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Original article

Scientific Assessment of the Anti-Inflammatory and Wound Healing Potential of *Campanula lyrata* subsp. *lyrata*, A Turkish Folk Remedy

Ipek SUNTAR[1], Esra KUPELI AKKOL[1*], Tuba MERT GONENC[2], Tugce FAFAL ERDOGAN[2], Hikmet KELES[3], Bijen KIVCAK[2]

[1]Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Etiler 06330 Ankara, TURKEY, [2] Ege University, Faculty of Pharmacy, Department of Pharmacognosy 35100 Izmir, TURKEY, [3] Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pathology, 03200 Afyonkarahisar, TURKEY

The aim of the present study is to evaluate the anti-inflammatory and wound healing activities of the extracts prepared from the aerial parts of *Campanula lyrata* Lam. subsp. lyrata (Campanulaceae) by using *in vivo* methods in order to confirm the traditional utilization. *n*-Hexane, diethyl ether, ethyl acetate (EtOAc), methanol (MeOH) and aqueous extracts were separately prepared from the air-dried and powdered plant materials. Carrageenan-, and serotonin- induced hind paw edema, 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema and acetic acid-induced increase in capillary permeability models were employed in mice for the anti-inflammatory activity assessment. Wound-healing activity was investigated by using incision and excision wound models along with hydroxyproline determination and histopathological analyses. MeOH extract displayed significant anti-inflammatory effect in the carrageenan- and serotonin- induced hind paw edema model and in acetic acid-induced increase in capillary permeability model with the values of 25.3, 27.8 and 31.8%, respectively. MeOH extract was also found to have significant wound healing potential in the incision and excision wound models with the values of 26.9 and 39.6%, respectively. MeOH extract ointment treated group tissues also showed enhaced hydroxyproline content. The present study confirms the anti-inflammatory and wound healing activities of *C. lyrata* subsp. *lyrata*.

Key words: *Campanula* spec., Campanulaceae, Carrageenan, Excision, Incision, Serotonin, TPA

Türk Halk İlacı *Campanula lyrata* subsp. *Lyrata*'nın Anti-enflamatuvar ve Yara İyileştirici Etkilerinin Bilimsel Olarak Değerlendirilmesi

Bu çalışmada, *Campanula lyrata* Lam. subsp. lyrata (Campanulaceae) bitkisinin halk arasındaki kullanımını bilimsel olarak doğrulamak amacıyla, bitkinin toprak üstü kısımlarından hazırlanan ekstrelerin anti-enflamatuvar ve yara iyileştirici aktivitelerinin *in vivo* yöntemler kullanılarak değerlendirilmesi hedeflenmiştir. Bitkinin kurutulmuş ve toz edilmiş toprak üstü kısımlarından ayrı ayrı *n*-hekzan, dietil eter, etil asetat (EtOAc), metanol (MeOH) ve sulu ekstreler hazırlanmıştır. Anti-enflamatuvar aktivitenin değerlendirilmesi için, karragen-, ve serotonin- nedenli arka ayak ödemi,12-*O*-tetradekanoilforbol-13-asetat (TPA)-nedenli kulak ödemi ve asetik asit-nedenli kapiller permeabilite artışı modelleri kullanılmıştır. Yara iyileştirici etki, insizyon ve eksizyon yara modelleri kullanılarak, hidroksiprolin miktar tayini ve histopatolojik analizlerle beraber değerlendirilmiştir. Metanol ekstresinin karragen-, ve serotonin- nedenli arka ayak ödemi ve asetik asit-nedenli kapiller permeabilite artışı modellerinde sırasıyla %25.3, %27.8 ve %31.8 değerleri ile anlamlı derecede anti-enflamatuvar etki gösterdiği tespit edilmiştir.Metanol ekstresinin benzer şekilde insizyon ve eksizyon yara modellerinde sırasıyla %26.9 ve %39.6 değerleri ile anlamlı derecede yara iyileştirici etki gösterdiği ve metanol ekstresi ile hazırlanan merhemle tedavi edilen dokularda hidroksiprolin miktarının arttğı belirlenmiştir. Bu çalışma, *C. lyrata* subsp. *lyrata* bitkisinin halk arasında anti-enflamatuvar ve yara iyileştirici amaçlarla kullanımını doğrulamaktadır.

Anahtar kelimeler: *Campanula* spec., Campanulaceae, Karragen, Eksizyon, Insizyon, Serotonin, TPA

Correspondence: E-mail: esrak@gazi.edu.tr; Tel: +90 312 2023185.

INTRODUCTION

The genus *Campanula* L. (Campanulaceae) is represented by 113 native species, 95 of which are growing in Turkish flora and 61 of them are endemic to Turkey (1). *Campanula* species have been used to treat various diseases such as tonsillitis, laryngitis, bronchitis, and warts in traditional medicine (2). In Turkey, *Campanula* species are known as "çançiçeği" and have been used for wound healing and for the treatment of inflammatory diseases (3). *Campanula* species possess stimulant, refreshing, antiallergic, antiphlogistic, antioxidant, spasmolytic, antiviral, cytotoxic, antinociceptive and antimicrobial properties and are used as an emetic (4-6). However, there are no reports on the anti-inflammatory and wound healing activities of *Campanula lyrata* Lam. subsp. *lyrata.*

Campanula species contain a variety of chemical compounds such as polyphenols (flavonoid aglycones and their glycosides, phenolic acids and their esters, as well as phenylpropanoid derivates), steroids, triterpenes and polyacetylenes (5,7-9).

In this study, the anti-inflammatory and wound healing activities of the *n*-hexane, diethyl ether, EtOAc, MeOH and aqueous extracts obtained from *C. lyrata* subsp. *lyrata* were evaluated by using *in vivo* experimental models.

EXPERIMENTAL

Plant material

Campanula lyrata Lam. subsp. *lyrata* was collected from İzmir-Ödemiş in May, 2009 and identified by B. Kıvçak from Ege University. The voucher specimen (No: 1310) is deposited in the herbarium of the Faculty of Pharmacy, Ege University, Izmir.

Preparation of the plant extract

n-Hexane, diethyl ether, EtOAc, MeOH and aqueous extracts were separately prepared from 20 g batches of the air-dried and powdered plant materials by extracting with 200 mL solvent at room temperature for 24 h. Then the solvents were evaporated to dryness in vacuo (60°C). The yields of *n*-hexane, diethyl ether, EtOAc, MeOH and aqueous extracts were 0.78, 0.98, 5.09, 8.32, and 9.80%, respectively. All the extracts were stored at -20°C.

Pharmacological procedures

Animals

Male Sprague-Dawley rats (160-180 g) and Swiss albino mice (20-25 g) were purchased from Kobay Experimental Animals Laboratory, Ankara, Turkey.

The animals were left for 3 days for acclimatization into animal room conditions and were maintained on standard pellet diet and water *ad libitum*. For the anti-inflammatory activity assessment the food was withdrawn on the day before the experiment, but free access to water was allowed. A minimum of six rats were used in each group for wound healing experiments, while ten mice were used in anti-inflammatory studies. The present study was performed according to the international rules considering the animal experiments and biodiversity rights. (Gazi University Ethical Council Project Number: G.U.ET-08.037).

Preparation of test samples for bioassay

For the anti-inflammatory test model, samples were given orally to test animals after suspending in a mixture of distilled water and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Indomethacin (10 mg/kg) in 0.5% CMC was used as a reference drug.

For the assessment of wound-healing activity, an ointment prepared with the test materials were topically applied onto the wounded area of test animals. The test ointments were prepared by mixing the extracts or with a mixture of ointment base consisting of glycol stearate: propylene glycol and liquid paraffin (3:6:1) in a mortar thoroughly. Treatments were started immediately after the production of wound by daily application of the test ointments on the wounded area. The control group animals were topically treated with blank vehicle base, while the animals in negative control group

were not treated with any product. Madecassol® (Bayer) (0.5 g) was used topically as the reference drug.

Anti-inflammatory activity

Carrageenan-induced hind paw edema model

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity (10). Sixty min after oral administration of a test sample or dosing vehicle, each mouse was injected with freshly prepared suspension of carrageenan (0.5 mg/25 µL) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. Saline solutions (25 µL) were injected into that of the left hind paw. Paw edema was then measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with those of the control group and analyzed by using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

Serotonin-induced hind paw edema model

The method of Kasahara et al. (1985) was used (11). Sixty min after oral administration of test sample or dosing vehicle each mouse was injected with serotonin (serotonin creatinin sulfate, Merck, Art. 7768) in Tyrode's solution (0.5 µg/5 µL) into subplantar tissue of the right hind paw and 5 µl of Tyrode's solution into that of the left as secondary control. Measurements were done and evaluated as described above in every 6 min during 30 min.

TPA-induced mouse ear edema

Each mouse received 2.5 µg of TPA dissolved in 20 µl of EtOH 70%. This was applied by an automatic pipette in 20 µl volumes to both anterior and posterior surfaces of the right ear. The left ear (control) received the same volume of solvent (EtOH 70%), simultaneously with TPA. Indomethacin (0.5 mg/ear) was used as reference drug. For the evaluation of the activity, two different measurements were taken as given below**.**

The thickness of each ear was measured 4 h after induction of inflammation using gauge calipers (Ozaki Co., Tokyo, Japan). The edema was expressed as the difference between the right and left ears due to TPA application and consequently inhibition percentage was expressed as a reduction thickness with respect to the control group.

After 4 h of the administration the animals were sacrificed. Discs of 6 mm diameter were removed from each ear and weighed in balance. The swelling was estimated as the difference in weight between the punches from right and left ears and expressed as an increase in the ear thickness (12).

Acetic acid-induced increase in capillary permeability

Effect of the test samples on increased vascular permeability induced by acetic acid in mice was determined according to Whittle Method with some modifications (13). Each test sample was administered orally to a group of 10 mice in 0.2 mL/20 g body weight. Thirty min after administration, tail of each animal was injected with 0.1 mL of 4% Evans blue in saline solution (i.v.) and waited for 10 min. Then, 0.4 mL of 0.5% (v/v) AcOH was injected i.p. After 20 min. incubation, mice were killed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 mL volumetric flasks through glass wool. Each flask was made up to 10 mL with distilled water, 0.1 mL of 0.1N NaOH solution was added to the flask, and the absorption of the final solution was measured at 590 nm (Beckmann Dual Spectrometer; Beckman, Fullerton, CA, USA). A mixture of distilled water and 0.5% CMC was given orally to control animals, and they were treated in the same manner as described above.

Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and morbidity or mortality was recorded.

Gastric-ulcerogenic effect

After the employment of serotonin-induced hind paw edema model, mice were sacrificed and the stomachs of each mouse were removed. Then the abdomen was opened

through the greater curvature and examined under dissecting microscope for lesions or bleedings.

Wound healing activity
Incision wound model

All the animals were anaesthetized with 0.05 cm^3 Xylazine (2% Alfazine®) and 0.15 cm^3 Ketamine (10% Ketasol®) and the back hair of the rats was shaved by using a shaving machine and cleaned with 70% alcohol. Two 5 cm length linear-paravertebral incisions were made with a sterile blade through the skin at the distance of 1.5 cm from the dorsal midline on each side. Three surgical interrupted sutures were placed each 1 cm apart.

The test ointments and the reference drug (Madecassol®) were topically applied on the dorsal wounds once daily for 9 days. All the sutures were removed on the last day and tensile strength of previously wounded and treated skin was measured by using a tensiometer (Zwick/Roell Z0.5, Germany) (14).

Excision wound model

This model was used to monitor wound contraction and wound closure time. Each mouse was anaesthetized with 0.02 cm^3 Xylazine (2% Alfazine®) and 0.08 cm^3 Ketamine (10% Ketasol®). The back hairs were depilated by shaving. A circular wound was created on the dorsal interscapular region of each animal by excising the skin with a 5 mm biopsy punch (Nopa instruments, Germany); wounds were left open. Test samples, the reference drug (Madecassol®, Bayer) and the vehicle ointments were applied topically once a day till the wound completely healed. The progressive changes in wound area were monitored by a camera (Fuji, S20 Pro, Japan) every other day. Later on, wound area was calculated by using AutoCAD program. A specimen sample of tissue was isolated from the healed skin of each group of mice for the histopathological examination (14).

Histopathology

The skin specimens from each group were collected at the end of the experiment (on day 12). Samples were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 micrometer sections and stained with hematoxylin & eosin (HE) and Van Gieson (VG) stains. The tissues were examined by light microscope (Olympus CX41 attached Kameram® Digital Image Analyze System) and graded as mild (+), moderate (++) and severe (+++) for epidermal or dermal re-modeling. Re-epithelization or ulcus in epidermis; fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neo-vascularization and collagen depositions in dermis were analyzed to score the epidermal or dermal re-modeling. Van Gieson stained sections were analyzed for collagen deposition. At the end of the examination, all the wound healing processes were combined and staged for wound healing phases as inflammation, proliferation, and re-modeling in all groups.

Hydroxyproline estimation

Tissues were dried in hot air oven at 60-70°C until consistent weight was achieved. Afterwards, samples were hydrolyzed with 6 N HCl for 3 h at 130°C. The hydrolyzed samples were adjusted to pH 7 and subjected to chloramin T oxidation. The colored adduct formed with Ehrlich reagent at 60°C was read at 557 nm. Standard hydroxyproline was also run and values reported as µg/mg dry weight of tissue (15).

Statistical Analysis

Data obtained from animal experiments were expressed as the mean standard error (± SEM). Statistical differences between the treated and the control groups were evaluated by ANOVA and Students-Newman-Keuls *post-hoc* tests. $P < 0.05$ was considered to be significant [*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$]. Histopathologic data were considered to be nonparametric; therefore, no statistical tests were performed.

RESULTS AND DISCUSSION

The anti-inflammatory activity of the extracts prepared from *C. lyrata* subsp. *lyrata* were evaluated by using the *in vivo* experimental methods *i.e.* carrageenan- and serotonin-

induced hind paw edema, TPA-induced mouse ear edema and acetic acid-induced increase in capillary permeability models at doses of 100 and 200 mg/kg. The results of the anti-inflammatory activity experiments were presented in Tables 1-4. MeOH extract displayed significant anti-inflammatory effect on carrageenan-induced hind paw edema model with the inhibition value of 25.3% at 180 min (Table 1). The carrageenan-induced paw edema model is a biphasic event, involves several chemical mediators such as histamine, serotonin, bradykinin, and prostaglandins (16). In the early phase (90-180 min) of the inflammation histamine, serotonin and similar substances are released. The later phase (270-360 min) kinin-like substances such as prostaglandins, proteases and lysosome are activated (17).

According to the results of the present study, the MeOH extract of *C. lyrata* subsp. *lyrata* exerted anti-inflammatory activity in the early phase of inflammation. Serotonin-induced hind paw edema model was employed to clarify the the anti-inflammatory mechanism of the extracts. *C. lyrata* subsp. *lyrata* displayed significant inhibition value of 24.8% at 12 min at 200 mg/kg dose (Table 2).

However, none of the extracts, including the MeOH extract, were found to be effective in the TPA-induced ear edema model (Table 3) which is closely related with the infiltration of macrophages and neutrophil, the induction of TNF-α and IL-1 (18). On the other hand, MeOH extract was found to be active on the acetic acid-induced increase in capillary permeability model with the remarkable inhibition value of 31.8% (Table 4). In all of the anti-inflammatory activity models, reference drug indomethacin exerted high activity potential.

In the present study, *in vivo* wound-healing

Table 1. Effect of the extracts from *Campanula lyrata* subsp. *lyrata* on carrageenan-induced paw edema model in mice

Material	Dose (mg/kg)	Swelling thickness (x 10^{-2}mm)±SEM (Inhibition %)			
		90 min	180 min	270 min	360 min
Control		49.2 ±3.5	50.2±2.8	51.7±2.9	56.7±3.0
n-Hexane extract	100	40.6±3.1 (2.0)	48.6±3.0 (3.2)	55.4±3.2 -	54.6±3.5 (3.7)
	200	45.2±3.3 (8.1)	40.6±2.5 (19.1)	42.6±2.4 (17.6)	49.0±3.6 (13.6)
Diethyl ether extract	100	48.7±3.0 (1.0)	49.6±2.9 (1.2)	46.4±3.6 (10.3)	51.3±3.7 (9.5)
	200	42.6±2.9 (13.4)	44.6±2.4 (11.2)	48.8±3.9 (5.6)	55.3±3.8 (2.5)
EtOAc extract	100	48.6±2.7 (1.2)	45.4±2.9 (9.6)	45.7±2.6 (11.6)	49.9±3.5 (11.9)
	200	42.6±3.3 (13.4)	53.6±3.9 -	43.3±2.5 (16.2)	52.4±2.7 (7.6)
MeOH extract	100	40.6±3.7 (17.5)	43.7±2.7 (12.9)	50.4±3.1 (2.5)	53.6±2.8 (5.5)
	200	37.5±3.2 (23.8)	37.5±2.8 **(25.3)***	49.1±3.5 (5.0)	52.4±3.1 (7.6)
Aqueous extract	100	41.8±3.6 (15.0)	55.6±3.5 -	50.7±3.6 (1.9)	50.3±2.9 (11.3)
	200	39.6±2.8 (19.5)	50.6±2.7 -	58.8±3.8 -	57.4±3.7 -
Indomethacin	10	30.8±2.0 **(37.4)****	34.6±2.1 **(31.1)****	37.6±1.9 **(27.3)***	32.7±2.0 **(42.3)*****

S.E.M.: Standard error of the mean

* $p<0.05$. ** $p<0.01$. *** $p<0.001$ significant from the control

effect of the extracts of *C. lyrata* subsp. *lyrata* was also evaluated. Ointment formulation prepared from the MeOH extract exerted a significant wound healing activity on the incision wound model with the tensile strength value of 26.9% at day 10 and on the excision wound model with the contraction value of 39.6% at day 12 (Tables 5, 6).The results of hydroxyproline estimation were in accord with the data those obtained from both incision and excision wound models (Table 7).

Histopathologically, well-considered re-modeling, particularly re-epithelization was detected in the reference, MeOH and aqueous extracts treated groups and lesser in the *n*-hexane, ethyl acetate and diethyl ether extracts treated groups. In comparison with these groups, limited re-modeling was noticed in the vehicle, and negative control groups (Table 8). Histopathological results are supported with figures (Figure 1), which stained with HE and VG.

According to the published literatures, *Campanula* species were reported to have antiallergic, antiphlogistic, antioxidant, spasmolytic, antiviral, cytotoxic, antinociceptive and antimicrobial properties (4-7). In a previous study, the antinociceptive potential of *C. punctata* extract were examined in mice. *C. punctata* extract administered orally (200 mg/kg) showed an antinociceptive effect as measured by the tail-flick and hot-plate tests. In addition, *C. punctata* extract attenuated the writhing numbers in the acetic acid-induced writhing test. The results suggested that *C. punctata* extract showed an antinociceptive property, which may be mediated by α2-adrenergic receptor, but not opioidergic, and serotonergic receptors (6). In the phytochemical studies on *Campanula* species, it was revealed that delphinidin-3-rutinoside-7-(tri-*p*-hydroxybenzoyltriglucoside) was isolated from *C. medium* (19). Phenylpropanoid and flavonoid derivatives, barbatosides and barbatoflavan were isolated from the whole plant of *C. barbata*, and barbatoflavan was found to possess $DPPH^{\bullet}$ scavenging activity (8). Furthermore, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, lobetyolin (9-*O*-*β*-D-glucopyranosyl-2,10-tetradecadien-4,6-diyne-8,14-diol) and lobetyol (2,10-tetradecadien-4,6-diyne-8,9,14-triol), were isolated from the MeOH extract of *C. alliariifolia.* The MeOH extract was shown to have antioxidant capacity. Lobetyol and lobetyolin showed significant antioxidant activity more than both MeOH extract and other purified compounds (5).

Flavonoids are the group of compounds that have diverse beneficial activities and antioxidant effects. These type of compounds exhibits *in vitro* and *in vivo* anti-inflammatory potential (20). Recently, scientists have proven that some antioxidants have anti-inflammatory and wound healing properties. In wound healing process, they have capacity to scavenge free radicals (21). Furthermore, they have capacity to block inflammation by lowering the activation of inflammatory signals. Indeed, certain flavonoids are potent inhibitors of the production of prostaglandins. Studies have shown that this effect is due to flavonoid inhibition of key enzymes such as lipoxygenase, phospholipase, and cyclooxygenase. Flavonoids also inhibit phosphodiesterases (22).

CONCLUSION

According to the results the significant anti-inflammatory and wound healing activities of the MeOH extract of *C. lyrata* subsp. *lyrata* could be attributed to the flavonoid content of the plant. The present research confirms the traditional utilization of *Campanula lyrata* subsp. *lyrata.*

Table 2. Effect of the extracts from *Campanula lyrata* subsp. *lyrata* on serotonin-induced paw edema in mice

Material	Dose mg/kg	Swelling thickness (x 10^{-2}mm)± S.E.M. (Inhibition%)					
		0 min	6 min	12 min	18 min	24 min	30 min
Control		5.0±0.8	8.8±1.4	15.3±1.8	16.1±0.9	22.1±1.3	26.9±1.8
n-Hexane extract	100	4.9±1.0 (2.0)	9.3±1.1 -	15.6±1.4 -	19.6±1.9 -	23.5± 1.6 -	21.9±1.3 (18.6)
	200	4.5±0.6 (10.0)	8.5±1.3 (3.4)	13.7±1.4 (10.5)	15.3±1.1 (4.9)	19.2±1.2 (13.1)	22.5±1.4 (16.4)
Diethyl ether extract	100	4.9±0.7 (2.0)	9.5±1.2 -	12.8±1.5 (16.3)	14.5±1.6 (9.9)	20.5±1.2 (7.2)	27.8±1.6 -
	200	4.4±1.4 (12.0)	9.0±1.1 -	16.4±1.2 -	16.2±1.3 -	21.4±1.8 (3.2)	23.1±1.5 (14.1)
EtOAc extract	100	4.5±0.9 (10.0)	8.2±0.8 (6.8)	14.8±1.7 (3.3)	14.8±1.5 (8.1)	18.9±1.3 (14.5)	22.5±1.1 (16.4)
	200	4.0±0.9 (20.0)	7.6±1.0 (13.6)	16.8±1.3 -	15.6±1.1 (3.1)	17.6± 0.9 (20.4)	25.1±2.2 (6.7)
MeOH extract	100	4.2±1.5 (16.0)	7.2±1.6 (18.2)	14.9±1.6 (2.6)	13.9±1.5 (13.7)	19.6± 1.6 (11.3)	20.6±0.7 **(23.4)***
	200	5.1±0.9 -	8.3±0.9 (5.7)	11.5±1.3 **(24.8)***	13.3±1.0 (17.4)	16.59 ±1.5 **(24.9)***	19.4±1.2 **(27.8)****
Aqueous extract	100	4.3±0.7 (14.0)	7.6±1.5 (13.6)	18.0±1.9 -	17.4±1.2 -	25.1±1.4 -	24.1±1.6 (10.4)
	200	4.8±0.8 (4.0)	7.8±1.6 (11.4)	15.2±1.2 (0.7)	17.1±1.2 -	20.5±1.1 (7.2)	23.8±1.5 (11.5)
Indomethacin	10	3.8±0.4 **(24.0)***	5.8±0.7 **(34.1)***	10.3±0.9 **(32.7)***	12.0±1.1 **(25.5)***	13.4±0.7 **(39.4)****	17.4±0.9 **(35.3)****

S.E.M.: Standard error of the mean

* $p<0.05$. **$p<0.01$. *** $p<0.001$ significant from the control

Table 3. Effect of the extracts from *Campanula lyrata* subsp. *lyrata* against TPA-induced ear edema in mice as measurement swelling thickness and weight measurement of edema

Test samples	Dose (mg/ear)	Swelling thickness (μm) ± SEM	Inhibition %	Weight edema (mg) ± SEM	Inhibition %
Control		342.7 ± 28.3		30.6 ± 3.0	
n-Hexane extract	0.5	328.5 ± 23.8	4.1	24.3 ± 2.9	20.6
Diethyl ether extract	0.5	298.3 ±27.6	12.9	31.2 ± 3.2	-
EtOAc extract	0.5	338.6± 30.7	1.19	32.6 ± 3.4	-
MeOH extract	0.5	285.0± 26.4	16.8	24.4 ± 2.8	20.3
Aqueous extract	0.5	336.5 ± 22.8	1.8	29.0 ± 2.6	5.2
Indomethacin	0.5	100.6 ± 12.5	**70.6*****	13.9 ± 1.5	**54.6*****

S.E.M.: Standard error of the mean
* p<0.05. **p<0.01. *** p<0.001 significant from the control

Table 4. Inhibitory effect of the extracts from *Campanula lyrata* subsp. *lyrata* on acetic acid-induced increase in capillary permeability

Material	Dose (mg/kg)	Evans blue concentration (μg/mL) ± SEM	Inhibition (%)
Control		12.82 ± 1.16	
n-Hexane extract	100	11.81 ± 0.91	7.9
	200	11.65 ± 0.78	9.1
Diethyl ether extract	100	12.95 ± 1.20	-
	200	11.97 ± 1.12	6.6
EtOAc extract	100	10.86 ± 0.99	15.3
	200	10.33 ± 0.87	19.4
MeOH extract	100	9.81 ± 0.86	23.5
	200	8.74 ± 1.11	**31.8****
Aqueous extract	100	12.60 ± 1.08	1.7
	200	12.38 ± 1.13	3.4
Indomethacin	10	6.08 ± 0.52	**52.6*****

S.E.M.: Standard error of the mean
* p<0.05. **p<0.01. *** p<0.001 significant from the control

Table 5. Effect of the extracts from *Campanula lyrata* subsp. *lyrata* on incision wound model

Material	Statistical Mean ± S.E.M.	(Tensile strength %)
Vehicle	10.08 ± 1.88	1.3
Negative Control	9.95 ± 1.75	-
n-Hexane extract	10.80 ± 1.92	7.1
Diethyl ether extract	10.44 ± 2.02	3.6
EtOAc extract	10.60 ± 1.26	5.2
MeOH extract	12.79 ± 0.79	**26.9***
Aqueous extract	11.41 ± 1.61	13.2
Madecassol®	15.38 ± 0.67	**52.6*****

* : p < 0.05; ** : p < 0.01; *** : p < 0.001; S.E.M.: Standard error of the mean
Percentage of tensile strength values: Vehicle group was compared to Negative control group; Extracts were compared to Vehicle group

Table 6. Effect of the extracts from *Campanula lyrata* subsp. *lyrata* on excision wound model

Material	Wound area ± S.E.M. (Contraction %)						
	0	**2**	**4**	**6**	**8**	**10**	**12**
Vehicle	19.52 ± 2.13	17.27 ±1.59 (0.63)	15.93± 1.50 (0.99)	14.28 ± 1.19 (4.22)	10.98 ± 1.27 (11.09)	7.42± 1.14 (8.85)	3.33 ± 0.47 (8.01)
Negative Control	19.46 ± 2.05	17.38 ± 1.62	16.09 ± 1.81	14.91 ± 1.60	12.35 ± 1.31	8.14 ± 1.22	3.62 ± 1.14
n-Hexane extract	19.39± 2.17	16.82 ± 1.46 (2.61)	15.75 ± 2.04 (1.13)	13.48 ± 1.73 (5.60)	10.26 ± 1.48 (6.56)	7.10 ± 1.24 (4.31)	2.91 ± 0.32 (12.61)
Diethyl ether extract	19.51 ± 2.04	17.34 ± 1.36 -	16.44 ± 1.90 -	14.02 ± 1.78 (7.00)	11.23 ± 1.41 -	7. 35± 1.23 (0.94)	3.40 ± 0.89 -
EtOAc extract	19.28 ± 2.15	16.95 ± 1.51 (1.85)	15.81 ± 2.03 (0.75)	13.77 ± 1.24 (3.57)	10.06 ± 1.43 (8.38)	6.49 ± 1.04 (12.53)	3.01 ± 0.39 (9.61)
MeOH extract	19.45 ± 2.24	15.91 ± 1.13 (7.87)	14.13 ± 1.27 (11.29)	12.31 ± 1.07 (13.79)	8.22 ± 1.04 (25.14)	5.01 ± 0.85 **(32.48)***	2.01 ± 0.21 **(39.64)***
Aqueous extract	19.38 ± 2.06	16.75 ± 1.47 (3.01)	15.22 ± 1.76 (4.46)	13.56 ± 1.28 (5.04)	9.01 ± 1.02 (17.94)	6.13 ± 0.93 (17.39)	2.63 ± 0.37 (21.02)
Madecassol®	19.50 ± 1.95	14.86 ± 1.29 (13.95)	13.18 ± 1.45 (17.26)	9.17 ± 0.57 **(35.78)***	3.49 ± 0.81 **(68.21)****	1.37 ± 0.39 **(81.54)*****	0.00±0.00 **(100.00)*****

*: $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$; S.E.M.: Standard error of the mean

Percentage of contraction values: Vehicle group was compared to Negative control group; Extracts were compared to Vehicle group.

Table 7. Effects of the test ointments prepared from the extracts of *Campanula lyrata* subsp. *lyrata* on hydroxyproline content

Material	Hydroxyproline (µg/mg) ± S.E.M.
Vehicle	11.25 ± 1.88
Negative Control	9.14 ± 1.64
n-Hexane extract	14.27 ± 1.49
Diethyl ether extract	11.61 ± 1.93
EtOAc extract	15.16 ± 1.75
MeOH extract	**26.41 ± 0.96***
Aqueous extract	20.46 ± 1.78
Madecassol®	**47.22 ± 0.51****

* : p < 0,05; ** : p < 0,01; *** : p < 0,001; S.E.M.: Standard error of the mean

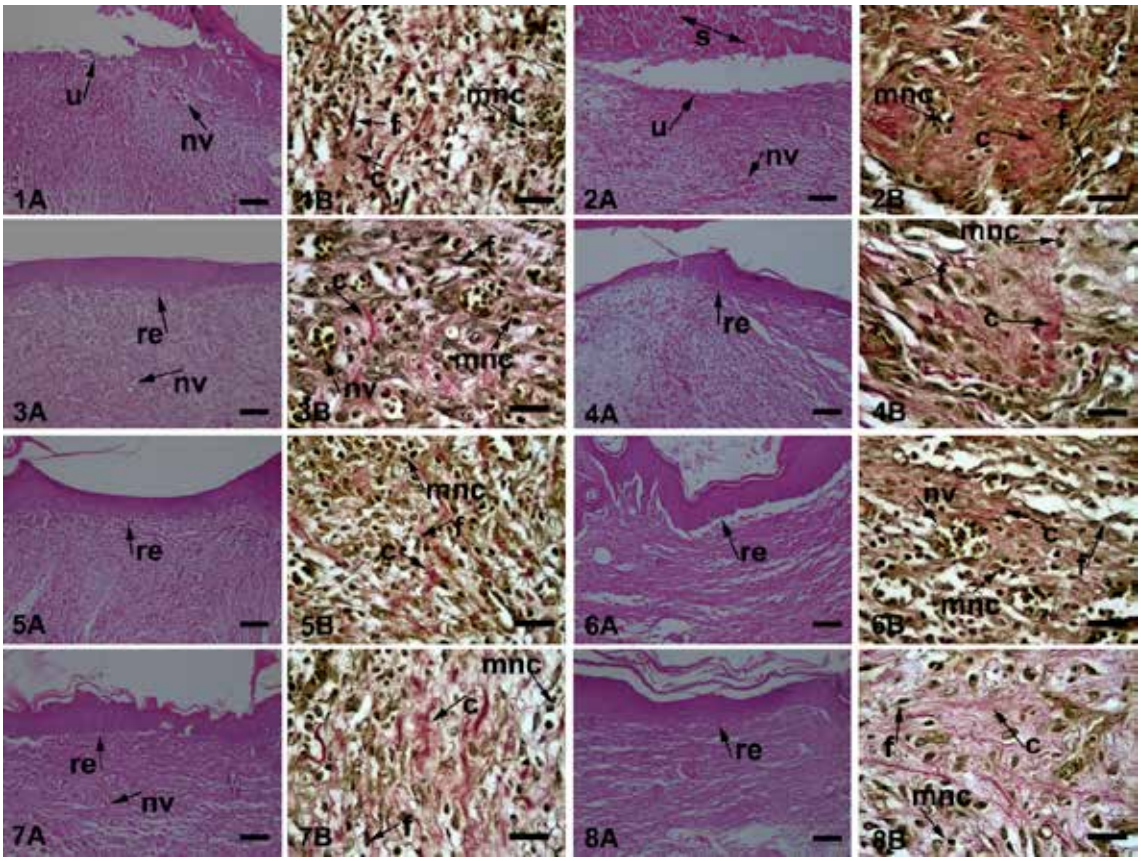

Figure 1. Histopathological view of wound healing and epidermal/dermal re-modeling in the vehicle, negative control, extract ointments and Madecassol® administered animals.

Skin sections show the hematoxylin & eosin (HE) stained epidermis and dermis in A, and the dermis stained with Van Gieson (VG) in B. The original magnification was x 100 and the scale bars represent 120 µm for figures in A, and the original magnification was x 400 and the scale bars represent 40 µm for B. Data are representative of 6 animal per group. 1) Vehicle; 2) Negative Control; 3) *n*-Hexane extract; 4) Diethyl ether extract; 5) EtOAc extract; 6) MeOH extract; 7) Aqueous extract; 8) Reference

Table 8. Wound healing processes and healing phases of the experimental group animals

Groups	Wound Healing Processes								Healing Phases		
	S	U	RE	FP	CD	MNC	PMN	NV	I	P	R
Vehicle	++/+++	+/++	+	++/+++	++/+++	++/+++	++	++/+++	++/+++	++/+++	+
Negative Control	++/+++	+/++	-/+	+++	++/+++	++/+++	++	+++	++/+++	+++	-/+
n-Hexane extract	++	-/+	+/++	++	++	+/++	+/++	+/++	+/++	++	+/++
Diethyl ether extract	++/+++	+	+/++	++/+++	++/+++	++/+++	++	++/+++	++	++/+++	+/++
EtOAc extract	++	+	+/++	++	++	++	+/++	++	+/++	++	+/++
MeOH extract	+	-	++	++	++	++	+	++	+	+/++	++
Aqueous extract	+/++	-	+/++	++	+/++	+/++	+	++	+/++	++	++
Madecassol®	+	-	++	++	++	++	+	++	+	+/++	++

HE and VG stained sections were scored as mild (+), moderate (++) and severe (+++) for epidermal and/or dermal re-modeling. S: Scab, U: Ulcus, RE: Re-epithelization, FP: Fibroblast proliferation, CD: Collagen depositions, MNC: Mononuclear cells, PMN: Polymorphonuclear cells, NV: Neovascularization, I: Inflammation phase, P: Proliferation phase, R: Re-modeling phase.

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