ASSESSMENT OF WOUND HEALING AND ANTIMICROBIAL POTENTIAL OF *CLERODENDRUM PANICULATUM* LEAF EXTRACT AND ITS FORMULATION

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ABSTRACT

In this work, the ethanolic extract of *Clerodendrum paniculatum* leaves was evaluated for its antibacterial, antifungal, and wound-healing properties in order to create a topical herbal ointment. The crude extract was obtained by Soxhlet extraction with ethanol and subsequently submitted to antibacterial investigations, wound healing (scratch assay), and in vitro cytotoxicity (MTT assay). Its safety was confirmed by the cytotoxicity experiment on L929 fibroblast cells, which showed dose-dependent action with a maximal viability of 76.93% at 100 µg/mL. Significant wound closure was demonstrated by the scratch assay results, with treated groups showing full healing in 72 hours as compared with controls. While antifungal activity against *Candida albicans* was relatively stronger, with zones of inhibition of 1 cm and 1.2 cm at 200 µg and 400 µg, respectively, antimicrobial testing revealed poor antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The physicochemical properties of the herbal ointment made with the extract were assessed, and it showed good washability, low LOD (0.67 g), spreadability (83.41 g·cm/s),

acceptable color, odor, pH (5.9), and non-irritating nature. These results support *C. paniculatum's* traditional use and demonstrate its many applications in herbal formulations for dermatological uses by indicating that it has excellent wound healing and antifungal capabilities.

Keywords: *Clerodendrum paniculatum*, wound healing, antimicrobial activity, antifungal activity, herbal ointment

1. INTRODUCTION

The term "pharmacognosy," which describes studies of drugs made from natural products, has been used for about 200 years as part of the scientific discipline of pharmacy[1]. Traditional medicines, particularly herbal medicine, continue to be the main source of healthcare in many countries and cultures throughout [2]. Often referred to as the "Red Pagoda plant" (Family *Verbenacea*), *Clerodendrum paniculatum* Linn is a semi-woody shrub that grows naturally in shaded spots all across India and can grow up to 1-2 meters tall. It has traditionally been used to treat inflammation, ulcers, rheumatism, neuralgia, and wound healing in China, Japan, and India [3]. The herb is considered to have ethnomedical importance and is used to treat wounds, typhoid, snakebite, jaundice, giddiness, malaria, anemia, and hemorrhoids [4].

Injury is one of the primary causes of physical limits. A towel condition known as a crack is one that has been changed by physical, chemical, microbiological, or immunological attacks; it is typically associated with a loss of function [5]. The loss or interruption of the cellular, anatomical, or functional continuity of living tissue is one way to define a wound [6]. Plants have long been used by humans to treat common infectious diseases, and some of these age-old treatments are still in use today to treat a range of ailments [7]. Plants are used medicinally in many countries and are the source of promising and effective treatments [8].

Among all forms of medicine, herbal medicine is the oldest [9].In addition to standard dose forms, herbal drugs can also be found in ointment formulations. Medication may or may not be present in an ointment [10]. Herbal ointments made from plants have many advantages, such as being readily available, having fewer side effects, and being a powerful treatment [11].

2. METHDOLOGY

2.1 Plant collection, authentication and drying:

Fresh leaves of *Clerodendrum paniculatum* Linn were collected from Vengasseri in Palakkad, Kerala. Dr. Ranjusha A.P., the head of the botany department of NSS College in Ottapalam, Palakkad, Kerala, confirmed and taxonomically identified the item. The plant material was processed into a powder using a mixer grinder and stored in an airtight container after being dried in the shade for 18 to 20 days.

2.2 Extraction

The leaves of *C. paniculatum* Linn. were subjected to hot continuous extraction using a Soxhlet apparatus with ethanol as the solvent. Thirty grams of powdered marc were placed in the extractor, while 400 mL of solvent was taken in a round-bottom flask. The extraction was carried out for 12 hours, after which the solvent was carefully evaporated to obtain the extract. The concentrated extract was then weighed and preserved in a desiccator for subsequent analysis [12].

2.3 Invitro cytotoxicity study

L929 mouse fibroblast cells were cultured in DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and antibiotics at 37°C in a humidified 5% CO_2 incubator. Cells (5×10^3/well) were seeded into 96-well plates and allowed to attach for 24 h. The test sample (1 mg/mL in 0.1% DMSO, sterile-filtered) was diluted with medium to obtain concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL, which were added to the cells in triplicates, while untreated and vehicle controls were maintained. After 48 h of incubation, cell morphology was

examined under an inverted microscope, and cytotoxicity was evaluated by the MTT assay. Briefly, 30 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 h at 37°C. The supernatant was then removed, and 100 µL of DMSO was added to dissolve the formazan crystals. Absorbance was recorded at 540 nm using a microplate reader, and the percentage of cell viability was calculated as: (Mean OD of sample/Mean OD of control) × 100.[13,14].

2.4 Invitro wound healing activity

L929 mouse fibroblast cells were cultured in DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and antibiotics at 37°C in a humidified 5% CO_2 incubator. For the assay, exponentially growing cells were seeded into 12-well plates at a density of 2×10^5 cells/well and allowed to form a confluent monolayer. A sterile 1 mL pipette tip was used to create straight-line scratches across the monolayer, and the detached cells were removed by rinsing with PBS. The wells were then treated with the test sample (25 µg/mL, prepared in 0.1% DMSO and sterile-filtered) and incubated for 0, 24, 48, and 72 h. Wound closure was observed under an inverted microscope (4× magnification, Olympus CKX41), and images were captured at the intersection of the scratch and pre-marked line. The percentage of wound healing was quantified by measuring the wound area using MRI-Image analysis software [15].

2.5 Invitro antimicrobial activity

2.5.1 Antibacterial activity-agar disc diffusion method

The antibacterial activity of the ethanol extract of *Clerodendrum paniculatum* leaves was evaluated by the disc diffusion method using *Pseudomonas aeruginosa* and *Staphylococcus aureus* as test organisms. Muller Hinton agar plates were prepared and uniformly lawned with bacterial cultures using sterile swabs, followed by a 15 min drying period. Sterile discs were impregnated with the plant extract, while 30 µg gentamycin discs served as the positive control and

ethanol (99%) as the negative control. The plates were incubated at 37°C for 18–24 h, after which the zones of inhibition were measured using calipers. All experiments were performed in triplicates to ensure reliability [16].

2.5.2 Antifungal activity-agar disc diffusion method

The antifungal activity of the ethanol extract of *Clerodendrum paniculatum* leaves was evaluated against *Candida albicans* using the disc diffusion method. Nutrient agar plates were lawned with the fungal inoculum and left at room temperature for 30 min (pre-diffusion time). Sterile discs impregnated with the plant extract at concentrations of 200 and 400 µg were placed on the plates, with fluconazole discs as the positive control and ethanol as the negative control. After allowing the discs to diffuse into the agar for 1 h at room temperature, the plates were incubated in the dark for 48 h, and the resulting zones of inhibition (ZOI) were measured and recorded [17].

2.6 Preparation and evaluation of wound healing ointment

2.6.1 Preparation of ointment

SL NO	INGREDIENTS	QUANTITY(10g)
1	Ethanolic extract of *Clerodendrum paniculatum* leaves	0.5g
2	Emulsifying wax	0.5g
3	White soft paraffin	4.8g
4	Liquid paraffin	3.9g

Table no;1 formulation of ointment

Procedure: Each component was weighed and heated with a heating mantle to 70°C in a beaker. After five minutes of gently mixing the materials, the temperature was maintained at 70°C. The ethanolic extract of the leaves of *Clerodendrum paniculatum* was then added, mixed well, and placed into the closed container [18].

2.6.2 Evaluation of ointment

- ✓ **Colour and odour:** Examine the ointment visually to determine its color and scent.
- ✓ **pH:** The ointment's pH was measured with a digital pH meter. After dissolving 1 g of ointment in 50 ml of purified water, the pH was measured[19].
- ✓ **Spreadability:** To assess spreadability, a particular weight is applied for a predetermined amount of time to compress the sample between two slides to a uniform thickness. The length of time required to separate two slides is referred to as spreadability. When two slides are separated in less time, spreadability improves.

  The formula used to determine spreadability was[20]

  $$S = MxL/T$$
- ✓ **Evaluation of Loss on Drying (LOD):** The formulation was put in a petri dish in a water bath and dried at 105°C to determine LOD.
- ✓ **Washability:** The combination was applied to the skin of two volunteers, and the ease of washing with water was then evaluated.
- ✓ **Test for Non-Irritation:** A prepared herbal ointment was applied to the skin of two individuals, and the outcomes were tracked[21].

3. RESULTS AND DISCUSSION

Invitro cytotoxicity study

Sample Concentration (µg/mL)	OD value I	OD value II	OD value III	Average OD	Percentage Viability
Control	0.9331	0.9248	0.913	0.9236	100.00
Sample Code: Sample					
6.25	0.9045	0.9187	0.9057	0.9096	98.49
12.5	0.8878	0.8748	0.8716	0.8781	95.07
25	0.8453	0.8365	0.8378	0.8399	90.93
50	0.8002	0.7956	0.7935	0.7964	86.23
100	0.7073	0.7118	0.7125	0.7105	76.93

Table no:2 Cytotoxic assay

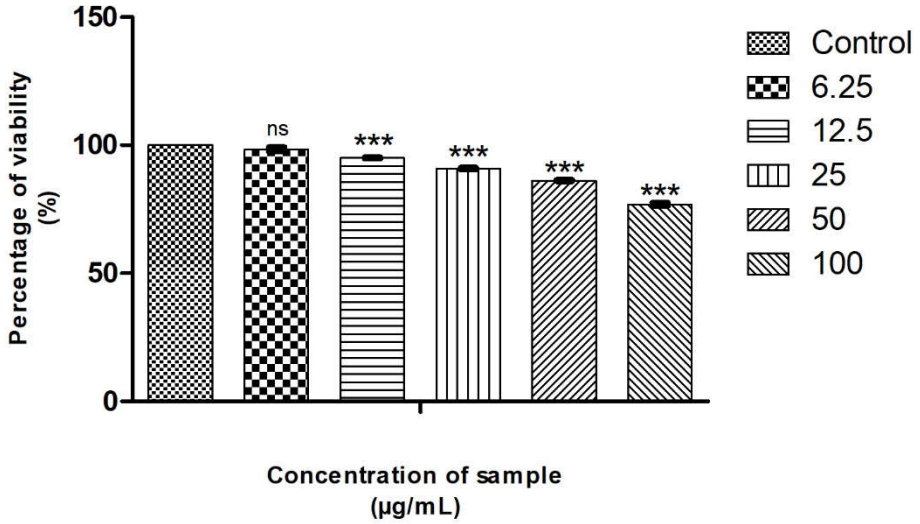

Figure no:1 Percentage of viability

A graphic representation along the Y axis that shows the sample's cytotoxic effect using the MTT assay percentage viability, with the concentration of the sample changing along the X axis. Three duplicates of each experiment were conducted, and the findings are shown as Mean+/- SE. The data was analyzed using the Dunnet's test and one-way ANOVA. $P < 0.001$ in comparison to the control group, and ns < non-significant.

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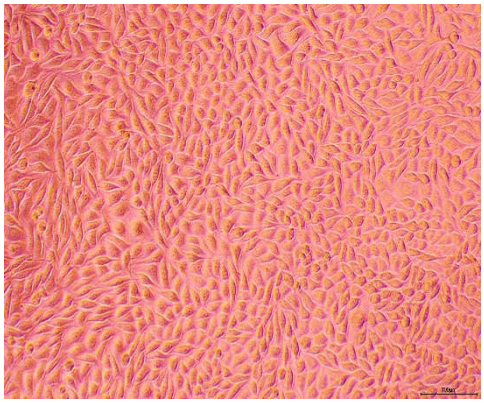

Figure no:2 sample 6.25µg/ml

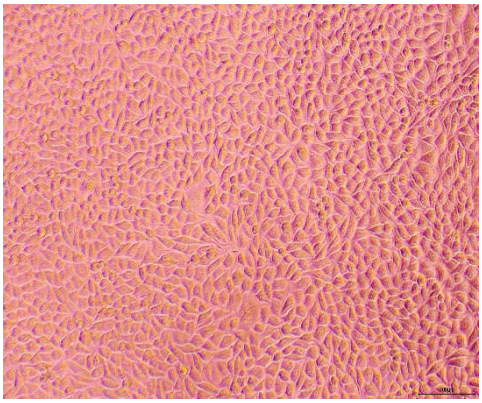

Figure no:3 sample 12.5µg/ml

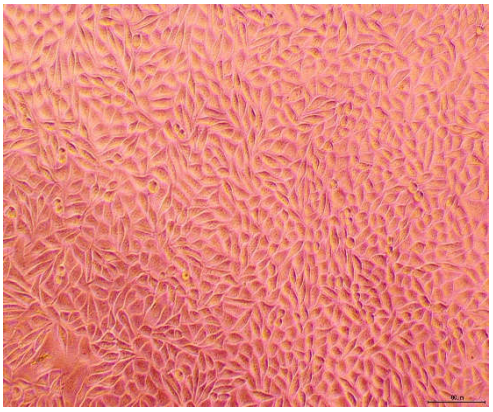

Figure no:4 sample 25µg/ml

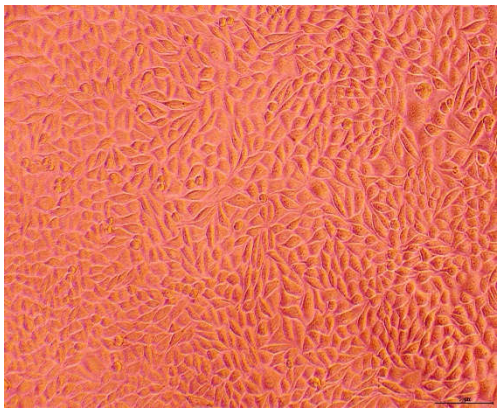

Figure no:5 sample 50µg/ml

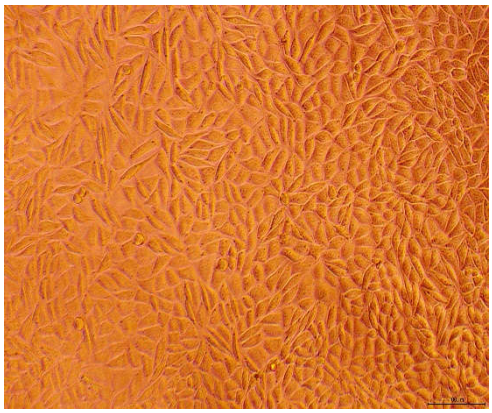

Figure no:6 sample 100µg/ml

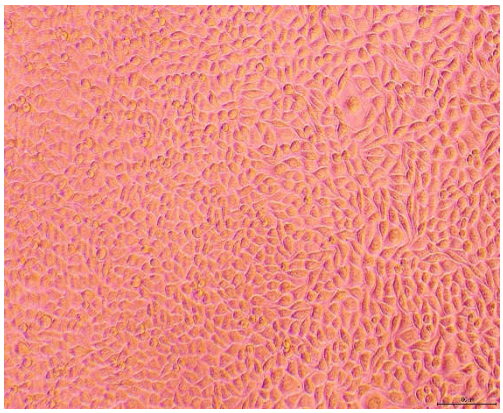

Figure no:7 Control

Invitro wound healing activity

Time Interval (hrs)	Scratch area (px)
CONTROL 0 HR	1428459
SAMPLE 0 HR	1426702
CONTROL 24 HR	1034415
SAMPLE 24 HR	828330
CONTROL 48 HR	577161
SAMPLE 48 HR	283455
CONTROL 72 HR	310032
SAMPLR 72 HR	0

Table no:3 Scratch assay

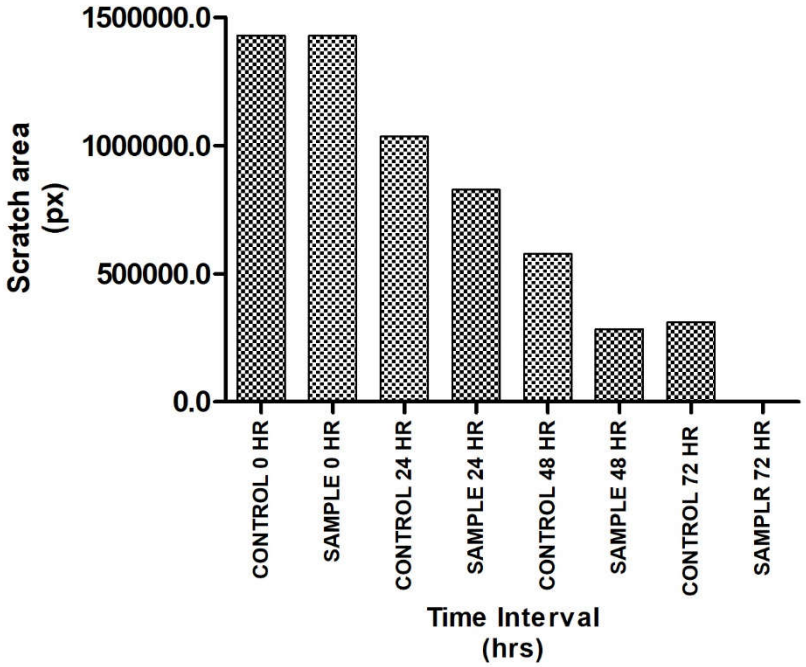

Figure no:8 Scratch wound healing area

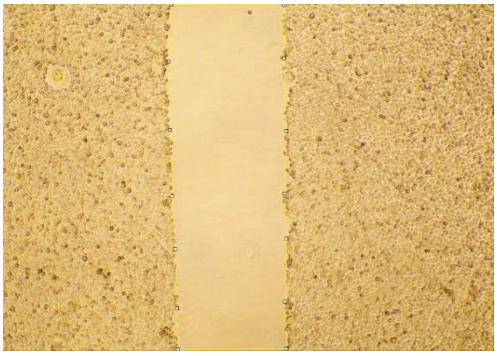

Figure no:9 Control at 0th hour

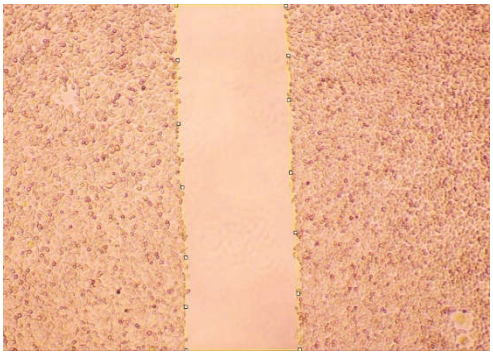

Figure no:10 sample at 0th hour

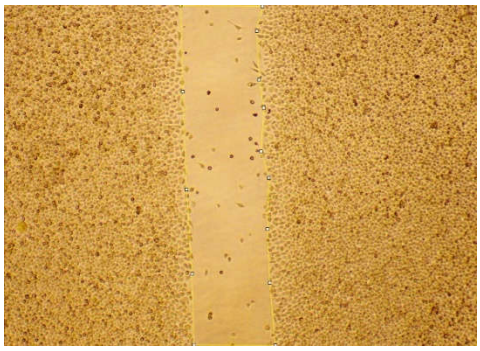

Figure no:11 Control at 24th hour

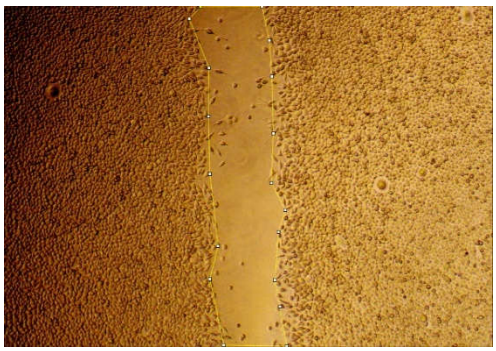

Figure no:12 sample at 24th hour

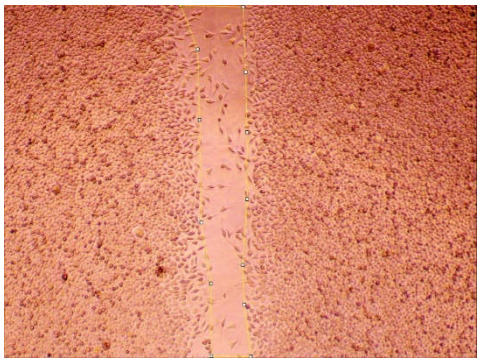

Figure no:13 Control at 48th hour

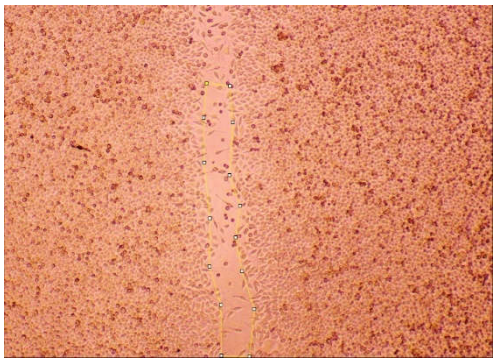

Figure no:14 sample at 48th hour

Figure no:15 Control at 72nd hour

Figure no:16 sample at 72nd hour

The scratch wound healing test revealed that the leaf extract from *Clerodendrum paniculatum* linn promoted a faster wound closure than the control. The treated cell's wound area shrank in just a day. After 48 hours, the treated cells had repaired considerably more. The wound on the treated cell was completely closed after 72 hours, whereas there was still a gap in the control. Consequently, the sample may hasten the wound-healing capacity of the cells.

Invitro antimicrobial activity

Antibacterial activity

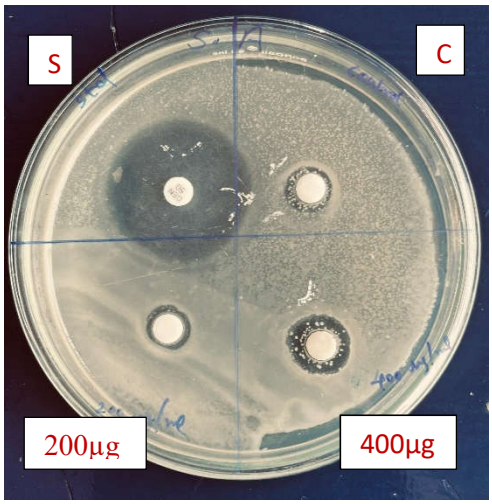

Figure no:17 Antibacterial activity of *S. aureus* in extract

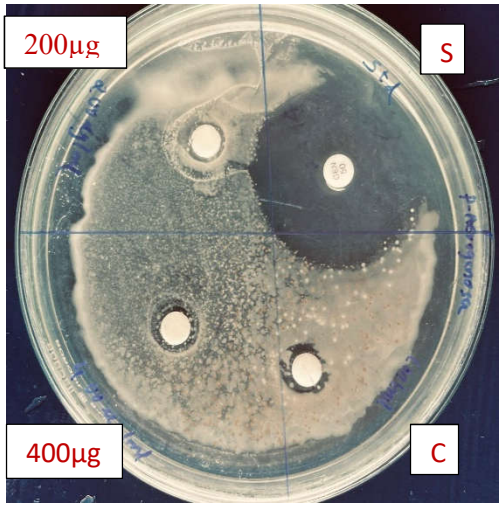

Figure no:18 Antibacterial activity of *P. aeruginosa* in extract

Sl no	Organisms	ZOI of control (cm)	ZOI of standard (cm)	ZOI of extract (200µg)	ZOI of extract (400µg)
1	*S. aureus*	0.9	3.1	0.2	0.7
2	*P. aeruginosa*	0.8	3.6	0.1	0.2

Table no:4 Anti- bacterial activity of *C.paniculatum* leaf

The antibacterial activity of the ethanolic extract of *Clerodendrum paniculatum* leaves was not as strong as that of the conventional medication. The extract demonstrated a mild dose-dependent activity against *Staphylococcus aureus*, producing a zone of inhibition (ZOI) of 0.2 cm at 200 µg and 0.7 cm at 400 µg. This was significantly less than the standard (3.1 cm). Comparing the extract to the standard (3.6 cm), *Pseudomonas aeruginosa* showed negligible inhibition, measuring 0.1 cm at 200 µg and 0.2 cm at 400 µg. The lack of solvent action was

confirmed by the control, which displayed very little activity in either organism. In summary, the findings indicate that although the extract has some modest antibacterial properties, its efficacy is significantly lower than that of the standard.

Antifungal Activity

Sl no	Organism	ZOI of control (cm)	ZOI of standard (cm)	ZOI of extract (200µg)	ZOI of extract (400µg)
1	*C. albicans*	**0**	**1.5**	**1**	**1.2**

Table no:5 Antifungal activity of *C. paniculatum* Leaf

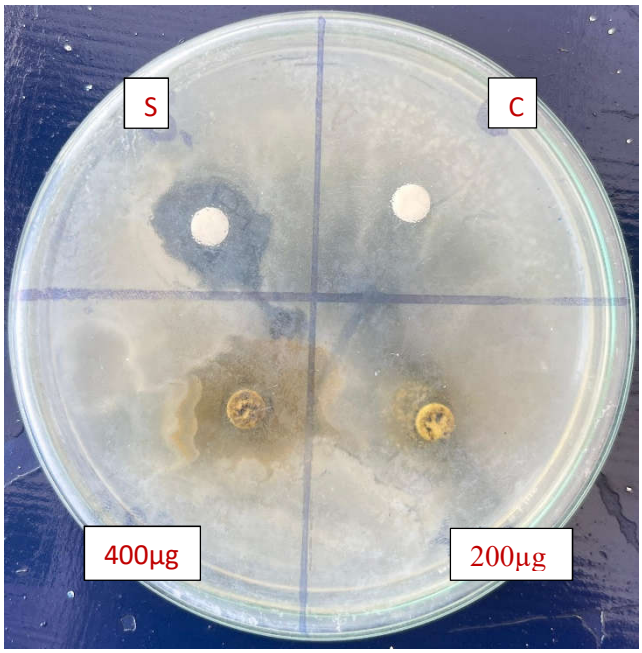

Figure no:19 Anti-fungal activity of *C.albicans* in extract

Clerodendrum paniculatum leaf ethanolic extract showed strong antifungal efficacy against *Candida albicans*. The extract showed a small dose-dependent increase in activity, producing a zone of inhibition (ZOI) of 1 cm at 200 µg and 1.2 cm at 400 µg. The extract had significant antifungal potential in comparison to the control, which showed no inhibition, even though the conventional

medication displayed a larger inhibition zone of 1.5 cm. Although being less effective than the standard, these results imply that the extract includes bioactive chemicals with antifungal activities.

Preparation and evaluation of wound healing ointment

Preparation of ointment

Figure no:20 10g wound healing Ointment

Evaluation of wound healing ointment

Sl no	Parameters	Observations
1	Colour	Light green
2	Odour	Not strong
3	pH	5.9
5	Spreadability	83.41gcm/s
6	LOD	0.67g
7	Washability	Good

8	Non irritation	Non irritant

Table no:6 Evaluation parameters of wound healing ointment

The physicochemical properties of the herbal ointment made from the ethanolic extract of *Clerodendrum paniculatum* leaves were assessed, and the findings suggest that it is suitable for topical use. The ointment was visually acceptable due to its faint odor and light green color. The pH of 5.9 is safe and has a low chance of irritating skin because it is within the skin-compatible range. With a spreadability of 83.41 g·cm/s, it was found to be easy to apply over the skin's surface. Low moisture content and good stability were confirmed by the loss on drying (LOD), which was 0.67 g. The ointment's promise for safe dermatological usage was further supported by its good washability and non-irritating properties. All things considered, these findings imply that the formulation has favorable properties for efficient topical administration.

4. CONCLUSION

Clerodendrum paniculatum leaves have long been used in herbal medicine, and its ethanolic extract showed encouraging antifungal and wound-healing properties. The extract demonstrated antifungal potential against *Candida albicans* and significantly accelerated wound closure in vitro, considering its comparatively low antibacterial efficiency. Favorable physicochemical properties, such as good spreadability, stability, washability, and non-irritating nature, demonstrated the produced herbal ointment's appropriateness for topical use. The study's findings demonstrate the possibility of *C. paniculatum* leaf extract as a natural, safe option for dermatological formulations.

5. ABBREVIATIONS

C.paniculatum : Clerodendrum paniculatum

ZOI: Zone of inhibition

S. aureus: staphylococcus aureus

P. aeruginosa: pseudomonas aeruginosa

C.albicans: Candida albicans

6. ACKNOWLEDGEMENT

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