

**FORMULATION AND IN VITRO EVALUATION OF GARLIC AND CLOVE
ESSENTIAL OIL CREAMS AGAINST CANDIDA ALBICANS****Lochan Markande*¹, Dr. Anandmahalwar², Vikas Kumar Sahu³**¹Student of M Pharmacy, (Pharmaceutics).²Professor Faculty of Health and Allied Sciences.³Assistant Professor Faculty of Health and Allied Sciences.

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ABSTRACT

This study investigated the formulation characteristics and preliminary in vitro antifungal activity of topical creams containing garlic (*Allium sativum*) and clove (*Syzygium aromaticum*) essential oils. Five oil-in-water formulations were described: 2% garlic oil (F1), 2% clove oil (F2), 1% of each oil (F3), 2% of each oil (F4), and an oil-free cream base (F5). Evaluation included physicochemical measurements, agar diffusion, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and checkerboard testing. The supplied report provided numerical antifungal results for *Candida albicans* only. Formulation pH ranged from 5.6 to 6.1, viscosity from 8,500 to 14,200 cP, and spreadability from 7.5 to 8.4 g·cm/s. Reported inhibition zones were 15.4 ± 0.5 , 18.6 ± 0.6 , 24.5 ± 0.7 , and 27.2 ± 0.8 mm for F1–F4, respectively. F3

had a reported MIC of 125 µg/mL and MFC of 250 µg/mL, although the concentration basis requires clarification. A fractional inhibitory concentration index of 0.50 was reported; the component concentrations needed to independently verify this result were unavailable. Qualitative observations suggested physical stability during three months of storage, but numerical time-course data were absent. The findings support further investigation of combined garlic–clove creams against *C. albicans*. They do not establish dermatophyte efficacy, confirmed synergy, shelf life, dermal safety, or clinical effectiveness.

KEYWORDS: *Allium sativum*; *Syzygium aromaticum*; *Candida albicans*; topical cream;

essential oils; antifungal screening.

I. INTRODUCTION

Plant-derived essential oils are being investigated as sources of antifungal compounds and as ingredients in topical delivery systems. For a combination formulation, the relevant questions are whether the oils can be incorporated into a reproducible semisolid base, whether antifungal activity is retained, and whether the combination performs differently from its individual components. These questions require separate formulation, microbiological, and interaction assessments; a larger inhibition zone alone cannot resolve all three.

Garlic essential oil contains volatile organosulfur constituents, including diallyl disulfide and diallyl trisulfide.^[1] Allicin is an important but unstable compound formed after damage to garlic tissue.^[2] Consequently, findings concerning fresh garlic or purified allicin should not automatically be attributed to a distilled garlic oil. Chemical profiling is necessary to identify the constituents present in a particular experimental batch.

Clove essential oil and its principal constituent, eugenol, have shown antifungal activity against *Candida* strains in published laboratory studies.^[3] Eugenol-associated effects on fungal growth and virulence have also been reviewed.^[4] This evidence supports investigating clove-containing formulations, while leaving the activity, safety, and interaction profile of a specific garlic–clove cream to be established experimentally.

A cream vehicle can influence dispersion, release, rheology, and diffusion of essential oil constituents. Physical appearance and initial pH are therefore useful formulation measurements but cannot independently establish chemical stability or therapeutic suitability.^[5] This study examined five creams with a common base and different essential oil compositions. The present manuscript focuses on the numerical *C. albicans* results available in the supplied report and identifies the limits affecting their interpretation.

II. MATERIALS AND METHODS

2.1 Study materials and scope

The source report describes garlic bulbs and dried clove flower buds procured from a supplier in Chhattisgarh, India, and authenticated by a taxonomist. Essential oils were isolated using a Clevenger-type apparatus, dried over anhydrous sodium sulfate, filtered, and stored in amber airtight vials at $4 \pm 1^\circ\text{C}$. Supplier details, voucher numbers, extraction yield, distillation

duration, and chromatographic composition were not provided. The exact steam-distillation or hydrodistillation arrangement requires confirmation from laboratory records.

The cream base contained stearic acid, cetyl alcohol, beeswax, liquid paraffin, Tween 80, glycerin, triethanolamine, methyl paraben, and purified water. The report identifies *C. albicans* ATCC 10231 and also lists *Trichophyton rubrum* ATCC 28188, *T. mentagrophytes* ATCC 9533, and *Microsporum gypseum* ATCC 24102. Numerical outcomes for the three dermatophytes were not supplied; they are excluded from efficacy conclusions. Culture provenance and strain records remain to be confirmed.

2.2 Formulation composition and preparation

Five formulations were described, as summarized in Table 1. F1 and F2 each contained a total essential oil concentration of 2% w/w. F3 also contained 2% total oil, divided equally between garlic and clove, whereas F4 contained 4% total oil. F5 contained the common base without essential oils. This design allows an equal-total-oil descriptive comparison of F1, F2, and F3, but the higher total loading in F4 must be considered when interpreting its activity.

Table 1: Composition of the experimental creams.

Ingredient (% w/w)	F1	F2	F3	F4	F5
Garlic oil	2.0	—	1.0	2.0	—
Clove oil	—	2.0	1.0	2.0	—
Stearic acid	15.0	15.0	15.0	15.0	15.0
Cetyl alcohol	4.0	4.0	4.0	4.0	4.0
Beeswax	3.0	3.0	3.0	3.0	3.0
Liquid paraffin	8.0	8.0	8.0	8.0	8.0
Tween 80	3.0	3.0	3.0	3.0	3.0
Glycerin	5.0	5.0	5.0	5.0	5.0
Triethanolamine	1.5	1.5	1.5	1.5	1.5
Methyl paraben	0.20	0.20	0.20	0.20	0.20
Purified water	q.s. to 100	q.s. to 100	q.s. to 100	q.s.*	NR*

= no essential oil added. *The report gives only “q.s.” for F4 and leaves the F5 water entry blank; these entries have not been silently completed. Confirm final batch mass and water quantities. NR = not reported.

Stearic acid, cetyl alcohol, beeswax, and liquid paraffin were heated to $70 \pm 2^\circ\text{C}$. The essential oils were incorporated into this phase. Tween 80, glycerin, methyl paraben, and triethanolamine were prepared separately with water at the same temperature. The report describes adding the aqueous phase gradually to the oil phase while stirring at 1,000–1,200 rpm for approximately 20–30 minutes, followed by homogenization and cooling with gentle

stirring. Creams were packed in laminated tubes and stored at $25 \pm 2^\circ\text{C}$. The report classifies the products as oil-in-water emulsions, but no emulsion-type verification test is documented. Batch size, homogenizer settings, and the duration of oil exposure to heat were not specified.

2.3 Physicochemical evaluation

Appearance and homogeneity were assessed visually and by checking for grittiness and phase separation. For pH measurement, approximately 1 g of cream was dispersed in 10 mL of distilled water and equilibrated for 30 minutes before measurement with a calibrated pH meter. Viscosity was measured using a Brookfield digital viscometer at $25 \pm 2^\circ\text{C}$. Spindle identity and rotational speed were not reported, limiting comparison and reproduction of the viscosity results.

Spreadability was assessed using a parallel-slide procedure and calculated as $S = M \times L/T$, where M is the applied mass, L is the slide travel distance, and T is travel time. The report does not give the load, distance, or loading duration. Extrudability was graded qualitatively after applying force to filled tubes; the force and grading thresholds were not documented. Washability was described using application to the back of a hand, but no numerical results or participant/ethics details were supplied. This observation is not treated as a dermal safety assessment.

2.4 Antifungal screening

For agar well diffusion, the report describes *C. albicans* inoculum adjusted to approximately 0.5 McFarland turbidity and spread on Sabouraud dextrose agar. Wells of 6 mm diameter received approximately 100 μL of test material. After 30 minutes of diffusion at room temperature, plates were incubated at $35 \pm 2^\circ\text{C}$ for 24–48 hours, and inhibition zones were measured in millimetres. The report states that assays were performed in triplicate. Preparation of the cream dispersion, delivered mass, and whether the well diameter was included in the measured zone require clarification.

A separate disc-diffusion procedure using 6 mm discs and 20 μL of material was also described. A commercial 1% clotrimazole cream was identified as the positive control and F5 as the vehicle control. Separate disc-diffusion and clotrimazole results were not supplied. Consequently, no comparison with clotrimazole or equivalence to a standard antifungal treatment is made.

2.5 MIC and MFC measurements

Broth microdilution was described using two-fold dilutions in RPMI-1640 medium over 0.03125–8.0 mg/mL. MIC was defined in the report as the lowest concentration with no visible growth. MFC was determined by transferring 10 μ L from wells without visible growth onto fresh agar and recording the lowest concentration yielding no colonies. The report cites CLSI methodology; however, the final inoculum, solvent or emulsifier controls, sample dispersion, and assay-specific incubation details are insufficient to confirm compliance with the yeast reference method.^[6]

The original table combines formulation labels with MIC and MFC values but does not establish whether μ g/mL denotes cream mass, total essential oil mass, or an individual oil concentration. Values are retained as reported and must not be interpreted as interchangeable measures of intrinsic oil potency until this basis is resolved.

2.6 Combination interaction assessment

The report describes a checkerboard assay using garlic and clove oil dilutions in MOPS-buffered RPMI-1640 medium, with growth and sterility controls. Fractional inhibitory concentrations were defined as $FIC_{garlic} = MIC_{garlic} \text{ in combination} / MIC_{garlic} \text{ alone}$ and $FIC_{clove} = MIC_{clove} \text{ in combination} / MIC_{clove} \text{ alone}$. The index was calculated as $FICI = FIC_{garlic} + FIC_{clove}$. Interpretation of checkerboard experiments depends on the method and chosen thresholds.^[7] The report used $FICI \leq 0.50$ for synergy. Component concentrations and the underlying checkerboard matrix were unavailable for independent verification.

2.7 Storage observations and data treatment

The report describes storage at $4 \pm 2^\circ\text{C}$, $25 \pm 2^\circ\text{C}/60 \pm 5\%$ relative humidity, and $40 \pm 2^\circ\text{C}/75 \pm 5\%$ relative humidity for three months, with evaluations at months 0, 1, 2, and 3. Planned outcomes included appearance, phase separation, pH, viscosity, application properties, and retained antifungal activity. Only qualitative stability conclusions were supplied. These observations are treated as preliminary storage observations rather than a completed ICH stability package or a basis for assigning shelf life.^[8]

Continuous measurements are reproduced as mean $\pm$ standard deviation, with $n = 3$ as stated in the report. Whether these represent independent batches, biological replicates, or repeated technical measurements requires confirmation. The report describes one-way ANOVA and Tukey testing, but raw observations, degrees of freedom, and pairwise results were

unavailable. No new inferential analysis was performed, and statistical significance is not asserted in this manuscript.

III RESULTS

3.1 Physical characteristics

All five creams were described as smooth or homogeneous. Table 2 shows pH values from 5.6 to 6.1, viscosity from 8,500 to 14,200 cP, and spreadability from 7.5 to 8.4 g·cm/s. These ranges are taken from the source table rather than the inconsistent wider ranges in its narrative. F3 had the highest reported spreadability, while F4 had the highest viscosity and was graded “Good” for extrudability. The other formulations were graded “Excellent”. These observations characterize the products under the reported tests but do not establish skin tolerability.

Table 2: Physicochemical measurements reported for the creams.

Code	pH	Viscosity (cP)	Spreadability (g·cm/s)	Extrudability	Appearance
F1	5.8 ± 0.12	9,200 ± 145	7.8 ± 0.21	Excellent	Smooth
F2	5.7 ± 0.10	9,800 ± 160	7.5 ± 0.18	Excellent	Smooth
F3	5.9 ± 0.14	11,800 ± 170	8.4 ± 0.20	Excellent	Homogeneous
F4	6.1 ± 0.11	14,200 ± 190	7.9 ± 0.22	Good	Homogeneous
F5	5.6 ± 0.13	8,500 ± 130	8.1 ± 0.19	Excellent	Smooth

Numerical values are mean ± SD; n = 3 according to the source report. Replicate independence and qualitative grading criteria need confirmation.

3.2 Activity against *Candida albicans*

The reported mean inhibition zone increased from 15.4 mm for F1 and 18.6 mm for F2 to 24.5 mm for F3 and 27.2 mm for F4 (Table 3). F3 therefore showed a larger zone than either single-oil formulation at the same nominal total oil loading. F4 produced the largest zone, but contained twice the total oil concentration of F3. This ranking is descriptive; neither statistical significance nor synergy follows from zone size alone.

Table 3: Reported antifungal results against *Candida albicans*.

Sample	Inhibition zone (mm)	MIC (µg/mL)*	MFC (µg/mL)*
F1 — 2% garlic oil	15.4 ± 0.5	500	1,000
F2 — 2% clove oil	18.6 ± 0.6	250	500
F3 — 1% of each oil	24.5 ± 0.7	125	250
F4 — 2% of each oil	27.2 ± 0.8	NR	NR
F5 — oil-free base	ND†	NR	NR

*Concentration basis and whether the assay tested cream dispersions or isolated oils require confirmation. Zone values are mean ± SD, n = 3 as reported. NR = not reported. †ND is

reproduced from the source table, which does not explicitly define it; it is not converted to zero.

F3 had lower reported MIC and MFC values than F1 and F2. The ratios of MFC to MIC were 2 for all three samples, calculated directly from the tabulated values. These ratios do not resolve the ambiguous sample concentration basis or replace validation of the fungicidal endpoint. No numerical efficacy results were available for the dermatophytes.

3.3 Interaction and storage findings

The source checkerboard table gives $FIC_{garlic} = 0.25$ and $FIC_{clove} = 0.25$, producing $FICI = 0.50$ for *C. albicans*. This value lies exactly at the source-defined synergy threshold, rather than below 0.50 as stated elsewhere in the report. It is therefore recorded as a reported threshold-level interaction result requiring verification, not as independently confirmed synergy. Dermatophyte FIC and FICI results were marked NR.

For F3, the report describes retained appearance and no visible phase separation during storage, with minor pH changes and a slight viscosity reduction at accelerated temperature. Without measurements at each time point, the size of these changes and retention of antifungal activity cannot be quantified. Absence of visible contamination does not establish microbiological quality or preservative effectiveness.

IV DISCUSSION

The main finding is the higher reported *C. albicans* inhibition zone of the 1% garlic plus 1% clove formulation relative to creams containing 2% of either oil alone. Equal nominal total oil loading makes this comparison useful for prioritizing a candidate formulation. Nevertheless, diffusion also depends on the cream matrix, constituent solubility, and release. The experiment therefore supports further study of F3 but does not establish the magnitude or mechanism of a pharmacological interaction.

Published work on clove oil and eugenol provides a biological rationale for anticandidal activity.^[3] Garlic oil composition provides a separate rationale for examining organosulfur-containing combinations.^[1] The present report lacks chromatographic measurements, so activity cannot be assigned to allicin, eugenol, or a defined constituent ratio. Membrane injury, oxidative stress, and biofilm inhibition remain possible explanations from the literature rather than measured mechanisms in this study.^[4]

The reported checkerboard index needs particular attention. If the F1 and F2 MIC values represent garlic and clove oils alone, FIC values of 0.25 imply combination concentrations of 125 µg/mL garlic oil and 62.5 µg/mL clove oil. That is a calculated total of 187.5 µg/mL at a 2:1 mass ratio. In contrast, F3 contains a nominal 1:1 oil ratio and is assigned an MIC of 125 µg/mL. These entries may describe different experiments or concentration conventions, but the report does not explain the relationship. The original checkerboard matrix and sample definitions are needed before the FICI can be used to claim synergy for F3.

F3 also warrants attention because its reported spreadability exceeded that of the other formulations while its viscosity was below F4. This balance makes it a candidate for further optimization; it does not establish a validated optimum. F4 may have a stronger diffusion signal because of its higher total oil content. Comparing formulations at matched oil exposure and evaluating release, rheology, and batch reproducibility would help distinguish dose effects from formulation effects.

Physical stability is distinct from chemical stability. A cream can retain its appearance while volatile constituents are lost or degraded. Emulsion characterization and appropriate chemical assays are therefore needed alongside pH and viscosity measurements.^[5] The stated three-month observation period and absence of numerical time-course results prevent shelf-life assignment under a formal stability framework.^[8] Similarly, plant origin and an apparently suitable pH do not establish safety. No skin irritation, sensitization, permeation, clinical efficacy, or resistance-development outcomes were supplied.

4.1 Limitations

The available evidence consists of summary tables and narrative methods rather than auditable laboratory records. Key limitations are missing dermatophyte and positive-control results, an undefined ND entry, incomplete water quantities, ambiguous MIC/MFC concentration units, insufficient checkerboard detail, and absent numerical stability data. Extraction conditions, oil composition, instrument settings, assay controls, and the nature of the triplicates also require clarification. The source statistical summary cannot support inferential claims without reconciliation with the underlying measurements. These limitations restrict the findings to preliminary descriptive evidence for *C. albicans*.

V. CONCLUSION

The reported results support continued investigation of garlic–clove essential oil creams as

experimental antifungal formulations. F3, containing 1% of each oil, showed a larger reported *C. albicans* inhibition zone and lower reported MIC and MFC values than the individual-oil samples, together with the highest recorded spreadability. F4 produced the largest inhibition zone at a higher total oil loading. Confirmation of sample concentrations, replicate data, and checkerboard results is required before attributing these findings to synergy. Dermatophyte efficacy, shelf life, dermal safety, and clinical usefulness remain unestablished.

Declarations

Author contributions: [Insert the actual contributions of each author and confirm author order.]

Funding and competing interests: [Provide the factual funding statement and each author's competing-interest declaration.]

Ethics and consent: [Confirm whether hand-application assessments involved human participants and provide the applicable approval, consent, exemption, or institutional determination. No approval or waiver is inferred here.]

Data availability: [State where the underlying measurements, assay images, checkerboard data, and storage records can be accessed. Raw data were not included in the supplied report.]

Acknowledgements: [Identify actual contributors or facilities, with their permission, or remove this entry.]

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