

Assessment evaluation of ointments based on *Lavandula angustifolia* and *Cymbopogon nardus* on wound healing

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Abstract

The use of medicinal plants in veterinary medicine has increased in recent years, particularly in treating skin wounds. This trend is promising, mainly due to the phenomenon of resistance, which diminishes the effectiveness of certain conventional drugs. The objective was to develop ointments using volatiles from *Lavandula angustifolia* Mill. and *Cymbopogon nardus* (L.) Rendle and evaluate their effectiveness on surgical wounds in mice. Volatiles were analyzed by GC-MS, with the major as linalool (36.3%) and linalool acetate (30.4%) for *L. angustifolia*, and citronellal (45.8%) and geraniol (22.3%) for *C. nardus*. Ointments were produced at 2% and 5% concentrations, and physical-chemical tests were conducted to assess their stability. Animals were divided into five groups: G5-control, G1-*L. angustifolia* 5%, G2-*C. nardus* 5%, G3-*L. angustifolia* and *C. nardus* 2%, and G4-5%. Surgical injuries were performed, with daily treatments administered; on the 21st day, the animals were euthanized, and tissue samples were collected for histopathology analysis. The group treated with the 5% ointment containing *L. angustifolia* and *C. nardus* showed the most significant reduction in wound-healing time (82.5%), achieving it in 17 days, and exhibited no secretion or crusting during healing. Histological findings revealed mild recruitment of mononuclear cells, marked to moderate vascular proliferation, and organized collagen fibers of adequate intensity in this group. This research presents opportunities for further advances in the therapeutic properties of volatiles derived from medicinal plants.

Introduction

Skin injuries involve anatomical rupture that compromises functional integrity, resulting from an external or internal wound caused by mechanical, physical, or chemical agents (Andjić et al., 2022).

Healing is a coordinated process of cellular and molecular events that occurs in three stages: inflammation, cell proliferation, and remodeling (Eriksson et al., 2022).

Herbal medicines have been used in veterinary medicine since ancient times. One of their main benefits is the ability to treat diseases with natural products, often at lower cost and with fewer side effects than conventional medicines. Additionally, Brazil's remarkable biodiversity offers significant potential for developing new treatments from native plants (Latip et al., 2022).

Research into active ingredients that promote healing has recently increased the use of medicinal plants. Volatiles are derived from aromatic and medicinal plants and possess various properties, including anti-inflammatory, antiseptic, healing, and bactericidal effects (Mori et al., 2016). *Lavandula angustifolia* Mill. (Lamiaceae) has been used empirically to treat pain and support healing processes since 1928 (Gnatta et al., 2016). However, side effects such as sedation have been reported (Kylie et al., 2018). *Cymbopogon nardus* (L.) Rendle (Poaceae) volatile is frequently used as a repellent, antimicrobial, and antifungal agent (Santos et al., 2020; Cunha et al., 2020).

The current study aimed to develop ointments with *L. angustifolia* and *C. nardus* volatiles and evaluate their healing effects on incisional wounds in mice.

Material and methods

Volatile oil and Gas-chromatographic analysis

Lavandula angustifolia and *Cymbopogon nardus* volatile oil were obtained from the company Bysamia[®] and chemically characterized by gas chromatography, aiming to identify the stability of the components. To separate, detect, and quantify the constituents of the volatile, it was diluted in dichloromethane at 10 µL/mL. This solution was injected into a Gas Chromatograph (GC), specifically the Hewlett-Packard 5890 Series II. The equipment utilized flame ionization detection and featured split/splitless injectors with a split ratio of 1:20, facilitating the separation and detection of volatile compounds in the oil.

The compounds were separated using a fused silica capillary column (5% phenyl, 95% dimethylpolysiloxane), with dimensions of 30m × 0.25mm (i.d.) × 0.25µm (film thickness). Helium gas was used as carrier gas at a 1 mL/min flow rate. The column temperature was programmed as follows: 60°C for 2 min, followed by heating at 5°C/min to 110°C, then at 3°C/min to 150°C, and finally at 15°C. Increase the temperature at a rate of °C/min to 290°C, then maintain it for 15 min. The injector temperature was 220°C and the detector temperature was 290°C. To separate and identify the substances, 1 µL of the volatile oil samples diluted in dichloromethane (10 µL/mL) at the defined times was injected into the gas chromatograph coupled to a mass spectrometer.

The helium gas carrier flow, capillary column, and temperature conditions for GC-MS analysis were the same as those described for GC. The injector temperature was 220°C and the interface temperature was 250°C. Mass spectra were obtained with a quadrupole detector operating at 70 eV, with a mass range of 40 to 400 m/z and a scan rate of 0.5 scan/s. The identification of volatile compounds in volatile oil has been based on linear retention indices (LRI) and mass spectra of samples, compared with authentic standards injected under the same conditions, with the NIST database (2008) and the Linear retention index. The LRI was calculated based on the co-injection of the alkane series (Adams, 2007).

Development of volatile-based ointment

Ointments based on volatiles from *L. angustifolia* and/or *C. nardus* were formulated following the Practical Guide of the National Health Surveillance Agency (ANVISA, 2010). Each ointment was composed of anhydrous lanolin (30 g), butylhydroxytoluene – BHT (0.02 g), and solid petrolatum *q.s.p.* – 100 g.

Incorporation of *Lavandula angustifolia* and *Cymbopogon nardus* volatile oils

Four ointment samples, each weighing 60 g, were prepared for use in the test groups. In each sample, volatiles were added with continuous stirring until a uniform mixture was achieved. The ointments were

produced with the following concentrations: *L. angustifolia* 5%, *C. nardus* 5%, *L. angustifolia* 2.5% + *C. nardus* 2.5%, and *L. angustifolia* 5% + *C. nardus* 5%. After mixing, the ointments were packaged in amber glass containers and underwent physical-chemical characterization.

The stability study was conducted in accordance with the guidelines of the National Health Surveillance Agency and the Brazilian Pharmacopoeia. The stability of the formulations was tested under different conditions (refrigerator at 8 – 2°C, room temperature at 20–25°C, and oven at 35°C), with parameters such as appearance, color, pH, and density monitored. The samples were removed from the oven and the refrigerator 30 min before analysis (ANVISA, 2010; Pharmacopeia, 2024).

Centrifugal test

Five grams of each formulated ointment was used in the analog centrifuge (Daiki©) 80-2B. It was placed in test tubes and centrifuged at 3000 RPM for 30 min at room temperature to verify the formation of phases (ANVISA, 2010; Pharmacopeia, 2024).

Determination of apparent pH

The pH of the ointments was measured using a pH meter, benchtop pHmeter range 0–14 ATC Incoterm, after calibrating the equipment with standard solutions of pH 7.0 and 4.0. For the test, 2g of each ointment was mixed with 20 mL of distilled water, homogenized using a vortex and transferred to the glass tube where the pH of the mixture was measured (9). The pH values of each ointment were obtained through the arithmetic averages of three consecutive measurements (ANVISA, 2010; Pharmacopeia, 2024).

Density

A Gay-Lussac pycnometer with a clean, dry and calibrated metallic side outlet was used. Calibration was improved by determining the mass of the empty pycnometer and the mass of the pycnometer with its contents of water, freshly distilled and boiled at 20 °C. After this phase, the samples were transferred to the pycnometer at 20 °C. The difference in mass of the whole and empty pycnometer determined the weight of the samples (ANVISA, 2010; Pharmacopeia, 2024).

In vivo wound healing assay

Ethical and animal guidelines

The study was approved by the Animal Use Ethics Committee (CEUA) of the University of Vassouras under number 009/2023. It involved 24 heterogenic (outbred) Swiss lineage male mice (*Mus musculus*), 90 days old, with body weights ranging from 80 to 100 grams, obtained from the Vivarium of the University of Vassouras, Rio de Janeiro. The animals were randomly assigned to five groups (G1, G2, G3,

G4, and G5). Groups 1, 2, 3, and 4 were treated with ointments based on *L. angustifolia* and/or *C. nardus* at 2% and 5% concentrations. Group 5 (8 animals) PBS control, and Nebacetin[®] ointment (neomycin sulphate 5mg/g e bacitracin zinc250 UI/g) was a positive control. Group 1 (5 animals) received *L. angustifolia*-based ointments at 5%, and G2 (5 animals) received *C. nardus*-based ointments at 5%. Group 3 (5 animals) was treated with ointments containing *L. angustifolia* and *C. nardus* at 2%, while Group 4 (5 animals) received the combination at 5%. The animals were housed in polypropylene boxes lined with wood shavings and provided with pelleted food and water ad libitum, maintained at an ambient temperature between 22–25°C throughout the study.

Surgical wound

The procedure for skin wounds was performed as described by Labib et al. (2019). On day zero (D0), each animal was previously medicated with a subcutaneous injection of meloxicam 2% (2.0 mg/kg), marketed as Mexican[®] 0.2Ouro Fino, along with acepromazine 0.2% (2.0 mg/kg), Acepran[®] 0.2%, Vetnil[®], and atropine 1% (0.04 mg/kg), Atrovene 1%, VilaVetSaúde Animal. Next, the animals were sedated with 2% xylazine (10 mg/kg), 2% Anasedan[®], CEVA, and 10% ketamine (100 mg/kg), 10% Dopalen[®], CEVS, administered intraperitoneally. After confirming sedation by assessing respiratory rate and amplitude, body immobility, and absence of corneal reflex, a trichotomy of the flank region was performed. The site was antiseptically cleaned with 10% iodinated alcohol, and, using a digital caliper, a 4 cm² area was marked for the surgical wound. With a scalpel, scissors, and rat-tooth forceps, a skin fragment of 4 cm² was excised, exposing the muscular fascia. Hemostasis was achieved using sterile gauze soaked in 0.9% saline. Immediately after creating the skin defect, treatments commenced: ointments were applied to groups G1, G2, G3, and G4, whereas the control group received only filtered water.

Treatment

All groups were treated for 20 days with a topical application (2 mm) of a thin layer of the respective ointment, using an acrylic spatula after cleaning the entire length of the surgical wound with filtered water and sterile gauze. In the control group, only the cleaning procedure was performed, as in the treated groups. Every three days from D0 (D3, D6, D9, D12, D15, D18, D19, and D21), a clinical evaluation form was filled out for each animal, recording data such as weight, hair condition, dyspnea, arched posture, half-open eyes, and behavioral response (aggressive or apathetic) to prevent unnecessary suffering. In the macroscopic evaluation of the wounds, hyperemia was scored as follows: 0 = absent, 1 = mild, 2 = moderate, and 3 = intense. Additionally, the presence or absence of secretion, hematoma, crusts, or necrosis was assessed. Photographs of the wounds were taken, and measurements were obtained using a digital caliper to evaluate wound retraction.

Histopathology

On the 21st (D21), the animals were euthanized using twice the sedative doses of xylazine, Anasedan[®] (20 mg/kg), and ketamine, Dopalen[®] (200 mg/kg). After confirming death through cardiac and

respiratory parameters, the granulation tissue region was collected, along with the margin of healthy tissue, using blunt scissors and rat-tooth forceps, carefully elevating the tissue. When diuresis was performed, the carcasses of the animals were discarded following the standards of the institution's vivarium. The material was then fixed in 10% (v/v) formaldehyde.

The collected fragments were processed using routine histological methods, and 4- μ m-thick sections were obtained with a manual rotating microtome (NEUK226 rotating handwheel). The glass microscope slides, 26x76mm, with smooth cuts and high precision, were stained with hematoxylin-eosin, Larboclin (primarily used to evaluate cellular responses [infiltrates]), and Mallory's trichrome stain (Biooptica) to analyze connective tissue proliferation and evidence of collagen. They were then photographed at 20x magnification using a Leica DM2500 photomicroscope. The histological sections from each group were evaluated for vascular density and the relative abundance of mononuclear and polymorphonuclear cells and fibroblasts. Additionally, the organization of collagen fibers in the healing and re-epithelialization areas was assessed. The data were organized in a table, with scores assigned to the intensity levels of each parameter: absent, discrete, moderate, and accentuated.

Statistical analysis

The collected data underwent a normality test before being subjected to Analysis of Variance (ANOVA) using the Tukey test. These tests were conducted at the 95% significance level ($P \leq 0.05$). Statistical analyses were performed using GraphPad Prism v.9.5.1.

Results and discussion

Chemical analysis

The chemical analysis of volatiles revealed 18 compounds in the *L. angustifolia* oil and 13 in *C. nardus*. The primary compounds were linalool (**1**, 36.3%) and linalool acetate (**2**, 30.4%) in *L. angustifolia*, and citronellal (**3**, 45.8%) and geraniol (**4**, 22.3%) in *C. nardus* (Fig. 1, Table 1).

Table 1
Chemical compounds of *Lavandula angustifolia* and *Cymbopogon nardus*
volatile.

EO	Compounds	AL _C	AL _L	CNEO	LAEO
1	α -thujone	922	924	0.1	-
2	α -pinene	930	932	–	2.7
4	β -pinene	971	974	–	0.2
5	α -terpinene	1015	1014	–	11.2
6	<i>o</i> -cymene	1019	1022	–	0.3
7	Limonene	1024	1024	0.9	-
8	<i>trans</i> - β -Ocimene	1032	1032	–	0.1
9	γ -terpinene	1055	1054	–	0.3
10	<i>cis</i> -linalool oxide	1064	1065	–	2.1
11	Terpinolene	1082	1086	–	0.5
12	Linalool	1093	1095	–	36.3
13	Allo-ocimene	1138	1140	–	1.0
15	Citronellal	1148	1148	45.8	-
14	Borneol	1167	1165	–	0.3
16	α -terpineol	1189	1186	–	2.9
19	Citronellol	1221	1223	11.4	-
17	Linalool acetate	1230	1231	–	30.4
21	Geraniol	1248	1249	22.3	-
26	Eugenol	1355	1356	2.5	-
20	Neryl acetate	1357	1359	–	4.6
27	Geraniol acetate	1378	1379	3.0	–
28	Methyl eugenol	1402	1403	–	–
30	β -caryophyllene	1415	1417	–	4.3
33	γ -gurjunene	1477	1477	1.5	-
29	γ -muurolene	1478	1478	–	1.3
34	Germacrene D	1485	1484	–	0.7

EO	Compounds	AL _C	AL _L	CNEO	LAEO
37	α -(E,E)-farnesene	1505	1505	0.4	–
25	Cadinene	1522	1522	–	0.2
38	Elemol	1548	1548	2.6	–
39	Germacrene-D-4-ol	1574	1574	1.9	–
41	<i>epi</i> - α -muurolol	1642	1642	1.2	–
43	α -cadinol	1652	1652	4.0	–
	Monoterpenes hydrocarbons			1.0	15.2
	Monoterpenes oxygenated			84.0	77.7
	Sesquiterpenes hydrocarbons			0.4	6.5
	Sesquiterpenes oxygenated			9.7	0.0
	Phenylpropanoid			2.5	0.0
	Total			97.6	99.4

*EO – elution order; AL_C - Arithmetic index calculated; AL_L – Arithmetic index literature; CNEO – *Cymbopogon nardus* volatile oil; LAEO – *Lavandula angustifolia* volatile oil.

The chemical compounds identified are consistent with those reported in the literature. Citronellal and geraniol are generally the main compounds in *C. nardus* (Andrade et al., 2012; Kaur et al., 2021), while linalool and linalool acetate are the primary compounds for *L. angustifolia* (Mantovani et al., 2013).

The ointment formulation used in the study was evaluated using physical-chemical stability and characterization tests. After 30 days of production, the homogeneous aspect characteristic of an oil-water phase (O/W) of the emulsion was verified without lumps, phase separation, or color change. The formulations' physicochemical characterization presented satisfactory results in line with the Pharmacopoeia guidelines. Regarding pH, the most significant variations were observed between groups G1 and G3.

Stabilization studies are essential for guiding formulation development and selecting packaging components. It provides guidance on necessary improvements to the formulations. Assessing organoleptic and physicochemical stability is fundamental to ensuring product balance and safety (Chow et al., 2023). The tests showed that the product remained stable after 30 days, even after evaluations at various temperatures, including freezing and heating, and chemical characterization of the oils used. This process highlights the importance of care and safety in formulation development.

The topical wound treatment method allows for even distribution of the formulation across the wound bed, ensuring proper adhesion, protecting the site, reducing pain, and creating optimal conditions for healing (Lipsky and Hoey, 2014). The formulations were designed to match the skin's pH, which generally ranges from 4.5 to 5.8 and is closest to the natural skin pH (Jesus et al., 2021). In this study, all formulations showed acceptable pH levels in their analysis.

On the 9th day, a significant wound reduction of approximately 77.5% is observed in G2, followed by reductions of 62.5% in G4, 55% in G1, and 35% in G3. By the 12th day, G2 shows a lasting reduction of around 77% on average, while G4 exhibits a significant reduction of 82.5%, G1 decreases by 80%, and G3 decreases by 65% (Fig. 2).

The total wound healing time in the treated and control groups ranged from 17 to 21 days. The shortest healing time was observed in G4 (*L. angustifolia* + *C. nardus* 5%), while the longest was in G1 (*L. angustifolia* 5%). In addition to the healing duration, macroscopic features such as hyperemia, crusts, secretions, and hematomas were evaluated during and at the end of healing (Table 2).

Table 2
Macroscopic characteristics of the healing according to the days.

Group	Full healing (days)	Macroscopic features
G1	19	Marked hyperemia (vascularization), absence of secretion and crusts
G2	19	Mild hyperemia, but also with absence of secretion and crusts
G3	21	Mild hyperemia, presence of some crusts
G4	17	Marked hyperemia (vascularization), absence of secretion and crusts
G5	21	Discreet or absent hyperemia in some individuals, discreet secretion and bruises in others; 4.0 mm was missing for complete healing.

G1 – ointment based on *Lavandula angustifolia* 5%; G2 – ointment based on *Cymbopogon nardus* 5%; G3 – ointment based on *Lavandula angustifolia* + *Cymbopogon nardus* 2%; G4 - ointment based on *Lavandula angustifolia* + *Cymbopogon nardus* 5%; G5 – Control PBS.

The most significant reductions in wound size were visually observed on D6, D9, and D12. To better explain these observations, D8 was selected to compare the treated groups with the control group (G5). When using the formulation ointment of *Lavandula angustifolia* + *Cymbopogon nardus* 5% (G4), there was approximately a 35% reduction in wound size compared to the control group (G5).

Several factors, including vascular density, were evaluated in assessing histopathological parameters. The formulation used in group G4 showed the highest level of vascularization, which is vital for accelerating healing. In contrast, groups G1 and G2 exhibited moderate vascularization, while groups G3 and G5 showed mild vascularization.

Mononuclear cells were prominently observed in groups G1, G2, and G3, indicating a strong cellular defense response to potential antigens. In contrast, the cellular recruitment in the treated group G4 was moderate. For polymorphonuclear cells, there was slight recruitment in the treated groups G1, G2, and G4 and moderate recruitment in G3 and G5 (Fig. 3).

Fibroblasts were also identified. However, there was no difference among the groups in the relative abundance of fibroblast cells; all groups exhibited a moderate level of intensity.

The organization of collagen fibers in the wound-healing process is shown in Fig. 4. The treated group G1 showed a marked intensity, with fiber deposition forming acidophilic bundles that were densely packed; for the treated groups G2 and G4, the presence was moderate, with thick acidophilic fibers interspersed with areas of loose connective tissue and fibroblasts; and for the treated groups G3 and G5, the deposition of discrete fibers was observed, with fewer acidophilic fibers deposited in the fibroblast media. For all groups, re-epithelialization was mild (Fig. 4).

The use of complementary and alternative medicines and therapies (CAMs) has grown worldwide, and, as part of this trend, aromatherapy has become increasingly popular. Aromatherapy uses plant-derived volatiles, which can be applied in various ways for various health purposes. One reason for its increasing popularity is that these oils are generally less toxic and produce fewer side effects (Mori et al., 2016).

L. angustifolia + *C. nardus* 5% is a blend of volatiles, and numerous studies in human and veterinary literature have examined its active components, such as healing, anti-inflammatory, antibacterial, and antifungal properties (Mori et al., 2016; Cunha et al., 2020). These active ingredients may have contributed to the higher concentration compared to *L. angustifolia* + *C. nardus* 2%, which has the same formulation but at a lower concentration. These factors may have favored the formulation that yielded the best healing outcomes.

From a macroscopic perspective of the response during treatment, the lesions in this group showed significant vascularization; therefore, increased blood supply benefits and a shorter healing time, as observed in 17 days. Histologically, the body's response to the formulation occurred through organized stages of the healing process, with moderate recruitment of the first line of defense—mononuclear cells—accompanied by only small numbers of lymphocytes and neutrophils, which are responsible for the immune response. These cells are attracted to a lesion in the endothelium to achieve hemostasis and then initiate the inflammation process (Heitman et al., 2018), but in an organized manner and without excess in response to this treatment.

Citronellal is one of the main compounds in *C. nardus* volatile. Some studies on pain and inflammation report that this substance can reduce leukocyte migration and provide antioxidant effects (Heitman et al., 2018) with low toxicity levels (Quintans-Júnior et al., 2011). Among the few studies on the antimicrobial activity of *C. nardus* volatile (Batubara et al., 2015), it was observed that the minimum inhibitory concentration to inhibit microorganisms such as *S. aureus* was 31.25 µg/mL; *E. coli* was not inhibited at 250 µg/mL, *P. aeruginosa* required 250 µg/mL, and *S. choleraesuis* needed 125 µg/mL,

indicating medium to high concentrations for inhibition. In the G2 formulation group (*C. nardus* 5%), local pain was less intense compared to G3 (*L. angustifolia* + *C. nardus* 2%) and the control group, probably due to the ointment's concentration level and the absence of macroscopically significant bacterial growth that could delay healing. The lesions showed slight hyperemia, no secretion or crusts, and histologically, the mononuclear cell response was moderate during the inflammatory phase.

In group G1, which received *L. angustifolia* at 5%, the animals exhibited calmer, less stressed behavior during the daily checklist manipulation than groups G3 (*L. angustifolia* + *C. nardus* 2%), G4 (*L. angustifolia* + *C. nardus* 5%), and G5 (control group). The animals in these treated groups were more agitated. Additionally, individuals in G1 showed less local pain when the ointment was applied. Lisboa et al. (2023) conducted a literature review on the use of *L. angustifolia* for pain relief in women. The review highlighted that volatiles are frequently used in hospital and outpatient settings due to their properties, which reduce local pain and provide calming effects. These benefits have been noted in various studies related to childbirth, menstrual cramps, and dysmenorrheasymptoms (Lisboa et al., 2023).

In the literature (Mori et al., 2016) it was reported that in the group treated topically with *L. angustifolia* oil, fibroblasts synthesized type III collagen, which is essential for the formation of granulation tissue during the early stages of wound healing. A greater number of P4H-positive fibroblasts accompanied this increase in collagen production. In comparison, the control group did not show these results. Additionally, RT-PCR analysis confirmed a significant increase in type I and type III collagen expression in the treatment group compared with the control group. This indicates that *L. angustifolia* oil accelerates granulation tissue formation during the initial phase of wound healing. Among the treated groups, G1 showed the second-best collagenization, while G2 and G4 showed moderate responses, and G3 showed a mild response.

However, excess collagenization is also not beneficial, as observed by Ogawa et al. (2021), because when there are too many collagen cells, it can cause an accumulation of extracellular matrix that results in dermal nodules, and collagen becomes thick, eosinophilic, hyalinizing bundles. These are called keloidal collagen, which subsequently causes a pathology called keloids, an abnormal manifestation of healing, also driven by the increased durability of the connective tissue inflammation process.

Conclusion

The search for herbal products that aid healing has recently intensified, mainly due to their fewer side effects. The present study examined four formulations and developed an ointment containing a blend of 5% volatiles from *Lavandula angustifolia* and *Cymbopogon nardus*. This ointment speeds up the healing process and encourages the development of more vascularized tissue and appropriate collagen levels. This research presents promising opportunities for further advancements in healing using volatiles from medicinal plants.

Declarations

Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. The Ethics Committee of Vassouras University approved number 009/2023.

Data availability

Data sharing does not apply to this article.

Acknowledgment

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Conflict of interest

Authors declare no conflict of interest.

Authors Contributions

Contribution	IAOT	SPA	ECRR	MAJS	NPF	BAS	YPC	DSAC
Concepts or Ideas	X		X					X
Design	X		X					X
Definition of intellectual content	X			X			X	X
Literature search	X	X			X	X		X
Clinical trial		X	X					
Experimental studies	X	X			X	X	X	
Data acquisition	X	X	X		X	X		X
Data analysis	X				X	X	X	X
Statistical analysis	X	X	X					X
Manuscript preparation	X		X		X	X		X
Manuscript editing	X				X	X	X	X
Manuscript review			X				X	X

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Figures

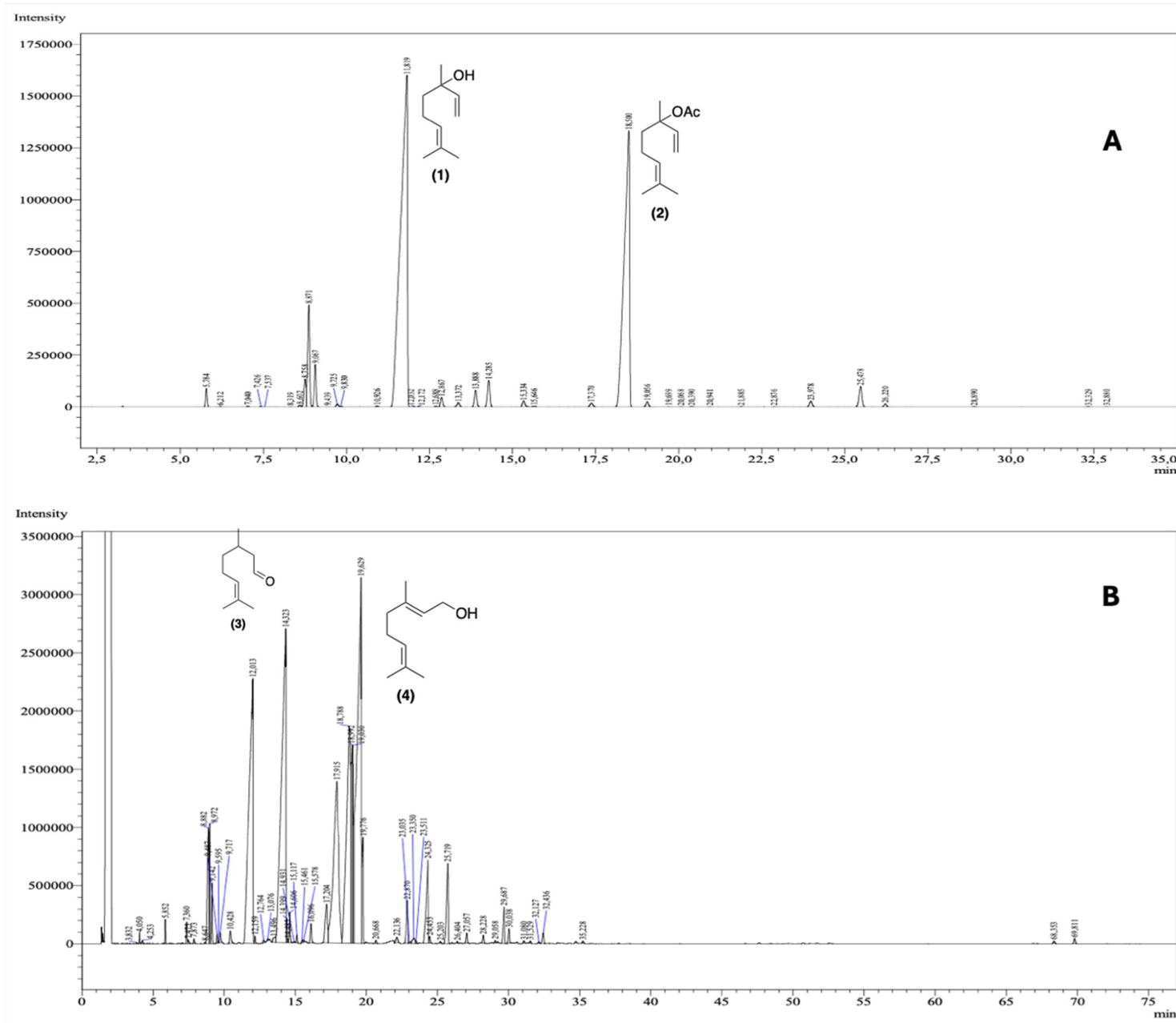


Figure 1

Gas chromatography-mass spectrometer analysis of *Lavandula angustifolia* (A), *Cymbopogon nardus* (B), and essential oil. (1) linalool – ($R_T = 11.819$, 36.3%), (2) linalool acetate ($R_T = 18.500$, 30.4%), (3) citronellal ($R_T = 14.323$, 45.8%), and (4) geraniol ($R_T = 19.629$, 22.3%).

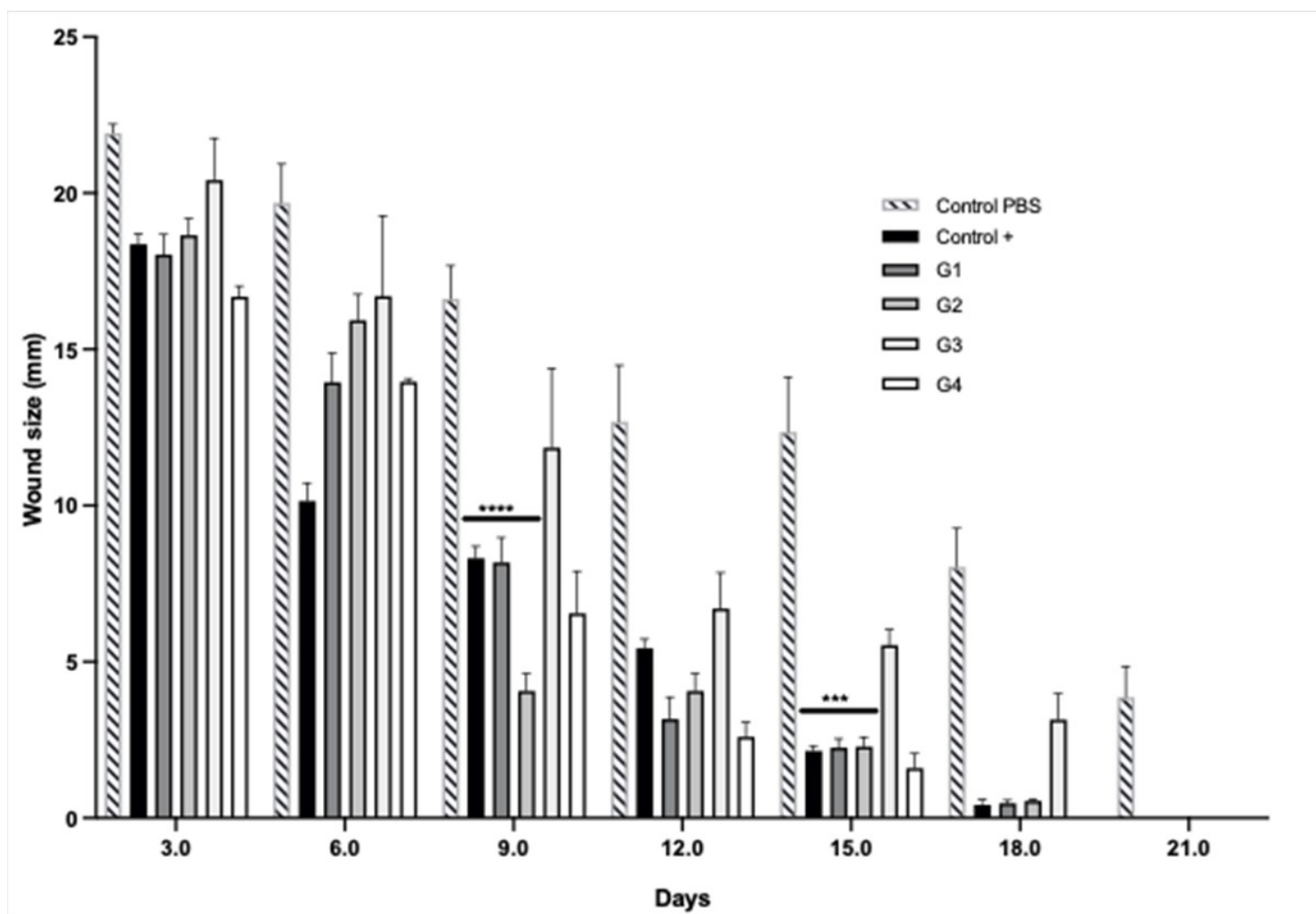


Figure 2

Healing evolution during 21 days of treatment for of the different formulations (G1 – ointment based on *Lavandula angustifolia* 5%; G2 – ointment based on *Cymbopogon nardus* 5%; G3 – ointment based on *Lavandula angustifolia* + *Cymbopogon nardus* 2%; G4 - ointment based on *Lavandula angustifolia* + *Cymbopogon nardus* 5%). Positive control (Control +) – Nebacetin®. Data are presented as mean $\pm$ standard deviation (SD) and *** $P < 0.001$, **** $P < 0.0001$, carried out by ANOVA.

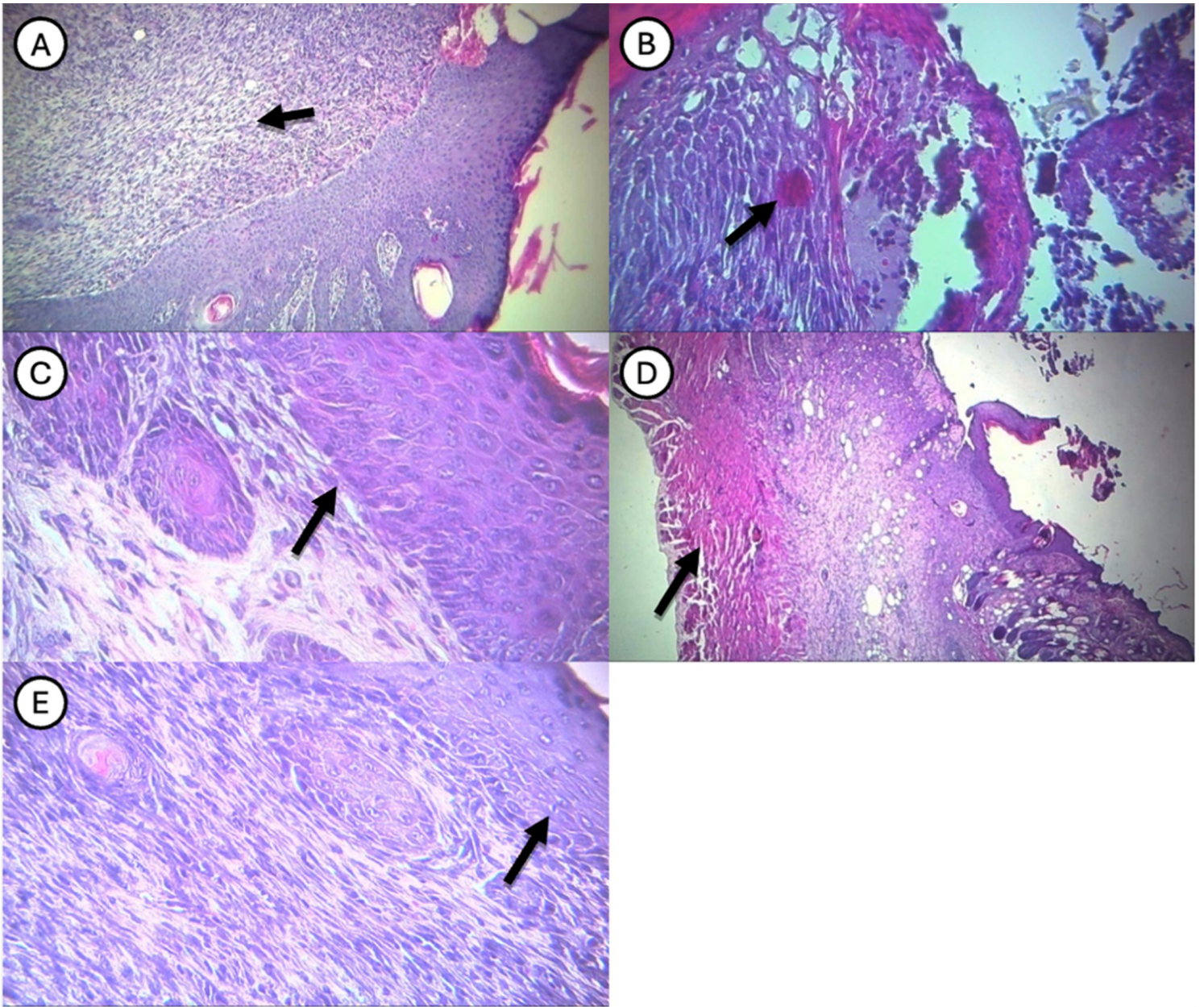


Figure 3

Histological analyses in hematoxylin-eosin staining, a cellular investigation of the treated and control groups. A - represents G5 (control). The arrow is mononuclear cells; B- represents G1, the arrow is polymorphonuclear cells (*L. angustifolia*-based ointments of 5%); C - represents G2 (*C. nardus*-based ointment of 5%) arrow is a thick layer of fibroblasts; D - represents G3 (*L. angustifolia* + *C. nardus* 2%) arrow demonstrates a region of vascularization; E - represents G4 (*L. angustifolia* + *C. nardus* 5%) the arrow points to the area with fibroblast cells forming a well-united network, without interstitial space.

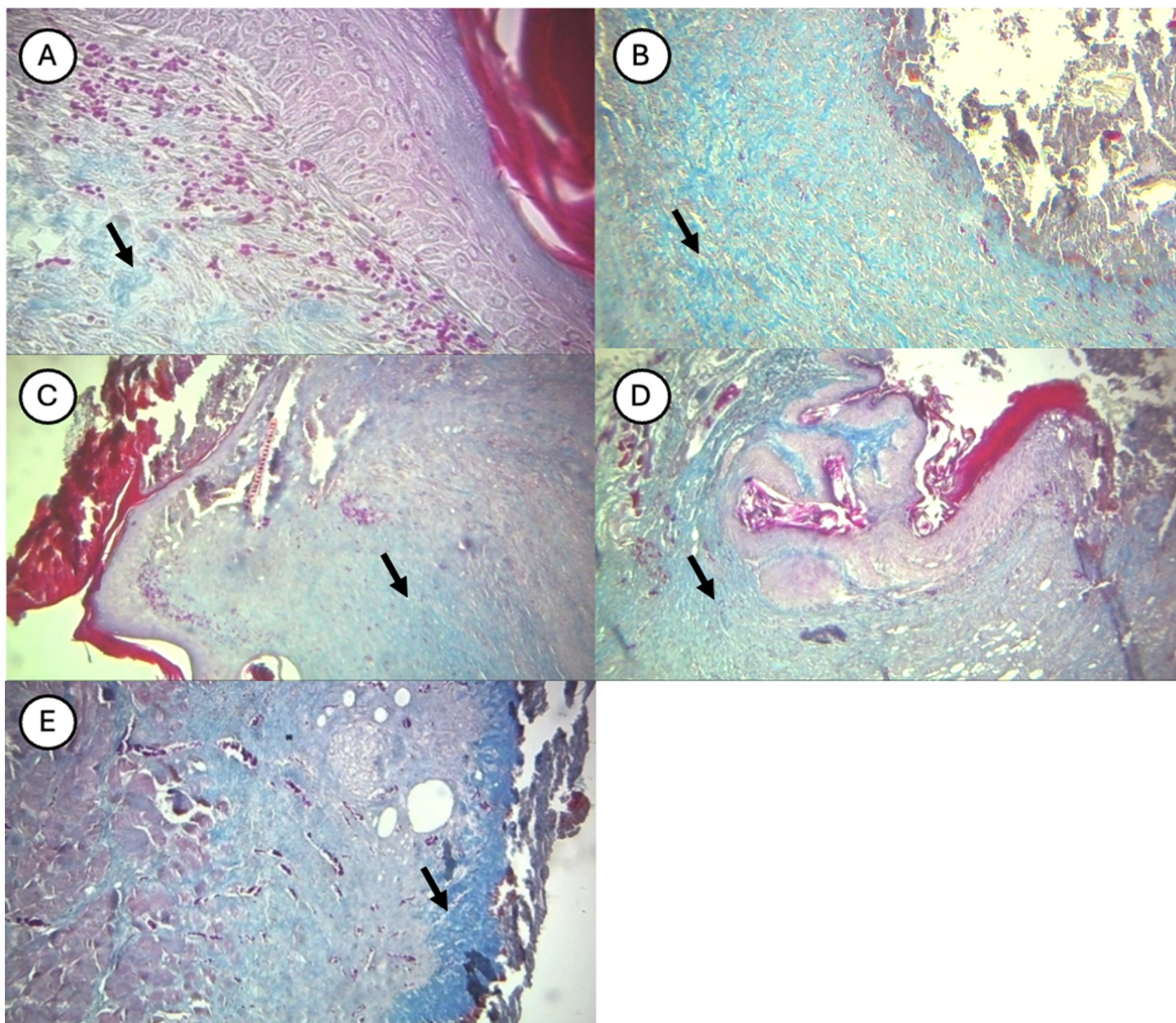


Figure 4

Wound collagenization process in different formulations in Mallory coloring. A - represents G1 (*L. angustifolia*-based ointments of 5%); B - represents G2 (*C. nardus*-based ointment of 5%); C - represents G3 (*L. angustifolia* + *C. nardus* 2.5%); D - represents G4 (*L. angustifolia* + *C. nardus* 5%); E - represents G5 (control). The arrows represent the collagenization process.

Supplementary Files

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