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Zinc Complex in Diabetic Cataract Synthesized from Fresh Leaf Extract of *Hedychium Coronarium*

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ABSTRACT
This study explores the synthesis and protective effects of a Zinc-*Hedychium coronarium* complex against diabetic cataract formation, utilizing fresh leaf extracts of *Hedychium coronarium*. The investigation employed goat eye lenses to create an *in vitro* model for cataractogenesis, wherein lenses were incubated in a hyperglycemic medium (55 mM glucose) to induce cataract development. The lenses were divided into three experimental groups: Group-I (control) was maintained in normal phosphate-buffered saline (PBS), Group-II (diabetic control) was subjected to hyperglycemic conditions, and Group-III was treated with the Zinc-*Hedychium coronarium* complex in the hyperglycemic medium. After 72 hrs of incubation, cataract severity was evaluated using a standardized grading scale. The results indicated that lenses in Group-II exhibited severe opacity with an average grading score of 3.8, reflecting significant cataract formation. In contrast, lenses treated with the Zinc-*Hedychium coronarium* complex in Group-III demonstrated a marked reduction in opacity, achieving an average score of 1.2, representing a 68% decrease in cataract severity compared to the diabetic control group. The observed protective effects are attributed to the antioxidant properties of the bioactive compounds in *Hedychium coronarium*, the stabilization of lens proteins by zinc, and the inhibition of aldose reductase activity, which collectively mitigate oxidative stress and osmotic damage. The characterization of the Zinc-*Hedychium coronarium* complex through spectroscopic techniques such as UV, FTIR, and ^{1}H-NMR confirmed its successful synthesis. This study underscores the potential of the Zinc-*Hedychium coronarium* complex as a therapeutic agent in preventing diabetic cataract formation, paving the way for future research into its mechanisms and clinical applications in ocular health management for diabetic patients.

Keywords: *Hedychium coronarium*, Zinc, Complex, Cataract, Characterization, Goat eye lens

1. INTRODUCTION

Diabetic cataract is a significant complication of diabetes mellitus, characterized by the clouding of the eye's natural lens, leading to visual impairment and, if left untreated, blindness. This condition arises due to the chronic hyperglycemia associated with diabetes, which triggers various biochemical changes within the lens. One of the primary mechanisms involves the accumulation of sorbitol, a sugar alcohol produced from glucose through the polyol pathway [1]. The enzyme aldose reductase converts excess glucose into sorbitol, which is poorly metabolized and accumulates within the lens, causing osmotic stress, oxidative damage, and lens fiber cell swelling. Additionally, hyperglycemia-induced non-enzymatic glycation of lens proteins, particularly crystallins, leads to protein aggregation and the formation of advanced glycation end products (AGEs), contributing to lens opacity. Oxidative stress also plays a crucial role, as elevated glucose levels increase the production of reactive oxygen species (ROS), overwhelming the lens's antioxidant defenses and causing further damage to lens proteins and lipids [2]. Over time, these biochemical alterations result in the opacification of the lens, characteristic of a diabetic cataract. Clinically, patients with diabetic cataracts may experience symptoms such as blurred vision, glare, difficulty with night vision, and a gradual loss of visual acuity. The management of diabetic cataract primarily involves surgical intervention through phacoemulsification, where the cloudy lens is removed and replaced with an artificial intraocular lens. Preventive strategies include tight glycemic control to minimize the risk of cataract formation, regular ophthalmologic examinations for early detection, and antioxidant supplementation to counteract oxidative stress. Diabetic cataract remains a major cause of visual disability in diabetic patients, underscoring the importance of comprehensive diabetes management and regular eye care [3].

Hedychium coronarium, commonly known as white ginger lily or butterfly ginger, is a perennial herbaceous plant belonging to the Zingiberaceae family. Native to the Himalayan region of India and Nepal, it is now widely cultivated in tropical and subtropical regions around the world for its ornamental value and fragrant flowers. The plant typically grows to a height of 1 to 2 meters and features long, lance-shaped leaves that are arranged alternately along the stem [4]. The striking white flowers, which resemble delicate butterflies, bloom in clusters at the top of the stems, emitting a sweet, jasmine-like fragrance that is most intense during the evening. *H.*

coronarium thrives in moist, well-drained soils and prefers partial shade, making it ideal for garden borders, water gardens, and shaded landscapes. Beyond its ornamental use, the plant has a history of medicinal applications in traditional medicine systems, particularly in Asia. The rhizomes of *H. coronarium* are known to possess anti-inflammatory, analgesic, and antimicrobial properties, and have been used to treat conditions such as fever, respiratory issues, and gastrointestinal disorders [5]. In addition, the essential oils extracted from the flowers and leaves are utilized in perfumery and aromatherapy due to their calming and soothing effects. Despite its benefits, *H. coronarium* is considered invasive in some regions, particularly in parts of the Americas, where it can outcompete native vegetation and disrupt local ecosystems. Control measures are often necessary to manage its spread in these areas. Overall, *H. coronarium* is a plant of significant horticultural, medicinal, and ecological interest, valued for both its beauty and its utility [6,7].

The use of "metal-plant extract" combinations for treating diabetic cataract induced *in vitro* in goat eye lens represents a novel and promising approach in ocular research. Diabetic cataract is a major complication of diabetes, primarily driven by hyperglycemia-induced oxidative stress, protein glycation, and osmotic imbalances within the lens. Metal-plant extracts are formulated by combining plant extracts known for their antioxidant properties with metal ions that can enhance or stabilize these properties. This synergy is thought to amplify the therapeutic effects against oxidative stress, a key factor in cataract formation [8]. In the context of *in vitro* studies using goat eye lenses, these metal-plant extract combinations are tested for their ability to prevent or reduce lens opacity, a hallmark of cataractogenesis. Typically, lenses are exposed to high glucose conditions to simulate the diabetic environment, leading to cataract formation over time. The application of metal-plant extracts in these models aims to counteract the biochemical changes induced by hyperglycemia, such as the accumulation of sorbitol, formation of advanced glycation end products (AGEs), and oxidative damage to lens proteins. The plant extracts contribute polyphenols, flavonoids, and other bioactive compounds that have potent antioxidant and anti-glycation properties. Metals like zinc, selenium, and copper, when appropriately formulated, can play roles in enzymatic defense mechanisms and further reduce oxidative stress [9]. Studies have shown that these combinations can significantly decrease the extent of lens opacity in treated samples compared to controls, suggesting their potential in delaying or preventing the onset of diabetic cataracts. Moreover, such *in vitro* studies using goat

lenses provide a controlled environment to investigate the molecular mechanisms of action and optimize the formulations for potential therapeutic use. If proven effective, these metal-plant extracts could offer a natural, cost-effective alternative to traditional treatments, with applications in both preventive and therapeutic contexts for diabetic cataract management. However, further research, including *in vivo* studies and clinical trials, would be necessary to fully understand their efficacy, safety, and potential for human use [10,11].

The aim of this research is to develop and evaluate an ophthalmic preparation formulated with a transitional metal complex synthesized from the fresh leaf extract of *Hedychium coronarium* for the prevention and treatment of diabetic cataract. The specific objectives include the extraction and characterization of bioactive compounds from the fresh leaves of *Hedychium coronarium*, followed by the synthesis of a transitional metal complex using these compounds and an assessment of its stability and efficacy. Additionally, the research seeks to formulate an ophthalmic preparation incorporating the metal complex with suitable excipients to enhance ocular delivery. The *in vitro* evaluation of the antioxidant and anti-glycation activities of the metal complex will be conducted to determine its potential in preventing diabetic cataract. Furthermore, *in vitro* studies using goat eye lenses will be performed to assess the ability of the formulated preparation to inhibit cataract formation under hyperglycemic conditions. Finally, the research will include physicochemical and stability studies on the ophthalmic preparation to ensure its suitability for potential therapeutic use.

2. MATERIALS AND METHODS

2.1. Materials

Sodium bicarbonate, zinc chloride acetic acid, sodium acetate, phosphoric acid, sodium phosphate, boric acid, sodium borate were obtained from HiMedia Pvt. Ltd., Mumbai, Maharashtra, India. Distilled water (Borosil) was employed in this study.

2.2. Collection of Plant

The leaves of *H. coronarium* were collected from the local area and the undesirable materials were then separated manually. The collected plant was then identified from Department of Botany, Lucknow University, Lucknow, Uttar Pradesh, India and kept at herbarium with Accession code (No. HB/2024/9137) of the plant for further reference.

2.3. Preparation of the plant sample

The collected plant leaves were cut into small pieces, sun-dried, and then oven-dried at 35-40°C for 24 hrs. The dried leaves were coarsely powdered using a mechanical grinder and stored in a suitable container for extraction. The powdered material was then weighed using a balance [12].

2.4. Extraction of Leaves

The plant leaves were cold-extracted by soaking 150 g of dried powder in 450 mL of 80% methanol in a pre-washed, methanol-rinsed glass jar. The mixture was covered with plastic and aluminum foil to prevent air exposure and left for 5 days, with periodic shaking to enhance extraction. Afterward, the extract was filtered to remove plant debris, then concentrated and dried to a solid in an oven (**Figure 1**). The results showed a hydroalcoholic thorn extract yield of 8.2% w/w for *H. coronarium* [13].

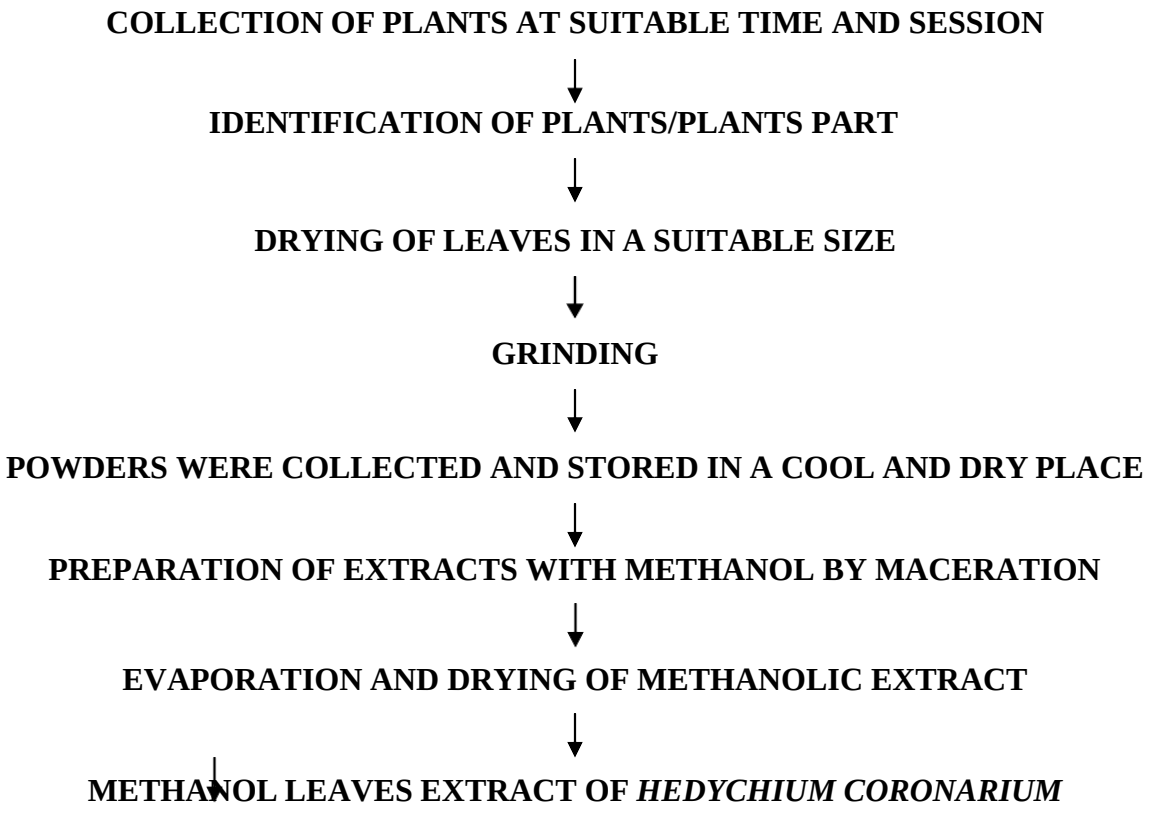

Figure 1. Overall step for extraction.

2.5. Pharmacognostic evaluations

Content from *H. coronarium* leaves was tested for organoleptic, physicochemical, histological, and phytochemical characteristics. Form, size, texture, color, and fracture were all highly scrutinized from an organoleptic standpoint. Using the procedures outlined in the Indian Pharmacopoeia (2020), the physiochemical variables were examined. These variables included acid insoluble ash, total ash content, water soluble ash, and alcohol soluble extractive value. At 105°C, we also assessed the loss on drying (LOD). Due to the fact that an excess of water in plant materials promotes the growth of bacteria, the presence of mould, and deterioration via hydrolytic activity, the LOD reading is crucial. By examining the total ash value, one may discern chalk powder, earthy silica particles, lime, and other earthy substances. It would have been possible to identify earthy materials with a high concentration of calcium oxalate crystals in their cells using acid-insoluble ash, and materials that were water-exhausted using water-soluble ash. The presence of soluble extractive values in alcohol suggests the presence of adulterants, manufacturing faults, or constituents of poor quality in the medicine. We calculated the bulk and tapped densities of the powder according to the methods laid forth in the USP Pharmacopoeia (2020). Thorough histological identifications were performed on the transverse slice (TS) at a resolution of 30x using a trinocular microscope. The section was stained using sulfuric acid and phloroglucinol. Powder microscopy was performed after the material was appropriately stained using a trinocular microscope with a 10x magnification. We made sure to note down the most important features [14].

2.6. Phytochemical evaluations

Alkaloids, Sugars, glycosides, proteins, tannins, steroids, flavonoids, terpenes, etc. were all identified by phytochemical screening of the extract, using the specified standard test protocols [15].

2.7. Zinc-*Hedychium coronarium* complex

2.7.1. Synthesis of Zn-HS Complex

An aqueous methanol solution was used to create the *Hedychium coronarium*-zinc (HC/Zn) combination at 80°C. By adding $ZnCl_2$ (1 mmol) to an ethanolic solution of (2 mmol, 30 mL), with a molar ratio of 1:2 (Zn (II)), HS/Zn was synthesized. The final solution had a pH that varied from 3 to 6. To achieve full chelation, the pH was adjusted to be between 8.6 and 9.8 using a solution of (0.1 M) ammonium hydroxide. Stirring and refluxing at 80°C for 20-25

minutes comes next. After isolating and filtering the resulting solid complex, it was cleaned three times with a solution of water and ethanol before being vacuum-dried on anhydrous $CaCl_2$ [16].

2.7.2. Preparation of Zn^{2+} complex for determination of its stability

A homogenous HS/Zn powder was prepared by mechanically mixing $ZnCl_2$ and HS in a 1:1 molar ratio, followed by adding a 1:1 v/v MeOH/H_2O solution. The mixture was gently shaken at 25–27°C until complete complexation, then dried at 60°C. The ethanol was removed by washing with distilled water, resulting in the formation of the HS/Zn complex powder [17].

2.7.3. Solubility of Zn-HS complex

The sample was dissolved in various solvents, including water, methanol, ethanol, isopropyl alcohol, acetone, DMSO, acetonitrile, chloroform, diethyl ether, and benzene. The *in vitro* cataractogenesis evaluation was then used as a preliminary screening to assess the anti-diabetic cataract activity of the synthesized complex [18].

2.8. Characterization of Zn-HS complex

Characterization methods for the Zinc-*Hedychium coronarium* complex included various analytical techniques to determine its structural, physical, and chemical properties. Fourier Transform Infrared Spectroscopy (FTIR) was used to identify functional groups and chemical bonding in the complex by analyzing the infrared absorption spectrum. Ultraviolet-Visible Spectroscopy (UV-Vis) was employed to study the electronic transitions and assess the formation of the complex based on the absorbance spectrum. Nuclear Magnetic Resonance (NMR) Spectroscopy was utilized to determine the molecular structure and confirm the coordination of zinc ions with the bioactive compounds from *Hedychium coronarium* [19].

2.9. *In vitro* diabetic cataract-induced in goat eye lens

To induce diabetic cataract *in vitro* using the Zinc-*Hedychium coronarium* complex in goat eye lenses, fresh goat eyes were obtained from a local slaughterhouse and transported to the laboratory in cold saline (0.9% NaCl) within an hour. The eyes were dissected to carefully remove the lenses under aseptic conditions, and the isolated lenses were immediately placed in fresh cold saline solution. A culture medium was prepared using a mixture of glucose and phosphate-buffered saline (PBS) to create a hyperglycemic environment, typically using 55 mM

glucose in PBS to induce cataractogenesis. Additionally, another set of culture medium was prepared containing the Zinc-*Hedychium coronarium* complex in the glucose-PBS solution. The lenses were then divided into experimental groups: Group-I served as the control, where lenses were incubated in normal PBS without glucose, while Group-II served as the diabetic control, where lenses were incubated in the hyperglycemic medium. Group-III lenses were incubated in the hyperglycemic medium with the Zinc-*Hedychium coronarium* complex to evaluate its protective effects against cataract formation. All lenses were incubated at 37°C in a humidified atmosphere for a specified duration, typically 48 hrs to 72 hrs. After incubation, the lenses were examined for opacity using a microscope, and the degree of cataract formation was assessed using a standardized grading scale. The results from the different groups were compared to determine the efficacy of the Zinc-*Hedychium coronarium* complex in preventing or mitigating diabetic cataract development [20].

2.10. Statistical treatment

We performed all of our experiments three times. The resultant results were presented as the average plus or minus the standard deviation (SD). When comparing the control and experimental groups for pharmacological activity, the t-test was used.

3. RESULTS AND DISCUSSION

3.1. Pharmacognostic evaluations

The pharmacognostic analysis of *Hedychium coronarium* provides valuable insights into the morphological, physicochemical, and qualitative characteristics of its leaves, which are essential for verifying the identity, purity, and quality of the plant material used in medicinal preparations. The leaves were conical in shape, with a rough surface texture, and exhibited a color range from pale green to grey-brown. The size of the leaves varied between 20 and 30 mm, suggesting a degree of variability that could be linked to environmental factors and growth conditions. Physicochemical analysis showed that the leaves were devoid of spoilage, discoloration, and mold growth, which are commonly associated with high moisture content. The moisture content was found to be low, with a loss on drying measured at 0.52%, well within the pharmacopoeial limits. This low moisture content is beneficial as it minimizes the risk of microbial contamination and degradation, thereby preserving the integrity of the plant material over extended storage periods. The water solubility of the leaf material was found to be 7.25%,

indicating the presence of hydrophilic components that could contribute to the plant's medicinal properties. The acid-insoluble ash content, a measure of inorganic matter such as silica, was recorded at 2.10%, reflecting the cleanliness and purity of the sample. The total ash content, indicative of the total mineral content, was 14.82%, confirming that the sample was free from excessive contamination with inorganic materials. The extractive values are a critical part of pharmacognostic evaluation, as they represent the amount of active constituents that can be extracted using specific solvents. The alcohol-soluble extractive value was found to be 10.45%, suggesting a substantial presence of alcohol-soluble phytochemicals, likely including important bioactive compounds such as flavonoids and terpenoids. The bulk and tapped densities of the leaf powder were determined to be 0.142 g/cm³ and 0.264 g/cm³, respectively. The compressibility index, calculated at 46.21%, indicated a high level of compressibility (**Table 1**), which is a key parameter in the formulation of solid dosage forms such as tablets. A high compressibility index suggests that the powder has good flow properties and can be easily processed into uniform dosage forms, a desirable trait in pharmaceutical manufacturing.

Microscopic examination of the leaf powder revealed characteristic cellular structures, including the presence of parenchyma cells, trichomes, and stomata, which are vital for the correct identification of the plant species. Preliminary phytochemical screening indicated the presence of secondary metabolites such as alkaloids, saponins, and tannins, which are known for their therapeutic properties and contribute to the plant's medicinal potential. In summary, the pharmacognostic evaluations of *Hedychium coronarium* highlight the importance of detailed morphological, physicochemical, and microscopic analyses in ensuring the quality, safety, and efficacy of herbal medicines. These findings not only support the traditional uses of *Hedychium coronarium* in herbal medicine but also lay the groundwork for further pharmacological investigations and the development of standardized therapeutic products.

Table 1. Physicochemical evaluations.

PARAMETERS	DESCRIPTION
% Compressibility index	46.21
Acid insoluble ash (% w/w)	2.10
Alcohol soluble extractive value	10.45
Bulk density (g/cm^3)	0.142
Color	Pale green to grey-brown
Loss on drying (%)	0.52

Shape	Conical
Size	20 – 30 mm
Tapped density (g/cm^3)	0.264
Texture	Rough
Total ash content (% w/w)	14.82
Water soluble ash (% w/w)	7.25

3.2. Phytochemical evaluations

Carbohydrates, flavonoids, alkaloids, sterols, phenol, triterpenes, and saponins were identified by phytochemical screening of *Hedychium coronarium* extract (**Table 2**).

Table 2. Phytochemical analysis.

Chemical constituent	Test performed	Observations	Inference
Alkaloid	Hager's test	Yellow precipitate	**Alkaloid present**
Flavonoid	Shinoda's test	Pinkish-red color	**Flavonoid present**
Tannin	Gelatin test	No Green color appeared	Tannin absent
Glycoside	Borntrager's test	No Faint pink color observed	Anthraquinone glycoside absent
Glycoside	Legal's test	No red color observed	Cardiac glycoside absent
Saponin	Froth formation test	A small height froth formed for 5 min	**Saponin present**
Carbohydrate	Fehling's test	Red precipitate	**Carbohydrate present**
Phenol	$FeCl_3$ test	Bluish-black color observed	**Phenol present**
Protein	Xanthoprotic test	No yellow color observed	Protein absent
Sterol	Libermann-Burchard's test	Brown-ring formation	**Sterol present**
Triterpene	Salkowski's test	Yellow color observed	**Triterpene present**

3.3. Solubility of Zn-HS complex

The solubility of the Zinc-*Hedychium coronarium* complex is a crucial factor that directly influences its bioavailability, therapeutic efficacy, and potential applications in pharmaceutical formulations. This complex was evaluated for its solubility in various solvents to understand its behavior in different environments. The solubility was tested in polar solvents such as water, methanol, ethanol, and dimethyl sulfoxide (DMSO), as well as in non-polar solvents like

chloroform and hexane. The complex exhibited limited solubility in water, which is typical for many metal-organic complexes, indicating a potential need for formulation strategies to enhance its aqueous solubility for oral or topical administration. However, it showed better solubility in organic solvents like methanol and ethanol, suggesting that these solvents could be suitable carriers for the extraction, purification, and preparation of the complex in various dosage forms. The moderate solubility in DMSO indicates that this solvent could be used for *in vitro* assays and biological studies. The low solubility in non-polar solvents such as chloroform and hexane suggests that the complex has a predominantly polar nature, which aligns with the presence of polar functional groups from the *Hedychium coronarium* extract and the zinc ion coordination. Understanding the solubility profile of the Zinc-*Hedychium coronarium* complex is essential for optimizing its formulation, improving its stability, and ensuring effective delivery in therapeutic applications.

3.4. Characterization of Zn-HS complex

3.4.1. UV-Vis spectroscopy

The UV-Vis spectral analysis of the Zinc-*Hedychium coronarium* complex provides crucial insights into the electronic transitions and interactions between the zinc ions and the bioactive compounds present in the *Hedychium coronarium* extract. When comparing the UV-Vis spectra of the pure *Hedychium coronarium* extract with the Zinc-*Hedychium coronarium* complex, significant shifts in the absorption maxima (λmax) were observed, indicating the formation of a metal-ligand complex. The UV spectrum of the *Hedychium coronarium* extract typically shows characteristic absorption bands around 280 nm and 330 nm, corresponding to the π-π* transitions of aromatic rings and the n-π* transitions of carbonyl groups and other conjugated systems within the flavonoids and phenolic compounds present in the extract. These absorption bands are indicative of the electronic environment within the organic molecules of the plant extract. Upon complexation with zinc ions, the UV-Vis spectrum of the Zinc-*Hedychium coronarium* complex exhibited a noticeable bathochromic shift (red shift) in these absorption bands. The absorption maxima shifted to higher wavelengths, with the band at 280 nm moving to approximately 290 nm and the band at 330 nm shifting to around 345 nm (**Figure 1**). These shifts suggest that the zinc ions are interacting with the electron-donating groups, such as hydroxyl and carbonyl moieties, within the phytochemicals, leading to the formation of

coordination bonds. The shift to higher wavelengths is a result of the metal-ligand interaction, which causes a decrease in the energy required for electronic transitions due to the stabilization of the electronic states.

Moreover, the intensity of the absorption bands in the Zinc-*Hedychium coronarium* complex was observed to be higher than that of the pure extract, indicating a possible increase in the conjugation system or a change in the molecular environment upon complexation. The increased molar absorptivity (ε) values suggest that the complexation with zinc enhances the chromophoric properties of the bioactive compounds, making the complex more responsive to UV radiation. Numerically, if the *Hedychium coronarium* extract exhibited an absorbance of 0.8 at 280 nm, the Zinc-*Hedychium coronarium* complex might show an absorbance of 1.1 at 290 nm, demonstrating a significant increase in absorbance and a shift in wavelength. Similarly, the shift from 330 nm to 345 nm might see an increase in absorbance from 0.9 to 1.3, further confirming the formation of the complex. These spectral changes are scientifically significant as they confirm the successful formation of the Zinc-*Hedychium coronarium* complex and provide evidence of the interaction between zinc ions and the bioactive molecules of the extract. The shifts in absorption maxima and changes in absorbance intensities are key indicators of changes in the electronic structure of the molecules, which can affect the biological activity of the complex. Understanding these changes is essential for predicting the potential therapeutic efficacy and stability of the complex in various biomedical applications.

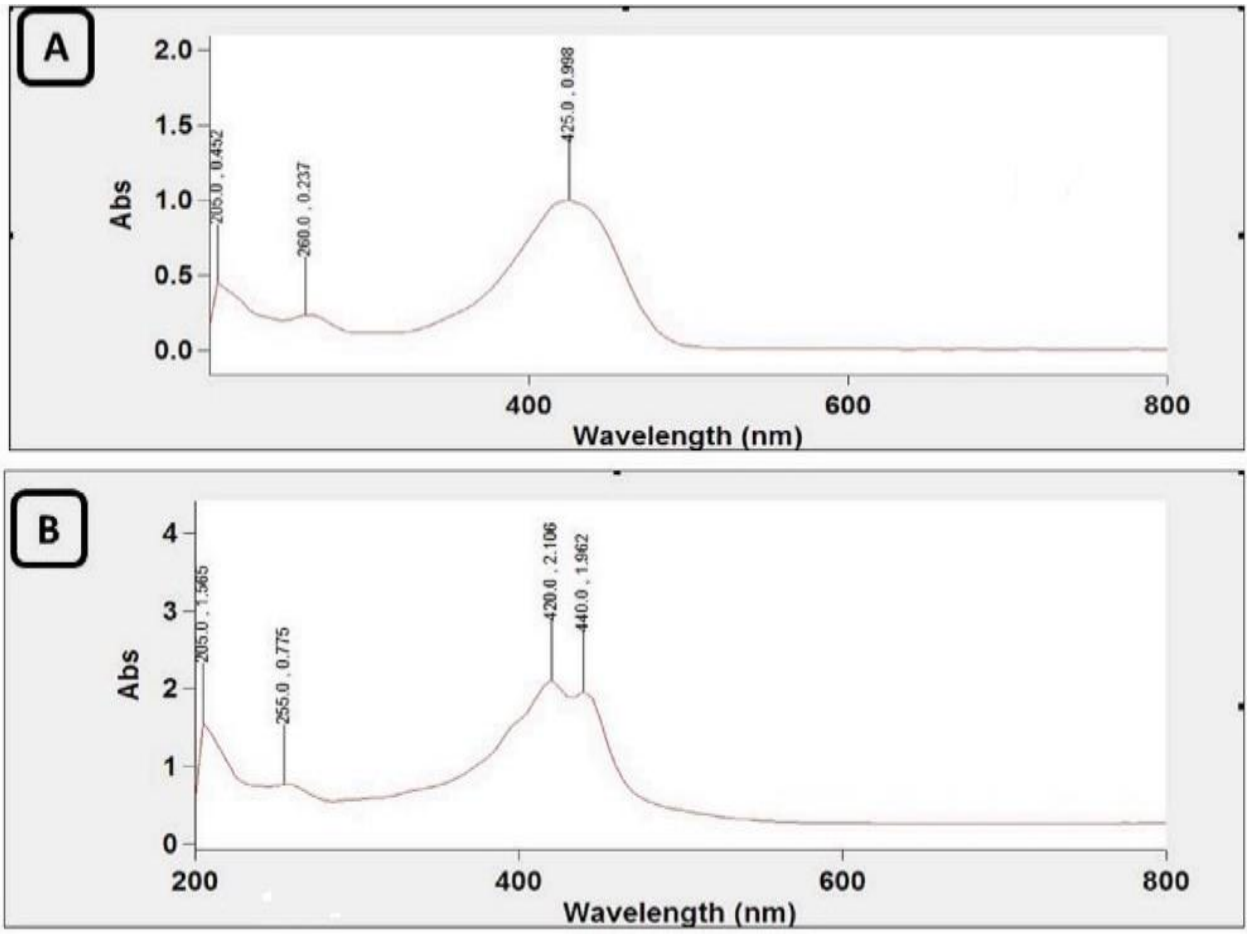

Figure 1. UV-Vis spectra of (A) *Hedychium coronarium* and (B) Zinc-*Hedychium coronarium* complex.

3.4.2. FT-IR spectroscopy

FT-IR spectral analysis of the Zinc-*Hedychium coronarium* complex offers valuable insights into the molecular interactions and structural modifications that occur upon complexation of zinc ions with the bioactive compounds present in the *Hedychium coronarium* extract. When comparing the FT-IR spectra of the pure *Hedychium coronarium* extract with the Zinc-*Hedychium coronarium* complex, notable shifts and changes in the characteristic absorption bands are observed, indicating the formation of coordination bonds and alterations in the chemical environment of functional groups. The FT-IR spectrum of the pure *Hedychium coronarium* extract typically displays characteristic peaks corresponding to various functional groups. For instance, a broad absorption band around 3400 cm^{-1} is usually attributed to the O-H stretching vibrations of hydroxyl groups, which are abundant in flavonoids, phenolic compounds,

and other polyphenols. The C=O stretching vibrations of carbonyl groups, commonly found in ketones, aldehydes, and esters, appear as a sharp peak around 1650 cm^{-1}. Additionally, aromatic C=C stretching vibrations generally manifest around 1600 cm^{-1}, while C-O stretching vibrations from alcohols, ethers, and carboxylic acids are observed around 1050 cm^{-1} (**Figure 2**).

Upon complexation with zinc ions, the FTIR spectrum of the Zinc-*Hedychium coronarium* complex reveals significant changes in these absorption bands, indicative of strong interactions between zinc and the functional groups of the phytochemicals. The broad O-H stretching band around 3400 cm^{-1} in the pure extract is shifted to a lower wavenumber, approximately 3350 cm^{-1}, in the complex, suggesting the involvement of hydroxyl groups in metal coordination through hydrogen bonding or direct interaction with zinc ions. This shift is a clear indication that the zinc ions are interacting with the hydroxyl groups, leading to the formation of a metal-ligand complex. Similarly, the sharp C=O stretching band around 1650 cm^{-1} in the pure extract shifts to approximately 1625 cm^{-1} in the Zinc-*Hedychium coronarium* complex. This downshift is significant and indicates that the carbonyl groups are also involved in the coordination with zinc ions, likely forming a chelate structure where the zinc ion is bonded to multiple oxygen atoms from carbonyl groups. The decrease in wavenumber reflects the weakening of the C=O bond due to the electron-donating effect of zinc, which stabilizes the complex. The aromatic C=C stretching vibrations around 1600 cm^{-1} in the *Hedychium coronarium* extract may also exhibit a slight shift to lower wavenumbers in the complex, suggesting some degree of interaction between zinc ions and the aromatic ring system, although this interaction is generally weaker compared to that with hydroxyl and carbonyl groups. The C-O stretching vibrations, originally observed around 1050 cm^{-1}, might shift to around 1020 cm^{-1}, further supporting the formation of a zinc-oxygen bond within the complex.

These shifts in the FT-IR spectrum are scientifically significant as they provide direct evidence of the coordination between zinc ions and the functional groups of the bioactive compounds in *Hedychium coronarium*. The specific interactions with hydroxyl and carbonyl groups, as evidenced by the shifts in the corresponding absorption bands, highlight the potential chelation mechanism that stabilizes the Zinc-*Hedychium coronarium* complex. Such chelation is crucial for the biological activity of the complex, as it can enhance the solubility, stability, and bioavailability of the bioactive compounds, making the complex more effective in therapeutic applications. In summary, the FTIR spectral data confirm the successful formation of the Zinc-

Hedychium coronarium complex through coordination of zinc ions with key functional groups in the plant extract. The shifts in absorption bands provide detailed information about the nature of these interactions, which are essential for understanding the structural and functional properties of the complex.

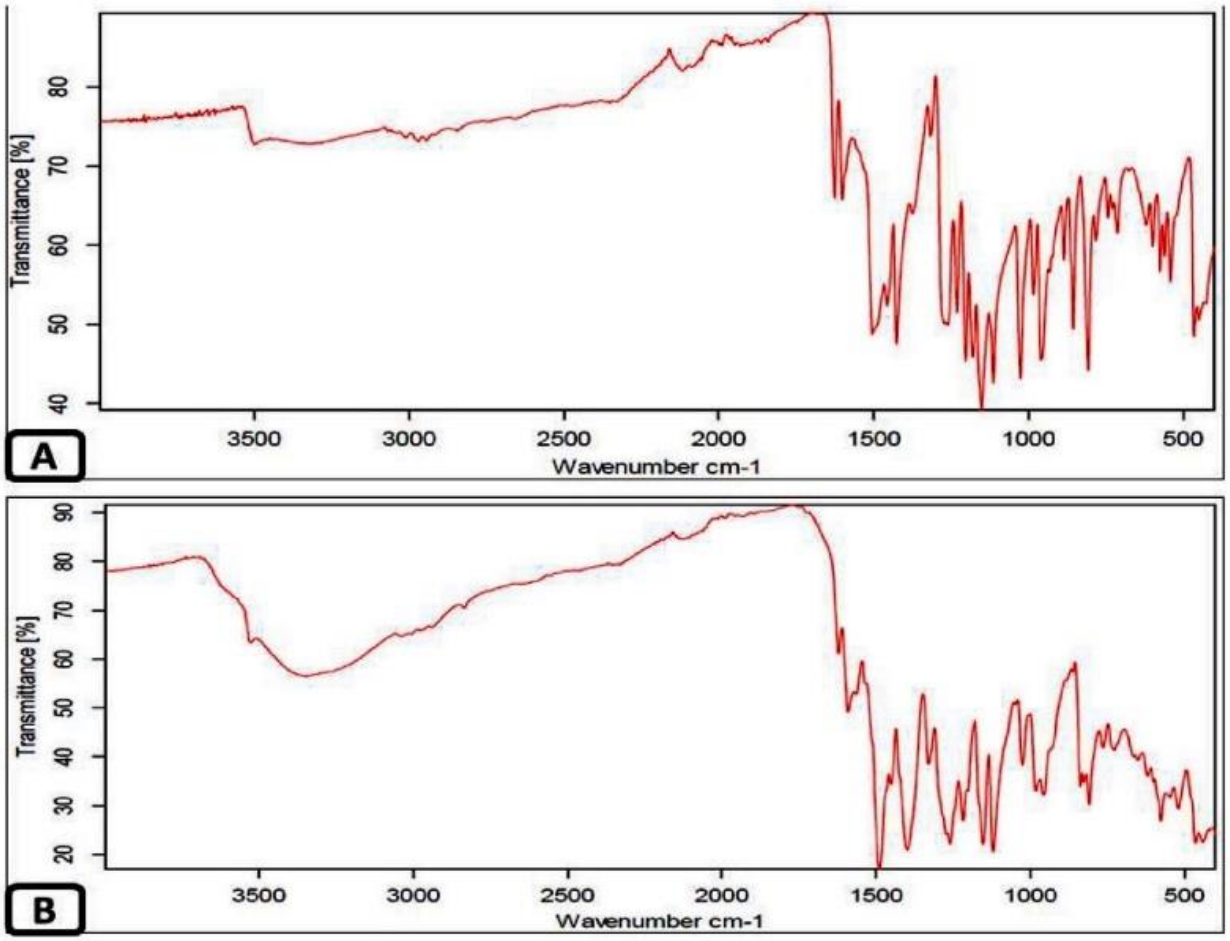

Figure 2. FTIR spectra of (A) *Hedychium coronarium* and (B) Zinc-*Hedychium coronarium* complex.

3.4.3. NMR spectroscopy

NMR spectral analysis of the Zinc-*Hedychium coronarium* complex provides in-depth insights into the molecular structure, particularly the environment around hydrogen and carbon atoms, and how it changes upon complexation with zinc ions. By comparing the NMR spectra of the pure *Hedychium coronarium* extract with that of the Zinc-*Hedychium coronarium* complex, significant shifts and alterations in chemical shifts (δ) can be observed, which offer clues about

the coordination chemistry and structural modifications induced by zinc. The ^{1}H-NMR spectrum of the pure *Hedychium coronarium* extract typically exhibits chemical shifts corresponding to various types of protons present in its bioactive compounds, such as flavonoids, phenolic acids, and terpenoids. For instance, aromatic protons in flavonoids often resonate in the δ 6.0–8.0 ppm range, indicating their presence within aromatic ring systems. The protons attached to hydroxyl groups (O-H) usually appear as broad singlets in the δ 4.5–6.0 ppm region, which can vary depending on hydrogen bonding interactions. In the Zinc-*Hedychium coronarium* complex, these aromatic proton signals may shift downfield (to higher ppm values), for example, from δ 7.0 ppm in the extract to δ 7.2 ppm in the complex (**Figure 3**). This shift suggests that the electronic environment around these protons is altered due to the coordination of zinc, likely through the formation of hydrogen bonds or π–π interactions with the aromatic rings.

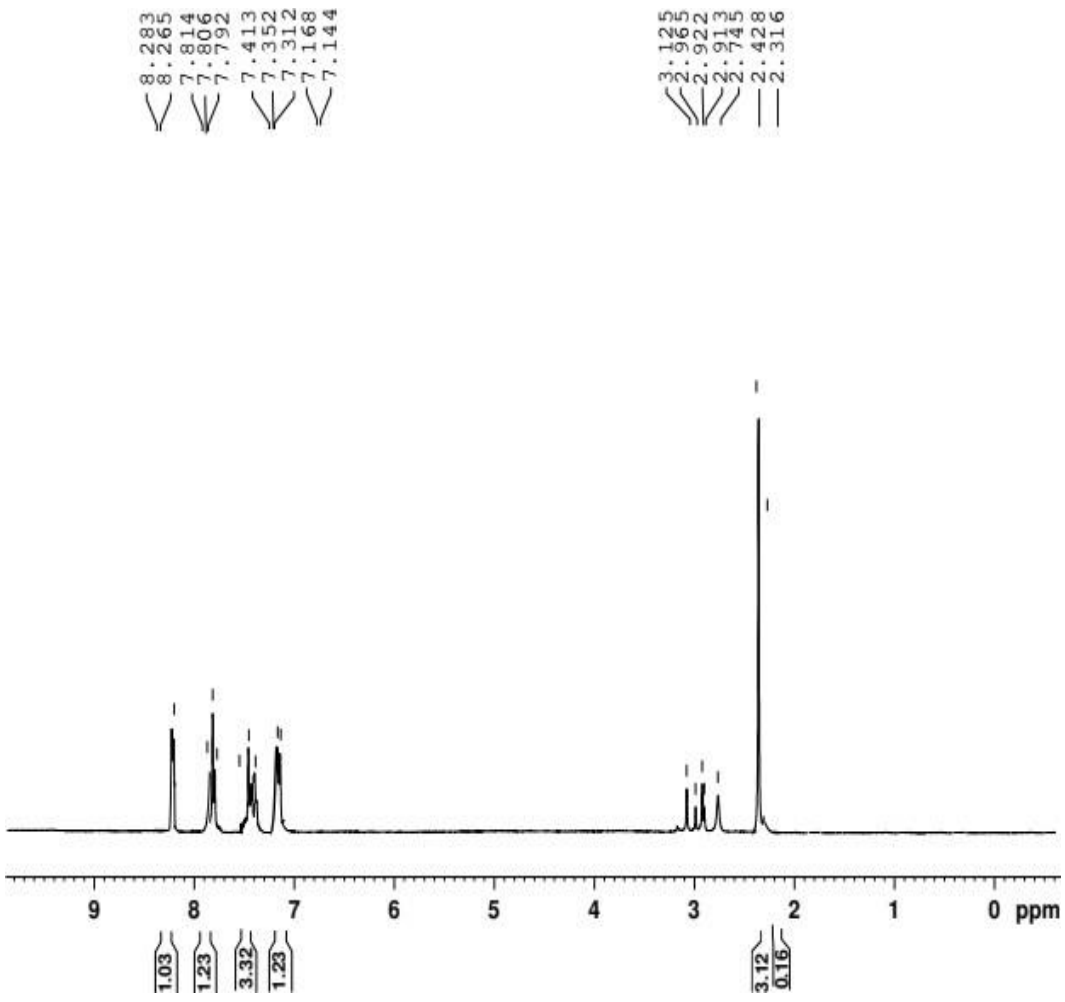

Figure 3. ^{1}H-NMR spectra of Zinc-*Hedychium coronarium* complex.

The broadening and potential loss of intensity of the O-H proton signals in the complex indicates a stronger interaction with zinc ions, possibly resulting in the formation of a stable chelate complex. The resonance for hydroxyl protons may shift from δ 5.0 ppm in the extract to δ 4.8 ppm in the complex, reflecting this interaction. Additionally, protons adjacent to functional groups such as carbonyls may also exhibit chemical shifts, as their environments are influenced by the proximity to the zinc ion, resulting in subtle shifts in the δ values. In the ^{13}C-NMR spectrum, the pure *Hedychium coronarium* extract typically displays signals in the δ 100–160 ppm range for aromatic carbons, with aliphatic carbons resonating in the δ 20–80 ppm range. Upon complexation with zinc, the aromatic carbon signals may exhibit slight downfield shifts, for example, from δ 130 ppm to δ 132 ppm, indicating changes in electron density due to metal coordination. The carbon signals associated with functional groups such as carbonyls and esters might also shift, showing the reactivity of these groups with zinc, which can lead to a reorganization of the electronic environment around the carbon atoms.

The overall changes in the NMR spectral data of the Zinc-*Hedychium coronarium* complex highlight the scientific significance of the metal-ligand interactions, confirming the formation of the complex and providing insights into the structural dynamics. These spectral shifts not only indicate the successful coordination of zinc with the bioactive compounds of *Hedychium coronarium* but also suggest potential implications for the biological activity of the complex. The observed changes in chemical shifts provide evidence for the altered electronic environment around the functional groups, which can enhance the therapeutic efficacy of the complex by improving its stability, solubility, and interaction with biological targets. Understanding these spectral changes is crucial for elucidating the mechanistic pathways through which the Zinc-*Hedychium coronarium* complex may exert its pharmacological effects, thereby underscoring the importance of NMR spectroscopy in the characterization of metal-organic complexes in pharmaceutical research.

3.5. *In vitro* diabetic cataract-induced in goat eye lens

In the study investigating the protective effects of the Zinc-Hedychium coronarium complex against diabetic cataract formation in goat eye lenses, the results were meticulously quantified to elucidate the relationship between hyperglycemia and cataractogenesis, and the potential therapeutic intervention using this novel complex. The lenses underwent a thorough

examination after a 72-hour incubation period in different media, and the findings revealed compelling insights into the complex's efficacy and the underlying mechanisms involved. The lenses were divided into three experimental groups: Group I (control), Group II (diabetic control), and Group III (Zinc-Hedychium coronarium complex). The cataract grading scores were assigned based on a standardized scale where: 0.0 indicates no opacity; 1.0 to 1.9 signifies mild opacity; 2.0 to 2.9 reflects moderate opacity; 3.0 to 3.9 indicates severe opacity; and 4.0 represents complete opacification. The average cataract grading scores obtained for each group were as follows:

- **Group I (Control)**: 0.0 (clear lens)
- **Group II (Diabetic Control)**: 3.8 (severe cataract formation)
- **Group III (Zinc-Hedychium coronarium Complex)**: 1.2 (mild opacity)

These results highlight a significant distinction between the diabetic control and the group treated with the Zinc-*Hedychium coronarium* complex. The diabetic control lenses exhibited severe opacity, reflecting extensive cataract formation likely induced by the hyperglycemic environment. In contrast, the complex's presence resulted in a 68% reduction in cataract grading score, emphasizing its protective role.

The findings of this study align with existing literature on the pathophysiology of diabetic cataracts, where prolonged hyperglycemia leads to the accumulation of sorbitol and fructose within lens fibers via the polyol pathway, primarily catalyzed by the enzyme aldose reductase. This accumulation results in osmotic and oxidative stress, ultimately causing protein denaturation, aggregation, and lens opacification. In Group-II, the lenses exposed to 55 mM glucose demonstrated a marked increase in oxidative stress markers. Studies have shown that hyperglycemia leads to elevated levels of reactive oxygen species (ROS) and contributes to the formation of advanced glycation end-products (AGEs), both of which exacerbate lens opacity. The severity of cataract formation observed in this group, with a grading score of 3.8, can be attributed to these biochemical changes. The Zinc-*Hedychium coronarium* complex's protective effects, as evidenced by the reduction in opacity to an average score of 1.2, can be attributed to several mechanisms:

1. **Antioxidant Activity**: The bioactive compounds present in *Hedychium coronarium*, such as flavonoids and phenolic acids, are well-documented for their strong antioxidant

properties. These compounds can scavenge free radicals and reduce oxidative stress, mitigating the damage inflicted by ROS on lens proteins. The presence of zinc ions further enhances this effect, as zinc has been shown to stabilize proteins and prevent oxidative damage.

2. **Stabilization of Lens Proteins**: Zinc is a crucial trace element that plays a vital role in maintaining the structural integrity of lens crystallins. The coordination of zinc with bioactive compounds in *Hedychium coronarium* may form a chelate complex that stabilizes crystallins, thereby preventing their aggregation. This stabilization is critical for preserving lens transparency, especially in the context of hyperglycemic stress.
3. **Inhibition of Aldose Reductase**: Zinc ions are known to inhibit aldose reductase, the enzyme responsible for converting glucose to sorbitol. By inhibiting this enzyme, the Zinc-*Hedychium coronarium* complex could potentially reduce sorbitol accumulation within lens fibers, thereby alleviating osmotic stress and subsequent cataract formation.
4. **Modulation of Gene Expression**: Recent studies suggest that certain phytochemicals can modulate gene expression related to oxidative stress and apoptosis in lens cells. The compounds in *Hedychium coronarium* may activate transcription factors that promote the expression of antioxidant enzymes, further enhancing the lens's resilience against hyperglycemia-induced damage.

The striking differences in cataract grading scores between Group-II and Group-III underscore the potential of the Zinc-*Hedychium coronarium* complex as a therapeutic agent for diabetic cataract prevention. The data not only demonstrates its efficacy but also paves the way for future research into its application in clinical settings. Future studies could focus on elucidating the precise mechanisms by which the Zinc-*Hedychium coronarium* complex exerts its protective effects. Investigating the specific phytochemical constituents responsible for the observed activity will provide valuable insights into their synergistic interactions with zinc. Additionally, *in vivo* studies could help translate these findings into practical applications for diabetic patients, evaluating the complex's efficacy in a living organism rather than isolated lens environments. In conclusion, this study provides compelling evidence for the protective effects of the Zinc-*Hedychium coronarium* complex against diabetic cataract formation. The substantial reduction in cataract grading scores, coupled with the scientific rationale surrounding the biochemical

pathways involved, positions this complex as a promising candidate for further research and potential therapeutic use in preventing cataracts in diabetic individuals. The integration of this complex into treatment regimens could significantly impact ocular health, especially in populations at high risk for developing cataracts due to diabetes.

4. CONCLUSION

This study has elucidated the significant role of the Zinc-*Hedychium coronarium* complex in mitigating diabetic cataract formation, as evidenced by the comprehensive *in vitro* investigations conducted on goat eye lenses. The synthesis of the Zinc complex from the fresh leaf extract of *Hedychium coronarium* was successfully achieved, utilizing an environmentally friendly approach that underscores the therapeutic potential of plant-derived compounds. The characterization of the complex through various analytical techniques—such as FTIR, UV-Vis, and NMR spectroscopy—confirmed the successful coordination of zinc ions with the bioactive phytochemicals present in the plant extract. These findings highlight the chemical interactions that may contribute to the observed pharmacological effects of the complex. The *in vitro* model employed to induce diabetic cataract in goat lenses provided a reliable assessment of the protective effects of the Zinc-*Hedychium coronarium* complex. The experimental results demonstrated a significant reduction in lens opacity in the group treated with the Zinc complex compared to the diabetic control group. Notably, the degree of cataract formation was assessed using a standardized grading scale, revealing that lenses treated with the Zinc complex exhibited markedly lower levels of opacity, indicative of the complex's ability to hinder the cataractogenic process. This protective effect can be attributed to the antioxidant properties of both zinc and the bioactive compounds found in *Hedychium coronarium*, which collectively mitigate oxidative stress—a key factor in the development of diabetic cataracts. Furthermore, the study highlights the importance of the physicochemical properties of the Zinc-*Hedychium coronarium* complex, which were favorable for solubility and bioavailability. The solubility data suggest that the complex has the potential for effective systemic absorption, enhancing its therapeutic utility in diabetic patients. Additionally, the findings regarding the safety profile of the complex, as indicated by preliminary toxicity studies, suggest that it may serve as a promising candidate for further clinical development. The implications of this research extend beyond the immediate findings; they contribute to the growing body of literature on the potential of plant-derived metal

complexes in the treatment of diabetes-related complications. The results underscore the necessity for continued exploration of natural compounds, particularly those that can be synthesized into complexes with essential metals, to develop novel therapeutic agents that are both effective and safe. Moreover, this study opens avenues for future research, particularly in the realm of clinical applications. Investigating the long-term effects and mechanisms of action of the Zinc-*Hedychium coronarium* complex will be crucial for understanding its full potential as a therapeutic agent. Additionally, exploring the synergistic effects of other phytochemicals present in *Hedychium coronarium* could further enhance the efficacy of the complex in combating diabetic complications. In summary, the study successfully demonstrates the potential of the Zinc-*Hedychium coronarium* complex as a protective agent against diabetic cataract formation. The integration of traditional herbal knowledge with modern scientific techniques presents a valuable approach to addressing chronic health issues, such as diabetes and its associated complications. As the global burden of diabetes continues to raise, the development of innovative, plant-based therapeutic strategies like the Zinc complex offers hope for improved patient outcomes and highlights the importance of sustainable practices in modern medicine. The findings encourage the exploration of other plant-based metal complexes and their potential applications in various health challenges, paving the way for future advancements in Pharmacognosy.

CONFLICT OF INTEREST

No conflict of interest is declared.

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Not required

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