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Original Article

Wound healing and antiulcer activities of *Solenostemma oleifolium* (Nectoux) Bullock & E.A.Bruce ex Maire essential oil in rats

[Actividad cicatrizante y antiulcerosa del aceite esencial de *Solenostemma oleifolium* (Nectoux) Bullock & E.A.Bruce ex Maire en ratas]

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Abstract

Context: *Solenostemma oleifolium* is a Saharan plant part of the traditional pharmacopoeia to treat wound and gastric problems. This work supports the presence of active compounds in the essential oil of *S. oleifolium*, facilitating the healing process.

Aims: To evaluate the wound healing and antiulcer effects of the essential oil of *S. oleifolium* (EOSO).

Methods: EOSO was prepared and analyzed by gas chromatography and mass spectrometry (GC-MS). The toxicity of the product was evaluated by an acute toxicity test and a skin toxicity test. A 1% EOSO cream was prepared and used. The percentage of wound contraction was monitored in an excision model in rats. A histopathological study was conducted on the damaged tissue throughout the experiment. For the ulcer study, two assays were performed in rats: the aspirin-induced gastric ulcer and the pyloric ligation-induced gastric ulcer assays. In the aspirin test, the ulcer index and the percentage of ulcer protection were calculated.

Results: The major compounds of the EOSO were linalool (57.1%), terpineol (12.95%), and trans-geraniol (12.66%). EOSO was not considered toxic. In the excision rat model, from day 2 to day 10, the wound contraction was significantly higher ($p<0.05$) for the EOSO-treated wound than for the non-treated wound. In the aspirin-induced ulcer test, the ulcer index was significantly reduced in the EOSO-treated rats compared to the control rats ($p<0.05$).

Conclusions: The present study demonstrated the wound healing and antiulcer properties of the essential oil of *S. oleifolium*.

Keywords: Algeria; volatile oils; histology; wounds and injuries; linalool.

Resumen

Contexto: *Solenostemma oleifolium* es una planta sahariana que forma parte de la farmacopea tradicional para tratar heridas y problemas gástricos. Este trabajo apoya la presencia de compuestos activos en el aceite esencial de *S. oleifolium*, facilitando el proceso de cicatrización.

Objetivos: Evaluar los efectos cicatrizantes y antiulcerosos del aceite esencial de *S. oleifolium* (EOSO).

Métodos: Se preparó EOSO y se analizó mediante cromatografía de gases y espectrometría de masas (GC-MS). La toxicidad del producto se evaluó mediante una prueba de toxicidad aguda y una prueba de toxicidad cutánea. Se preparó y utilizó una crema de EOSO al 1%. Se controló el porcentaje de contracción de la herida en un modelo de escisión en ratas. Se realizó un estudio histopatológico del tejido dañado a lo largo del experimento. Para el estudio de la úlcera, se realizaron dos ensayos en ratas: el de la úlcera gástrica inducida por aspirina y el de la úlcera gástrica inducida por ligadura pilórica. En el ensayo de la aspirina, se calcularon el índice de úlcera y el porcentaje de protección de la úlcera.

Resultados: Los principales compuestos del EOSO fueron linalol (57,1%), terpineol (12,95%) y trans-geraniol (12,66%). El EOSO no se consideró tóxico. En el modelo de rata con escisión, desde el día 2 hasta el día 10, la contracción de la herida fue significativamente mayor ($p<0,05$) en la herida tratada con EOSO que en la no tratada. En la prueba de úlcera inducida por aspirina, el índice de úlcera se redujo significativamente en las ratas tratadas con EOSO en comparación con las ratas de control ($p<0,05$).

Conclusiones: El presente estudio demostró las propiedades cicatrizantes y antiulcerosas del aceite esencial de *S. oleifolium*.

Palabras Clave: Argelia; aceites volátiles; histología; heridas y lesiones; linalool.

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INTRODUCTION

A wound can be defined as an acute or chronic rupture of the cellular and anatomical or functional continuity of living tissues caused by internal or external factors (Teixeira et al., 2020). Wound healing is a dynamic natural process of restoring the integrity of dermal and epidermal tissues, which occurs in three interdependent and overlapping phases: the inflammatory phase, the proliferative phase, and the remodeling or epithelization phase (Gushiken et al., 2016). The wound treatment must avoid complications and achieve rapid and quality healing. Natural products are widely used in wound healing, especially essential oils, which have shown promising results in preclinical studies on wound healing (Gushiken et al., 2016). Gastric ulcers are a public health problem worldwide generally due to an imbalance between aggressive factors (pepsin and hydrochloric acid) and mucosa defenses, such as blood flow, mucus, and bicarbonate secretion (Souza et al., 2011). In a meta-analysis of 31 published studies, the pooled incidence of uncomplicated peptic ulcer disease (PUD) was approximately one case per 1000 person-years in the general population, and the incidence of ulcer complications was approximately 0.7 cases per 1000 person-years (Lin et al., 2011). Several factors can favor this imbalance, among which long treatment with non-steroidal anti-inflammatory drugs (NSAIDs), stress, the intensity of physical activity, inappropriate nutrition management, genetic predisposition, systemic disease, *Helicobacter pylori* infection, and ingestion of poison are the most important (Parrah et al., 2013). A wide range of drugs is used for peptic ulcer treatment, including histamine receptor antagonists, proton pump inhibitors and prostaglandin analogues (Sumbul et al., 2011). The undesirable effects of these drugs are numerous, hindering their proper use. Various plants are being used in complementary and alternative medicines for the management of gastric ulcers, because of their minimum toxicity and high effectiveness (Afroza et al., 2014).

Solenostemma oleifolium (Nectoux) Bullock & E.A.Bruce ex Maire is a plant belonging to the *Asclepiadaceae* family growing in the desert zones of Algeria, Libya, and Egypt (Benhouhou, 2005). The leaves of this plant are traditionally used to treat gastric and intestinal problems (Benmaarouf et al., 2020), while the juice is used for wound healing (Innocenti et al., 2005). In this context, *S. oleifolium* essential oil might be a good candidate for the treatment of wounds and ulcers in animals.

The present study aimed to chemically characterize the composition of the essential oil of *S. oleifolium* (EOSO) and to test the wound healing and antiulcer properties of EOSO on rat models.

MATERIAL AND METHODS

Chemicals and reagents

The materials used were Cremophor (Sigma-Aldrichr, USA), cetyl alcohol (Sigma-Aldrichr, USA), paraffin oil (Sigma-Aldrichr, USA), Madecassol® (Bayer, Germany), ranitidine (Sigma-Aldrich, USA).

Sampling and identification

S. oleifolium was collected in the first trimester of 2020 at Abalessa (22° 47′ 13″ North, 5° 31′ 38″ East) in the south of Algeria. The identification of the plant was carried out in the botanical department of the higher national agronomic school in Algiers (Algeria), which delivered an identification voucher deposited in the herbarium of the laboratory "Santé et production animale" of the higher national veterinary school of Algiers (SPA.031).

Extraction

Extraction of the essential oil from *S. oleifolium* leaves was conducted by hydro-distillation using a distilling device. One hundred and fifty grams of crushed leaves were put in a 1000 mL round flask and added distilled water. The mixture was hydrodistilled for 4 hours. The resulting oil was stored in an amber-sealed bottle in the refrigerator at 4°C until use. The percentages of essential oils were calculated as volume (mL) of essential oil per 100 g of plant material (v/w).

Water was eliminated by settling using anhydrous sodium sufate.

Chromatography coupled with mass spectrometry analysis

The oil was analysed by GC/MS. The GC-MS analysis was performed using an Agilent Technologies 7890A gas chromatography interface with an Agilent 5975 C mass selective detector. Data acquisition was performed with Chem-station software. The analytical capillary column was HP5-MS (30 m × 0.25 mm i.d, 0.25 mm film thickness). The injector temperature was 250°C, the injection volume 0.2 µL, and the split ratio 1:50. The oven temperature program was 60°C for 8 min, 2°C/min to 250°C for 20 min. The temperatures of the MS source and quadrupole source were respectively 230°C and 150°C, and the impact of ionization mode was 70 eV with scan ranges of 29 to 550.

The compounds were identified based on the GC retention indices calculated from a series of alkane injected with the sample and analysed together in the same conditions and by comparing the mass spectral fragmentation patterns and their IR with those stored in the database NIST Mass spectral and Wiley Registry of Mass Spectral Data (McLafferty, 2011).

Animals

The skin tolerance and healing activity of EOSO were assessed on Wistar rats (160-180 g) of both sexes (Pasteur Institute of Algiers, Algeria). All animals were housed under standard temperature conditions (25 ± 2°C), relative humidity (50 ± 1%), 12 hours light/ dark cycle, feeding with pellets and water *ad libitum*.

The study was authorized by the scientific council of the animal health and production research laboratory of the higher veterinary school of Algiers in accordance with the ARRIVE guidelines 2.0 (Percie du Sert et al., 2020).

Acute toxicity study

The acute oral toxicity test was performed as described previously (Benmaarouf et al., 2020) and in accordance with international guidelines (OECD, 2002). A single dose of EOSO (500, 1000, and 2000 mg/kg) was administered to fasted rats overnight, while the control group received vegetable oil (10 mL/kg). The animals were observed individually for 14 days for any behavioural and neurological changes. The number of deaths was counted.

Skin toxicity

The dermal toxicity study was performed following the guidelines of the OECD (OECD, 2017). A total of 10 healthy nulliparous, non-pregnant female rats with intact skin were used. The animals were acclimated to laboratory conditions for five days before the start of the study. The day before the test, 10% of the body surface was shaved in the back of the tested animals. A limit test dose of the 1% formulation was applied to the shaved area for 24 hours. The animals were observed for 14 days to determine any manifestation of skin toxicity and to report cases of morbidity and mortality.

Wound healing activity

Dermal cream formulation

The basic formula of the prepared cream was a semi-solid preparation of hydrophilic emulsion type (lipophilic/hydrophilic). The protocol was that of a conventional emulsion with the preparation of two phases under agitation. The cream contained purified water, paraffin oil (18%), Cremophor® EL (6%), cetyl alcohol (5%), and EOSO (1%). The 1% concentration was based on preliminary results. The formulation was based on the recommendations of the Belgian Federal Association for Medicines and Health Products (AFMPS, 2010).

Excision wound model and wound contraction

The healing activity evaluation test, according to the circular excision wound model, was based on a planimetric study, which allows a direct quantitative evaluation by calculating the area of the wound and its evolution over time (Jha et al., 2012).

The rats were divided into three groups of six animals and fasted the day before the test. The animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal), and their back regions were shaved and cleaned with ethanol. A circular area of 10 mm in diameter was created in the inter-scapular region of each rat by excising the skin with a biopsy punch. Then, the rats were caged individually. The animals were treated daily: the control group received the vehicle (basic formula), the EOSO group received the 1% EOSO cream, and the standard group received a commercial cream (Madecassol 1%). The used cream amount was based on the wound surface. The idea was to cover all the surface of the wound. The cream was applied each day until healing.

The progressive development of the wounds was monitored daily by photography. In addition, the wound prints were taken on transparent papers according to the following schedule: day zero (D_0), D_2, D_4, D_6, D_8, D_{10}, D_{12}, and D_{14}. Based on the fingerprints taken, the wound areas were measured using AutoCAD software and the percentages of contraction were calculated.

Wound contraction represented the progressive changes in the planimetric wound area. The area obtained was then used to calculate the percentage of wound contraction using the following equation [1] (George et al., 2014).

$$\text{Wound contraction (\%)} = \frac{WA\ D_0 - WA\ D_n}{WA\ D_0} \times 100 \quad [1]$$

Where WA: wound area; D_0: day of wounding and beginning treatment; D_n: n days after treatment.

Epithelialization period

The period of epithelization was calculated as the time (in days) necessary for the falling of the dead tissue without any residual raw wound (Nayak et al., 2007).

Histopathology

At the end of the experimental period (D_{14}), the skin fragments from each group were removed and stored in 10% formalin for the purpose of anatomopathological studies. Once fixed, the samples were dehydrated, thinned, and then embedded in paraffin blocks. Slices (4 µm thick) were made and stained by hematoxylin-eosin (HE) and Masson's trichrome (TC) techniques. The slides were observed under an optical microscope, and the evaluation of the healing quality focused on demonstrating fibroblastic proliferation, collagen maturation, angiogenesis, and epithelialization (George et al., 2014).

Antiulcer activity

Aspirin-induced gastric ulcer

In the aspirin-induced gastric ulcer experiment (Hegde et al., 1994), three groups of 6 albino rats (150-200 g) were used. The first group was the control group receiving distilled water (10 mL/kg), the second group was the standard group receiving ranitidine (50 mg/kg), and the third group was the test group treated with EOSO (250 mg/kg), orally for 8 days. After 8 days of treatment, the animals fasted for 24 hours. The ulcer was induced by the oral administration of an aqueous solution of aspirin (200 mg/kg). The rats were sacrificed 4 hours later, and the stomach was opened to calculate the ulcer index (Kunchandy et al., 1985). The stomachs were recovered from being the subject of an anatomopathological study and examined macroscopically to highlight pathomorphological changes such as congestion, oedema, haemorrhage, and erosion.

Pyloric ligation-induced gastric ulcer

The test was performed as described previously (Shay et al., 1945). Animals fasted for 24 hours. Three groups of 6 rats were treated orally with EOSO (250 mg/kg), ranitidine (50 mg/kg), and distilled water (10 mL/kg), respectively, 30 minutes prior to pyloric ligation. After the fasting period, the rats were anesthetized with ether. The abdomen was opened by a midline incision below the xiphoid process, and the pyloric end was ligated. Ligation was performed without modification of the blood supply of the stomach. The rats were deprived of food and water during the postoperative period. Four hours after surgery, all animals were euthanized. The stomachs were opened, and the contents were collected. The volume of the gastric contents was measured and then centrifuged at 1000 rpm for 10 minutes. Free acidity and total acidity were estimated by titrating 1 mL of supernatant with 0.01 N NaOH, using phenolphthalein as an indicator. The ulcer index was calculated (Kunchandy et al., 1985). The stomachs were recovered from being the subject of an anatomopathological study and examined macroscopically to highlight pathomorphological changes such as congestion, oedema, haemorrhage, and erosion.

Statistical analysis

The statistical treatment of the data was performed using XLSTAT version 7.1 software and the IBM SPSS Statistics version 20 software. The descriptive analysis was based on the calculation of the means of the percentages of the different parameters and the graphic illustrations. All values were expressed as means ± standard deviation. The results obtained were statistically analysed by a one-factor and two-factor Analysis of Variance (ANOVA) followed by a post hoc multiple comparison test of the differences between the groups by applying the Tukey HSD test. Results were considered statistically significant if the p-value <0.05.

RESULTS

GC-MS analysis

Quantitative analysis of the composition of EOSO was performed using the GC-MS method. Table 1 illustrates the most important data of the compounds (calculated IR, identification of compounds, theoretical IR, and % GC-MS).

The extraction of EOSO by hydro-distillation yielded a very fragrant yellow oil. The yield obtained per 100 g of the plant was 0.3% (v/w). Twenty components were characterized, representing 95.44% of the total oil components detected (Table 1, Fig. S1)

Acute toxicity study

Oral administration of EOSO at doses of 500, 1000, and 2000 mg/kg body weight did not cause visible signs of toxicity in the treated rats. No neurological toxicity or changes in the behaviour were notified. A normal body weight gain was observed throughout the experimental period. Thus, EOSO was found to be non-toxic up to the dose of 2000 mg/kg body weight.

Skin toxicity

Skin toxicity symptoms such as irritation, inflammation, redness, and rash were not observed when the animals were monitored at 24, 48, and 72 hours. In addition, neither toxicity nor mortality were recorded during the 14 days of observation. Consequently, the concentration of EOSO cream tested (1%) was found to be safe.

Table 1. GCMS data from the essential oil of *Solenostemma oleifolium*.

N°	IR[a]	Compounds	IR[b]	%GCMS [c]	Identification
1	983	cis-2,6-Dimethyl-2,6-octadiene		0.126	RI, MS
2	1000	trans-2-(2-Pentenyl)furan	1007	0.326	RI, MS
3	1024	D-Limonene	1025	0.903	RI, MS
4	1035	Z-β-Ocimene	1038	0.552	RI, MS
5	1045	E-β-Ocimene	1048	0.949	RI, MS
6	1083	α-Terpinolen	1085	0.885	RI, MS
7	1106	Linalool	1103	57.103	RI, MS
8	1107	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	1104	1.047	RI, MS
9	1173	Terpinene-4-ol	1174	0.245	RI, MS
10	1190	Terpineol	1189	12.954	RI, MS
11	1212	p-Menth-1-en-9-al	1232	0.430	RI, MS
12	1228	Nerol (cis-Geraniol)	1226	4.678	RI, MS
13	1257	trans-Geraniol	1258	12.658	RI, MS
14	1286	Edulan I, dihydro-	1289	0.679	RI, MS
15	1379	β-Damascenone	1382	0.487	RI, MS
16	1449	Geranyl acetone	1452	0.124	RI, MS
17	1480	β-Ionene	1483	0.184	RI, MS
18	1713	Pentadecanal	1713	0.441	RI, MS
19	1844	2-Pentadecanone, 6,10,14-trimethyl-	1843	0.21	RI, MS
20	1970	n-Hexadecanoic acid	1970	0.462	RI, MS
% Identification				**95.443**	

[a]Retention indices with respect to C5–C28 n-alkanes calculated on non-polar HP5-MS capillary column. [b]Retention indices given in the literature (NIST or Wiley on non-polar HP-MS or DB5-MS capillary column). [c]Percentage calculated from the peak's areas of GC chromatogram on non-polar HP5-MS capillary column.

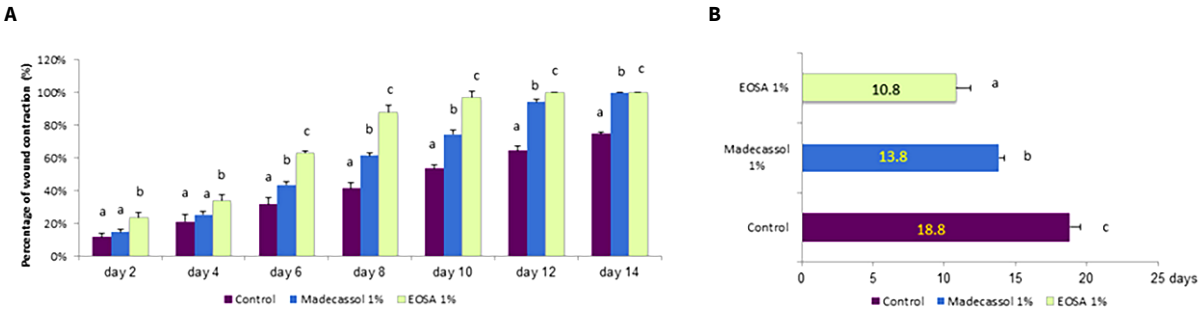


Figure 1. **(A)** % of wound contraction in the function of the post-inoculation days. **(B)** Epithelialization period in the function of the time (in days).

Results are expressed as mean ± standard deviation (n = 6). Bars with the same letter show no significant difference (p>0.05).

Wound healing activity

Wound contraction and epithelialization

In order to study the healing properties of the EOSO-based cream, the evolution of the percentages of contraction and the average surfaces of the wounds treated with EOSO 1%, the reference product (Madecassol), and the control were recorded during a period of 14 days (Fig. 1).

On D_0, no significant difference between the mean surfaces of the treated wounds was recorded (p=1). Between D_2 and D_6, a reduction in the mean surface areas of the wounds treated locally with EOSO 1% was recorded compared to the control, with contraction percentages ranging from 23.76 to 63.13%

(p<0.0001). However, no difference was noted between the reduction in the mean surface area of the wounds treated with Madecassol cream and that of the control group (p=1.00). From D_8, a significant reduction in the average surface area of the wounds treated with EOSO 1% began with reduction percentages of 88.16%, until the complete disappearance of the wounds from the eleventh day. The mean area of the wounds of the corresponding control group persisted beyond the fourteenth day, even if it gradually decreased between the eighth and the twelfth day.

Moreover, the average surface area of the wounds treated with Madecassol cream decreased moderately between the fourth and eighth days to reach a significant reduction on the twelfth day but persisted beyond the thirteenth day (Fig. 1). Wounds treated with EOSO 1% presented the shortest period of epithelialization, followed by wounds treated with the standard Madecassol (Fig. 1).

Fig. 2 shows the evolution in the pictures of the surfaces of the control wounds, those treated with EOSO 1%, and those receiving the Madecassol standard.

Histopathology

Histopathological examination of the control wounds showed delayed healing, highlighting a hypertrophic epidermis, small cell fibroblast proliferation, deposition of collagen fibres, and angiogenesis (Fig. 3A1, A2).

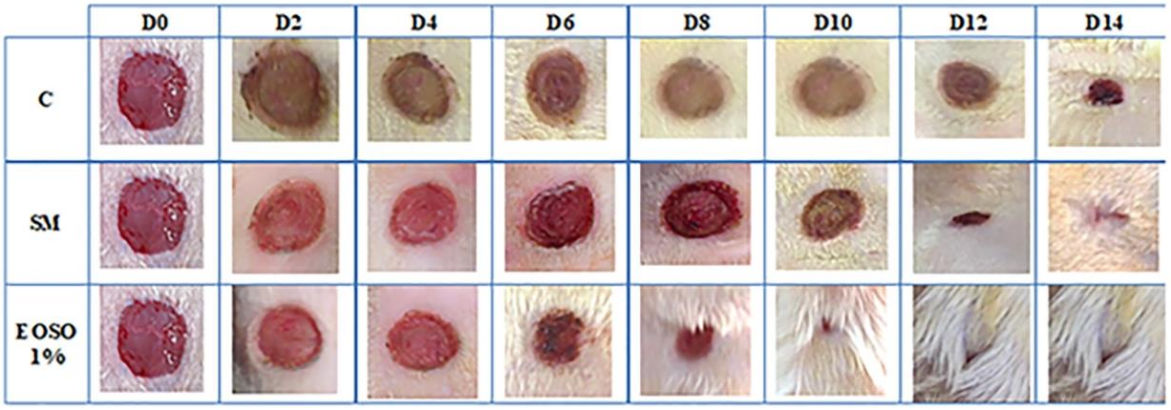


Figure 2. Evolution in images of the surfaces of the wounds during the healing activity of the *Solenostemma oleifolium* essential oil.
C: Control; SM: Standard Madecassol; Dn: n days post-treatment.

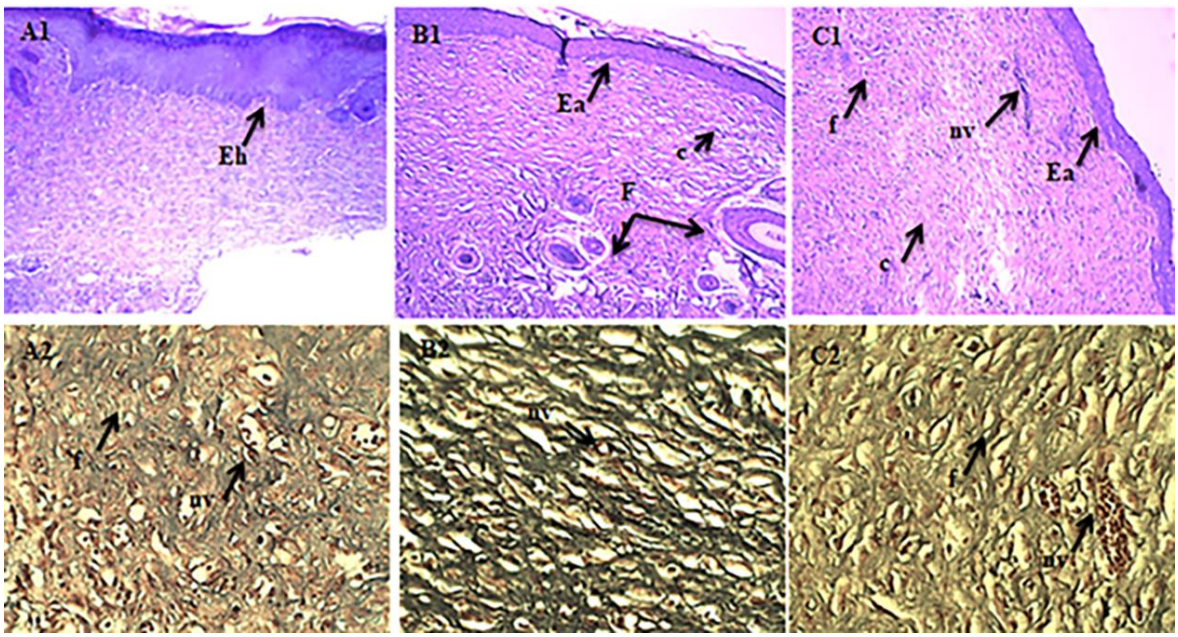


Figure 3. Micrograph of the skin, histological sections stained with hematoxylin & eosin (1) and trichrome (2).
(A1) Vehicle, magnification 10x; **(A2)** Vehicle, 40x; **(B1)** EOSO 1%, 10x; **(B2)** EOSO 1%, 40x; **(C1)** Madecassol, 10x; **(C2)** Madecassol 40x. **(Ea)** Atrophic epidermis, **(Eh)** Hypertrophic epidermis, **(f)** Fibroblast, **(c)** Collagen fibers, **(nv)** Neo-vessels, and **(Fr)** rudimentary hair follicles.

Table 2: Effect of essential oil of *Solenostemma oleifolium* on aspirin-induced gastric ulcer in rats.

Treatment	Dose (mg/kg bw.)	Ulcer index	% Ulcer protection
Control	NA	7.26 ± 1.44[a]	_
Standard Ranitidine	50	2.24 ± 0.66[b]	68.8[a]
EOSO	250	1.03 ± 0.66[b]	84.71[a]

Results are indicated as mean ± standard deviation (n = 6). The numbers with the same letter in the same column show no significant difference (P > 0.05). NA: not applicable.

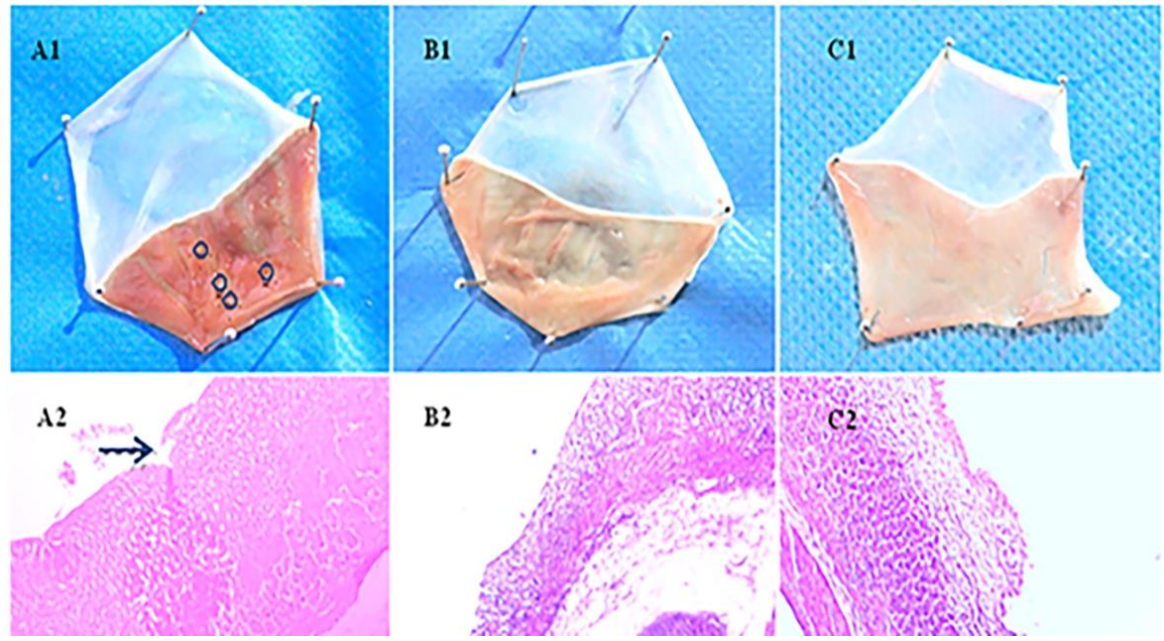


Figure 4. Photograph (1) and micrograph (2) of the stomach on aspirin-induced gastric ulcer in rats, histological sections stained with hematoxylin & eosin.

(A1) Control; **(A2)** Control, magnification 10x; **(B1)** Ranitidine 50 mg/kg, **(B2)** Ranitidine 50 mg/kg, 4x; **(C1)** EOSO 250 mg/kg; **(C2)** EOSO 250 mg/kg, 4x. Circles and arrows indicate the presence of ulcers.

Furthermore, histopathological examination of the wounds treated with the cream based on EOSO 1% revealed almost complete healing with an atrophic epidermal coating and a dermal scarring area rich in collagen fibres, mature fibroblasts, and neo-vessels, as well as the formation of rudimentary hair follicles (Fig. 3B1, B2). In addition, the examination of the wounds treated with the standard drug (Madecassol) revealed an atrophic epidermal coating with a dermal area characterized by dominant fibroblastic proliferation, collagen fibres, and neovascularization (Fig. 3C1, C2).

Antiulcer activity

Aspirin-induced gastric ulcer

The results of EOSO on aspirin-induced gastric ulcers are summarized in Table 2.

EOSO exhibited a significant gastro-protective effect on aspirin-induced ulceration in rats. The oral administration of EOSO at the dose of 250 mg/kg caused a significant reduction of the ulcer index, compared to the control group ($p<0.0001$), with a percentage of ulcer protection of 84.71%. In addition, the reference drug ranitidine (50 mg/kg) resulted in a significant gastro-protective effect (68.8% inhibition) comparable to that EOSO at a dose of 250 mg/kg.

Histopathological analysis of the stomachs of the experimental rats showed the presence of ulcerations in the untreated control group, unlike the EOSO and ranitidine groups, in which no lesion was demonstrated (Fig. 4). These results confirm the recorded gastro-protective effect.

Pyloric ligation-induced gastric ulcer

The result of the gastro-protective effect of EOSO on pyloric ligation-induced gastric ulcers in rats is shown in Table 3.

Oral administration of EOSO at doses of 250 mg/kg significantly attenuated the gastric volume, free acidity, total acidity, and ulcer index ($p<0.001$), considering the control group, with a percentage of ulcer protection of 85.54%. Likewise, the antiulcer effect due to EOSO at the dose of 250 mg/kg was similar to the effect of ranitidine (50 mg/kg), with an ulcer protection rate of 79.16%.

Histological examination revealed the absence of lesions in the groups treated with EOSO and ranitidine in contrast to the untreated control group, in which lesions of ulceration and exulceration were reported (Fig. 5).

DISCUSSION

In this study, the composition, wound healing, and antiulcer effects of S. *oleifolium* essential oil were evaluated. The chemical composition of EOSO recorded in our study is like that described previously; insofar as the two plants were harvested in the same region (Tamenrasset; Algeria) (Chikhi et al., 2019). However, the composition of EOSO from Egypt (Ibrahim et al., 2014) is different from that of Mascara (Algeria) (Chouitah et al., 2016). These differences in composition could be due to various environmental factors, including the nature of the soil, the climatic conditions, the altitude, and the plants growing nearby. Nevertheless, genetic variations can also participate in the appearance of different chemical types within the same species (Chikhi et al., 2019). Healing is the body's natural response to an injury, and it takes place in three successive phases, some of which overlap: the vascular and inflammatory phase, the proliferative phase, and the maturation phase (formation of scar tissue) (Honrado and Murakami, 2005). During this study, the healing activity of the EOSO was tested according to the excision wound model. This model is used for the assessment of wound contraction and epithelialization (Ximenes et al., 2013). The results obtained indicate that the topical treatment of wounds with EOSO 1% cream resulted in a significant healing effect.

Table 3: Effect of essential oil of *Solenostemma oleifolium* (EOSO) on pyloric ligation-induced gastric ulcer in rats.

Treatment	Dose (mg/kg bw)	Volume of gastric juice (mL)	Free acidity (mEq/L)	Total acidity (mEq/L)	Ulcer index	Ulcer protection (%)
Control	-	6.50 ± 1.16^{a}	58.33 ± 3.22^{a}	75.43 ± 3.37^{a}	6.93 ± 1.16^{a}	-
Standard ranitidine	50	2.13 ± 0.46^{b}	12.58 ± 0.54^{b}	28.61 ± 1.37^{b}	1.48 ± 0.94^{b}	79.16^{a}
EOSO	250	2.28 ± 0.15^{b}	12.98 ± 0.88^{b}	28.98 ± 0.91^{b}	1.59 ± 1.13^{b}	77.65^{a}

Results indicated as are mean ± standard deviation (n = 6). The numbers with the same letter in the same column show no significant difference ($P > 0.05$).

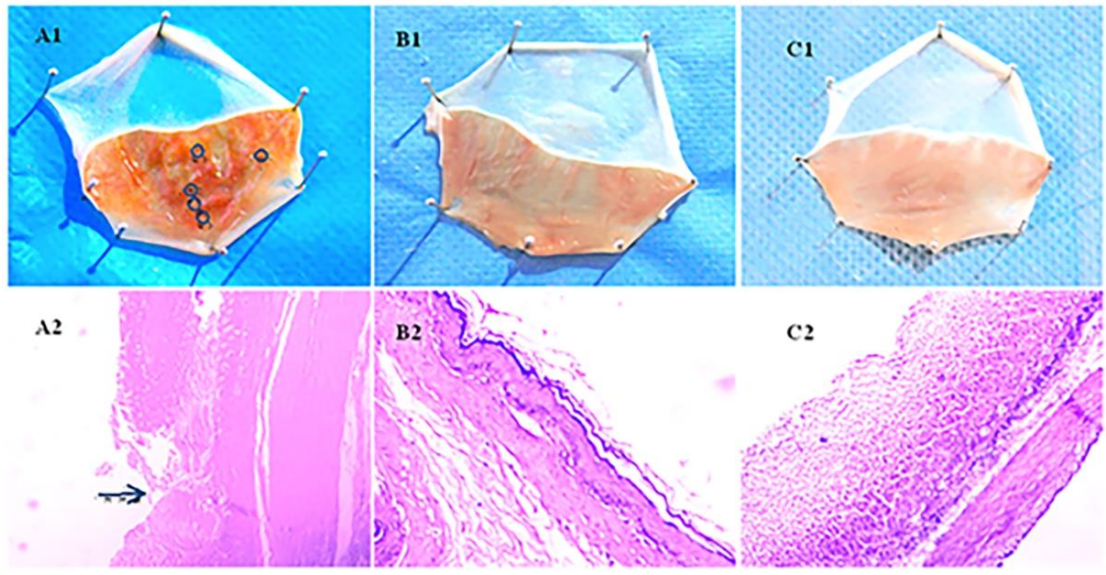


Figure 5. Photograph (1) and micrograph (2) of the stomach on pyloric ligation-induced gastric ulcer.

(A1) Control, **(A2)** Control, magnification 10×; **(B1)** Ranitidine 50 mg/kg, **(B2)** Ranitidine 50 mg/kg, 4×; **(C1)** EOSO (essential oil of *S. oleifolium*) 250 mg/kg; **(C2)** EOSO 250 mg/kg, 4×. Circles and arrows indicate the presence of ulcers. Histological sections were stained with hematoxylin and eosin.

The healing effect of EOSO 1% could be attributed to its antioxidant, anti-inflammatory, analgesic, and anti-infective properties. Indeed, free radicals and oxidative reactions damage tissue and are particularly present during wound healing (Jha et al., 2012). Therefore, any substance with antioxidant activity may enhance wound healing and participate in skin reparation by destroying free radicals (Peşin Süntar et al., 2010).

EOSO is mainly composed of monoterpenes, mainly linalool, α-terpineol, and geraniol, known for their antioxidant effects. Indeed, the antioxidant effect of the essential oil of *S. oleifolium* by free radical scavenging was previously demonstrated (Chikhi et al., 2019).

On the other hand, the inflammatory process is the normal reaction of the immune system to infection and/or injury, although it has been implicated in several pathologic processes development, such as ulcers and wounds (John-Africa et al., 2014). Several components in EOSO are at the origin of their anti-inflammatory and analgesic properties. Previous studies have demonstrated the anti-inflammatory and analgesic effects of linalool (Peana et al., 2002), α-terpineol (de Oliveira et al., 2012), geraniol (Wang et al., 2016), and nerol (González-Ramírez et al., 2016). These anti-inflammatory and analgesic properties would reduce pain and oedema in wounds, thus contributing to smooth wound healing.

Numerous studies have indicated that infection can drastically reduce the healing process by involving poor granulation and tissue formation, reducing connective tissue tensile strength, and perturbing epithelialization (Mulisa et al., 2015). EOSO has antimicrobial properties thanks to the synergistic action of its several components. Indeed, the antimicrobial effect of EOSO was demonstrated in a previous study (Chouitah et al., 2016).

EOSO has a chemical composition like that of lavender essential oil with the same major component, linalool. Therefore, EOSO could have the same mechanism of action during the wound-healing process. The growth factor TGF-β is expressed in wounds treated locally with the essential oil of lavender. Indeed, TGF-β is characterized by a wide spectrum of action during the healing process and is responsible for the stimulation of angiogenesis, induction of fibroblast proliferation and their conversion into myofibroblasts, and the synthesis of type I and III collagen (Mori et al., 2016). A previous study investigated the expression of growth factors (PDGF and EGF) during the wound healing process by lavender essential oil and suggested that PDGF might cause a rapid decrease in granulation tissue, thus accelerating wound closure, while EGF would be responsible for a progression of re-epithelialization (Koca Kutlu et al., 2013). Furthermore, high EGF levels have been demonstrated during the healing process of acetyl acetate and ethanolic extracts of *S. argel* (Abdel-Motaal et al., 2022). Confirmation of these mechanisms of action for EOSO will be the subject of our future study.

Gastric ulcer is a widespread gastrointestinal pathology. Typically, gastric ulcers are due to a discrepancy between hostile elements and the preservation of mucosal integrity by the interne defense mechanism (Ahmed et al., 2016). The antiulcer property of EOSO was studied according to the models of the ulcer induced by aspirin and the ulcer induced by the ligature of the pylorus. Gastric injury is a major restriction to the use of NSAIDs (Maruthappan and Sakthi Shree, 2010). Synthetic NSAIDs can induce mucosal damage by impairing prostaglandin synthesis, increasing acid secretion, neutrophil infiltration, changes in nitric oxide (NO) production, generation of ROS, initiation of lipid peroxidation, and a decrease in mucus production, thus leading to bleeding ulcers (Rozza et al., 2011). In the present study, EOSO showed a significant gastroprotective effect against aspirin-induced ulcers. These results indicate that it exhibits an effective cytoprotective effect, probably by increasing the secretion of mucus and/or bicarbonate, inhibiting the formation of ulcers. Effectively, the gastroprotective effect of the mucilage fraction of this plant on gastric ulcers induced by ethanol was previously shown (El-Shiekh et al., 2021).

The effect of EOSO on gastric secretion was studied in the pyloric ligation model. In this model, autodigestion of the gastric mucosa by the overload of gastric juices, interference with gastric blood flow, and destruction of the mucosa are responsible for the induction of ulcerations (Afroza et al., 2014). In this study, EOSO clearly reduced gastric volume, free acidity, total acidity, and ulcer index, thus preventing ulcer formation. These results suggest its anti-secretory activity.

Plant extracts constitute valuable sources for the development of new therapeutic molecules. These extracts showed interesting results against gastric ulcer pathology (Sannomiya et al., 2005). It has been demonstrated that certain monoterpenes present in essential oils of plant origin have gastroprotective effects against different ulcerogenic agents (de Carvalho et al., 2014). EOSO is mainly composed of monoterpenes. Its gastroprotective activity might be due to the synergy of different compounds of the oil, such

as linalool, geraniol, nerol, α-terpineol, terpinen-4-ol, and limonene.

The protective effect of EOSO can be partly due to its main compound, linalool. Indeed, the antioxidant and anti-inflammatory actions of linalool could prevent injuries due to aggressive factors. The gastroprotective activity of linalool is likely related to its antioxidant activity. Indeed, linalool significantly reduced lipid peroxidation, suggesting that it acts as a reactive oxygen species scavenger (da Silva et al., 2016).

The presence of geraniol in the composition of EOSO may also contribute to its gastroprotective effect. Indeed, geraniol increases the liberation of antioxidant substances and maintains the levels of PGE_2 and NO, contributing to the maintenance of gastric microcirculation (Périco et al., 2020). Geraniol also activates GABA-A and TRPV-1 receptors, thereby increasing the production of CGRP, which works by increasing the mucosal blood flow, the mucus production, and the intracellular pH of the stomach mucosa (Périco et al., 2020; Wang et al., 2016). Similarly, a beneficial effect of nerol after induction of gastric ulcer involving protection against gastric damage and modulation of the immunological system was shown, suggesting the gastroprotective potential of this monoterpene (González-Ramírez et al., 2016).

Furthermore, the α-terpineol present in EOSO could contribute to the gastroprotective effect. Indeed, a previous study indicated a substantial gastroprotective action against NSAIDs in experimental models (Souza et al., 2011). The gastroprotective actions of α-terpineol may include cytoprotective processes (Souza et al., 2011). Finally, the limonene present in EOSO can also participate in canceling the side effects of NSAIDs and thus prevent the formation of lesions via the establishment of a protective coat, increasing the integrity of the mucosa (Rozza et al., 2011).

In this study, EOSO recorded a remarkable anti-secretory effect by decreasing gastric volume and stomach acidity. According to the literature, the monoterpene components mentioned above would not have an anti-secretory effect (de Assis Oliveira et al., 2014). Therefore, the anti-secretory activity recorded could be secondary to the healing of the ulcer. Indeed, the anti-secretory effect of geraniol secondary to ulcer healing or an effect on the autoregulation of gastrin release was previously demonstrated in rats (Bhattamisra et al., 2019). However, the terpinene-4-ol from EOSO could participate in the anti-secretory effect. The anti-secretory effect of terpinen-4-ol present in the essential oil of *Cryptomeria japonica* during ulcers induced by several mediators was demonstrated (Matsunaga et al., 2000).

CONCLUSION

This work demonstrated the wound healing and the antiulcer properties of *S. oleifolium* essential oil. Future studies should focus on the mechanism of action of this oil during healing and gastroprotection processes.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Benmaarouf D	China B	Aliboudhar H	Boulahlib S	Zenia S	Bensedira H	Bouzid K	Slimani A	Ben-Mahdi M
Concepts or ideas	x								
Design	x								
Definition of intellectual content	x	x							x
Literature search	x								
Experimental studies	x		x	x		x	x	x	
Data acquisition	x		x	x		x	x	x	
Data analysis	x				x		x	x	
Statistical analysis					x				
Manuscript preparation	x	x							
Manuscript editing	x	x							
Manuscript review	x	x	x	x	x	x	x	x	x

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Supplementary data

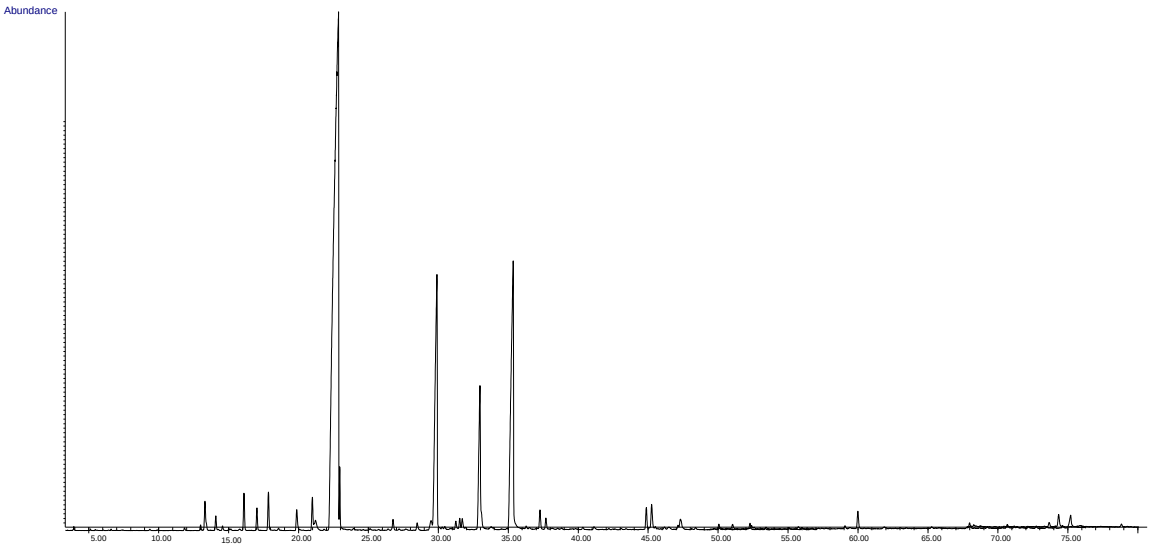


Figure S1. Chromatogram of *Solenostemma oleifolium* essential oil.