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Research Protocol

Ayurveda Section

Pharmaceutico-analytical Comparison of Shweta *Karvirapallavadya Taila* and Its Modified Dosage Form for Antioxidant, Anti-inflammatory, and Antimicrobial Activities: A Research Protocol for an In Vitro Study

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ABSTRACT

Introduction: Psoriasis is a chronic, immune-mediated inflammatory skin disorder characterised by erythema, scaling, and recurrent exacerbations. In Ayurveda, it is classified under *Kushtha* and is commonly associated with symptoms such as itching, scaling, and inflammation.

Need of the study: *Shweta Karvirapallavadya* (SKP) *Taila* is a classical Ayurvedic oil formulation traditionally indicated for psoriasis and itching. Although it holds therapeutic importance in Ayurvedic dermatology, its oily consistency may hinder patient compliance and aesthetic acceptability in present-day clinical practice. Reformulating SKP *Taila* into an ointment and evaluating both scientifically will help determine their comparative therapeutic potential and promote integrative use in modern dermatological care.

Aim: To modify SKP *Taila* into an ointment formulation and to comparatively evaluate the in-vitro antioxidant, anti-inflammatory, and antimicrobial activities of both SKP *Taila* and its ointment form.

Materials and Methods: An in-vitro study will be conducted at Mahatma Gandhi Ayurved College, Wardha, and Biocyte Laboratory, Sangli, Maharashtra, India, from January 2025 to November 2026. Both formulations will undergo organoleptic and physicochemical evaluation, along with Gas Chromatography-Mass Spectrometry (GC-MS) analysis to identify bioactive constituents. Antioxidant activity will be assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay, anti-inflammatory activity using the Human Red Blood Cell (HRBC) membrane-stabilisation method, and antimicrobial activity using the agar well diffusion method against selected Gram-positive, Gram-negative, and fungal strains. Statistical analysis will be performed using appropriate parametric and non parametric tests, including Student's t-test and Analysis of Variance (ANOVA), based on data distribution, and a p-value < 0.05 will be considered statistically significant.

Keywords: Gas chromatography, Plant preparations, Psoriasis, Skin diseases

INTRODUCTION

Psoriasis is a chronic, systemic autoimmune skin disorder affecting approximately 2-3% of the global population, with around 30% of cases involving the joints, a condition referred to as Psoriatic Arthritis (PsA) [1]. The management of psoriasis is challenging due to its rising prevalence, chronic course, significant disability in some patients, and associated health complications. The worldwide prevalence of psoriasis is estimated at over 100 million people [2], and in India, it ranges from 0.44% to 2.8% [3].

SKP *Taila* is one formulation mentioned in the *Charaka Samhita* and indicated in *Kushtha Roga*. It is an herbal combination in an oil dosage form containing ingredients such as *Karveer, Indrajava*, *Vidanga, Kushtha, Madar, Sarshapa, Shigru, Kutaki, Gomutra*, and Mustard Oil [4]. It is prepared using the classical Ayurvedic pharmaceutical process known as *Sneha Kalpana*, which involves the processing of medicinal herbs with oils or ghee. This method enhances the potency, stability, and transdermal absorption of the active constituents. *Sneha Kalpana* is especially beneficial in the treatment of skin disorders, as it facilitates deeper tissue penetration and delivers the therapeutic properties of the herbs effectively through the lipid medium [5].

Antioxidants are neutralising chemicals that minimise oxidative damage to biological processes by free radicals and pass them off as harmless. Psoriasis is associated with higher oxidative stress due to excessive free radicals in the skin, which can lead to more inflammation and worsen symptoms. Antioxidant oils help neutralise these free radicals, potentially reducing inflammation, promoting skin barrier repair, and improving symptoms [6]. Converting a medicated oil into an ointment may improve ease of application, skin adherence, and patient acceptability by reducing the inconvenience associated with oily preparations. Considering these aspects, an attempt has been made to standardise SKP *Taila* into SKP ointment and comparatively evaluate the in-vitro antioxidant, anti-inflammatory, and antimicrobial activities of both dosage forms.

REVIEW OF LITERATURE

The individual ingredients have been reviewed to provide a scientific basis for evaluating the antioxidant, anti-inflammatory, and antimicrobial activities of the complete formulation. A study by Vinayagam A and Sudha PN demonstrated that *Nerium indicum* has significant antioxidant activity. The methanolic extracts of the leaves and flowers showed 72.8% and 67.0% scavenging of DPPH radicals, respectively [7]. Another study by Saha S and Subrahmanyam EV showed that *Holarrhena pubescens* exhibits significant antimicrobial, antioxidant and anti-inflammatory activities. Among the successive solvent fractions, the chloroform fraction (HCF) exhibited the highest antimicrobial activity against both Gram-positive and Gram-negative bacteria. The same fraction also showed potent antioxidant activity, with 80.35±0.46% DPPH free radical scavenging activity (IC50 28.41±0.33 µg/mL). It also

showed significant anti-inflammatory activity by giving 68.37±1.11 % inhibition of bovine serum albumin denaturation in-vitro and 75.16 % inhibition of paw oedema (p-value <0.05) in the adjuvant-induced arthritis model, supporting its potential application in inflammatory skin disorders [8].

Lal B and Mishra N studied the antibacterial, antifungal, antioxidant, anti-inflammatory, wound healing and immunomodulatory activities of *Embelia ribes* are mainly due to embelin and other phenolic constituents. Embelin was experimentally shown to have potent free radical scavenging activity, suppress inflammatory mediators and have broad-spectrum antimicrobial activity, which supports the traditional use of *E. ribes* in chronic inflammatory skin disorders [9]. Ansari S et al., reported that *Saussurea lappa* has remarkable antioxidant and antimicrobial activities. These activities are mainly attributed to sesquiterpene lactones such as costunolide and dehydrocostus lactone. The presence of these bioactive constituents justifies its traditional use for chronic inflammatory skin conditions [10]. Kumar V et al., studied that *Moringa oleifera* is rich in phenolic compounds and flavonoids that provide its antioxidant and antimicrobial activities [11]. Similarly, a study by Singh S showed that *Gomutra* (Cow's urine) has antioxidant, antimicrobial, immunomodulatory, wound-healing, and anti-inflammatory properties that may enhance host immune responses and tissue repair [12].

Further, Sharma P and Singh R concluded that *Brassica juncea* possesses significant antioxidant, antimicrobial and anti-inflammatory activities, mainly attributed to glucosinolates, flavonoids and other phenolic constituents [13]. Khatib R and Al-Makky K demonstrated that *Sinapis alba* has considerable free radical scavenging activity and antibacterial effects against both Gram-positive and Gram-negative microorganisms in experimental studies. These pharmacological properties support its potential role in reducing oxidative stress and secondary microbial infection associated with psoriasis [14]. A study by Singh G et al., showed *Picrorhiza kurroa* leaf extract has significant antioxidant and antibacterial activities. Phytochemical analysis identified six major bioactive compounds in the extract, and the formulation exhibited effective free radical scavenging activity along with antibacterial effects against selected pathogenic microorganisms [15].

The effects of individual ingredients may differ from the overall activity of the combined formulation due to possible synergistic or antagonistic interactions. Although the ointment may improve ease of application, skin retention, and patient compliance, comparative pharmaceutico-analytical and in-vitro studies are lacking. Previous studies have mainly evaluated methanolic or hydroalcoholic extracts; therefore, further evaluation of the present oil-based formulation is required. Future studies may also assess its safety and toxicity. Hence, the present study aims to evaluate and compare the pharmaceutico-analytical properties and antioxidant, anti-inflammatory, and antimicrobial activities of SKP *Taila* and its modified ointment dosage form.

Primary Objectives

1. To evaluate the in-vitro antioxidant, anti-inflammatory, and antimicrobial activity of SKP *Taila* and SKP ointment.

Secondary Objectives

1. To formulate a modified dosage form as SKP ointment (SKP ointment).
2. To carry out an analytical evaluation of SKP *Taila* and SKP ointment for standardisation.

Null hypothesis (H_0): SKP *Taila* and its modified form (SKP Ointment) will not show any significant difference in in-vitro antioxidant activity, anti-inflammatory activity, and antimicrobial activity.

Alternate hypothesis (H_1): SKP *taila* and its modified form (SKP ointment) will show a significant difference in in-vitro antioxidant activity, anti-inflammatory activity, and antimicrobial activity.

MATERIALS AND METHODS

An in-vitro study will be conducted at Mahatma Gandhi Ayurved College, Hospital and Research Centre (MGACH & RC), Salod (H.), DMIHER (DU), Wardha, Maharashtra, India, from January 2025 to November 2026. The study will be conducted in accordance with the Declaration of Helsinki. The research has been approved by the IEC of Datta Meghe Institute of Higher Education and Research under reference number MGACHRC/IEC/Oct-2024/907.

All raw drugs [Table/Fig-1], along with an ointment base with necessary excipients, will be procured from certified vendors with documented batch details and traceability. Experts from the Department of Dravyaguna will perform authentication, and quality control will be carried out in accordance with the standards of the Ayurvedic Pharmacopoeia of India (API) using organoleptic and physicochemical parameters.

S. No.	Dravya	Botanical name	Parts used	Quantity	
1.	*Karveer*	*Nerium indicum* Mill	Leaf, bark, root	1 Part	*Kalka Dravya* (Drug paste prepared in bolus form)
2.	*Indrajava*	*Holarrhena pubescens* Wall. Ex G.Don	Seeds	1 Part	
3.	*Vidanga*	*Embelia ribes* Burm. f	Fruits	1 Part	
4.	*Kushtha*	*Saussurea lappa* C.B. Clarke	Roots	1 Part	
5.	*Madar*	*Calotropis procera* (Aiton) W.T.Aiton	Roots	1 Part	
6.	*Sarshapa*	*Sinapis alba* L.	Seeds	1 Part	
7.	*Shigru*	*Moringa oleifera* Lam.	Bark	1 Part	
8.	*Kutaki*	*Picrorhiza kurroa* Royle ex Benth.	Roots	1 Part	
9.	Mustard oil	*Brassica juncea* (L.) Czern.	Oil	4 times of Kalka Dravyas	*Sneha Dravya* (fatty substance)
10.	*Gomutra*	Cow urine	-	16 times of *Kalka* Dravyas	*Drava Dravya* (liquid medium)

[Table/Fig-1]: Ingredients for *Shweta Karvirapallavadya* (SKP) *Taila* [4].

The raw drugs will be thoroughly cleaned to remove physical impurities and pounded individually in a *Khalwa Yantra* to obtain coarse powders. The coarse powder will then be pulverised using a mixer grinder at 1200 RPM to obtain a fine powder. Sieve the powdered drugs through an 80# mesh and store them separately in airtight containers until further use.

Sarshapa Taila (Mustard oil) will be subjected to the *Murchhana* (purification and preprocessing of oil) process to remove *Ama Dosha* (undesirable impurities/toxic metabolites) [16].

Preparation of *Shweta Karvirapallavadya* (SKP) *Taila* [4,5]: SKP *Taila* will be prepared following the classical *Sneha Paka* (medicated oil preparation) method in the proportion of 1:4:16 (*Kalka: Sneha: Gomutra*), where *Kalka* refers to herbal paste, *Sneha Dravya* refers to mustard oil, and *Gomutra* is used as *Drava Dravya* (liquid medium). The prescribed drugs will be powdered and mixed with a small quantity of potable water to prepare *Kalka* (herbal paste). Mildly heat mustard oil (*Sneha Dravya*) in a stainless-steel vessel over *Madhyama Agni* (moderate heat) to remove residual moisture, with continuous stirring, followed by the addition of *Gomutra* (*Drava Dravya*). Heat the mixture under controlled *Madhyama Agni* with intermittent stirring until *Sneha Siddhi Lakshanas* are achieved and the liquid component is completely evaporated.

Sneha Paka will be confirmed by classical parameters, including proper *Varti* formation, absence of crackling sound, and development of reddish foam on the oil surface. After completion, heating will be discontinued and the oil allowed to cool. The warm oil will be filtered through a clean muslin cloth, transferred into airtight PET containers, labelled, and stored in a clean, dry place. The same procedure will be followed for all three batches to ensure pharmaceutical standardisation.

Preparation of SKP ointment: All the ingredients [Table/Fig-2] will be taken. SKP *Taila* will be used as the active ingredient and gently heated on a hot plate using stainless steel vessels [17]. The base materials, namely paraffin wax, hard beeswax, cetostearyl alcohol, and stearic acid, will be accurately weighed, melted, and thoroughly mixed with the heated oil. The mixture will then be blended using a blender to achieve uniform consistency, followed by continuous stirring with a spatula or ladle until a smooth semi-solid mass is obtained. The molten mass will be allowed to cool to room temperature and subsequently transferred into clean, dry containers using a measuring cylinder. The final prepared formulation will be labelled as SKP Ointment.

The formulation will be analytically assessed to establish standardised quality parameters in the context of contemporary pharmaceutical chemistry.

S. No.	Ingredients	Quantity
1	Active Ingredients (*Shweta Karvirapallavadya* (SKP) *Taila*)	83%
2	Paraffin wax	8%
3	Hard beeswax	8%
4	Cetostearyl alcohol	0.5%
5	Stearic acid	0.5%

[Table/Fig-2]: Ingredients of SKP ointment [17].

1. Organoleptic Evaluation: The raw drugs will be evaluated and documented for *Sparsha* (touch), *Rupa* (appearance), *Rasa* (taste), and *Gandha* (odour) before processing [16].
2. Physicochemical parameters for raw material analysis [Table/Fig-3] [18].
3. Parameters for finished product analysis [Table/Fig-4,5] [19]:

Microbial Quality Assessment

Microbial limit test: The microbial quality of SKP *Taila* and SKP ointment will be assessed by the plate count method to determine Total Aerobic Microbial Count (TAMC) and Total Yeast and Mould Count (TYMC) under specified incubation conditions. The formulations will also be tested for the absence of specified microorganisms, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Results will be expressed as CFU/g or CFU/mL and compared with prescribed microbiological limits [19].

Modern Sophisticated Analysis

Gas Chromatography-Mass Spectrometry (GC-MS): GC-MS will be used for qualitative and quantitative analysis of volatile and trace constituents based on their retention times and mass spectral profiles. Analysis will be performed under standardised conditions, including a capillary column, helium as the carrier gas at a flow rate of approximately 1 mL/min, an injector temperature of 250°C, split injection (1:10), an oven temperature programme starting at 60°C and increasing to 280°C, Electron-Ionisation (EI) at 70 eV, and mass scanning over an appropriate mass-to-charge (m/z) range. Before analysis, the *Taila* sample will be diluted with n-hexane (1:10 v/v). The ointment will be extracted with n-hexane and filtered to remove waxy excipients, followed by membrane filtration. The resulting extracts will be subjected to GC-MS analysis according to established protocols [20].

S. No.	Parameter	Procedure
1	Foreign matter (%)	The raw drugs will be examined visually for the presence of extraneous organic or inorganic materials such as stones, soil, insects, or any other foreign matter. The impurities will be separated manually from a representative sample, weighed, and expressed as a percentage w/w.
2	Total ash (%)	Approximately 2-3 gm of the powdered raw drug will be accurately weighed in a silica crucible and incinerated at a temperature not exceeding 450°C until carbon-free ash is obtained. The crucible will be cooled, weighed, and the total ash value will be calculated as a percentage w/w, indicating the total inorganic residue present in the sample.
3	Water soluble Ash (%)	The total ash obtained will be boiled with distilled water, and the insoluble residue will be collected on an ashless filter paper, ignited, cooled, and weighed. The difference between the total ash and the insoluble residue will be considered as the water-soluble ash and expressed as percentage w/w.
4	Loss on drying (%)	Approximately, 10 g of the powdered raw drug will be accurately weighed and dried at 105°C until a constant weight is obtained. The percentage loss in weight will be calculated to determine the moisture and volatile matter present in the sample.
5	Water soluble extractive (%)	Approximately 5 gm of the air-dried powdered drug will be macerated with 100 mL of chloroform-water in a closed flask for 24 hours with intermittent shaking during the initial six hours. After filtration, a measured volume of the filtrate will be evaporated to dryness, dried to constant weight, and the water-soluble extractive value will be calculated as a percentage w/w.
6	Acid insoluble ash (%)	The total ash obtained will be treated with dilute hydrochloric acid and boiled for the prescribed duration. The insoluble residue will be collected, washed with hot water, ignited to constant weight, and expressed as acid-insoluble ash percentage, indicating the presence of siliceous matter in the sample.
7	Alcohol soluble extractive (%)	Approximately 5 gm of the air-dried powdered drug will be macerated with 100 mL of alcohol for 24 hours with intermittent shaking. After filtration, a measured volume of the filtrate will be evaporated to dryness, dried to constant weight, and the alcohol-soluble extractive value will be calculated as a percentage w/w.

[Table/Fig-3]: Raw material analysis parameters [18].

S. No.	Parameter	Procedure
1	Refractive index	The refractive index of the prepared *Shweta Karvirapallavadya* (SKP) *Taila* will be determined using a calibrated refractometer at room temperature. The observed value will be recorded to assess the identity and uniformity.
2	Specific gravity	The specific gravity of the formulation will be determined using a specific gravity bottle at a controlled temperature. The value will be calculated by comparing the weight of the formulation with that of an equal volume of distilled water.
3	Acid value	The acid value will be determined by titration of the oil sample with standard potassium hydroxide solution. The result will be expressed as the amount of potassium hydroxide required to neutralise the free fatty acids present in one gram of the formulation.
4	Iodine value	The iodine value will be determined by the standard iodometric method to estimate the degree of unsaturation of the formulation. The results will be expressed as grams of iodine absorbed by 100 gm of the sample.
5	Saponification value	The saponification value will be determined by refluxing the formulation with alcoholic potassium hydroxide followed by titration with standard hydrochloric acid. The obtained value will indicate the amount of alkali required for saponification of one gram of the oil.
6	Peroxide value	The peroxide value will be estimated by iodometric titration to determine the extent of primary lipid oxidation in the formulation. The result will be expressed as milliequivalents of active oxygen per kilogram of the sample.

7	Rancidity test	The formulation will be evaluated for rancidity using the prescribed qualitative test to detect oxidative deterioration during storage. The observations will be recorded and compared with the acceptable standards.

[Table/Fig-4]: Physicochemical parameters for *Shweta Karvirapallavadya Taila* [19].

S. No.	Parameter	Procedure
1	Loss on drying at 105°C [18]	The ointment will be weighed accurately and dried at 105°C until a constant weight is achieved. The percentage loss in weight will be calculated to determine the moisture and volatile matter present in the formulation.
2	pH [19]	A 1% aqueous dispersion of the ointment will be prepared using distilled water. The pH will be determined using a calibrated digital pH meter after standardisation with appropriate buffer solutions, and the observed value will be recorded.
3	Spreadability test [17]	The spreadability of the ointment will be determined by placing a measured quantity of the formulation between two glass slides under a specified weight. The time required to separate the slides will be recorded, and the spreadability will be calculated to assess the ease of application of the formulation.
4	Rancidity [19]	The ointment will be examined for signs of oxidative rancidity using the prescribed qualitative test. The observations will be documented to assess changes in the lipid components during storage.
5	Iodine value [19]	The degree of unsaturation in the ointment's lipid fraction will be assessed using the standard iodometric method. Results will be reported as grams of iodine absorbed by 100 g of the formulation.
6	Peroxide value [19]	Primary lipid oxidation in the ointment will be evaluated through iodometric titration, with the result expressed as milliequivalents of active oxygen per kilogram of the formulation.

[Table/Fig-5]: Physicochemical parameters for SKP ointment [17-19].

Outcomes

1. **Antioxidant activity:** DPPH Assay (96-Well Method) [21]

A fresh 0.1 mM DPPH solution will be prepared in methanol (3.94 mg/100 mL) and protected from light. Test samples and ascorbic acid (reference standard) will be prepared in methanol at concentrations of 10-250 µg/mL by serial dilution.

For the assay, 100 µL of each sample or standard will be mixed with 100 µL of DPPH solution in 96-well microplates in triplicate. Control wells will contain methanol and DPPH, while blank wells will contain methanol only. The plates will be incubated at room temperature for 30 minutes in the dark, and absorbance will be measured at 517 nm using a microplate reader. Antioxidant activity will be expressed as percentage inhibition and IC_{50}.

2. **Anti-inflammatory Activity [22]:** The anti-inflammatory activity of the test samples will be evaluated using the HRBC membrane stabilisation method. Fresh human blood will be collected from healthy volunteers through a local pathology laboratory after written informed consent, following aseptic and biosafety procedures. Approximately, 2 mL of blood will be collected in Alsever's solution (1:1) and centrifuged at 3000 rpm for 10 minutes. The packed RBCs will be washed three times with 0.9% saline, and a 10% RBC suspension will be prepared.

The reaction mixture will contain the test sample or standard, 10% RBC suspension, and hypotonic solution. Diclofenac sodium will serve as the reference standard, and distilled water as the control. The mixtures will be incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant will be measured at 540 nm using a UV-Visible spectrophotometer. All experiments will be performed in triplicate, and results will be expressed as mean ± SD. Anti-inflammatory activity will be assessed by calculating the percentage inhibition of haemolysis, with higher inhibition indicating greater membrane stabilisation.

3. **Antimicrobial study [23]**

A. Method of analysis: Antimicrobial activity will be evaluated by the agar well diffusion method. Sterile agar medium will be inoculated with standardised microbial suspensions and poured into sterile Petri plates. After solidification, 6 mm wells will be aseptically prepared and filled with predetermined concentrations of the test samples, standard antimicrobial agents, and controls. The plates will undergo prediffusion at 2-8°C, followed by incubation under suitable conditions for bacterial and fungal strains. After incubation, the zones of inhibition will be measured using a zone reader or vernier calliper. The activity will be assessed by comparing the inhibition zones of the test formulations with those of the respective standards. All experiments will be performed in triplicate, and results will be expressed as mean ± standard deviation.

B. Antimicrobial evaluation: The antimicrobial susceptibility of *SKP Taila* and SKP ointment will be evaluated against selected bacterial and fungal microorganisms [Table/Fig-6]. The selected strains represent Gram-positive bacteria Gram-negative bacteria and fungi enabling preliminary assessment of antimicrobial activity against diverse microbial groups. The sample codes and their details are presented in [Table/Fig-7].

Bacterial strain		Fungal strain
Gram (+)	Gram (-)	1. *Candida albicans* 2. *Aspergillus niger*
1. *Staphylococcus aureus* 2. *Bacillus subtilis*	1. *Escherichia coli* 2. *Pseudomonas aeruginosa*	

[Table/Fig-6]: Microorganisms used for antimicrobial study.

Sample code	Sample detail
Sample 494- SKP Ointment	*Shweta Karvirapallavadya* (SKP) Ointment
Sample 495- SKP *Taila*	*Shweta Karvirapallavadya* (SKP) *Taila*

[Table/Fig-7]: Sample codes and their details for experimental studies.

C. Media and reagents preparation [24]: Antibiotic Assay Medium No.19 will be prepared by dissolving Beef extract (2.4 g), Sodium chloride (10.0 g), Dextrose (10.0 g), and Agar (23.5 g) in 1000 mL of purified water. The medium will be heated until completely dissolved, and the pH will be adjusted to 6.1±0.1 using 1 M sodium hydroxide or 1 M hydrochloric acid if required. The prepared medium will be distributed into flasks and sterilised by autoclaving at 121°C (15 lbs pressure) for 15 minutes. After sterilisation, the medium will be cooled and used for antimicrobial analysis.

Test sample solutions will be prepared at concentrations of 5 mg/mL and 10 mg/mL, and 100 µL of each solution will be used for antimicrobial evaluation. Standard antimicrobial agents such as streptomycin for bacterial strains and fluconazole for fungal strains will be used, and 100 µL of standard solution will be inoculated into designated wells.

D. Preparation of test organism and suspension: The test organisms will include *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 15442, *Candida albicans* ATCC 14053, and *Aspergillus niger* ATCC 14414. Stock cultures will be maintained on nutrient agar slants and incubated at 30-35°C for 18-24 hours. The growth will be suspended in sterile saline, vortexed, and adjusted to an optical density of 0.6-0.7 at 530 nm (approximately 1×10^8 CFU/mL).

Approximately, 2 mL of the standardised suspension will be mixed with 200 mL of sterile molten agar cooled to 40-45°C, poured into Petri plates, and allowed to solidify. Wells of 6 mm diameter will be prepared and filled with test samples (100 and 200 µL), streptomycin (100 µL) as the antibacterial standard, fluconazole (100 µL) as the

antifungal standard, and DMSO (100 µL) as the negative control. Following prediffusion at 2-8°C, bacterial plates will be incubated at 30-35°C for 24 hours and fungal plates at 25-28°C for 48-72 hours.

STATISTICAL ANALYSIS

Data will be analysed using IBM Statistical Package for the Social Sciences (SPSS) Statistics, version 29.0, IBM Corp., Armonk, NY, USA. Data will be expressed as mean ± standard deviation. The Shapiro-Wilk test will be used to assess normality. For normally distributed data, the unpaired t-test will be used for two-group comparisons and One-way ANOVA for comparisons involving more than two groups, followed by Tukey's post-hoc test for pairwise comparisons. For non normally distributed data, the Mann-Whitney U test will be used for two-group comparisons and the Kruskal-Wallis test for more than two groups, followed by Dunn's post-hoc test with Bonferroni correction for multiple comparisons. A p-value <0.05 will be considered statistically significant.

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