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Anticandidal effect of *Eucalyptus* oil and three isolated compounds on cutaneous wound healing in rats

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Abstract. – OBJECTIVE: Poor healing is one of the major complications of microbial contamination of wounds. When the skin is damaged, microorganisms can quickly invade the underlying tissues and cause infections that are potentially life-threatening. As a result, effective therapies are required to handle such pathological disorders. Several bioactivities, including fungicidal and antibacterial properties, have been noted for *Eucalyptus* essential oils. This study aimed to investigate the effect of *Eucalyptus* oil (EO) and mixed oils (MO) of *Eucalyptus citriodora*, citronellol acetate, linalool, and α-pinene on the healing of *C. albicans* infected wounds in rats.

MATERIALS AND METHODS: Essential oils were extracted from the fresh areal parts of *Eucalyptus citriodora*, *Lavandula stricta*, and *Rosmarinus officinalis* then their active compounds were chromatographically isolated and identified using GC/Ms. The *in vitro* antifungal activities of EO and MO were evaluated against *Candida albicans* using the Agar well diffusion method. Further, their effect on the healing of *C. albicans* infected wounds was evaluated via the excision wound rat's model. Percentages of wound contraction, epithelialization period, wound *Candida* load, and the histopathology of wounded tissues were evaluated to confirm the progression of wound healing.

RESULTS: Results of the *in vitro* tests showed that MO has a potent activity against *C. albicans* evaluated by an inhibitory zone (IZ) diameter of 23.4 mm and a MIC value of 0.24 g/mL, compared to EO's corresponding values of 13.4 mm and 15.63 g/mL. The beneficial impacts of MO creams in improving the percentage of contraction of *C. albicans* contaminated wounds were better than those of EO creams. MO 10% cream showed the greatest proportion of wound contraction and epithelialization rate. The beneficial effect of MO was further confirmed by a significant reduction of the fungal load of wounds in addition to histopathological improvement compared to the NC group.

CONCLUSIONS: This study suggested the potential of 10% MO cream in enhancing the healing of *C. albicans* infected wounds upon topical application.

Key Words:
Candida, Essential oils, *Eucalyptus*, *Lavandula*, *Rosmarinus*, Wound healing.

Introduction

The skin serves as the body's first line of defense against many dangerous substances like UV radiation, heat, germs, and toxins. Excessive mechanical stress on the skin can result in major health problems for people like age-related skin tears, blisters, and pressure ulcers[1]. In case of skin deterioration, the protective function of the skin is lost, and therefore the body has to quickly generate new tissues to restore this function. However, if the wound is not promptly healed, bacteria can invade the tissues and infection may result.

The proliferation and colonization of microorganisms inside or around the wound area are one of the factors that delay wound healing. The degree of wound contamination depends on microbial virulence, colonization as well as microbial replication[2]. Opportunistic fungi are

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one of the principal microorganisms found on the skin's surface[3]. *C. albicans* one of the most prevalent opportunistic mycoses, can cause both systemic and superficial infections, especially in immunocompromised hosts, and can coexist with bacterial commensals in certain niches[4,5].

Wound care dates to initial civilizations, and numerous of these medications relied on herbal remedies. About 33% of folk medicines are used to treat wounds as well as skin disorders, in comparison to only 1% to 3% of modern pharmaceuticals[6]. The use of essential oils as natural antimicrobial agents can be an appropriate strategy to stop and prevent microbial growth and accelerate wound healing. Essential oils are highly concentrated extracts of leaves, flowers, stems, seeds, roots, resins, barks, or fruit rinds. These essential oils are frequently used in a variety of products, including medications and cosmetics due to their flavor and therapeutic or odoriferous properties.

The topical application of some essential oils, including Rosemary essential oil, accelerated the healing of infected wounds in the animal models by reducing the tissue microbial colonization, inflammation, and wound contraction ratio[7]. *Eucalyptus* essential oils, as well as leaves, are used for therapeutic purposes. Numerous varieties of soap, lotion, and toothpaste contain *Eucalyptus* oil as an antibacterial ingredient[8]. Numerous laboratory studies[9,10] have shown components in *Eucalyptus* oil that have potent antibacterial properties on both fungi and bacteria.

Unfortunately, the majority of the wound treatments that are currently available come with their own drawbacks, such as high costs, the emergence of germ resistance, and allergic responses[11]. Finding a safe and efficient alternative to synthetic chemicals from natural resources that are thought to have only few negative side effects is necessary. These encouraged us to investigate the potential activity of pure *Eucalyptus* oil and a mixture of *Eucalyptus* oil with citronellol, pinene, and linalool on the healing of *C. albicans* contaminated wounds in rats.

Materials and Methods

Plant Materials

Fresh aerial parts of *Eucalyptus citriodora* (Myrtaceae), *Lavandula stricta* Delile (Lamiaceae), and Rosmarinus officinalis L. (Lamiaceae) were collected from Dakhla Oasis (Western Egyptian desert), Saint Catherine (Sinai), Wadi Hagul (Cairo) respectively during 2019. The identification and authentication of these plants were done by plant taxonomists at the Herbarium, Desert Research Center (DRC), Cairo, Egypt.

Extraction of Essential Oils

Essential oils of *Eucalyptus citriodora, Lavandula stricta,* and Rosmarinus officinalis were extracted using hydrodistillation in an apparatus (Clevenger-type) as follows; 500 g of fresh leaves and 1,000 mL of water were added to a flask with a rounded bottom and boiled for 5 h. The collected oils were placed in small opaque vials. Yields of the preserved oils were evaluated compared to the plant materials as weight per weight[12,13]. Oil-opaque sample vials were kept in the refrigerator for additional investigations.

Essential oils of plants under investigation and their fractions were chromatographed using different columns and solvents. Oils of *Eucalyptus citriodora*, and *Lavandula stricta* were subjected to further isolation of their main components. From these columns, volatile oils were extracted from three plants under investigation. Isolated compounds Linalool from *Lavandula oil,* α-pinene from Rosmarinus oil, whereas Citronellol acetate isolated from *Eucalyptus* oil.

GC/MS Analysis

The isolated compounds were identified using GC/Ms by comparison peaks reported in the computerized information bank by the National Institute of Standards and Technology (NIST). The gas chromatography-mass spectrometry analysis of the prepared isolated compounds was performed on an Agilent 7890A/5975C system outfitted with nonpolar column (HP-5MS) consuming He 5 at a septum purge the rate of flow of 3.0 milliliter per minute.

Test Microorganism

A clinical sample of *Candida albicans* was isolated from human cutaneous candidiasis and stored at 4°C for further tests. It was isolated during 2021 from clinical samples and maintained on yeast malt agar (YMA) media at 4°C. The isolated species were identified by chromogenic agar, germ tube test, and other biochemical tests as *C. albicans*[14]. The identification was performed at the Medical Mycology Lab, Medical Laboratory Sciences Department, College of Applied Medical Sciences (CAMS), Prince Sattam bin Abdulaziz University (PSAU).

In Vitro Anticandidal Activity

The *in vitro* antifungal activity of the α-pinene, EO, Citronellol acetate, Linalool, and MO (EO, Citronellol acetate, Linalool, and α-pinene), as well as Amphotericin B (as a positive control), were determined in accordance with agar well diffusion method[15]. The isolated *Candida albicans* test organism was aseptically subcultured on yeast malt medium at 37°C for 24 h. The yeast suspension was adjusted to 0.5 McFarland Standard by inserting a loopful of the fungal colonies from an overnight culture of *Candida albicans* test organism aseptically into a sterilized normal saline test tube[16]. For the agar-well diffusion method, the *Candida albicans* test organism was seeded in an agar medium, 6 millimeters in diameter wells were cut and removed aseptically from agar plates, and then 30 microliters of each sample were transported aseptically into wells. After the incubation period, plates were observed, zones of inhibition were measured and sensitivity tests were performed in triplicate, and the mean of diameters was calculated.

Evaluation of Minimum Inhibitory Concentration (MIC)

MICs of different samples were evaluated according to microbroth dilution method[17]. Two-fold serial dilutions (using DMSO) of each sample (stock solution) were placed in Eppendorf tubes. The samples were relocated into microtiter plates (96-well). One hundred microliters of candidal suspension were added to each prepared well to obtain serial dilution. The mixtures were gently mixed and incubated at 37°C. Final *Candida* inoculum adjusted to 300×10^2 CFU per ml, dimethyl sulfoxide is a control negative, candidal broth suspension and sterilized broth is a growth control. After the incubation period, candidal growth was measured by the degree of tube turbidity, MIC was determined by the lowest sample concentration that made the tube clear and after reading, the MIC was recorded.

Experimental Animals

Male Wistar rats weighing 190±10 g were used in the current study. Animals were housed and bred in ventilated cages in the Pharmacy College, Prince Sattam bin Abdulaziz University. The animals were kept in a controlled environment (24± 2°C and a 12/12 h dark/light cycle) with free access to food and water. All environmental cage conditions were adjusted in Rat Individually Ventilated Cages Blue Line, Techniplast (Buguggiate, VA, Italy). The rats handling and care were in accordance with the internationally accepted standard use of animal guidelines[18]. Further, the BERC at Prince Sattam bin Abdulaziz University approved the animal experiments (Reference No. BERC 008-04-21).

Acute Toxicity Test in Mice

The animals used in the acute toxicity test were handled and cared in accordance with the internationally accepted standard animal guidelines. LD_{50} of *Eucalyptus* oil and mixed oils was determined in mice as described by Kerber[19]. For this purpose, albino mice (28-30 g of both sexes) were divided into different groups, each group composed of five animals. To determine the range of doses used in the experiment, preliminary trials were conducted to explore the starting dose (the highest dose that cannot kill any animal) and the end dose (the smallest dose that can kill all mice). Graded increased doses at equal intervals were chosen in between these doses (1,000-4,000 mg/kg). Each dose was given orally to a group of five mice. The control group received the vehicle (3% Tween 80) *via* the same route. The animals were kept under observation for 24 hours, during which the rate of mortality in each group was recorded.

Wound Healing Study

Preparation of creams

In the current study, wound healing treatments were used after mixing with base cream. The tested oils were mixed with cream (Lipobase™) composed of purified water, paraffin (liquid and white soft), cetostearyl alcohol, sodium citrate, cetomacrogol 1,000, citric acid, and methyl hydroxybenzoate to concentrations of 1% (0.1 g/10 g cream) and 10% (1 g/ 10 g cream) and kept at 4°C until use. Amphotericin B (Fungizone®) 1% (0.1 g/10 g cream) and cream only without any additives were utilized as a reference and negative test control, respectively.

Induction and contamination of excision wound

Four days prior to experimental infection with *Candida*, subcutaneous injections of 500 µg/rat of estradiol benzoate (Folone ampoules) were used to suppress the immune systems of rats[20]. *Eucalyptus* oil and mixed oils (*Eucalyptus* with Linalool, α- pinene, and Citronellol acetate oils) were assessed for their potential for wound healing in experimental animals utilizing a wound

excision model[21]. Thirty rats were anesthetized by intraperitoneal injection of ketamine hydrochloride (5 mg/kg) and xylazine hydrochloride (2 mg/kg). Each animal's dorsal skin was shaved with an electrical clipper and cleaned with 70% ethanol. With the help of sterilized tools (dissecting forceps toothed, sharp-point surgical scissor), a full-thickness wound measuring eleven millimeters in diameter (Figure 1) was excised carefully from the same position of the shaved area of each rat[22].

For the experimental infection of the created wound, one milliliter of *C. albicans* broth suspension (adjusted to 4×10^5 CFU/ ml) was flooded on the wound by sterilized Pasteur pipettes. To reduce other microbial contamination, fresh sterile dressings were used to cover the wounds and the entire procedure was performed in a biosafety cabinet (class II). All animals under investigation were located individually in a clean and sterile cage to reduce contamination of external microbes. To ensure candidal infection and colonization were established, the contaminated wounds were left untreated for 24 hours after contamination. Six treatment groups (n = 5) were treated as follows:

Group I: Negative control (NC) group; topically treated with a cream base.
Group II: Reference (REF) group; treated topically with Amphotericin B.
Groups III and IV: Treated topically with EO cream at 1% and 10%, respectively.
Groups V and VI: Treated topically with MO cream at 1% and 10%, respectively.

Starting on day zero (day 0), various creams were applied topically to the wound region once daily until full wound healing was accomplished.

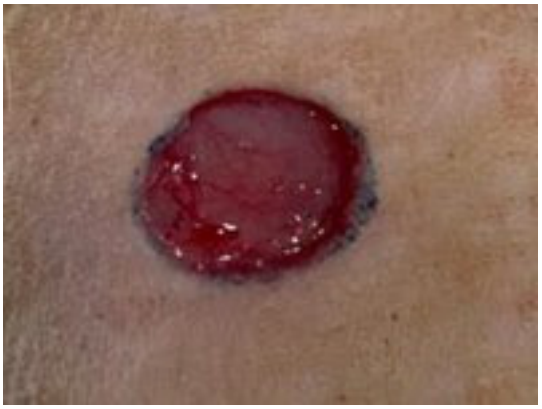

Figure 1. Photographic of a wounded skin lesion on 0th day.

Parameters Evaluated for Wound Healing

The percentage of wound contraction

Percentage of wound contractions were noted on days 7, 14, and 21 post-wounding by putting a transparent sterilized paper over the wound and tracing it out. The tracing transparent paper was put over a 1-mm^2 sheet graph and traced out to measure the values of contraction[23].

$$\text{Wound contraction \%} = \frac{\text{Wound area on (day 0 – day “no”)}}{\text{Wound area on day 0}} \times 100$$

*No = number of days (7th, 14th, and 21st day).

Epithelialization period

The number of days needed for Escher to fade away leaving no visible wound was used to determine the epithelialization period[24].

Evaluation of wound fungal load

The one-point method[25] was used to collect yeast after treatment at 3, 10, and 14 days; the center of each incision was swabbed gently by rotating the sterile swab three times anticlockwise with equal mild pressure. The totally viable yeast counting of inoculated swabs was measured by the serial dilution method; each inoculated swab was placed into a 2 mL sterile saline tube and vortexed for 10 seconds to make stock solutions. By sterile tubes, five consequent steps of dilution 10 times using 1 mL of stock solutions, then 100 µl of dilution was inoculated into yeast malt agar (YMA) plates. The sterile loop was used to spreading each yeast suspension on the surface of YMA plates to achieve separated colonies. The inoculated plates were incubated for 24-48 hours at 37°C and the number of yeasts on each plate was counted by Manual Colony Counter [aCOLade Colony Counter, Synbiosis (Cambridge, England)].

Histopathological study of wounds

After the previous treatments in this study, all the animals were intraperitoneally injected for anesthetization with Ketamine (hydrochloride form, 50 mg/kg) and Xylazine (hydrochloride form, 10 mg/kg) and then sacrificed. Away from outside the margin of the healed wound by five millimeters in normal skin, tissue samples were taken from the healed wounds and then gathered from each rat. Specimens of tissue samples were fixed right away in neutral buffered formalin (100ml/L), and at 40±2 to 60±2 °C were embed-

Table I. IZ (inhibition zone) values of the tested substances against *C. albicans*.

Samples	Mean of inhibition zone (IZ) expressed by mm
Amphotericin B	19.9 ± 0.19
α-pinene	20.2 ± 0.58
EO	13.4 ± 0.20
Citronellol acetate	12.9 ± 0.58
Linalool	15.4 ± 0.22
MO	23.4 ± 0.32

Mean of IZ in mm ± SD (standard deviation), well diameter 6 mm and tested sample 30 µl, IZ: inhibition zone, EO: *Eucalyptus citriodora* oil, MO: Mixed oils.

ded in paraffin for blocking and processing. By using a microtome, five millimeters thick sections were cut, and Eosin and Haematoxylin (E&H) were used as staining solutions. All microscope skin slides were observed under a light microscope for histopathological changes[26].

Statistical Analysis

The data were presented as Mean ± SE (standard error of the mean). SPSS version 19 (IBM Corp., Armonk, NY, USA) was used to analyze the data, which included a one-way ANOVA and then Dunnett's multiple comparison tests. Microsoft Excel (2010) was used to create the graphical representation. The mean value differences were significantly deemed at $p < 0.05$.

Results

Essential Oils and Isolated Compounds

The three isolated oils (isolates compounds) were extracted from three essential oils; Citronellol acetate (13.68%), Linalool (34.1%), and α-pinene (36.42%) isolated from *Eucalyptus*, Lavender, and from *Rosemary* essential oils, respectively.

In Vitro Anticandidal Activity

Inhibition zone values of Amphotericin B, α-pinene, EO, Citronellol acetate, Linalool, and MO against *C. albicans* are displayed in Table I. The combined oils (MO) sample was the most potent against *Candida albicans* with a clear zone diameter measured 23.4±0.32 mm, whereas citronellol acetate had the lowest *in vitro* anticandidal activity with a clear zone diameter measured 12.9±0.58 mm. The inhibition zone value of the positive control against *C. albicans* was 19.9± 0.19 mm (Table I).

Evaluation of MIC

Minimum inhibitory concentration (MIC) values of oils (crude essential and isolated compound), as well as Amphotericin B as a positive control, ranged from 0.24-30.23 mg/mL. According to the results of Table II, MO and Amphotericin B show the highest activity against clinically isolated *Candida albicans* strain.

Determination of LD50

The tested oils, *Eucalyptus* oil alone, and mixed oils at doses up to 4 g/kg failed to kill mice within 24 h. The present results indicate to a certain extent that these oils are safe, as their LD50 values are very high.

Table II. MIC values of the tested substances against *C. albicans*.

Samples	Mean of MIC (µg/mL) values
DMSO (Negative Control)	ND
Amphotericin B	01.95
α-pinene	09.56
EO	15.63
Citronellol acetate	30.23
Linalool	13.52
MO	00.24

EO: *Eucalyptus citriodora* oil, MO: Mixed oils, ND: no inhibition halo detected.

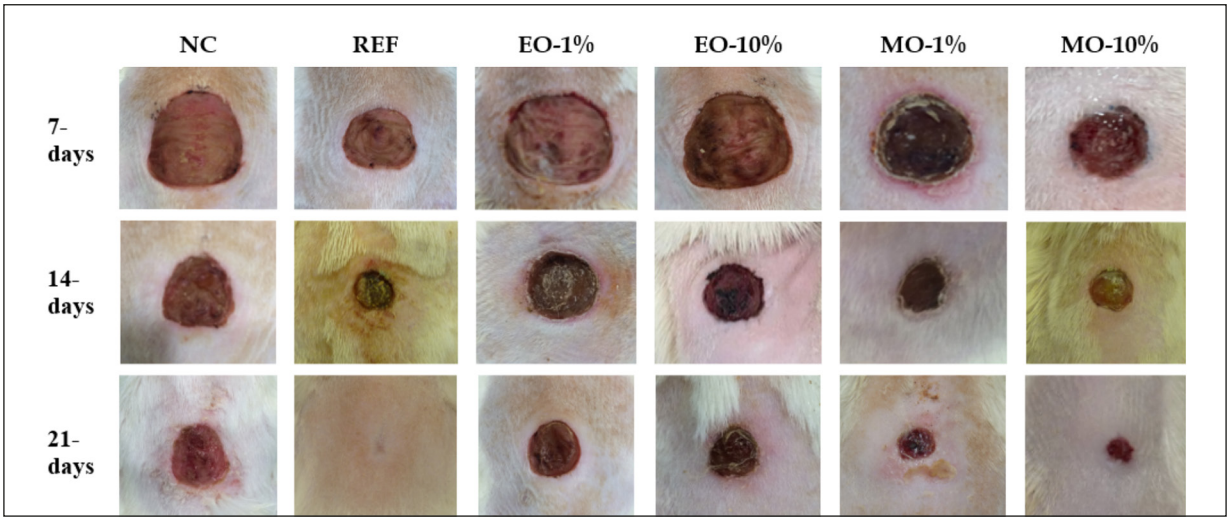

Figure 2. Photographic elucidation of a wounded skin lesion following topical application of cream base (NC), 1% Amphotericin B (REF), 1% *Eucalyptus* oil cream (EO-1%), 10% *Eucalyptus* oil cream (EO-10%), 1% mixed oils cream (MO-1%) and 10% mixed oils cream (MO-10%).

Wound Healing Activity

On day 0, there were no noticeable variations among the various groups, and the wound areas were essentially identical in all of them (Figure 1). Visual observation on days 7, 14, and 21 after wounding revealed that the infected wounds exposed to Amphotericin B and 10% MO creams appeared to show improved healing and were little in size relative to the normal control group wounds (Figure 2). The wound contraction (%) was estimated in the current study at the day's 7th, 14th, and 21st post wounding (Figure 3). In the present study, infected wounds of the NC rats showed slow healing as their percentage of contraction increased from 31.40±2.39% on day 7 to 62.28±2.21% on day 21 after wounding. By contrast, infected wounds of the REF rats had a percentage of contraction of 47.02±2.12% on day 7 and 100.0±0.00% on day 21 post-wounding. When compared to the REF group, the infected wounds

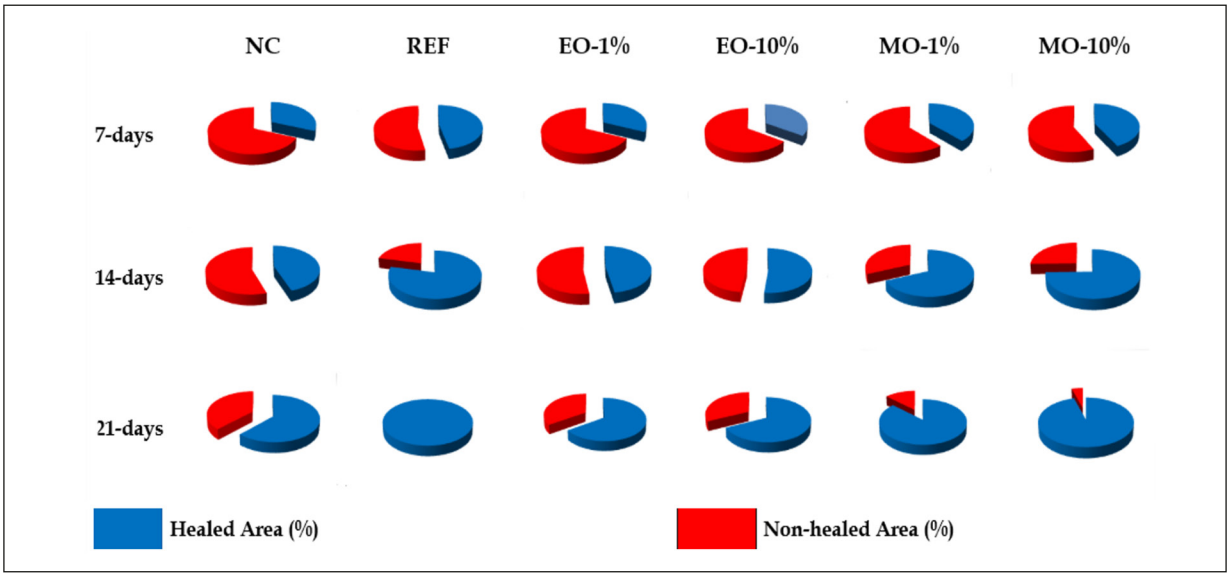

Figure 3. Effect of topical application of cream base (NC), 1% Amphotericin B (REF), 1% *Eucalyptus* oil cream (EO-1%), 10% *Eucalyptus* oil cream (EO10%), 1% mixed oils cream (MO-1%) and 10% mixed oils cream (MO-10%) on the percent. (%) of rat wound contractions.

treated topically with 1% or 10% EO cream displayed reduced percentages of contraction. The percentages of wound contraction in these groups were 31.93±2.70% and 34.60±3.80%, respectively on day 7 and 65.44±1.71% and 67.90±2.44%, respectively on day 21 post-wounding. Furthermore, the percentages of contraction of the infected wounds of MO-treated rats were significantly higher than that of the NC rats at each evaluation time point. Interestingly, the healing patterns of the infected wounds treated topically with 10% MO and REF creams were similar. The infected wounds of animals treated with 10% MO cream achieved 95.61±1.99% contraction by day 21, which is the same day that 100.0±0.00% wound contraction of rats treated with the REF cream occurred (Figure 3).

These findings were supported by the results of the epithelialization period (Figure 4). The infected wounds treated with the cream base took the longest time to complete epithelialization achievement (30.83±1.19 days). In the case of the REF-treated group, complete epithelialization of the infected wounds was observed on the 19.83±1.40 post-wounding day. Moreover, the period needed for complete epithelialization of infected wounds exposed to 1% and 10% EO extended to 29.67±1.56 and 29.17±1.45 days, respectively. The results indicated that the times required for epithelialization of infected wounds treated topically with 1% and 10% MO creams were significantly decreased (26.50±1.34 and 23.17±1.66 days, respectively).

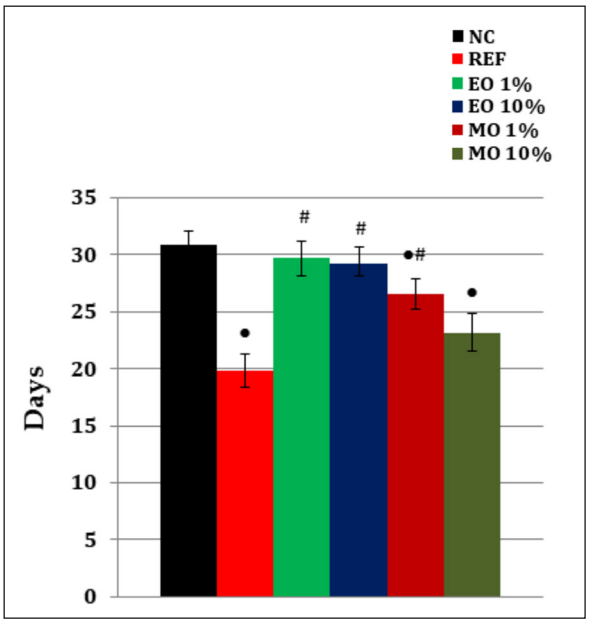

Figure 4. Topically application effect of the normal control group (NC), 1% Amphotericin B (REF), 1% *Eucalyptus* oil cream (EO-1%), 10% *Eucalyptus* oil cream (EO10%), 1% mixed oils cream (MO-1%) and 10% mixed oils cream (MO-10%) on the animal epithelialization period. Values are as stated by mean ± SE, n = 6 rats/group. •Indicates significance in comparison to the negative control group at $p < 0.05$. #Indicates significance in comparison to the reference group at $p < 0.05$.

Wound Fungal Load

The mean total viable count obtained three days after treatment was significantly lower ($p<0.05$) in the REF, EO 1%, EO 10%, MO 1%, and MO 10%-treated groups than in the NC group. A similar pattern was seen on days 3, 7, and 14 after treatment (Table III).

Histopathological Findings

Histopathological examination was used as an important parameter for evaluating wound healing. Tissue samples of the wounds from the NC group showed necrosis accompanied by severe infiltration of inflammatory cells, congestion, and edema. Furthermore, there is poor epithelialization associated with the presence of *Candida* in the epidermis and dermis layers of the skin and around hair follicles (Figure 5). Rats treated with the reference drug showed normal skin structures

Table III. Total viable yeast load in the wounds after treatment.

Treatment group	Mean of total viable count (CFU) at day post treatment		
	3	7	14
Cream base (NC)	12.80	9.30	4.17
1% Amphotericin B (REF)	4.20	1.15	0.00
1% *Eucalyptus* oil cream (EO-1%)	6.30	4.25	1.60
10% *Eucalyptus* oil cream (EO-10%)	6.10	3.95	1.18
1% mixed oils cream (MO-1%)	4.50	2.18	0.25
10% mixed oils cream (MO-10%)	3.90	1.29	0.00

EO: *Eucalyptus citriodora* oil, MO: Mixed oils, NC: Normal control.

and the absence of candidal growth. Photomicrographs of the healed wounds that were treated with 1% EO cream showed necrosis and moderate infiltration of inflammatory cells, congestion, and moderate epithelialization associated with the presence of *Candida* in the skin dermis as well as epidermis layers. The infected wound tissues of 10% EO-treated rats showed necrosis and slight infiltration of inflammatory cells and low epithelialization associated with the presence of *Candida*. Infected wounds of rats that were treated with 1% MO showed necrosis, mild infiltration of inflammatory cells, and slight epithelialization associated with the presence of *Candida* in the epidermis and dermis layers. Wound tissues of infected rats that were treated with 10% MO showed normal skin with the absence of candidiasis (Figure 5).

Discussion

The prevalence of invasive fungal infections has steadily elevated in recent decades, resulting in significant morbidity and mortality[27]. Infections by bacteria and fungi are still regarded as one of the most widespread and unpleasant conditions that significantly increase mortality and morbidity[28]. This is true despite recent advancements in the therapy of wounds. Effective and focused treatments are still required because of the unique microbiologically contaminated wound environment and the incredibly complex mechanism of wound healing. Therefore, the search for more effective treatments for infected wounds is now stimulated by experimentation[29]. Nowadays, there is an increasing interest in traditional, complementary, and alternative medicines. Essential oils are secondary metabolites found in plants that have regenerative, antimicrobial, anti-inflammatory, and antioxidant activities[30]. Numerous studies[31,32] have suggested that the various compounds of essential oils are what give them their antimicrobial properties.

The current study exhibited three isolated compounds as constituents of the chemical content of the essential oils of the tested plants. The three isolated compounds namely citronellol acetate (13.68%), linalool (34.1%), and α-pinene (36.42%) isolated from *Eucalyptus*, Lavender, and Rosemary essential oils, respectively. The chemical constituents of *Eucalyptus* essential oil differ greatly within the genus *Eucalyptus* but only slightly between species, which means that the different compounds in *Eucalyptus* essential oil are related to the species as well as the genus[33]. In previous studies[34], the citronellol content present

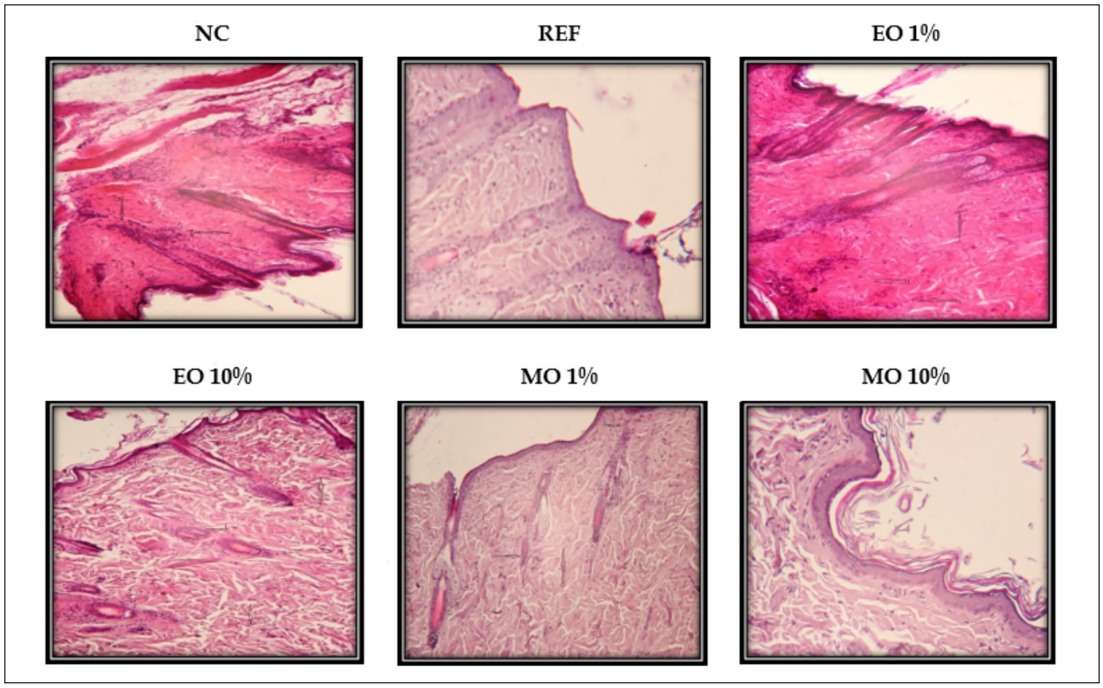

Figure 5. Histological sections of the animal wound tissues after topical application of different treatments; base cream (NC), Amphotericin B cream (REF), 1% *Eucalyptus* oil cream (EO 1%), 10% *Eucalyptus* oil cream (EO 10%), 1% Mixed oil cream (MO 1%) and 10% Mixed oil cream (MO 10%). Magnification: ×400.

in some *Eucalyptus* oils was in different concentrations. Its content in citriodora *Eucalyptus* essential oil ranged from 8 to 14.1%. In addition, essential oils of lavender flowers contain linalool in the range of 25.3 to 43.0%[35,36]. Further, α-pinene was recorded as the main compound isolated from *Rosmarinus officinalis* (93%)[37].

Numerous essential oils, including those from *Eucalyptus*, have been studied for their therapeutic characteristics and utilized in traditional medicine throughout the world[38]. Many bioactive and biochemical studies[39,40] of *Eucalyptus* essential oils for instance antimicrobial, antioxidant, antifungal, and antibacterial activities have been recorded. In the present study, we examined the *in vitro* antifungal susceptibility of Amphotericin B, α-pinene, EO, citronellol acetate, linalool, and MO against clinically isolated *Candida albicans*. The most active sample was the mixed oils, while the least was citronellol acetate. With respect to the agar-well diffusion method, with the exception of MO, which has not been previously tested, our results did not differ significantly from those reported previously[41,42]. The present results, regarding the more potent effect of MO compared to other oils, confirm the synergistic effect of mixed antimicrobial agents against microbes[43].

According to the MIC values of the crude essential oil and isolated compounds, as well as the reference drug, MO and Amphotericin B were the most active against the clinically isolated *Candida albicans* strain. The MIC results of the current study did not significantly differ from those that had already been published[41,42].

In a number of animal models, various essential oils have been investigated and studied for potential pharmacological activities. These substances have been found to have significant analgesic, anti-inflammatory, or wound healing properties[44-46]. The wound healing properties of *Eucalyptus* essential oil have been reported[47]. Wound healing is a natural physiological response to tissue damage that helps to repair the function and integrity of harmed skin tissues[48]. The wound healing process requires integrated and sequential steps to repair cells and tissues to restore their pre-injury condition[49]. The process of wound healing can be slowed down by a number of factors. One of the main factors is microbial contamination[50]. Healing of infected wounds is more difficult and is influenced by the degree of tissue damage and the kind of infection. According to Negut et al[28], infected wounds require a long period of time to heal because uncontrolled microbial growth prolongs the wound infection period and thus delays the wound healing period.

The current study, however, appears to be the first to investigate the efficacy of EO and MO (*Eucalyptus citriodora,* citronellol acetate, linalool, and α-pinene) for the treatment of candidiasis in a rat model. The wound area and the percentage of wound contraction are crucial factors in the wound healing process[51], therefore monitoring these stages is critical when evaluating the MO's capacity to cure wounds. Wound areas and wound contraction (%) in the current study were recorded at 7, 14, and 21 days after wound creation. According to the study's findings, 10% MO cream can help treat wounds that have become infected with *C. albicans* and can also hasten the healing process. It is interesting that the percentage of wound contraction in the MO 10%-treated group hasn't significant results and equivalent to that observed in the REF-treated group. This means MO 10% cream indicated the same wound healing effects as the standard Amphotericin B cream.

Additionally in excision wounds, the required time to achieve epithelialization completely is a crucial factor when evaluating the wound healing process. The success of wound healing is determined by the degree of epithelialization, which is a crucial component of the healing process. The process of epithelialization involves the migration and multiplication of epithelial cells over the wounded bed. According to the present findings, the time needed for re-epithelialization was shorter in groups treated topically with Amphotericin B and 10% MO creams than in other groups because their contraction values were higher. The presence of α-pinene, which aid in the formation of collagen may be the cause of the wound healing capacity because the wound healing process depends on the production, deposition as well as maturation of collagen[52].

In the current study, the mean total viable count obtained after three days of treatment is significantly ($p< 0.05$) lower in treated groups compared to the NC group. The *in vivo* anticandidal activity using a rate model revealed that *Candida albicans* was sensitive to the substances tested in the following order: Amphotericin B, 10% mixed oils, 1% mixed oils, 10% *Eucalyptus* oil, and 1% *Eucalyptus* oil. The current study, however, appears to be the first to investigate the efficacy of these mixed oils against candidiasis in a rat model.

Histopathological examination was used as an important wound-healing parameter for the evaluation of wound healing[53]. Rats treated with Amphotericin B showed normal skin structures and the absence of candidiasis. Photomicrographs of the skin of a rat infected with *Candida albicans* and were treated with EO 1%, 10%, and MO 1% creams showed similar results in histopathological examination parameters of the skin dermis as well as epidermis layers. Wound tissues of infected rats that were treated with MO 10% showed normal skin and the absence of candidiasis.

The results of this work during *in vitro* and *in vivo* presented that, the combination of the isolated compounds from different essential oils showed promising activities against clinically isolated *Candida albicans* in wound healing properties, these results confirmed the synergetic combination of natural products in other studies[54].

Together, these results indicated that topical application of 10% MO cream accelerated the healing of wounds infected with *Candida albicans* at a higher rate than EO creams. The ability of 10% MO to improve wound healing may be due to the rapid rate of epithelialization, increased rate of wound contraction, and inhibition of fungal contamination. Finally, the use of MO cream for topical application has promising results in infected wound healing by candida as the reference Amphotericin B cream.

Conclusions

This study indicates that 10% MO cream prepared from *Eucalyptus citriodora*, citronellol acetate, linalool, and α-pinene speeds up the healing of *C. albicans*-infected wounds in rats, as evidenced by the increase in the rate of wound contraction, shortening of the epithelization period, and skin histological parameters improved. Additionally, clinical investigations must be conducted to demonstrate that the promising properties of the MO may be used safely, efficiently, and with clinical relevance.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Data Availability

All relevant data supporting the study findings are included in the article.

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Ethics Approval

The rats handling and care were in accordance with the internationally accepted standard use of animal guidelines18. Further, the BERC at Prince Sattam bin Abdulaziz University approved the animal experiments (Reference No. BERC 008-04-21).

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