches can enhance performance over pure drugs, they all have drawbacks, most of which

are linked to the drug's chemical stability and physical stability against crystallization [17].

A general approach to improve drug solubility can be demonstrated by incorporating such active molecules into appropriate Metal-Organic Frameworks (MOFs) [15]. Since 1999, when Yaghi et al. named materials connected by metal-ligand coordination as Metal-Organic Frameworks (MOFs), they have rapidly become a hot topic of research [18]. Indeed, MOFs are novel porous materials with ultrahigh surface areas, tunable pores, high crystallinity, designable crystal structures, permanent porosity, and abundant metal sites which have shown huge potential applications in various fields, including gas sorption and separation, catalysis, luminescence, biomedicine, environmental pollution, sensor, and so on [19-22]. In recent years, MOFs have been investigated for the encapsulation of organic or organometallic molecules as well as for the development of potential drug carriers [23]. To this end, they ensure the structural, chemical, and thermal stability of the active molecules through confinement in their pores and they experience rapid hydrolytic decomposition, resulting in the release of the drug molecule [15]. Recently, a concept of "edible" MOF named cyclodextrin metal-organic framework (CD-MOF) has been reported, it is synthesized by using γ-CD as an organic ligand, and low-toxic alkali metal potassium ions (K^+) as metal ions [24, 25]. CD-MOFs which have the empirical formula $[(C_{48}H_{80}O_{40})(KOH)_2]_n$ can be employed as a versatile host to encapsulate enough biological agents and convert crystal pharmaceuticals into molecular form, significantly increasing the solubility and bioavailability of poorly soluble drugs [26, 27].

One of the reasonable and helpful strategies in drug discovery is the quantitative structure-activity relationship (QSAR), which is important for the development and optimization of drug candidates as well as for improving their biological activities [28]. The development of a new medicine is a time-consuming, costly, difficult, and ineffective procedure that can take up to 15 years [29]. The concept of computational chemistry such as computer-aided drug design (CADD) has recently achieved a remarkable revolution in medicinal chemistry as well as the model for discovering pharmacologically interesting molecules [30]. This study aimed to investigate in silico, the inclusion of betulinic acid into CD-MOF-1 by quantum mechanics (QM) calculations, and molecular docking simulation, to indicate the possibility of forming the host-guest inclusion complex between BA and CD-MOF-1 which may enhance the solubility of BA and then its efficiency for drug delivery. To the best of our knowledge, this work has not yet been done in the literature, but it has been reported some similar work using protein as a receptor like myeloid differentiation 2 (MD2) [31], phospholipase A2 (PLA2) [32], human serum albumin (HSA) [33], and so on to show the biological properties of betulinic acid.

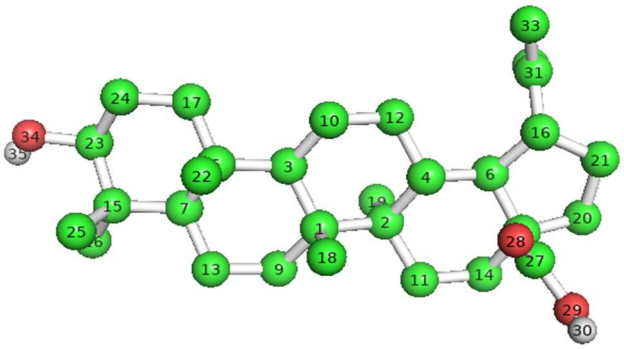

Figure 1. Molecular structure of betulinic acid retrieved after adding atomic partial charges based on B3LYP/6-31+G(d) level, (carbon atoms in green, oxygen atoms in red, and hydrogen atoms in grey)

Materials and Methods

Quantum mechanics (QM) calculations

Density functional theory (DFT) developed by Hohenberg and Kohn [34] and Kohn and Sham [35], is a method for calculating the electronic structures of molecules using the first principles of quantum

mechanics [29]. DFT calculations are a functional tool for determining the correct molecular structure corresponding to the lowest energy state at the potential energy surface. This approach is useful in exploring the structure-activity relationship since accurate molecular structure determination is required in this field. Researchers can utilize DFT calculations to obtain reliable information about molecules' electronic structure and properties, which can then predict their behavior in chemical reactions and interactions with other compounds [36]. In this study, all geometry optimizations were calculated by using the function of Becke, Lee, Yang, and Parr (B3LYP) with a basis set of 6-311+G(d). Atomic partial charges of CD-MOF-1 and betulinic acid were calculated by CHarges from ELectrostatic Potentials using a Grid-based (CHelpG) method based on B3LYP/6-31+G(d) level, the cluster structures used for those calculations are given in Figures 1 and 2 and their values in Tables 1 and 2 for betulinic acid and CDMOF-1 respectively. Thus, we were allowed to analyze some properties such as frontier molecular orbital (FMO) and molecular electrostatic map (MEP).

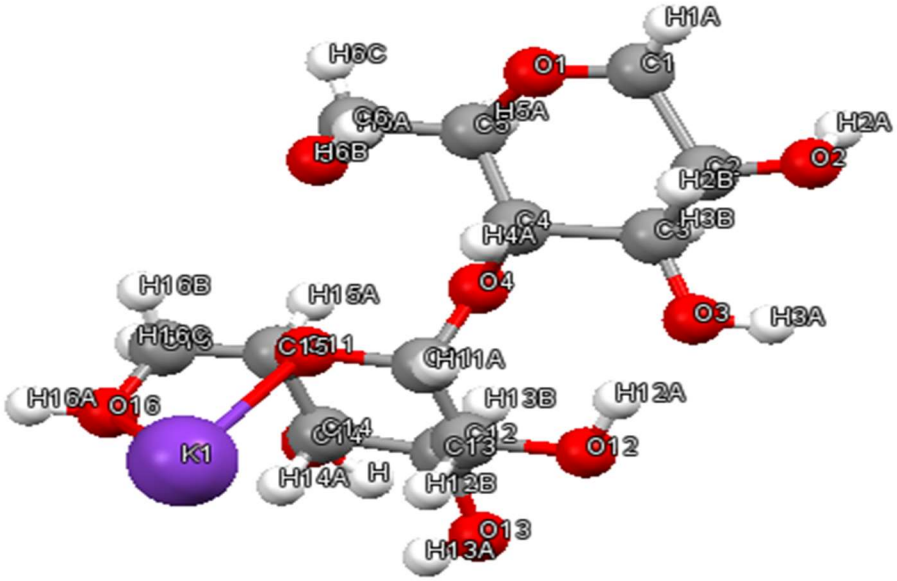

Figure 2. The cluster was created by cutting directly a piece of framework used for the calculation of atomic charges. One hydrogen (H) has been added to O14 to saturate the structure. (K, O, C, and H atoms are represented as light blue, red, grey, and white respectively)

Table 1. The partial atomic charge values of CD-MOF-1 as given by CHelpG calculation based on the B3LYP/6-31+G(d) method

Atoms	Charges	Atoms	Charges	Atoms	Charges
K1	0.070	C4	0.104	O13	-0.849
O1	-0.318	C5	0.434	H13A	0.480
C1	0.538	O6	-0.468	C13	0.254
O2	-0.701	H6A	0.375	O14	-0.616
H2A	0.492	C6	0.172	C14	0.341
C2	0.176	O11	-0.093	C15	-0.304
O3	-0.660	C11	-0.709	O16	-0.576
H3A	0.371	O12	-0.678	H16A	0.350
C3	0.472	H12A	0.333	C16	0.142
O4	-0.763	C12	0.605	H	0.418

Table 2. The partial atomic charge values of betulinic acid as given by CHelpG calculation based on the B3LYP/6-31+G(d) method

Atoms	Charges	Atoms	Charges	Atoms	Charges
C1	0.283	C13	-0.047	C25	- 0.177
C2	0.388	C14	-0.133	C26	-0.152
C3	0.181	C15	0.462	C27	0.728
C4	-0.249	C16	0.038	O28	-0.607
C5	0.098	C17	-0.191	O29	-0.603
C6	0.346	C18	-0.205	H30	0.409
C7	-0.058	C19	-0.191	C31	0.185
C8	-0.017	C20	0.081	C32	-0.052
C9	-0.072	C21	-0.118	C33	-0.182
C10	-0.030	C22	-0.134	O34	-0.759
C11	0.007	C23	0.305	H35	0.415
C12	-0.067	C24	0.054	Total	0.000

Molecular docking simulation: Receptor preparation

The CIF (crystallographic information files) of CD-MOF-1 was retrieved from the Cambridge Crystallographic Data Centre (CCDC) (https://www.ccdc.cam.ac.uk/structures/) using the reference code 773709 with the name catena-((μ8-γ-cyclodextrin)-di-potassium dihydroxyde) [37]. Atomic partial charges were added, then the AutoDock Tool was used to add hydrogen atoms and Kollman charges and the file has been saved in pdbqt format.

Ligand preparation

Betulinic acid molecule was obtained in Structure Data Format (sdf) from PubChem web platform (https://pubchem.ncbi.nlm.nih.gov/) in 3D conformation (CID 64971) and converted to the PDB file using OpenBabel software [38] of the Python Prescription (PyRx) suite, a virtual screening tool. Betulinic acid geometry was optimized, and atomic charges added, then, the preparation of the pdbqt file was done using the AutoDock Tool.

Molecular docking processes

Molecular docking simulations were carried out using the AutoDockTool 1.5.7 and AutoDock Vina v1.1.2 program. The number of points in the grid map of 42×46×40 Å was set to cover whole the framework spacing of 1.000 Å, which was centered at x = 12.971, y = 18.216, and z = 14.482. Grid-based energy evaluation methods combined with a Lamarckian Genetic Algorithm (LGA) were considered for pre-calculating grid maps according to the interatomic potentials of all-atom types present for each CD-MOF-1 pores and BA molecules. The docking has also been performed using the packed 1×1×1 CD-MOF-1 structure to have more accurate results. The exhaustiveness value was set as 8 to obtain an efficient binding conformation pose of the CD–MOF–drug complex. Using an AutoDock Vina, the docking analysis was performed, and the receptor-drug complex's binding affinity was observed as a negative score with a unit of kcal/mol and the same value for all 9 poses. Discovery Studio 16.1 software packages and Pymol were assisted to analyze and visualize the host-guest interactions.

Results and Discussion

Frontier molecular orbital (FMO) analysis

The most important orbitals in a molecule also called frontier molecular orbitals (FMOs) are the lowest unoccupied molecular orbital (LUMO), which can accept electrons, and the highest occupied molecular orbital (HOMO), which can donate electrons [36]. These orbitals are important for determining a compound's electrical properties, kinetic stability, optical polarizability, and chemical reactivity [29]. To understand the chemically reactive site of BA, the HOMO, and LUMO are obtained, where the negative region is presented in red and the positive one in green (Figure 3). As we can see from the given figure, HOMO is located over the alkene group, the cyclohexane and cyclopentane rings, which can be useful for donating electrons to the electrophilic sites of CD-MOF-1. On the other side, the formation of LUMO in the carboxylic group is important for accepting electrons given by

nucleophilic sites of CD-MOF-1.

The HOMO and LUMO energy values of betulinic acid were calculated as well as the global chemical parameters, like energy gap (Egap), ionization potential (I), electronic affinity (EA), chemical potential (μ), electronegativity (χ), chemical hardness (η), softness (S), electrophilicity (ω) and nucleophilicity (ε) according to equations (1 to 9) below (Table 3) [29].

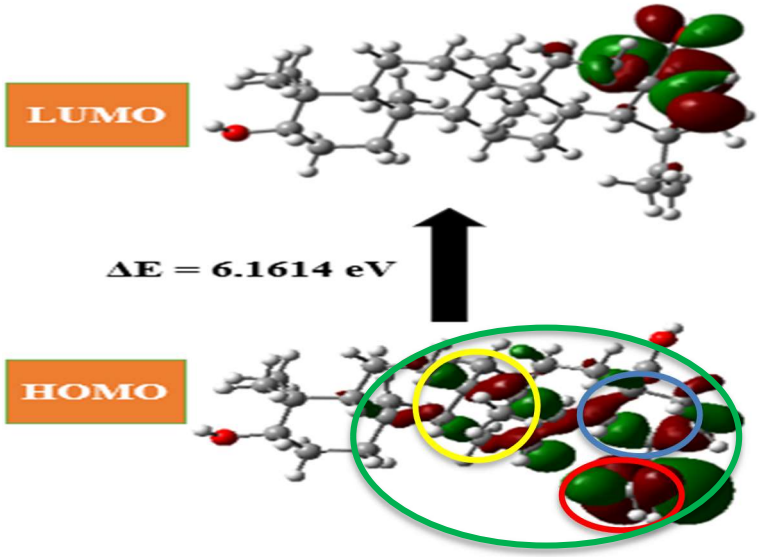

Figure 3. HOMO and LUMO orbitals of betulinic acid, the circles in green, yellow, blue, and red show the location of HOMO, cyclohexane, cyclopentane, and alkene groups respectively

Table 3. Electronic properties of betulinic acid based on B3LYP/6-31G+ (d)

Electronics properties	Values
E_{HOMO}	-6.5558 (eV)
E_{LUMO}	-0.3943 (eV)
Energy gap (E_{gap})	6.1615 (eV)
Ionization potential (I)	6.5558 (eV)
Electronic affinity (EA)	0.3943 (eV)
Electronegativity (χ)	3.4750 (eV)
Chemical potential (μ)	-3.4750 (eV)
Chemical hardness (η)	3.0807 (eV)
Chemical softness (S)	0.3246 (eV^{-1})
Electrophilicity (ω)	1.9599 (eV)
Nucleophilicity (ε)	0.5102 (eV^{-1})

The energy gap (Egap) was calculated by the difference of energy between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) shown in equation 1. For the molecular system containing π electrons, smaller Egap increases the mobility of π electrons to higher energy levels, with better energy distribution and higher reactivity [39]. According to Table 3, the value of Egap of betulinic acid is 6.1615 eV in the gas phase, which is less than 6.378 eV in water as reported by Khan et al. (2018). Khan et al. (2018) performed the DFT calculations of betulinic acid using the Solvation Model on Density (SMD) involving several solvents like water, dimethyl sulfoxide (DMSO), acetonitrile, n-octanol, chloroform [32]. That

model is called SMD, where the "D" stands for "density" to denote that the full solute electron density is used without defining partial atomic charges [40]. This means that betulinic acid is less stable and more reactive and its electrons can easily flow from the HOMO orbital to the empty LUMO orbital until reaching the equilibrium state.

The ionization potential (I) and electronic affinity (EA) are obtained from the reverse sign of the calculated energies of HOMO and LUMO as follows in equations 2 and 3 respectively.

$$Egap = ||E_{HOMO}|| - ||E_{LUMO}|| \quad (1)$$

$$I = -E_{HOMO} \quad (2)$$

$$EA = -E_{LUMO} \quad (3)$$

Electronegativity (χ) (equation 4) is a measure of an atom's ability to attract electrons towards itself in a chemical bond, it is a fundamental property of atoms that affects the nature of chemical bonding and the resulting properties of compounds. Electronic chemical potential (μ) (equation 5) which is the absolute reversed value of electronegativity means escaping the tendency of electron cloud from the ground state. Betulinic acid can be capable of forming polarized covalent bonds with other atoms having both relatively low electronegativity, such as alkali metals or alkaline earth metals, or strong electronegativity like the hydroxide group. The polarity of the bonds in the molecule can have effects on the physical properties of betulinic acid, such as solubility and polarizability, BA may tend to interact with other molecules with complementary electronic properties, which may affect its chemical reactivity.

$$\chi = \frac{I+EA}{2} \quad (4)$$

$$\mu = -\chi \quad (5)$$

Hardness (η) (equation 6) is a measure of the resistance of a molecule to electron transfer and is defined as the energy required to add or remove an electron from the molecule's HOMO or LUMO respectively. In order words, a molecule with a high hardness requires a large amount of energy to donate or accept an electron. On the other hand, softness (S) (equation 7) is the inverse of hardness and represents the ease with which a molecule can donate or accept electrons.

$$\eta = \frac{-Egap}{2} \quad (6)$$

$$S = \frac{1}{\eta} \quad (7)$$

The concept of hardness (η) and softness (S) is related to a compound's reactivity, so, to the energy gap of a molecule. As the energy gap increases, the molecule becomes harder (less soft), significantly less polarizable, and less reactive. The calculated values are 3.0807 eV and 0.3246 eV^{-1} for chemical hardness and softness respectively, as compared to 3.189 eV and 0.314 eV^{-1}. As shown above, betulinic acid has a smaller energy gap, so it is less soft, more reactive, and polarizable. The same behavior has been demonstrated by Muya and collaborators, they did the DFT calculations of betulinic acid using the continuum solvation model based on density (SMD) at B3LYP and M062X with 6-311 + G(d,p) basis sets, they conclude that betulinic acid appears soft and can interact with soft acceptor molecules [41].

Other important descriptors are the electrophilicity index (ω) and nucleophilicity (ε) used to describe the ability of a molecule or atom to accept or donate electrons in a chemical reaction. Electrophilicity refers to the tendency of a molecule to attract electrons, it is typically associated with electron-deficient species that have a partially positive charge and nucleophilicity refers to the tendency of a molecule to donate electrons, it is associated with electron-rich species that have a partially negative charge. Their values are defined as follows in equations 8 and 9:

$$\omega = \frac{\mu 2}{2\eta} \quad (8)$$

$$\varepsilon = \frac{1}{\omega} \quad (9)$$

Electrophilicity included both the ability of an electrophile to acquire additional electronic charge and a system's resistance to exchange electronic charge with the environment. Betulinic acid electrophilicity is 1.9599 eV at the B3LYP/6-31G+ (d), 1.48 eV at the B3LYP/6-31G(d) using SMD solvation model [32] and

1.15 eV at B3LYP/6-31G+ (d,p) and 1.19 eV at M062X/6-311 + G(d,p) using SMD model [41]. We notice that, with atomic partial charges, the electrophilicity value of BA increases at the B3LYP/6-31G+ (d). The electrophilicity character of betulinic acid proves that the active site of the latter molecule may be the carboxylic group.

Molecular electrostatic potential (MEP) and contours analyses

The MEP maps give an idea about the chemical reactivity of the compounds by determining electrophilic and nucleophilic attack regions in the molecule according to charge distributions [42]. The maps of electrostatic potential (MEPs) surfaces and contours were used to display the electron density on surfaces that surround the atoms within the molecules and sites for nucleophilic and electrophilic attacks. To predict the electrophilic and nucleophilic center of betulinic acid, MEP for optimized geometry was calculated at the DFT/B3LYP/6-31G+ (d). According to Figure 4a, potential decreases in the following order of colors red < orange < yellow < green < blue. Red and yellow regions indicate negative regions (rich electron) which promote nucleophilic site while the positive regions represented by the blue color promote electrophilic site. The green color represents a region of zero potential which indicates the neutral region [36]. In addition, MEP contours are more significant for illustrating the reactive nature of outermost orbital electrons (Figure 4b) [29]. The reactivity of betulinic acid is observed with red-colored contours and green lines show that electrons are not moving in the area. Figure 4 shows that the negative potentials are generated over the electronegative oxygen atom of the hydroxide group and the positive potentials are located in the oxygen atom of the carboxylic group. As shown above, for the molecular system containing π electrons, a smaller energy gap increases the mobility of π electrons to higher energy levels, which corroborates with MEPs showing that the carboxylic group of BA is preferred active site. This may be confirmed or not following this work by performing molecular docking simulation between betulinic acid and CD-MOF-1.

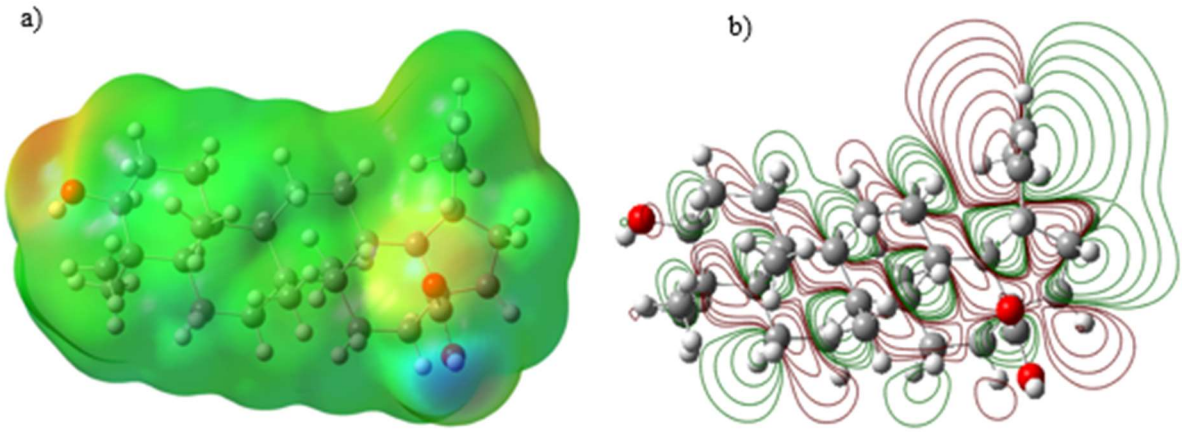

Figure 4. MEP of (a) surface and (b) contours of betulinic acid

Molecular docking analysis

CD-MOF has spherical voids with a diameter of 0.95 nm and pore size of 0.78 nm, which are body-centered cubic extended structures with surface areas of approximately 1200 m^2/g (Figure 5) [43]. The possible conformations of betulinic acid molecules were done by docking BA into CD-MOF-1, the hydrogen bond interactions are given in Table 4. The blind docking was done in triplicate and the binding affinity of all of the nine poses was -8.2 kcal/mol, the specific docking has also been performed thrice and the binding affinity varying between -8.2 and -8.1 kcal/mol, a very low difference. The best-docked conformation of betulinic acid, located in the center of the cyclodextrin pore has been analyzed by Discovery Studio as shown in Figure 6. We noticed that betulinic acid bound in the hydrophobic inner cavity of CD and its electrophilic side (carboxylic group) interacted with a hydrophilic outer surface of CD (constituted by D-glucopyranose units) and the nucleophilic side of BA (hydroxyl group) can just form

the unfavorable interaction. This confirms that the carboxylic group (COOH) of betulinic acid is its active site as predicted by the global descriptors and the MEPs. So, betulinic acid and CD-MOF-1 exhibit a stronger affinity examined by the hydrogen bond interactions, which means that CD-MOF-1 can stabilize BA against oxidation by tailoring its physical properties.

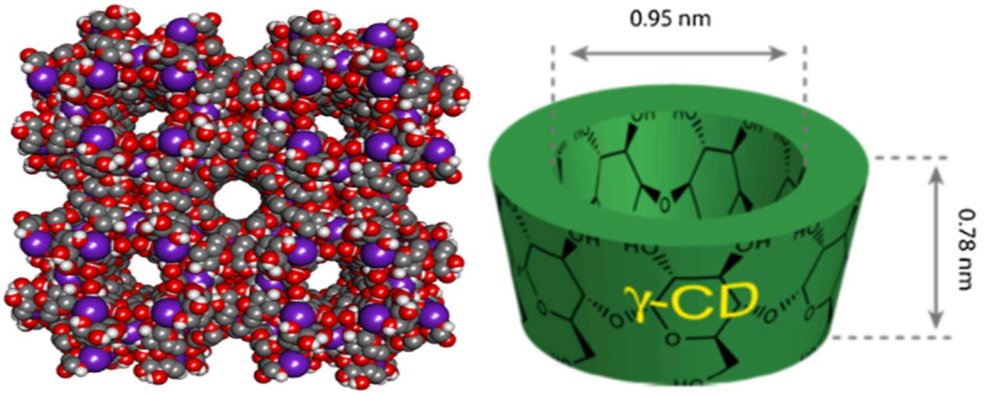

Figure 5. A space-filling representation of the extended solid-state structure, showing the $(\gamma CD)_6$ repeating motifs adopting a body-centered cubic packing arrangement (C gray, O red, K purple)

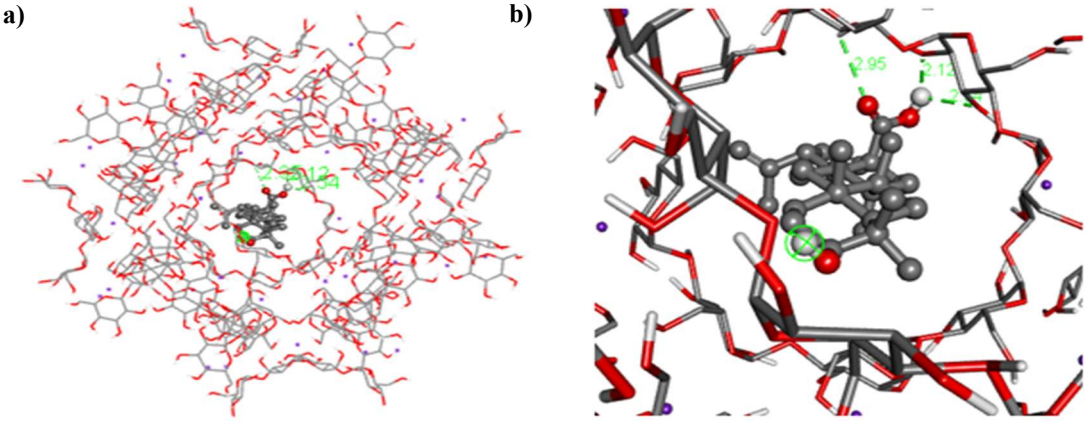

Figure 6. (a) The best binding affinity of BA into CD-MOF-1, (b) the zoom of the cyclodextrin pore where we have hydrogen bonds interactions, the green lines are hydrogen bonds between CD-MOF-1 and BA

Many researchers noticed that the drug candidate may be most effective when the binding energy is lowest and the number of H-bond donors with the receptor is largest [30]. Figure 6 shows three hydrogen bonds between BA and CD-MOF-1, which are [CD-MOF-$1_{OH13}\cdots_{O28}$BA (2.95 Å)], [CD-MOF-$1_{O13}\cdots_{H30}$BA (2.54 Å)], and [CD-MOF-$1_{OH12}\cdots_{H30}$BA (2.12 Å)]. So, CD-MOF-1 may be a good carrier of betulinic acid in drug delivery and can enhance its bioavailability and solubility, by improving its biological efficiency.

To have more accurateness, molecular docking simulation has also been done with the packed 1×1×1 of CD-MOF-1 structure, the binding affinities of the blind and specific docking of all of the nine poses were -11.3 kcal/mol more significant than -8.2 kcal/mol. All the poses are located in the cavities of the cyclodextrin, Figure 7 shows hydrogen interactions between BA and CD-MOF-1 which are [CD-MOF-$1_{OH12A}\cdots_{O28}$BA (3.00 Å)], [CD-MOF-$1_{H13A}\cdots_{H30}$BA (2.72 Å)] and [CD-MOF-$1_{OH2A}\cdots_{H30}$BA (2.66 Å)]. These interactions are not similar to the ones above, but they are longer and more significant in terms of energy and distance, so the CD-MOF-1 packed structure is the best to have accurate results.

The observed binding affinities in molecular docking studies provide valuable insights into the strength of interaction between betulinic acid and CDMOF-1, these binding affinities can have several implications for stabilizing betulinic acid against oxidation:

- Identification of stable binding interactions: High binding affinity often indicates strong and stable interactions between the ligand and receptor. In the context of stabilizing betulinic acid molecules against oxidation, this suggests that betulinic acid is tightly bound to CDMOF-1, which may shield it from interacting with oxidizing agents in the surrounding environment.
- Prevention of oxidation-induced conformational changes: Binding to the CDMOF-1 molecule in a specific conformation may prevent betulinic acid from adopting alternative conformations that are more susceptible to oxidation. By stabilizing betulinic acid in a particular orientation, CDMOF-1 may help shield vulnerable functional groups from oxidation.
- Design of antioxidant compounds: Insights gained from molecular docking studies can aid in the design of small molecules with enhanced antioxidant properties. By understanding the structural features that contribute to high binding affinity and stability against oxidation, we can modify betulinic acid or design novel molecules that better fulfill the desired criteria.

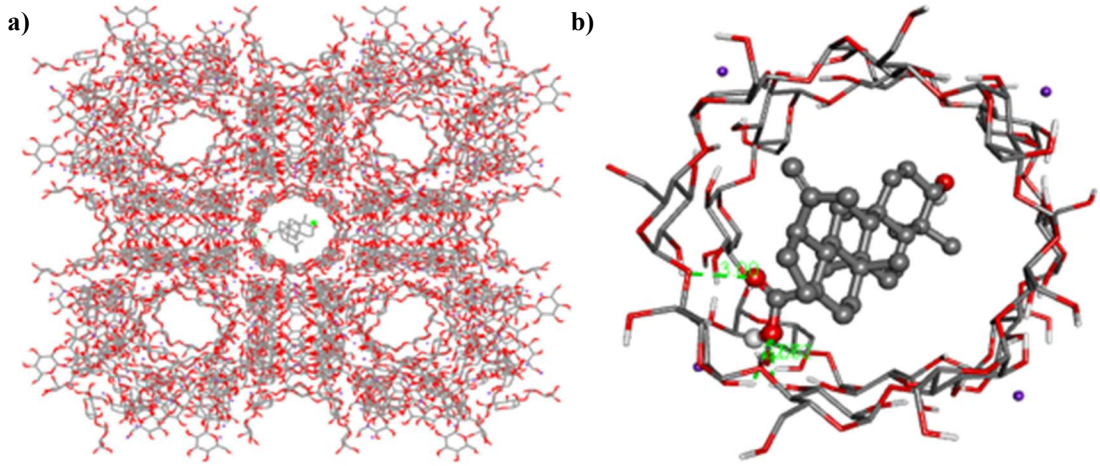

Figure 7. The best binding affinity of BA into CD-MOF-1 using the packed 1×1×1 structure of CD-MOF-1, the green lines are hydrogen bonds between CD-MOF-1 and BA

Table 4. Hydrogen bond interactions between CD-MOF-1 and betulinic acid

CD-MOF-1 Structure	**Hydrogen bond interactions between CD-MOF-1 and BA (Interaction distance)**		
Simple	CD-MOF-1_{OH13}··· $_{O28}$BA (2.95 Å)	CD-MOF-1_{O13} ···$_{H30}$BA (2.54 Å)	CD-MOF-1_{OH12} ··· $_{H30}$BA (2.12 Å)
Packed 1×1×1	CD-MOF-1_{OH12A} ···$_{O28}$BA (3.00 Å)	CD-MOF-1_{H13A} ··· $_{H30}$BA (2.72 Å)	CD-MOF-1_{OH2A} ··· $_{H30}$BA (2.66 Å)

Conclusion

CD-MOF-1 is a porous coordination compound, that exhibits many unique characteristics such as high surface area, high porosity, and tunability leading to numerous applications in many fields including drug delivery. In the present work, we have theoretically obtained the host-guest interactions between betulinic acid and CD-MOF-1 by performing QM calculation to understand the behavior of BA inside the CD-MOF-1, and molecular docking simulation to determine the best

binding affinity of BA into CD-MOF-1. The global descriptors such as energy gap, electronegativity, chemical hardness and softness, and electrophilicity show that betulinic acid has a smaller energy gap which involves less stability, more reactivity, and more polarizability. The MEPs map shows that the active site of betulinic acid is the carboxylic group and the latter molecule preferred electrophilic attacks. Molecular docking revealed that BA bound in the hydrophobic cavities of CD-MOF-1 through hydrogen bonds [CD-MOF-$1_{OH13\cdots O28}$BA (2.95 Å)], [CD-MOF-$1_{O13\cdots H30}$BA (2.54 Å)], and [CD-MOF-$1_{OH12\cdots H30}$BA (2.12 Å)] for the simplest structure and [CD-MOF-$1_{OH12A\cdots O28}$BA (3.00 Å)], [CD-MOF-$1_{H13A\cdots H30}$BA (2.72 Å)] and [CD-MOF-$1_{OH2A\cdots H30}$BA (2.66 Å)] for the packed structure 1×1×1. The packed CD-MOF-1 structure is the best with accurate results, this may be the factor of enhancement of betulinic acid solubility and bioavailability. So, CD-MOF-1 may be investigated and validated as an efficient carrier to enhance the solubility and bioavailability of betulinic acid. However, translating theoretical findings from molecular docking studies into practical applications can face several challenges and limitations such as accuracy of predictions, validation, flexibility and dynamics, solvent effects, chemical accessibility, and synthesis. In forthcoming research, the integration of molecular docking and molecular dynamic simulations unveils potential mechanisms governing binding affinities, enabling virtual observation. This integrated approach guides experimental synthesis and characterization validation from in vitro to preclinical studies, offering cost-effective methods to advance drug discovery, particularly in nanomedicine for cancer treatment, ensuring sustainable research practices.

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