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Research Article

Design, Formulation & Characterisation of An Effective Anti-Fungal & Anti-Microbial Poly-Herbal Formulation

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ABSTRACT

Objective: Momordica charantia, Cassia, Calendula, and Abelmoschus are traditional medicinal plants known for their antifungal and antimicrobial properties. This study aims to formulate and evaluate a polyherbal gel combining these plants to enhance both antifungal and antimicrobial activity. Methods: The antifungal and antimicrobial activities of extracts from M. charantia, Cassia, Calendula, and Abelmoschus were assessed using the agar well diffusion method. A polyherbal topical gel was then prepared using Sodium carboxymethyl cellulose as a gelling agent at concentrations of 1%, 1.5%, and 2%. The gel's efficacy was evaluated against Candida albicans for antifungal activity and various microbial strains for antimicrobial activity. Parameters tested included organoleptic properties, pH, extrudability, spreadability, diffusion, and stability. Results: The combined extracts of M. charantia, Cassia, Calendula, and Abelmoschus demonstrated significant antifungal and antimicrobial activities. The gel formulation with optimal concentrations of these extracts exhibited a synergistic effect against Candida albicans and other tested microbes. Stability tests confirmed that all gel formulations remained stable after 30 days. Conclusion: This study provides the first scientific evaluation of a polyherbal gel combining extracts from M. charantia, Cassia, Calendula, and Abelmoschus for antifungal and antimicrobial use. The results indicate that this gel formulation is effective against fungal and microbial infections and maintains satisfactory physicochemical properties.

INTRODUCTION

In India, traditional medicine systems such as Ayurveda, Unani, Siddha, and various tribal practices have long incorporated herbal remedies, including those with antifungal properties. Ayurveda, in particular, is the most widely practiced and respected among these systems. Over the past few decades, there has been a notable shift in how herbal medicine is viewed in developed countries. Once seen as outdated and unreliable, herbs with antifungal benefits, such as neem and turmeric, are now recognized for their

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potential in treating fungal infections. This shift began in the 1960s with the 'hippie' movement, which emphasized natural living and alternative therapies [1,2]. The rise of the conservation movement and the establishment of companies that focus on eco-friendly, natural products also played a role in this change. Today, a variety of herbs with antifungal properties are available in different forms, including fresh, dried, and as ingredients in cosmetics, perfumes, and over-the-counter medications. While conventional medicine continues to advance, the resurgence of interest in herbal remedies highlights their historical importance and effectiveness. For most of human history, plants have been the primary treatment for a range of ailments, including fungal infections, and their relevance is growing once more as resistance to conventional antifungal treatments increases [2,3].

Plants like *Momordica charantia* L., *Cassia fistula* L., *Calendula officinalis* L., and *Abelmoschus moschatus* Medik. have been utilized for their medicinal properties across various traditional systems.

- *Momordica charantia L.* (Bitter melon) is known for its potential in managing diabetes and its anti-inflammatory properties.

-*Cassia fistula L.* (Golden shower tree) is celebrated for its laxative effects and its role in treating digestive disorders.

- *Calendula officinalis L.* (Marigold) is widely used for its anti-inflammatory and wound-healing properties.

- *Abelmoschus moschatus Medik.* (Musk mallow) is recognized for its antimicrobial and anti-inflammatory benefits [4,5,6].

These plantsare rich in various phytochemicals such as flavonoids, saponins, and alkaloids, which contribute to their therapeutic effects. The study of these plants aims to explore their antifungal activities and their potential use in combined formulations to enhance efficacy. Gel formulations are particularly suitable for these applications due to their ease of application, prolonged contact time, and minimal side effects compared to other topical or oral treatments.

Antifungal medications, including azoles, polyenes, echinocandins, and pyrimidine analogs, are commonly used to treat denture stomatitis. However, the effectiveness of these treatments is diminishing, largely due to the development of antifungal resistance, varying resistance profiles among Candida species, and issues with patient adherence to treatment regimens [7,8].

There has been a growing shift towards using natural substances, such as phytochemicals, to address fungal infections. Advances in science have led to the identification of more pharmacologically active Ayurvedic medicines that offer effective treatment options. The beneficial effects of these herbal medicines are attributed to their bioactive compounds, including saponins, tannins, alkaloids, and flavonoids [9].

Terpenoids and sesquiterpenes are examples of compounds that work together to produce beneficial effects. Herbal treatments are increasingly favored for their compatibility with biological systems and their reduced side effects compared to synthetic medications.

For many years, essential oils and herbal extracts have been used in pharmaceuticals, alternative medicine, and natural therapies. These oils and extracts are well-known for their antimicrobial properties and other uses, such as in perfumes (cedarwood and rosewood), flavoring agents (lime and juniper oil), and preservatives (lemongrass oil).

Herbal preparations consist of active substances derived from one or more herbs, including various plant parts such as leaves, flowers, fruits, seeds, stems, bark, or roots, either fragmented or powdered. When multiple herbs are combined, the result is a mixed herbal product or polyherbal formulation [10,11]. These polyherbal

formulations benefit from the synergistic effects of their active constituents, often providing enhanced therapeutic outcomes and better patient compliance compared to single-herb formulations. Additionally, they are generally more affordable, eco-friendly, and safe.

Topical biphasic dosage forms offer properties between solid and liquid dosage forms and possess distinctive rheological properties. They are advantageous for their ease of application and prolonged presence at the site of application. Gels, in particular, are valued for their compatibility with various excipients, thixotropic nature, easy spreadability, and non-staining, greaseless characteristics.

Emulsion hydrogels, also known as emulgels, are typically created by either dispersing an oil phase into an aqueous phase or by inducing gelation within the oil phase of an emulsion. Oil-in-water (O/W) emulsions are commonly used for water-soluble drugs and cosmetic bases, while water-in-oil (W/O) emulsions are preferred for treating dry skin conditions. Polymers function as emulsifiers and thickening agents in these formulations [12,13]. By altering surface and interfacial tension, and enhancing the viscosity of the water phase, polymers help maintain the stability of the formulation. The gelling agents in the water phase are crucial in converting standard emulsions into emulgels, which offer several advantages over both novel and traditional drug delivery systems. Emulgels can be enhanced with various penetration enhancers, making them a superior option for topical drug delivery.

This study aims to develop and evaluate a polyherbal gel containing extracts from *Momordica charantia* L., *Cassia fistula* L., *Calendula officinalis* L., and *Abelmoschus moschatus* Medik. The focus is on comparing the antifungal activity and clinical efficacy of this gel against commercially available options for treating antifungal antimicrobial action [14,15].

METHODS & MATERIALS

2.1. Material selection

It was decided that the studies could be made for further research on those plants which are potentially anti-fungal and anti-microbial for fungal treatment as per prior art and traditional knowledge and practice.

Collection of plant materials, Identification and primary Processing of the materials

1. *Momordica Charantia L*. collected locally from the Bilaspur Market and store in glass container. Identity was confirmed by Dept. of Pharmacy, CEC SOP, Bilaspur, Chhattisgarh.Tinospora cordifolia (Guduchi) growing on neem trees collected from Koni village, Bilaspur. Identity was confirmed by Dept. Of Botany, CEC SOP, Bilaspur, Chhattisgarh. Stem immediately processes for cold Extraction.
2. *Cassia Fistula L.* collected from Bilaspur and Identity was confirmed by Dept. of Pharmacy, CEC SOP, Bilaspur, Chhattisgarh, Purified arial plant part with water &Distil water, sun dried & powder immediately sieve under 60 mesh. Store in air tightContainer.
3. *Calendula Officinalis L.,* collected from Ameri road, nearby Institute of Pharmacy & Technology, SVITS, Bilaspur. Leaves of the plant separated, purified with water & distil water and dried under sun. Powder immediately sieves under 60 meshes. Store in air tight container.
4. *Abelmoschus MoschatusMedik.,* collected from Herbal Garden, Koni, Bilaspur. Leaves of the plant separated, purified with water & distil water and dried under sun. Powder immediately sieves under 60 meshes. Store in air tight container.

2.2. Preparation of extract

Theaboveplantsandmodifiedherbalmaterialswere mixedindifferentcompositionandratiotogetextract formulations. The fresh leaves were collected, air-

dried in the shade, and then stored in polyethylene bags. The dried leaves were mechanically powdered. The powdered leaves were subsequently extracted using a hydro-methanol solution through the maceration method.

2.3. Preformulation study

2.3.1. Incompatibility Study

Identifying potential chemical or physical interactions is crucial as they can influence the drug's bioavailability and stability.

a) **Fourier Transform Infrared Spectroscopy**

Compatibility studies were conducted at room temperature using FTIR spectroscopy. This technique helped assess interactions between drugs, as well as between drugs and excipients or polymers used in the formulation.

2.4. Preparation of Polyherbal Gel

Plants are considered a valuable source of potentially beneficial compounds for developing new therapeutic medications due to their generally low risk of adverse effects. Topical gels, in particular, offer significant advantages over creams or ointments by delivering medication more quickly and effectively to the targeted area. This mode of administration is increasingly preferred for its efficacy. Gels can be formulated with various plant and herb extracts that possess specific therapeutic properties, enhancing their effectiveness. In this study, a polyherbal gel was formulated using an optimized 2% Carbopol gel base. The ethanolic extract was tested at varying concentrations (1%, 1.5%, and 2%), while the concentration of another component was consistently maintained at 5 mL in each gel formulation. Details of these polyherbal gel formulations are provided in Table 1.

Table 1: Formulation of Polyherbal gel

Ingredients	F1	F2	F3
Polyherbal extract	1%	1.5%	2%
Carbopol 940	2%	2%	2%
Propylene glycol	5ml	5ml	5ml
Methyl paraben	0.15gm	0.15gm	0.15gm
Propyl paraben	0.30gm	0.30gm	0.30gm
Triethanolamine	5ml	5ml	5ml
Water	q.s.	q.s.	q.s.

2.5. Phytochemical Evaluation Parameters

The alcoholic extract of plants was screened qualitatively for the presence of various phytoconstituents, such as flavonoids, proteins, amino acids, phenol, and organic acid.

2.6. Evaluation parameters: Evaluation Parameters for the Gel

Table 2: Physical appearance of the formulated gel

Characteristics	F1	F2	F3
Physical appearance	Transparent gel	Transparent gel	Transparent gel
Colour	Pale Yellow	Pale Yellow	Pale Yellow
Homogeneity	Absence of aggregates	Absence of aggregates	Slight aggregates



Figure 1: Prepared Polyherbal Gel Formulation

2.6.1. Homogeneity

Each gel was visually inspected for uniformity once it had set in the container. The appearance of the gels was assessed to ensure consistent texture and lack of any visible separation or clumping.

2.6.2. pH of the Gel

The pH level of the gel was measured using a digital pH meter to ensure it was within the desired range.

2.6.3. Extrudability

For the extrudability test, 20 grams of the gel formulation were placed into standard collapsible aluminum tubes, which were then sealed by crimping. The initial weight of each tube was recorded. The tubes were positioned between two glass slides and clamped securely. A 500-gram weight was applied on top of the slides, and the cap of the tube was removed. The gel that was extruded was collected, weighed, and the percentage of extruded gel was calculated.

2.6.4. Spreadability

To assess spreadability, the gel formulation was sandwiched between two glass plates measuring 5 cm x 2 cm. A 100-gram weight was evenly distributed over the plates to ensure uniform application of pressure. After removing the weight, any excess gel was scraped off. The two glass plates were then positioned at a 45° angle, with one plate held firmly in place by a clamp and the other allowed to move freely. A 20-gram weight was attached to the upper plate to facilitate its movement. The time taken for the upper plate to separate from the lower plate was recorded. The spreadability was calculated using the formula:

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

where (S) is the spreadability, (L) is the length of the glass plate, (W) is the weight tied to the upper plate, and (T) is the time taken (in seconds). The experiment was conducted in triplicate for accuracy, results are shown in Table 3.

Table 3: Measurement of pH, Viscosity, spread ability

Formulation code	pH	Viscosity (cps)	Spread ability (g/cm/s)
F1	5.9	1428	16.37
F2	5.7	1425	23.45
F3	5.8	1356	22.16

2.6.5. In Vitro Diffusion Study

An in vitro diffusion study was conducted using a Franz diffusion cell with a 25 ml volume. A 1-gram portion of the gel was applied evenly onto the surface of an egg membrane within the cell. The receptor chamber was filled with freshly prepared phosphate buffer (pH 5.8), and the solution was stirred with a magnetic stirrer. At specified time intervals, 1.0 ml aliquots were withdrawn from the receptor chamber and replaced with fresh buffer solution. The samples were analysed for drug content using a UV-visible spectrophotometer at 280 nm and 342 nm for eugenol and piperine, respectively, after appropriate dilution. The cumulative amount of drug released through the egg membrane was calculated as a function of time.

2.6.6. Antifungal Activity

The antifungal activity of hydro-methanolic leaf extracts of *Piper betel* and *Piper nigrum* incorporated into a gel was evaluated. A fungal culture of *Candida albicans* was spread on a plate with Potato Dextrose Agar media. Wells were filled with different concentrations of the leaf extract (1 mg/ml) in dimethyl sulfoxide (DMSO) and a standard solution of 2% ketoconazole. The plate was incubated at room temperature for 2-3 days. The zone of inhibition around each well was measured in millimetres.

2.6.7. Stability Study

Both placebo and medicated gels were assessed for their thermostability.

3. RESULTS AND DISCUSSION

3.1. Organoleptic Evaluation of Extract

Organoleptic evaluation is a qualitative assessment method where the unique characteristics of substances, especially plant-based drugs, are examined using the senses—sight, smell, taste, touch, and hearing. This approach involves recording attributes such as size, shape, colour, and texture to provide a comprehensive description of the extract's sensory qualities, results are shown in Table 4.

Table 4: Evaluation of organoleptic characteristics of the extract

Characteristics	Observation
Physical state	Semisolid
Colour	Green
Odor	Characteristics
Taste	Characteristics

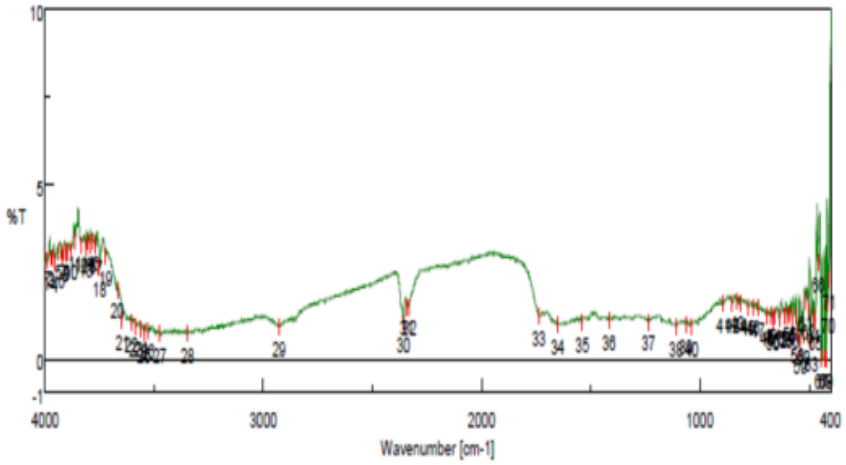
3.2. Phytochemical Analysis of the Extract

Phytochemical analysis plays a crucial role in identifying and characterizing various plant-derived compounds. This process involves examining and isolating different classes of phytoconstituents present in plant extracts. Such analysis helps in determining which compounds are most prevalent and in identifying bioactive substances that may be useful in developing therapeutic agents. Key phytochemicals such as alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, and terpenoids are important for their industrial and medicinal applications. For the *Vigna radiata* extract, the phytochemical analysis detected the presence of flavonoids, phenolics, and other constituents known for their antimicrobial, antidiabetic, and antihyperlipidemic properties. Detailed results are summarized in Table 5.

Table 5: Phytochemical analysis results of the polyherbal extract

Constituents	Test	End Point	Results
Flavonoids	Ferric chloride Lead Acetate	Green to yellow precipitate	++
Protein	Xanthoprotein Ninhydrin	Yellow precipitate	++
Amino acids	Ninhydrin Tyrosine	Dark Red	++
Phenol	Ferric chloride	Blue or red yellow	++
Organic acid	Phosphoric acid	Light Yellow precipitate	++

3.1. Incompatibility study: The pre-formulation study was carried out by FTIR method. The results were shown below.



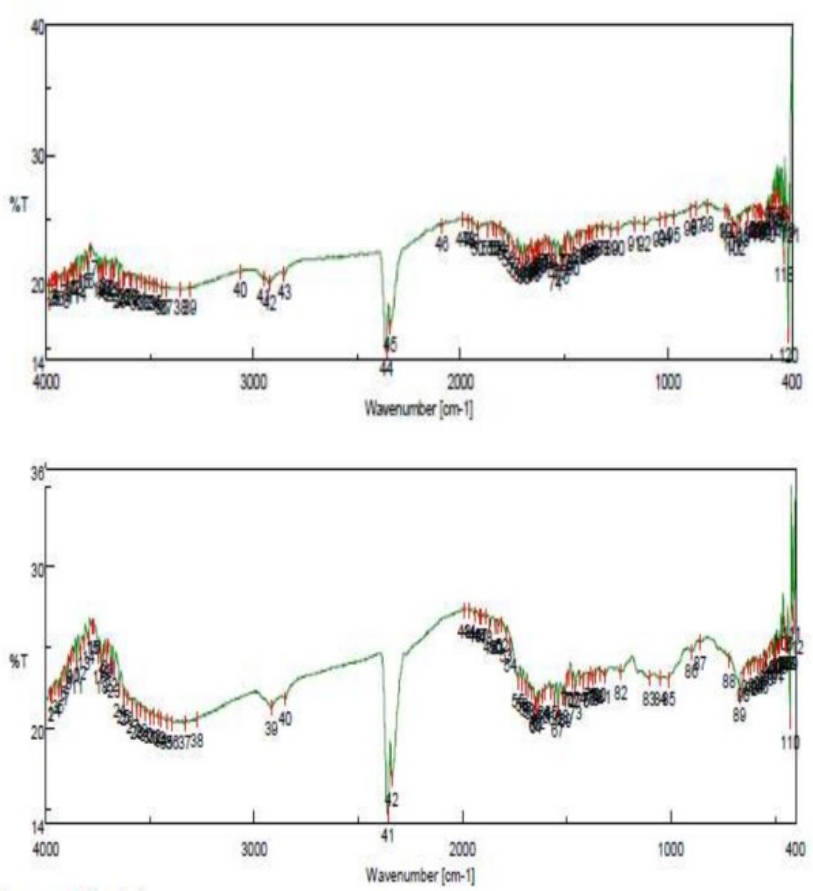


Figure 2: FTIR Graph (Poly Extract)

Antimicrobial Activity of the Extract

The antimicrobial activity of the gel was assessed by measuring the diameter of inhibition zones (in mm), which reflects the efficacy of the antimicrobial agents. The gel's effectiveness against acne-causing organisms, such as *Staphylococcus aureus* (ATCC-6538P), *Escherichia coli* (ATCC-8739), and *Candida albicans* (ATCC-18804), was evaluated and compared with standard antibiotics, including Gentamicin (10 µg) and Fluconazole (25 µg). The observed antimicrobial effects are likely attributed to the high flavonoid content in the extract, which contributes to the gel's efficacy. Additionally, the gel's antioxidant properties, which help prevent photodegradation and oxidative damage, further enhance its stability and effectiveness. The presence of polyphenols in the extract may also contribute to anti-acne activity by affecting mitochondrial permeability transition pores (mPTP). Furthermore, the antioxidant properties of the gel protect epidermal cells from damage induced by UVA radiation, partly due to the presence of orthophosphoric acid, detailed results are summarized in Table 6.

Table 6: Anti-microbial activity of the polyherbal extract

Sample	Staphylococcus aureus (ATTC-6538P)	Escherichia coli (ATCC-8739)	Candida albicans (ATCC-18804)
Polyherbal gel (F2)	12.3	13.5	12.6
Gentamicin (10 mcg)	22.5	22.6	-
Fluconazole (25 mcg)	-	-	22.5

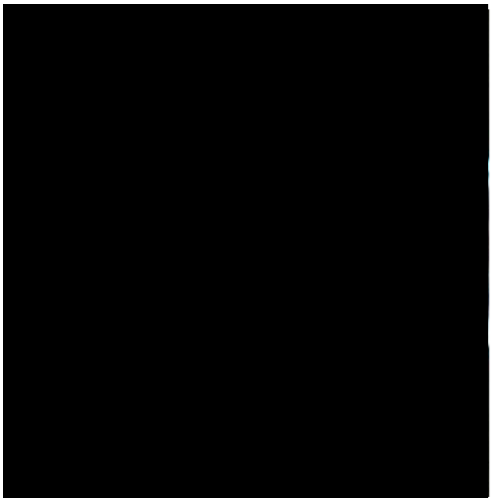


Figure 3: Zone of inhibition of Momordica Charantia L., Cassia Fistula L. extract towards Staphylococcus aureus (1) 12.4 mm, (2) 12.3 mm, (3) 12.1 mm and (c) control. Gels 2023, 9, x FOR PEER REVIEW

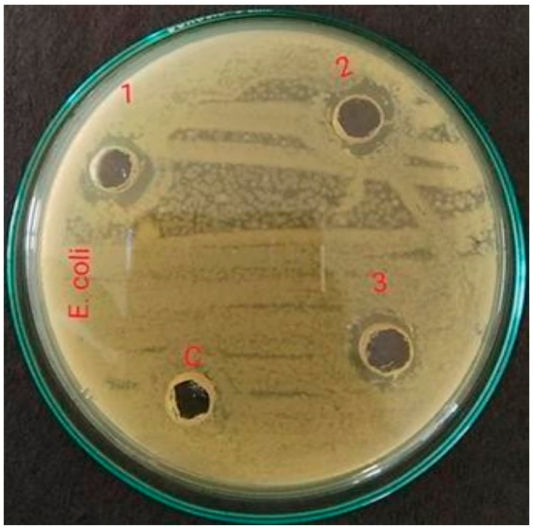


Figure 4: Zone of inhibition of Calendula Officinalis L., Abelmoschus Moschatus Medik., extract toward Escherichia coli (1) 13.2 mm, (2) 13.1 mm, (3) 13.1 mm and (c) control.



Figure 5: Standard Gentamycin on Staphylococcus aureus at 10 mcg in duplicate at ZOI of 22.2 mm.

Gel strength analysis

The development of products such as gels, capsules, and contact lenses relies on factors like strength, flexibility, and rupture force. Gel strength is a key parameter for assessing a colloidal dispersion's ability to form and maintain a gel. In the gelatin industry, this is referred to as Bloom strength, which measures the amount of force, in grams, needed to compress a gelatin gel's surface by 4 mm using a standard 0.5-inch diameter probe. This metric indicates the force required to break the gel. For the polyherbal gel developed, the Bloom strength is 0.0163 kg, as detailed in Tables 6 & 7 and Figures 3,4 & 5. This characteristic impacts how effectively the drug can penetrate through the gel matrix.

Table 7: Test data of polyherbal gel strength

Gel Strength/ m value (g) Force 1	Force at target (cycle:1) (kg)	Radiant to positive peak (cycle:1) kg/s
0.696	0.015	0.003

CONCLUSION

A hydrogel formulation combining hydro-methanolic extracts of *Piper betle* (betel leaf) and *Piper nigrum* (black pepper) (1:1) was created using sodium carboxymethyl cellulose. This formulation exhibited satisfactory physicochemical properties. *Piper nigrum* demonstrated over 90% drug release within 24 hours, while *Piper betle* achieved over 70% drug release within the same period. Stability studies of the hydrogel formulations were positive. Antifungal testing against *Candida albicans*

indicated that both individual extracts and their combination exhibited antifungal activity. The observed antifungal effects are likely attributed to their alkaloid, phenol, and lignan content. Additionally, hydro-methanolic extracts of *Momordica charantia* (bitter melon), *Cassia fistula* (golden shower tree), *Calendula officinalis* (marigold), and *Abelmoschus esculentus* (okra) were also evaluated for their potential activities. *Momordica charantia* is known for its antiviral and antidiabetic properties, *Cassia fistula* has been recognized for its laxative and anti-inflammatory effects, *Calendula officinalis* is noted for its wound-healing and anti-inflammatory activities, and *Abelmoschus esculentus* is valued for its antioxidant and anti-diabetic properties. Further research is needed to elucidate the specific contributions of these phytoconstituents to the antifungal activity. This study represents the first scientific evaluation of a gel formulation combining *Piper betle* and *Piper nigrum* extracts for antifungal purposes. Overall, both extracts demonstrated strong antifungal properties, and their methanolic hydro extracts are suitable for formulation as hydrogels with desirable physicochemical characteristics.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none.

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