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Green Synthesis And Evaluation Of Antifungal Gel From The Leaves Of Aclypha Indica

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INTRODUCTION

Herbal medicines play a pivotal role in global healthcare, with an estimated 80% of the world's population relying on plant-based treatments for their primary health needs. Traditional medicinal systems like Ayurveda, Unani, and Siddha utilize plant-derived compounds for treating a wide range of diseases. In the modern era, plant-based medicines continue to contribute to drug discovery, with approximately 25% of modern medicines derived from plants. The use of herbal medicines, particularly in developing countries, is valued at over USD 100 billion globally and continues to grow by 6-8% annually.One such medicinal plant is Acalypha indica Linn., belonging to the Euphorbiaceae family, which is widely distributed across Asia, Africa, and South America. Studies have shown that Acalypha indica has potent medicinal properties, particularly in combating microbial infections[1]. According to recent research, about 30% of the population in tropical regions uses traditional remedies involving Acalypha indica for skin infections, respiratory conditions, and gastrointestinal issues. The global burden of fungal infections has also been on the rise, with Candida albicans and Aspergillus niger being among the most common fungal pathogens[2]. It is estimated that around 1 billion people globally suffer from fungal infections each year, with invasive fungal infections leading to over 1.5 million deaths annually. This study focuses on the green synthesis and evaluation of an antifungal gel formulated using the leaves of Acalypha indica. By adopting green extraction techniques, we aim to minimize environmental impact while harnessing the antifungal properties of the plant. The gel formulation is tested for antifungal activity against common fungal strain of Candida albicans . This studies have shown that plant-based antifungal agents can exhibit up to 60-80% efficacy compared to conventional treatments. The formulation is evaluated for its stability and physicochemical properties, with the potential to offer an eco-friendly alternative to synthetic antifungal medications, addressing both sustainability and health concerns. This work is aligned with the growing trend in natural product research, where the market for herbal antifungal treatments is expected to reach USD 2.5 billion by 2030, with a projected annual growth rate of 5%. This highlights the increasing demand for safer, plant-based solutions in combating fungal infections. Through this research, we aim to contribute to the development of a sustainable and effective antifungal gel that can cater to this demand.

Acalypha indica- Plant profile[3]:

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Figure 1 : *Acalypha indica*

Botanical name : *Acalypha indica L*
Family : Euphorbiaceae
Synonym : Acalypha spicata , Acalypha ciliate
Vernacular name :

Hindi	Tamil	English	Sanskrit
Khokli	Kuppaimeni	Indianacalypha	Harita manjari

Plant description :
Nature : annual herb
Height : 30cm1m
Shape : pubescent, leaves simple, alternate, petiolate, rhombic ovate.
Flowers : unisexual, monoecious, bracteate, capsules one seeded, seedovoid
Distribution : throughout India
Properties : bitter, acrid, astringent, thermogenic
Medicinal part : whole plant
Propagation : seeds
Medicinal uses : antibacterial, antifungal, anthelmintic,asthma , diuretic, emetic , expectorant etc..
Phytoconstituents : kaempferol, HCN(27mg/100g) , acalyphamide and other amides, quinine, sterols and cyanogenic glycosides.

MATERIALS AND METHODS:

Preparation of *Acalypha indica* Extract

Collection and Preparation:

Acalypha indica leaves were collected from Arulmigu kalasalingam college of pharmacy garden,Krishnankoil and the gathered plants were rigorously cleaned with distilled water to eliminate contaminants. The plants were then shade-dried for 10 days at room temperature. The dried plants were pulverized in an electric blender to create a fine powder

Extraction Process[4]:

100 grams of the powdered leaves were weighed and taken in a suitable container.
500 ml of ethanol was added to the powdered leaves and macerate for 48 hours with intermittent stirring. The mixture was filtered through a sterile filter paper and the crude extract were obtained. The filtrate was concentrated using a rotary evaporator at 40°C to remove the ethanol and a concentrated extract were obtained. The concentrated extract were stored in a refrigerator at 4°C until use.

Figure 2: Extract of *Acalypha indica*

Formulation of the Gel[5]

Preparation of HPMC Gel Base:

HPMC (hydroxyl propyl methyl cellulose) was allowed to soak for 24hours until fully hydrated and a gel like consistency is achieved.

Add sodium hydroxide to adjust the pH to a range of 6.0 to 7.0 and add DMSO (dimethyl sulfoxide) for further enhance gel permeability.

Incorporate 5% Propylene glycol into the gel to act as a humectant and maintain moisture. Add 0.1% Methylparaben as preservatives to prevent microbial growth.

Preparation of SCMC Gel Base:

SCMC (sodium carboxy methy cellulose) was allowed to soak for 24hours until fully hydrated and a gel like consistency is achieved. Add sodium hydroxide to adjust the pH to a range of 6.0 to 7.0 and add DMSO (dimethyl sulfoxide) for further enhance gel permeability. Incorporate 5% Propylene glycol into the gel to act as a humectant and maintain moisture.Add 0.1% Methylparaben as preservatives to prevent microbial growth.

Incorporation of Extract:

The concentrated *Acalypha indica* extract was added gradually into the prepared gel base with stirring continuously to ensure even distribution. Adjust the final volume of the gel to 10 gm with distilled water if needed. Stirred continuously until a homogeneous gel is formed. The prepared gel was transferred into sterile containers and stored at room temperature.

Figure 3: *Acalypha indica* gel

Table 1: Composition of *Acalypha indica* Antifungal Gel

INGREDIENTS%	H1	H2	S1	S2
CRUDE EXTRACT OF ACALYPHA INDICA(gm)	1	1	1	1
HPMC%	0.5	1	-	-
SCMC%	-	-	0.5	1
DMSO(ml)	0.5	0.5	0.5	0.5
METHYL PARABEN(ml)	0.2	0.2	0.2	0.2
PROPYLENE GLYCOL(ml)	1	1	1	1
WATER TO PRODUCE(gm)	10	10	10	10

Characterization of *Acalypha indica* Antifungal Gel[6]

Physicochemical properties:

1. Spreadability:

The spreadability of the gel was determined using two glass slides. A measured amount of gel was placed between the slides, covering an area of 6 cm. A 100 g weight was placed on the upper slide to form a thin layer of gel, after which the weight was removed, and excess gel was scraped off. The upper slide was then tied to a 20 g weight, and the time taken for the slide to slip off and travel 6 cm under the weight was recorded. The experiment was repeated four times, and the average time was calculated.

Spreadability was calculated using the formula:

S =ml/t

Where:

- S = spreadability
- m = weight (50 g)
- l = length (6 cm)
- t = time taken

2. Homogenecity:

The formulations were tested for the homogenecity and visual appearance after the gels have been set in the container. The results are tabulated.

3. pH measurement:

The pH measurements were done by using a digital type of pH meter by dipping the glass electrode completely into the gel system so as to cover the electrode. The measured values are presented on the table 2

4. Extrudability:

The gels were filled into collapsible tubes after the formulations were set in the container. The extrudability of the formulations has been checked and the results are tabulated.

Invitro drug release studies:

The release of phytochemical drugs from the gels was studied using a permeation apparatus. A glass cylinder (10 cm height, 3.7 cm outer diameter) served as the permeation cell with a cellophane membrane fixed to one end. Ten grams of medicated gel were placed in the donor compartment, which was immersed in a beaker with 100 ml of 7.4 pH phosphate buffer, agitated at 37°C ± 1°C. Samples (1 ml) from the receptor compartment were taken at 30-minute intervals over 6 hours and analyzed at 313 nm. The amount of drug released was calculated and plotted over time. Results are summarized in the tables 3(a-d)

Antifungal activity of formulated antifungal gel[8]

Preparation of Fungal Cultures:

Obtain fungal strain Candida albicans from a reliable culture collection.Cultivate the fungal strain on appropriate agar media (e.g., Sabouraud Dextrose Agar) and incubate at 30°C for 48 hours.

Invitro Antifungal Testing:

Perform an agar diffusion method (e.g., disk diffusion or well diffusion) to assess the antifungal activity of the gel (H2 Formulation). Prepare agar plates inoculated with fungal cultures and apply the gel formulations(S1,S2,H1,H2) & standard antifungal agent(clotrimazole)to the plates using sterile disks or wells. Incubate the plates at 25°C for 48 hours and measure the zone of inhibition around the gel application area.

RESULTS AND DISCUSSION:

The physicochemical evaluation of the antifungal gels formulated with *Acalypha indica* leaves revealed various performance characteristics, which are summarized in Table 2.

Spreadability:

- FORMULATION H1: 54.54
- FORMULATION H2: 70.58
- FORMULATION S1: 52.17
- FORMULATION S2: 46.15

This indicates that H1 gel formulation (1% HPMC) spread more easily, which is crucial for effective application.

Homogeneity: Visual inspection of the gels after setting indicated that all formulations were homogeneous.

pH Measurements:

- FORMULATION H1: 6.6
- FORMULATION H2: 6.8
- FORMULATION S1: 6.3

- FORMULATION S2: 6.2

Indicating that the gels are likely to be compatible with skin application, as shown in Table 2.

Extrudability: The extrudability tests, conducted after filling the gels into collapsible tubes, confirmed that all formulations could be easily extruded.

Table 2: Characterization of *Acalypha indica* Antifungal Gel

FORMULATION CODE	EXTRUDABILITY	SPREADABILITY	pH	HOMOGENECITY
H1	*	54.54	6.6	**
H2	**	70.58	6.8	**
S1	**	52.17	6.3	**
S2	*	46.15	6.2	**

**-GOOD
*-SATISFACTORY

In Vitro Drug Release Studies: The cumulative (%) of drug release of various formulations are listed below

- FORMULATION S1: 67%
- FORMULATION S2: 72%
- FORMULATION H1: 68%
- FORMULATION H2: 78%

This indicates H2 formulation shows highest cumulative % drug release.

TABLE NO : 3(a)(INVITRO DRUG RELEASE FOR S1) (0.5% SCMC + 0.5 % DMSO)

S.NO	TIME IN HOURS	CUMULATIVE (%) OF DRUG RELEASE
1	0.5	8
2	1	12
3	1.5	18
4	2	20
5	2.5	23
6	3	25
7	3.5	28
8	4	35
9	4.5	43
10	5	48
11	5.5	55
12	6	67

TABLE NO: 3(b) (INVITRO DRUG RELEASE FOR S2) (1% SCMC + 0.5 % DMSO)

S.NO	TIME IN HOURS	CUMULATIVE (%) OF DRUG RELEASE
1	0.5	8
2	1	10
3	1.5	14
4	2	20
5	2.5	26
6	3	28
7	3.5	32
8	4	38
9	4.5	43
10	5	51
11	5.5	62
12	6	72

**TABLE NO : 3(c)
INVITRO DRUG RELEASE FOR H1
(0.5% HPMC + 0.5 % DMSO)**

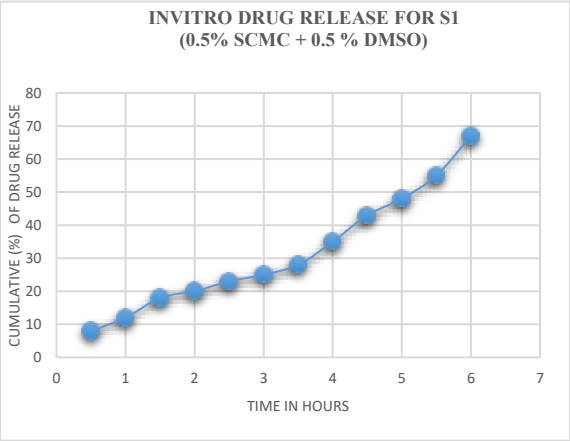
S.NO	TIME IN HOURS	CUMULATIVE OF DRUG RELEA
1	0.5	8
2	1	12
3	1.5	18
4	2	24

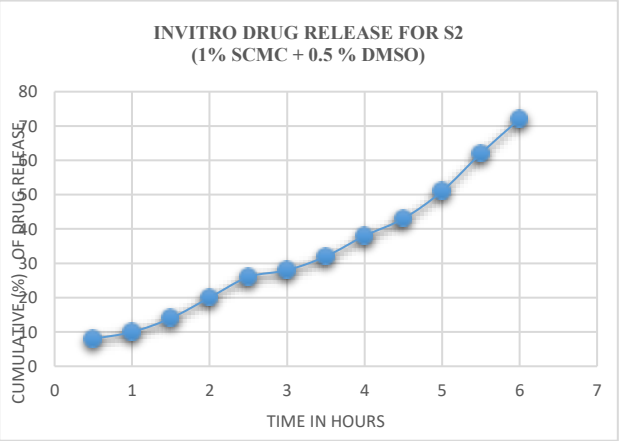
**TABLE NO : 3(d)
INVITRO DRUG RELEASE FOR H2
(1% HPMC + 0.5 % DMSO)**

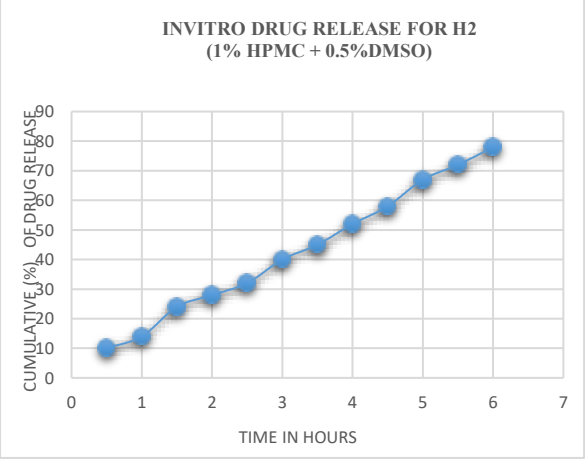
S.NO	TIME IN HOURS	CUMULATIVE (%) OF DRUG RELEASE
1	0.5	10
2	1	14

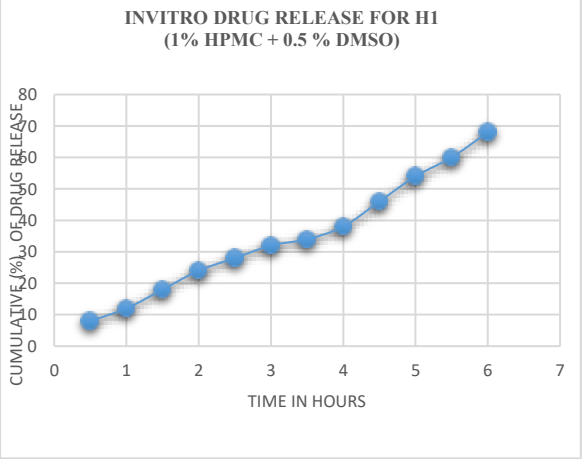
5	2.5	28
6	3	32
7	3.5	34
8	4	38
9	4.5	46
10	5	54
11	5.5	60
12	6	68

3	1.5	24
4	2	28
5	2.5	32
6	3	40
7	3.5	45
8	4	52
9	4.5	58
10	5	67
11	5.5	72
12	6	78

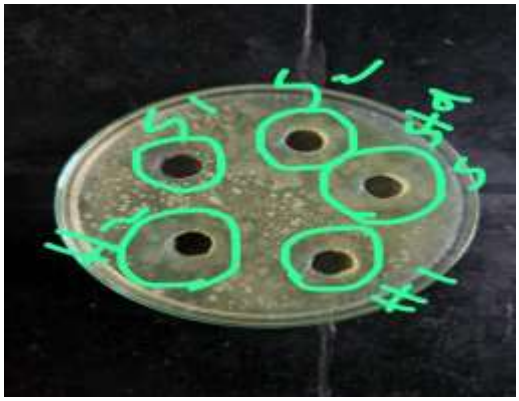








Antifungal Activity:Compare the zones of inhibition formulated gels with standard antifungal agents (clotrimazole) to determine the relative efficacy of the gel



Candida albicans- Zone of inhibition

I - S1 GEL FORMULATION (17mm)

II - S2 GEL FORMULATION(19mm)

III - H1 GEL FORMULATION(18mm)

IV - H2 GEL FORMULATION(20mm)

S - STANDARD ANTIFUNGAL AGENT(CLOTRIMAZOLE)(21mm)

figure 4: Zone of inhibition

This indicates that the H2 Formulation Acalypha indica gel is nearly as effective as the standard antifungal agents against Candida albicans fungal strains.

CONCLUSION:

The study demonstrates that the green synthesis of antifungal gels from Acalypha indica leaves is both effective and environmentally friendly. All formulations are having antifungal activity, Particularly the H2 formulation exhibit favorable physicochemical properties, including good spreadability, proper pH, and effective drug release profiles. Moreover, the antifungal activity of H2 formulation is nearly as effective as the standard antifungal agent(clotrimazole), confirming their potential as viable alternatives in antifungal treatments. Further research is recommended to explore the precise bioactive compounds responsible for these effects and to validate their clinical efficacy.

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