Analysis of Betulin Complex Formation with Polar Solvents Using DFT Methods

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Abstract. The efficiency of betulin extraction from plant material is highly dependent on the choice of solvent, particularly due to the role of hydrogen bonding in determining solvation behavior. This study aims to investigate the molecular interactions between betulin and polar solvents, focusing on hydrogen bond formation, to elucidate the mechanisms underlying solvent efficiency in betulin extraction. Using density functional theory calculations with 6-31++G(2d,2p) basis and IR spectral analysis, the interactions between betulin and polar solvents (acetonitrile, isopropanol, acetone, and ethanol) were examined. The results revealed that ethanol forms the strongest and most stable hydrogen bonds with betulin, which correlates with its experimentally observed superior extraction efficiency. This work underscores the importance of hydrogen bonding in solvation behavior and offers valuable insights for the development of efficient extraction protocols in natural product chemistry. Additionally, understanding betulin's molecular interactions with solvents can open new possibilities for its applications in biophotonics, where its unique structural and optical properties may be utilized.

Keywords: betulin; density functional theory; infrared spectra; polar solvents; extraction.

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1 Introduction

Betulin, a pentacyclic triterpenoid from the lupane group (Fig. 1), is widely distributed in nature, particularly in birch bark. Lupane series triterpenoids possess a complex polycyclic framework built upon a sterane core, which integrates four cyclohexane rings and a fully saturated cyclopentane ring. A key structural characteristic of betulin within this class is the inclusion of a five-membered ring along with an α-isopropenyl substituent [1]. Due to its potential biological properties, including antitumor and antiviral activity [2−4], this compound has generated interest in the fields of pharmacology and natural product chemistry.

Triterpenoids are crucial in enhancing plant defense systems against infections from a variety of pathogens. Their antiviral properties are commonly linked to hindering the initial stages of viral attachment and entry into host cells. This interference subsequently disrupts viral replication process once infection has occurred [5−6].

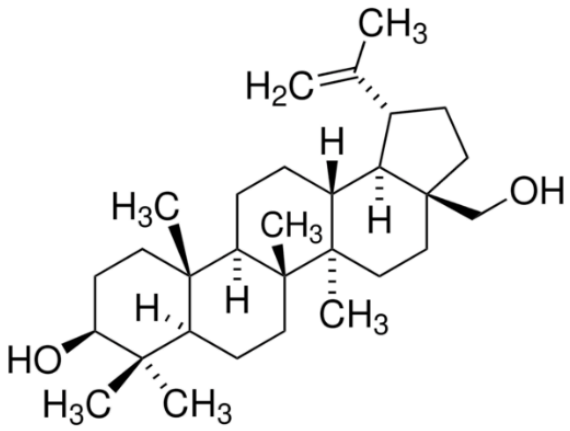

Fig. 1 The structural formula of betulin.

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While betulin therapeutic potential has been extensively explored, an emerging area of interest is its possible role in biophotonics. Betulin's unique chemical structure and biological effects suggest it could contribute to this field, particularly in enhancing light-mediated diagnostic and therapeutic approaches. Betulin's pentacyclic triterpenoid structure provides a robust platform for chemical modifications, enabling creation of derivatives with enhanced solubility, stability, and functionality. Key derivatives include betulinic acid, betulin esters, betulin-based polymers, and hybrid materials such as betulin-nanoparticle conjugates [7−8]. These modifications expand the applicability of betulin in biophotonic applications.

Betulinic acid, a derivative of betulin, can be conjugated with fluorescent dyes to create probes for bioimaging. These probes are useful in techniques such as fluorescence microscopy and optical coherence tomography (OCT), enabling high-resolution imaging of cellular and tissue structures. For example, betulinic acid-based probes have been used to track cancer cells *in vitro* and *in vivo*, providing valuable insights into tumor biology [9].

Betulinic acid and its esters have been explored as photosensitizers in photodynamic therapy. Upon light activation, these compounds generate reactive oxygen species (ROS), which can selectively destroy cancer cells. This approach minimizes damage to healthy tissues and offers a promising alternative to conventional cancer treatments [10−11].

Betulin esters and polymers can be incorporated into nanoparticles or hydrogels for light-triggered drug delivery. For instance, betulin-based hydrogels have been developed to release therapeutic agents in response to specific wavelengths of light, enabling precise control over drug release kinetics. This technology is particularly useful for targeted cancer therapy and wound healing [12−13].

The extraction of betulin from plant material is a critical process for obtaining this valuable compound in its pure form. This purified betulin can then be used in a variety of industries, including the pharmaceutical industry, where it serves as a precursor for the synthesis of betulinic acid, another compound with significant medicinal properties. To ensure the success of the extraction process, it is important to carefully select a solvent that is compatible with the solvation interactions and polarity of the material being extracted. The choice of solvent has a significant impact on the efficiency of the extraction [14], as it determines not only the yield but also the purity and stability of the extracted compound. Factors such as solvent polarity, temperature, and extraction time must be optimized to achieve the best results.

In a study conducted by Levdansky et al. [15], the extraction of betulin from birch bark using various organic solvents with different polarities was investigated. The results demonstrated that the use of polar solvents allows for the production of extracts with yields up to 28.8% and betulin contents ranging from 90% to 95%. (Fig. 2). Researchers have noted that as the temperature and polarity of the solvent increase, the total volume of the extract increases, but the percentage of betulin in the extract decreases [15, 16]. While these experimental findings provide valuable insights into the extraction process, the molecular-level mechanisms governing the interactions between betulin and solvents remain unclear. To address this gap, density functional theory (DFT) calculations can be employed to investigate the nature and strength of these interactions.

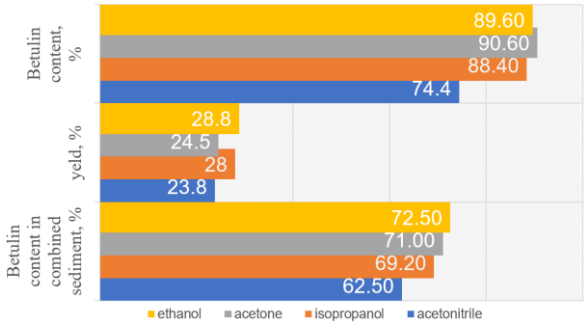

Fig. 2 Content of betulin in the extract, yield, and betulin content in the combined precipitate depending on the solvent, %, The data was taken by Levdansky et al. [15].

Hydrogen bonds represent a critical class of intermolecular forces, exerting substantial influence on the structural and functional characteristics of biological molecules. These bonds may be especially significant in the context of betulin, as they can influence its behavior in different chemical and biological settings. For instance, hydrogen bonding can affect the solubility, stability, and reactivity of betulin, which in turn impacts its extraction efficiency and subsequent applications.

This study aims to employ advanced computational methods, particularly DFT, to simulate and analyze the molecular interactions between betulin and various polar solvents. By systematically examining hydrogen bond formation, binding energy, and vibrational spectral changes, we seek to elucidate the molecular mechanisms governing betulin solvation. Understanding these interactions is crucial for improving solvent selection in betulin extraction, thereby enhancing both yield and purity.

2 Methods

To theoretically substantiate the extraction ability of various polar solvents, molecular modeling was performed, and IR spectra were calculated using the DFT method [17] in Orca [18]. B3LYP functional [19, 20] and 6-31++G(2d, 2p) basis set [20] were used for the calculations. The DFT calculations provided detailed insights into the energetics and geometries of hydrogen bond formation, while IR spectroscopy validated the theoretical findings by identifying

characteristic vibrational modes associated with betulin − solvent interactions.

Since intermolecular interaction is based on the formation of hydrogen bonds through the hydroxyl groups of betulin, there are only two potential possibilities for the attachment of a polar solvent, which we will consider (Fig. 3). Explicit solvation was performed, but to increase the accuracy of calculations, we also used implicit solvation of water using the conductor-like polarizable continuum model (CPCM) [21] method.

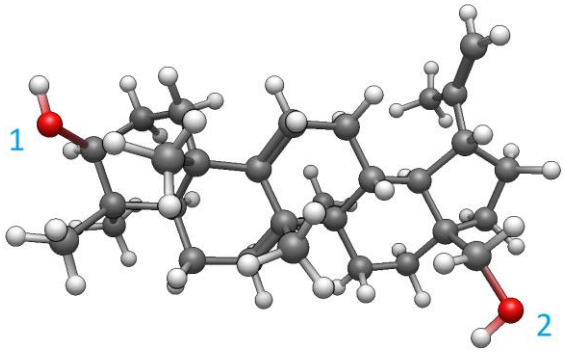

Fig. 3 Betulin structure. 1, 2 – sites for the attachment of a polar solvent.

The Avogadro editor − visualizer of molecular structures [22] and the author's program for displaying infrared spectra, which allows recreating the IR spectrum based on numerical values obtained by Orca program, were used for the work.

To calculate the energy of hydrogen bonds between betulin and solvents, methods related to computation of the isolated fragments energies and Johansen's empirical formula will be employed. The method energies of isolated fragments (Eq. (1)) will be calculated to determine the interaction energy, while the Johansen formula [23] will be applied to further analyze and validate the results. Isolated fragment approach is based on computational analysis of the betulin-solvent complex (Eq. (1)). Simultaneously, the approach based on Johansen's formula (Eq. (2)) employs an empirical formula, which is commonly used by experimental researchers, with primary focus placed on determining the bond energy through analysis of frequency shifts.

$$E_{interaction} = E_{complex} - (E_{betulin} + E_{solvants}), \quad (1)$$

$$-\Delta H = 0.3\sqrt{\Delta v - 40}. \quad (2)$$

For the evaluation of hydrogen bonds, the classification presented in Table 1 of reference [24] will be utilized. This classification provides a systematic framework for analyzing and categorizing hydrogen bond interactions based on established criteria.

Finally, the calculated frequencies are multiplied by an anharmonicity correction factor [25−27]. The following scaling factors were chosen: 0.97 for frequencies between 0 and 2000 cm^{-1}, and 0.95 for frequencies between 2000 and 4000 cm^{-1}.

Table 1 Classification of hydrogen bonds by bond length and energy.

Strength of hydrogen bond	Hydrogen bond length, Å	Energy, kcal/mol
Strong	2.2−2.5	14.34−28.65
Medium	2.5−3.2	3.82−14.43
Weak	3.2−4.0	Less 2.87

3 Results and Discussion

The optimized structures of betulin complexes with polar solvents (acetonitrile, isopropanol, acetone, ethanol) are presented in Fig. 4. The results of this study demonstrate that ethanol forms the shortest hydrogen bond with betulin (1.86 Å), followed by isopropanol (1.88 Å), acetone (1.90 Å), and acetonitrile (1.99 Å). These findings correlate with the experimentally observed extraction efficiencies, where ethanol yielded the highest betulin content [15]. The shorter hydrogen bond length observed for ethanol can be attributed to its ability to act as both a hydrogen bond donor and acceptor, facilitating stronger interactions with betulin. In contrast, acetonitrile, which primarily acts as a hydrogen bond acceptor, forms weaker and longer bonds due to its lower polarity and reduced ability to participate in hydrogen bonding.

The IR spectra of betulin and betulin complexes with various polar solvents such as acetonitrile, isopropanol, acetone, and ethanol are illustrated in Fig. 5. A comparison calculated IR spectrum with experimentally obtained IR spectrum data from the articles [28] reveals a high degree of correspondence. Minor variations in band shape or intensity may be attributed to differences in sample preparation (e.g., KBr pellet vs. Nujol mull), or instrumental resolution, or supramolecular effects in most cases. The main characteristic bands (OH, C–O, C–C, C=C CH_2/CH_3) match in both position and relative intensity, confirming the structural features of betulin. The calculated IR spectrum is compared with literature data or results from related triterpenoids [29] in Table 2 to validate the assignments main vibrational modes.

Since hydrogen bonds manifest as resonances in the high-frequency region of the IR spectrum, within the range from 3000 to 4000 cm^{-1}, we will investigate the spectral characteristics of betulin molecular complexes with the polar solvents in this range (Fig. 6). In the frequency range of 2800 to 3100 cm^{-1}, peaks corresponding to the vibrational frequencies of C–H bonds are observed. Peaks within the interval of 3400 to 3500 cm^{-1} are associated with the vibrations of hydrogen bonds in betulin-solvent complexes. The stretching vibrations of free O–H bonds are located in the range of 3500 to 4000 cm^{-1}.

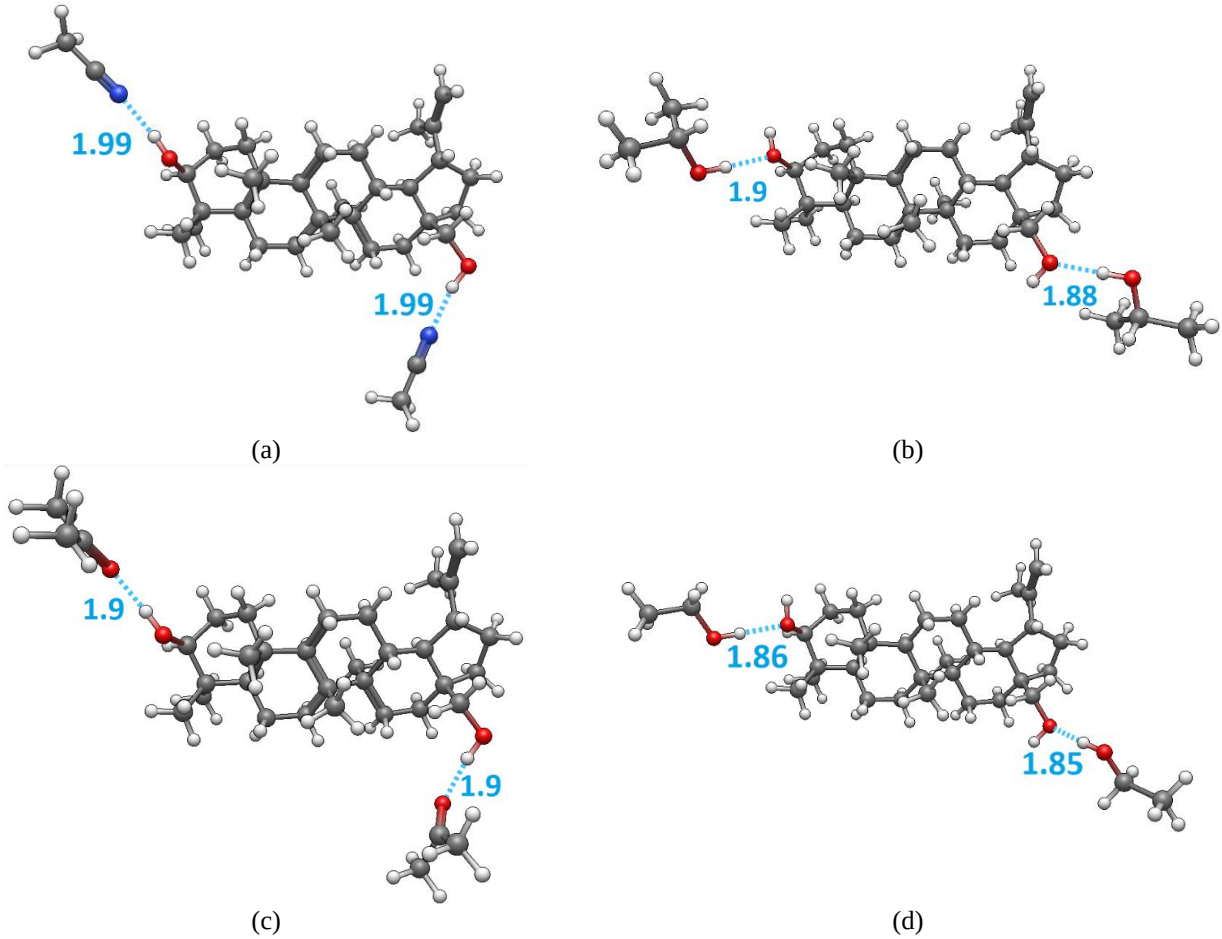

Fig. 4 Optimized structures of betulin with various solvents: (a) acetonitrile, (b) isopropanol, (c) acetone, (d) ethanol. Hydrogen bond lengths are marked in blue color.

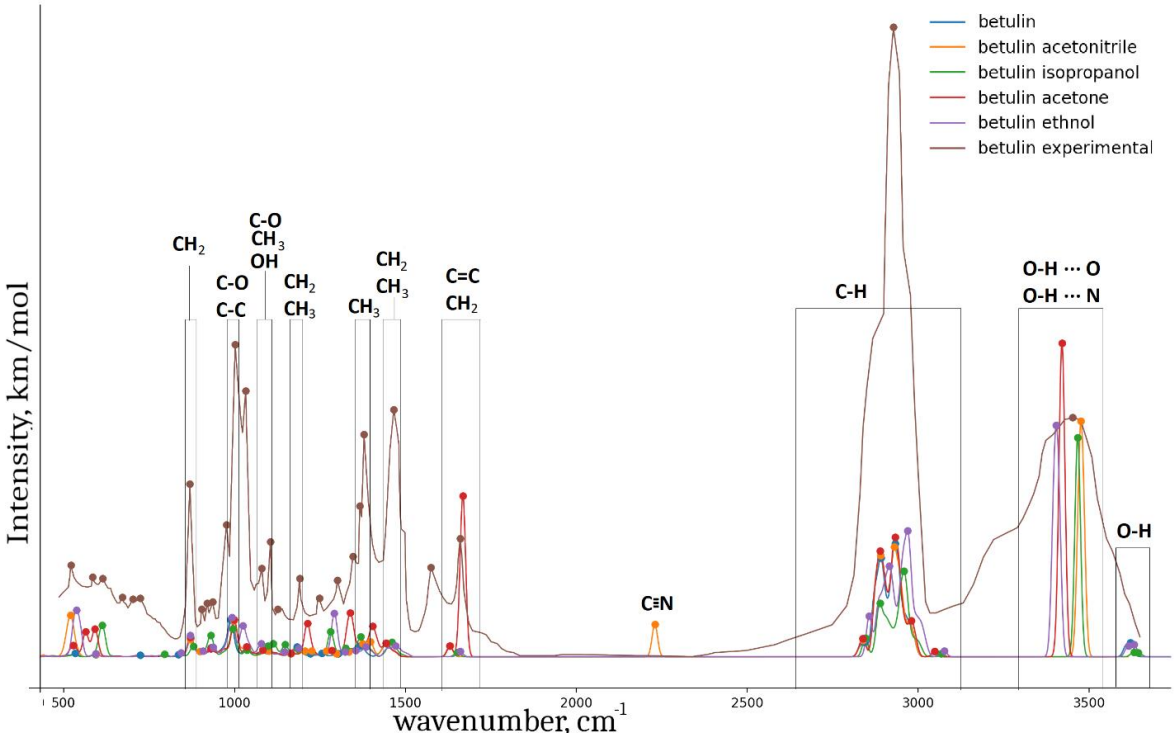

Fig. 5 IR spectra of betulin and complexes of betulin with various polar solvents (blue – betulin, orange – acetonitrile, green – isopropanol, red – acetone, purple – ethanol, brawn – betulin experimental [28]).

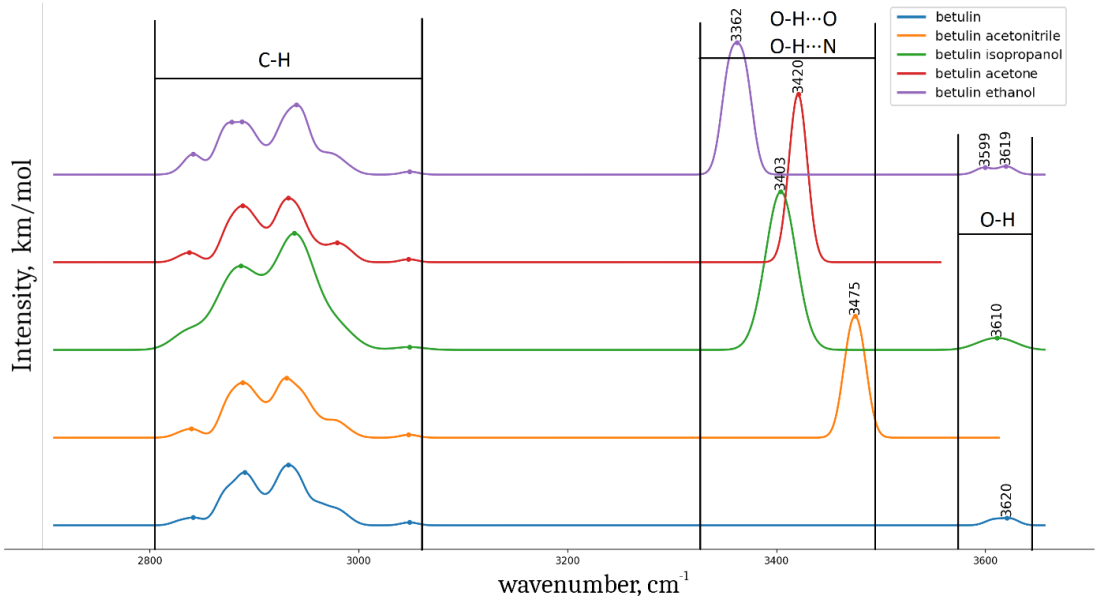

Fig. 6 High-frequency region of the IR spectra of betulin and complexes of betulin with various polar solvents (blue – betulin, orange – acetonitrile, green – isopropanol, red – acetone, purple – ethanol).

Table 2 Measured and calculated bond stretching vibrations frequencies of betulin depending on the solvent.

Bond number	Bond type	Frequency, cm^{-1}		
		Calculated		Experiment
		Solvent	Frequency	
1	CH_2	Acetone	870	875
		Acetonitrile, Ethanol, Isopropanol	871	
2	C–O, C–C	Acetone	1003	1006
		Acetonitrile	997	
		Ethanol	974	
		Isopropanol	981	
3	C–O, CH_2, OH	Acetone	1036	1032
		Acetonitrile	1036	
		Ethanol	1032	
		Isopropanol	1032	
4	CH_2, CH_3	Acetone	1204	1190
		Acetonitrile	1203	
		Ethanol	1190	
		Isopropanol	1192	
5	CH_3	Acetone, Acetonitrile	1376	1373
		Ethanol	1371	
		Isopropanol	1364	
6	CH_3, CH_2	Acetone	1445, 1470	1450 1485
		Acetonitrile	1446, 1473	
		Ethanol	1445, 1474	
		Isopropanol	1449, 1474	
7	C=C, CH2	Acetone	1629	1642
		Acetonitrile	1630	
		Ethanol, Isopropanol	1631	

Table 3 Energy of hydrogen bonds by isolated fragment method.

Solvent name	Complex betulin, hartree	Solvents Energy, hartree	Bond energy, kcal/mol	Dipole moment, D
Acetonitrile	1587.46	132.64	17.61	3.92
Acetone	1708.13	192.97	17.45	2.84
Ethanol	1631.95	154.89	15.64	1.69
Isopropanol	1710.47	194.15	14.08	1.66

Table 4 Calculated parameters of hydrogen bonds.

Bond number	Bond type	Hydrogen bridge length, Å	Bond length, Å	Frequency, cm^{-1}	Frequency shift Δv, cm^{-1}	Enthalpy ΔH, kcal/mol	Intensity , km/mol
Betulin acetonitrile							
1	O–H ••• N	2.96	1.99	3470	137	2.96	872
2	O–H ••• N	2.97	1.99	3480	143	3.05	846
Betulin isopropanol							
1	O ••• O–H	2.88	1.91	3406	228	4.12	906
2	O ••• O–H	2.85	1.88	3403	245	4.3	908
Betulin acetone							
1	O–H ••• O	2.87	1.9	3417	190	3.68	1042
2	O–H ••• O	2.85	1.9	3424	200	3.79	1037
Betulin ethanol							
1	O ••• O–H	2.84	1.86	3426	222	4.05	1124
2	O ••• O–H	2.82	1.85	3440	208	3.89	1103

The results of the interaction energy calculations between betulin and its polar solvents, based on the isolated fragment method, are presented in Table 3. The highest binding energy was observed for acetonitrile (17.61 kcal/mol), while the energies for the remaining solvents were ranked as follows: acetone (17.45 kcal/mol), ethanol (15.64 kcal/mol) and isopropanol (14.08 kcal/mol).

A clear correlation with the dipole moment of the solvents was observed, indicating that the strength of the interaction is influenced by the solvent's polarity. Specifically, acetonitrile, which has the highest dipole moment among the studied solvents, exhibited the strongest binding energy. This trend suggests that solvents with larger dipole moments facilitate stronger interactions with betulin, likely due to enhanced electrostatic forces and improved hydrogen bonding capabilities.

The intensity and frequency of the stretching vibrations of hydrogen bonds between solvents and betulin are presented in Table 4. The hydrogen bond energies were calculated using Johansen's empirical formula.

According to the calculation results, the hydrogen bond energy of the complexes formed by various polar solvents and betulin increases in the following order: acetonitrile (2.96 and 3.05 kcal/mol) < acetone (3.68 and 3.79 kcal/mol) < isopropanol (4.12 and 4.3 kcal/mol) < ethanol (4.05 and 3.89 kcal/mol). Using the classification criteria for hydrogen bonds presented in Table 1, the observed interactions can be characterized as moderate-strength hydrogen bonds. The trend correlates with the experimental data was taken by Levdansky et al. [15], as shown in Fig. 2. This is also evidenced by the increase in the intensity of hydrogen bond vibrations.

We propose that the empirical Johansen formula, which is based on experimental observations of characteristic frequency shifts, provides a more accurate description of the interactions between betulin and polar solvents. This is because the isolated fragment method does not account for supramolecular effects, such as collective solvent-solvent and solvent-solute interactions, which play a significant role in determining the overall binding behavior.

The findings of this study, particularly the detailed understanding of hydrogen bonding interactions between betulin and polar solvents, have significant implications for the field of biophotonics, which focuses on how light interacts with biological materials, relies heavily on the precise control of molecular interactions and the optical

properties of biomolecules. The IR spectral data, which show distinct vibrational modes for betulin-solvent complexes, could serve as a basis for developing label-free optical sensing techniques. These techniques could be used to monitor molecular interactions in real-time, providing valuable information for diagnostic applications [30].

In addition, insights into solvent interactions can inform the design of betulin-loaded nanoparticles. Nanoparticles have been extensively studied in biophotonics for their applications in imaging and therapy. By selecting solvents that form strong hydrogen bonds with betulin, such as ethanol, it is possible to enhance the encapsulation efficiency and stability of betulin within nanoparticle carriers. These nanoparticles can be engineered for targeted drug delivery, photodynamic therapy, or as contrast agents in imaging applications [31].

The results of our study on the binding energy of betulin with polar solvents provide valuable insights into the efficiency of these solvents in green extraction processes. Betulin, a naturally occurring triterpenoid, exhibits strong interactions with polar solvents due to its hydroxyl groups, which facilitate hydrogen bonding. Ours findings can be extended to other structurally similar compounds, such as triterpenoids and polyphenols, which also contain polar functional groups. The reuse of polar solvents after recrystallization is a promising strategy to enhance the economic efficiency of extraction processes. Recrystallization, a common purification technique, often involves the use of polar solvents to dissolve and subsequently crystallize target compounds. After the process, the solvent can be recovered through distillation or evaporation, allowing for its reuse in subsequent extraction cycles. This not only reduces solvent waste but also minimizes the environmental footprint of the process. Studies have shown that solvent like ethanol can be effectively recycled multiple times without significant loss of efficiency, provided that proper purification steps are implemented to remove impurities [32].

Beyond extraction, polar solvents play a critical role in the synthesis and functionalization of carbon-based quantum dots (QDs), such as fullerenes and graphene quantum dots. These nanomaterials, which consist primarily of carbon, have gained significant attention for their applications in optoelectronics, bioimaging, and energy storage. The principles underlying the interactions between polar solvents and betulin, as demonstrated by our binding energy calculations, can also be extended to the synthesis and stabilization of carbon-based QDs [33]. Polar solvents, such as water and ethanol are often used as reaction media in the synthesis of carbon-based QDs due to their ability to dissolve carbon precursors and stabilize nanoparticles during growth. For example, aqueous-phase synthesis of graphene quantum dots has been explored as a greener alternative to organic-phase synthesis, reducing the use of toxic solvents and enabling the production of biocompatible QDs for medical applications [34]. Additionally, polar solvents facilitate the functionalization of carbon-based QDs with hydrophilic ligands, enhancing their dispersibility in aqueous environments and expanding their applicability in biological systems.

4 Conclusions

As a result of the study, it can be concluded that the strongest molecular interaction, primarily driven by hydrogen bonding, occurs with ethanol. This conclusion is supported by experimental data demonstrating ethanol's superior extraction efficiency for betulin compared to other solvents. The enhanced extraction properties of ethanol can be attributed to its ability to form robust hydrogen bonds with betulin. Ethanol’s polar hydroxyl group (–OH) facilitates the formation of these hydrogen bonds, which are critical for solubilizing and extracting betulin from the plant.

The strength of hydrogen bonding in ethanol is further evidenced by the observed increase in the intensity of vibrational modes and hydrogen bond lengths. This phenomenon underscores the significance of hydrogen bonding as a key intermolecular force in the extraction process. Additionally, ethanol’s relatively small molecular size and favorable polarity enable it to penetrate the plant more effectively, thereby enhancing its interaction with betulin molecules.

Beyond its role in extraction, understanding the hydrogen bonding interactions of betulin with different solvents may also have implications for its potential use in biophotonics. The ability of betulin to form stable hydrogen-bonded complexes can influence its electronic properties, fluorescence behavior, and stability in various environments. These properties could be exploited in applications such as fluorescence-based biosensing, bioimaging, and light-mediated therapeutic strategies. Future research should explore how solvent interactions modify betulin’s optical characteristics, potentially broadening its applications in biophotonics and related fields.

Disclosures

The authors declare no conflict of interest.

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