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# Investigation of the Therapeutic, Physicochemical, and Pharmaceutical Properties of Bioactive Compounds from *Citrullus colocynthis*

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## Abstract

*Citrullus colocynthis* is a perennial plant found in arid regions with medicinal properties. Its fruits contain bioactive compounds that offer laxative, anti-diabetic, and anti-cancer effects. However, there are toxicity risks associated with its use, potentially leading to gastrointestinal issues and kidney damage if not used properly, necessitating professional guidance. A literature review was conducted on *Citrullus colocynthis*, also known as bitter cucumber, to explore its traditional medicinal uses. The team searched databases for information on its herbal therapy potential and predicted properties using the SwissADME platform, focusing on solubility and permeability. Cucurbitacins, with larger molecular weights ranging from 516 to 718 daltons, have poor solubility and absorption compared to smaller alkaloids like Kaempferol and Quercetin. These alkaloids can cross the blood-brain barrier more effectively due to their higher Fraction Csp3 indices. Solubility is influenced by factors such as rotatable bond counts, hydrogen bond interactions, polar surface area, and lipophilicity, as indicated by Ilogp and Consensus Log P. Structural characteristics play a significant role in the solubility of flavonoids and phenolic acids. To improve bioavailability, it is recommended to use intravenous solutions and simpler structures. Smaller drug molecules can enhance bioavailability and cross biological barriers, such as the blood-brain barrier. Alkaloids adhere to Lipinski's rule and exhibit better permeability. Molecular properties play a crucial role in drug efficacy. *Citrullus colocynthis* demonstrates anti-diabetic effects by improving glucose regulation, with previous trials confirming its safety and efficacy for further research.

*Keywords*: *Citrullus colocynthis*, Cucurbitacins, Absorption, Physicochemical, Diabetes Mellitus.

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## 1. Introduction

*C. colocynthis* is a perennial plant with angular stems, triangular leaves, yellow flowers, and fruits containing oval seeds. It primarily grows in dry and tropical areas, especially in West Asia, Africa, and the deserts of Pakistan. The plant's bioactive substances, including essential oils, glycosides, flavonoids, alkaloids, and fatty acids, provide medicinal properties such as laxative, antidiabetic, and anti-cancer effects. The fruit extract has antioxidant and anti-inflammatory properties, aiding in disease prevention. In poultry, natural phytobiotics from *C. colocynthis* are used to promote gut health and improve growth performance instead of traditional antimicrobial

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Figure 1. It shows a *C. colocynthis* fruit (29).

growth promoters (1, 2).

The fruit acts as a strong laxative and helps remove intestinal parasites due to a glycoside called coloside. The dried pulp helps with indigestion and gastroenteritis, while plant extracts can relieve pain and reduce inflammation due to a compound named colofacine. *C. colocynthis* is also used for skin infections like scabies and eczema. It may assist with respiratory issues and has potential for diabetes management, liver and kidney support, and obesity management, but more studies are needed to confirm its safety and effectiveness (Figure 1) (1-3).

### *1.1. Bioactive Compounds of C. colocynthis*

This plant contains bioactive compounds, particularly cucurbitacins. These compounds are structurally complex and exhibit a diverse range of biological activities. The cucurbitacins found in *C. colocynthis* are derived from the tetracyclic skeleton [19-(10→9β)-abeo-10α-lanost-5-en]. This genus of triterpenoids is categorized into 12 classes, but not all of these classes are present in *C. colocynthis*. The primary class of compounds found in this species is cucurbitacins, which are known for their bitter taste. Notable examples include cucurbitacins A to L, along with their glycosides. Cucurbitacin E is the predominant member of this class in the fruit pulp (4). These cucurbitacins have been the subject of extensive research for their pharmacological properties, such as anti-inflammatory, antidiabetic, antitumor, and hepatoprotective effects (Figure 2) (3-6).

In addition to cucurbitacins, the fruits of *C. colocynthis* have been found to contain a variety of other bioactive constituents. Alkaloids present in the species include quinoline, nicotinamide, and their respective hydroxy and methyl derivatives Among these, 4-methylquinoline is noteworthy for its natural insecticidal properties against pests such as weevils in grain storage and spider mites. This suggests potential uses of *C. colocynthis* in agricultural applications for pest management (7-9).

Furthermore, the plant is rich in other secondary metabolites such as flavonoids and phenolic acids, which are known for their antioxidant properties. The presence of these compounds may contribute to the plant's overall therapeutic profile, as they are commonly associated with various health benefits in humans, including cardiovascular protection, anti-cancer activity, and neuroprotective effects (8, 10).

Volatile compounds, ketones, epoxy compounds, and hydrocarbons have also been detected in *C. colocynthis*. These substances contribute to the aromatic profile of the plant and may have roles in plant defense mechanisms or ecological interactions. For instance,

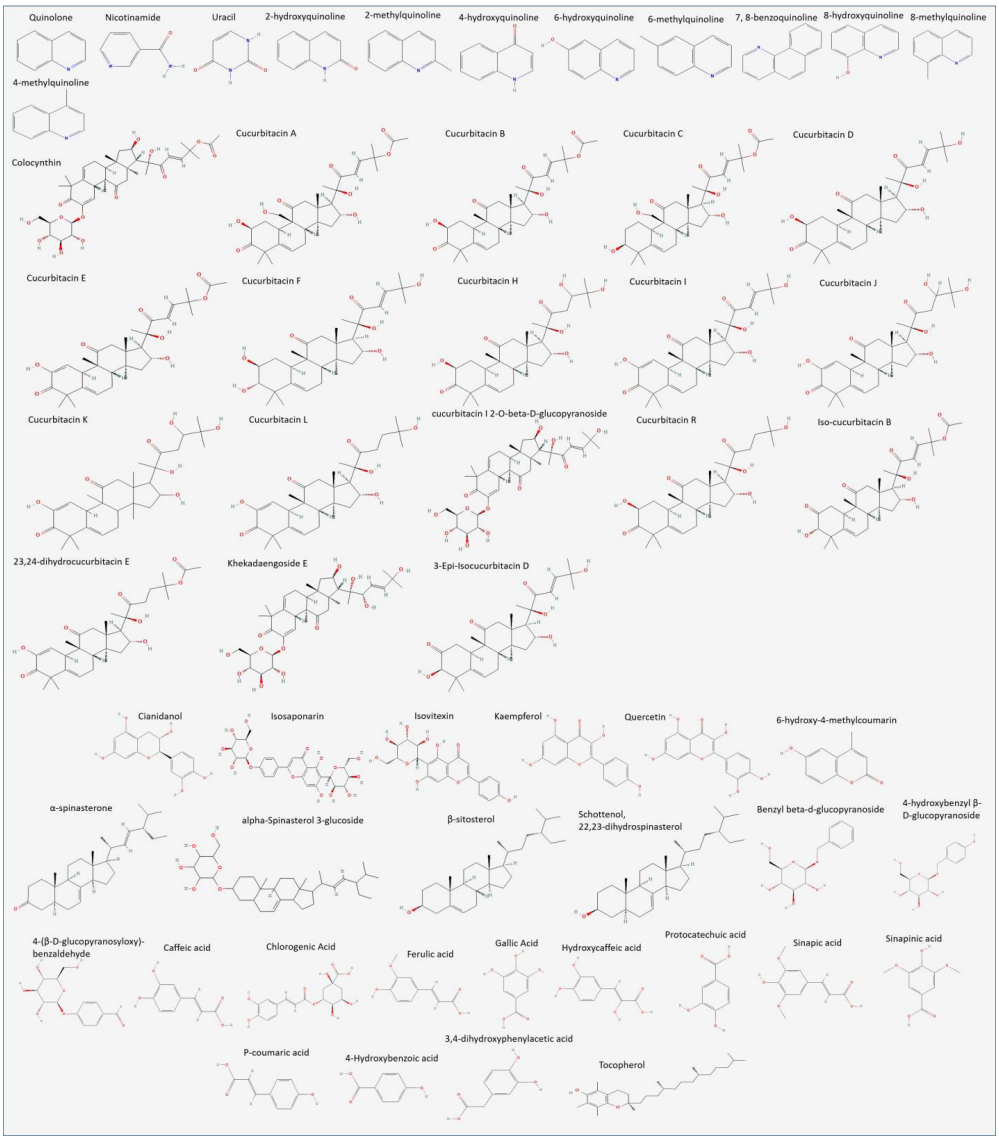


Figure 2. It shows the 2-D structure of bioactive compounds in *C. colocynthis*.

volatile organic compounds (VOCs) can act as chemical signals to deter herbivores or attract pollinators, while ketones and epoxy compounds can serve as structural components of the plant's natural defense system (7, 8).

Fatty acids, which are essential components of plant lipids, have been identified in *C. colocynthis* as well. These fatty acids can be used for various purposes, such as nutritional value or the production of bioactive molecules (11).

## *1.2. Cucurbitacin*

Cucurbitacin A is a triterpenoid compound with potential cytotoxic and anti-inflammatory effects, found in the fruit of *C. colocynthis*. Cucurbitacin B is another triterpenoid with potential therapeutic uses due to its biological activities and is also present in the fruit. Cucurbitacin C, D, E, I, J, and K are triterpenoids found in the fruit of *C. colocynthis*. Cucurbitacin L is another triterpenoid compound located in the same fruit (4, 6, and 12). Cucurbitacin E 2-O-β-D-glucopyranoside, cucurbitacin I 2-O-β-D-glucoside, cucurbitacin J 2-O-β-D-glucoside, cucurbitacin K 2-O-β-D-glucoside, and cucurbitacin L 2-O-β-D-glucoside are glycosides found in the fruit. Colocynthosides A and B are also glycosides present in the fruit (12-14). Deoxocucurbito-

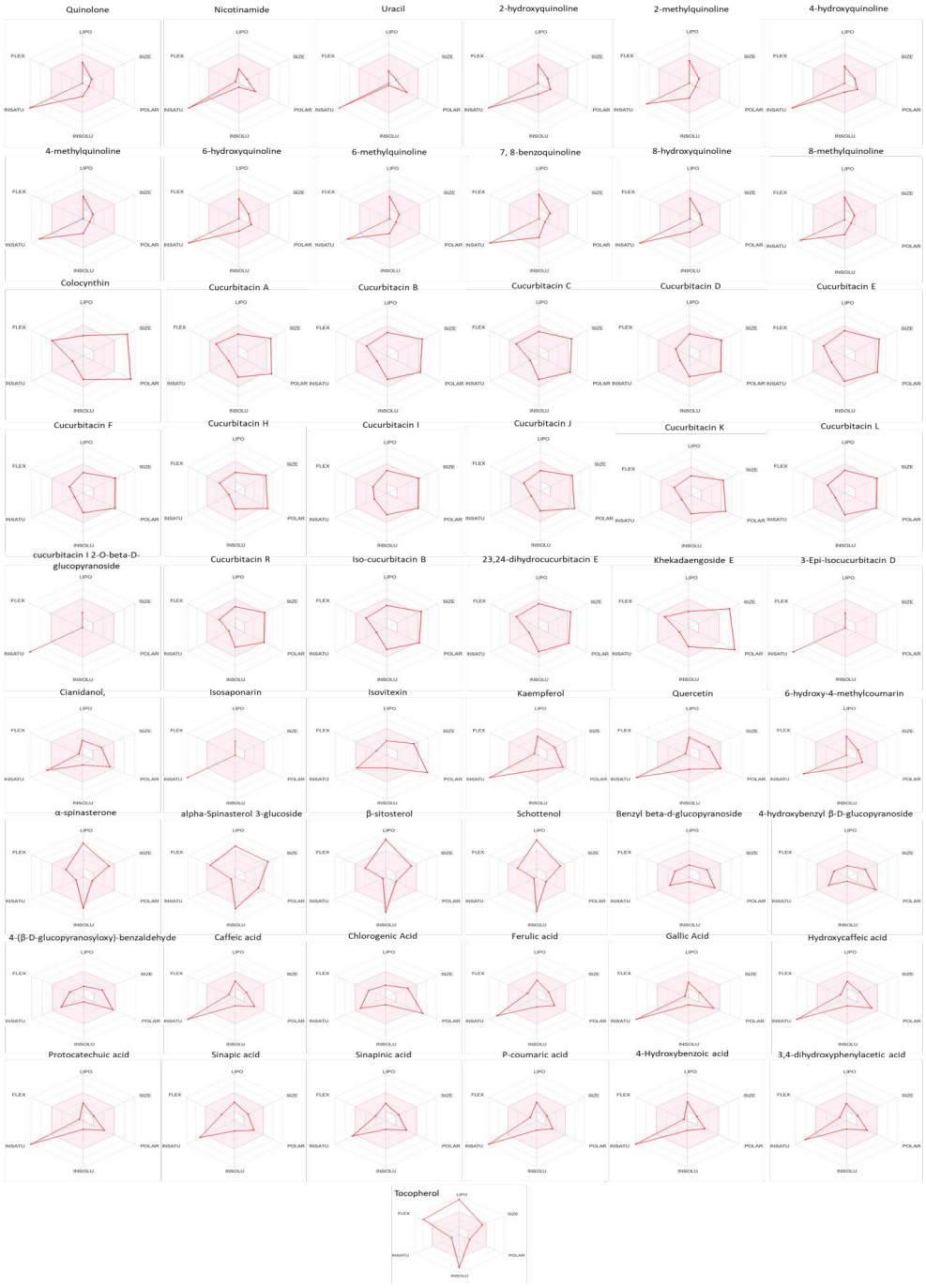


Figure 3. It illustrates a radar scale depicting the physicochemical properties and bioavailability of bioactive compounds in C. colocynthis. This scale highlights crucial properties like lipophilicity and molecular weight, which play a significant role in drug bioavailability. It assists in evaluating drug-likeness, as violations of the Rule of Five are often linked to poor oral bioavailability. Case studies have proven the efficacy of this scale in optimizing compounds.

side B is a triterpene glycoside isolated from the fruit, and Iso-cucurbitacin B is found in the fruit as well. Dihydrocucurbitacin E, a hydrogenated form of cucurbitacin E, is present in the fruit. Hexanocucurbitacin I 2-O-β-D-glucopyranoside and Khekadaengoside E are glycosides in the fruit. Other compounds, including dihydro-epi-iso-cucurbitacin D and derivatives of cucurbitacin E and I, can also be found in the leaves and fruit (5, 13-15).

### *1.3. Alkaloids*

Alkaloids are organic compounds found in the fruit, including several types of quinoline. Quinoline itself is an aromatic heterocyclic compound. Nicotinamide is a pyridine derivative also present in the fruit. Other derivatives include uracil, 2-hydroxyquinoline, 2-methylquinoline, 4-hydroxyquinoline, 4-methylquinoline, 6-hydroxyquinoline, and 6-methylquinoline. Additionally, 7,8-benzoquinoline, 8-hydroxyquinoline, and 8-methylquinoline are found in the fruit, each having distinct chemical structures related to quinoline (2, 5, 9).

### *1.4. Flavonoids*

Flavonoids include catechin, a type of flavan-3-ol found in *C. colocynthis* fruit. Isosaponarin is a saponin and flavonoid from *C. colocynthis* seeds. Isovitexin and isoorientin 3-O-methyl ether are found in the seeds. Kaempferol, myricetin, quercetin, and 6-C-p-methylbenzoylvitexin are flavonols present in the fruit (1, 3, and 6).

### *1.5. Steroid and its saponins*

α-spinasterone is a steroid found in the fruit. α-spinasterol-3-O-β-D-glucopyranoside is a glycoside of α-spinasterol isolated from the fruit. β-sitosterol is a sterol present in the fruit. 22,23-dihydrospinasterol is a hydrogenated derivative of spinasterol found in the fruit (5, 17).

### *1.6. Aromatic rings*

Benzyl β-D-glucopyranoside is an aromatic glycoside from the fruit. 4-hydroxybenzyl β-D-glucopyranoside is another glycoside found in the fruit. 4-(β-D-glucopyranosyloxy)-benzaldehyde is an aromatic aldehyde glycoside in the fruit. 4-(β-D-glucopyranosyloxy)-benzoic alcohol is an aromatic alcohol glycoside present in the fruit (13).

### *1.7. Phenolic acids*

Caffeic acid is a hydroxycinnamic acid found in the leaves of *C. colocynthis*. Chlorogenic acid is a polyphenol in the fruit. Ferulic acid is an aromatic carboxylic acid in the leaves. Gallic acid is a phenolic acid in the leaves, while gallic acid monohydrate is found in the roots. Hydroxycaffeic acid comes from seeds. Protocatechuic and sinapic acids are in the fruit. Syringic acid is found in seeds, and p-coumaric and p- hydroxybenzoic acids are present in the leaves. 3,4-dihydroxyphenylacetic acid is isolated from the leaves (18).

### *1.8. Other compounds include*

6-hydroxy-4-methylcoumarin, a coumarin found in the fruit (22), and alpha, beta, gamma, and delta-tocopherol, fat-soluble antioxidants and another form of vitamin E found in seeds (7, 19).

### *1.9. Toxicology and Safety*

The fruit contains cucurbitacins, which can cause severe stomach issues such as colic, nausea, vomiting, and diarrhea. Eating too much may lead to serious problems like cerebral congestion and hypothermia. Therefore, it is important to consult a healthcare provider before using it for medicinal purposes. *C. colocynthis* has purgative and anti-inflammatory effects. When combined with Nerium oleander, it can increase toxicity, potentially causing death in rats during experiments. The negative effects stem from its irritant properties, causing symptoms such as severe vomiting, stomach pain, and bloody diarrhea. This can lead to dehydration and electrolyte imbalances, especially with self-medication. It can also harm kidneys and disrupt normal organ functions (3, 7).

## 2. Materials and Methods

### *2.1. Literature Review*

A literature review was conducted to assess the medicinal use of *C. colocynthis*, also known as bitter cucumber or colocynth, in traditional medicine. The goal was to determine its potential as a therapeutic agent in

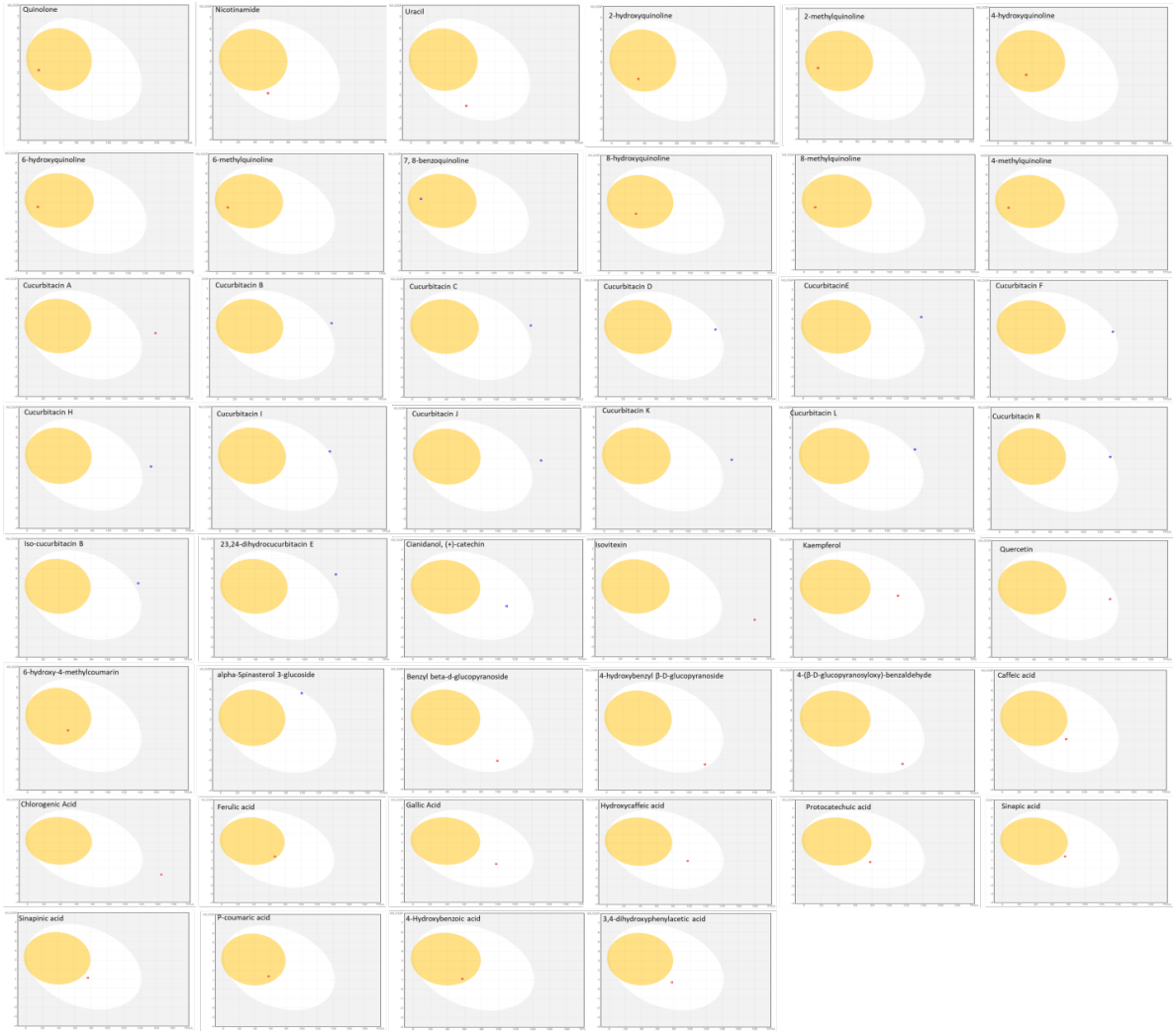


Figure 4. It illustrates the egg plot, a visual tool that shows how a compound interacts with biological barriers such as the blood-brain barrier (BBB) and the digestive system. The yellow area indicates the compound's ability to passively cross the BBB, while the white area represents its potential for passive absorption in the digestive system. Blue dots on the plot symbolize the entry of the compound into the central nervous system (CNS) via P-glycoproteins, while red dots indicate its exit from the CNS, impacting its efficacy and duration.

herbal medicine. The review involved searching several databases such as PubMed, ScienceDirect, and Scopus using relevant keywords to gather information on its medicinal properties.

### *2.2. Predicting Physicochemical Properties and Pharmacokinetics*

The physicochemical properties of these compounds were calculated using SwissADME, focusing on solubility, permeability, and lipophilicity. The pharmacokinetic profiles were also assessed to understand their bioavailability, absorption, distribution, metabolism, and excretion (ADME). SwissADME is an advanced web platform for predicting ADME (Absorption, Distribution, Metabolism, and Excretion) properties of drugs, integrating multiple validated models for free use. It features a Consensus Modeling Approach with five different log P prediction methods, helping to overcome variability issues in lipophilicity predictions. This approach is recognized as best practice in cheminformatics. The platform includes a novel visualization tool called the BOILED-Egg model, which graphi-

Table 2. It displays the Pharmaceutical Properties of the Studied Compounds. Key pharmaceutical characteristics include GI absorption for oral bioavailability, BBB permeability for CNS drugs, P-glycoprotein substrate risks for bioavailability, and CYP inhibition profiles for drug interactions. Strategies for optimization involve prodrugs, structural modifications, and scaling considerations, guiding rational drug candidate prioritization in development.

| Molecules | GI absorption | BBB permeant | Pgp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | log Kp (cm/s) | Bioavailability Score | Synthetic Accessibility |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Quinolone | High | Yes | No | Yes | No | No | No | No | -5.65 | 0.55 | 1.00 |
| Nicotinamide | High | No | No | No | No | No | No | No | -7.31 | 0.55 | 1.00 |
| Uracil | High | No | No | No | No | No | No | No | -7.74 | 0.55 | 1.35 |
| 2-hydroxyquinoline | High | Yes | No | Yes | No | No | No | No | -6.29 | 0.55 | 1.36 |
| 2-methylquinoline | High | Yes | No | Yes | No | No | No | No | -5.33 | 0.55 | 1.00 |
| 4-hydroxyquinoline | High | Yes | No | Yes | No | No | No | No | -6.77 | 0.55 | 1.06 |
| 4-methylquinoline | High | Yes | No | Yes | No | No | No | No | -5.32 | 0.55 | 1.00 |
| 6-hydroxyquinoline | High | Yes | No | Yes | No | No | No | No | -5.91 | 0.55 | 1.00 |
| 6-methylquinoline | High | Yes | No | Yes | No | No | No | No | -5.35 | 0.55 | 1.00 |
| 7, 8-benzoquinoline | High | Yes | Yes | Yes | No | No | No | No | -4.96 | 0.55 | 1.03 |
| 8-hydroxyquinoline | High | Yes | No | Yes | No | No | No | No | -5.75 | 0.55 | 1.07 |
| 8-methylquinoline | High | Yes | No | Yes | No | No | No | No | -5.33 | 0.55 | 1.00 |
| Colocynthin | Low | No | No | No | No | No | No | No | -9.66 | 0.17 | 8.21 |
| Cucurbitacin A | Low | No | No | No | No | No | No | Yes | -8.41 | 0.55 | 6.88 |
| Cucurbitacin B | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin C | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin D | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin E | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin F | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin H | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin I | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin J | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin K | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| Cucurbitacin L | Low | No | Yes | No | No | No | No | Yes | -7.83 | 0.55 | 6.79 |
| cucurbitacin I 2-O-beta-D-glucopyranoside | Low | No | No | No | No | No | No | No | -9.81 | 0.17 | 8.02 |
| Cucurbitacin R | High | No | Yes | No | No | No | No | Yes | -7.96 | 0.55 | 6.68 |
| Iso-cucurbitacin B | Low | No | Yes | No | No | No | No | Yes | -7.92 | 0.55 | 6.66 |
| 23,24-dihydrocucurbitacin E | Low | No | Yes | No | No | No | No | Yes | -7.36 | 0.55 | 6.69 |
| Khekadaengoside E | Low | No | No | No | No | No | No | No | -10.05 | 0.17 | 8.16 |

**Continued Table 2.**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-Epi-Isocucurbitacin D | High | No | Yes | No | No | No | No | Yes | -8.07 | 0.55 | 6.49 |
| Cianidanol | High | No | Yes | No | No | No | No | No | -7.82 | 0.55 | 3.5 |
| Isosaponarin | Low | No | Yes | No | No | No | No | No | -11.06 | 0.17 | 6.28 |
| Isovitexin | Low | No | No | No | No | No | No | No | -8.79 | 0.55 | 4.99 |
| Kaempferol | High | No | No | Yes | No | No | Yes | Yes | -6.7 | 0.55 | 3.14 |
| Quercetin | High | No | No | Yes | No | No | Yes | Yes | -7.05 | 0.55 | 3.23 |
| 6-hydroxy-4-methylchromen-2-one | High | Yes | No | Yes | No | No | No | No | -6.03 | 0.55 | 2.47 |
| α-spinasterone | Low | No | No | No | No | Yes | No | No | -3.21 | 0.55 | 5.97 |
| alpha-Spinasterol 3-glucoside | High | No | Yes | No | No | No | No | No | -5.04 | 0.55 | 7.81 |
| β-sitosterol | Low | No | No | No | No | No | No | No | -2.2 | 0.55 | 6.3 |
| Schottenol | Low | No | No | No | No | No | No | No | -2.38 | 0.55 | 6.18 |
| Benzyl beta-d-glucopyranoside | High | No | No | No | No | No | No | No | -8.45 | 0.55 | 4.2 |
| 4-hydroxy-benzyl β-D-glucopyranoside | High | No | No | No | No | No | No | No | -8.8 | 0.55 | 4.13 |
| 4-(β-D-glucopyranosyloxy)-benzaldehyde | High | No | No | No | No | No | No | No | -8.68 | 0.55 | 3.99 |
| Caffeic acid | High | No | No | No | No | No | No | No | -6.58 | 0.56 | 1.81 |
| Chlorogenic Acid | Low | No | No | No | No | No | No | No | -8.76 | 0.11 | 4.16 |
| Ferulic acid | Low | No | No | No | No | No | No | No | -8.76 | 0.11 | 4.16 |
| Gallic Acid | High | No | No | No | No | No | No | Yes | -6.84 | 0.56 | 1.22 |
| Hydroxycaffeic acid | High | No | No | No | No | No | No | No | -6.77 | 0.56 | 2.09 |
| Protocatechuic acid | High | No | No | No | No | No | No | Yes | -6.42 | 0.56 | 1.07 |
| Sinapic acid | High | No | No | No | No | No | No | No | -6.63 | 0.56 | 2.17 |
| Sinapinic acid | High | No | No | No | No | No | No | No | -6.63 | 0.56 | 2.17 |
| P-coumaric acid | High | Yes | No | No | No | No | No | No | -6.26 | 0.85 | 1.61 |
| 4-Hydroxybenzoic acid | High | Yes | No | No | No | No | No | No | -6.02 | 0.85 | 1.00 |
| 3,4-dihydroxyphenylacetic acid | High | No | No | No | No | No | No | No | -6.63 | 0.56 | 1.27 |
| Tocopherol | Low | No | Yes | No | No | No | No | No | -1.33 | 0.55 | 5.17 |

cally represents key drug disposition factors by showing gastrointestinal absorption, blood-brain barrier penetration, and P-glycoprotein substrate status in a single plot, achieving high predictive accuracy. SwissADME also integrates proprietary algorithms, such as iLOGP and Bioavailability Radar, that enhance its capabilities compared to other free tools. Extensive validation of machine learning classifiers ensures reliability in the predictions, utilizing rigorous methods to prevent data leakage and optimize performance metrics like accuracy (21-24).

### *2.4. Methods*

Names and identifiers, along with

computed descriptors, IUPAC names, and SMILES of the studied compounds and molecular models of compounds from *C. colocynthis* were obtained from the PubChem database, providing essential structural details for further analysis. The IUPAC names and SMILES of the studied compounds were used for analyzing and predicting physicochemical properties and pharmacokinetics. The compounds from *C. colocynthis* were studied using SwissADME software, and the output results were reported. The investigation aimed to clarify its potential as a therapeutic agent in herbal medicine (25-28).

## 3. Results

### *3.1. Physicochemical Properties of Studied Compounds*

The compounds studied include Cucurbitacins, which weigh between 516 and 718 daltons and contain 37 to 51 atoms. Other compounds weigh below 432 daltons, indicating good digestive absorption. Alkaloid compounds with 8-14 atoms weigh less than 180 daltons and exhibit high digestive absorption and permeability through the blood-brain barrier.

Compounds like Kaempferol, Quercetin, Isovitexin, Isosaponarin, 7,8-benzoquinoline, and Cianidanol have the highest number of aromatic heavy atoms, ranging from 16 to 12. Compounds such as β-sitosterol, Schottenol, alpha-Spinasterol 3-glucoside, α-spinasterone, and Cucurbitacin R have the highest Fraction Csp3 index, with a value above 0.8. The Fraction Csp3 index indicates the percentage of carbon atoms in a molecule that are sp3 bonded, which is crucial for determining a compound's lipophilicity and its ability to penetrate cell membranes, affecting its bioavailability (23).

Tocopherol, Colocynthin, and alpha-Spinasterol 3-glucoside have the highest number of rotatable bonds, ranging from 8-12. Isosaponarin, Colocynthin, Khekadaengoside E, and Cucurbitacin I 2-O-beta-D-glucopyranoside have the highest number of hydrogen bond acceptors, ranging from 10-15. Isosaponarin, Khekadaengoside E, Cucurbitacin I 2-O-beta-D-glucopyranoside, and Isovitexin have the highest number of hydrogen bond donors, with 7-10 hydrogen bond donor groups.

The lowest MR values are related to Chlorogenic Acid, Ferulic Acid, Quercetin, Kaempferol, and Cianidanol, ranging from 74-83. The highest MR values are for Colocynthin, Khekadaengoside E, and Cucurbitacin I 2-O-beta-D-glucopyranoside, ranging from 173-182. The compounds Isosaponarin, Colocynthin, Khekadaengoside E, and Cucurbitacin I 2-O-beta-D-glucopyranoside have the highest polar surface area, with a value of 211-260 Angstroms squared.

On the other hand, compounds like 7,8-benzoquinoline, 2-methylquinoline, 4-methylquinoline, 6-methylquinoline, 8-methylquinoline, and Quinolone have the lowest polar surface area, with a value of 12.88 Angstroms squared.

The lowest Ilogp values are related to compounds like Nicotinamide, Protocatechuic Acid, Benzyl beta-D-glucopyranoside, Uracil, and Gallic Acid, ranging from 0.7 to -0.4. The highest Ilogp values are for Tocopherol, alpha-Spinasterol 3-glucoside, Schottenol, and β-sitosterol, with a value of 5.05 to 6.04.

The compounds Tocopherol, β-sitosterol, Schottenol, alpha-Spinasterol, and alpha-Spinasterol 3-glucoside have the highest Consensus Log P values, ranging from 5.23 to 8.29. The lowest values are for Isosaponarin, 4-hydroxybenzyl beta-D-glucopyranoside, 4-(β-D-glucopyranosyloxy)-benzaldehyde, Benzyl beta-D-glucopyranoside, Chlorogenic Acid, and Ferulic Acid, ranging from -0.30 to -1.48.

The compounds Tocopherol, β-sitosterol, Schottenol, alpha-Spinasterol, and alpha-Spinasterol have the lowest ESOL Log S values, ranging from -7.02 to -8.6. The highest values are for the compounds 4-(β-D-glucopyranosyloxy)-benzaldehyde,

4-hydroxybenzyl beta-D-glucopyranoside, Nicotinamide, and Uracil, with values ranging from -0.99 to -0.42, indicating very high solubility in physiological fluids (Figure 3, and 4) (Table 1).

The study results provide a comparative analysis of various compounds based on their solubility, lipophilicity, and drug-like properties. It starts with small heterocycles like nicotinamide and uracil, which show high solubility due to favorable properties like low partition coefficients (logP) and moderate polar surface areas. These characteristics enhance their hydrophilicity and solubility. In contrast, methyl-substituted quinolines exhibit much lower solubility because of increased lipophilicity.

Next, regarding the cucurbitacins, which struggle with Lipinski's rules, specifically their high molecular weight and excessive hydrogen bonding capabilities. Cucurbitacins are generally poorly soluble and have low $Csp^3$ fractions, contributing to their hydrophobic nature and flexible structures, which do not help improve solubility.

The section on phenolic acids and flavonoids reveals that compounds like caffeic acid and chlorogenic acid have excellent solubility due to their favorable structural properties, while flavonoids such as quercetin and kaempferol suffer from low solubility because of their aromatic structures promoting stacking.

Sterols are highlighted as being highly lipophilic, resulting in poor solubility due to their long alkyl chains and low polar surface areas, making them unsuitable for oral use.

In terms of drug design implications, compounds that meet Lipinski's criteria show better bioavailability. Most phenolic acids and small heterocycles qualify, while cucurbitacins do not. A polar surface area of under 140 $Å^2$ suggests good absorption potential for most compounds, except for colocynthin, which has higher values (Suppl 1. Table 1).

### *3.2. Pharmaceutical Properties of Studied Compounds*

Most cucurbitacins, steroid compounds, and some flavonoid compounds have low gastrointestinal absorption. However, compounds like 7,8-benzoquinoline, 4-methylquinoline, 8-methylquinoline, 2-methylquinoline, 6-methylquinoline, 6-hydroxy-4-methylchromen-2-one, 8-hydroxyquinoline, Quinolone, 6-hydroxyquinoline, 2-hydroxyquinoline, 4-Hydroxybenzoic Acid, P-coumaric Acid, and 4-hydroxyquinoline can cross the blood-brain barrier.

Additionally, compounds like Kaempferol, Quercetin, 7,8-benzoquinoline, 4-methylquinoline, 8-methylquinoline, 2-methylquinoline, 6-methylquinoline, 6-hydroxy-4-methylchromen-2-one, 8-hydroxyquinoline, Quinolone, 6-hydroxyquinoline, 2-hydroxyquinoline, and 4-hydroxyquinoline inhibit the CYP1A2 enzyme. None of the compounds inhibit the CYP2C19 enzyme, while only α-spinasterone can inhibit the CYP2C9 enzyme. Kaempferol and Quercetin can inhibit CYP2D6. Moreover, Kaempferol, Quercetin, 23,24-dihydrocucurbitacin E, Cucurbitacin B, Cucurbitacin C, Cucurbitacin D, Cucurbitacin E, Cucurbitacin F, Cucurbitacin H, Cucurbitacin I, Cucurbitacin J, Cucurbitacin K, Cucurbitacin L, Iso-cucurbitacin B, Cucurbitacin R, 3-Epi-Isocucurbitacin D, Cucurbitacin A, Protocatechuic Acid, and Gallic Acid can inhibit CYP3A4 (22).

Lastly, α-spinasterone, Schottenol, β-sitosterol, and Tocopherol have the highest skin absorption, with log Kp (cm/s) ranging from -3.21 to -1.23. The log Kp value indicates how well a chemical can pass through the skin, with a higher value indicating better skin permeability and potential absorption and toxicity risks (Table 2) (22, 24).

The analysis of pharmaceutical properties of various compounds leads to the identification of promising candidates and risks for therapeutic use. For CNS-targeting drugs, quinolone derivatives, such as 2-methylquinoline

and others, show advantages like high absorption and ability to cross the blood-brain barrier. However, they have risks like the need for cardiac monitoring due to CYP1A2 inhibition. p-Coumaric acid and 4-hydroxybenzoic acid stand out as non-quinoline compounds with high oral bioavailability and potential neuroprotective effects.

In peripheral-acting therapeutics, the cucurbitacin series faces significant limitations such as low bioavailability and high risk of drug interactions. It is suggested that intravenous formulations may be necessary, along with structural simplifications to improve synthetic accessibility. Kaempferol and quercetin could pose polypharmacology concerns but offer moderate synthetic accessibility for optimization.

Transdermal delivery candidates like α-spinasterone have optimal skin permeability but low gastrointestinal absorption, making topical development essential. β-sitosterol and schottenol display promising transdermal potential but require enhancers due to their crystalline nature.

Key development candidates include 2-methylquinoline for CNS infections, p-coumaric acid for neurodegeneration, β-sitosterol for dermatology, and cucurbitacin R for oncology, with several strategies recommended for risk mitigation. The analysis concludes that prioritizing certain compounds and validating data can significantly reduce clinical risk in drug development.

## 4. Discussion

Smaller molecules are generally favored in drug design due to their increased bioavailability and ability to cross biological barriers such as the blood-brain barrier. Alkaloids, including quinoline and nicotinamide derivatives, exhibit lower molecular weights, consistent with Lipinski's rule of five, a set of guidelines used to determine the likelihood of a molecule being an orally active drug. These compounds typically have fewer heavy atoms, contributing to their smaller size and potential for better cell permeability (22-24).

The number of aromatic heavy atoms is significant in assessing the aromaticity of molecules. Flavonoids such as Kaempferol, Quercetin, and Isovitexin have higher aromaticity, indicative of their planar, conjugated structures that confer antioxidant properties. These structures can delocalize electrons, scavenging free radicals, a critical mechanism for antioxidants (23).

The fraction of sp3 hybridized carbons (Fraction Csp3) measures a molecule's saturation and is linked to its lipophilicity. Lipophilic compounds are generally more stable and can penetrate biological membranes more readily, essential for drugs to reach their target sites in the body. The presence of rotatable bonds affects the molecule's flexibility, impacting its ability to interact with biological macromolecules, potentially affecting binding affinity and selectivity (23).

Molecular polarizability, indicated by molecular refractivity (MR) values, reflects electron distribution and size of molecules. Larger and more complex molecules like Colocynthin, Khekadaengoside E, and Cucurbitacin I 2-O-beta-D-glucopyranoside have higher MR values, suggesting greater polarizability. Polar surface area (TPSA) is another critical factor influencing water solubility and, consequently, a molecule's ability to interact with hydrophilic environments such as biological membranes (23).

Octanol-water partition coefficients (iLOGP and XLOGP3) provide insights into the hydrophobicity or lipophilicity of molecules, essential for understanding their distribution and potential for crossing biological barriers like the blood-brain barrier (BBB). High TPSA values, found in compounds like Isosaponarin and Colocynthin, indicate higher polarity and potentially greater water solubility despite their relatively high lipophilicity. This balance between hydrophobicity and hydrophilicity can be advantageous for com-

pounds needing to interact with both aqueous and lipid environments (23, 24).

The low absorption of most cucurbitacins and steroid compounds in the gut suggests they are not easily taken up orally, potentially lowering their effectiveness for systemic treatments. In contrast, some flavonoids like Kaempferol and Quercetin, along with certain quinoline derivatives, can cross the blood-brain barrier (BBB). The BBB protects the brain from harmful substances while allowing necessary nutrients to pass. Compounds penetrating the BBB are crucial for treating neurological disorders because they can directly act on brain cells (24).

Cytochrome P450 (CYP) enzymes are key for metabolizing many drugs and compounds. Inhibiting these enzymes can alter drug metabolism and effectiveness. For example, the inhibition of CYP1A2 by compounds like 7,8-benzoquinoline and others can affect the metabolism of medications, including antidepressants and anticancer drugs. The inhibition of CYP2D6 by Kaempferol and Quercetin can impact drugs like SSRIs and beta-blockers, potentially raising their levels in the blood and enhancing their effects or side effects. Additionally, CYP3A4 metabolizes many commonly prescribed drugs, and its inhibitors such as Kaempferol and Cucurbitacins can decrease the clearance rate of substrate drugs, leading to drug accumulation and increased toxicity or effectiveness (22).

Log Kp (cm/s) measures a compound's ability to penetrate the skin. Compounds with higher log Kp values, like α-spinasterone and β-sitosterol, may be suitable for transdermal drug delivery systems due to their effective skin barrier crossing ability, crucial for topical or transdermal treatments (22).

The aqueous extract of C. colocynthis can lower blood sugar levels in diabetic rats. Doses of 250 and 500 mg/kg not only reduced blood glucose but also improved body weight, while lowering glycosylated hemoglobin levels showed better long-term blood sugar control. The research examined the extract's impact on liver enzymes essential for glucose metabolism, such as hexokinase, glucose-6-phosphatase, and fructose-1,6-bisphosphatase. The findings showed increased hexokinase activity and decreased levels of the other enzymes, hinting that *C. colocynthis* might help manage diabetes through these enzymes (11, 30, and 31).

The previous study suggests that *C. colocynthis* has anti-diabetic effects beyond promoting insulin release from the pancreas.

Researchers studied plant extracts, notably ethyl acetate fractions from the seeds and pulp, and found that these could enhance insulin action in a model for insulin resistance. The pulp extract was more effective than the seed extract at facilitating the movement of GLUT4 to the cell surface, crucial for glucose uptake. This didn't affect the cells' sensitivity to insulin but may enhance insulin signaling pathways post-receptor activation (27, 28, 30, and 32).

At the molecular level, the *C. colocynthis* pulp extract significantly increased protein kinase B phosphorylation, vital for glucose metabolism, without altering insulin receptor phosphorylation. This suggests a specific action that might involve pathways like insulin receptor substrate-1 or phosphatidylinositol 3-kinase, important for insulin-related glucose uptake. Furthermore, the pulp extract was non-toxic and helped restore cell health during nutrient scarcity, likely beneficial for those with insulin resistance (27, 28, and 32).

A previous study examined the effects of C. colocynthis supplementation at a daily dose of 125mg over 60 days in patients with Type 2 diabetes (T2DM). Key results included a 0.6% decrease in HbA1c and an 11.6 mg/dL reduction in fasting blood glucose, while the placebo group showed no changes. The supplement worked better in patients with higher baseline HbA1c and BMI. No adverse effects were reported at this dosage, unlike higher doses that can cause gastrointestinal problems.

The study suggests that 125mg/day is effective and safe for managing T2DM, especially in high-BMI patients. Future research should explore long-term safety and combinations with other diabetes treatments (33).

The *C. colocynthis* Schrad has revealed important findings regarding its toxicity and medicinal properties. The acute toxicity profile shows that immature fruits are the most toxic at 95.8 mg/kg, while stems are the least toxic at 3903.2 mg/kg. This high toxicity in immature fruits aligns with reported adverse effects such as gastrointestinal irritation and neurotoxicity. In terms of analgesic activity, the extract from immature fruits shows the highest effectiveness with 84% inhibition at 4 mg/kg. Seed extracts also demonstrate strong inhibition at 78%, while root and stem extracts have weaker effects (34).

## 5. Conclusion

The physicochemical analysis of *C. colocynthis* molecules reveals a diverse array of compounds that could be of pharmacological interest in treating type II diabetes. Previous studies support the potential of these molecules in modulating glucose and lipid metabolism and suggest that they may be suitable for further development as therapeutic agents. However, comprehensive pharmacological studies are necessary to fully elucidate their mechanisms of action, efficacy, and safety before they can be considered for clinical use. The integration of these natural compounds into pharmaceutical or nutraceutical formulations requires careful consideration of their physicochemical properties and potential interactions with other medications or biological systems.

## Authors contributions

The study's conception and design, data collection, analysis, interpretation, drafting of the

manuscript, and critical review were conducted by Roohallah Yousefi.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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