

***In vivo* anti dermatophytic study of topical formulation containing essential oil of *Ocimumamericanum*Linn**

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Abstract:

In the current study, an attempt was made to create a gel containing essential oil, and its antifungal effects on several microorganisms were assessed. Hydrodistillation was used to collect and extract the plant's leaves. Various percentage of extracts and polymer concentrations were used to prepare gels. An innovative study focused on phytoconstituents of extracts containing flavonoids and polyphenolic chemicals, pushes to develop the novel drug delivery method for herbal pharmaceuticals. However, their inability to traverse lipid-rich biological membranes is limited by their poor lipid solubility and greater molecular size, leading to poor bioavailability. According to many reports, gel has a bright future as an improved herbal supplement that outperforms traditional herbal extracts in terms of absorption and use. It was determined that *Ocimumamericanum* gel has improved stability and dispersion characteristics, making it a desirable carrier for the administration of the numerous phytoconstituents found in it. The application of gel formulation as topical pharmaceutical agent and cosmetics with better safety and efficacy leads in proper utilisation of herbal medications and cost-effective pharmaceutical product. Zone of inhibition was determined using the cup plate method, and MIC was determined using the two fold dilution method (Minimum Inhibitory Concentration). Moreover, the minimum fungicidal concentration was determined. It was also investigated how sensitive microorganisms are to commercial items. The gel's antifungal efficacy against different research strains was encouraging. Also, the *in vivo* therapeutic benefits of a gel preparation containing *Ocimumamericanum* essential oil on dermatophytosis in mice brought on by *A. varis* and *A. niger* were assessed. Clotrimazole considerably treated *A. varis* and *A. niger* infection on days 15 of an *in vivo* antidermatophytic assay, while a gel formulation containing *Ocimum americanum* essential oil significantly cured both infections on days 19 of the assay. At ambient temperature, the gel remained stable.

Key words: *Ocimumamericanum*, Carbopol 934, Triethanolamine, Propyl paraben, Mice, Ethanol and Distilled Water.

Introduction:

Herbal medicine is one of the oldest and most universal system of health care system. The advancement in the field of herbal drug delivery started recently with the aim to manage human diseases efficiently. World Health Organization (WHO) estimates that 80% of the world populations presently use herbal medicine for primary health care. Every nation is seeking health care beyond the traditional boundaries of modern medicine; turning to self medication in the form of herbal remedies. ¹ Modern herbal medicine is

based upon the combination of traditional knowledge, clinical experience, understanding of medicinal science and scientific evidence of herbal medicine. People are slowly and gradually switching to alternative forms of medicine. One of these many alternative therapies include herbal system of medicine. It is made of from an extract taken from the plant parts (leaf, root, flower and bark). They are absolutely natural and safe form of curing illness form occurring repeatedly. They help in curing the ailment and are also known to prevent the illness from occurring repeatedly. Herbal medicines may have long curing periods, but they eradicate the illness from it and prevent any future episodes of the same.²

Novel Herbal Drug Delivery System

Novel herbal drug delivery system opens new way for delivery of herbal drugs at right place, at right concentration, for right period of time and also gives scientific evidence to verify the standardization of herbal drug. With the progress in all fields of science and technology, the dosage forms have evolved from simple pills to highly sophisticated technology intensive drug delivery systems, which are known as Novel Drug Delivery System (NDDS). In the past decades considerable attention has been focused on the development of novel drug delivery systems for herbal drugs.⁵ Herbal drugs are becoming more popular in the modern world for their ability to cure various diseases with less toxic effects and better therapeutic effects.^{3,4}

Animal

Inbred male mice (weighing 150–180 g) were raised in the Jeeva Life Sciences Animal House in Hyderabad with IAEC approval number CPCSEA/IAEC/JLS/18/07/22/025. They were treated in accordance with the accepted procedures for the handling of laboratory animals and kept in a colony room with a 12:12 hour light/dark cycle at 21°C. Depending on the type of assay, they were randomly chosen and housed alone or in groups for 5 days before to the test's commencement so they could get used to the conditions. Also, the control and test groups were randomly assigned to the animals.

***Invivo*antidermatophytic activity**

Dermal infection of animals

To assess the in vivo antidermatophytic activity of the essential oil and antidermatophytic gel in the current investigation, 25 male mice were employed. They were sorted into several treatment groups at random. They were split up into five groups of five animals each. One control group, a positive control group treated with the reference antifungal medication clotrimazole at 10 mg/kg of bodyweight, was employed as a basic control with no infection and no therapy (BW). Two groups received treatment by ingesting 100 ml and 150 ml of essential oil, respectively. A prepared gel is used to treat one group.^{6,7}

A singleuse scalpel was used to shave a 5 cm² section of each mouse's back hair and softly scrape the skin. The surface of a 3 cm² area within the shaved zone was then infected with 50 l of the suspension of *A. varis* or *A. niger*. Direct inspection of the diseased area, followed by the culture of scrapings from the area on agar, and microscopic examination of the resultant fungi from the scrapings, provided proof of the infection. From the Sambalpur University's Biotechnology Department, all of the fungus cultures were gathered.⁸

Treatment for animals with infection

Starting on the sixth day following animal infection and continuing daily (every morning) until full recovery, animals were treated by topical application of gel at concentrations of 1g/kg and 2g/kg BW (selection of these concentrations was based on the primary experiments which also showed that gel had no toxicity at these concentrations).

By cultivating skin scrapings and hair on SDA (Sabouraud Dextrose Agar) for the recovery of viable dermatophytes, the gel's effectiveness against dermatophytes was assessed.^{9,10} Every other day, scrapings and hair were gathered from the infection site's active border.

The cultures were kept at 28°C for 15 days.¹¹

Pharmacological evaluation of gel

Final selection of the optimal formulation involved pharmacological assessment of prepared gel at concentrations of 1gm/kg and 2gm/kg.

Study of antidermatophytic activity

The antidermatophytic activity in mice was carried out as per following procedure.

Calculating Wound Contraction





















Using clear paper and a permanent marker, the wound was traced on days 4, 8, 12, 16, 18, and 20 to determine the wound closure rate. A new way in order to determine the pace of wound contraction and the length of the epithelialization process, changes in the wound area were assessed. When calculating the percentage (%) of wound contraction, the evaluated area (surface area) was employed and the wound's initial size was assumed to be 100%.

Table-1 Gel's impact on wounds with fungal infections:

Treatment	Infected Wound Model Wound area(mm ²) ± SEM(Standard Error Mean)on post wounding days							
	0 day	4 th day	8 th day	12 th day	16 th day	18 th day	20 th day	MTE - days
Control	440.63 ± 22.17	425.32 ±16.77	394.07 ± 17.34	324.56 ± 13.96	292.37 ±7.48	253.36 ±6.35	186.64 ±3.66	-
Standard Clotrimazole	461.04 ± 17.35	321.53 ± 16.92	121.62 ± 6.05	30.16 ±2.29	0.0	0.0	0.0	14.31 ± 0.33
100 µl	371.52 ± 15.78	284.53 ± 19.87	173.82 ± 11.13	76.55 ±7.12	26.15 ±2.90	16.36 ±2.01	3.05	19.63 ± 0.43
150 µl	353.82 ±9.43	239.13 ±7.85	120.42 ± 6.12	49.14 ±4.1	12.44 ±1.36	5.27 ±0.89	0.00	18.68 ± 0.18
Prepared Gel	348.23 ±9.43	229.61 ±7.85	109.67 ± 6.12	41.24 ± 4.1	11.87 ±1.36	2.27± 0.89	0.0	18.83 ± 0.16

[Values are mean± S.E. of 5 animals in each group. . **Control**:no infection no treatment, **Standard**: Clotrimazole , 100 µl and 150 µl oil, **Gel**: Prepared gel , **MTE**: Mean Time of Epithelialization

Figure-1. % Wound contraction of infected wounds:

Days	Untreated Control	Standard Control (Clotrimazole)	100 µl Oil	150 µl Oil	Prepared Gel
0					
8th					
16th					
20th					

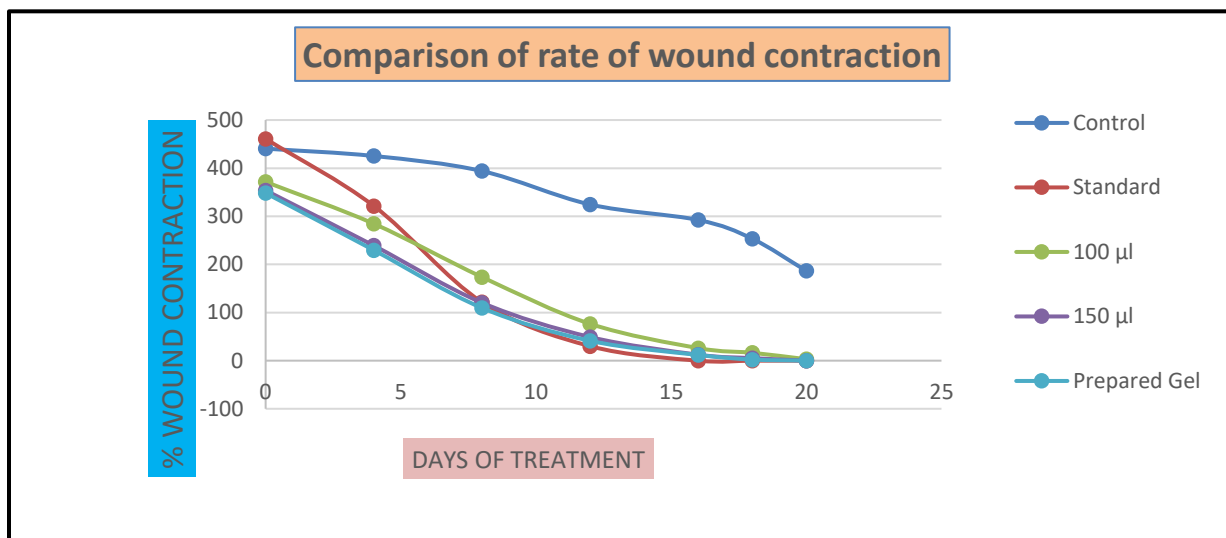


Figure-2 Graph showing comparison of rate of wound contraction

Discussion: From the infected wound healing investigation, it was noted that the manufactured gel showed maximal effect with 100% wound contraction before 19th day with mean time of epithelialization approximately 18.83 days. Similar to how the 150 μ l *Ocimumamericanum* oil demonstrated greatest efficacy with 100% wound contraction before 19th day with mean time of epithelialization about 18.68 days, the 100 μ l *Ocimumamericanum* oil demonstrated maximum effect with 100% wound contraction before 20th day with mean time of epithelialization around 19.63 days. Contrarily, the MTE for the common medication clotrimazole was 14.31 days.^{12,13} Hence, it was determined that the plant's leaf extracts (oil) have powerful antifungal properties. The plant's extract (oil) has wound-healing properties. Phytoconstituents such as flavonoids, phenols, alkaloids, tannins, and saponins are abundant in the extract (oil).^{14,15}

Analyses of medicinal gels

By using toxicological and antidermatophytic screening, gel bases and medicinal gels were assessed.

Evaluation of the produced gel's toxicity:

To determine if bases and extracts in bases are compatible with the skin of rabbits and humans, the Draize Rabbit Patch Test and Human Patch Test were undertaken. The gels that were used in a toxicological investigation on rabbits as well as on volunteers from the human population did not cause any skin reactions. Hence, it was determined that using the formulations topically was safe.¹⁶

Pharmacological evaluation of selected drug and the prepared gel:

The optimal formulation was ultimately chosen after pharmacological assessment of gel formulations like 2gm/kg BW. To assess wound healing activity, the current study used rats and an infected wound model.

Measurement of Wound Contraction: The wound closure rate was assessed by tracing the wound on days 48", 12", 16", 18" and 20 day using transparent paper and a permanent marker. The wound areas were measured using 1 mm scale of graph paper. Changes in wound area were evaluated, giving an indication of the rate of wound contraction and epithelialization period. The evaluated surface area was used to calculate the percentage of wound contraction, taking initial size of the wound as 100%. According to the study on the healing of infected wounds, the produced gel had the greatest impact, causing a 100% wound contraction before the 19th day and an average epithelialization duration of about 18.83 days. The 150 μ l of Plant *OcimumAmericanum* oil similarly demonstrated maximum effect with 100% wound contraction after 19th day with mean time of epithelialization around 18.68 days, and the 100 μ l of oil demonstrated maximum effect with 100% wound contraction before 20th day with mean time of epithelialization around 19.63 days. The MTE for the usual medication, however the standard drug had taken MTE of 14.31 days. Due to their astringent and antibacterial qualities, which are assumed to be responsible for wound contraction and a higher rate of epithelialization, such as flavonoids are known to facilitate the healing process of wounds. Nevertheless other elements like phenolics and tannins play a vital function in wound healing. Hence, it was determined that the oil from the *Ocimumamericanum* plant has strong antifungal properties.¹⁷⁻²⁰

Statistical Analysis

All the results of the experiments were analysed using ANOVA followed by student 't' test. The result expressed as mean \pm SEM. Statistical significance on comparison with the control group were considered by $p < 0.01$ highly significant and $p < 0.05$ significant.

Results

The study on the healing of infected wounds shows an average epithelialization of about 18.83 days and a 100% wound contraction by formulated gel. The 100 μ l of *ocimumamericanum* oil demonstrated a maximum effect with 100% wound contraction on 20th day with mean time of epithelialization around 19.63 days, while the 150 μ l of oil shows 100% wound contraction on 19th day with mean time of epithelialization (MTE) around 18.68 days. The MTE for the conventional drug had taken MTE of 14.31 days. Flavonoids are known to speed up the healing of wounds due to their astringent and antibacterial

properties, which are thought to be responsible for wound contraction and a quicker rate of epithelialization. Yet, other substances, such as tannins and phenolics, are essential for the healing of wounds. So it was discovered that the *ocimumamericanum* plant's oil had potent antifungal effects.

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Conflicts of interest

The authors do not have any conflict of interests to declare.

Ethical approvals

The experimental procedures carried out in this survey were in compliance with Guidelines of Jeeva Lifesciences, Hyderabad for the care and use of laboratory animals in line with Animal Ethics Committee

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