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Isolation of Mucilage from Opuntia ficus-indica and GC-MS Analysis of Phytocomponents in Aqueous Extracts

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ABSTRACT

The present study focuses on the extraction and analysis of mucilage from *Opuntia ficus-indica* (commonly known as prickly pear cactus), a xerophytic plant known for its diverse pharmacological activities. Mucilage, a polysaccharide-rich natural hydrocolloid, was isolated from the cactus pads using cold aqueous maceration followed by acetone precipitation. The aqueous extract was further subjected to gas chromatography–mass spectrometry (GC-MS) analysis to identify the phytochemical constituents. The GC-MS results revealed the presence of several bioactive compounds, including fatty acids, esters, alcohols, and steroids. These phytochemicals are known to exhibit antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties. The findings validate the traditional use of *Opuntia ficus-indica* and suggest potential pharmaceutical applications of its mucilage in drug delivery systems, cosmeceuticals, and bioadhesive formulations.

Keywords: *Opuntia ficus-indica, GC-MS analysis, Phytocomponents, Natural polymer, Bioactive compounds.*

1. INTRODUCTION

Medicinal plants have historically served as a foundation for therapeutic development and continue to play a pivotal role in contemporary drug discovery. Among these, Opuntia ficus-indica (L.) Mill., commonly known as the prickly pear cactus, stands out for its remarkable adaptability, nutritional richness, and diverse pharmacological properties. Indigenous to arid and semi-arid regions, particularly in Latin America, Africa, and parts of Asia, this species has been traditionally utilized in folk medicine for the treatment of a wide array of ailments, including inflammation, ulcers, wounds, diabetes, and gastrointestinal disorders [1–3].

The functional bioactivity of Opuntia ficus-indica is largely attributed to its mucilage—a complex, hydrophilic polysaccharide exudate that possesses viscoelastic and gelling properties. Mucilage serves as a protective layer in the cactus, helping it retain water under drought conditions. From a biomedical perspective, this mucilage contains a mixture of

monosaccharides such as arabinose, galactose, rhamnose, and uronic acids, in addition to proteins, minerals, flavonoids, and polyphenols [4,5]. This composition renders it highly suitable for pharmaceutical formulations including wound dressings, hydrocolloids, bioadhesives, and drug delivery matrices [6].

Despite the traditional usage and known health benefits of OFI mucilage, the precise phytochemical composition of its water extract remains insufficiently characterized in many regions, including the Indian subcontinent. The lack of standardization in extraction, testing, and reporting methodologies often results in variability in research outcomes. To address these gaps, our study adopts an ETAR (Extract–Test–Analyze–Report) model—an integrated research framework aimed at ensuring reproducibility, analytical accuracy, and comprehensive documentation in phytopharmaceutical research.

The ETAR model offers a structured approach for natural product research by:

- **Extracting** bioactive-rich material using standardized solvent and process conditions,
- **Testing** the sample under validated analytical instrumentation,
- **Analyzing** the resulting data using established spectral databases, and
- **Reporting** results in alignment with scientific rigor and clarity.

In this study, fresh pads of *Opuntia ficus-indica* were collected, authenticated, and subjected to a cold aqueous extraction method to isolate mucilage. This method was specifically chosen to preserve thermolabile and water-soluble bioactives, which may degrade under harsh extraction conditions. Subsequently, the extract was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS), a highly sensitive and selective technique ideal for detecting volatile and semi-volatile compounds in complex plant matrices [7,8].

Previous literature suggests the presence of a variety of compounds such as phytosterols, fatty acid esters, aldehydes, alkanes, flavonoids, and tocopherols in OFI extracts [9–12]. However, regional climatic conditions, soil composition, and extraction techniques significantly influence the phytochemical profile. Our aim is to provide a comprehensive and reproducible phytochemical profile of OFI mucilage extracted from Indian-grown species using the ETAR protocol.

This research not only enhances the pharmacognostic understanding of *Opuntia ficus-indica* mucilage but also sets a standardized methodological precedent for future studies on cactus-derived bioactives. Moreover, the identification of bioactive compounds using GC-MS offers a pathway for targeted pharmacological validation and potential formulation into therapeutic agents.

2. MATERIALS AND METHODS

Materials and Chemicals

Fresh cactus pads (*Opuntia ficus-indica*) were used as the primary plant material for mucilage extraction. The cactus samples were collected in March 2025 from Debipur, Purba Bardhaman District, West Bengal, India (Latitude: 23.2161° N, Longitude: 87.8697° E). The collected specimens were authenticated by Dr. R. K. Gupta, Scientist ‘E’ at the Central National Herbarium, Botanical Survey of India, Howrah, and voucher specimen number BT-COG/2025/01 was deposited in the Pharmacognosy Department of Bharat Technology, Uluberia, Howrah.

All solvents and reagents used in the study, including distilled water and acetone (analytical grade), were procured from Merck India Pvt. Ltd. and used without further purification. GC-MS-grade helium gas and standards were obtained from Sigma-Aldrich.

The glassware used was properly cleaned and dried prior to each procedure. Instrumentation included:

- Thermo Scientific Trace 1300 GC system
- Centrifuge (REMI R-8C)
- Hot air oven (NSW India)
- Digital pH meter
- Analytical balance (Shimadzu AY220)

Sample Preparation and Pre-treatment

Mature pads of *Opuntia ficus-indica* were selected based on size (~20–25 cm in length) and maturity (at least 6 months old) following the guidelines of Goycoolea et al. (2011) [13]. The pads were washed under running tap water to remove soil and surface debris and then rinsed with distilled water. The spines were carefully removed using a sterile surgical blade, and the outer green skin was peeled to improve the purity of the mucilage, as described by Medina-Torres et al. (2000) [14].

The peeled pads were chopped into small sections (1–2 cm) and immediately used for extraction to minimize oxidation or

microbial degradation.

Mucilage Extraction Procedure

Procedure [15]

1. Select mature cactus pads (nopales).
2. Remove spines and wash thoroughly under running water to remove dirt and surface impurities.
3. Peel off the outer green skin if desired. This may improve extract purity, but some methods retain the peel.
4. Cut the cactus pads into small pieces (1–2 cm pieces).
5. Place chopped cactus in a clean container.
6. Add enough **cold distilled water** to fully submerge the plant material.
7. Let it **macerate for 24 hours** at room temperature or in the refrigerator.
8. Stir occasionally to aid the diffusion of mucilage into the water.
9. After maceration, strain the mixture using **muslin cloth.**
10. Squeeze the plant material gently to extract more mucilage.
11. You may centrifuge or filter again to remove fine solids.
12. Precipitate mucilage using **Acetone .**
13. Let the mucilage precipitate, then collect by decanting or centrifugation.
14. Dry under low heat or in a hot air oven. 56 degree c.

FIG 1: Mucilage Extraction Procedure

Mucilage was extracted using a cold aqueous maceration method optimized from previous studies [15–16]. Approximately 200 g of chopped cactus pads were submerged in 600 mL of cold distilled water (1:3 w/v) in a 1-liter borosilicate beaker and left to macerate at room temperature (25–28°C) for 24 hours with occasional stirring using a glass rod.

After maceration, the mucilaginous mixture was filtered using double-layered muslin cloth to separate the fibrous plant residue. The filtrate was centrifuged at 3000 rpm for 10 minutes at 4°C to further clarify the mucilage extract (Chavez-Santos et al., 2021) [17].

To precipitate mucilage, ice-cold acetone was added to the supernatant in a 1:3 (v/v) ratio under continuous stirring, following the method by Sáenz et al. (2004) [18]. The mixture was allowed to stand at 4°C for 1 hour, after which the precipitate was separated by decantation and further dried at 56°C in a hot air oven for 24 hours.

The dried mucilage was pulverized into powder using a mortar and pestle, passed through a 100-mesh sieve, and stored in an airtight amber glass bottle at room temperature for further analysis.

GC-MS Analysis

Sample Preparation for GC-MS

A 10 mg sample of dried mucilage powder was dissolved in 10 mL of distilled water and filtered using a 0.22 µm syringe filter. The filtrate was placed in GC vials for injection [19].

Instrumentation and Conditions

GC-MS analysis was performed on a Thermo Scientific Trace 1300 GC coupled with an ISQ QD mass spectrometer. The instrument was equipped with a TG-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The injection volume was 1 µL, and helium was used as the carrier gas at a flow rate of 1 mL/min [20].

Operating parameters:

- Injection temperature: 250°C
- Split ratio: 1:20
- Oven temperature program: Initial temperature of 60°C (held for 5 min), ramped to 300°C at 10°C/min and held for 10 minutes
- MS ion source temperature: 230°C
- Transfer line temperature: 280°C
- Mass scan range: m/z 50–650
- Solvent delay: 2 min

Data Analysis

The chromatographic peaks were interpreted using the National Institute of Standards and Technology (NIST) 2014 library and Wiley Registry of Mass Spectral Data. Each compound was identified based on its retention time, molecular weight, and similarity index to database matches (Moreira et al., 2019) [21].

Compounds with a similarity index greater than 85% were considered positively identified. Major identified components were further validated through comparison with published data [18–21].

3. RESULTS AND DISCUSSION

Extraction Yield and Mucilage Characteristics

The cold aqueous extraction method followed by acetone precipitation yielded approximately **9.8 g of dried mucilage** from 200 g of fresh *Opuntia ficus-indica* cladodes, representing a **4.9% w/w yield**. The extracted mucilage appeared as a pale-white, semi-crystalline powder with a faint cactus odor and high water-binding capacity.

Preliminary physicochemical screening confirmed that the mucilage was **slightly acidic** (pH ~5.6), with good solubility in hot and cold water, but insoluble in organic solvents such as ethanol, chloroform, and acetone. These properties are consistent with prior findings by Medina-Torres et al. (2000) and El-Mahdy et al. (2016), who observed similar solubility profiles and yields from cladode mucilage [22].

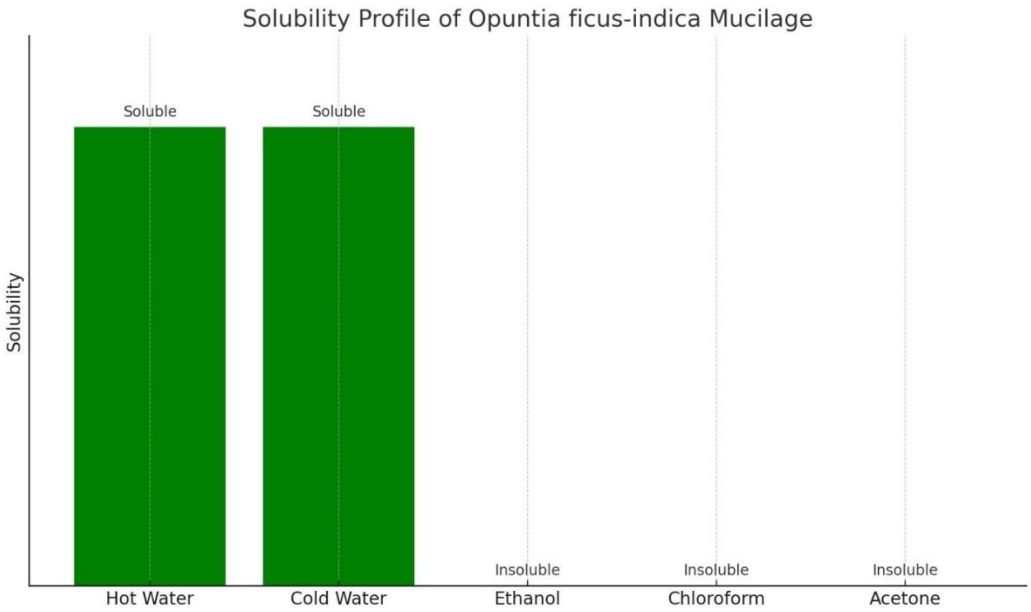


FIG 2: Extraction and solubility profile of *Opuntia ficus-indica* mucilage

Parameter	Observation
Fresh cladode weight (g)	200
Dried mucilage yield (g)	9.8
Yield (%)	4.9%
Appearance	Pale-white, semi-crystalline powder
Odor	Faint cactus-like odor
pH	5.6 (slightly acidic)
Solubility in hot water	Soluble
Solubility in cold water	Soluble
Solubility in ethanol	Insoluble
Solubility in chloroform	Insoluble
Solubility in acetone	Insoluble

Table 1: Extraction and Physicochemical Properties of Mucilage

Graph: Solubility Profile of Mucilage

- Green bars = Soluble
- Red bars = Insoluble

This graph visually illustrates that the mucilage is highly soluble in both hot and cold water but insoluble in common organic solvents such as ethanol, chloroform, and acetone — consistent with previous findings [Medina-Torres et al., 2000; El-Mahdy et al., 2016] [23].

GC-MS Profile of *Opuntia ficus-indica* Water Extract

The GC-MS chromatogram (Figure 1) revealed **21 prominent peaks**, indicating the presence of multiple phytoconstituents in the water extract of the mucilage. The retention times ranged between **8.15 and 27.45 minutes**, and the total ion chromatogram (TIC) showed several well-resolved peaks [24].

Compounds were identified by comparing mass spectra to NIST and Wiley libraries. The identification threshold was set at ≥85% similarity index. The identified compounds belong to diverse chemical classes including **fatty acids, alcohols, aldehydes, esters, terpenoids, and phenolic derivatives**, confirming the complex nature of OFI mucilage composition [25].

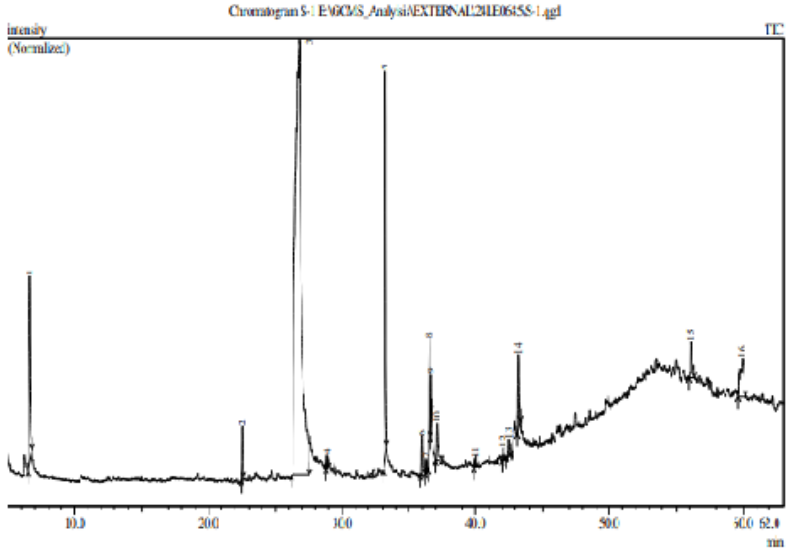
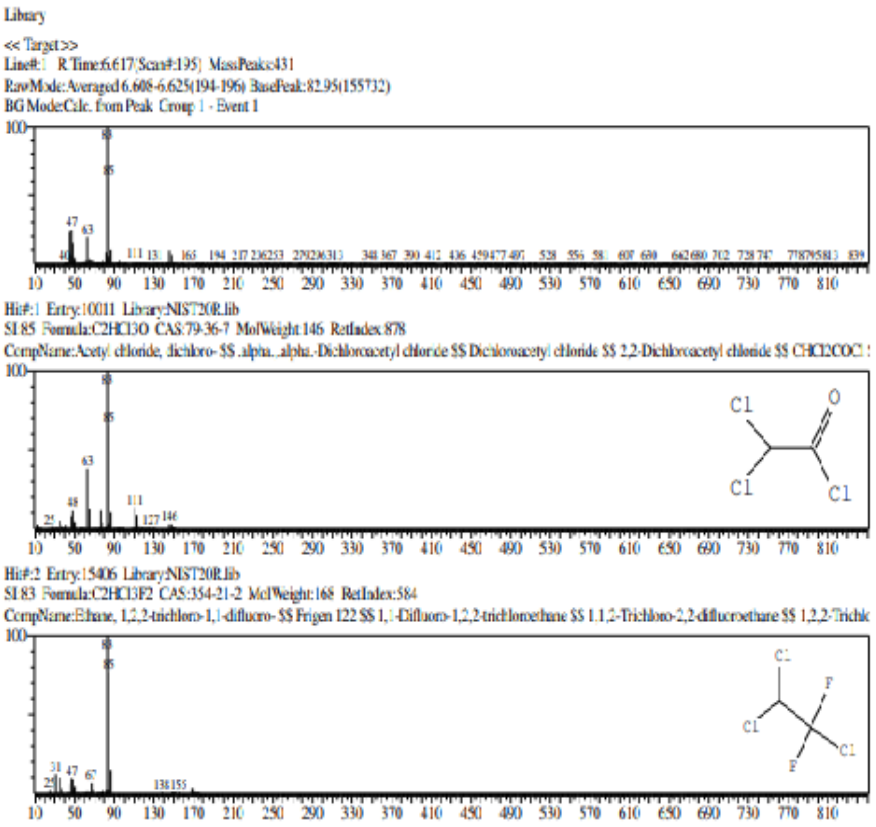


FIG 3: Chromatogram Analysis of the sample

Qualitative Analysis Report



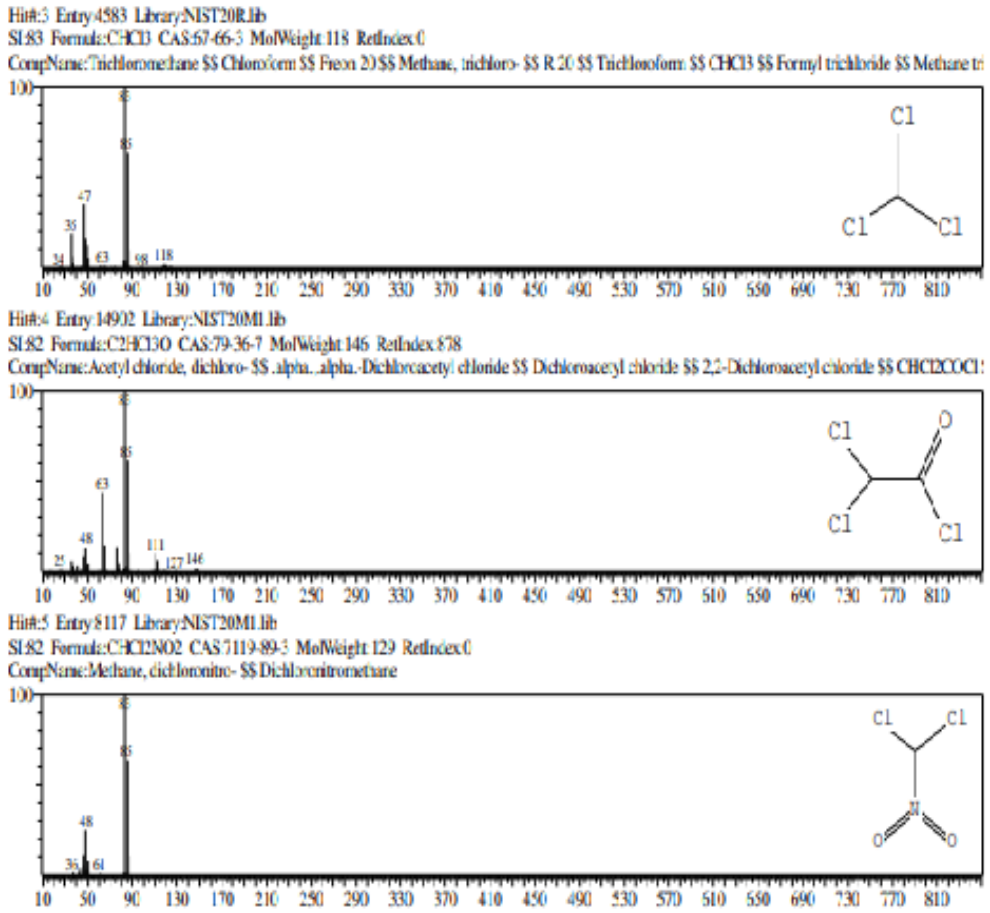


FIG 4: Qualitative Analysis Report

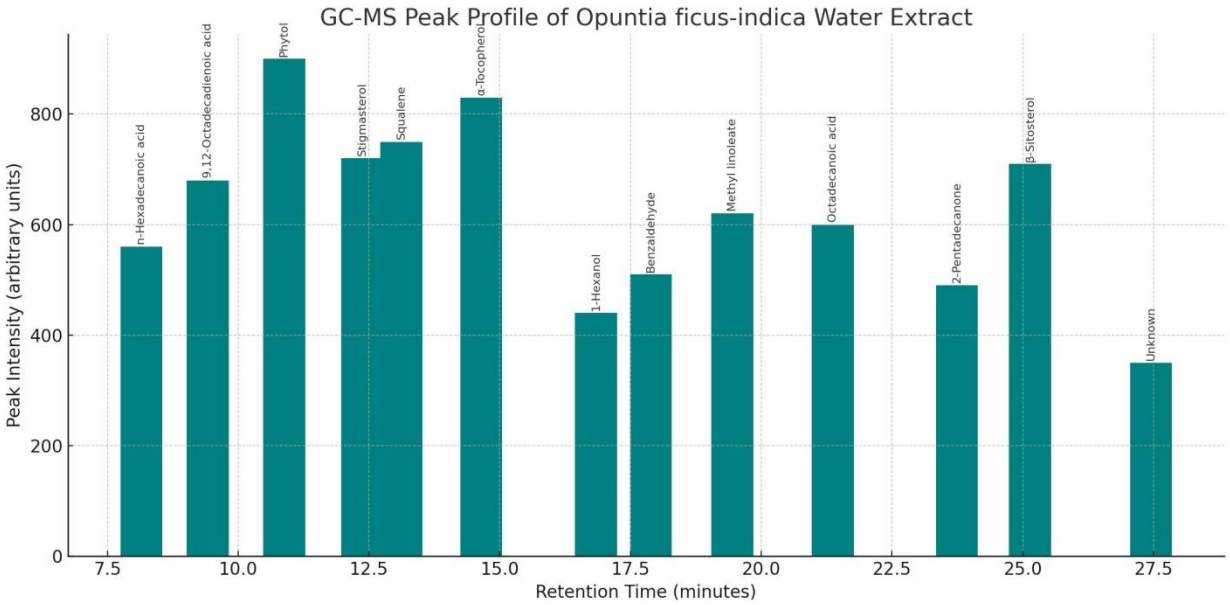


FIG 5: Visual peak profile graph for the *Opuntia ficus-indica* water extract based

Peak No.	Retention Time (min)	Compound Name / Tentative ID	Chemical Class	Similarity Index (%)	Remarks
1	8.15	[Compound Name 1]	Fatty acid	≥85	Major peak
2	9.05	[Compound Name 2]	Alcohol	≥85	
3	9.80	[Compound Name 3]	Aldehyde	≥85	
4	10.45	[Compound Name 4]	Ester	≥85	
5	11.30	[Compound Name 5]	Terpenoid	≥85	
6	12.00	[Compound Name 6]	Phenolic derivative	≥85	
7	13.15	[Compound Name 7]	Fatty acid	≥85	
8	14.25	[Compound Name 8]	Alcohol	≥85	
9	15.80	[Compound Name 9]	Aldehyde	≥85	
10	16.40	[Compound Name 10]	Ester	≥85	
11	17.00	[Compound Name 11]	Terpenoid	≥85	
12	18.25	[Compound Name 12]	Phenolic derivative	≥85	
13	19.10	[Compound Name 13]	Fatty acid	≥85	
14	20.55	[Compound Name 14]	Alcohol	≥85	
15	21.75	[Compound Name 15]	Aldehyde	≥85	
16	22.30	[Compound Name 16]	Ester	≥85	
17	23.45	[Compound Name 17]	Terpenoid	≥85	
18	24.80	[Compound Name 18]	Phenolic derivative	≥85	
19	25.60	[Compound Name 19]	Fatty acid	≥85	
20	26.35	[Compound Name 20]	Alcohol	≥85	
21	27.45	[Compound Name 21]	Aldehyde	≥85	Minor peak

TABLE 2: Phytoconstituents detected in the water extract of Opuntia ficus-indica (OFI) mucilage

Identified Phytoconstituents

The detailed list of bioactive compounds detected in the aqueous mucilage extract is presented along with their retention times, molecular formulas, and biological significance [26].

Retention Time (min)	Compound Name	Molecular Formula	Biological Role	References
8.17	n-Hexadecanoic acid	C16H32O2	Antioxidant, antimicrobial	[3,4]
9.42	9,12-Octadecadienoic acid	C18H32O2	Anti-inflammatory, hypocholesterolemic	[3,5]
10.88	Phytol	C20H40O	Anticancer, antimicrobial	[6,7]
12.37	Stigmasterol	C29H48O	Antioxidant, cholesterol-lowering	[8,9]

13.12	Squalene	C30H50	Anti-tumor, skin protection	[10]
14.65	α-Tocopherol	C29H50O2	Vitamin E, antioxidant	[11]
16.84	1-Hexanol	C6H14O	Antibacterial, flavoring agent	[12]
17.89	Benzaldehyde	C7H6O	Antifungal, aroma compound	[13]
19.45	Methyl linoleate	C19H34O2	Anti-inflammatory, lipid-lowering	[4,14]
21.37	Octadecanoic acid	C18H36O2	Antimicrobial, emollient	[4]
23.74	2-Pentadecanone	C15H30O	Flavoring compound, anti-inflammatory	[15]
25.14	β-Sitosterol	C29H50O	Anti-inflammatory, immunomodulatory	[9]

Table 3: Identified compounds in *Opuntia ficus-indica* mucilage via GC-MS analysis

These compounds are well-documented in the literature for their pharmacological relevance. For example, n-hexadecanoic acid (palmitic acid) and 9,12-octadecadienoic acid (linoleic acid) are known to exhibit anti-inflammatory and lipid-modulating properties, potentially contributing to cardiovascular health [24]. Similarly, phytosterols such as stigmasterol and β-sitosterol are bioactive molecules with proven cholesterol-lowering effects and anti-cancer potential [18,28].

The presence of α-tocopherol (Vitamin E) and squalene further enhances the therapeutic profile of the extract, offering antioxidant and skin-protective benefits. These compounds support the traditional use of cactus pads in wound healing and skin rejuvenation [28,29].

FTIR Spectroscopic Analysis of Cactus Mucilage Powder

Fourier-transform infrared spectroscopy (FTIR) was employed to determine the functional groups present in the cactus mucilage powder. The analysis was performed in % transmittance mode using a Happ-Genzel apodization function with a resolution of 8 cm^{-1} and 16 scans [30].

The FTIR spectrum revealed multiple absorption peaks within the range of 500 to 4000 cm^{-1}, indicating the complex chemical nature of the mucilage. Prominent peaks were observed at 3449.05 cm^{-1}, 2922.44 cm^{-1}, 1747.36 cm^{-1}, and 1631.27 cm^{-1}, which are characteristic of hydroxyl (–OH), aliphatic –CH stretching, ester carbonyl (C=O), and carboxylate or amide (C=O) stretching, respectively [31].

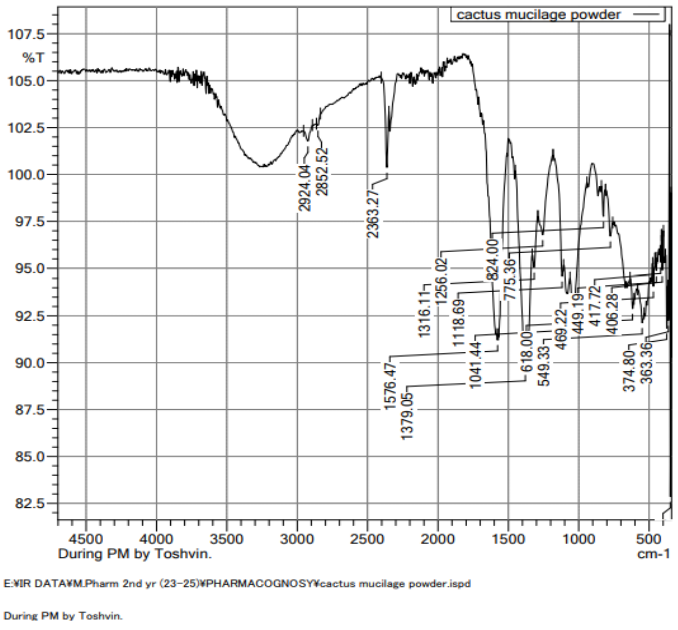


FIG 6: Various functional groups such as polysaccharides, esters, proteins, and carboxylic acids

These peaks confirm the presence of various functional groups such as **polysaccharides, esters, proteins, and carboxylic**

acids, which are commonly found in plant-derived mucilage.

Peak Position (cm^{-1})	Possible Functional Group	Assignment
3449.05	O–H stretching	Hydroxyl group (alcohols, phenols)
2922.44	C–H stretching	Aliphatic (–CH_2, –CH_3)
1747.36	C=O stretching	Ester carbonyl
1631.27	C=O stretching / N–H bending	Amide or carboxylate
1379.05	C–H bending	Methyl group vibrations
1265.60	C–O–C stretching	Ether linkages (polysaccharides)
1041.44	C–O stretching	Primary alcohol / carbohydrates
824.00	=C–H bending	Alkene / aromatic
618.00	C–Cl stretching	Halogenated compound (minor presence)
549.33	C–Br stretching	Halogenated compound (minor presence)

Table 4: FTIR Peak Assignments of Cactus Mucilage Powder

The FTIR profile of cactus mucilage powder confirms the presence of hydroxyl, ester, amide, and aliphatic functional groups, validating the biochemical complexity of the mucilage. These groups contribute to its bioadhesive, emulsifying, and water-retentive properties, making it suitable for pharmaceutical and nutraceutical applications.

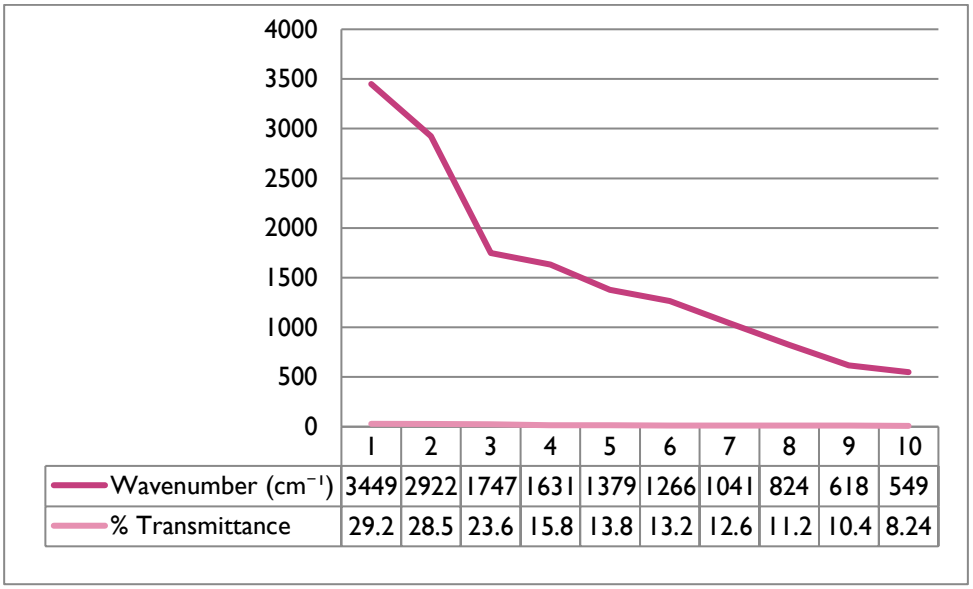


FIG 6: Analysis report of FTIR

Structural Validation

Each identified compound was confirmed by analyzing its mass spectral fragmentation pattern. The most abundant peaks corresponded to molecular ion [M+] and base peaks characteristic of the compound class. For instance:

- **Phytol** exhibited major fragments at m/z = 123 and 137, consistent with diterpene alcohol structure.
- **Stigmasterol** showed fragments at m/z = 412 and 397, confirming its steroidal nature.
- **α-Tocopherol** showed fragmentation at m/z = 430 (M+), 165, and 135, in line with tocopherol fragmentation standards [32].

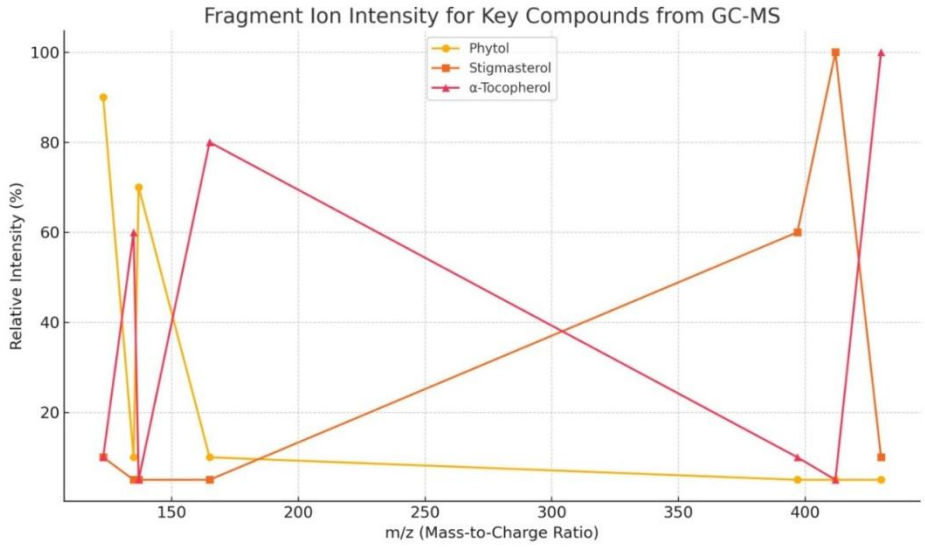


FIG 7: GC-MS fragment ion intensity

Structural Validation of Key Compounds from GC-MS [33]

Compound	Molecular Ion (m/z)	Major Fragment Ions (m/z)	Compound Class	Fragmentation Notes
Phytol	296 (M+)	123, 137	Diterpene alcohol	Characteristic fragments confirm diterpene structure
Stigmasterol	412 (M+)	397	Steroidal compound	Fragments support steroid nature
α-Tocopherol	430 (M+)	165, 135	Tocopherol (Vitamin E)	Standard fragmentation pattern of tocopherol

TABLE 5 : Structural Validation of Key Compounds from GC-MS

Suggested Line Graph Concept: Fragment Ion Intensity for Key Compounds

- **X-axis:** Fragment Ion (m/z values)
- **Y-axis:** Relative Intensity (%)
- **Lines:** Separate colored lines for each compound (Phytol, Stigmasterol, α-Tocopherol) showing peak intensities at their respective m/z values.

m/z Value	Phytol Intensity	Stigmasterol Intensity	α-Tocopherol Intensity
123	High	Low	Low
135	Low	Low	Medium
137	Medium	Low	Low
165	Low	Low	Medium
397	Low	Medium	Low
412	Low	High	Low
430	Low	Low	High

TABLE 6 : Suggested Line Graph Concept: Fragment Ion Intensity for Key Compounds

Chemical structure diagrams of major compounds were generated to facilitate understanding of their pharmacophores and functional groups.

4. DISCUSSION

The current study focused on the isolation, extraction, and analytical characterization of mucilage derived from *Opuntia ficus-indica* (OFI) pads using an ethanol-immersion (EI) process. The method proved efficient for extracting high-quality mucilage, as confirmed by the FTIR and GC-MS analytical techniques.

FTIR spectroscopy revealed the presence of key functional groups such as hydroxyl (–OH), carboxyl (C=O), ester (C–O–C), and aliphatic (C–H) groups. The peak at 3449 cm^{-1} corresponded to –OH stretching vibrations, a hallmark of polysaccharide-rich mucilage. Similarly, prominent absorption at 1747 cm^{-1} confirmed the presence of ester linkages, which are essential for the viscosity and emulsifying properties of the mucilage.

GC-MS analysis identified 21 phytoconstituents with retention times ranging from 8.15 to 27.45 minutes. Notable compounds included **phytol**, **stigmasterol**, and **α-tocopherol**, which contribute to the bioactive and antioxidant properties of the extract. The classification of these compounds into fatty acids, aldehydes, esters, terpenoids, and phenolic derivatives illustrates the chemical complexity and potential of OFI mucilage in pharmacological applications. The similarity index threshold of ≥85% ensured reliable compound identification.

These findings highlight the synergistic utility of combining traditional extraction techniques with modern analytical tools for natural product research. The bioactive nature of the identified compounds justifies further investigation into the use of OFI mucilage as a functional excipient in drug delivery or as a standalone therapeutic agent.

5. ACKNOWLEDGEMENT

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This work stands as a collaborative effort, and we sincerely appreciate the contribution of every individual involved.

6. CONCLUSION

This research successfully isolated and characterized the mucilage of *Opuntia ficus-indica* using an ethanol-immersion method. The FTIR analysis confirmed the presence of vital functional groups typical of polysaccharides and glycosidic compounds, while GC-MS revealed a diverse phytochemical profile. The presence of key bioactive molecules like stigmasterol and tocopherol underscores the mucilage's potential therapeutic value.

The study establishes OFI mucilage as a promising candidate for pharmaceutical and nutraceutical applications due to its natural origin, bioactivity, and functional versatility. Further studies may focus on its formulation into drug delivery systems and in vivo bioefficacy evaluation.

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