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Antifungal Activity of a Gel Formulation Containing Plant-Derived Compounds against Fungal Pathogens.

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Abstract:

Fungal infections pose a tremendous health burden globally, frequently handled with synthetic antifungal agents that may be related to unfavourable consequences and the emergence of resistant strains. Herbal remedies offer a promising alternative, providing a wealthy supply of bioactive compounds with powerful antifungal activity. This study aimed to prepare and compare a hydroalcoholic leaf extract gel for its antifungal capacity. Leaves of Musa Paradiasica had been extracted through hydroalcoholic extraction. The extract was characterised phytochemically, and its antifungal activity was assessed in vitro towards fungal pathogens. The extract was then incorporated into a gel form using carbapol 940 gelling agent The ensuing gel was evaluated for its rheological properties, textural characteristic, stability, and in vitro antifungal efficacy. The results proven that the preparation of gel from hydro alcoholic extract exhibited tremendous antifungal activity. The prepared gel showed suitable physical traits and stability. In vitro research showed the gel's potential to inhibit fungal infections, suggesting its capability as a topical antifungal agent. This examine highlights the capability of leaf Musa Paradiasica hydroalcoholic extract gel as a herbal and powerful alternative for treating fungal infections.

Keywords:HERBAL GEL,MUSA PARADIASICA HYDROALCOHOLIC LEAF EXTRACT,ANTIFUNGAL STUDY,CANDIDIASIS,ASPERGILLUS,CARBOPOL 940.

1.Introduction.

Fungal infections, affecting both superficial and internal organs, are a substantial health problem, especially in immunocompromised people. Common fungal pathogens like Candida, and Aspergillus species are responsible for a extensive range of infections, including skin, nail, and respiratory tract infections . While diverse synthetic antifungal medications are available, their prolonged use can lead to undesirable consequences, including hepatotoxicity and drug interactions, as well as the development of drug resistance.

The emergence of drug-resistant fungal strains and the adverse consequences associated with conventional antifungal agents have necessitated the exploration of alternative therapeutic modalities, particularly those leveraging phytochemicals derived from botanical sources.

The increasing prevalence of antifungal resistance and the undesirable outcomes linked to synthetic antifungal medications have prompted a paradigm shift towards investigating plant-based remedies as potential adjuncts or alternatives to conventional treatments.

Herbal drugs have a protracted records of use in traditional medicine for treating diverse illnesses, including fungal infections Plants are rich resources of bioactive compounds, along with alkaloids, flavonoids, terpenoids, and phenols, which frequently possess potent antifungal activity.

Musa Paradiasica belonging to the family Musacea has been traditionally used for its medicinal residences, which includes its said antifungal activity Previous studies have indicated the presence of various bioactive compounds in Musa Paradiasica leaves, contributing to its healing capability.Topical formulations, including gels, offer several advantages for treating localized fungal infections, imparting direct effect of the energetic ingredient to the affected area , minimizing systemic exposure, and improving patient compliance. This study aimed to put together a hydroalcoholic extract of Musa Paradisaica leaves and contain it into a gel formulation. The extract and the ensuing gel had been then evaluated for their physicochemical , balance, and in vitro antifungal activity in opposition to common fungal pathogens.

2.Material and methods

TABLE NO 1:MATERIALS AND METHOD

Sr no.	Reagent
1	MUSA Paradiasica extract
2	Carbopol940
3	Methylparaben
4	EDTA
5	Propylene glycol
6	Triethylamine
7	Distilled water

THE PHYSICOCHEMICAL PARAMETERS OF MUSA Paradiasica LEAF HYDROALCOHOLIC EXTRACT.

TABLE NO 2: PHYSICOCHEMICAL PARAMETERS OF MUSA Paradiasica LEAF HYDROALCOHOLIC EXTRACT.

SR NO	TEST	OBSERVATION	STANDARD	REMARK
1	COLOUR	BLACK	BLACK	PASSES THE TEST
2	ODOUR AND TASTE	AROMATIC	AROMATIC	PASSES THE TEST
3	SOLUBILITY	MISCIBLE	MISCIBLE	PASSES THE TEST
4	%MOISTURE CONTENT	2.25%W/W	1-3%W/W	PASSES THE TEST
5	%ASH CONTENT (AS PER IP)	2.47%W/W	2-7%W/W	PASSES THE TEST
6	NATURE	VISCOUS	VISCOUS	PASSES THE TEST

3. Procedure –PREPARATION OF HERBAL TOPICAL GEL

Mix Carbopol 940, methylparaben, and distilled water in a beaker. Stir the mixture constantly for 1 hour with a magnetic stirrer. Leave the mixture at room temperature for 24 hoursNeutralization Process* Mix EDTA and triethanolamine with the carbopol 940 base to allow neutralization. Preparation of Propylene Glycol Solution* Heat the propylene glycol to 65°C. Add distilled water to the hot propylene glycol solution slowly in a total volume of 12 ml.

Propylene Glycol Solution Incorporation* Pour the propylene glycol solution into the Carbopol 940 base dropwise while stirring pH Adjustment and Gel Base Trituration* Adjust the pH of carbopol940 base to a value of 6.7-6.9PH by using a mortar and a pestle.
Triturate the gel base to make it uniform. Incorporation of Herbal Extract* Combine the herbal extract with the produced gel basis and thoroughly mix to ensure compatibility and correct the PH.

4.Formulation table:

table no 3:formulation of gel

ingredient	quantity	role
musaparadisiaca	**30 mg**	**active ingredient**
carbopol 940	5 gm	gelling agent
methyl paraben	**0.6 gm**	**preservative**
edta	**0.8 gm**	stabilizer
trietyhanolamine (tea)	**q.s**	**gelation aid, ph adjuster**
propylene glycol	**15 ml**	**humactant**
distilled water	**upto 240 ml (q.s)**	**solvent**

4. EVALUATION OF GEL:

A] PHYSICAL EXAMINATION.
The prepared gel formulation was inspected visually for their colour,odour,appearance and consistency.

B] PH MEASUREMENT.
The PHof herbal gel was checked using PH meterby dispersing the glass electrode in gel and making sure it is covered in electrode .Prepared gel 1GM was mixed in distilled watertill uniform suspension is obtained.Note the PH.

C] RHEOLOGY STUDY OF GEL.
The viscosity and torque of all gels was determined using Brookfield digital viscometer (DV II + Pro viscometer).The
Gels (100 g) were taken in a beaker and subjected to increasing and decreasing shears using spindle no. 94 at different rotational
Speeds of 1, 5, 10, 20, 50, and 100 rpm at room temperature(30°C ± 2°C) in Difference between measured responses. The samples
were held at each rotational speed for 1 min [39] and then readings were recorded. The viscosity (η) and torque (Ƭ) was measured
in centipoises (cP).

D] SPREADABILITY STUDIES

The gels were tested for spreadability using Brookfield TextureAnalyzer (Model-CT3). The gels were taken in the sample
Holder, previously aligned with the probe (TA3/100). The probe traveled through the sample at a speed of 1 mm/s to a
distance of 25 mm at a trigger load of 1000g. The responseswere measured in terms of hardness and adhesive force.

E] SYNERESIS MEASUREMENT

For the syneresis measurement, gels were stored at 30°C ±2°C and 65%RH±5%RH for 1 month [40]. In this method,
Gels were centrifuged (Remi, Type C854/4, R-302) at 2000Rpm for 15 min. Supernatant was decanted and weight of residue was taken. Readings for syneresis was taken at , 15, and 30 days. The percentage syneresis was then calculatedusing following formula:
% Syneresis = Weight of separated liquid from gel /Total Weight of gel before centrifusing X 100

F] STABILITY STUDIES

The prepared gels were packed in aluminum collapsible tubes (5 g) and subjected to stability studies At 5°C, 25°C / 60% RH, 30°C /65% RH, and 40 °C /75% RH for a period of 3 months .Samples were
withdrawn at 15-day time intervals and evaluated for physical appearance, pH, rheological properties and Drug content (Harmonized Tripartite Guidelines.

G]ANTIMICROBIAL ACTIVITY(KIRBY-BAUER METHOD)

Antimicrobial activity test was performed by the commonly used Agar diffusion method which is designed to determine the smallest amount of the antibiotic needed to inhibit the growth of microorganisms.

Culture preparation-
One loop of mother culture inoculated in Sterile 15 ml NA broth and incubated at 37 ^{0} C for 24 hrs.
Procedure.
1.Take a sterile cotton swabs and dip it into a culture of Test organism suspension
2.Inoculate the entire agar surface of each plate first in horizontal and then in vertical direction to ensure the even distribution of the organism over the agarsurface using the swab.
3.Allow the agar surface to dry for 5 min.
4.Sterilize a cork borer by autoclaving or disinfect it by rinsing in alcohol followed by sterile water. Obtain a Mueller –Hinton agar plates and aseptically punch (8mm) holes in the agar using a cork borer. Using a wax pencil, mark the underside of the Petri to label the wells.
5.With the help of a micropipette add a test solution in the well. Repeat the procedure for all wells.
6.Incubate all plates at 37^{0}C for 24-48 hrs in an incubator.
Microorganisms used –
***Candidaalbicans* (NCIM 3100) AND *Aspergillus niger (ATCC504*)**

H] MINIMUM INHIBITORY CONCENTRATION BY BROTH DILUTION METHOD

Procedure:-

1.In one sterile test tube add 2 ml antibiotic compound to be tested know concentration.

2.Add 1ml sterile broth to all 6 test tubes. (NB for bacteria and PDB for fungus.)

3.Add 1ml DMSO in each tubes.

4.Add 500 ul, 250ul, 125ul, 62.5ul, 31.2ul sample respectively in tubes. After that add 50 ul culture in each tubes.

5.Set 1 tube as negative control without adding sample.

6.Incubated at 37^0 C for20 -24 hrs.

Note the result. Examine tubes for visible signs of bacterial growth.

The highest dilution without growth is the minimal inhibitory concentration.

Microorganisms used –

Candidaalbicans* (NCIM 3100) AND *Aspergillus niger (ATCC504)

Result and discussion:

A] PHYSICAL EXAMINATION.

TABLE NO 4: PHYSICAL EXAMINATION

Colour	**Brown**
Odour	**Characteristic**
Appearance	**Excellent**
Consistency	**Smooth and light to spread**

B]PH MEASUREMENT : THE PH OF GEL WAS FOUND TO BE NEUTRAL 6.5.

.C] RHEOLOGICAL STUDIES :

TABLE NO 5: RHEOLOGICAL STUDY BY USING BROOKFIELD VISCOMETER

RPM	TORQUE (CP)	%
1.0	70000CP	3.5
2.0	51000CP	5
4.0	33000CP	6.0
5.0	28000CP	7.0
10	17200CP	8.6
20	10400 CP	10.4
50	4920CP	12.3
100	2960CP	14.8

D]SPREADABILITY STUDIES

TABLE NO6:SPREDABILITY STUDIES

HARDNESS	**12.40GM**
ADHESIVE FORCE	**6.30G**

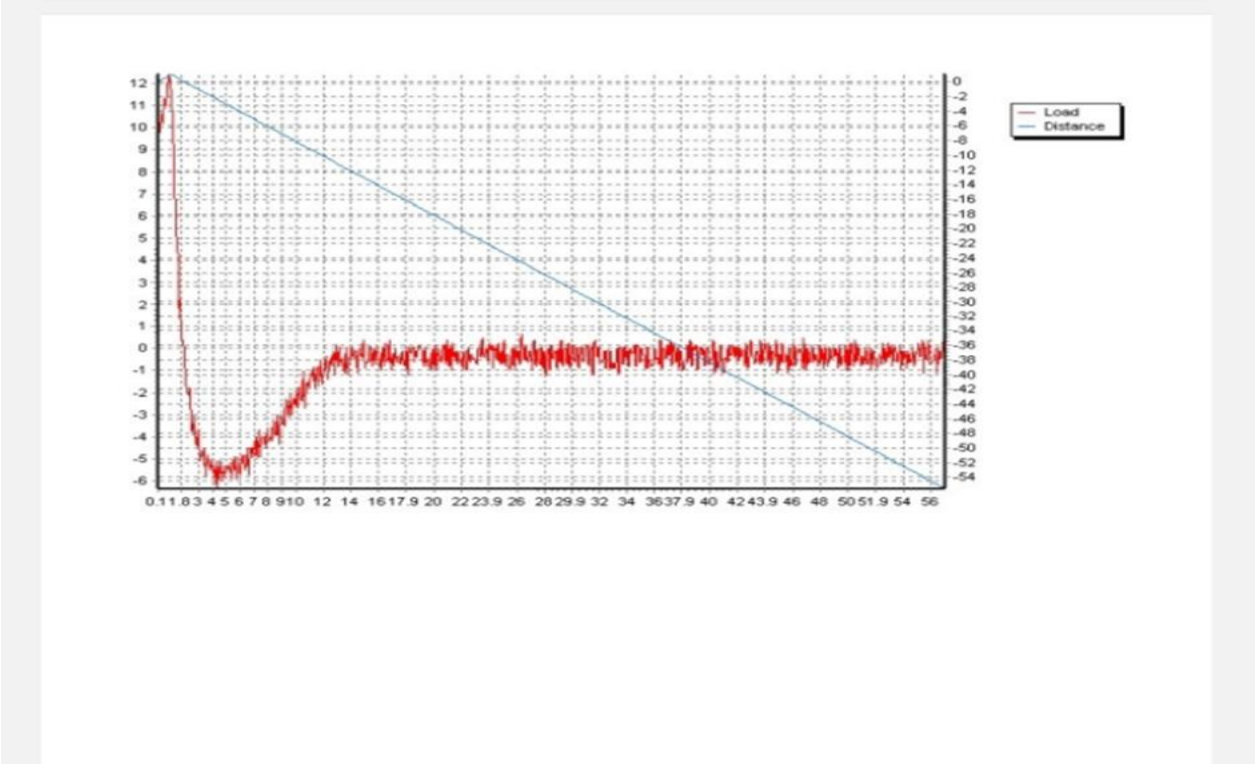

FIGURE NO 1: TEXTURE ANALYZER OF GEL FORMULATION[i]

E] SYNERESIS MEASUREMENT

TABLE NO 7 :SYNERESIS STUDY OF PREPARED GEL.

DAYS	%SYNERESIS
15DAYS	0.54
30 DAYS	1.52

F] STABILITY STUDIES

Physical Characteristics:
No discernible changes in physical appearance, including color, texture, or consistency, were observed across all samples stored at various temperatures and humidity levels.
PH Stability:
The pH values of the gel samples remained remarkably consistent, falling within the acceptable range of 6.7-6.9 throughout the 3-month study period.
Rheological Properties:
The viscosity and texture of the gel samples demonstrated remarkable consistency throughout the study, indicating no significant alterations in rheological properties.
Drug Content Stability:
The drug content of the gel samples remained well within the acceptable range of 90-110% of the labeled claim throughout the 3-month study period.

G] Antimicrobial activity

Examine all the plates for the clear zone of inhibition surrounding the discs/ well.
Measure the diameter of zone of inhibition in mm using a ruler on the underside of the plate Record the zone size .
the prepared gel significantly showed good antimicrobial activity against microorganisms *Candidaalbicans* (NCIM 3100) and *Aspergillus niger (ATCC504*)

TABLE NO 8: DETERMINATION OF ZONE OF INHIBITION OF FORMULATED GEL

	ZONE OF INHIBITION(MM) (VALUES ARE MEAN TRIPLICATE)					
	MICROGRAM STUDIED(SAMPLE IN DMSO) (CONC.MG/ml)					
SAMPLE	mgsample	1mlDMSO	ASPERGILLIUS NIGER(MM) (ATCC504)	MEAN(MM)	Candida albicans (NCIM3100)	Mean(MM)
Musa Paradiasica PREPARED GEL	1	1	13,13,14	13.3	11,13,12	12
DMSO NEGATIVE CONTROL	-	1	9,9,9	9	-	-
ITRACONAZOLE	1	1	20,20,19,20	19.75	18,16,17,16	16.75
FLUCONAZOLE	1	1	18,18,19,19	18.3	18,	19.5

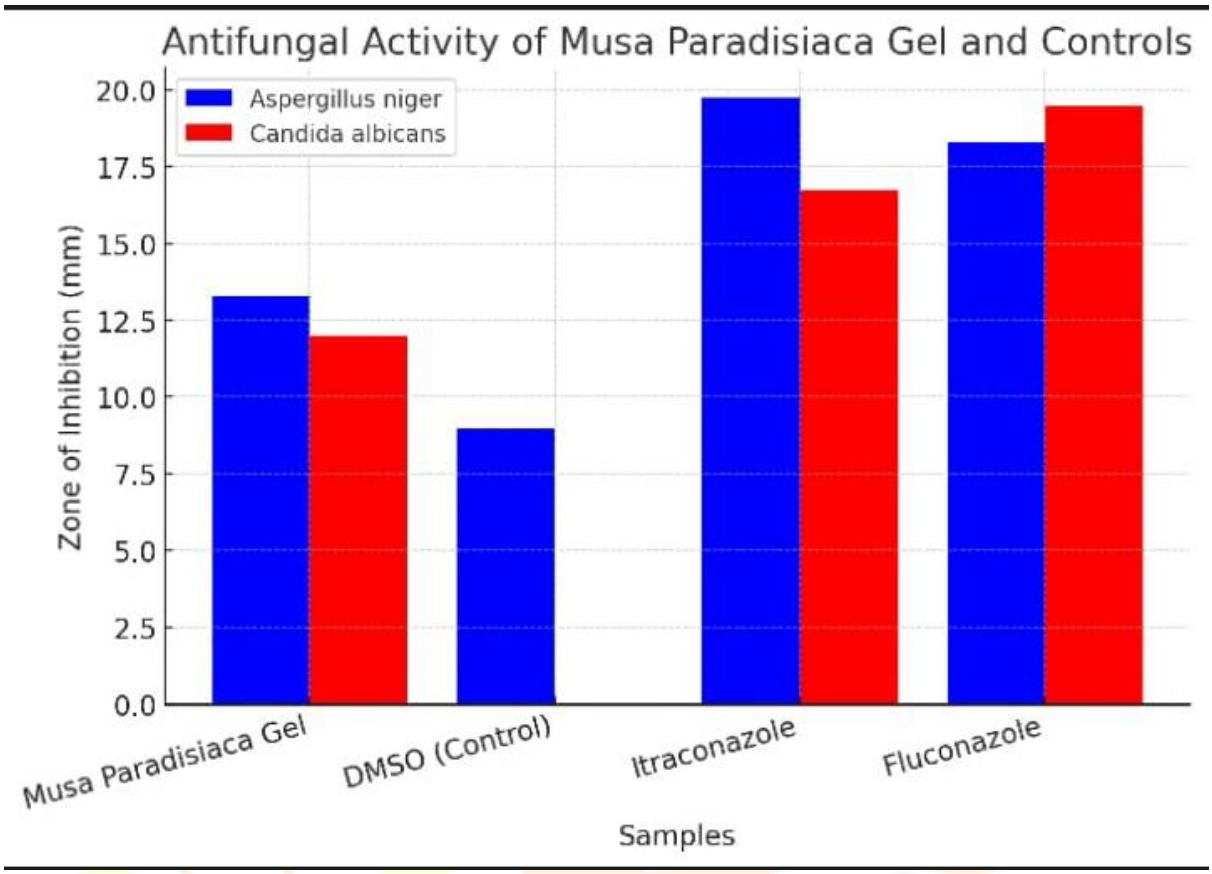

FIGURE NO 2: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITIONOF GEL

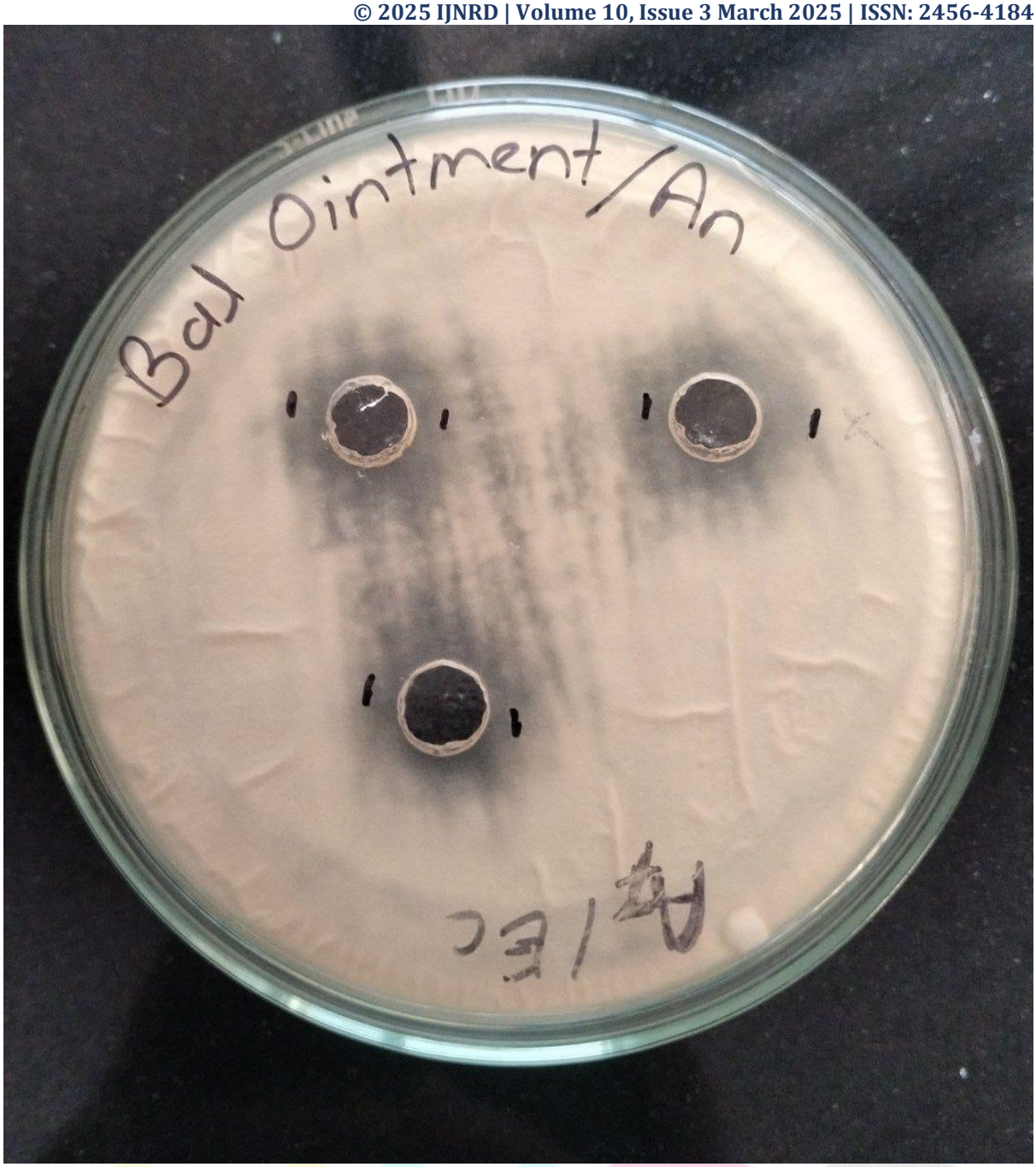

FIGURE NO3: ZONE OF INHIBITION OF PREPARED GEL AGAINST microorganisms *Aspergillus niger (ATCC504*)

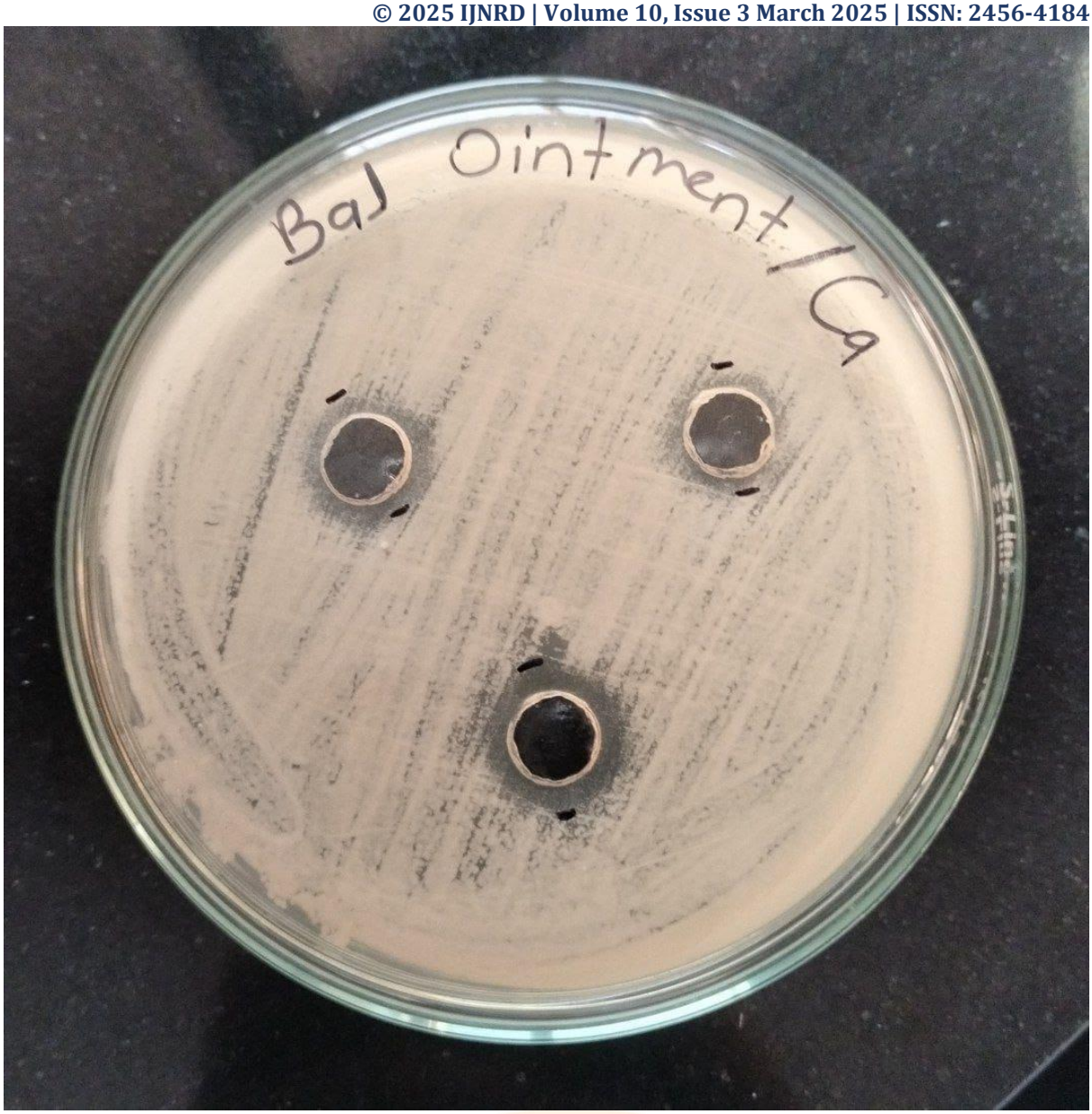

Figure no 4: ZONE OF INHIBITION OF PREPARED GEL AGAINST microorganisms *Candidaalbicans* (NCIM 3100)

H] MINIMUM INHIBITORY CONCENTRATION BY BROTH DILUTION METHOD

The hydroalcoholic extract of Musa Paradisiaca, which was prepared in gel form, was found to have antifungal activity against Candida albicans [NCIM3100] and Aspergillusniger [ATCC504]speciesminimum inhibitory concentration (MIC) of 500 µg/mL.

CONCLUSION:

In the quest for efficient and natural antifungal therapies, the present study has proven the high potential of the hydroalcoholic leaf extract of Musa paradisiaca. The herbal gel preparation has exhibited outstanding antifungal activity, which makes it a possible candidate for antifungal infection therapy. Detailed stability and safety analyses of the gel have been conducted, which

confirm its acceptability for human application. With the increasing issue of antifungal resistance globally, our research is a beacon of hope. With the natural benefits of nature, it is possible to deliver efficient, eco-friendly, and affordable therapies for the benefit of humanity. Even though further study and development are essential to ultimately unlock the full potential of the Musa paradisiaca hydroalcoholic leaf extract, the present study has undoubtedly opened the door to a promising future in antifungal therapy.

CONFLICT OF INTEREST : THE AUTHORS HAVE NO CONFLICTS OF INTEREST.

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