RESEARCH ARTICLE

Development and Evaluation of Antifungal Gel from Garlic Powder

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ABSTRACT

Introduction: Garlic has long been recognized for its antifungal properties, but could not find strong place in formulation and development due to the unstable nature of allicin, a sulfur-rich compound present in garlic, referred to as di-allyl thiosulfinate, plays a key role in inhibiting fungal growth.

Objectives: Purpose of the study was to use microwave-processed garlic powder in formulation and development of multipurpose antifungal gel using carbopol as a gelling polymer.

Materials and Methods: Antifungal Susceptibility testing was performed as per CLSI guidelines using processed garlic powder against *Candida albicans (MTCC 277)* and *Malassezia furfur (MTCC No-1374)* as prominent species involved in fungal infections of hair, skin and vagina. Carbopol 934 was used to develop and optimize the gel formulations. A skin irritation assessment was conducted using the HET-CAM test method.

Results and Discussion: The antifungal activity of gel was observed for 24, 48, 72 hours and further observations were continued for seven days' period and effective concentration was identified, wherein formulation prepared with 1% concentration of garlic powder was found to be prominently effective. The results from the HET-CAM test confirmed the non-irritating properties of the formulated product. In the positive control, coagulation occurred within 0.5 seconds of application, while no such reaction was observed with the developed antifungal gel formulation, indicating its lack of irritancy.

Conclusion: The developed antifungal gel formulation using microwave-processed garlic powder was found to be stable and effective for longer with no skin irritation observed.

Keywords: Microwave assisted drying, garlic, carbopol, HET-CAM, antifungal gel

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Conflict of interest: None

INTRODUCTION

Allium sativum L. (Liliaceae), also known as garlic, is a spice that is used in contemporary traditional healing and alternative medicinal approaches, where various remedies are utilized to treat a wide array of health issues. Because of its active components, alliin and allicin, Garlic is utilized for its properties as an antifungal, antibacterial, antiviral, anti-inflammatory, and antiseptic agent. Studies have suggested that garlic may also have medical applications. Published research suggests that garlic extracts have potential antibacterial properties wherein it is observed that many bacterial strains are significant contributors to global morbidity.[1,2] Research indicates that garlic bulbs are home to a diverse range of phytochemicals, among which are compounds abundant in sulfur, including sulphides, vinyldithiins, thiosulfinates (allicin), and ajoenes, which account for 82% of the sulphur content in garlic overall. Once the parenchyma is ruptured and the garlic is sliced, the enzyme allinase converts alliin, the primary cysteine sulfoxide, into allicin. Freshly milled garlic homogenates primarily contain three odoriferous ingredients: allicin, S-methyl cysteine-sulfoxide, and S-propyl cysteine-sulfoxide.[3,4] Garlic has been shown to have antibacterial qualities, however because allicin, an antibiotic component present in garlic bulbs, is unstable, clinical trials including garlic have never been conducted.[5] In a study on allicin's stability, researchers found it was most stable in aqueous extract between pH 5 and 6 but highly unstable below pH 1.5 or above pH 11. Higher temperatures, especially above 40°C and 70°C, accelerated its deterioration. Light had no significant impact. Storing garlic aqueous extract in a dark, cool environment at -20℃, with a pH of 5 to 6, is recommended for preservation.[6]

The objective of this study was to develop an antifungal gel utilizing carbopol, as the gelling agent and to enhance the stability of allicin, an antibacterial compound found in garlic, through a revised drying and processing method for garlic powder.[5,7] The developed gel formulation proves effective in treating various fungal infections, including those of the vaginal area, skin, hair, and scalp. This research seeks to establish a safe, long-lasting antifungal gel suitable for skin application.

EXPERIMENTAL

Preparation of Garlic Powder

The garlic powder was made by our own reported method using microwave and flower drying techniques[5,8] and Allicin quantification was conducted following the protocol established by our own laboratories.[7]

Antifungal susceptibility testing against *Candida albican*[9-11]

Broth Dilution for Yeast *(Candida albican*)

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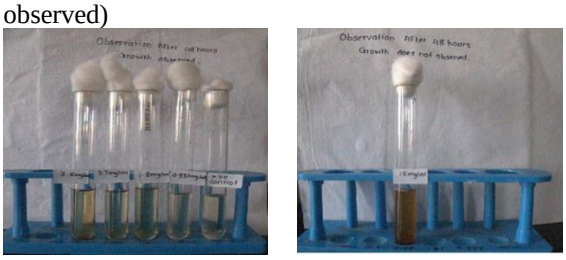
Table 1: Determination of MIC of *Garlic powder*

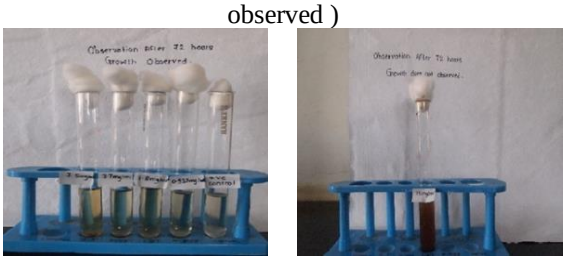
Organism			*Candida albicans* (MTCC 227)		
Sample			Garlic powder prepared by updated method		
Inoculum Standardization			(1-5×10^4 CFU/ml)		
% Transmittance			85%. (1× 10^6).....by digital colorimeter		
Tube no.	Concentration mg/ml	µg/ml	MIC readings After 24hrs	After48 hrs	After72 hrs
1	0.9375	2.37534375	0	3+	3+
2	1.875	4.7506875	0	3+	3+
3	3.75	9.501375	0	2+	2+
4	7.5	19.00275	0	2+	2+
5	15	38.0055	0	0	0
6	20	50.674	0	0	0
7	Positive growth control		4+	4+	4+
8	Negative growth control		0	0	0

Note : Considering yield of 2533.7 µg per gram i.e for 15 mg it is (38.0055 µg/ml) and so on. Table 1 shows the results of MIC value obtained and it was found to be 15 mg/ml. (38.0055 µg/ml) Photographs in (Figure 1) indicate the observations for MIC study.

Observation after 24 hours – (No growth was observed)



Observation After 48 hours (No growth was observed)



Observation After 72 hours (Growth was observed)

Figure1: Observation after 24, 48 and 72 hours of Incubation-*Candida albicans* (MTCC 227)

Principle

Testing for antifungal susceptibility via broth dilution is one of the often employed methods. In this procedure, the in vitro drug activity against the test organism is assessed in broth media at a known drug concentration. A specific amount of test organism inoculum is employed, along with a test tube containing a standard medium and an antifungal agent diluted twice serially. The endpoint of this assay

Table 2: (Worksheet 2B)- MFC Determination of Garlic powder prepared by updated method- *Candida albicans (MTCC 227)*

Plate no.	Concentration µg/ml	MFC readings after 24 hrs	after 48 hrs	after 72hrs
1	2.37534375	0	3+	3+
2	4.7506875	0	3+	3+
3	9.501375	0	2+	2+
4	19.00275	0	2+	2+
5	38.0055	0	0	0
6	50.674	0	0	0
10	Positive growth control	+ 4	+ 4	+ 4
11	Negative growth control	0	0	0

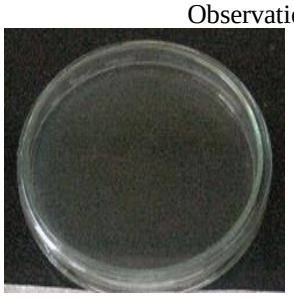
method is determined by the degree of reduction in the growth of the test organism achieved through the specified drug concentration.

[10] Based on preliminary trials and literature, the range selected for antifungal screening studies was 15-0.9375 mg/ml (15, 7.5, 3.75, 1.875, 0.9375 mg/ml). With a yield of 2533.7 µg of allicin per gram of garlic powder, the dilutions represent microgram values as follows: 38.0055, 19.00275, 9.501375, 4.7506875, and 2.3753437 µg/ml.

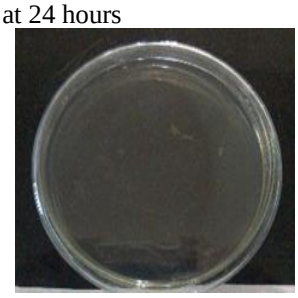
Stock Solution and Working Dilutions

To create working dilutions within the designated range of 15-0.9375 mg/ml, a stock solution of 150 mg/ml was created. This involved dissolving 1500 mg of microwave-assisted garlic powder in ten milliliter of sterile distilled water. Furthermore, the initial set of five test tubes was designated as Set I, covering a spectrum of concentrations from 150-9.375 mg/ml (i.e., 150, 75, 37.5, 18.75, 9.375 mg/ml). Each test tube, except the one labeled with 150 mg/ml, received 5 ml of Sabouraud Dextrose Broth (SDB). In tube 1, labeled with 150 mg/ml, 10 ml of the prepared

Observation at 24 hours

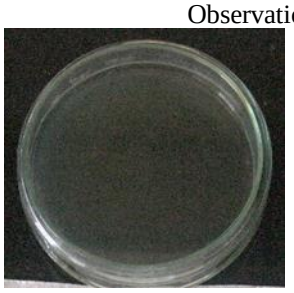


20 mg/ml (Growth was not observed)

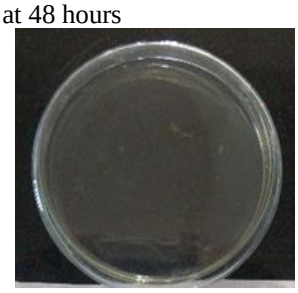


15mg/ml (Growth was not observed)

Observation at 48 hours

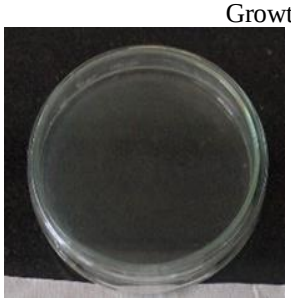


20 mg/ml (Growth was not observed)

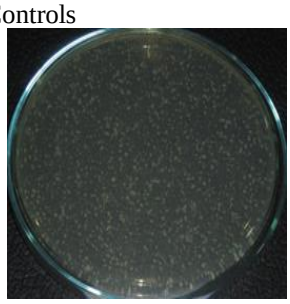


15mg/ml (Growth was not observed)

Growth Controls



Negative growth control



Positive growth control

Figure 2: Observations of the MFC Study- *Candida albicans (MTCC 227)*

solution containing 150 mg/ml drug concentration was added. Serial dilution was then performed from tube 1 to tenth number tube by transferring 5 ml of solution dispensed from tube 1 to tube 2, followed by tube 2 to tube 3, and so on, until the fifth test tube was reached.

Inoculum Standardization

The standardized inoculum suspension containing 1 x 10^5 to 5 x 10^5 CFU/ml of *candida albicans* was utilized throughout the study[10].

Inoculation and incubation

A second set, Set II, comprising five test tubes, was labeled with concentrations varying from 15 to 0.9375 mg/ml. Each test tube received 9 ml of broth solution and 0.9 ml of inoculum. Starting from the stock dilution tube containing 9.375 mg/ml, 0.1 ml of the drug solution was withdrawn and transferred to the test tube labeled with 0.9375 mg/ml, which already contained 9 ml of broth solution and 0.9 ml of inoculum. This process continued, sequentially diluting the inoculum from 1 x 10^5 - to 5 x 10^5 -CFU/ml suspension to 1 x 10^4 to 5 x 10^4 CFU/ml in each subsequent test tube. The procedure was replicated until all five test tubes were supplied with 0.1 ml of the desired concentration, ranging from 0.9375 to 15 mg/ml. Additionally, two empty tubes were designated for positive and negative growth controls, positioned at the extreme right of the setup. For the positive growth control, 0.9 ml of the final inoculum was combined with 0.1 ml of broth. For the negative growth control, 1 ml of broth was added to the tube. Inoculum (0.01 ml) was introduced into the tube containing solvent diluted in broth, serving as the solvent control in the study.

Reading MICs

MICs were interpreted at both 24 and 48 hours, and scored accordingly: 0 indicated optically clear results, 1+ represented slightly hazy outcomes, a noticeable reduction in turbidity compared to the drug-free growth control was denoted by 2+, Compared to the drug-free growth control, 3+ indicated a slight reduction in turbidity, and Compared to the drug-free growth control, 4+ indicated no reduction in turbidity. Results mentioned in (Table 1 and Figure1).

Determination of Minimum Fungicidal Concentration (MFC)

Using a micropipette, 100-µl samples were extracted from the MIC tube, each higher concentration tube, and the positive growth control tube. With the micropipette tip, each 100-µl sample was spread over half the surface of an SDA plate. These plates were then placed in an incubator at 35°C. Plate readings were conducted when colonies on the growth control plate became visible, typically within 24 hours, 48 hours and 72 Hours. Any plate containing five colonies or fewer was considered negative. The lowest concentration at which subculture resulted in a negative outcome was identified as the Minimum Fungicidal Concentration (MFC). Results mentioned in Figure 2 and Table 2.

Determination of MIC and MFC in Air dried Garlic Extract against *Candida albicans*

MIC Determination by Broth Dilution Method

To form a drug solution, twenty milligrams (20 mg) of air-dried garlic powder were dissolved in 20 ml of sterile distilled water, which was subsequently filtered through a filter paper. Based on literature indicating the MIC range of allicin against Candida albicans species, a corresponding range of concentrations was selected for the study. Serial dilutions of the drug solution were prepared in labeled test tubes, ranging from 0.015 mg/ml (15µg/ml) to 3.84 mg/ml (3840µg/ml). Inoculum preparation, inoculation, incubation, and MIC readings followed the previously described procedure. The results are given in Table 3.

Antifungal Susceptibility Testing against *Malassezia furfur*

MIC Determination by Broth Dilution Method for Species: *Malassezia furfur*

Axenic culture of *Malassezia furfur* (MTCC No-1374) was obtained from the Institute of Microbial Technology, Sector 39 (A), Chandigarh. Emmon's medium, also known as Modified Sabourd Dextrose Agar Medium, was utilized for the subculturing of *Malassezia furfur*. These strains were cultured on Emmon's Medium at 30^0 C. All the cultures maintained at 30 ± 2ºC in incubator for seven days. Innoculum standardized to $1x10^5$ to 5 x 10^5 CFU/ml was used during the study. In sterile tubes, 10 ml of medium

Table 3: Antifungal Susceptibility Testing of Air Dried Garlic Powder- *Candida albicans* (MTCC 227)

Organism	*Candida albicans* (MTCC 227)
Sample	Aqueous Garlic Extract.
Inoculum Standardization	($1-5\times10^4$ CFU/ml)
% Transmittance	85%. (1×10^6).....by digital colorimeter

Tube no.	Concentration (Garlic powder) µg/ml	Concetration of Allicin In mg/ml considering 1395 µg/gram yeild	MIC readings After 24hrs	After48 hrs	After72 hrs
1	25.6	35.71	4+	4+	4+
2	12.8	17.85	4+	4+	4+
3	6.4	8.92	3+	3+	3+
4	3.2	4.46	2+	2+	2+
5	1.6	2.23	-	-	-
6	0.8	1.11	2+	2+	2+
7	0.4	0.55	2+	3+	3+
8	0.2	0.27	4+	4+	4+
9	0.1	0.13	4+	4+	4+
10	0.05	0.06	4+	4+	4+
11	Positive growth control		4+	4+	4+
12	Negative growth control		0	0	0

1000 µg/ml

500 µg/ml

250 µg/ml

125 µg/ml

62.5 µg/ml

Negative Growth Control

Positive Growth Control

Figure 3: Antifungal susceptibility testing: Broth dilution Method *Malassezia furfur* (MTCC No-1374)

with inoculum was poured for the positive control, while 10 ml of medium alone was poured for the negative control.

Preparation of Stock Solution and working dilutions

In order to establish working dilutions spanning from 1000

Table 4: Antifungal Susceptibility Testing of updated Garlic Powder-*Malassezia furfur* (MTCC No-1374)

Organism	*Malassezia furfur* (MTCC No-1374)
Sample	.
Inoculum Standardization	(1-5×10^4 CFU/ml)
% Transmittance	85%. (1× 10^6).....by digital colorimeter

Tube no.	Concentration (In terms of garlic powder) µg/ml	Concentration in terms allicin content In µg /ml considering 2533.7 µg per gram yield	MIC readings		
			After 24hrs	After48 hrs	After72 hrs
1	1000	2.5337	2+	2+	2+
2	500	1.26685	0	0	0
3	250	0.63342	2+	2+	2+
4	125	0.3167	3+	3+	3+
5	62.5	0.1583	4+	4+	4+
11	Positive growth control		4+	4+	4+
12	Negative growth control		0	0	0

Table 5: Comparative MIC values

S.No	Sample	Species	MIC value(In terms of Allicin content)
1	Updated Microwave assisted garlic powder	*Candida albicans* (MTCC 227)	38.0055 µg/ml
2	Air dried garlic powder	*Candida albicans* (MTCC 227)	2.23 µg/ml
3	Updated Microwave assisted garlic powder	*Malassezia furfur* (MTCC No-1374)	1.26685 µg/ml

µg/ml to 62.5 µg/ml, a stock solution of 10,000 µg/ml was prepared. This involved dissolving 100 mg of microwave-assisted garlic powder in 10 ml of Emmon's nutrient broth media. This solution contained 25.337 µg/ml of allicin. From the stock solution, working dilutions were prepared by adding 1, 0.5, 0.25, 0.125, and 0.625 ml to 0.9 ml of inoculum, then diluting up to 10 ml of Emmon's broth media. This yielded concentrations of 1000, 500, 250, 125, and 62.5 µg/ml of garlic powder, equivalent to 2.5337, 1.26685, 0.633425, 0.3167, and 0.1583 µg/ml of allicin content, respectively. The turbidity score of each resulting solution was recorded according to Table 4 and Figure 3, and comparative MIC values were determined and presented in Table 5.

Development of Gel Formulation

Calculation based on the allicin content of 2533.7 µg in 1 gram of garlic powder revealed a MIC of 15 mg/ml (38.0055 µg/ml) for *Candida albicans* (MTCC 227) species and 1.26685 µg/ml for *Malassezia furfur* (MTCC No-1374). Considering minimum gel applications of 1-5 mg, the selected concentration range for batch optimization was 0.125%, 0.25%, 0.5%, and 1% gel formulations. Four formulations were prepared using 0.125, 0.25, 0.5, and 1 gram of garlic powder, with some containing preservatives

Table 6: Formulation compositions of the Screening batches.

Batches Ingredients	F1	F2	F3	F4
Garlic powder %	1%	1%	1%	1 %
Carbopol 934(%)	0.5	1	1.25	2
Triethanolamine(ml) Volume required to adjust pH 5-7	1.65	1.65	1.65	1.65
Methylparaben (mg)	0.2	0.2	0.2	0.2

Table 7: Results for preliminary screening batches

T. No.	Formulations (stored at 4°C)			
	F1	F2	F3	F4
1	22.27 ±0.0264	20.04 ±0.0057	23.21 ±0.268	21.94 ±0.0057
2	6.65 ±0.015	6.88 ±0.115	6.58 ±0.075	6.57 ±0.02
3	11899.33 ±1884.30	17321.33 ±2153.3	13657 ±7736878	18993 ±2215.30

*Mean± S.D., (n=3)

1. Spreadability test (gm.cm/sec)2.pH determination
3. Viscosity measurement (cps)

to assess stability and efficacy. Preliminary screening batches, ranging from 0.5% to 2% carbopol concentration, were tested. Among these, a carbopol 934 concentration of 1.25% was deemed suitable for further trials. The composition of the preliminary screening batches and results are detailed in Table 6, Table 7 and Figure 4 respectively in the Results and Discussion section.

Evaluation Parameters of Antimicrobial Gel

Determination of pH

The gel formulation, weighing 50 grams (50 g), was moved to a beaker to measure its pH using a digital pH meter. Prior calibration of the pH meter was conducted with buffer solutions of pH 4, 7, and 9. For optimal efficacy in treating skin infections, the pH of the topical gel formulation should

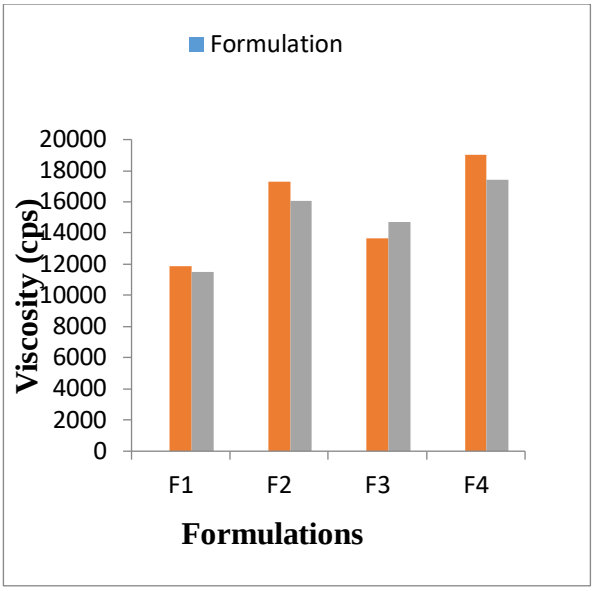


Figure 4: Viscosity of Preliminary Screening batches

ideally fall within the range of 5 to 7. Results from the pH measurements are summarized in Table 7.

Assessment of Viscosity

The viscosity of each formulation was assessed using a Brookfield viscometer equipped with a T-bar spindle (T 95).[13] The results are given in Table 7 and Figure 4

Spreadability Test[14]

To assess the spreadability of the gel formulations, two glass slides of standard dimensions (10cm×10cm) were chosen. One slide had 0.1 grams of gel formulation applied to it, and then another slide was placed on top to sandwich the gel between them. The slides were pressed together to remove any trapped air, and excess gel was wiped away. One slide was secured in place with a clamp, while the other had a weight of 32 gm attached to it. The time taken for the upper slide to detach completely from the lower slide was noted. Spreadability (s) was calculated using the formula: s = (m × l) / t, where 'm' represents the weight tied to the upper slide, 'l' denotes the length of the glass slide, and 't' signifies the time taken. Results from these measurements are presented in Table 7.

Experimental Batches for antifungal and stability Evaluation

On the basis of previous screening batches, further four formulations were designed with varying concentrations of garlic powder. Keeping rest of the concentrations of ingredients constant. The formulations were evaluated for antifungal activity and stability in terms of long term effect on priority. Formulation compositions of the Experimental batches shown in Table 8. Initially, propyl paraben (0.00005% or 0.05 mg in 100 ml) and methyl paraben (0.002% or 0.2 mg per 100 ml) were dissolved in water at 80ºC. Meanwhile, a precisely measured amount of carbopol 934 (1.25%) was dissolved in water at 40ºC with continuous stirring for 30 minutes. Triethanolamine (approximately 1.65 ml) was subsequently added to adjust the pH of the gel base to the range of 5–7. Since the skin's pH is around 5.5, this pH range is considered suitable to prevent skin irritation. Subsequently, an accurately weighed quantity of garlic powder, prepared using the updated method, was dissolved in water. This garlic powder solution was gradually incorporated into the gel base with constant mixing with stirring until a uniform gel consistency was achieved, typically taking around 5 to 10 minutes.

Antifungal Study of Experimental batches

First, 6500 mg of Sabouraud dextrose agar were measured and then moved into a 250 ml conical flask containing 100 ml of distilled water. Heat was applied to dissolve the agar completely, and then the mixture was sterilized for 15 minutes at 121°C at 15 Ib pressure in an autoclave for about 20 minutes. Once sterilized, it was cooled to room temperature. Standardized inoculum was utilized as discussed previously. The Sabouraud dextrose agar medium was poured into sterile petri plates and allowed to cool at room temperature until it solidified. Following this, a loopful suspension of fungal species was inoculated onto each petri dish using a sterile nichrome wire loop. After inoculating the plates, we spread 1 mg of garlic powder-containing gel, without preservative, onto each dish using a sterile spreader. The petri plates were then incubated for up to 72 hours at 37°C in an incubator. Positive and negative growth controls were included to monitor growth. All samples were observed for effective antifungal activity at 24, 48, and 72 hours, with further observations continued for a period of seven days. The effective concentration was identified based on these observations. Results are depicted in Figure 5

Skin irritation study

The HET CAM test[15] evaluates the potential irritancy of a test substance by examining its toxicity on the chorioallantoic membrane of a chicken egg, focusing on the onset of hemorrhage, coagulation, and vessel lysis, with results scored to classify irritancy. Solvents include 0.9% NaCl, 1% SDS, and 0.1 N NaOH. Negative controls use 0.9% NaCl; positive controls use 0.1 N NaOH. Test

Table 8: Formulation compositions of the Experimental batches

Batches Ingredients	F1	F2	F3	F4
Garlic powder %	0.125% (316.7125 µg Allicin)	0.25% (633.425 µg Allicin)	0.5% (1266.85 µg Allicin)	1 % (2533.7 µg Allicin)
Carbopol 934(%)	1.25	1.25	1.25	1.25
Triethanolamine(ml)	1.65	1.65	1.65	1.65
Methylparaben (mg)	0.2	0.2	0.2	0.2
Propylparaben(mg)	0.05	0.05	0.05	0.05
Purified water upto…(ml)	100	100	100	100

substance (tablet dissolved in 10 ml 0.9% NaCl) is prepared. Three eggs per group, including negative and positive controls, as well as the test substance, are utilized. Eggs are incubated, candled, and CAMs prepared. Negative control (0.9% NaCl) and positive control (0.1 N NaOH) are applied, incubated. Test substance (0.3 ml) is applied, and reactions observed for 300 seconds, noting hemorrhage, vascular lysis, and coagulation times.

Evaluation of Test Results

In the evaluation of HET CAM test results, the irritation potential of the test substance is determined by cumulative numerical scores for lysis, hemorrhage, and coagulation. These scores are derived from observations at 0.5, 2, and 5 minutes. Lysis is scored 5, 3, and 1 at these respective time points, while hemorrhage is scored 7, 5, and 3, and coagulation is scored 9, 7, and 5. The maximum cumulative score achievable is 21, with higher scores indicating greater irritation potential. (ICCVAM Test Method Evaluation Report *Appendix B*3). Results are discussed in Figure 6

RESULTS AND DISCUSSION

Preparation of Garlic Powder

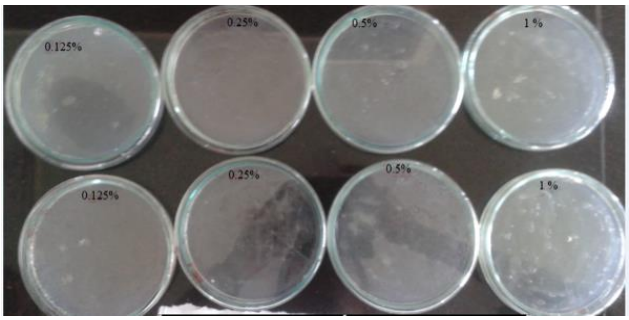
Garlic powder was prepared by the method described by us in our previous literature using microwave drying and floral drying techniques[5]. Allicin yield. 2533.7 µg per gram of garlic powder was considered for preparing stock and working dilutions for the antifungal susceptibility testing study.

Antifungal Susceptibility Testing

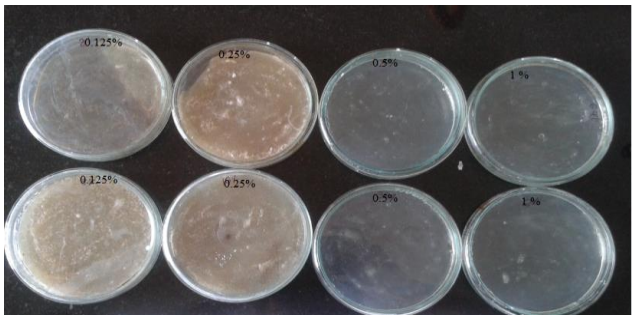
***Candida albican* (MTCC 227): Broth Dilution Method**

Determination of Minimum Inhibitory Concentration (MIC)

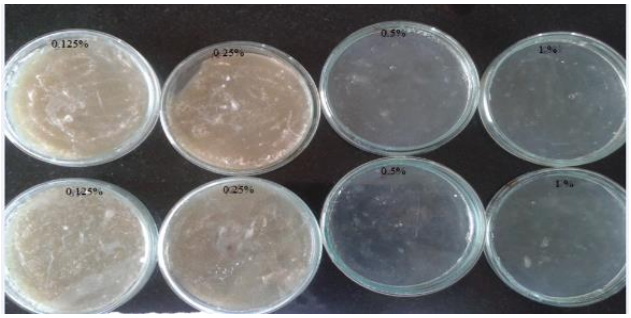
Determination of Minimum Fungicidal Concentration (MFC)-*Candida albicans*



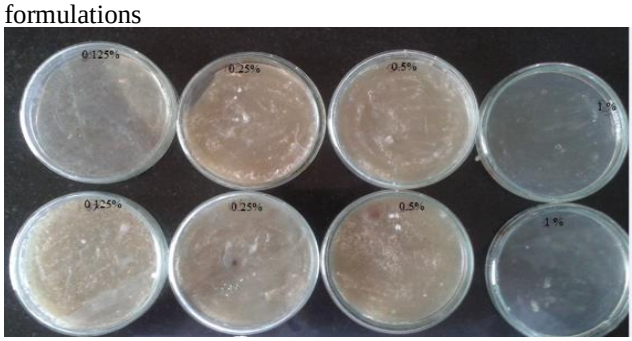
Antifungal testing of gel formulation No growth observed after 24 hours in all the four concentrations under trial.



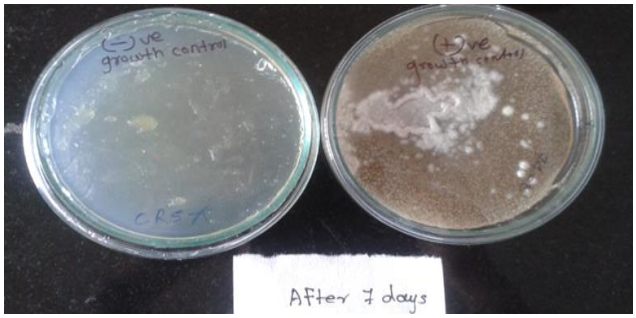
Antifungal testing of gel formulation No growth observed after 48 hours in 0.5 and 1% formulations where growth was observed after 72 hours in 0.125% and 0.25% gel formulations



Antifungal testing of gel formulation No growth observed after 72 hours in 0.5 and 1% formulations where growth was observed after 72 hours in 0.125% and 0.25% gel formulations



Antifungal testing of gel formulation: No growth observed after 7 days in 1% formulations where growth was observed after 7 days in 0.125% and 0.25% and 0.5% gel formulations.



Negative and Positive Growth Control plates. No growth was observed in the negative growth control plate indicating a controlled sterile environment throughout the study

Figure 5: Antifungal testing of gel formulation

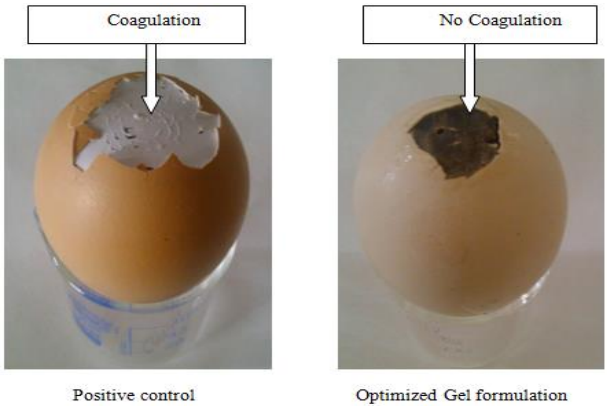


Figure 6: Results of HET CAM Test of Optimized Gel

MFC was determined in order to verify whether the drug is only fungistatic or fungicidal also. MIC concentration was evaluated for MFC study along with one higher concentration than that of MIC on safer side.

Plating and Reading MFC

The figure 2 indicates the observations of MFC Study

Determination of MIC and MFC in Air dried Garlic Powder against *Candida albicans*

MIC Determination by Broth Dilution Method.

The results are given in following **Table 3 (Worksheet 2A)**

From these results, it has been seen that there was prominent reduction in turbidity in tube containing 1.6 µg/ml garlic powder that is (2.23mg/ml) Allicin concentration of air dried garlic powder which is considered as a minimum inhibitory concentration for air dried garlic powder.

Antifungal susceptibility testing: Broth dilution Method *Malassezia furfur* (NCCLS, M100-S12): The sample was garlic powder prepared by updated method. The prominent reduction in turbidity was observed in tube with concentration 500 µg/ml (1.26685 µg/ml) (Figure 3).

Formulation and development of Garlic Gel

Evaluation of screening batches

The formula composition of preliminary batches is shown in Table 6. Garlic gel formulations were prepared for preliminary screening to select an appropriate concentration of carbopol giving appropriate spread ability viscosity and pH range.(Table 6, Table 7, Figure 4).

All the concentrations tested were found to have similar characteristics. But considering the optimum spread ability of the gel formulation and on the basis of appearance, F3, Formulation concentration was further considered to test the varying concentrations of the garlic powder. F3 Formulation with 1.25% for carbopol 934 was found to be suitable for further trials.

Experimental Batches for Antfungal and stability Evaluation: The formula composition for experimental batches for antifungal and stability evaluation is given in Table 8.

Antifungal Evaluation of Experimental Batches

All the samples were observed for effective antifungal activity for 24, 48, 72 hours and further observations were continued for seven days period and effective concentration was identified, wherein formulation prepared with 1% concentration was found to be effective for further 7 days extended study. Results shown in Figures 5.

HET CAM Test

In the HET CAM test, Incubated Hen's Eggs were subjected to observation for either Coagulation, Hemorrhage, or Hemolysis after 300 seconds (5 minutes) of applying the test substance. The results, as discussed in Figure 6, revealed significant findings. Coagulation occurred within 0.5 seconds of applying the positive control (0.1N NaOH), while no observable coagulation or any viable reaction was noted with the test substance, which was an optimized gel formulation. The observations for the HET CAM test are depicted in accompanying photographs. (Figure 6)

CONCLUSION

In conclusion, the study successfully utilized microwave-processed garlic powder to develop a stable and effective antifungal gel using Carbopol as a gelling agent. Through rigorous testing following CLSI guidelines, the gel demonstrated significant antifungal activity against *Candida albicans* and *Malassezia furfur*, common fungal pathogens affecting the skin, hair, and vagina. The optimized formulation containing 1% garlic powder exhibited prominent effectiveness over a prolonged period, with no observed skin irritation according to the HET-CAM test. This innovative approach offers promise for addressing fungal infections with a natural and well-tolerated solution.

Acknowledgements

Grateful for all the helping hands during the work.

Abbreviations

MCSO: S-methyl cysteine-sulfoxide; **PCSO**: S-propyl cysteine-sulfoxide; **SDB**: Sabouraud Dextrose Broth; **MTCC**: Microbial Type Culture Collection and Gene Bank; **CFU:** colony forming unit; MIC: Minimum inhibitory concentration; **MFC:** Minimum Fungicidal Concentration; **NaCl**: Sodium Chloride; **HET-CAM Test:** Hen's Egg Test – Chorioallantoic Membrane

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