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Adventist Medical Center College

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Phytochemical Profiling, Formulation, and Evaluation of Diclofenac Diethylamine Gel Using *Diplazium esculentum* Fronds (Athyriaceae) Mucilage as Gelling Agent

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APPROVAL SHEET

This thesis entitled:

"PHYTOCHEMICAL PROFILING, FORMULATION, AND EVALUATION OF DICLOFENAC DIETHYLAMINE GEL USING *Diplazium esculentum* FRONDS (Athyriaceae) AS GELLING AGENT"

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CERTIFICATE OF ORIGINALITY

We hereby declare that this submission is our own work and that, to the best of our knowledge and belief, it contains no materials previously published or written by another person nor material to which to a substantial extent has been accepted for award of any other degree or diploma of a university or other institute of higher learning, except where due acknowledgement is made in the text.

We also declare that the intellectual content of this thesis is the product of our work, even though we have received assistance from others on style, presentation, and language expression.

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ABSTRACT

Diplazium esculentum **(locally known as "pako") was investigated for its potential application as a natural gelling agent in topical pharmaceutical formulations. Traditionally recognized as an edible fern in the Philippines, *D. esculentum* contains mucilage rich in polysaccharide components that contribute to gel formation, viscosity, and water-retention properties. This study aimed to formulate and evaluate Diclofenac diethylamine topical gels using *D. esculentum* mucilage as a natural alternative to the synthetic gelling agent Carbopol 940. The fern fronds were collected, authenticated, cleaned, air-dried, powdered, and extracted using hot water. The extracted mucilage was precipitated with 95% ethanol, dried, pulverized, and incorporated into gel formulations at varying concentrations of 2.5%, 5%, and 7.5% w/w. Comparative formulations using Carbopol 940 at 1%, 1.5%, and 2% w/w served as synthetic controls.**

The extracted mucilage showed an average percentage yield of 13.86% ± 1.61%, loss on drying of 9%, and swelling index of 223.33%, indicating favorable water-retention and gel-forming properties. Phytochemical screening revealed the presence of alkaloids, flavonoids, total phenolics, saponins, steroids, tannins, and terpenoids. Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of functional groups associated with polysaccharide-rich mucilage and showed compatibility between *D. esculentum* mucilage and Diclofenac diethylamine, with no significant drug–excipient interaction observed. The formulated gels were evaluated for pH, viscosity, spreadability, extrudability, homogeneity, and drug content. Results showed that all formulations had acceptable pH values ranging from 7.2 to 7.9, uniform homogeneity without phase separation, and drug content within the acceptable range. Viscosity increased with higher mucilage concentration, with the 7.5% natural gel showing the strongest viscosity among the natural formulations and improved extrudability compared with lower concentrations. Overall, the findings suggest that *D. esculentum* mucilage demonstrates promising potential as a sustainable, biocompatible, and cost-effective natural gelling agent for Diclofenac diethylamine topical gel formulations.

Keywords: *Diplazium esculentum*, Mucilage, Natural Gelling Agent, Diclofenac Diethylamine, Topical Gel, Carbopol 940, Phytochemical Profiling, FTIR Spectroscopy.

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CHAPTER ONE
INTRODUCTION OF THE STUDY

➢ *Introduction*

Topical gels are semi-solid formulations that were applied to the mucous membranes of the skin. They deliver drugs in a localized fashion and are not subjected to first-pass metabolism. The ease of administration, improved patient compliance, and direct delivery of the active pharmaceutical ingredients (API) to the location of action made these gels popular (Bagmar et al. 2024). Diclofenac diethylamine is an anti-inflammatory medication (NSAID) that was commonly prepared as a topical gel for use in treating musculoskeletal pain, arthritis, sprains, and inflammatory conditions. Nevertheless, synthetic polymers served as gelling agents in most commercially available topical gels. Such materials were expensive to produce, could cause skin irritation, and posed environmental problems (Pawar et al. 2020). This prompted the search for safer, biodegradable, and cheaper natural alternatives.

Plant mucilages emerged as promising gelling agents due to their unique, hydrophilic, and complex polysaccharide structure. This structure allowed swelling and the ability to coexist with living tissues. The vegetable fern, *Diplazium esculentum*, was a prime example of a plant with these properties. It was indigenous to the Philippines, was commonly found on gravel bars within the banks of streams, and was known to contain mucilaginous material in complex polysaccharides (Ibrahim et al. 2019). When present as mucilage, these complex polysaccharides acted as natural gelling agents in pharmaceutical preparations by increasing viscosity and retaining water (Goksen et al. 2023). These features suggested that the mucilage from *D. esculentum* could serve as a plant-based alternative to synthetic gelling agents in topical drug delivery systems.

Despite the known presence of mucilaginous polysaccharides in *D. esculentum*, no study had investigated its potential as a gelling agent in topical formulations. In contrast, Sundari et al. (2025) successfully used natural mucilages from plants such as okra and hibiscus as gelling agents in diclofenac diethylamine gels at concentrations of 2.5%, 5%, 7.5%, and 10%. However, *D. esculentum*, despite its abundance and known mucilage content, remained unstudied for this pharmaceutical application. To address this research gap, the present study formulated diclofenac diethylamine gels using *D. esculentum* mucilage at 2.5%, 5%, and 7.5% concentrations by applying the fusion method. The goal of this study was to evaluate the effectiveness of *D. esculentum* mucilage as a natural gelling agent by comparing the prepared formulations with a standard synthetic base in terms of pH, spreadability, homogeneity, extrudability, viscosity, and drug content.

➢ *Background of the Study*

Topical gels are widely used as semisolid drug delivery systems. They allowed localized drug application, improved patient compliance, and facilitated sustained drug release (Bagmar et al. 2024; Choudhary 2021). Gelling agents were essential in topical formulations because they provided structure, thickness, and consistency. This ensured that the active pharmaceutical ingredients were delivered effectively. They also improved the product's spreadability, maintained the drug's stability, and increased patient compliance. While synthetic polymers like Carbopol were commonly used due to their effectiveness, concerns about cost, potential skin irritation, and environmental impact had led to the search for safer, more sustainable, and natural alternatives (Bagmar et al. 2024; Pawar et al. 2020). Natural mucilages are hydrophilic complex polysaccharides extracted from plants. They showed excellent gelling properties, such as water retention, swelling, and thickening, which made them strong candidates for natural gelling agents (Cakmak et al. 2023; Prajapati et al. 2022). *Diplazium esculentum*, known as vegetable fern, was a member of the Athyriaceae family and was commonly found in the Philippines. It held nutritional and traditional value (Bhutia et al. 2025; Vélez-Gavilán 2024). The fronds of *D. esculentum* contained mucilaginous polysaccharides, which were natural substances responsible for the plant's gelling ability and viscosity. These polysaccharides help form stable gels by contributing to water retention, swelling, thickening, and gel stability. Although specific monosaccharides such as arabinose and galactose were not directly measured in this study, plant mucilage was generally known to contain polysaccharides composed of uronic acids and other sugar units. These sugar chains formed the structural backbone of mucilage, allowing it to produce high-viscosity gels with good stability. Because of these functional properties, including emulsion stability and potential antioxidant activity, *D. esculentum* mucilage was considered a promising natural gelling agent for pharmaceutical and food applications (Borah et al.; Hayati et al. 2018; Semwal et al. 2021).

The physical and chemical properties of gels were key to their effectiveness. To assess these properties, standard tests were performed. These included measuring pH with a calibrated digital pH meter to ensure skin compatibility, checking spreadability through the glass slide method to evaluate ease of application, inspecting homogeneity visually and through centrifugation to look for phase separation, testing extrudability by measuring how easily the gel came out of collapsible tubes, assessing viscosity with a viscometer to confirm proper gel thickness, and analyzing drug content to verify uniform distribution of the active ingredient (Choudhary 2021). Natural mucilages mainly consist of complex polysaccharides, which help maintain drug stability and formula effectiveness without causing adverse reactions (Parhi et al. 2023). Recent studies on natural mucilages, specifically from okra and hibiscus, in diclofenac diethylamine gels showed that these polysaccharides were compatible with the drug and commonly used excipients, including Carbopol, propylene glycol, methylparaben, methanol, and water, while preserving gel stability and performance (Sundari et al. 2025). This suggested that *D. esculentum* mucilage might have been compatible when used as a gelling agent. Testing its performance at concentrations of 2.5%, 5%, and 7.5% w/w using the fusion method helped evaluate its potential as a stable and effective topical gel formulation (Choudhary 2021).

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While natural mucilages were often used as gelling agents in topical formulations, ferns like *D. esculentum* had not been extensively studied (Semwal et al., 2021; Bhutia et al., 2025). This study aimed to address that gap by examining the potential of *D. esculentum* mucilage as a natural gelling agent for diclofenac diethylamine gels. The findings might have provided an environmentally friendly, cost-effective, and biocompatible alternative to synthetic gelling agents, supporting further research on mucilage-rich ferns in semisolid pharmaceutical formulations (Ajala et al., 2022; Sundari et al., 2025).

➢ *Conceptual Framework*

Phytochemical Profiling, Formulation, and Evaluation of Diclofenac Diethylamine Gel Using *Diplazium esculentum* (Athyriaceae) as Gelling Agent.

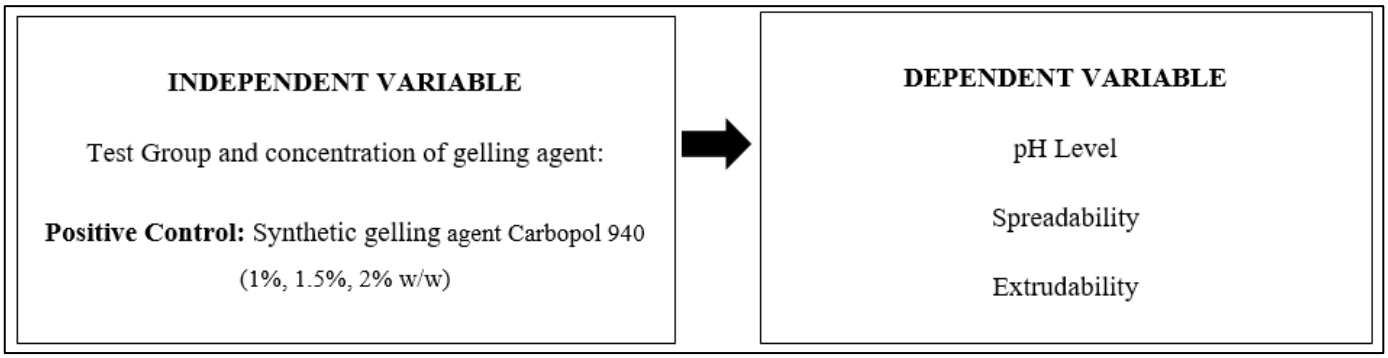

Fig 1 Conceptual Framework of the Study

This study explored the potential of mucilage extracted from *Diplazium esculentum* as a natural gelling agent in the formulation of a topical diclofenac diethylamine gel. The independent variable in this research was the different formulations of concentrations of *D. esculentum* mucilage as the primary natural gelling agent. The dependent variables included the physical and chemical properties of the gel, which assessed the pharmacopeial standard of the gel, such as pH, spreadability, homogeneity, extrudability, viscosity, and drug content. These properties were evaluated and compared against a standard synthetic gelling agent, Carbopol 940, which served as the control variable following the study of Sundari et al. (2025).

➢ *Objective of the Study*

This study aimed to formulate and evaluate the effectiveness of mucilage derived from *D. esculentum* as a natural alternative to synthetic gelling agents in the formulation of topical diclofenac diethylamine gels.

- *Specifically, the Aim of the Study was to:*

✓ Determine the phytochemical profile of the mucilage from *D. esculentum.*
✓ Determine the percentage mucilage yield via triplicate analysis of 30-gram plant samples.
✓ Extract mucilage from *D. esculentum* fronds using hot water extraction and confirm the presence of polysaccharides through Fourier Transform Infrared (FTIR) spectroscopy.
✓ Prepare topical diclofenac diethylamine gels using *D. esculentum* mucilage at 2.5%, 5%, and 7.5% w/w concentrations as the natural gelling agent and a standard synthetic gelling agent (Carbopol 940) through the fusion method.
✓ Determine the compatibility of *D. esculentum* mucilage with diclofenac diethylamine gel through Fourier Transform Infrared (FTIR) spectroscopy.
✓ Evaluate and compare the physical properties of both gel formulations, including pH, spreadability, extrudability, homogeneity, viscosity, and drug content.
✓ Determine whether the *D. esculentum* mucilage gel formulation meets pharmacopeial standards for topical gels and performs comparably to the synthetic gel formulation.

➢ *Research Hypothesis*

- H0: There is no significant difference in the physical properties (pH, spreadability, extrudability, homogeneity, viscosity) and performance parameters (drug content and compatibility) of topical diclofenac diethylamine gels formulated with *Diplazium esculentum* mucilage at 2.5%, 5%, and 7.5% w/w compared to gels formulated with a standard synthetic gelling agent.

- H1: There is a significant difference in the physical properties (pH, spreadability, extrudability, homogeneity, viscosity) and performance parameters (drug content and compatibility) of topical diclofenac diethylamine gels formulated with *Diplazium esculentum* mucilage at 2.5%, 5%, and 7.5% w/w compared to gels formulated with a standard synthetic gelling agent.

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➢ *Significance of the Study*

This research evaluated the potential of *D. esculentum* mucilage as a natural gelling agent in topical diclofenac diethylamine gels and may benefit the following sectors:

- Pharmaceutical Industry. The study's findings suggested an eco-friendly, cost-effective, and locally available alternative to synthetic gelling agents. Using *D. esculentum* mucilage may have helped reduce reliance on synthetic polymers, cut production costs, and support the development of sustainable topical pharmaceutical formulations.
- Clinical Practice. The study may have provided pharmacists, formulators, and healthcare professionals with a safe and natural option for topical gel formulations. This could have reduced the risks associated with synthetic excipients and provided a more cost-effective alternative for patients with financial constraints.
- Community and Agriculture. Promoting the use of *D. esculentum* in pharmaceuticals created new opportunities for local farmers and small-scale producers. This may have promoted agricultural sustainability and added economic value to a common plant resource that was widely available in the area.
- Academia. The research may have added to the growing body of literature on natural excipients used in gel formulation. It served as a reliable reference for pharmacy students, faculty members, and future researchers interested in plant-derived materials for topical drug delivery.
- Future Research and Development. The methods and findings from this study may have guided further investigations into optimizing *D. esculentum* mucilage concentration, its use in other topical formulations, and its potential combination with other natural excipients for better performance.
- Adventist Medical Center College. The study served as a valuable resource for the college library, enriching the collection of research on natural pharmaceutical formulations. This study guided and inspired future academic work in the field of pharmaceutics, particularly in the exploration of plant-based excipients and sustainable drug delivery systems.
- Department of Pharmacy (AMCC). The research served as a model for future formulation studies, encouraging the exploration of natural and accessible alternatives in drug delivery systems. The use of *D. esculentum* mucilage highlighted the department's commitment to applied research, innovative formulation, and the sustainable use of local resources in pharmaceutical development.

➢ *Scope and Limitations*

This study was conducted at Adventist Medical Center College, Department of Pharmacy Laboratory, and focused on the extraction of *D. esculentum* mucilage using only hot water extraction, with the goal of evaluating its potential as a natural gelling agent in topical diclofenac diethylamine gels. Phytochemical profiling was conducted to identify the presence of various phytochemicals in *D. esculentum*, while Fourier Transform Infrared (FTIR) spectroscopy was used to confirm the presence of polysaccharide components. The mucilage was incorporated into topical gels at concentrations of 2.5%, 5%, and 7.5% w/w, prepared using the fusion method. These formulations were evaluated and compared to a standard synthetic gel formulated with Carbopol 940 at concentrations of 1%, 1.5%, and 2%. FTIR compatibility tests were performed to assess the interaction between *D. esculentum* mucilage and diclofenac diethylamine, which helped establish preliminary pharmacopeial compliance. Key physical and chemical parameters such as pH, spreadability, homogeneity, extrudability, viscosity, and drug content were assessed to evaluate the natural mucilage gel as a potential topical drug delivery system.

However, the scope of this research was limited to fixed mucilage concentrations (2.5%, 5%, and 7.5% w/w) without further optimization across different levels. Long-term stability testing, skin irritation assessments, or comparisons with other synthetic gelling agents beyond Carbopol 940 were not included. The study focused on hot water extraction and phytochemical profiling, with FTIR spectroscopy confirming the presence of polysaccharide components. Advanced chemical standardization and confirmatory tests were not part of the research scope. More importantly, this study did not evaluate the therapeutic effects of the formulated gels. Instead, it served as a preliminary study that provided valuable insights into the potential of *D. esculentum* mucilage as a natural, sustainable, and pharmaceutically acceptable alternative to synthetic gelling agents in topical formulations.

➢ *Definition of Terms*

- Carbopol 940 - This refers to a synthetic gelling agent used as the control in gel formulation for comparison with *D. esculentum* mucilage.
- Compatibility Test – This refers to the assessment of possible interactions between *D. esculentum* mucilage and other formulation components like diclofenac, propylene glycol, methylparaben, and distilled water.
- Diclofenac Diethylamine – This refers to the active drug incorporated into both gel formulations to assess drug content and stability.
- *Diplazium esculentum* - This refers to the plant source of mucilage used as a natural gelling agent. The fronds are subjected to hot water extraction to obtain mucilage for gel formulation.
- Extrudability – This refers to how the gel is released from a tube, indicating ease of application.
- Fusion Method - This refers to the method employed in this study to prepare both natural and synthetic gel formulations. It involves heating the ingredients to form a uniform mixture and then cooling to achieve gel consistency.

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- Gelling Agent – This refers to a substance responsible for gel structure, either Carbopol 940 or *D. esculentum* mucilage, in this study.
- Homogeneity – This refers to the uniform distribution of components in the gel, checked visually and via centrifugation.
- Mucilage – This refers to the polysaccharide-rich, gel-forming substance extracted from the fronds of *D. esculentum* using hot water.
- pH – This refers to the acidity or alkalinity of the gel formulations. It is evaluated to ensure the gels are within the acceptable skin-compatible range (typically 6.75–7.9).
- Phytochemical Profiling – This refers to the procedure used in the study to identify and characterize the major polysaccharides in the extracted D. esculentum mucilage, determining its suitability as a natural gelling agent in topical formulations.
- Spreadability – This refers to how the gel spreads on the skin, which reflects its user-friendliness and application behavior.
- Topical Gel – This refers to a semisolid preparation applied to the skin, formulated with either natural or synthetic gelling agents to deliver diclofenac diethylamine directly to the affected area.
- Viscosity – This refers to the gel's resistance to flow, measured using a viscometer. Used to assess the gel's consistency, stability, and appropriateness for topical application and ease of spreading on the skin.

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CHAPTER TWO
REVIEW OF LITERATURE AND RELATED STUDIES

Topical gels rely on effective gelling agents to maintain their structure, viscosity, and overall stability, ensuring that the formulation remains uniform and acceptable for use (Bagmar et al. 2024; Choudhary 2021). This need has driven interest in natural gelling agents that can provide stability while improving patient safety and environmental sustainability.

Natural mucilages have gained attention because they are inexpensive, biodegradable, non-toxic, widely available, and highly compatible with biological systems (Villegas Calero et al. 2021). Their polysaccharide-rich composition allows them to function as gelling, thickening, and stabilizing agents, making them promising substitutes for synthetic polymers (Bhutia et al. 2025).

Among these natural sources, the Athyriaceae family, particularly the genus *D. esculentum* has been identified as a significant reservoir of hydrocolloids. According to Ibrahim et al. (2019) and Semwal et al. (2021), *D. esculentum* mucilage contained complex polysaccharides made up of uronic acids and other sugars, which contributed to gel formation and stability. These properties make *D. esculentum* a strong candidate for use as a natural gelling agent in pharmaceutical formulations.

➢ *Diplazium esculentum*

Diplazium esculentum is a native fern of the family Athyriaceae. It is present in the Philippines, particularly in Laguna, Bukidnon, Iligan, and Negros Oriental. Locally, it is known as pako. This fern grows in damp, dark locations, such as riverbanks, wetlands, and forests. It is one of the most widely consumed edible ferns in the country. This species is tender and young-felted with a slippery feel and is one of the most popular local vegetables (Aguilar et al. 2023; Ang et al. 2022). In Visayas and Mindanao, pako is commonly used as a fresh salad of fern. It can also be served as a vegetable side dish. The fern exhibits a distinctly bitter taste and a slightly greasy texture, attributable to its mucilaginous content. It contains a high amount of nutrients and has a light taste (Agarwal et al. 2022).

- *Taxonomy of Diplazium esculentum*

The taxonomic classification of *D. esculentum* according to the Global Biodiversity Information Facility.

✓ Kingdom: Plantae
✓ Phylum: Tracheophyta
✓ Class: Polypodiopsida
✓ Order: Polypodiales
✓ Family: Athyriaceae
✓ Genus: *Diplazium*
✓ Species: *Diplazium esculentum*

- *Morphology of Diplazium esculentum*

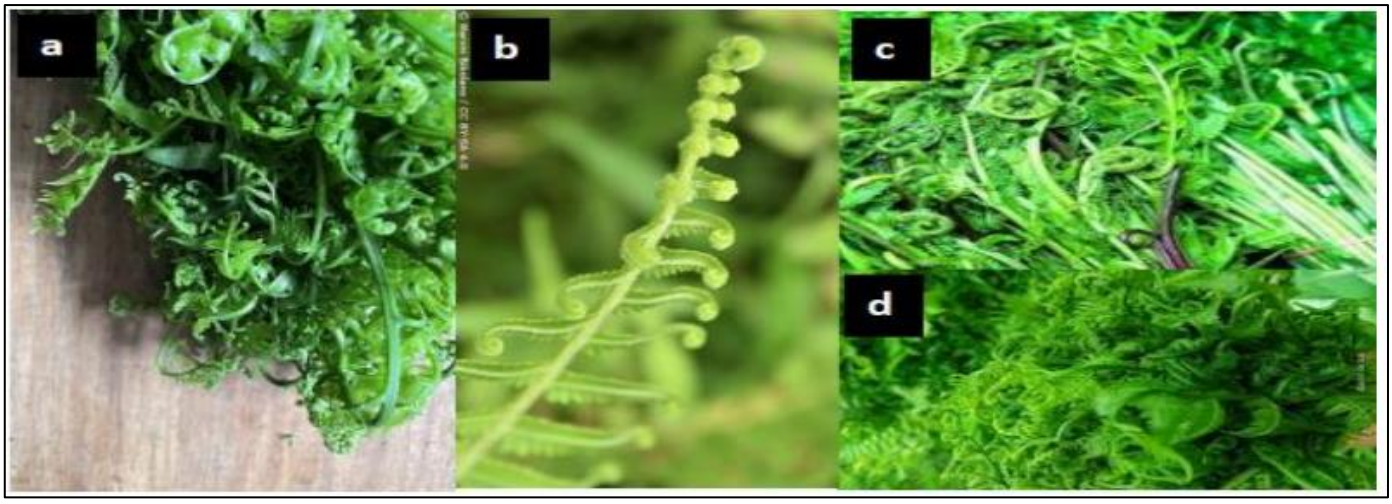

Fig 2 *Diplazium esculentum* (Pako) Plant

Morphological characteristics of *D. esculentum* from NParks (2025), the specimen observed includes the whole plant with erect rhizomes and bipinnate fronds (a), a close-up linear, detailed view of papery leaflets (b), sori aligned along the veins (c), and the mature rhizome structure in habitat (d).

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D. esculentum is a straggling terrestrial fern that typically attains up to 1 m in height, featuring erect rhizomes from which emerge robust, bipinnate fronds that may exceed 1 m in length. The fronds are supported by long stripes approximately 0.7 m long and bear linear, papery-textured leaflets with lobed to serrated margins, pointed apices, and bases that are subcordate to auriculate. The rhizome is erect or sometimes slightly leaning, bearing dark brown, narrowly lanceolate scales with toothed black margins. The sori are elongated and arranged diagonally along the veins of the leaflets, often covering a substantial length of the veinlets. Basal scales are dark brown with toothed, black-edged margins. Habitually, *D. esculentum* grows in moist, shaded terrestrial environments such as paddy fields, riverbanks, or light shade habitats below 800 m elevation. (NParks 2025).

- *Phytochemical Constituents of Diplazium esculentum Plant*

Diplazium esculentum contains mucilaginous polysaccharides, which are natural substances responsible for its gelling ability and viscosity. These polysaccharides are crucial because they allow the plant to form stable gels with a high viscosity. The structure of the mucilage from *D. esculentum* provides important functional properties, such as emulsion stability and antioxidant activity, making it a potential natural gelling agent for pharmaceutical and food applications. While specific monosaccharides such as arabinose and galactose were not directly measured in this study, it is known that plant mucilage generally contains polysaccharides made up of uronic acids and other sugars (Borah et al.). These sugar chains form the backbone of gels and contribute to the thickening and water retention that are necessary for forming stable gels. This structure has been widely observed in plant mucilage research and is critical for making gels that are useful in various applications (Hayati et al. 2018; Semwal et al. 2021).

In addition to the polysaccharides, *D. esculentum* also contains other compounds such as flavonoids, phenolic compounds, saponins, tannins, and alkaloids (Bhutia et al. 2025; Aguilar et al. 2023). These compounds are important because they contribute to the overall properties of the mucilage. For example, flavonoids and phenolic compounds help to stabilize the gel by protecting it from oxidation and helping to maintain its strength over time. Saponins help make the gel more uniform and improve its foaming and emulsifying abilities, while alkaloids and tannins support the gel by stabilizing its pH and improving its overall performance (Tongco et al. 2014). Recent studies have confirmed that the polysaccharides in *D. esculentum* mucilage is not just responsible for gel formation and stability but also contributes to the functional properties of the mucilage. These polysaccharides, along with the other compounds, work together to make *D. esculentum* mucilage as a promising material for use in gelling agent drug delivery systems, offering both structural stability and practical benefits for topical formulations (Ibrahim et al. 2019; Semwal et al. 2021).

- *Ethnobotanical Uses of Diplazium esculentum*

In many Asian countries, *D. esculentum* is valued primarily as a food source. Local communities seasonally gather their young fronds from damp and shaded locations like paddy fields, riverbanks, and wood margins (NParks 2025). *D. esculentum* continues to serve as a bridge between community traditions and plant knowledge. Semwal et al. (2021) demonstrated how local groups will harvest its young fronds seasonally, preserve them, and pass on techniques for preparation through generations, thereby embedding the species into both everyday diets and cultural identity.

D. esculentum has uses in traditional community rituals in addition to its function as a nutritious dish. According to Philippine Medicinal Plants (2025), the seasonal gathering and communal processing of pako fosters cultural connections because the plant will be used for more than just its physical qualities, a source of food as well as a representation of standard practices and the strength of rural communities. Indigenous groups in Bukidnon traditionally collect young fronds of pako for food and cultural practices. The plant is valued as a nutritious vegetable that is high in fiber, antioxidants, and minerals. It is also used in communal rituals, where its seasonal harvest represents unity and sustenance (Ang et al. 2022; Philippine Medicinal Plants 2025). In these communities, pako serves as a staple food and symbolizes cultural identity, connecting traditional diets to local heritage.

- *Diplazium esculentum Mucilage Composition*

In *D. esculentum*, the mucilage present in the ferns has been reported to contain a high proportion of polysaccharides, which are complex plant-derived polymers responsible for their gelling properties and viscosity. These polysaccharides are crucial for the plant's ability to form viscous, stable gels. The polysaccharide structure in *D. esculentum* mucilage contributes significantly to its functional properties, including high viscosity, emulsion stability, and antioxidant activity, making it a promising natural gelling agent for pharmaceutical and food applications. While specific monosaccharides were not directly quantified in this study, it was well established that the polysaccharides in plant mucilage, including those in *D. esculentum*, were made up of uronic acids and other sugars. These polysaccharide chains form the backbone of gel-forming polymers, contributing to the thickening and water retention properties that are essential for gel formation and stability. The structural characteristics of these polysaccharides, which are commonly observed in plant mucilages, enhance the functional behavior of gels (Hayati et al. 2018; Semwal et al. 2021). Meanwhile, uronic acids play a critical role in imparting anionic character to the polysaccharide structure, allowing for electrostatic interactions with other formulation components, which can improve gel stability and pH buffering (Bhutia et al. 2025). For topical applications, the physicochemical properties of mucilage directly influence its suitability as a gelling agent. Plant mucilages rich in complex polysaccharides have been reported to enhance viscosity, prevent phase separation, and improve spreadability when incorporated into gel formulations (Goksen et al. 2023).

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✓ *Uronic Acid*

Uronic acids are acidic monosaccharides, mainly galacturonic and glucuronic acids, which possess free carboxyl groups that impart a negative charge to plant mucilages (Cakmak et al. 2023). This characteristic improves pH buffering capacity, gel consistency, and bioadhesion to the skin surface (Villegas Calero et al. 2021). Uronic acid increased mucilage concentration, increased viscosity dramatically, and enhanced antioxidant activity, demonstrating a clear functional role of uronic acid in viscosity and bioactivity (Puligundla & Lim 2022). In mucilage-based gels, this ionic interaction substantially modifies the three-dimensional structure, stiffness, and rheology of the gel, which are essential for achieving pharmaceutically acceptable viscosity and spreadability (Wang et al. 2024). Figure 3 illustrates the chemical structure of galacturonic acid, highlighting its uronic acid backbone and the functional carboxyl group responsible for its gelling and binding characteristics.

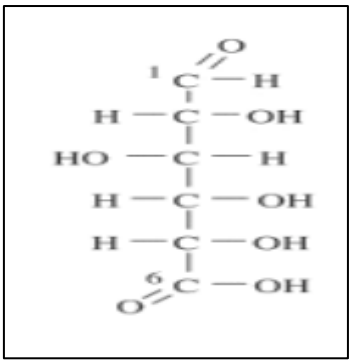

Fig 3 Uronic Acid Structure

➢ *Members of the Athyriaceae Family Utilized as Pharmaceutical Excipients*

The family Athyriaceae, comprising around 50 genera and more than 700 species, is distributed extensively in tropical and subtropical parts of the world. Among its members, *D. esculentum* has been recognized as a leafy vegetable with local traditional uses in Southeast Asia and the Philippines, particularly in regions such as Bukidnon, Davao, and Lanao del Sur (Barcelona and Pelser 2024). *D. esculentum* is also known to have high fiber, protein, and polyphenolic composition, with antioxidant activity and functional properties such as swelling and water retention (Sareen et al. 2020). Reviews also accentuate *D. esculentum* with antioxidant, antimicrobial, and immunomodulatory activities, several attributed to its polysaccharide and mucilage composition (Roy and Chaudhuri 2020).

Although no direct research verifies Athyriaceae mucilage as a pharmaceutical excipient, *D. esculentum* mucilage was isolated and found to display high viscosity, thixotropic flow, and stability of emulsion (Ibrahim et al. 2019). More generally, plant mucilage is known as a biodegradable biopolymer with known pharmaceutical applications (Góźdź et al. 2021; Archana et al. 2024). Experimental research has confirmed okra and hibiscus mucilage as gelling agents in diclofenac diethylamine gels (Sundari et al. 2025) and fenugreek mucilage as a mucoadhesive excipient for drug adhesion improvement, release control, and bioavailability enhancement (Kurien et al. 2024). Athyriaceae research has been encapsulated in Table 1, while Table 2 represents mucilage research from other plant families.

Table 1 Members of Athyriaceae Studies Demonstrating Polysaccharide/Mucilage Potential and Pharmacological Activities

Species used	Study Title	Results/Conclusion	Citation
Diplazium esculentum	Antioxidant Activities and Functional Properties of Complex Polysaccharide (Mucilage) in Vegetable Fern (*Diplazium esculentum*)	Mucilage yields via hot-water (2.6%) and alkaline (2.5%) extraction; high viscosity, thixotropic flow, emulsion stability, and antioxidant activity indicate hydrocolloid potential.	Ibrahim et al. 2019
Diplazium maximum	Nutritional characterization and chemical composition of Diplazium maximum (D. Don) C. Chr.	High protein, fiber, and polyphenols (epicatechin, myricetin, catechin, and protocatechuic acid) show antioxidant activity and water absorption/swelling functional properties.	Sareen et al. 2021
Diplazium esculentum	Determination of Phytochemicals, Minerals, and Evaluation of Antioxidant, Antidiabetic Activities of Young Fronds of *Diplazium esculentum*: A Vegetable of Tripura, India	Young fronds contain alkaloids, phenols, tannins, and minerals; they show potent antioxidant and antidiabetic activity and support the functional and pharmacological potential of polysaccharides/mucilage.	Manna et al. 2023
Diplazium esculentum	Phytochemical profiling and biological activities of *Diplazium esculentum* (Retz.) Sw.: an edible vegetable fern	Contains polysaccharides, antioxidants, and bioactive compounds; supports antioxidant, antimicrobial, and immunomodulatory effects.	Raina et al. 2023

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➢ *Gel Formulations*

Gel formulations are semisolid systems comprising a three-dimensional polymeric network that entraps a liquid phase, thereby ensuring stability and desirable rheological properties (Bagmar et al., 2024). Natural mucilages and gums, in particular, have garnered attention as eco-friendly and biocompatible gelling agents, offering advantages such as biodegradability, safety, and renewable sourcing (Munot et al. 2021). Broad reviews have also highlighted the increasing role of long-acting gel formulations in various therapeutic areas, including oncology, wound healing, and infectious disease management, due to their ability to provide sustained release, localized action, and enhanced patient compliance (Hossein Omidian & Wilson 2024).

- *Types of Gels*

Gels can be broadly classified into different categories depending on their origin and properties. Synthetic gels, such as carbopol and poloxamers, are widely used due to their ability to impart clarity, viscosity, and stability to formulations (George et al. 2025).

Table 2 Classification of Gelling Agents

Types of Gel	Examples of Gelling Agents	Characteristics	Citation
Synthetic	Carbopol, Poloxamers, Polyvinyl alcohol	Provide high viscosity, clarity, and long-term stability; widely used in pharmaceutical gels.	Chelu & Musuc. 2023
Semi-synthetic	Hydroxypropyl methylcellulose (HPMC), Carboxymethyl cellulose (CMC), Methylcellulose	Chemically modified derivatives of natural polymers; improved solubility, consistency, and reproducibility.	Satchanska et al. 2024
Natural	Plant mucilages (okra, Plantago, *Diplazium esculentum*), Gums (guar gum, xanthan gum, acacia), Alginates, Gelatin	Biodegradable, biocompatible, eco-friendly, and sustainable alternatives to synthetic polymers.	Zhuang et al. 2023
Inorganic	Bentonite, Aluminum hydroxide	Have good suspension properties and strong transport ability in porous media; used in specific pharmaceutical and industrial gel formulations.	Cao et al. 2021

Table 3 Advantages and Disadvantages of Gels

Type of Gel	Advantage	Disadvantage	Citation
Synthetic	• High viscosity and clarity • Good stability and reproducibility • Easily modified for desired properties • Non-greasy and cosmetically elegant	• Possible irritation (acidic nature of Carbopol) • Risk of microbial contamination due to water content • Poor solubility for hydrophobic drugs • Higher cost and manufacturing complexity	Metta et al. 2023; Mashabela et al. 2022; Hamdi 2023
Semi-synthetic	• High viscosity and smooth texture • Biocompatible and less irritating • Stable over wide pH range • Clear, spreadable gels with good film-forming property	• Slow hydration or dissolution • Limited mechanical strength • Higher production cost • Risk of microbial growth if not preserved	Kumar and Sharma 2025; Metta et al. 2023
Natural	• Biocompatible and biodegradable • Non-toxic and eco-friendly • Readily available and cost-effective • Excellent film-forming and mucoadhesive properties	• Batch-to-batch variability due to natural origin • Poor mechanical strength and stability • Susceptible to microbial contamination • Limited pH and temperature stability	Abdalla and Wilson 2021.
Inorganic	• Form thixotropic gels (semisolid at rest, liquid when shaken) • Provide good viscosity and suspension stability • Chemically stable and suitable for antacid and dermatological formulations	• May exhibit syneresis (liquid separation) • Often less transparent than organic gels • Limited drug release control and flexibility • Can settle over time if not properly stabilized	Sharma et al. 2022

- *Formulation Components of Topical Gels*

Components for formulating gels typically include pharmaceutical ingredients (APIs), natural gelling agents, excipients, solvents or vehicles, and pH adjusters or stabilizers (Bagmar et al. 2024; Parhi et al. 2023; Choudhary 2021). These components are essential for the stability and efficacy of a topical gel.

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Table 4 Components in the Formulation of Topical Gels

Components	Role in Formulation	Concentration/Amount
Active Pharmaceutical Ingredient (API)	Provides therapeutic effect as an NSAID for anti-inflammatory and analgesic action.	Diclofenac diethylamine 1% w/w
Natural Gelling Agent	Provides viscosity, gel consistency, and spreadability.	*D. esculentum* mucilage at 2.5%, 5%, 7.5% w/w
Synthetic Gelling Agent (Control)	Standard gelling agent for comparison.	Carbopol 940 at 1%, 1.5%, 2% w/w
Humectant	Enhances the solubility of the drug and skin penetration; retains moisture.	Propylene glycol 3.5 ml
Preservatives	Prevents microbial growth, especially important with natural mucilage.	Methylparaben 0.045%
Vehicle / Solvent	Disperses all ingredients; ensures uniform gel matrix.	Distilled water q.s. to 100%
Neutralizer / pH Adjuster	Maintains skin-compatible pH (5.5–7.0).	Sodium hydroxide / Citric acid (q.s. to pH) 20 - 30% solutions

- *Advantages of Topical Gels*

Topical gels present several advantages over other semisolid dosage forms due to their unique physicochemical properties. They provide localized drug delivery at the site of application, which minimizes systemic side effects and improves therapeutic efficacy (Bagmar et al. 2024). Another key advantage is the avoidance of first-pass metabolism, which allows drugs to maintain higher bioavailability compared to oral administration (Parhi et al. 2023). Gels are also easy to apply, cosmetically acceptable, and provide better patient compliance, particularly in chronic conditions such as arthritis and skin-related disorders (Barnes et al. 2021). Furthermore, their aqueous nature enhances drug release and penetration into the skin. At the same time, their cooling and soothing effects make them preferable over ointments or creams according to Institute for Quality and Efficiency in Health Care 2021.

- *Disadvantages of Topical Gels*

One major drawback is the limited penetration of drugs through the stratum corneum, which can reduce therapeutic efficacy, especially for poorly soluble or high-molecular-weight compounds (Parhi et al. 2023). Gels may also cause skin irritation, allergic reactions, or sensitization, particularly when synthetic polymers or preservatives are used (Tang and Du 2024). Another limitation is their short residence time on the skin surface, as gels can be easily removed by washing, sweating, or friction, resulting in reduced drug availability at the target site (Bagmar et al. 2024). Moreover, natural gelling agents, such as mucilages and gums, often face challenges of microbial contamination and poor long-term stability, which restrict their direct use in formulations without appropriate processing (Amiri et al. 2021).

➢ *Mucilage*

Mucilage is an odorless, colorless, and tasteless polysaccharide complex exuded from various plant parts, including seeds, leaves, stems, and roots. It consists mainly of highly branched carbohydrates such as L-arabinose, D-xylose, D-galactose, L-rhamnose, and galacturonic acid and may also contain glycoproteins, tannins, and other bioactive components. When hydrated, mucilage forms a viscoelastic, gelatinous hydrocolloid. This non-toxic, biodegradable plant exudate serves as a natural thickener, emulsifier, and film-forming agent. Its industrial applications are expanding in the food, pharmaceutical, cosmetics, and agricultural sectors, including its use in edible coatings, nanocarrier delivery systems, and hydrogels (Tosif et al. 2021).

Recent investigations have underscored the versatile functional properties of plant-derived mucilages as gelling agents, highlighting their influence on rheology, stability, and drug delivery performance. These properties are demonstrated by their ability to maintain a favorable pH, viscosity, spreadability, extrudability, homogeneity, and drug content while enabling controlled drug diffusion (Gupta et al. 2019). The functional versatility of mucilage, due to its high water-binding capacity, viscoelastic, and gelling behaviors, makes it suitable as an emulsifier, stabilizer, film-forming agent, and natural thickener across food and pharmaceutical applications (Tosif et al. 2021).

➢ *Gelling Agents*

In pharmaceutical technology, gels, being semisolid dosage forms, offer several advantages, including ease of application, uniform distribution of active ingredients, enhanced drug release, and improved patient compliance. They are characterized by a three-dimensional polymeric network that entraps a liquid phase, providing both stability and desirable rheological properties. Because of these features, gels are widely used in topical, oral, and transdermal preparations.

Naturally, gel formulations have relied on synthetic polymers such as carbopol, hydroxypropyl methylcellulose (HPMC), and poloxamers, which provide desirable viscosity, clarity, and stability. However, concerns regarding toxicity, cost, and environmental impact have encouraged the exploration of natural alternatives. In this context, plant-derived mucilages have attracted significant attention as promising gelling agents. Mucilages are polysaccharide-rich substances that swell in water to form viscous colloidal dispersions, making them suitable for gel preparation. Their natural origin ensures biocompatibility, biodegradability, and non-

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toxicity, while their ability to form stable gels provides an eco-friendly substitute for synthetic polymers (Rajeswari 2017; Singh et al. 2021).

The use of mucilage in gel formulation provides not only the essential viscosity and spreadability but also supports the development of innovative and sustainable dosage forms. Exploring underutilized plant sources of mucilage, such as *D. esculentum* highlights the potential of natural materials in pharmaceutical technology. This study, therefore, focused on the utilization of plant-derived mucilage in gel formulation, with emphasis on its gelling properties rather than its therapeutic activity.

- *Types of Gelling Agents*

According to Bhurat and Kawtikwar (2013), gelling agents are broadly classified into synthetic, semi-synthetic, natural, and inorganic categories depending on their source and functionality. Synthetic polymers such as Carbopol, poloxamers, and polyvinyl alcohol are commonly employed in gel formulations due to their ability to impart high viscosity, clarity, and long-term stability. Semi-synthetic polymers, including hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC), and methylcellulose, are chemically modified derivatives of natural polymers that provide improved solubility, consistency, and reproducibility, making them suitable for both oral and topical gel preparations. On the other hand, natural gelling agents derived from plants and animals such as gums (guar gum, xanthan gum, acacia gum), mucilages (okra, Plantago, *D. esculentum*), alginates, and gelatin are gaining increasing importance due to their biodegradability, safety, and eco-friendly nature, offering sustainable alternatives to synthetic excipients (Saju and Sivaraman 2021).

- *Factors Affecting Actions of Gelling Agents*

The actions of gelling agents are mainly affected by concentration, pH, temperature, and the presence of other formulation components (Kolawole & Cook 2023). The concentration of the gelling agent determines viscosity and gel strength, while pH influences gel stability and compatibility with the skin. Temperature can alter the gelation behavior and rheological properties of a gel, and additives such as humectants, preservatives, or salts may enhance or hinder its spreadability, homogeneity, and extrudability. Altogether, these factors control key parameters, including viscosity, spreadability, extrudability, homogeneity, and drug content, in topical gels (Slavkova et al. 2023).

➢ *Diclofenac Diethylamine*

Diclofenac is a frequently used drug that has additional pain-relieving and antipyretic action. It performs a crucial task in the management of MSK disorders caused by a variety of etiological illnesses, such as rheumatoid arthritis and osteoarthritis. The inhibition of an enzyme, specifically cyclooxygenase (COX) 1 or COX 2, assists in the anti-inflammatory process by reducing prostaglandin synthesis at the inflammation site. Diethylammonium salt of diclofenac (diclofenac diethylamine) is said to be utilized for topical treatments (Sharma and Verma 2022).

- *Parameters Used in Evaluating Diclofenac Diethylamine Gels*

✓ *pH Determination*

Measuring the pH in gel formulations plays a vital role in ensuring the active ingredient remains stable and effective. The pH level influences multiple aspects, such as chemical stability, uniformity, and overall quality, all of which are critical for consistent delivery and minimizing irritation when applied to the skin. To achieve accurate pH readings, it is essential to use a properly calibrated pH meter and follow rigorous testing procedures. Maintaining the pH within an optimal range helps prevent product degradation, improving its shelf life and user experience. The pH is found in the range of 6.75 to 8.0. This slightly acidic to slightly basic range helps ensure the stability and efficacy of the medication (Sundari et al. 2025).

✓ *Spreadability*

In mucilage-based gel formulations, spreadability becomes particularly critical because natural gelling agents often yield viscosities that differ markedly from those of synthetic polymers. Striking the right balance was essential: an overly viscous gel may be difficult to spread and feel sticky, while one that is too fluid may not stay in place at the application site. Assessing spreadability, therefore, enables researchers to determine whether gels formulated with mucilage possess rheological properties that are comparable to or even exceed those of traditional synthetic gels. As emphasized by Bakhrushina et al. (2022), spreadability is a fundamental attribute of semisolid dosage forms, governing drug delivery efficiency, ease of application, and even extrudability from packaging, all of which significantly influence user preference and compliance. Accordingly, in this study, spreadability was evaluated as a key performance characteristic of gels formulated with *D. esculentum* mucilage, in line with the importance highlighted by Bakhrushina et al. (2022), ensured that the natural polymer not only formed a stable gel but also provides favorable application properties for patient use.

✓ *Extrudability*

For mucilage-based formulations, such as those prepared with *D. esculentum*, extrudability is particularly significant because natural polymers often exhibit variations in viscosity compared to synthetic ones, which can alter their flow and dispensing characteristics. According to Khan et al. (2022), extrudability can be quantitatively assessed by recording the weight required to extrude at least a 0.5 cm ribbon of gel from an aluminum collapsible tube within 10 seconds. It assesses the ease with which the

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product can be dispensed from its container. This property directly impacts user convenience, dosing accuracy, and overall product acceptability. A gel that is too stiff may be difficult to extrude, reducing compliance, while an overly fluid formulation results in wastage and inconsistent dosing.

✓ *Homogeneity*

The homogeneous distribution of the formulation components, in this case the active drug, within the gelatinous gel is imperative in providing consistency in both physical appearance and performance as a therapeutic agent. Any lack of homogeneity in the topical gel can result in dose variability and decrease the efficacy. Assessment of homogeneity is achieved through visual inspection of the item (no lumps, aggregates, or phase segregation) with or without a content uniformity test to approximate the proportion of the batch (Das et al. 2022). In the study of Sundari et al. (2025), natural mucilage obtained from utilized in diclofenac diethylamine gel shows the usefulness of plant-based mucilages with gelling properties. Furthermore, gels based on ethanolic extract of *Pandanus amaryllifolius* formulation, which supports the use of plant-based excipients in semi-solid formulations due to the achievement of dose uniformity (Forestryana et al. 2022).

✓ *Viscosity*

A critical property for semisolid formulations like topical gels, as it defines the formulation's resistance to flow under applied force. Ideal viscosity ensures that the gel remains well-placed at the application site, preventing leakage while still being manageable during application, which supports both formulation stability and patient compliance. As highlighted by Vilimi et al. (2024), viscosity measurements provide essential insight into formulation quality, including stability, drug release potential, and overall applicability. Moreover, modern techniques such as microfluidic rheology, offered alongside traditional rotational viscometers, enable fast, accurate, and small-volume analysis (under 500 µL) across a wide range of shear rates, making them exceptionally useful for evaluating gel systems, particularly when working with natural polymers like mucilage. Effective in analyzing gels and pharmaceutical formulations with higher precision and efficiency. (Vilimi et al. 2024).

In the context of gels prepared with *Diplazium esculentum* mucilage, assessing viscosity via such techniques ensures that the formulation strikes the right balance: viscous enough to stay in place but not so stiff that it hinders spreadability or usability.

✓ *Drug Content*

Drug content determination in diclofenac diethylamine gels involves dissolving a measured amount of gel in an appropriate solvent, filtering the solution, and measuring the drug concentration using UV spectrophotometry. This method ensures formulation quality and compliance with pharmaceutical standards by comparing the drug content against a calibration curve derived from known drug concentrations. Typically, the drug content should be within 85-115% of the labeled claim, confirming uniformity and stability. The analysis also evaluates whether natural excipients like *D. esculentum* mucilage impact drug distribution or stability relative to synthetic excipients such as Carbopol 940. Diclofenac diethylamine was detectable in UV analysis over a concentration range of approximately 0.005% to 0.02%, corresponding to 0.05 to 200 µg/mL in solution, enabling sensitive and reliable quantification in topical gel formulations (Sundari et al. 2025; Singh and Dabre 2020).

The study implies the Beer-Lambert law in UV spectrophotometry, which states that the absorbance of diclofenac diethylamine in solution is directly proportional to its concentration and the path length of light through the solution. This foundational principle was the basis for the quantitative determination of drug content by correlating absorbance values with concentrations using a calibration curve (Mayerhöfer et al. 2020).

➢ *Related Studies*

Table 5 Related Studies on the Phytochemical Profiling, Formulation, and Evaluation of Diclofenac Diethylamine Gel Using *Diplazium esculentum* (Athyriaceae) as Gelling Agent.

Study/ Journal Article	**Relevant/ Related Information**	**Citation**
Studies/ Related Literature on the Use of Natural Mucilages as Gelling Agents in Diclofenac and Other Topical Gel Formulations		
Formulation and Evaluation of Diclofenac Diethylamine Gel Using Natural Mucilages	The hibiscus and okra mucilages exhibited a supporting ability to form gels at a range of 2.5, 5%, 7.5%, and 10% w/w, when compared to Carbopol 934. A 10% w/w of hibiscus mucilage formed the best gel formulation with desirable clarity, homogeneity, pH, spreadability, extrudability, and drug content. Natural mucilage gels also had significantly greater exactness and efficacy relative to Carbopol 934, yet general performance in the capacity to	Sundari et al. 2025

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	serve as gelling agents was superior	
Natural Gums and Mucilage as Gelling Agents in Topical Gel Formulation	The study has determined that natural gums and mucilages have the potential to serve as gelling agents in topical gels. The resulting polymers were natural, thereby allowing for pH, spreadability, and homogeneity in formulations, and affording them as alternatives to synthetic agents in gel systems.	Kumari et al. 2022
Formulation and Evaluation of Diclofenac Emulgel Using Natural Permeation Enhancers	Although the study focused on enhancing permeation, the researchers emphasized the use of natural excipients in formulations containing diclofenac. This suggests that the topical biomass of plant-based mucilages and associated biopolymers can enhance drug delivery.	Sekhar et al. 2025
Design, Formulation, and Evaluation of Diclofenac Diethylamine Gel	The optimization and formulation of diclofenac diethylamine gel were completed using well-known gelling agents. Although synthetic polymers were used, the experiment offers a reference point against which natural mucilage-based gel formulations can be compared regarding their clarity, stability, and drug content	Das et al. 2022
Functional Properties of Cactus Pear Mucilage: Gelling and Spherification	The research has investigated the selective characteristics of cactus pear mucilage, demonstrating its gelation capacity and its application in the spherification process. These structures highlight the flexibility of this natural polymer, which can be utilized in pharmaceutical gel formulations.	Mushanganyisi et al. 2022
Development and Evaluation of Emulgel Formulation of Diclofenac Sodium Utilizing *Lepidium sativum* as a Gelling Agent	*Lepidium sativum* seed mucilage has been used as a gelling agent in diclofenac emulgels. The optimized formulations exhibited good rheological behavior, spreadability, drug release, and stability, making them a viable natural alternative to synthetic gels.	Sonule et al. 2023
The Gelling Properties of *Dillenia indica* Mucilage in Benzyl Benzoate Emulgel Formulations	*Dillenia indica* mucilage has been shown to exhibit its appropriate gelling behavior in benzyl benzoate emulgel formulae. It has comparable qualities to Carbopol, making it a likely alternative for natural gelling agents in topical preparations.	Ajala et al. 2022
The Renaissance of Plant Mucilage in Health Promotion and Industrial Applications: A Review	Chia mucilage and other mucilages of plants are renewable, biodegradable, biocompatible, and nontoxic. They find extensive applications in pharmacological preparations as binders, disintegrants, and gel-forming agents. They are helpful as these mucilages enable long-standing and localized drug delivery, liquid suspension thickening, and the creation of gels, which can be used in pharmaceutical and cosmetic products.	Aguilar-Toala et al. 2021
Exploitation of *Borassus flabellifer* fruit	Diclofenac sodium gels have used	Ravi Kumar et al. 2012

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mucilage as a novel natural gelling agent	*Borassus flabellifer* fruit mucilage as a natural gelling agent. The gels, with a 4 % w/w mucilage content, exhibit good stability, drug content, viscosity, and permeation characteristics, making them suitable substitutes for commercial and synthetic formulations. Thus, 4 % w/w mucilage gel is a prospective alternate natural excipient.	

Table 6 Studies on Plant Mucilage Used as a Pharmaceutical Excipient

Plant/Source	Study Title	Results/ Conclusion	Citation
Hibiscus rosa-sinensis	Formulation and Evaluation of Diclofenac Diethylamine Gel Using Natural Mucilages	Mucilage acted as a gelling agent with good viscosity, spreadability, and stability in gel formulations.	Sundari et al. 2025
Fenugreek *(Trigonella foenum-graecum)* mucilage	Isolation and Characterization of Fenugreek Seed Mucilage, A Natural Mucoadhesive Polymer	Provided high-yield (31.78%), carbohydrate-rich mucilage with excellent mucoadhesive strength, swelling index, and pH compatibility—ideal for drug delivery systems. Used to enhance drug adhesion, control release, and improve bioavailability.	Kurien et al. 2024
Okra (*Abelmoschus esculentus*)	Formulation and Evaluation of Diclofenac Diethylamine Gel Using Natural Mucilages	Provided gel stability, viscosity, and thixotropic properties suitable for topical delivery.	Sundari et al. 2025
Fern polysaccharides	Characterisation of novel polysaccharide extracts from indigenous New Zealand ferns	Demonstrated hydrocolloid-like behavior and extractable polysaccharides; supports mucilage potential in Athyriaceae.	Peh et al. 2025

Mucilage was also reported to have promising gelation properties in pharmaceutical preparations, specifically the mucilage of different plant sources such as *Mimosa pudica*, *Lepidium sativum*, *Dillenia indica*, cactus pear, okra, and hibiscus. Due to increasing interest in natural mucilages, researchers formulated a diclofenac diethylamine gel using *D. esculentum* mucilage as a gelling agent. It was selected because it was natural and native to the Philippines, non-toxic, and environmentally friendly, making it an alternative to synthetic gelling agents, such as Carbopol.

➢ *Theoretical Framework*

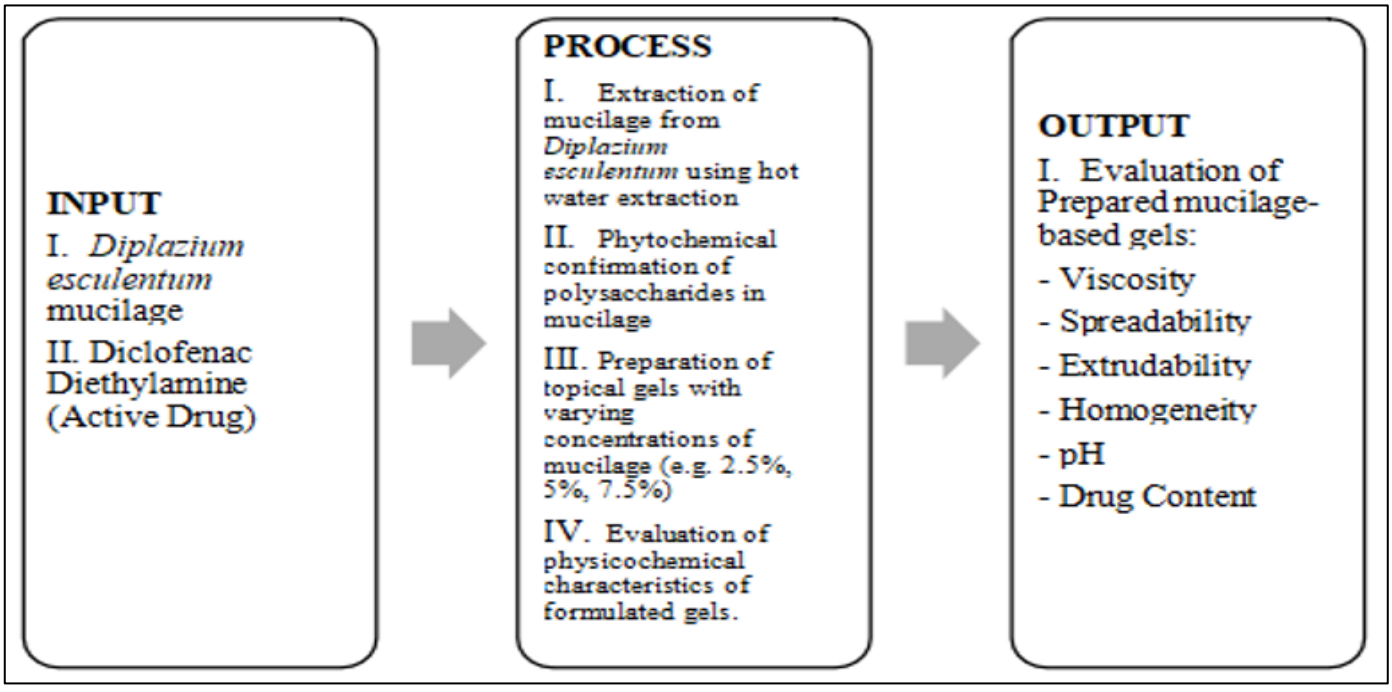

Fig 4 Relationship of the Input, Process, and Output of the Research Study

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The input includes the resources necessary for the preparation of topical gel formulations utilizing *D. esculentum* mucilage as a natural gelling agent. Extraction of the mucilage was carried out through hot water extraction, followed by phytochemical screening to confirm the presence of polysaccharides. Different concentrations of the mucilage (2.5%, 5%, and 7% w/w) are incorporated into diclofenac diethylamine gel formulations to evaluate their suitability as gelling agents. The output was assessed based on key physicochemical parameters, including viscosity, spreadability, extrudability, homogeneity, and pH, which are critical in determining the quality and applicability of topical gel.

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CHAPTER THREE
METHODOLOGY

This chapter presented the methods and materials that were utilized in the phytochemical profiling, formulation, and evaluation of diclofenac diethylamine gel using *Diplazium esculentum* (Athyriaceae) as a gelling agent.

➢ *Plant Material Authentication, Collection, and Preparation*

A sample of *D. esculentum* (pako) was obtained from Barangay Tipanoy, Iligan City [8.1902° N, 124.2634° E]. The plant specimen was submitted to a licensed botanist at Mindanao State University–Iligan Institute of Technology, in the Biology Department under the College of Science and Mathematics, for verification and authentication of the species.

After authentication, *D. esculentum* (vegetable fern) was collected from Barangay Tipanoy, Iligan City [8.1902° N, 124.2634° E]. The ferns were washed thoroughly with distilled water to remove soil, debris, and any impurities. This step was essential to prevent contamination during extraction (Ibrahim et al. 2019). The cleaned fronds were air-dried in shade at 25–30°C. Shade drying helped preserve polysaccharides and phenolic compounds better than drying in direct sunlight (Semwal et al. 2021; Goksen et al. 2023). After drying, the material was ground using a mechanical grinder, specifically a Hanabishi blender, and passed through a 120-mesh sieve (0.25 mm particle size) to produce fine, uniform powder suitable for efficient extraction (Cakmak et al. 2023; Prajapati et al. 2022). The pulverized plant material was then kept in an airtight container stored in a cool, dry place, away from sunlight, at 4°C–25°C to prevent possible phytochemical content degradation. The pulverized plant material was stored until future use for phytochemical profile analysis and mucilage extraction.

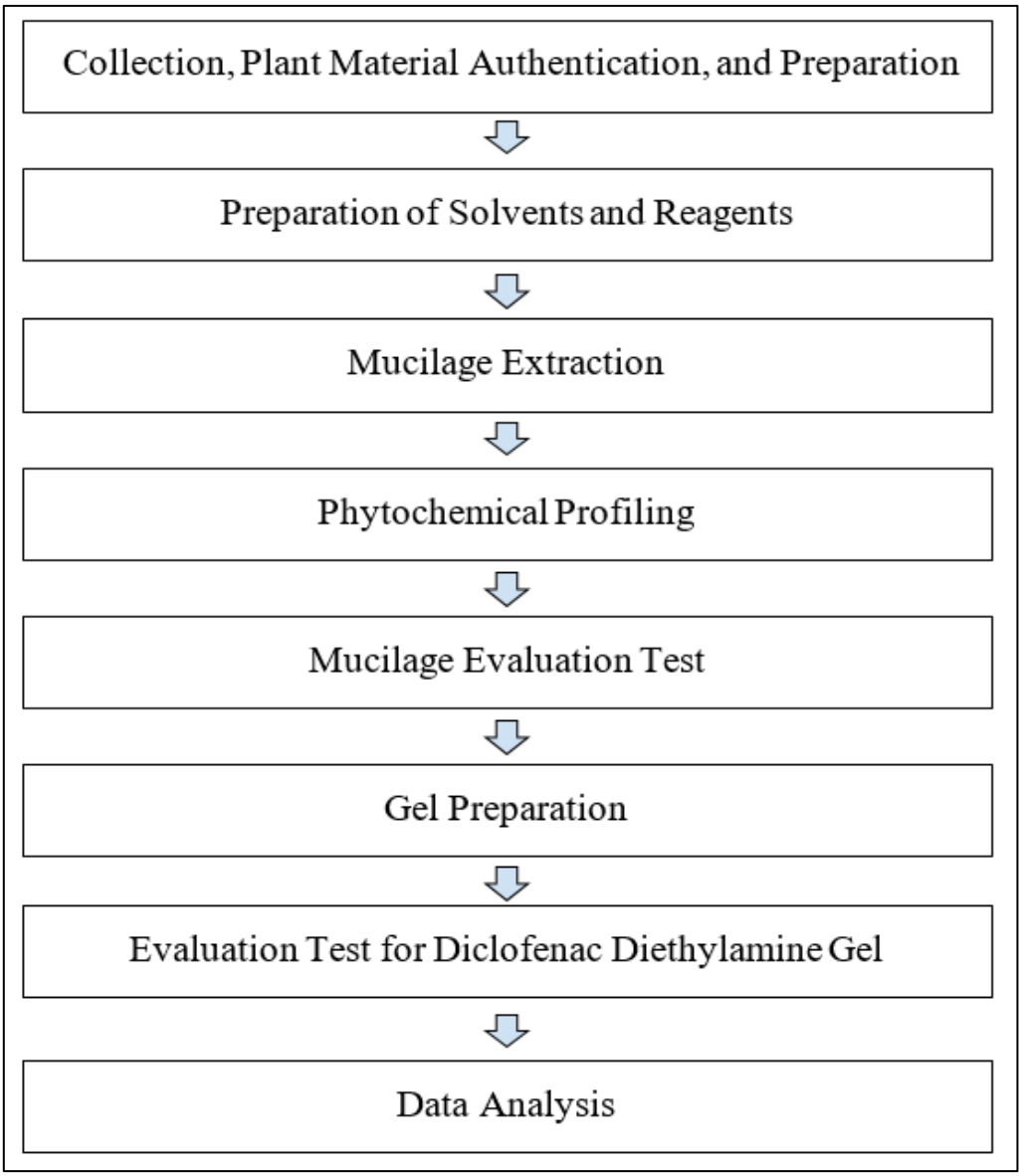

Fig 5 Research Flow of the Study

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➢ *Preparation of Solvents and Reagents*

All solvents and reagents that were utilized in this study were of analytical and laboratory grade. They were obtained from the Department of Pharmacy Laboratory at Adventist Medical Center College, which were originally sourced from Christian Health Marketing (Lanumbaan St., Capistrano Complex, Gusa, Cagayan de Oro City, Misamis Oriental). Reagents that were not available in the laboratory were acquired from Forever Pharmacy and JBL Chemicals, both based in Luzon.

➢ *Mucilage Extraction*

The mucilage from *D. esculentum* ferns was extracted using hot water extraction following the method described by Syed et al. (2022) adapted, modified, and is further supported by the method of Hayati et al. (2018). The fronds of *D. esculentum* were collected, washed thoroughly with distilled water to remove dirt and impurities, and air-dried at room temperature (25–30°C) for 5–7 days. The dried plant material was then ground into coarse powder using a blender and stored in an airtight container for further extraction. It was then boiled in distilled water not exceeding 70°C for 45 minutes because this temperature is sufficient to soften the plant tissue and promote the release of mucilage from the cell wall without exposing the polysaccharide-rich mucilage to excessive heat (Hong et al. 2021), and then a ratio of 1:10 w/v (10 g plant with 100 mL distilled water) was used. This helped release mucilage from the plant cell walls. After this, the mixture was filtered through a muslin cloth to remove fibrous matter and solid residues in a 1000 mL beaker; the filtrate and the extracted mucilage were precipitated with 95% ethanol at a ratio of 1:3 v/v and allowed to stand for 12 hours. The precipitated mucilage was then collected and transferred to an evaporating dish. It was then dried in a hot-air oven for 12 hours not exceeding 60°C (Sonule et al. 2023). After it was completely dried, the mucilage was passed through a 120-mesh sieve (125-micrometer particle size) to produce a fine, uniform powder (Cakmak et al. 2023; Prajapati et al. 2022). The material was then stored in an evaporating dish covered with aluminum foil to keep out moisture and prevent microbial growth (Ajala et al. 2022; Parhi et al. 2023). This method ensured that the obtained mucilage retained its physical and chemical properties for use as a natural gelling agent in the formulation of diclofenac diethylamine gel.

➢ *Phytochemical Profiling*

In this study, the phytochemical profiling of *Diplazium esculentum* mucilage was conducted to identify and confirm the natural chemical compounds found in the fronds of *D. esculentum*, particularly those that contribute to the gelling properties of the mucilage. Three hundred grams of the powdered plant material were sent to the MSU-IIT Department of Chemistry for testing. A series of standard phytochemical screening tests were performed to identify key components, such as flavonoids, phenolics, alkaloids, steroids, saponins, and tannins, which are known to enhance the texture, thickness, stability, and gelling ability of the mucilage. To confirm the polysaccharide content in the mucilage, Fourier-Transform Infrared Spectroscopy (FTIR) was utilized. FTIR helped identify key functional groups associated with the polysaccharides, particularly those that contribute to the gelling properties. Specific absorption bands related to C–O stretching, O–H bending, and C=O stretching of polysaccharide components were observed. These groups are indicative of the presence of sugars such as arabinose and galactose, as well as uronic acids, which are critical for gel formation and stability. FTIR provided detailed insights into the chemical composition of the mucilage, further supporting its potential as a natural gelling agent. The following table presented the specific tests to be conducted, the procedures to be followed, and the expected outcomes.

Table 7 Standard Phytochemical Screening Tests for *Diplazium esculentum* Mucilage

Phytochemicals	Test / Method	Procedure	Positive Indication
Flavonoids	$AlCl_3$ Colorimetric Assay	~1 mL of extract mixed with 1 mL of 2% $AlCl_3$ in methanol, incubated for 10 minutes	Yellow coloration confirms flavonoid content.
Phenolic Compounds	Folin–Ciocalteu Method	~1 mL of extract mixed with 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 7.5% Na_2CO_3; incubate for 30 minutes.	Blue color intensity corresponds to total phenolic concentration.
Saponins	Vanillin–Sulfuric Acid Assay	~1 mL of extract reacted with 0.5 mL of 8% vanillin and 5 mL of 72% H_2SO_4; incubate at 60 °C for 10 minutes, cool	Red color indicates saponin presence.
Alkaloids	Dragendorff's Reagent Method	~1 mL of acidified extract (1% HCl) treated with Dragendorff's reagent; precipitate collected	Orange or reddish-brown precipitate confirms alkaloids.
Phenols	Ferric Chloride Test	~ Dissolve a small amount of the extract in distilled water or ethanol. Add 2–3 drops of 5% ferric chloride solution.	Formation of a deep blue, or black coloration indicates the presence of phenolic compounds.
Steroids	Salkowski Test	~ Dissolve the extract in chloroform. Carefully add concentrated sulfuric acid along the side of the test tube to form a lower layer.	Formation of a reddish-brown or golden yellow ring at the interface

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			indicates the presence of steroids.
Tannins	Ferric Chloride Test	~ Add a few drops of 1% ferric chloride solution to the extract dissolved in distilled water.	Development of a blue-black or greenish-black coloration indicates the presence of tannins.
Terpenoids	Salkowski Test	~ Mix the extract with chloroform and carefully add concentrated sulfuric acid to form a layer.	Appearance of a reddish-brown coloration at the interface indicates the presence of terpenoids.

➢ *Mucilage Evaluation Test*

- *Percentage Yield*

The extracted *D. esculentum* mucilage weight was determined, and the percentage yield was calculated using the following formula based on triplicate analysis of a 30-gram sample of the powdered *D. esculentum* mucilage (Berhe et al., 2023). The weight of the *D. esculentum* mucilage was taken, and the percentage yield was calculated using the following formula.

$$(\text{Percentage Yield}) = \frac{\text{Weight of extracted } D.\ esculentum \text{ mucilage}}{\text{Weight of powdered fronds}} \times 100\%$$

- *Identification Test for Mucilage Content*

The mucilage extracted from *D. esculentum* underwent initial identification tests commonly used for plant-based polysaccharides. The identification and quality evaluation of the sample were further supported by the Molisch test, swelling index, and loss on drying. The Molisch test confirmed the presence of carbohydrates through the formation of a violet ring at the interface, indicating that the sample contained polysaccharides or mucilage components responsible for its swelling properties. The swelling index was determined to evaluate the ability of the plant material to absorb water and swell, which reflects the presence and quality of mucilage; the obtained value met the minimum acceptable requirement, indicating good quality and the moisture content, an important parameter for stability and storage. The result was functionality of the sample. Meanwhile, loss on drying was performed to determine within the acceptable pharmacopoeial limit of not more than 10%, suggesting low moisture content and reduced risk of microbial growth and degradation. Overall, these findings supported the identity, purity, and quality of the material used in the study (Ibrahim et al. 2019; Semwal et al. 2021).

✓ *Swelling Index*

The swelling index of the mucilage extracted from *D. esculentum* was determined to evaluate its water absorption capacity and hydrophilic nature, which are essential characteristics for a gelling agent in topical formulations, a swelling index of not less than 78% may be considered acceptable for plant mucilage intended for gel formulation (Sabale et al. 2023). A 1g dried mucilage was placed in a 50 mL beaker, and 25 mL distilled water was added. The mixture was allowed to stand for a set period, typically 24 hours, at room temperature to enable complete swelling. After the swelling period, the excess water was carefully removed, and the swollen mucilage was weighed. The swelling index was then calculated using the following formula:

$$\text{Swelling Index (\%)} = \frac{Wf - Wi}{Wi} \times 100$$

Where *Wf* is the weight of the swollen mucilage after 24 hours and *Wi* is the initial weight of the dry mucilage. The test was performed in triplicate to ensure accuracy and reproducibility of results. This swelling capacity reflected the mucilage's ability to absorb and retain water, forming a gel matrix that enhanced the viscosity and stability of topical gel preparations. These findings demonstrated the potential of *D. esculentum* mucilage as an effective natural gelling agent for topical drug delivery systems (Kumari et al.).

✓ *Loss of Drying*

This test helped determine the moisture content in the extracted *D. esculentum* mucilage. This measurement showed the stability and storage quality of the mucilage, as excessive moisture can encourage microbial growth, lead to degradation, and lower

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gelling efficiency over time. About 1 gram of the dried mucilage sample was weighed precisely using an analytical balance and placed in a clean, pre-weighed 10 mL beaker. The sample was dried in a hot-air oven at 105°C for 24 hours (Berhe et al., 2023). It was then determined by the following formula:

$$\text{Loss of Drying (\%)} = \frac{\text{Initial weight of sample - Final weight of sample}}{\text{Initial weight of sample}} \text{ x100\%}$$

✓ *Molisch Test*

A small amount of the extracted mucilage from *D. esculentum* was dissolved in 2 mL of distilled water and placed in a clean test tube. Two (2) drops of Molisch reagent (α-naphthol solution) were added and mixed gently. Subsequently, 1–2 mL of concentrated sulfuric acid (H_2SO_4) was carefully added along the side of the test tube to form a separate layer. The formation of a violet or purple ring at the interface indicated a positive result for carbohydrates, confirming the presence of polysaccharides in the mucilage extract (Kumari et al., 2021; Singh & Sharma, 2022).

✓ *Determining the Degree of Esterification via Fourier Transform Infrared (FTIR) Spectroscopy*

The FTIR analysis of the *D. esculentum* mucilage was carried out using a Bruker Alpha II FTIR spectrophotometer (400 to 2000 cm^{-1}). The extent of esterification (DE) of the samples was determined by comparing the peak area values of both carboxyl groups and the esterified groups using a specific equation. The FTIR was conducted at Mindanao State University – Iligan Institute of Technology at the Center of Sustainable Polymers (CSP) laboratory.

$$\text{Degree of Methyl Esterification (DME)} = \frac{A1740}{A1740 + A1630} \text{ X 100}$$

Where A1740 is the area of the ester carbonyl peak and A1630 is the area of the carboxylate peak.

Based on reported studies on plant mucilages, including *D. esculentum*, the expected esterification value (degree of methyl esterification) typically ranged from 20% to 70%. This variability depended on the extraction method and sample preparation but provided a reference window for functional properties such as gelling and viscosity. This estimated esterification degree captured the polysaccharide methylation level in the mucilage from *D. esculentum*, which is crucial for understanding its pharmaceutical and functional potential as a natural excipient (Hayati et al. 2018).

➢ *Gel Preparation*

The diclofenac diethylamine gels were prepared using the fusion method. This method was commonly used for semisolid preparations to ensure even distribution of the drug in the gel base (Choudhary 2021; Sundari et al. 2025). The necessary concentrations of *D. esculentum* mucilage (2.5%, 5%, and 7.5% w/w) were mixed with distilled water and allowed to hydrate. Meanwhile, Carbopol 940 (1%, 1.5%, and 2% w/w) was prepared separately as a synthetic control (Bagmar et al. 2024). Diclofenac diethylamine was dissolved in distilled water along with propylene glycol, which served as a co-solvent and penetration enhancer to improve solubility and dermal absorption (Parhi et al. 2023). Next, the drug solution was added to the hydrated gelling base with continuous stirring until it became uniform. Methylparaben was then included as a preservative to prevent microbial growth (Ajala et al. 2022). The pH of the formulations was adjusted to 6.75–8 for skin compatibility (Sundari et al. 2025). Finally, the completed gels were placed into collapsible tubes, sealed, and stored at room temperature in a desiccator until further evaluation.

- *Materials*

Pure diclofenac diethylamine, Carbopol (synthetic agent), methylparaben, propylene glycol, and ethanol were bought from Forever Pharmacy and JBL Scientific. The distilled water (Nature's Spring) was obtained from a convenience store. All other reagents used were of analytical grade.

- *Methods*

The diclofenac diethylamine gels were prepared using the fusion method (Pawar et al. 2020; Bagmar et al. 2024; Parhi et al. 2023; Sundari et al. 2025). The gels were made by mixing the required amount of *Diplazium esculentum* mucilage (2.5%, 5%, and 7.5% w/w) in distilled water and allowing it to hydrate completely. Diclofenac diethylamine (1% w/w) was dissolved separately in distilled water with propylene glycol, then incorporated into the hydrated mucilage under continuous stirring until a uniform gel was obtained. Methylparaben (0.045% w/w) was added as a preservative, and the final weight was adjusted with distilled water. For

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the control formulations, the same procedure was followed using Carbopol 940 (1%, 1.5%, and 2% w/w) as the gelling agent. The prepared gels were stored in airtight containers for further evaluation under laboratory conditions

Tables 8, 9, 10, and 11 summarized all the diclofenac diethylamine gel formulations prepared using the fusion method and *D. esculentum* mucilage as the natural gelling agent, along with Carbopol 940 as the synthetic control. Table 8 presented the independent variable concentrations of *D. esculentum* mucilage at three levels—2.5%, 5.0%, and 7.5% w/w. Table 9 showed the independent variable concentrations of *D. esculentum* mucilage and Carbopol 940 used as gelling agents in both the test and control gel formulations. Each formulation underwent compatibility testing with diclofenac diethylamine and other excipients to ensure homogeneity, stability, and absence of any physical or chemical incompatibilities. Table 10 outlined the complete composition (in percentage values) of each formulation utilizing *D. esculentum* mucilage, specifying the amounts of diclofenac diethylamine, *D. esculentum* mucilage, propylene glycol, methylparaben, and distilled water used in the preparation. Lastly, Table 11 presented the composition and formulation codes for diclofenac diethylamine gels utilizing Carbopol 940 as the synthetic gelling agent, detailing the same components in percentage values to allow comparison between natural and synthetic formulations.

Table 8 The Variable for *Diplazium esculentum* Mucilage and Carbopol 940 as Gelling Agent

Independent Variable (Factor)	**Levels**		
	2.5%	5%	7.5%
Gelling Agent *Diplazium esculentum* mucilage	0.25 g	0.5g	0.75 g
	Levels		
	1%	1.5%	2%
Gelling Agent Carbopol 940	0.1 g	0.15 g	0.2 g

Table 9 Compatibility Test and Gelling-Agent Composition

Independent Variable	Formulation Code		
	F1	F2	F3
Gelling Agent *Diplazium esculentum* mucilage	2.5%	5%	7.5%
Gelling Agent Carbopol 940	1%	1.5%	2%

Table 10 Composition and Formulation Codes for Diclofenac diethylamine Utilizing *D. esculentum* as Gelling Agent

Components	**Formulation Code**		
	F1	**F2**	**F3**
Diclofenac diethylamine (g)	0.116	0.116	0.116
Propylene Glycol (g)	3.5	3.5	3.5
Methylparaben (g)	0.045	0.045	0.045
Ethanol (mL)	2	2	2
Distilled Water (mL)	3	3	3

Table 11 Composition and Formulation Codes for Diclofenac Diethylamine Utilizing Synthetic Gelling Agent

Components	**Formulation Code**		
	F1	**F2**	**F3**
Diclofenac diethylamine (g)	0.116	0.116	0.116
Propylene Glycol (g)	3.5	3.5	3.5
Methylparaben (g)	0.045	0.045	0.045
Ethanol (mL)	2	2	2
Distilled Water (mL)	3	3	3

Table 12 Functions of Each Component in the Gel Formulation

Components	**Uses**
Diplazium esculentum mucilage	Natural gelling agent
Carbopol 940	Synthetic gelling agent (Sundari et al.)
Diclofenac diethylamine	Active Pharmaceutical Ingredient (Sundari et al.)
Propylene Glycol	Humectant (Sundari et al.)
Methylparaben	Preservative (Sundari et al.)
Ethanol	Solvent (Kongrawa et al.)
Distilled water	Vehicle (Sundari et al.)

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➢ *Evaluation Test for Diclofenac Diethylamine Gel*

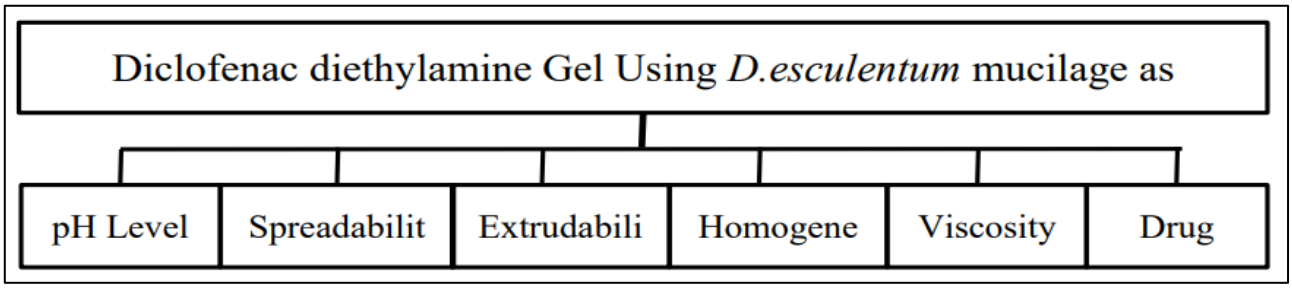

Fig 6 Evaluation Tests to be Done for the Prepared Diclofenac Diethylamine Gels.

Table 13. The Quality Control Target Product Profile for a Gel Formulation

Characteristics/Parameters	Ideal Quality Range
pH	6.75 to 8.0 (Sundari et al. 2025)
Spreadability	4.1 cm to 4.8 cm (Sundari et al. 2025)
Viscosity	35000-45000 cP (Haryanti et al. 2025)
Homogeneity	Uniform color and smooth consistency without lumps or phase separation (Sundari et al. 2025)
Drug Content	85-115% (Sundari et al. 2025)
Extrudability	>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair (Gowda et al. 2016)
Swelling Index	≥78% (Sabale et al. 2023)

- *Morphological Evaluation*

The gels were visually inspected for color, transparency, and consistency. Visual inspection was carried out with the gel inside a non-transparent container, which was placed on a clean, flat surface with a white background to evaluate the color and transparency. The consistency was assessed by observing the uniformity and smoothness of the gel's appearance. The evaluation was performed by an independent panel of three trained evaluators to ensure unbiased observation.

- *pH Measurement*

The pH of the formulated diclofenac diethylamine gels was determined to ensure compatibility with the skin and to maintain the stability of both the drug and the gelling agent. Approximately 1 g of gel was dispersed in 100 mL of distilled water, and the pH was measured using a calibrated digital pH meter at room temperature (Shiehzadeh 2023); calibration was performed in the Adventist Medical Center College Pharmacy Laboratory. Each formulation was tested in triplicate, and the mean ± SD pH value was recorded.

- *Spreadability*

To assess the ease of application of diclofenac diethylamine gels to the skin, their spreadability was tested. The spreadability of the formulated gels was determined using the glass slide method. Three glass slides were placed separately on top of a glass plate. A measured amount of the gel (1 g) was placed at the center of a clean, dry glass slide, and a second glass slide was carefully placed on top of the three glass slides to form a uniform film of the sample between the two slides. A standard weight of 500 g was then placed over the upper slide for 10 seconds to ensure uniform spreading of the gel and to eliminate any entrapped air. After removing the weight, the lower slide was then taped to hold its position as the glass plate was positioned slantly to observe the upper slide to fall slowly in vertical movement. The time required for the upper slide to move was recorded using a stopwatch. The spreadability was calculated using the following formula:

$$S = \frac{M \times L}{T}$$

Where S represents the spreadability (cm/sec), M represents the applied weight on the slide (which is 500 g), L represents the distance moved by the slide (in cm), and *t* represents the time taken (in seconds). Each formulation was tested three times to ensure consistency. Gels that spread over a larger area were considered easier to apply and more user-friendly (Sundari et al. 2025).

- *Extrudability*

About 10 g of gel was placed in collapsible tubes by incorporating the gel formulations prepared during the study after the gels had set in the container. Extrudability was measured by determining the percentage of gel extruded, as well as the mass in

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grams required to form a minimum ribbon of 0.5 cm in thickness within the tube in 10 seconds. The extrudability percentage was calculated using the following formula:

Where Mass of Gel Extruded is the difference between the initial mass (before extrusion) and the final mass (after extrusion), and Total Mass of Gel is the 10 g of gel used in the test. Each formulation was subjected to three independent replicates to ensure consistency and repeatability. The extrudability was recorded as the average percentage of gel extruded, along with the average mass required, thus providing an estimate of the ease of application of the gel. This protocol followed the methodology proposed by Sundari et al. (2025), one of the most widely used methods for mechanically assessing topical gel formulations.

$$\text{Extrudability \%} = \frac{\text{Mass of the gel Extruded}}{\text{Total Mass of the gel}} \text{ X } 100$$

- *Homogeneity*

The homogeneity of the formulated gels was evaluated to ensure even distribution of the active ingredient within the formulation. About 1 g of gel was placed in a centrifuge tube. The sample was then subjected to centrifugation at 3000 rpm for 30 minutes to allow any undissolved particles, aggregates, or phase separation to settle. After centrifugation, the sample was visually inspected for the presence of any layering, particle aggregation, or phase separation (Das et al. 2022). The absence of these irregularities indicated that the formulation had a uniform color and smooth consistency without lumps or phase separation, meeting the ideal quality range for homogeneity (Sundari et al. 2025). Each gel sample was tested in triplicate to confirm the consistency of the results (Sundari et al. 2025; Forestryana et al. 2022).

- *Viscosity*

The viscosity of the formulated gels was measured to determine their flow properties using a Brookfield viscometer. The viscosity was assessed at different shear rates using 10 g of gel. The results were recorded in millipascal-seconds (mPa·s), which is equivalent to centipoise (cP). Higher viscosity values indicated thicker gels, which could affect the release rate of the active ingredient and the ease of application. Each formulation was tested in triplicate to ensure consistency and reliability of the results (Kumari et al. 2022; Sundari et al. 2025).

- *Drug Content*

The drug content of the formulated diclofenac diethylamine gels was determined using a UV-visible spectrophotometric method to ensure formulation accuracy and compliance with pharmaceutical standards. A measured amount of 1 g of gel from the 10 g formulated gel was transferred into a 250 mL beaker and diluted with distilled water up to 80 mL. The beaker was placed in a water bath at 37°C and manually stirred every 5 minutes for 2 hours to ensure complete dissolution of the drug. The volume was then brought up to 100 mL with distilled water and homogenized. An aliquot (20 mL) of the solution was withdrawn and further diluted to 100 mL with distilled water. The absorbance of the test samples was measured at 224 nm. For the standard, 5 mg of diclofenac diethylamine was dissolved in 1,000 mL of distilled water, and the solution was placed in a water bath at 37°C for 2 hours, following the same procedure as the formulated gel. Two (2) mL of both the standard solution and the test sample were placed in a cuvette for absorbance measurement at 224 nm. The absorbance readings of the test samples were compared against a calibration curve prepared from known concentrations of diclofenac diethylamine within the detectable range of 0.05 to 200 µg/mL, following the Beer–Lambert law. The percentage concentration was calculated using the following formula:

Each formulation was analyzed in triplicate, and the mean ± standard deviation (SD) was reported. The acceptable drug content ranged from 85 to 115% of the labeled claim, confirming the uniformity and stability of the formulation. This procedure followed the method described by Kumari et al. (2022) and Sundari et al. (2025) and further evaluated whether the use of *D. esculentum* mucilage as a natural gelling agent affected drug distribution compared with synthetic Carbopol 940.

➢ *Data Analysis*

All evaluations of the diclofenac diethylamine gels, including pH, spreadability, homogeneity, viscosity, extrudability, and drug content, were conducted in triplicate to ensure accuracy and reproducibility. The collected raw data were compiled and submitted to a professional statistician for analysis and interpretation. The statistician computed the mean values and standard deviations (SD) and performed appropriate statistical tests, such as one-way ANOVA, to compare the performance of gels prepared with different concentrations of *D. esculentum* mucilage and the synthetic control, Carbopol 940. The study involved only one independent variable—the gelling agent concentration. Although the concentrations differed between Carbopol 940 and *D. esculentum* mucilage, they were considered levels under a single factor; thus, one-way ANOVA was appropriate. When significant differences were observed, Tukey's HSD was applied to determine which specific group means differed significantly. Statistical significance was set at $p < 0.05$. Graphical representations of the data were prepared using Microsoft Excel, IBM SPSS Statistics, or GraphPad Prism to visually summarize the comparative performance of the formulations.

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CHAPTER FOUR
RESULTS AND DISCUSSION

➢ *Extraction of Mucilage and Percentage Yield*

The percentage yield of mucilage extracted from *D. esculentum* was determined to evaluate the efficiency of the extraction process. The extraction was performed in triplicate using 30 g of powdered plant material, and the resulting mucilage was collected, dried, and weighed. The obtained yield reflected the amount of mucilage present in *D. esculentum* and indicated its potential as a natural gelling agent. A higher percentage yield suggested that the plant material was a good source of mucilage, making it suitable for pharmaceutical applications such as gel formulation. Variations in the yield were attributed to factors such as extraction temperature, duration of heating, and efficiency of precipitation using ethanol. Additionally, the natural composition of the plant and environmental conditions during growth also influenced the amount of mucilage obtained (Berhe et al. 2023). Figure 7 showed its appearance before drying, Figure 8 showed the dried mucilage, and Figure 9 showed the pulverized form of the mucilage.

Fig 7 Extracted Mucilage of *D. Esculentum*

Fig 8 Dried Mucilage of *D. Esculentum*

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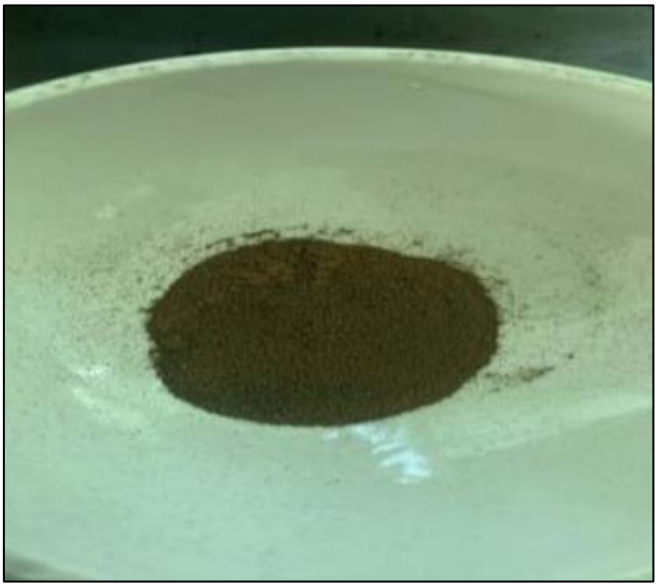

Fig 9 Pulverized Mucilage of *D. esculentum* Passed Through #120 Sieve

Table 14 presents the individual yields obtained from each trial, along with the average (mean) yield of pectin extracted from *D. esculentum*. The average percentage yield, calculated from the triplicate analysis of a 10-gram sample, is 13.86% ± 1.61%.

Table 14 Percentage Yield Using Triplicate Analysis of the Mucilage Extract of *D. esculentum* fronds.

Trial	**Weight of Dried *D. esculentum* Fronds**	**Weight of Dried *D. esculentum* Mucilage extracted**	**Percentage**
1	10g	4.65	15.50
2	10g	3.5	11.67
3	10g	4.32	14.40
Mean ± SD			13.86% ± 1.61%

➢ *Phytochemical Profiling of D. esculentum*

A phytochemical screening test was carried out at the MSU-Iligan Institute of Technology (MSU-IIT) by Miss Enjelyn Calleno Gomez, a licensed chemist from the institution, to identify the presence of specific phytochemical constituents in the sample. The analysis targeted the detection of alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids.

Table 15 Phytochemical Constituents Present in *D. esculentum* Fronds Extract

Phytochemical Test	**Visual Result**	**Interpretation**
Alkaloids (Dragendorff's Reagent Method)		The result of a reddish-brown color in the Wagner test for alkaloids indicates a positive result, confirming the presence of alkaloids in the sample.

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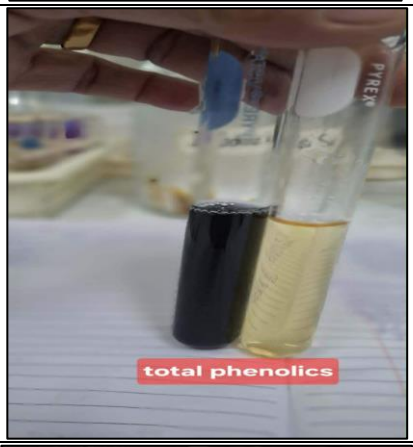
Flavonoids ($AlCl_3$ Colorimetric Assay)		A dark reddish color develops in the left test tube during the flavonoids test, indicating a positive result and confirming the presence of flavonoids in the sample.
Phenols (Ferric Chloride Test)		The development of a dark blue to black color in the left test tube during the phenolic compounds test shows a positive result, confirming the presence of total phenolics in the sample.
Saponins (Vanillin–Sulfuric Acid Assay)		A stable frothy layer, at least 1 cm thick, appears in the left test tube during the saponins test, indicating a positive result and confirming the presence of saponins in the sample.
Steroids (Salkowski Test)		The presence of an orange-yellow liquid with a dark reddish-brown layer concentrated at the bottom indicates a positive result, confirming the presence of steroid compounds in the sample.

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Tannins (Ferric Chloride Test)		The sample forms a dark blue-black solution with a thin layer at the top, indicating a positive reaction and confirming the presence of tannins in the sample.
Terpenoids (Salkowski Test)		The positive result obtained in the Terpenoids test, when a reddish-brown layer was formed in the left test tube, confirms the presence of terpenoids in the sample.

The findings of the phytochemical screening are provided below, outlining the specific tests conducted, the corresponding visual observations, and their respective interpretations.

The presence of these phytochemicals was consistent with findings in *D. esculentum*. Compounds such as flavonoids, phenolic compounds, saponins, tannins, and alkaloids were detected in *D. esculentum*. These compounds contributed to the properties of the mucilage, which was used in the formulation of gelling agents. Flavonoids and phenolic compounds helped maintain the gel's stability, while saponins improved its uniformity and foaming abilities. Alkaloids and tannins aided in stabilizing the gel's pH, enhancing its overall performance (Tongco et al. 2014). The presence of these phytochemicals suggested that *D. esculentum* mucilage could be utilized as a valuable material in the formulation of gelling agents. The table 16 summarizes the results of the phytochemical screening of the *D. esculentum* powder.

Table 16 Phytochemical Constituent Concentration Detected in *D. esculentum*

Phytochemical Constituent	**Result**
Alkaloids	Positive
Flavonoids	Positive
Total Phenolics	Positive
Saponins	Positive
Steroids	Positive
Tannins	Positive
Terpenoids	Positive

➢ *Compatibility Studies Analysis on FTIR Results*

Fourier Transform Infrared (FTIR) spectroscopy was employed to evaluate the compatibility between diclofenac diethylamine and *D. esculentum* mucilage was used in the gel formulations. This analysis aimed to identify the functional groups present and determine whether any chemical interaction occurred between the drug and excipients. The FTIR spectra were obtained for all formulations, including synthetic gels at 1%, 1.5%, and 2% concentrations and natural gels containing *D. esculentum* mucilage at 2.5%, 5%, and 7.5%.

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- *FTIR Spectra of Diclofenac Diethylamine*

The FTIR spectrum of diclofenac diethylamine was used as the reference spectrum for identifying the characteristic functional groups of the drug in the prepared gel formulations. Diclofenac diethylamine showed distinct absorption bands at 3381.57 cm^{-1}, 1572.66 cm^{-1}, and 745.35 cm^{-1}. The peak at 3381.57 cm^{-1} was attributed to N–H stretching vibration of the secondary amine group, confirming the presence of the amine structure in diclofenac diethylamine. The peak at 1572.66 cm^{-1} corresponded to the C=O stretching vibration of the carboxyl ion, indicating the carboxylate-related functional group of the drug. Meanwhile, the peak at 745.35 cm^{-1} was assigned to C–Cl stretching vibration, indicating the chlorine-containing aromatic structure of diclofenac diethylamine. These characteristic bands served as reference indicators in determining whether diclofenac diethylamine remained chemically identifiable after incorporation into the synthetic and natural gel formulations. In the compatibility assessment, the retention of these drug-related bands, together with the absence of major new peaks, disappearance of important characteristic peaks, or marked peak shifting, supported the compatibility of diclofenac diethylamine with the gel-forming components (Sengupta et al. 2015). Figure 10 below shows the FTIR spectrum of diclofenac diethylamine, while the major FTIR absorption bands and their corresponding functional group assignments are presented in Table 17.

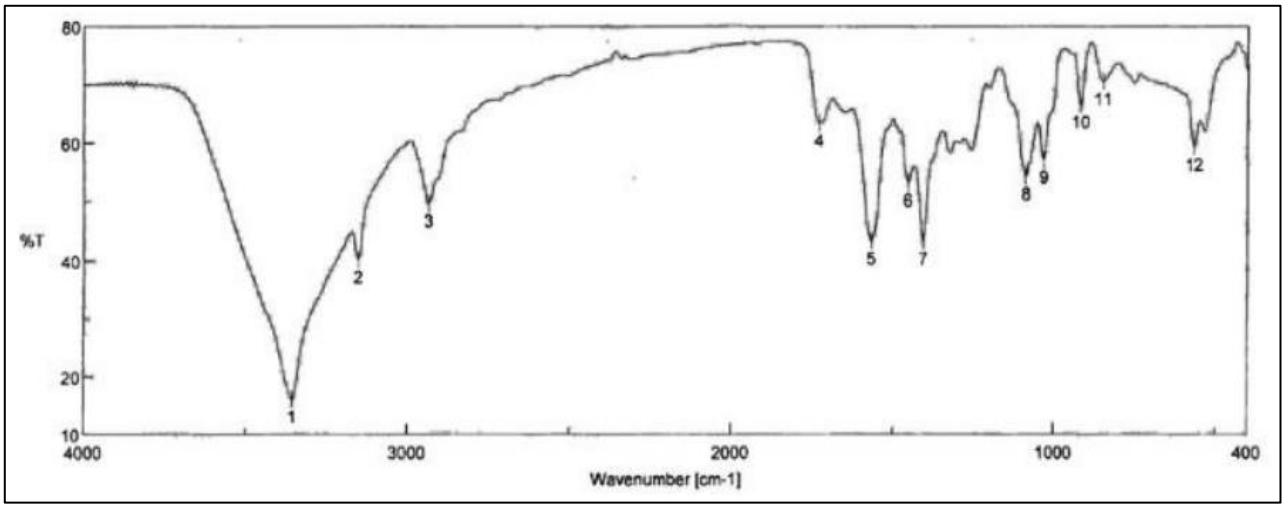

Fig 10 FTIR Spectrum of Diclofenac Diethylamine Gel (Sengupta et al. 2015)

Table 17 Interpretation of FTIR Spectra of Diclofenac diethylamine

Functional Group	Wave number (cm^{-1})	Interpretation in the Spectrum
N–H stretch	3381.57 cm^{-1}	Indicated the secondary amine group of diclofenac diethylamine (Sengupta et al. 2015)
C=O stretching of carboxyl ion	1572.66 cm^{-1}	Indicated the carboxylate-related functional group of diclofenac diethylamine (Sengupta et al. 2015)
C–Cl stretching	745.35 cm^{-1}	Indicated the chlorine-containing aromatic structure of diclofenac diethylamine (Sengupta et al. 2015)

- *FTIR Analysis of D. esculentum Mucilage Alone*

The FTIR analysis of *D. esculentum* mucilage revealed key absorption bands, providing valuable insights into its chemical composition as a potential gelling agent for pharmaceutical formulations. A broad O–H stretching vibration was observed around 3405.87 cm^{-1}, indicating the presence of hydroxyl (-OH) groups. These groups are commonly found in carbohydrates, polysaccharides, and mucilage-like substances, suggesting a strong hydrophilic nature that facilitates water binding and moisture retention. These properties are essential for the effectiveness of a gelling agent, particularly in the formation of hydrophilic gels used in pharmaceutical applications (Kumari et al. 2022). Additionally, a smaller absorption in the 2921.12 cm^{-1} range corresponded to C–H stretching vibrations, pointing to sugar units –CH and $–CH_2$ groups—typically present in polysaccharides. This further indicates that *D. esculentum* mucilage is rich in carbohydrate-based units, which are crucial for its gelling properties. A significant peak around 1701.30 cm^{-1} was observed, corresponding to C=O stretching vibrations, associated with uronic acids or other acidic sugar derivatives commonly found in plant-derived mucilage. This suggests that *D. esculentum* mucilage contains these acidic sugars, contributing to the structural backbone of its polysaccharide matrix, which enhances its ability to form gels in pharmaceutical formulations (Borade et al. 2022). Furthermore, sharp peaks in the fingerprint region (1006.44–1199.98 cm^{-1}) confirmed the presence of glycosidic linkages, a hallmark of polysaccharide structure. Peaks below 900 cm^{-1} likely represent β-glycosidic bonds, typical of complex polysaccharides, contributing to the cohesive network necessary for gel formation (Kumari et al. 2022). These features are crucial for ensuring that *D. esculentum* mucilage performs effectively as a gelling agent in pharmaceutical excipient applications. Figure 11 below shows the FTIR result of *D. esculentum* mucilage. Also, key FTIR results are summarized in Table 18

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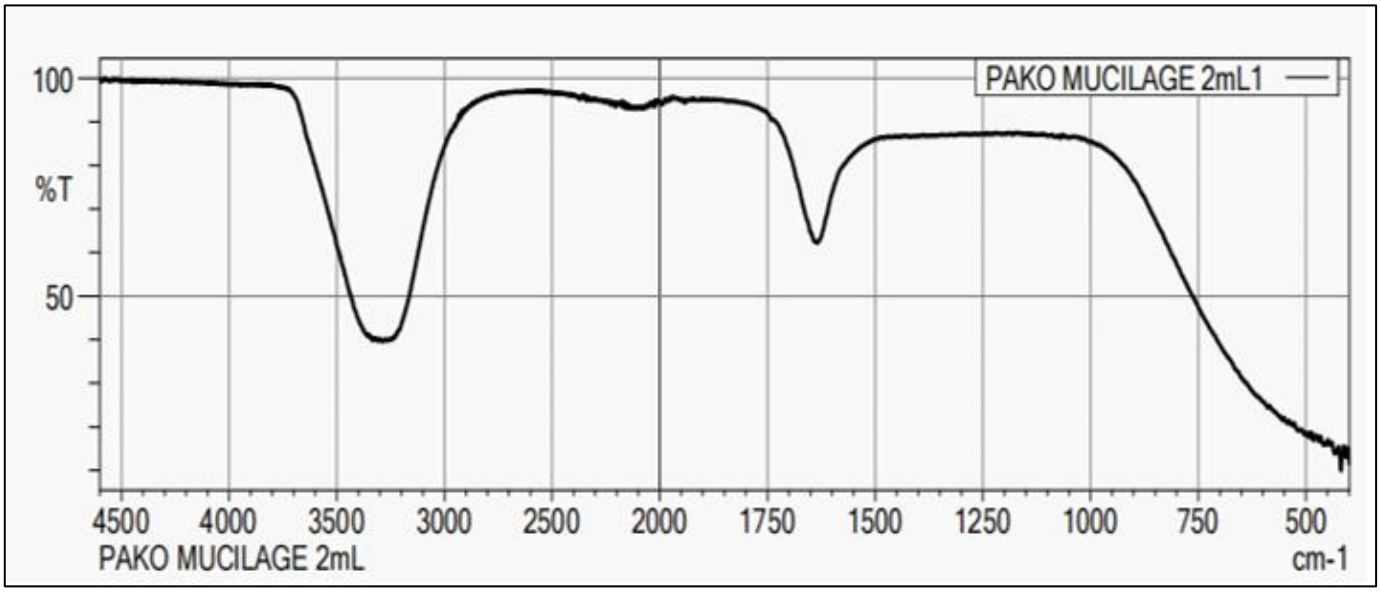

Fig 11 FTIR Spectra of *D. esculentum* Mucilage

Table 18 Interpretation of FTIR Spectra of *D. esculentum* mucilage

Functional Group	Wave number (cm^{-1})	Interpretation in the Spectrum
O–H Stretch	3405.87 cm^{-1}	Broad peak indicating the presence of hydroxyl (-OH) groups. These groups are commonly found in carbohydrates, polysaccharides, and mucilage-like substances, suggesting a strong hydrophilic nature that facilitates water binding and moisture retention, which are essential properties for an effective gelling agent (Kumari et al. 2022).
C–H Stretch	2921.12 cm^{-1}	Weak peak indicating the presence of sugar units (–CH and $–CH_2$ groups) that are typically found in polysaccharides. This suggests that *D. esculentum* mucilage is rich in carbohydrate-based units, essential for its gelling properties (Hong et al. 2021).
C=O Stretch	1701.30 cm^{-1}	Significant peak corresponding to C=O stretching vibrations, associated with uronic acids or other acidic sugar derivatives commonly found in plant-derived mucilage. This suggests that *D. esculentum* mucilage contains these acidic sugars, which contribute to the structural backbone of its polysaccharide matrix and enhance its ability to form gels in pharmaceutical formulations (Borade et al. 2022).
C–O–C Stretch	1006.44–1199.98 cm^{-1}	Multiple peaks confirming the presence of glycosidic linkages, a hallmark of polysaccharide structure. These are essential for the cohesive network required for gel formation, indicating that *D. esculentum* mucilage has a polysaccharide structure (Kumari et al. 2022).
Glycosidic Bonds	<900 cm^{-1}	Peaks corresponding to β-glycosidic bonds, typical of complex polysaccharides, confirming that *D. esculentum* mucilage contains the necessary structural components for gel formation (Hong et al. 2021).

- *FTIR Compatibility of Synthetic Gel Formulations with Diclofenac Diethylamine*

The FTIR analysis of the 1% to 2% synthetic gel formulations containing diclofenac diethylamine showed characteristic absorption bands that indicated the presence of major functional groups from the gel system and the incorporated drug. A broad peak at 3405.61 cm^{-1} was assigned to O–H stretching, indicating the hydrophilic nature of the gel, which is important for water interaction and gel formation (Kumari et al. 2022). The peak at 2920.45 cm^{-1} corresponded to C–H stretching from methyl and methylene groups, while the peak at 1700.92 cm^{-1} was attributed to C=O stretching of carbonyl-containing groups, suggesting the presence of carboxyl-related structures (Hong et al. 2021; Borade et al. 2022). Another peak at 1630.45 cm^{-1} was associated with C=C stretching or carboxylate anion (COO^-) bending, and the peak at 1050.23 cm^{-1} corresponded to C–O stretching, which may indicate ether linkages or glycosidic-type bonds in the gel structure (Kumari et al. 2022). The retention of these major peaks, together with the absence of major new peaks, disappearance of important bands, or marked peak shifting, suggested that no strong chemical interaction occurred between diclofenac diethylamine and the synthetic gel components. Therefore, the FTIR findings supported the compatibility of diclofenac diethylamine with the synthetic gel formulations (Sengupta et al. 2015). Figure 12 below shows the FTIR spectra of the synthetic gel formulations containing diclofenac diethylamine.

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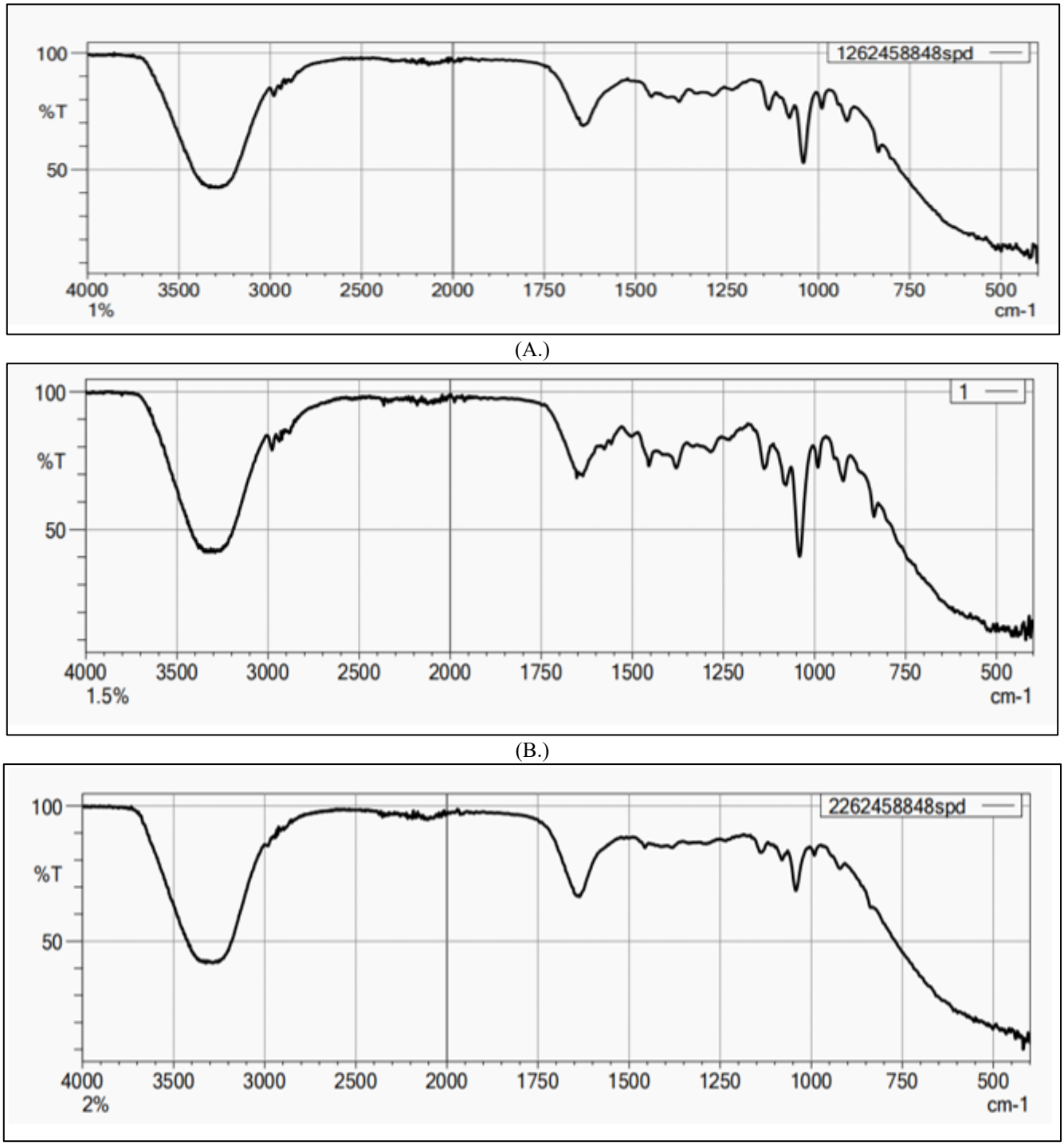

Fig 12 FTIR Spectra of Synthetic Gel Containing Diclofenac Diethylamine.
a.) 1% Synthetic gel containing the diclofenac diethylamine drug
b.) 1.5% Synthetic gel containing the diclofenac diethylamine drug
c.) 2% Synthetic gel containing diclofenac diethylamine drug

- *FTIR Compatibility of Natural Gel Formulations with Diclofenac Diethylamine*

The FTIR spectra of the natural gel formulations containing 2.5% to 7.5% *D. esculentum* mucilage and diclofenac diethylamine revealed similar key absorption peaks, indicating the presence of functional groups associated with the mucilage-based gel system and the incorporated drug. A broad peak at 3405.87 cm^{-1} was assigned to O–H stretching, suggesting the hydrophilic nature of *D. esculentum* mucilage, which is important for water retention and gelation properties (Kumari et al. 2022). The peak at 2921.12 cm^{-1} corresponded to C–H stretching from methyl and methylene groups in the sugar backbone of the mucilage, confirming its polysaccharide-rich structure (Hong et al. 2021). The peak at 1701.30 cm^{-1} was attributed to C=O stretching vibrations, which may be associated with acidic sugars or uronic acid groups that contribute to the structural integrity and gelling capacity of the mucilage

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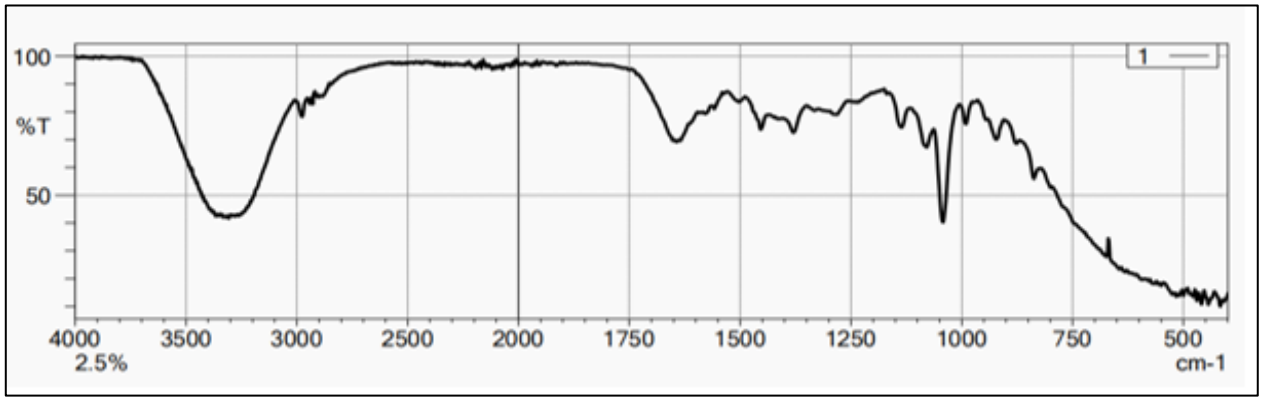
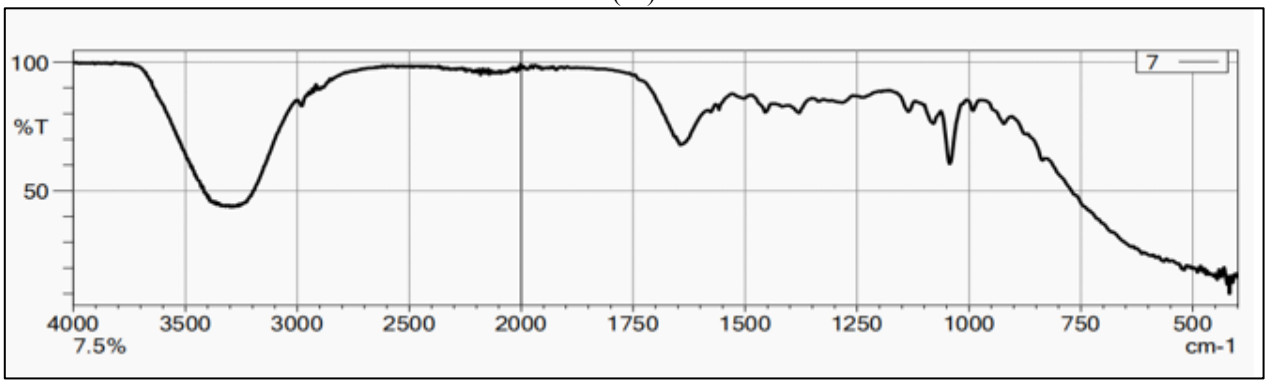
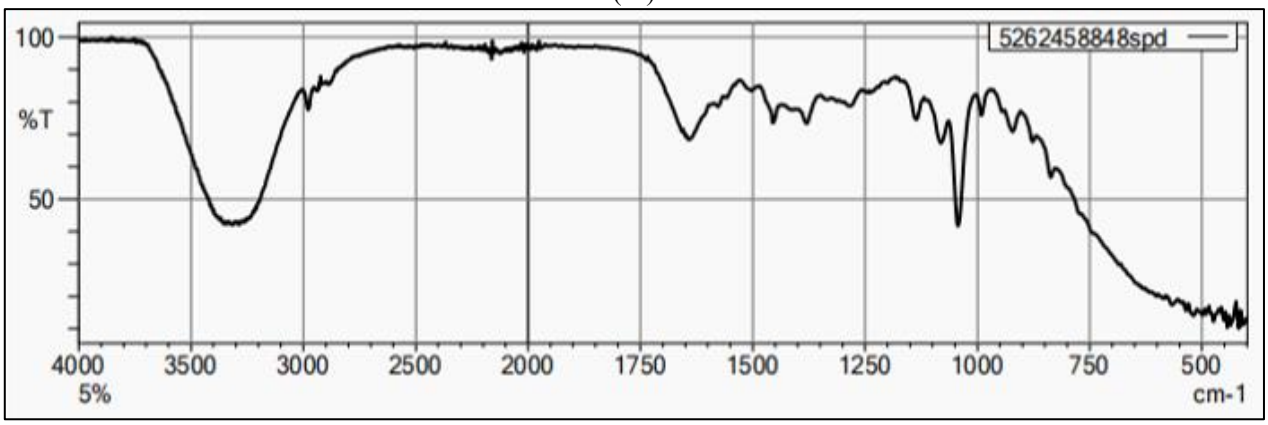
(Borade et al. 2022). The peak at 1630.45 cm^{-1} corresponded to C=C stretching or carboxylate anion bending vibrations, further supporting the presence of carboxyl-related functionalities, while the peak at 1050.23 cm^{-1} was assigned to C–O stretching, confirming glycosidic linkages and the polysaccharide structure needed for gel formation (Kumari et al. 2022). The retention of these major functional groups, together with the absence of major new peaks, disappearance of important bands, or marked peak shifting, suggested that no strong chemical interaction occurred between diclofenac diethylamine and *D. esculentum* mucilage. Therefore, the FTIR findings supported the compatibility of *D. esculentum* mucilage with diclofenac diethylamine in the natural gel formulation (Sengupta et al. 2015). Figure 13 below shows the FTIR spectra of the natural gel formulations containing *D. esculentum* mucilage and diclofenac diethylamine.



(A.)



(B.)



(C.)

Fig 13 FTIR Spectra of Natural Gel Containing Diclofenac Diethylamine.
a.) 2.5 % Natural gel containing diclofenac diethylamine drug
b.) 5% Natural gel containing diclofenac diethylamine drug
c.) 7.5% Natural gel containing diclofenac diethylamine drug

- *Comparative FTIR Interpretation of Diclofenac Diethylamine, D. esculentum Mucilage, and Gel Formulations*

The comparative FTIR results showed that the characteristic functional groups of diclofenac diethylamine, *D. esculentum* mucilage, and the prepared gel formulations were retained, supporting the compatibility of the drug with the gel-forming components. Diclofenac diethylamine showed drug-related peaks at 3381.57 cm^{-1}, 1572.66 cm^{-1}, and 745.35 cm^{-1}, corresponding to N–H stretching, C=O stretching of the carboxyl ion, and C–Cl stretching, respectively (Sengupta et al. 2016). Meanwhile, *D.*

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esculentum mucilage showed polysaccharide-related peaks, including O–H stretching at 3405.87 cm^{-1}, C–H stretching at 2921.12 cm^{-1}, C=O stretching at 1701.30 cm^{-1}, C–O–C stretching at 1006.44–1199.98 cm^{-1}, and glycosidic bond-related peaks below 900 cm^{-1}, which supported its hydrophilic and polysaccharide-based structure as a gelling agent (Kumari et al. 2022; Hong et al. 2021; Borade et al. 2022). In the formulated gels, the synthetic gel showed peaks at 3405.61 cm^{-1}, 2920.45 cm^{-1}, 1700.92 cm^{-1}, and 1005.10–1200.02 cm^{-1}, while the natural gel showed peaks at 3405.87 cm^{-1}, 2921.12 cm^{-1}, 1701.30 cm^{-1}, and 1006.44–1199.98 cm^{-1}. The presence of diclofenac diethylamine in the formulated gels was supported by the retention or representation of drug-related absorption regions, particularly the N–H/O–H region, carbonyl/carboxylate-related region, and fingerprint region associated with C–Cl vibration, together with the characteristic polysaccharide-related peaks of the gel-forming components. The close similarity and retention of these major absorption bands indicated that the functional groups of the drug and gel-forming components remained present after formulation. Since no major new peak formation, disappearance of important characteristic peaks, or significant shifting of characteristic peaks was observed, no strong chemical interaction occurred between diclofenac diethylamine and *D. esculentum* mucilage. Therefore, the FTIR comparison supported the compatibility of *D. esculentum* mucilage with diclofenac diethylamine and further supported its potential use as a natural gelling agent in the formulation (Sengupta et al. 2016; Kumari et al. 2022; Borade et al. 2022). The comparative FTIR peaks and their compatibility interpretations are presented in Table 19.

Table 19 Comparative FTIR Peaks of Diclofenac Diethylamine, *D. esculentum* Mucilage, Synthetic Gel, and Natural Gel Formulations

Functional Group	Diclofenac diethylamine	*D. esculentum* Mucilage Alone	Synthetic Gel with DDEA	Natural Gel with DDEA	Compatibility Interpretation
N–H stretch	3381.57 cm^{-1}	—	Present within the broad O–H/N–H region	Present within the broad O–H/N–H region	The drug-related amine region was represented in the gel spectra, supporting the presence of diclofenac diethylamine in the formulations (Sengupta et al. 2016).
O–H stretch	—	3405.87 cm^{-1}	3405.61 cm^{-1}	3405.87 cm^{-1}	The hydroxyl peak was retained, indicating that the hydrophilic gel and mucilage structure remained present after drug incorporation (Kumari et al. 2022).
C–H stretch	—	2921.12 cm^{-1}	2920.45 cm^{-1}	2921.12 cm^{-1}	The C–H peaks were retained, indicating the presence of methyl and methylene groups associated with organic and polysaccharide related structures (Hong et al. 2021).
C=O / carboxyl-related stretching	1572.66 cm^{-1}	1701.30 cm^{-1}	1700.92 cm^{-1}	1701.30 cm^{-1}	Carbonyl and carboxyl-related bands remained present, suggesting that important drug- and mucilage-related functional groups were not lost during formulation (Sengupta et al. 2016; Borade et al. 2022).
C–O–C stretching / glycosidic linkage	—	1006.44–1199.98 cm^{-1}	1005.10–1200.02 cm^{-1}	1006.44–1199.98 cm^{-1}	The polysaccharide fingerprint region was retained, confirming that the mucilage-based gelling structure remained intact (Kumari et al. 2022).

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C–Cl / fingerprint region vibration	745.35 cm^{-1}	<900 cm^{-1}	<900 cm^{-1}	<900 cm^{-1}	The fingerprint region remained represented in the formulation spectra, supporting the retention of drug-related structural characteristics and the absence of major incompatibility (Sengupta et al. 2016).
Overall compatibility	Reference drug spectrum	Reference mucilage spectrum	Compatible with DDEA	Compatible with DDEA	The retention of major absorption bands, together with the absence of major new peaks, peak disappearance, or significant peak shifting, supported the compatibility of diclofenac diethylamine with both synthetic and natural gel formulations.

- *Degree of Esterification of D. esculentum mucilage*

The Degree of Methyl Esterification (DME) of *D. esculentum mucilage* was calculated based on the FTIR spectra obtained for the synthetic and natural gel formulations. The absorbance values were derived from peaks at 1740 cm^{-1} (esterified C=O) and 1630 cm^{-1} (carboxyl group). The transmittance values at these peaks were found to be 87% for the esterified C=O and 83% for the carboxyl group.

Using the standard equation:

$$A = \log \frac{(100)}{T}$$

For the 1740 cm^{-1} peak, which corresponds to the esterified C=O group, the absorbance was calculated as:

$$A_{1740} = \log_{10} \frac{(100)}{87} \approx 0.060$$

For the 1630 cm^{-1} peak, corresponding to the carboxyl group, the absorbance was calculated as:

Substituting the absorbance values:

This DME of 60.2% classifies *D. esculentum* mucilage as high methyl esterification mucilage (HME). According to Sundari et al. (2025), a DME above 50% indicates high methyl esterification, suggesting that more than 50% of the carboxyl groups in the mucilage are methyl-esterified, a characteristic of high methyl esterification mucilages (HME). HME mucilages are known for forming strong gels and typically require higher sugar concentrations and low pH (typically between 2.8 and 3.5) for gelation. However, for topical gel formulations that have a pH range of 6.75–8, the high methyl esterification indicates that the mucilage will still form stable gels under slightly alkaline conditions, providing high gel strength and viscosity necessary for pharmaceutical applications (Hayati et al. 2018).

With a DME of 60.2%, *D. esculentum mucilage* is classified as high methyl esterification mucilage (HME), making it suitable for topical gel formulations that require strong gel formation and stability at slightly alkaline pH conditions. This result suggests that *D. esculentum* mucilage is a promising natural gelling agent for pharmaceutical formulations that need high viscosity and gelation under higher pH conditions (Hayati et al. 2018; Sundari et al. 2025).

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➢ *Identification Tests for Mucilage*

The identification of *D. esculentum* mucilage was carried out through a series of tests to confirm its carbohydrate composition and assess its properties, which were crucial for its potential applications as a gelling agent. According to the study by Kumari et al. (2022) on natural gums and mucilages as gelling agents in topical gel formulations, the Molisch test was a reliable method for detecting polysaccharides, which are a major component of mucilage. The Molisch test involved the addition of α-naphthol solution followed by concentrated sulfuric acid, resulting in the formation of a violet or purple ring at the interface, indicating the presence of carbohydrates. The results of the Molisch test for *D. esculentum* mucilage are shown in Table 20.

In addition to the Molisch test, the Loss on Drying test was conducted to determine the moisture content of the mucilage, an important factor for its stability and usability in gel formulations. The Loss on Drying results, presented in Table 21, showed an acceptable moisture loss, ensuring that the mucilage was properly dried for further use, consistent with the standards suggested by Kumari et al. (2022).

Furthermore, the swelling index was measured to assess the water absorption capacity of the mucilage, which was essential for its gelling properties. Table 22 showed favorable swelling index results, indicating that the mucilage had excellent water retention properties, which were critical for its use in gel formulations. These findings aligned with the work of Kumari et al. (2022), who emphasized that high water absorption was a desirable trait in gelling agents.

Table 20 Molisch Test for *D. esculentum* Mucilage

Trial	Test	Observation	Visual	Result
1	Mucilage + Molisch reagent (α-naphthol solution) + H_2SO_4 (Sulfuric Acid)	Formation of a violet/purple ring at the interface		Positive
2	Mucilage + Molisch reagent (α-naphthol solution) + H_2SO_4 (Sulfuric Acid)	Formation of a violet/purple ring at the interface		Positive
3	Mucilage + Molisch reagent (α-naphthol solution) + H_2SO_4 (Sulfuric Acid)	Formation of a violet/purple ring at the interface		Positive

The consistent positive results from the Molisch test confirm the presence of carbohydrates, which are a key component of mucilage, as noted by Kumari et al. (2022).

Table 21 Loss on Drying for *D. esculentum* mucilage

Trial	Initial weight (g)	Final weight (g)	Weight Loss (g)	% Loss on Drying	Result
1	1.00	0.91	0.09	9.00%	Acceptable
2	1.00	0.90	0.10	10.00%	Acceptable
3	1.00	0.92	0.08	8.00%	Acceptable
Mean ± SD				**9% ± 0.1528**	

As per Kumari et al. (2022), the loss on drying test is essential to ensure the stability of mucilage, and the results here fall within the acceptable moisture loss range for dried mucilage.

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Table 22 Swelling Index for *D. esculentum* mucilage

Trial	Initial Weight (g)	Final Weight (g)	Percentage Yield	Interpretation
1	1 g	3.20 g	220 %	Favorable
2	1 g	3.15 g	215 %	Favorable
3	1 g	3.25 g	225 %	Favorable
Mean ± SD			**223.33% ± 0.1528**	

The Swelling Index results were favorable, with an average swelling yield of 220%. According to Kumari et al. (2022), high swelling index values indicate a high capacity for water absorption, which is essential for mucilage to function effectively as a gelling agent. As shown in the tables, this preliminary test further verifies and confirms the presence of mucilage on *D. esculentum.*

➢ *Preparation of Diclofenac diethylamine gel*

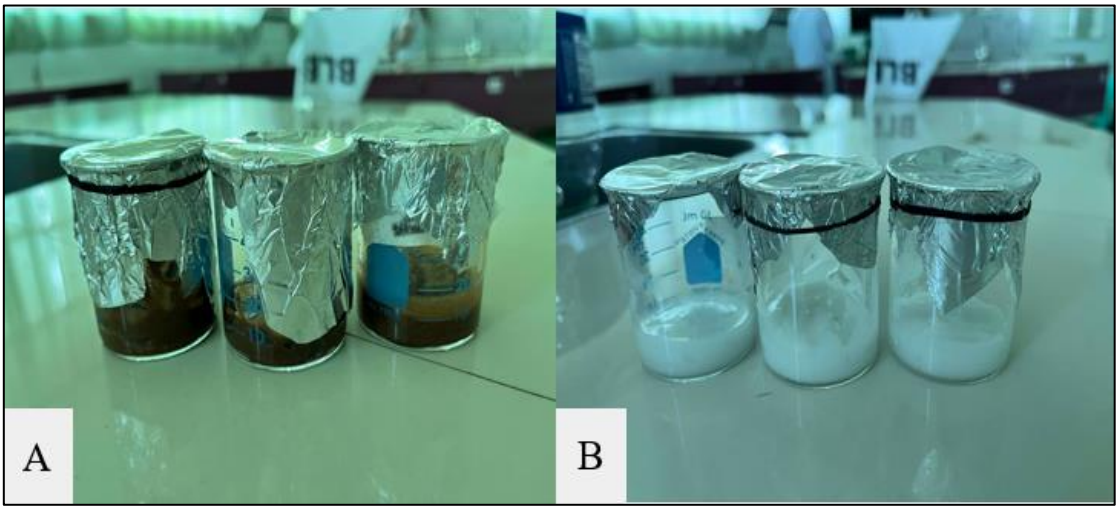

Fig 14 Physical Appearance of Diclofenac Diethylamine Using *D. esculentum* Mucilage
A.) Diclofenac diethylamine gel with *D. esculentum* as gelling agent
B.) Diclofenac diethylamine with synthetic gelling agent

The diclofenac diethylamine gels were successfully prepared using the fusion method, producing formulations with acceptable semisolid characteristics. Both the *D. esculentum* mucilage gels and Carbopol 940 gels formed uniform and stable preparations after mixing. The resulting gels showed smooth consistency, good appearance, and no visible phase separation immediately after preparation, indicating proper incorporation of the ingredients. Differences in texture and thickness were observed among formulations depending on the type and concentration of gelling agent used, with higher concentrations generally producing firmer and more viscous gels. These observations suggest that the extracted *D. esculentum* mucilage possessed suitable gel-forming ability and was capable of producing diclofenac diethylamine gels comparable to the synthetic control formulations. The successful preparation of all formulations provided the basis for further evaluation of their physical properties and overall performance as topical gels.

➢ *Evaluation and Characterization Tests Results on Formulated Gel*

The physical properties of topical gels are important in determining their quality, stability, ease of application, and overall performance. Parameters such as pH, spreadability, extrudability, homogeneity, viscosity, and drug content help assess whether the formulation is suitable for topical use and comparable to standard commercial preparations. In this study, gels prepared using *Diplazium esculentum* mucilage were evaluated alongside Carbopol 940 formulations to determine the effect of gelling agent type and concentration on these properties. Statistical analysis was then used to identify whether the observed differences among formulations were significant and to determine the potential of the natural mucilage as an alternative gelling agent.

- *Test for Evaluation*

Table 23 Assumptions test for One-Way ANOVA

Normal test-value (Shapiro-Wilk)			**Levene's test**	
Physical Properties	W	p-value	F	p-value
Absorption	0.977	0.919	1.67	0.216
Percentage	0.977	0.91	1.393	0.294
Viscosity	0.976	0.906	1.583	0.238
pH_Pmtr	0.924	0.151	0.565	0.726
Extrudability	0.874	0.021	0.284	0.913
Spreadability	0.819	0.003	6.580	0.004

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Note: p-value < 0.05 indicates a violation to normality or Equal of variances.

The assumptions of normality and homogeneity of variances were examined prior to conducting the one-way ANOVA. Results of the Shapiro–Wilk test indicated that all physical property variables, including absorption (W = 0.977, p = .919), percentage (W = 0.977, p = .910), viscosity (W = 0.976, p = .906), and pH parameter (W = 0.924, p = .151), were normally distributed, as all p-values exceeded the .05 threshold. Additionally, Levene's test showed that the assumption of homogeneity of variances was satisfied for absorption (F = 1.67, p = .216), percentage (F = 1.393, p = .294), viscosity (F = 1.583, p = .238), and pH parameter (F = 0.565, p = .726), with all p-values greater than .05. These results indicate that the assumptions for conducting a one-way ANOVA were met, specifically Fisher's.

The data were appropriate for one-way ANOVA. All Shapiro–Wilk p-values were above 0.05, showing that the results were normally distributed, and all Levene's test p-values were above 0.05, indicating equal variances among formulations. This confirms that the statistical comparisons are valid and dependable. These results are consistent with the methodology in Chapter 3 and support the main objective in Chapter 1 of comparing the physical properties of gels prepared with *D. esculentum* mucilage and Carbopol 940. Therefore, the succeeding ANOVA findings can be interpreted with confidence.

Table 24 One-Way ANOVA (Fisher's) Consolidated table

Physical Properties	F	df1	df2	p-value	Interpretation
Absorption	177.12	5	12	<.001	Sig.
Percentage	170.19	5	12	<.001	Sig.
Viscosity	1268.53	5	12	<.001	Sig.
pH_Pmtr	9.08	5	12	<.001	Sig.
Extrudability	12.7	5	5.59	<0.001	Sig.
Spreadability	16.4	5	(Kruskall-Wallis)	0.006	Sig.
Sig. at p-value <0.05					

A one-way ANOVA was conducted to determine whether there were significant differences in the physical properties of the gel formulations. The analysis revealed statistically significant differences across groups for all measured variables: absorption, $F(5, 12) = 177.12$, $p < .001$; percentage, $F(5, 12) = 170.19$, $p < .001$; viscosity, $F(5, 12) = 1268.53$, $p < .001$; and pH parameter, $F(5, 12) = 9.08$, $p < .001$. Since all p-values were less than the .05 significance level, the null hypothesis of no significant difference was rejected for each physical property. This indicates that at least one gel formulation differs significantly from the others in terms of absorption, percentage, viscosity, and pH, warranting further post hoc analysis to identify specific group differences.

The gel formulations differed significantly in absorption, percentage, viscosity, and pH, since all p-values were below 0.05. This means the type and concentration of the gelling agent affect the physical properties of the gels. The highest F-value was seen in viscosity, indicating that gel thickness was the most influenced parameter. These findings support the study objective in Chapter 1 to compare the performance of *D. esculentum* mucilage and Carbopol 940 formulations. It also agrees with the discussion in Chapter 2 that polymer source and concentration can greatly affect gel consistency, spreadability, and overall quality.

✓ *Drug Content Results and Discussion*

Drug content determination was performed to evaluate the amount of diclofenac diethylamine present in the different gel formulations. In topical gel formulations, drug content testing is important because it helps determine whether the active ingredient is properly distributed and whether the formulation meets acceptable pharmaceutical quality standards. Drug content may be measured using UV spectrophotometry by comparing the absorbance of the test sample with a standard solution, since absorbance is directly related to concentration based on the Beer-Lambert law (Mayerhöfer et al., 2020). This method also helps evaluate whether natural excipients, such as *D. esculentum* mucilage affects drug distribution compared with synthetic gelling agents such as Carbopol 940 (Sundari et al., 2025; Singh & Dabre, 2020). The formula followed was:

$$\% \text{ Concentration} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard sample}} \text{ x100}$$

Where absorbance of test sample refers to the absorbance reading obtained from each gel formulation containing diclofenac diethylamine. Absorbance of a standard sample refers to the absorbance reading of the prepared standard diclofenac diethylamine solution. This formula was used to determine the percentage concentration or drug content percentage of each gel formulation by comparing the absorbance of the test sample with the absorbance of the standard sample. Figure 15 presents the drug content percentage values of the different gel formulations after applying the formula. Based on the bar graph, the synthetic gel formulations showed higher drug content percentage values compared with the natural gel formulations. Among all formulations, SynthGel_F2 (1.5%) showed the highest drug content percentage of 109.22%, followed by SynthGel_F3 (2%) with 106.93% and SynthGel_F1

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(1%) with 102.73%. For the natural gel formulations, NtrlGel_F3 (7.5%) showed the highest drug content percentage of 100.49%, followed by NtrlGel_F2 (5%) with 99.78%, while NtrlGel_F1 (2.5%) showed the lowest value of 97.78%. This indicates that increasing the concentration of *D. esculentum* mucilage improved the drug content percentage of the natural gel formulations. All formulated gels fell within the acceptable drug content range of 85%–115%, indicating that the formulations contained an acceptable amount of diclofenac diethylamine based on the required drug content range for pharmaceutical preparations (Sundari et al. 2025; Singh and Dabre 2020). This indicated that increasing the concentration of *D. esculentum* mucilage improved the drug content percentage of the natural gel formulations while still maintaining values within the acceptable range.

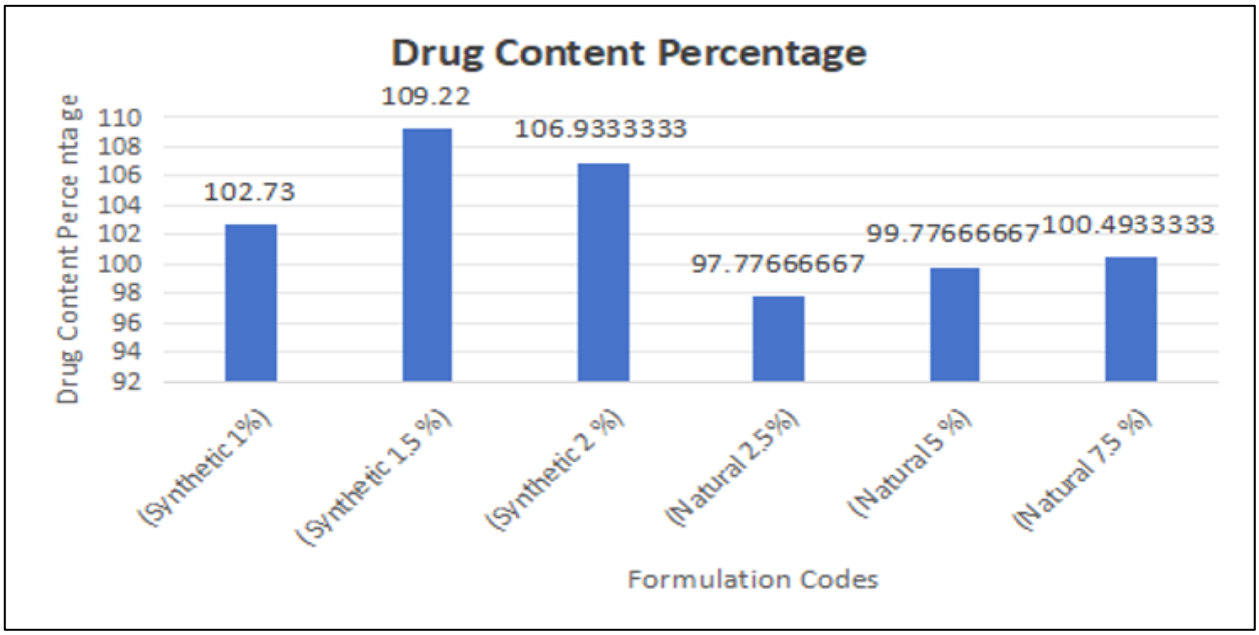

Fig 15 Bar Graph showing the Drug Content Percentage Values of the Different Gel Formulations.

Table 25 Post-Hoc Test (Tukey) for Drug Percentage

Treat A	Treat B	Mean Difference	p-value	Interpretation
NtrlGel_F1 (2.5%)	NtrlGel_F2(5%)	-2	0.013	Sig.
	NtrlGel_F3(7.5%)	-2.717	0.001	Sig.
	SynthGel_F1(1%)	-4.95	<.001	Sig.
	SynthGel_F2 (1.5%)	-11.44	<.001	Sig.
	SynthGel_F3(2%)	-9.16	<.001	Sig.
NtrlGel_F2 (5%)	NtrlGel_F3(7.5%)	-0.717	0.675	Not Sig.
	SynthGel_F1 (1%)	-2.95	<.001	Sig.
	SynthGel_F2 (1.5%)	-9.44	<.001	Sig.
	SynthGel_F3 (2%)	-7.16	<.001	Sig.
NtrlGel_F3 (7.5%)	SynthGel_F1(1%)	-2.24	0.006	Sig.
	SynthGel_F2 (1.5%)	-8.73	<.001	Sig.
	SynthGel_F3(2%)	-6.44	<.001	Sig.
SynthGel_F1 (1%)	SynthGel_F2 (1.5%)	-6.49	<.001	Sig.
	SynthGel_F3(2%)	-4.2	<.001	Sig.
SynthGel_F2 (1.5%)	SynthGel_F3(2%)	2.29	0.005	Sig.

A Tukey post hoc analysis was performed to identify pairwise differences in drug percentage among the gel formulations. The results revealed that NtrlGel_F1 (2.5%) had significantly lower percentage values compared to NtrlGel_F2 (5%) (MD = −2.00, p = .013), NtrlGel_F3 (7.5%) (MD = −2.717, p = .001), and all synthetic gel formulations (p < .001). Similarly, NtrlGel_F2 (5%) showed significantly lower percentage values than all synthetic formulations (p < .001), while no significant difference was found between NtrlGel_F2 (5%) and NtrlGel_F3 (7.5%) (MD = −0.717, p = .675). This indicated that the 5% and 7.5% natural gel formulations were statistically comparable in terms of drug percentage. Furthermore, NtrlGel_F3 (7.5%) also differed significantly from all

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synthetic gel formulations, although the difference with SynthGel_F1 (1%) was comparatively smaller (MD = −2.24, p = .006). Among the synthetic formulations, all pairwise comparisons were statistically significant, with SynthGel_F2 (1.5%) generally exhibiting the highest percentage values, followed by SynthGel_F3 (2%) and SynthGel_F1 (1%). Overall, the findings indicated significant differences in drug percentage values among most gel formulations. The synthetic gel formulations generally demonstrated higher drug percentage values than the natural mucilage gel formulations. Increasing the concentration of *D. esculentum* mucilage improved the drug percentage of the natural gels, although the 5% and 7.5% formulations were already statistically comparable. These results suggested that both the type and concentration of gelling agent affected formulation performance. The findings supported the objective of the study in comparing *D. esculentum* mucilage with Carbopol 940 and agreed with related studies stating that natural mucilages can improve gel properties, although synthetic polymers often provide more consistent formulation performance (Kumari et al. 2022; Sundari et al. 2025).

✓ *Viscosity Results and Discussion*

Viscosity testing was performed to evaluate the thickness and flow behavior of the different gel formulations. Viscosity was considered an important parameter in topical gel formulations because it affected the consistency, spreadability, stability, and ease of application of the product. In gel systems, increasing the concentration of gelling agents generally increased viscosity due to greater polymer interaction and formation of a stronger gel network (Kumari et al. 2022; Sundari et al. 2025).

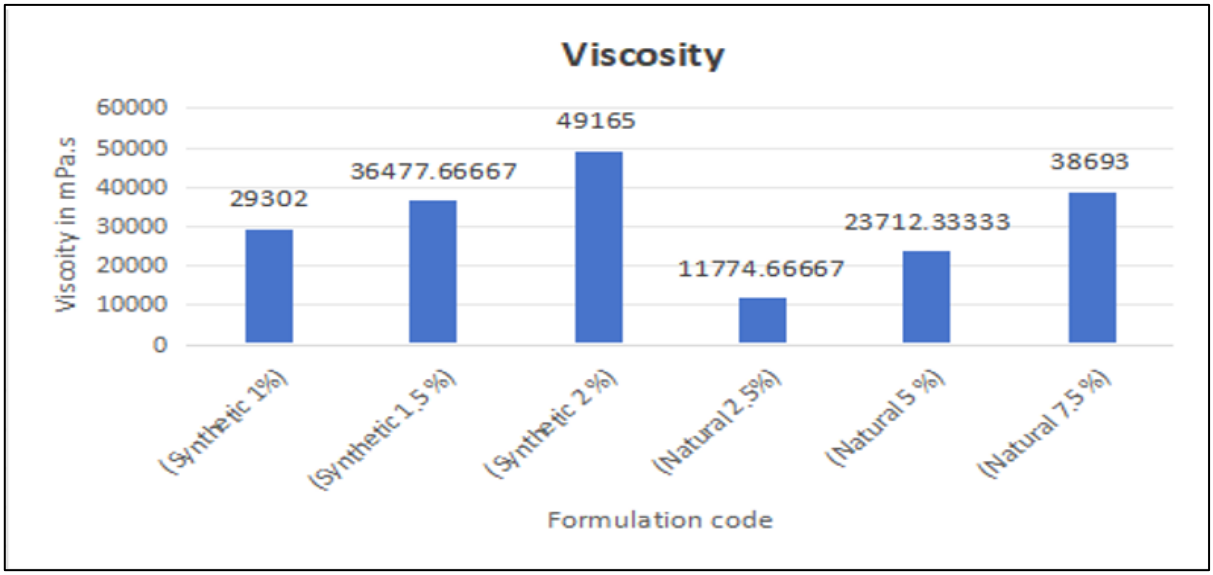

Fig 16 Bar Graph Showing the Viscosity Values of the Different Gel Formulations.

Figure 16 presented the viscosity values of the different gel formulations. Among the natural gel formulations, NtrlGel_F1 (2.5%) showed the lowest mean viscosity of 11,775 mPa.s, followed by NtrlGel_F2 (5%) with 23,712 mPa.s, while NtrlGel_F3 (7.5%) showed the highest viscosity among the natural gels with 38,693 mPa.s. For the synthetic gel formulations, SynthGel_F1 (1%) had 29,302 mPa.s, followed by SynthGel_F2 (1.5%) with 36,478 mPa.s, while SynthGel_F3 (2%) showed the highest viscosity overall with 49,165 mPa.s. The ideal viscosity range for gel formulation was reported to be 35,000–45,000 cP (Haryanti et al. 2025). Based on this range, NtrlGel_F3 (7.5%) and SynthGel_F2 (1.5%) were within the ideal viscosity range, indicating that these formulations had acceptable thickness and consistency. Meanwhile, SynthGel_F3 (2%) exceeded the ideal range, suggesting a thicker gel consistency, while the lower-concentration natural gels did not reach the ideal viscosity range. The results also showed that the 7.5% natural gel formulation produced viscosity values comparable to the synthetic gel formulations, particularly SynthGel_F2 (1.5%). In fact, NtrlGel_F3 (7.5%) showed higher viscosity than SynthGel_F1 (1%), indicating that increasing the concentration of *D. esculentum* mucilage improved gel thickness and consistency. This suggested that *D. esculentum* mucilage had the potential to provide viscosity performance comparable to Carbopol 940 when used at higher concentrations.

Table 26 Post-hoc test (Tukey) for Viscosity

Treat A	Treat B	Mean Difference	p-value	Interpretation
NtrlGel_F1 (2.5%)	NtrlGel_F2(5%)	-11938	<.001	Sig.
	NtrlGel_F3(7.5%)	-26918	<.001	Sig.
	SynthGel_F1(1%)	-17527	<.001	Sig.
	SynthGel_F2 (1.5%)	-24524	<.001	Sig.
	SynthGel_F3(2%)	-37390	<.001	Sig.
NtrlGel_F2 (5%)	NtrlGel_F3(7.5%)	-14981	<.001	Sig.
	SynthGel_F1(1%)	-5590	<.001	Sig.

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	SynthGel_F2 (1.5%)	-12586	<.001	Sig.
	SynthGel_F3(2%)	-25453	<.001	Sig.
NtrlGel_F3 (7.5%)	SynthGel_F1(1%)	9391	<.001	Sig.
	SynthGel_F2 (1.5%)	2394	0.006	Sig.
	SynthGel_F3(2%)	-10472	<.001	Sig.
SynthGel_F1 (1%)	SynthGel_F2 (1.5%)	-6997	<.001	Sig.
	SynthGel_F3(2%)	-19863	<.001	Sig.
SynthGel_F2 (1.5%)	SynthGel_F3(2%)	-12866	<.001	Sig.

The Tukey post hoc analysis revealed that all pairwise comparisons in viscosity were statistically significant ($p < .05$), indicating that each gel formulation differed from one another. NtrlGel_F1 (2.5%) had the lowest viscosity, followed by NtrlGel_F2 (5%), and both were significantly lower than the other formulations. Within the natural gel formulations, viscosity significantly increased as the concentration of *D. esculentum* mucilage increased, with NtrlGel_F3 (7.5%) showing significantly higher viscosity than the 2.5% and 5% formulations. It was also observed that NtrlGel_F3 (7.5%) had significantly higher viscosity than SynthGel_F1 (1%) and SynthGel_F2 (1.5%), but remained significantly lower than SynthGel_F3 (2%). This indicated that the 7.5% natural gel produced viscosity values comparable to some synthetic gel formulations, but the 2% synthetic gel still produced the highest viscosity overall. Among the synthetic gels, viscosity also increased as the Carbopol 940 concentration increased, with SynthGel_F3 (2%) showing the highest viscosity, followed by SynthGel_F2 (1.5%) and SynthGel_F1 (1%). Overall, the findings indicated that higher concentrations of both natural mucilage and synthetic gelling agent produced thicker gels. These results suggested that concentration greatly affected gel thickness and consistency and supported the objective stated in Chapter 1, which aimed to compare the formulation performance of *D. esculentum* mucilage and Carbopol 940 as gelling agents. The findings also agreed with related studies stating that polysaccharide-rich mucilages improved gel viscosity and stability through their water-binding and swelling properties (Kumari et al. 2022; Sundari et al. 2025).

✓ *pH Results and Discussion*

pH determination was performed to evaluate the acidity or alkalinity of the different gel formulations. This parameter was important because topical gels should have a pH that was suitable for skin application and should not cause irritation. The pH of a gel formulation may be affected by the type and concentration of the gelling agent and other formulation components. Related studies reported that plant-based mucilages and polymers could influence the pH and overall properties of gel formulations while maintaining suitability for topical use (Kumari et al. 2022; Sundari et al. 2025).

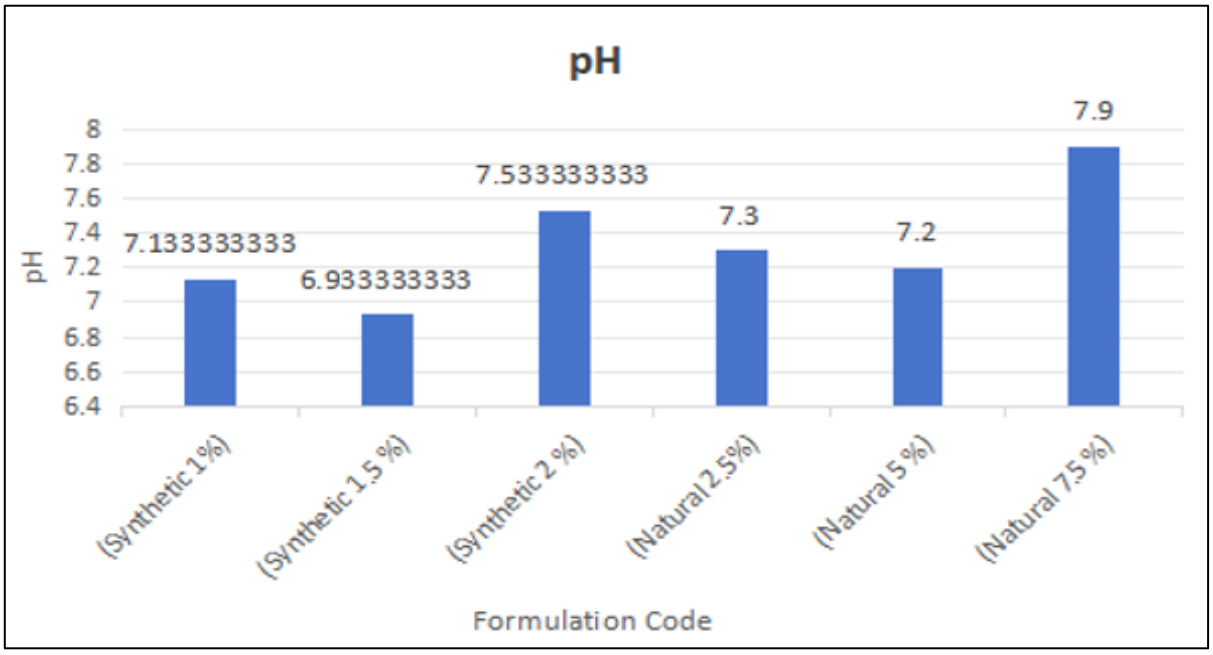

Fig 17 Bar Graph Showing the pH Values of the Different Gel Formulation

Figure 17 presented the pH values of the different gel formulations. Among the natural gel formulations, NtrlGel_F1 (2.5%) showed a mean pH value of 7.3, followed by NtrlGel_F2 (5%) with 7.2, while NtrlGel_F3 (7.5%) showed the highest mean pH value among the natural gels with 7.9. For the synthetic gel formulations, SynthGel_F1 (1%) showed a mean pH value of 7.1, followed by SynthGel_F2 (1.5%) with 6.9, while SynthGel_F3 (2%) showed the highest mean pH value among the synthetic gels

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with 7.5. The ideal pH range for topical gel formulations was reported to be 6.75–8.0, indicating suitability for topical application and reduced risk of skin irritation (Sundari et al. 2025). Based on this range, all formulated gels were within the acceptable pH range, suggesting that both the natural and synthetic gel formulations were suitable for topical use. However, the 7.5% natural gel showed the highest pH value among the formulations, indicating that increasing the concentration of *D. esculentum* mucilage slightly increased the pH of the formulation.

Table 27 Post-hoc test (Tukey) pH Parameter

Treat A	Treat B	Mean Difference	p-value	Interpretation
NtrlGel_F1 (2.5%)	NtrlGel_F2(5%)	0.1	0.987	Not Sig.
	NtrlGel_F3 (7.5%)	-0.6	0.026	Sig.
	SynthGel_F1 (1%)	0.1667	0.894	Not Sig.
	SynthGel_F2(1.5%)	0.367	0.268	Not Sig.
	SynthGel_F3 (2%)	-0.233	0.694	Not Sig.
NtrlGel_F2 (5%)	NtrlGel_F3 (7.5%)	-0.7	0.009	Sig,
	SynthGel_F1 (1%)	0.0667	0.998	Not Sig.
	SynthGel_F2(1.5%)	0.267	0.574	Not Sig.
	SynthGel_F3 (2%)	-0.333	0.355	Not Sig.
NtrlGel_F3 (7.5%)	SynthGel_F1 (1%)	0.7667	0.005	Sig.
	SynthGel_F2 (1.5%)	0.967	<.001	Sig.
	SynthGel_F3 (2%)	0.367	0.268	Not Sig.
SynthGel_F1 (1%)	SynthGel_F2(1.5%)	0.2	0.804	Not Sig.
	SynthGel_F3(2%)	-0.4	0.198	Not Sig.
	SynthGel_F3(2%)	-0.6	0.026	Sig.
	SynthGel_F3(2%)	-0.6	0.026	Sig.

A Tukey post hoc analysis was performed to identify pairwise differences in pH among the gel formulations. The results showed a combination of significant and non-significant differences. No significant difference was observed between NtrlGel_F1 (2.5%) and NtrlGel_F2 (5%) (MD = 0.10, p = .987), indicating that these two natural gel formulations had comparable pH values. NtrlGel_F1 (2.5%) and NtrlGel_F2 (5%) also showed no significant differences when compared with most synthetic gel formulations ($p > .05$), suggesting that their pH values were generally comparable with the synthetic gels. However, NtrlGel_F3 (7.5%) showed significant differences compared with NtrlGel_F1 (2.5%) (MD = −0.60, p = .026) and NtrlGel_F2 (5%) (MD = −0.70, p = .009). This indicated that the higher concentration of *D. esculentum* mucilage produced a distinct pH response compared with the lower natural mucilage concentrations. NtrlGel_F3 (7.5%) also showed significant differences when compared with SynthGel_F1 (1%) (MD = 0.767, p = .005) and SynthGel_F2 (1.5%) (MD = 0.967, $p < .001$), but it did not significantly differ from SynthGel_F3 (2%) (MD = 0.367, p = .268). Among the synthetic gel formulations, most comparisons were not statistically significant, except for the comparison between SynthGel_F1 (1%) and SynthGel_F3 (2%) (MD = −0.60, p = .026). This indicated that the pH values of most synthetic gel formulations were generally comparable, although the higher Carbopol 940 concentration showed a significant difference from the lowest concentration. Overall, the findings indicated that most gel formulations had comparable pH values, as many comparisons were not statistically significant. Significant differences were mainly observed in the 7.5% natural gel, suggesting that increasing mucilage concentration slightly affected the pH of the formulation. These findings addressed the objective stated in Chapter 1, which aimed to evaluate pH as one of the important quality parameters of the gel formulations. The results also agreed with related studies stating that plant mucilage components may influence gel pH and other formulation properties while still supporting their potential use in topical preparations (Kumari et al. 2022; Sundari et al. 2025).

✓ *Extrudability Results and Discussion*

Extrudability testing was performed to evaluate the ability of the gel formulations to be expelled from the container with minimal force. This parameter was important because it affected the ease of dispensing, convenience of application, and user acceptability of topical gels. Related studies reported that plant mucilages may improve semisolid formulation properties due to their hydration, swelling, and flow characteristics (Kumari et al. 2022; Sundari et al. 2025). The extrudability percentage was calculated using the following formula:

Where mass of the gel extruded referred to the amount of gel successfully expelled from the container, the total mass of the gel referred to the initial total amount of gel placed in the container. This formula was used to determine how easily each gel formulation could be extruded or dispensed.

$$\text{Extrudability \%} = \frac{\text{Mass of the gel Extruded}}{\text{Total Mass of the gel}} \text{ X } 100$$

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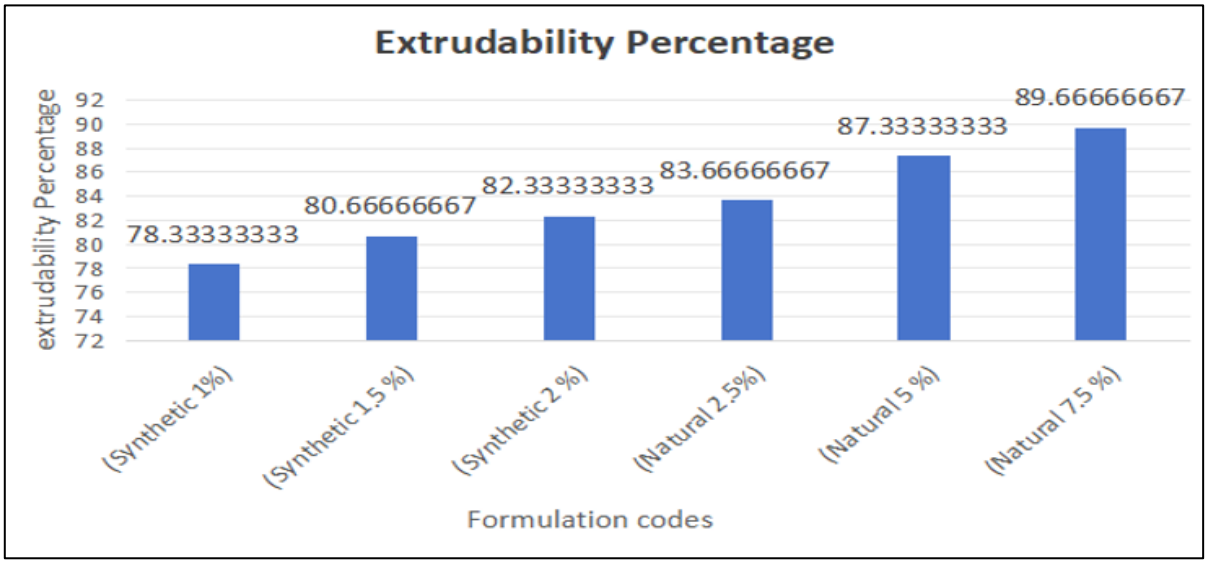

Fig 18 Bar Graph Showing the Extrudability Values of the Different Gel Formulations

Table 28 Post-Hoc Test for Extrudability

Treat A	Treat B	Mean Difference	p-value	Interpretation
Natural 2.5%	Natural 5%	-0.367	0.173	Not Sig.
	Natural 7.5%	-0.6	0.011	Sig.
	Synth 1%	0.533	0.025	Sig.
	Synth 2%	0.133	0.927	Not Sig.
	Synth1.5%	0.3	0.339	Not Sig.
Natural 5%	Natural 7.5%	-0.233	0.585	Not Sig.
	Synth 1%	0.9	<.001	Sig.
	Synth 2%	0.5	0.037	Sig.
	Synth1.5%	0.667	0.005	Sig.
Natural 7.5%	Synth 1%	1.133	<.001	Sig.
	Synth 2%	0.733	0.002	Sig.
	Synth1.5%	0.9	<.001	Sig.
Synth 1%	Synth 2%	-0.4	0.119	Not Sig.
	Synth1.5%	-0.233	0.585	Not Sig.
Synth 2%	Synth1.5%	0.167	0.839	Not Sig.

The Tukey post hoc analysis indicated both significant and non-significant differences in extrudability across gel formulations. Within the natural gels, no significant difference was observed between 2.5% and 5% (MD = −0.367, p = .173) or between 5% and 7.5% (MD = −0.233, p = .585), while a significant difference emerged between 2.5% and 7.5% (MD = −0.600, p = .011), suggesting improved extrudability at higher concentrations. Comparisons between natural and synthetic formulations showed that Natural 2.5% differed significantly only from Synth 1% (MD = 0.533, p = .025) but not from Synth 2% or Synth 1.5% (p > .05). In contrast, natural 5% and natural 7.5% exhibited significantly higher extrudability than all synthetic formulations (p < .05), highlighting a stronger performance at increased natural gel concentrations. Among the synthetic gels, no significant differences were found (p > .05), indicating relatively comparable extrudability within this group. Overall, the results suggest that extrudability tends to improve with increasing concentration in natural gels, while synthetic formulations display more uniform behavior across concentrations.

The extrudability of the gel formulations varied depending on the type and concentration of the gelling agent. Higher concentrations of the natural mucilage, especially 5% and 7.5%, showed significantly better extrudability than most synthetic gels, meaning the product can be pressed out of the tube more easily and applied more conveniently. This suggests that *D. esculentum* mucilage can produce gels with good user-friendly consistency. These findings support Chapter 1, which aimed to determine if the natural gel can perform comparably to Carbopol 940, and agree with Chapter 2, stating that plant mucilages have good hydration, swelling, and flow properties that improve semisolid formulations. Based on the results, the natural mucilage gel, particularly at higher concentrations, demonstrated promising performance as a topical gelling agent.

✓ *Spreadability Results and Discussion*

Spreadability testing was performed to assess how easily the gel spread during application. This parameter was important because good spreadability allowed easier skin application and more uniform drug distribution. Plant mucilages may improve gel texture and application properties due to their swelling and water-binding characteristics (Kumari et al. 2022; Sundari et al. 2025).

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Table 29 Dwass-Steel-Critchlow-Fligner Pairwise Comparison for Spreadability

Treatment A	Treatment B	W	p	Interpretation
Natural 2.5%	Natural 5%	56.4	0.013	Sig.
	Natural 7.5%	80.6	<.001	Sig.
	Synth 1%	-63.5	0.005	Sig.
	Synth 2%	71.81	0.002	Sig.
	Synth1.5%	48.25	0.035	Sig.
Natural 5%	Natural 7.5%	24.2	0.506	No Sig.
	Synth 1%	-120	<.001	Sig.
	Synth 2%	15.36	0.858	No Sig.
	Synth1.5%	-8.2	0.989	No Sig.
Natural 7.5%	Synth 1%	-144.2	<.001	Sig.
	Synth 2%	-8.84	0.984	No Sig.
	Synth1.5%	-32.4	0.232	No Sig.
Synth 1%	Synth 2%	135.33	<.001	Sig.
	Synth1.5%	111.77	<.001	Sig.
Synth 2%	Synth1.5%	-23.56	0.533	No Sig.

The DSCF pairwise comparison revealed several significant differences in spreadability among the gel formulations. Within the natural gels, Natural 2.5% showed significantly lower spreadability compared to both Natural 5% ($W = 56.40$, $p = .013$) and Natural 7.5% ($W = 80.60$, $p < .001$), while no significant difference was found between Natural 5% and Natural 7.5% ($W = 24.20$, $p = .506$). When comparing natural and synthetic formulations, Natural 2.5% differed significantly from all synthetic gels ($p < .05$), whereas Natural 5% and Natural 7.5% showed significant differences only when compared with Synth 1% ($p < .001$) but not with Synth 2% or Synth 1.5% ($p > .05$). Among the synthetic formulations, Synth 1% exhibited significantly lower spreadability than both Synth 2% ($W = 135.33$, $p < .001$) and Synth 1.5% ($W = 111.77$, $p < .001$), while no significant difference was observed between Synth 2% and Synth 1.5% ($W = -23.56$, $p = .533$). Overall, the findings indicate that spreadability generally improves with increasing concentration in natural gels, while synthetic formulations show variability, with Synth 1% performing lower than the other synthetic concentrations.

Spreadability differed among the gel formulations. The 2.5% natural gel had lower spreadability, while the 5% and 7.5% natural gels improved and became comparable to some synthetic formulations. This means increasing the concentration of *D. esculentum* mucilage enhances the ease of spreading on the skin. These findings support Chapter 1, which included spreadability as an important quality parameter, and agree with Chapter 2 that plant mucilage can improve gel texture, hydration, and application properties. The results suggest that higher concentrations of the natural mucilage can provide acceptable spreadability for topical use.

- *Organoleptic Properties (Homogeneity)*

Organoleptic properties refer to the sensory characteristics of the Diclofenac Diethylamine gel formulated with *D. esculentum mucilage* and the Carbopol 940 gel. These are evaluated to determine the acceptability, appearance, and overall quality of the formulations.

Table 30 Property of Homogeneity Results

Synthetic gel	Trial 1	Trial 2	Trial 3
F1 (1 %)	Uniform color, no signs of phase separation or aggregation, homogeneous texture.	Uniform color, no phase separation, smooth and consistent gel.	Uniform color, no phase separation, smooth and consistent gel.
F2 (1.5%)	Uniform color, no phase separation, consistent texture after centrifugation.	Uniform color, no phase separation, smooth and consistent gel.	Uniform color, no phase separation, smooth and consistent gel.
F3 (2 %)	Uniform color, no signs of phase separation or aggregation, homogeneous texture	Uniform color, no phase separation, consistent texture after centrifugation.	Uniform color, no phase separation, consistent texture after centrifugation.
Natural	**Trial 1**	**Trial 2**	**Trial 3**
F1 (2.5 %)	Slight aggregation was observed, uniform consistency after centrifugation.	No significant aggregation, smooth and consistent gel	No signs of phase separation or aggregation; homogeneous texture.
F2 (5%)	No visible phase separation; slight aggregation observed.	Slight aggregation, smooth and consistent gel after centrifugation.	Slight aggregation, smooth and consistent gel after centrifugation.

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F3 (7.5%)	No visible phase separation and uniform consistency after centrifugation.	No significant aggregation; smooth and consistent gel.	No signs of phase separation or aggregation; homogeneous texture.

- *Standard for D. esculentum and Synthetic mucilage gel*

Determine whether the *D. esculentum* mucilage gel formulation meets pharmacopeial standards for topical gels and performs comparably to the synthetic gel formulation.

Table 31 Natural and synthetic gel Pharmacopeial Standards

Properties	Treat	N	Mean	SD
Absorption	NtrlGel_F1(2.5%)	3	2.63	0.02066
	NtrlGel_F2(5%)	3	2.69	0.007
	NtrlGel_F3(7.5%)	3	2.71	0.01795
	SynthGel_F1(1%)	3	2.77	0.008
	SynthGel_F2(1.5%)	3	2.94	0.0115
	SynthGel_F3(2%)	3	2.88	0.02122
Percentage	NtrlGel_F1(2.5%)	3	97.78	0.76559
	NtrlGel_F2(5%)	3	99.78	0.26006
	NtrlGel_F3(7.5%)	3	100.49	0.66523
	SynthGel_F1(1%)	3	102.73	0.41073
	SynthGel_F2(1.5%)	3	109.22	0.42509
	SynthGel_F3(2%)	3	106.93	0.79198
Viscosity	NtrlGel_F1(2.5%)	3	11774.67	217.9916
	NtrlGel_F2(5%)	3	23712.33	580.0193
	NtrlGel_F3(7.5%)	3	38693	334.0314
	SynthGel_F1(1%)	3	29302	925.8396
	SynthGel_F2(1.5%)	3	36298.67	809.0478
	SynthGel_F3(2%)	3	49165	616.8728
pH_Prmtr	NtrlGel_F1(2.5%)	3	7.3	0.2
	NtrlGel_F2(5%)	3	7.2	0.2
	NtrlGel_F3(7.5%)	3	7.9	0.1
	SynthGel_F1(1%)	3	7.13	0.25166
	SynthGel_F2(1.5%)	3	6.93	0.15275
	SynthGel_F3(2%)	3	7.53	0.23094

➢ *Post Hoc Pairwise Comparisons*

Post hoc pairwise comparisons were conducted following the one-way Analysis of Variance (ANOVA) to determine which specific group differences contributed to the overall statistical significance. Since ANOVA only indicates the presence of a significant difference among groups, post hoc analysis is necessary to identify where these differences occur (Sharma et al. 2022).

In this study, the Dwass–Steel–Critchlow–Fligner (DSCF) pairwise comparison test was specifically applied to the spreadability test to compare the mean spreadability values among the different gel formulations containing varying concentrations of *D. esculentum* mucilage (2.5%, 5%, and 7.5% w/w) and the control group containing Carbopol 940. The DSCF post hoc test was used to identify which formulations significantly differed in spreadability performance after obtaining a significant ANOVA result (Metta et al. 2023).

Pairwise comparisons were performed between all possible treatment groups. A significance level of $p < 0.05$ was used to determine statistically significant differences. If the computed p-value for a pair exceeded 0.05, the difference between the two groups was considered not statistically significant, indicating comparable spreadability performance. Conversely, a p-value less than 0.05 indicated a significant difference between formulations (Hamdi 2023).

The results of the post hoc analysis provided a clearer understanding of the spreadability behavior of each gel formulation relative to one another and to the synthetic control. This enabled the identification of the formulation concentration that exhibited spreadability properties closest to or better than the standard formulation (Kumar and Sharma 2025; Abdalla and Wilson 2021).

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CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

➢ *Conclusion*

This study aimed to formulate and evaluate diclofenac diethylamine gel using *Diplazium esculentum* mucilage as a natural gelling agent and compare its performance with Carbopol 940, a widely used synthetic polymer. Topical gels are commonly used because they allow localized drug delivery, improve patient compliance, and avoid first-pass metabolism (Bagmar et al. 2024). However, concerns regarding the cost, possible irritation, and environmental impact of synthetic polymers have encouraged the search for safer, more sustainable, and natural alternatives (Pawar et al. 2020). In this study, the mucilage was successfully extracted from *D. esculentum* and incorporated into gel formulations at concentrations of 2.5%, 5%, and 7.5% w/w. The prepared formulations were evaluated based on pH, viscosity, homogeneity, spreadability, drug content, and extrudability.

The results showed that all formulations exhibited acceptable pH values ranging from 7.2 to 7.9, indicating their suitability for topical application. This range was consistent with the acceptable pH range for diclofenac diethylamine gel formulations, which is important in maintaining skin compatibility and reducing the risk of irritation (Sundari et al. 2025). These findings suggest that *D. esculentum* mucilage can be incorporated into topical gel formulations without producing pH values that may be unsuitable for skin application.

In terms of viscosity, the thickness of the gels increased as the concentration of *D. esculentum* mucilage increased. The 2.5% natural gel showed the lowest viscosity among the natural formulations at 11,774 cP, while the 5% natural gel increased to 23,712 cP. The 7.5% natural gel showed the highest viscosity among the natural formulations at 38,693 cP. This indicates that increasing the concentration of *D. esculentum* mucilage improved gel consistency and thickness. However, only the 7.5% natural gel showed viscosity that was comparable to selected synthetic gel formulations, particularly the 1% and 1.5% Carbopol 940 gels. This result is supported by studies stating that plant mucilages contain hydrophilic polysaccharides that contribute to swelling, water retention, thickening, and gel formation (Cakmak et al. 2023; Prajapati et al. 2022).

The homogeneity results showed that the formulated gels had smooth and uniform consistency, with no major signs of phase separation. This indicates that the active ingredient and excipients were properly incorporated into the gel base. Homogeneity is an important quality parameter because it helps ensure uniform distribution of the drug and consistency of the formulation (Das et al. 2022). The FTIR analysis also supported the compatibility between diclofenac diethylamine and *D. esculentum* mucilage, as no major interaction or incompatibility was observed between the drug and the natural mucilage. The retention of major FTIR absorption bands and the absence of significant peak changes support drug-excipient compatibility (Sengupta et al. 2015).

For spreadability and extrudability, the results showed that the type and concentration of the gelling agent affected the application properties of the gel. Lower-viscosity formulations were generally easier to spread, while higher concentrations of *D. esculentum* mucilage showed acceptable extrudability. Spreadability and extrudability are important parameters because they influence ease of application, user convenience, and proper dispensing of the product (Kumari et al. 2022; Sundari et al. 2025). These findings suggest that *D. esculentum* mucilage, particularly at higher concentrations, may provide acceptable application properties for topical gel formulation.

The statistical analysis showed significant differences among the formulations in the evaluated parameters. Since the p-values were below the 0.05 level of significance, the null hypothesis was rejected, and the alternative hypothesis was accepted. This means that there was a significant difference in the physical properties and performance parameters of diclofenac diethylamine gels formulated with *D. esculentum* mucilage compared with gels formulated using Carbopol 940. However, accepting the alternative hypothesis does not mean that the natural mucilage gel was better than the synthetic gel in all parameters. Rather, it indicates that the formulations differed significantly, and that the 7.5% natural gel showed viscosity that was comparable to selected synthetic formulations, while the other natural concentrations showed acceptable but lower viscosity.

Therefore, the findings suggest that *D. esculentum* mucilage has potential as a natural gelling agent for diclofenac diethylamine topical gel formulations. Although it did not consistently match Carbopol 940 in all parameters, the 7.5% natural mucilage gel showed the most promising performance among the natural formulations, particularly in terms of viscosity. Overall, *D. esculentum* mucilage may serve as a sustainable, locally available, and natural alternative gelling agent, especially when further optimized through additional formulation, stability, drug release, and safety studies.

➢ *Recommendations*

Based on the findings of this study, several recommendations are proposed for future research and development. It is recommended to conduct long-term stability studies on the formulated diclofenac diethylamine gels containing *D. esculentum* mucilage under various storage conditions. These studies will help assess the shelf life of the gels and determine their performance over time, ensuring their durability and effectiveness for extended use. Additionally, conducting *In vitro* testing, particularly focusing on drug release and skin permeability, is important for evaluating the therapeutic effectiveness of the gels. These tests will

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provide valuable information on how well the active ingredient, diclofenac diethylamine, is released and absorbed through the skin, ensuring that the gel will deliver the intended benefits in real-world applications.

Further studies could explore the combination of *D. esculentum* mucilage with other natural or synthetic polymers. This could enhance the physicochemical properties of the gel, improving its stability, texture, and overall performance. Such research would also enable more customized formulations that could cater to specific pharmaceutical needs. It is also recommended that safety evaluations, such as skin irritation and toxicity testing, be performed to establish the safety profile of *D. esculentum* mucilage. These studies would provide assurance that the mucilage is safe for prolonged topical use without causing adverse effects.

Finally, further exploration of *D. esculentum* mucilage in other pharmaceutical dosage forms, such as creams, ointments, and transdermal systems, should be pursued. This could broaden its application beyond gels, helping to position *D. esculentum* mucilage as a viable, sustainable alternative to synthetic gelling agents in the pharmaceutical industry. These recommendations aim to fully explore the potential of *D. esculentum* mucilage as a natural and effective gelling agent and encourage its broader use in pharmaceutical formulations.

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APPENDIX A

Schedule of Activities

SCHEDULE OF ACTIVITIES

Thesis Title: Phytochemical Profiling, Evaluation, and Formulation of Diclofenac Diethylamine using *Diplazium esculentum* Fronds *(Athyriaceae) as a Gelling Agent.*

Project Dates: December 2025 - May 2026

SCHEDULE OF OBJECTIVES		2025-2026					
		DEC	JAN	FEB	MAR	APR	MAY
1	Plant Collection						
2	Plant Authentication						
3	Requesting and Purchasing of Chemicals and Reagents						
4	Collection and Preparation of *D. esculentum* mucilage						
5	Phytochemical Profiling Test for mucilage						
6	Diclofenac Diethylamine Gel formulation						
7	Evaluation Tests for formulated Gelling agent						
8	Data Analysis						
9	Writing and Editing of paper						
10	Final Defense						
11	Editing of panelists suggestions, recommendations, and annotations						
12	Processing of Panelists signatures						
13	Manuscript Binding						

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APPENDIX B

Budget Schedule

BUDGET REPORT OF THE STUDY

Thesis Title: Phytochemical Profiling, Evaluation, and Formulation of Diclofenac Diethylamine using *Diplazium esculentum* Fronds *(Athyriaceae) as a Gelling Agent.*

Project Dates: December 2025 - May 2026

This budgetary schedule reflects more or less the necessary costs that are associated with the above-named research project. The estimated total cost for the research project is itemized below:

EXPENDITURES	**BUDGET**
Oral Defense Presentation Fee	*PHP 12,000*
Plant Collection	*PHP 2,900*
Hard Bound	*PHP 1,500*
Reagents and Chemicals	*PHP 28,635*
Pharmacy Laboratory Fee	*PHP 500*
Phytochemical Profiling	*PHP 1,500*
Lab Materials	*PHP 3,000*
FTIR Analysis	*PHP 7,900*
Plant Authentication	*PHP 300*
Statistician's Fee	*PHP 2,500*
TOTAL	*PHP 60, 735*

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APPENDIX C

Plant Authentication Certificate

DEPARTMENT OF BIOLOGICAL SCIENCES
COLLEGE OF SCIENCE AND MATHEMATICS
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csm.biosci@g.msuiit.edu.ph

CERTIFICATE OF AUTHENTICATION OF BOTANICAL SPECIMENS

This is to certify that the BOTANICAL specimen/s herein listed and presented by the persons/s herein noted were identified and authenticated by this office.

Name: **Quia Francine P. Buhian**
Nigel C. Cabil
Rdlyn C. Gemperoso
Josh Andre V. Gubalane
School/Institution: **Adventist Medical Center College – Iligan City**

Family	Scientific Name	Local/Common Name
Athyriaceae	*Diplazium esculentum* (Retz.) Sw.	Fiddlehead fern (Eng), pako(MnyLgs)

Collected from: Brgy. Tipanoy, Iligan City
Date of Collection: 12 December 2025

Muhmin Michael E. Manting, MSc.
Assistant Professor
Department of Biological Sciences
MSU-Iligan Institute of Technology

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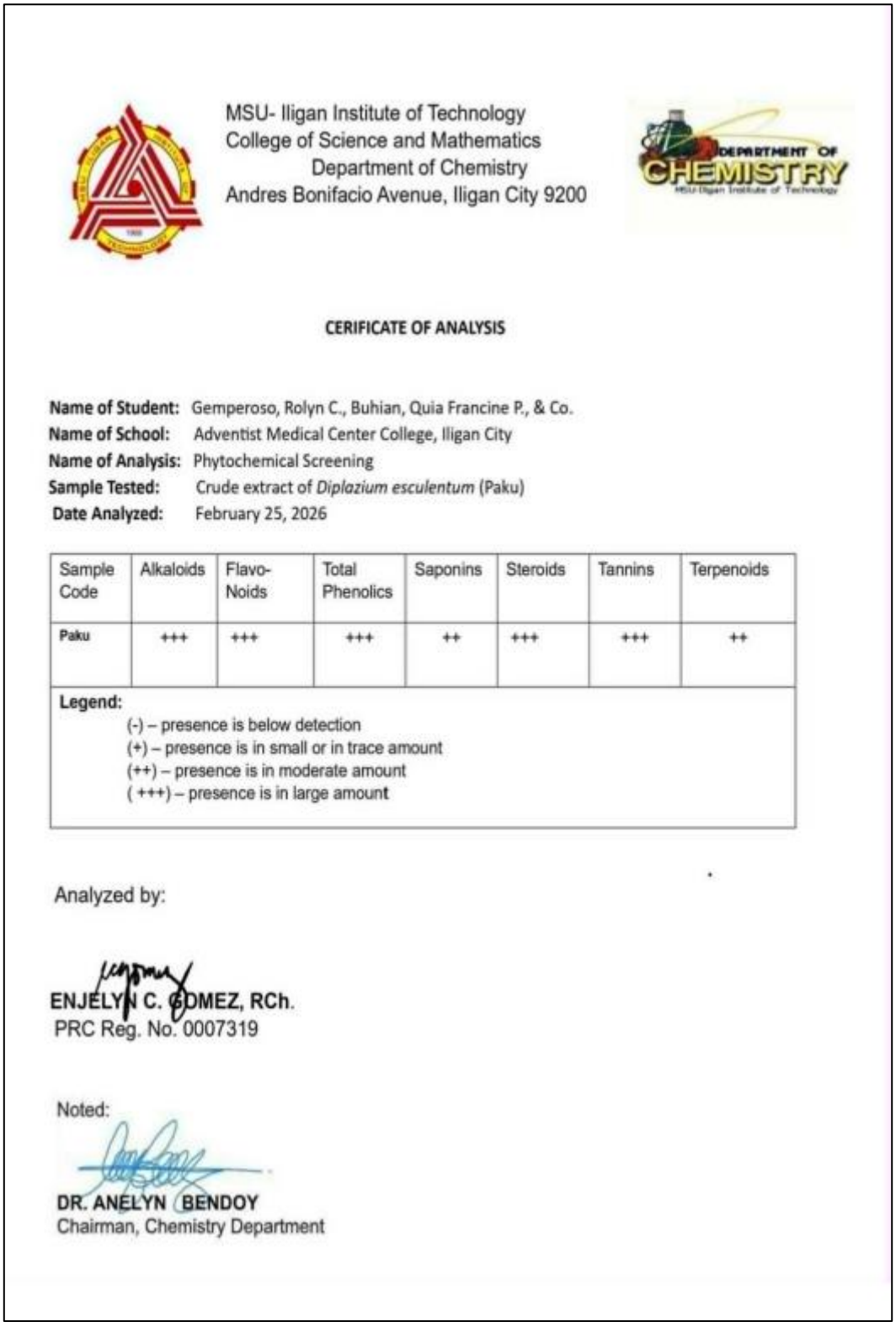
APPENDIX D

D. esculentum Phytochemical Screening Certificate of Analysis



MSU- Iligan Institute of Technology
College of Science and Mathematics
Department of Chemistry
Andres Bonifacio Avenue, Iligan City 9200

DEPARTMENT OF CHEMISTRY
MSU-Iligan Institute of Technology

CERIFICATE OF ANALYSIS

Name of Student: Gemperoso, Rolyn C., Buhian, Quia Francine P., & Co.
Name of School: Adventist Medical Center College, Iligan City
Name of Analysis: Phytochemical Screening
Sample Tested: Crude extract of *Diplazium esculentum* (Paku)
Date Analyzed: February 25, 2026

Sample Code	Alkaloids	Flavo-Noids	Total Phenolics	Saponins	Steroids	Tannins	Terpenoids
Paku	+++	+++	+++	++	+++	+++	++

Legend:
(-) – presence is below detection
(+) – presence is in small or in trace amount
(++) – presence is in moderate amount
(+++) – presence is in large amount

Analyzed by:

ENJELYN C. GOMEZ, RCh.
PRC Reg. No. 0007319

Noted:

DR. ANELYN BENDOY
Chairman, Chemistry Department

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APPENDIX E

Methyl Paraben Certificate of Analysis and Material Origin

FOREVER

南京九佳科技有限公司
Nanjing Forever Pharmacy Co., Ltd.
地址：南京市栖霞区新港开发区27号

Certificate of Analysis
检 验 报 告 书

产品名称 Product Name	Methyl Paraben	CAS No.	99-76-3
批号 Batch No	N03253609	数量 Quantity	25g
生产日期 Manufacture Date	2025-12-10	有效时间 Expired Date	2028-12-09
检测日期 Test Date	2025-12-11	储存 Storage	RT

检测项目 Inspection Items	检测标准 Standard	检测结果 Results
Appearance	Colorless to white to off-white powder or crystals	Conforms
Purity	≥99%	99.71%
SO42-/%	≤0.05	0.01
Water (by Karl Fischer)(%)	≤5	1.03
Pb (ppm)	≤5	1.26

结论: 按企业标准检验，符合公司标准
CONCLUSION: COMPLIED WITH COMPANY STANDARD

分析员 (ANALYST):
审核 (CHECKED):
质量经理 (QC MANAGER)

南京九佳科技有限公司 质检专用章 Quality Inspection Nanjing Forever Pharmacy Co., Ltd.

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APPENDIX F

Carbopol Certificate of Analysis and Material Origin

FOREVER

南京九佳科技有限公司
Nanjing Forever Pharmacy Co., Ltd.
地址：南京市栖霞区新港开发区27号

Certificate of Analysis
检 验 报 告 书

产品名称 Product Name	Carbopol 940	CAS No.	76050-42-5
批号 Batch No	N50706512	数量 Quantity	25g
生产日期 Manufacture Date	2025-11-23	有效时间 Expired Date	2028-11-22
检测日期 Test Date	2025-11-24	储存 Storage	RT

检测项目 Inspection Items	检测标准 Standard	检测结果 Results
Appearance	Loose white, slightly acidic powder; odorless or with a slight characteristic odor	Conforms
Purity	≥99%	99.81%
Acidity	pH of 0.5% aqueous solution is 2.7–3.5	Conforms
Equivalent Weight	76 ± 4	Conforms
Ash Content	≤ 0.2%	0.1%
Loss on Drying	≤ 2%	1.03%
Bulk Density	0.21 g/cm³	Conforms
Glass Transition Temperature	100–110°C	106.2°C
Lead Content	≤ 20 ppm	10.69ppm
Carboxyl Content (COOH%)	57.7–65.0	61.2

结论: 按企业标准检验，符合公司标准
CONCLUSION: COMPLIED WITH COMPANY STANDARD

分析员 (ANALYST): 程勇 审核 (CHECKED): 朱丽丽 质量经理 (QC MANAGER): 王博宇

南京九佳科技有限公司 质检专用章 Nanjing Forever Pharmacy Co., Ltd.

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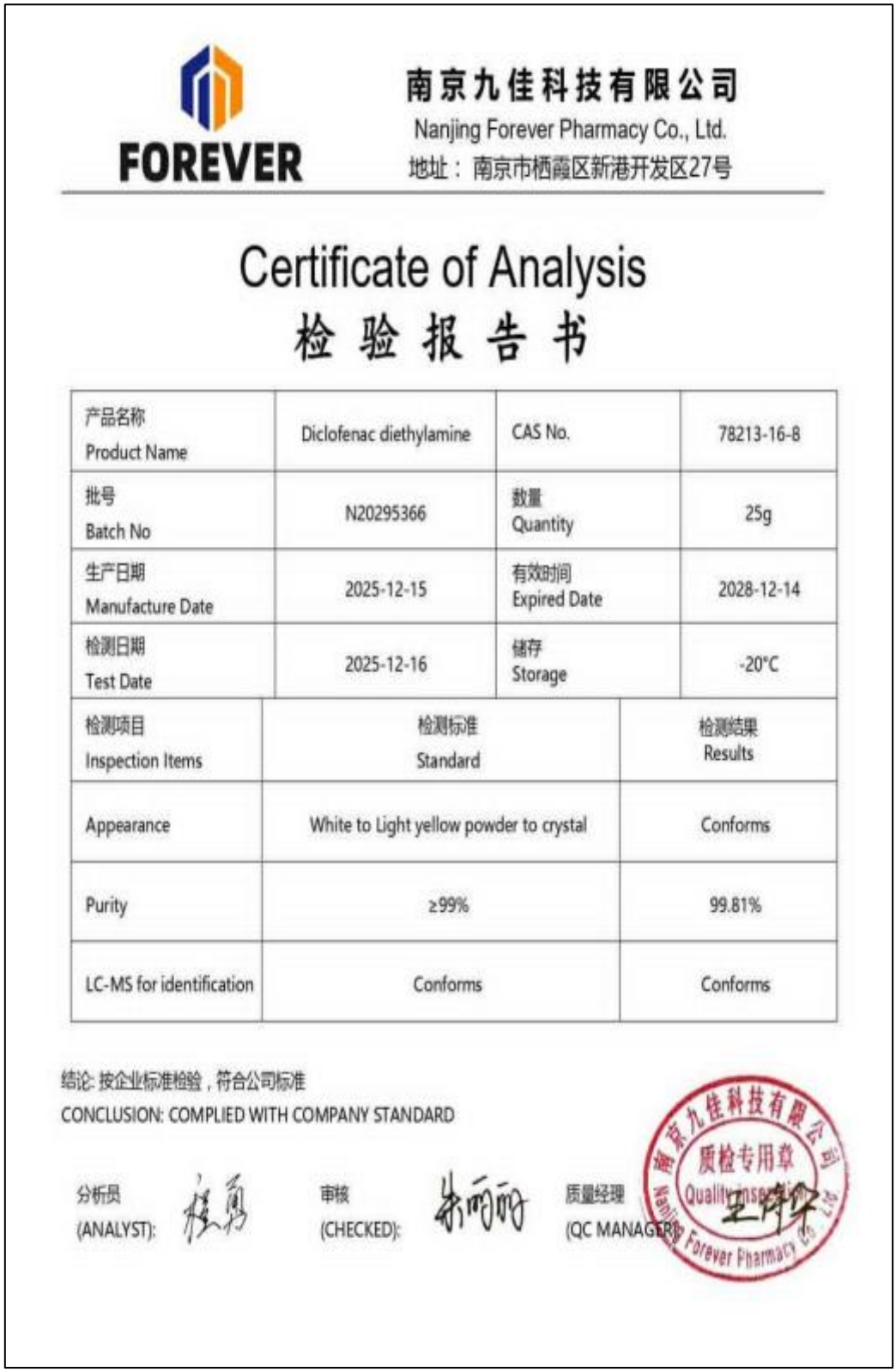
APPENDIX G

Diclofenac Diethylamine Certificate of Analysis and Material Origin



南京九佳科技有限公司
Nanjing Forever Pharmacy Co., Ltd.
地址：南京市栖霞区新港开发区27号

FOREVER

Certificate of Analysis
检 验 报 告 书

产品名称 Product Name	Diclofenac diethylamine	CAS No.	78213-16-8
批号 Batch No	N20295366	数量 Quantity	25g
生产日期 Manufacture Date	2025-12-15	有效时间 Expired Date	2028-12-14
检测日期 Test Date	2025-12-16	储存 Storage	-20°C

检测项目 Inspection Items	检测标准 Standard	检测结果 Results
Appearance	White to Light yellow powder to crystal	Conforms
Purity	≥99%	99.81%
LC-MS for identification	Conforms	Conforms

结论: 按企业标准检验，符合公司标准
CONCLUSION: COMPLIED WITH COMPANY STANDARD

分析员 (ANALYST):
审核 (CHECKED):
质量经理 (QC MANAGER):

南京九佳科技有限公司 质检专用章 Nanjing Forever Pharmacy Co., Ltd.

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APPENDIX H

Triethanolamine (TEA) Certificate of Analysis and Material Origin

ASTERIA APOTHECARY OPC
Artisan's Raw Materials Supplier
Mezzanine Level, Choice Market Ortigas
Brgy. Rosario Pasig City, Philippines 1609
Contact No.: 0915-097-0349 (Viber); 0962-950-4569
Email: hello@asteriaapothecary.com / sales@asteriaapothecary.com

www.asteriaapothecary.com

CERTIFICATE OF ANALYSIS (COA)

TRIETHANOLAMINE (TEA) AALQ15043

Representative Sample Certificate

CERTIFICATE OF ANALYSIS

TRIETHANOLAMINE (TEA)

2,2',2"-Nitrilotriethanol | AALQ15043

Product Name:	Triethanolamine AALQ15043	Order Number:	
INCI Name:	2,2',2"-nitrilotriethanol	Manufacturer:	
Product Code.:	AALQ15043	CAS No.:	102-71-6
Country Origin:	Taiwan	Brand:	
Lot No. / Batch No.:	ABL02A12	Batch Quantity:	50MT
Production Date:	July 2025	Package:	225Kg Metal drum
Expiration Date:	July 2028		

PARAMETERS	UNITS	STANDARD	RESULTS
Appearance		Transparent Liquid	Clear, Colourless
Triethanolamine (Purity, wt%)	%	99% min.	99.46%
Diethanolamine (Purity, wt%)	%	0.3% max.	0.097%
Color (Pt/Co)		50 max.	4.5
Moisture (wt, %)	%	0.2%	0.046%

TYPICAL PROPERTIES		
PARAMETERS	UNIT	TYPICAL
Boiling Point (760mmHg, °C)	°C	360 °C
Flash Point (PMCC, °C/ °F)		201 °C / 395 °F
Freezing Point (°C/ °F)		21.20 °C / 70.20 °F
Molecular Weight		149.19
Specific Gravity		1.122 to 1.126

The product has been evaluated by quality assurance and complies with all aspects of the agreed specifications.

Storage: Store in an airtight container; in a dark, cool place at room temperature; away from direct heat and sunlight.

**** Order number, manufacturer, and Brand Name have been withheld as a trade secret.*

"The test results in this certificate relate only to samples of product taken at the time of manufacture. Whilst systems are in place to the control of the manufacturer and distributor may affect the quality of the product. It is suggested that any user of this product should make his or her own assessment of product quality and suitability for use."

Disclaimer: The information above is obtained from reliable sources and the best information currently available to us. Please refer to all relevant technical information specific only to the product and may not be valid for such material used in combination with any other materials or in any other process. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting outside the control of Asteria Apothecary. Users should make their own investigations and independent judgement to determine the suitability of the information for their own purposes. In no event shall Asteria Apothecary be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Asteria Apothecary has been advised of the possibility of such damages prior to use or purchase of the specific product.

This is a computer printed document and is therefore not signed.

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Prepared by: RC
Asteria Apothecary
February 24, 2026

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APPENDIX I

Forever Pharmacy Quality Management System Certificate

QUALITY MANAGEMENT SYSTEM CERTIFICATE

Certificate No. 00123Q37062R1S/3200

We hereby certify that

Nanjing Forever Pharmacy Co., Ltd.

Business Registration Number: 91320115MAC12KG34XH

Registered / Operation / Production Address: 27th, Nanjing Xin'gang Development Zone, Nanjing City, China

by reason of its

Quality Management System

has been awarded this certificate for compliance with the standard

ISO 9001:2015

The Quality Management System Applies in the following area:

Production of laboratory equipment, laboratory consumables and laboratory reagents

Certified since: September 2, 2020 Valid from: September 6, 2023 Valid until: September 1, 2026

the expiry date of last certification cycle: September 1, 2023 the date of recertification audit: August 1, 2023 to August 2, 2023

After a surveillance cycle, the certificate is valid only when used together with an Acceptance Notice of Surveillance Audit issued by CQC.

Please access www.cqc.com.cn for checking validity of the certificate.

This certificate and its relevant information can query in the website of Certification and Accreditation Administration of the People's Republic of China (www.cnca.gov.cn).

IAF CNAS IQNET

CHINA QUALITY CERTIFICATION CENTRE

Section 9, No.188, Nansihuan(the South Fourth Ring Road) Xilu(West Road), Beijing 100070, China

http://www.cqc.com.cn

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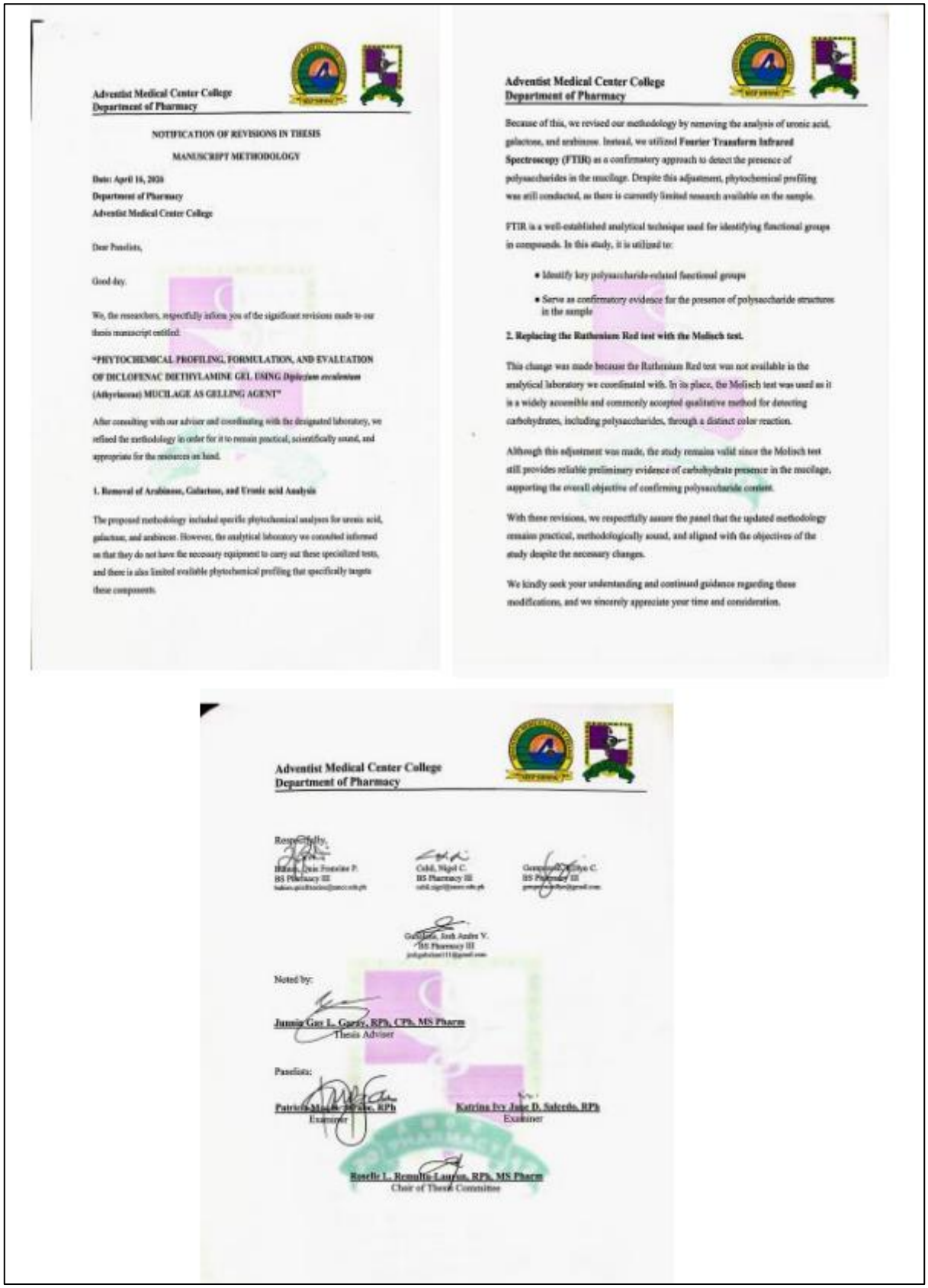
APPENDIX J

Letter for consideration



Adventist Medical Center College
Department of Pharmacy

NOTIFICATION OF REVISIONS IN THESIS

MANUSCRIPT METHODOLOGY

Date: April 16, 2026
Department of Pharmacy
Adventist Medical Center College

Dear Panelists,

Good day.

We, the researchers, respectfully inform you of the significant revisions made to our thesis manuscript entitled:

"PHYTOCHEMICAL PROFILING, FORMULATION, AND EVALUATION OF DICLOFENAC DIETHYLAMINE GEL USING *Dipterium cevalenium* (Athyriaceae) MUCILAGE AS GELLING AGENT"

After consulting with our adviser and coordinating with the designated laboratory, we refined the methodology in order for it to remain practical, scientifically sound, and appropriate for the resources on hand.

1. Removal of Arabinose, Galactose, and Uronic acid Analysis

The proposed methodology included specific phytochemical analyses for uronic acid, galactose, and arabinose. However, the analytical laboratory we consulted informed us that they do not have the necessary equipment to carry out these specialized tests, and there is also limited available phytochemical profiling that specifically targets these components.

Adventist Medical Center College
Department of Pharmacy

Because of this, we revised our methodology by removing the analysis of uronic acid, galactose, and arabinose. Instead, we utilized **Fourier Transform Infrared Spectroscopy (FTIR)** as a confirmatory approach to detect the presence of polysaccharides in the mucilage. Despite this adjustment, phytochemical profiling was still conducted, as there is currently limited research available on the sample.

FTIR is a well-established analytical technique used for identifying functional groups in compounds. In this study, it is utilized to:

- Identify key polysaccharide-related functional groups
- Serve as confirmatory evidence for the presence of polysaccharide structures in the sample

2. Replacing the Ruthenium Red test with the Molisch test.

This change was made because the Ruthenium Red test was not available in the analytical laboratory we coordinated with. In its place, the Molisch test was used as it is a widely accessible and commonly accepted qualitative method for detecting carbohydrates, including polysaccharides, through a distinct color reaction.

Although this adjustment was made, the study remains valid since the Molisch test still provides reliable preliminary evidence of carbohydrate presence in the mucilage, supporting the overall objective of confirming polysaccharide content.

With these revisions, we respectfully assure the panel that the updated methodology remains practical, methodologically sound, and aligned with the objectives of the study despite the necessary changes.

We kindly seek your understanding and continued guidance regarding these modifications, and we sincerely appreciate your time and consideration.

Adventist Medical Center College
Department of Pharmacy

Respectfully,

Noted by:

Junnie Gay L. Garay, RPh, CPh, MS Pharm
Thesis Adviser

Panelists:

Katrina Ivy Jane D. Salcedo, RPh
Examiner

Roselle L. Remulla-Laurea, RPh, MS Pharm
Chair of Thesis Committee

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APPENDIX K

Collection, Air Drying, and Preparation of *D. esculentum*

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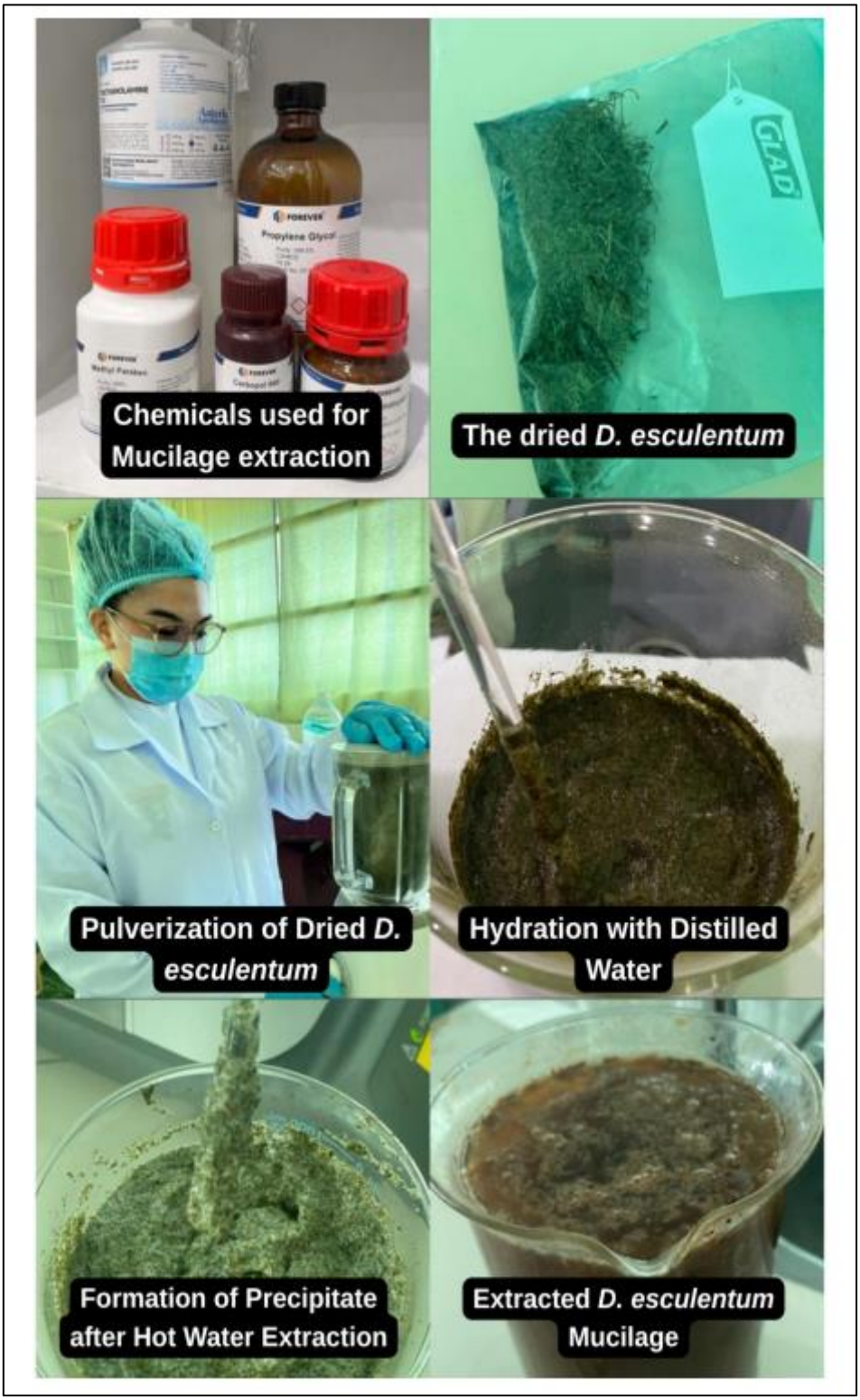
APPENDIX L

Mucilage Extraction



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APPENDIX M

Characterization and Evaluation

Tests for Diclofenac Diethylamine Gel Using D. esculentum Mucilage as a Gelling Agent: pH, Spreadability, Extrudability, Homogeneity, Viscosity, and Drug Content

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APPENDIX N

Raw Results/Data

UV-Visible spectrophotometer data for Drug Content		
Formulation (Synthetic 1%)	**Absorbance**	**Percentage**
Trial 1	2.779	103.19 %
Trial 2	2.763	102.60 %
Trial 3	2.771	102.40 %
Formulation (Synthetic 1.5 %)	**Absorbance**	**Percentage**
Trial 1	2.953	109.65 %
Trial 2	2.930	108.80 %
Trial 3	2.941	109.21 %
Formulation (Synthetic 2%)	**Absorbance**	**Percentage**
Trial 1	2.886	107.17 %
Trial 2	2.897	107.58 %
Trial 3	2.856	106.05 %
Formulation (Natural 2.5 %)	**Absorbance**	**Percentage**
Trial 1	2.627	97.55 %
Trial 2	2.616	97.15%
Trial 3	2.656	98.63 %
Formulation (Natural 5%)	**Absorbance**	**Percentage**
Trial 1	2.687	99.77 %
Trial 2	2.680	99.52 %
Trial 3	2.694	100.04%
Formulation (Natural 7.5%)	**Absorbance**	**Percentage**
Trial 1	2.720	101.00%
Trial 2	2.686	99.74%
Trial 3	2.713	100.74 %
STANDARD ABSORBANCE OF DDEA: 2.693 Abs.		
Viscosity Parameter		

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Formulation (Synthetic 1%)	**Viscosity**
Trial 1	30365 mPa.s
Trial 2	28869 mPa.s
Trial 3	28672 mPa.s
Formulation (Synthetic 1.5 %)	**Viscosity**
Trial 1	36929 mPa.s
Trial 2	37057 mPa.s
Trial 3	35447 mPa.s
Formulation (Synthetic 2%)	**Viscosity**
Trial 1	49391 mPa.s
Trial 2	48467 mPa.s
Trial 3	49637 mPa.s
Formulation (Natural 2.5 %)	**Viscosity**
Trial 1	11661 mPa.s
Trial 2	12026 mPa.s
Trial 3	11637 mPa.s
Formulation (Natural 5%)	**Viscosity**
Trial 1	23684 mPa.s
Trial 2	23147 mPa.s
Trial 3	24306 mPa.s
Formulation (Natural 7.5 %)	**Viscosity**
Trial 1	38310 mPa.s
Trial 2	38924 mPa.s
Trial 3	38845 mPa.s

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pH Parameter	
Formulation (Synthetic 1 %)	**pH**
Trial 1	6.9
Trial 2	7.1
Trial 3	7.4
Formulation (Synthetic 1.5%)	**pH**
Trial 1	6.9
Trial 2	6.8
Trial 3	7.1
Formulation (Synthetic 2%)	**pH**
Trial 1	7.8
Trial 2	7.4
Trial 3	7.4
Formulation (Natural 2.5 %)	**pH**
Trial 1	7.3
Trial 2	7.1
Trial 3	7.5
Formulation (Natural 5%)	**pH**
Trial 1	7.2
Trial 2	7.0
Trial 3	7.4
Formulation (Natural 7.5 %)	**pH**
Trial 1	8.0
Trial 2	7.8
Trial 3	7.9

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Spreadability					
Formulation	**Weight (g)**	**Trial 1**	**Trial 2**	**Trial 3**	**Average value**
Synthetic 1%	500	190.91	136.67	210.00	179.19
Synthetic 1.5%	500	62.12	72.41	67.74	67.42
Synthetic 2%	500	43.75	46.67	41.18	43.87
Natural 2.5%	500	105.00	113.89	128.13	115.67
Natural 5%	500	53.75	60.29	63.64	59.23
Natural 7.5%	500	35.00	37.27	32.81	35.03

Extrudability			
Synthetic F1 (1%)	Total Mass of gel	Mass of the gel extruded	Percentage
Trial 1	10g	7.9	79 %
Trial 2	10g	7.6	76 %
Trial 3	10g	8.0	80 %
Synthetic F2 (1.5%)	Total Mass of gel	Mass of the gel extruded	Percentage
Trial 1	10g	8.2	82 %
Trial 2	10g	7.9	79%
Trial 3	10g	8.1	81 %
Synthetic F3 (2%)	Total Mass of gel	Mass of the gel extruded	Percentage
Trial 1	10g	8.3	83 %
Trial 2	10g	8.0	80 %
Trial 3	10g	8.4	84 %
Natural F1 (2.5%)	Total Mass of gel	Mass of the gel extruded	Percentage
Trial 1	10g	8.5	85%
Trial 2	10g	8.2	82 %
Trial 3	10g	8.4	84 %
Natural F2 (5%)	Total Mass of gel	Mass of the gel extruded	Percentage
Trial 1	10g	8.7	87 %
Trial 2	10g	8.6	86 %
Trial 3	10g	8.9	89%
Natural F3 (7.5%)	Total Mass of gel	Mass of the gel extruded	Percentage
Trial 1	10g	9.0	90%
Trial 2	10g	8.8	88%
Trial 3	10g	9.1	91%

Identification Test for Mucilage

Loss of Drying				
Trial	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	% Loss on Drying
1	1.00	0.91	0.09	9.00%
2	1.00	0.90	0.10	10.00%
3	1.00	0.92	0.08	8.00%

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Swelling Index			
Trial	Initial weight	Final weight	Percentage Yield
1	0.5 g	1.55 g	210 %
2	0.5 g	1.60 g	220 %
3	0.5 g	1.70 g	240 %
Mean ± SD	0.5 g	1.62 g	