Wound Healing Effects from 3 *Hypericum* spp. Essential Oils

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ABSTRACT

Hypericum species have a long-term use as wound healing agents, with the most common preparation being the infused oil from the aerial parts. It contains naphthodianthrones, phloroglucinols, and essential oil. An extensive literature survey shows that, unlike napthodianthrones and phloroglucinols, essential oils from *Hypericum* spp. have not yet been evaluated for their wound healing efficacy. The present study aims to assess the wound healing efficacy of essential oils from *H. perforatum*, a plant recognized in European Pharmacopoeia for having wound healing properties, as well from 2 other *Hypericum* species commonly used in Greece as wound healing agents since classical antiquity, namely, *H. empetrifolium* and *H. triquetrifolium*. So far, only the wound healing effects of Hypericum oil are known, which is a different herbal preparation containing nonvolatile compounds, while the essential oils under investigation contain only volatile constituents. The essential oils were subjected to GC-MS analyses. Wounds were created on the upper back of hairless SKH-hr1 mice. Healing was evaluated by clinical, histopathological, and biophysical assessment. The essential oils showed a significantly faster wound healing rate in comparison to the controls and the vehicle-treated groups. *H. empetrifolium* possessed the most significant healing properties while for *H. perforatum* and *H. triquetrifolium* skin inflammation persisted. The essential oils from *Hypericum* spp. showed promising results as wound healing agents and are likely to contribute to the wound healing efficacy of the *Hypericum* preparations. *H. empetrifolium*, being the most potent anti-inflammatory and wound healing agent, confirms the traditional use of this plant in Greece for wounds and skin inflammations.

Introduction

The genus *Hypericum* L. (Hypericaceae) comprises more than 450 taxa worldwide [1] and 51 taxa in Greece, of which 15 are endemic [2]. *Hypericum* species have been used as wound healing agents since classical antiquity, and they have been described by Hippocrates [3], Dioscorides [4], and later on in the Medieval era by Nikolaos Myrepsos [5, 6].

In modern times, a monograph of *H. perforatum* L. was included in 2009 in the European Pharmacopoeia, mentioning wound healing properties (www.ema.eu) [7]. The translucent glands on the leaves of the plant look like perforations, and its preparations are red resembling blood. The use of the plant in wound treatment was suggested since ancient times [8].

The infused oil (Oleum *Hyperici*) is the most common preparation for the treatment of wounds and skin inflammation. It is obtained by macerating the fresh aerial parts under sunlight, usually in olive or sunflower oil for a period of 2 – 3 weeks. Oleum *Hyperici* has a red color, resulting from the degradation of naphthodianthrones, which are not extracted in the infused oil. The dark glands contain this group of compounds, which are important bioactive secondary metabolites of other preparations of the *Hyperici* herba, such as tincture, also used for wound healing [7]. The content of the translucent glands (phloroglucinols and essential oil) is extracted in the infused oil [9].

Phloroglucinols are reported to be sensitive metabolites that are quickly degraded in the presence of air, heat, and light [10], and there is much controversy in the scientific community regarding the composition and stability of the formulations containing this group of compounds [11].

The translucent glands also contain essential oils (EOs), which are partially derived from the same biosynthetic pathway [12]. According to the European Pharmacopeia, EO (Aetherolea) are odorous products, usually of complex composition, obtained mainly by steam distillation. Although *Hypericum* spp. are classified as EO-poor plants [9], studies have shown that their volatile oils possess antimicrobial, antioxidant, antiangiogenetic, and gastroprotective activities [13]. An extensive literature survey shows, that, unlike napthodianthrones and phloroglucinols, the wound healing efficacy of EOs from *Hypericum* spp. was not still evaluated and compared between species. Thus, the aim of the present study is the investigation of the wound healing efficacy of the EOs specifically used for this reason: *H. perforatum* L. (HP) and 2 other *Hypericum* species commonly used in Greece since classical antiquity, namely *H. empetrifolium* Willd. (HE) and *H. triquetrifolium* Turra (HT).

Results and Discussion

As presented in ▶ **Table 1**, the EOs of the under-investigation *Hypericum* spp., namely HE, HP, and HT, were complex mixtures. In total, 122 individual constituents were identified, representing 88.1–96.8 % of the EOs. Even the main constituents never exceeded 19.0 % (α-pinene in HE). Regarding HE, its chemical analysis has been recently completed [14] and is presented in ▶ **Table 1** to facilitate the comparison. The *Hypericum* spp. Under investigation yielded EOs 0.9 and 0.6 v/w % for HE and HT respectively, which were calculated in dry weight (▶ **Table 1**). It is noteworthy that when only inflorescences and leaves were carefully selected instead of total aerial parts of HE, the yielded EO reached 0.6 mL (13 v/w %). The main compounds have been identified as follows: HE: α-pinene, germacrene D, β-pinene, E-caryophyllene; HP: ishwarane, α-himachalene, α-pinene, β-pinene; and HT: α-pinene, 3-methyl-nonane, caryophyllene oxide, germacrene D. In comparison to different previous studies [9, 13, 15, 16], intraspecific-variability has been observed for all the under-investigation species, which could be explained by the different extraction methods that have been applied, as well as different collection times and sites.

Regarding the skin parameters, the most sensitive measurement is transepidermal water loss (TEWL), when it is cautiously measured in areas fully healed. TEWL has been recovered in several treatment groups, especially in HE 0.5 % and HP 0.05 % ointments, while the other remains at relatively higher levels (highest levels in the control groups, petrolatum, and the HT 0.5 %, ▶ **Fig. 1a**). Moreover, skin hydration has been recovered. Despite this, the healed skin generally shows higher hydration; however, this does not reflect the reality, as cysts or edema are frequently formed in wounds (▶ **Fig. 1b**). An overall increase of the erythema factor was observed for all treatments. This observation is inconsistent with previous observations of our laboratory, where the high sensitivity of the detector often leads to the evaluation of healed skin as inflamed. Furthermore, skin thickness was increased in all treatments, while it generally takes more than 2 years to recover completely. Regarding treatments HT 0.05 % and HT 0.5 %, as well as treatments HE 0.05 % and HE 0.5 %, the elasticity increased, probably as a result of neovascularization.

Based on the clinical evaluation of the mice, it became apparent that the treatment with the low dose of HP 0.05 % ointment led to almost complete wound healing (99.9 %) with expected scarring. This was also the criterion for terminating the experiment. Compared to the treatment with the same EO at the highest dose (0.5 %), a similar degree of healing was observed by day 8, but at the end of the experiment, for HP 0.5 %, the degree decreased and reached a healing rate of 96.6 %. This leads to the suspicion of possible dose-dependent toxicity, which is characteristic of EOs. Also, HP 0.05 % showed positive results compared to the control and the petrolatum, while HP 0.5 % resulted in less healing in comparison to the control and the petrolatum.

HT ointment showed 99.2 % healing at a low dose and 97.9 % at the highest (HT 0.05 % and HT 0.05 %, respectively). Therefore, it is evident, again, that the low dose has better results compared to the control group and the treatment with petrolatum, but the high dose showed a similar clinical impact with them. Treatments with 0.05 % and 0.05 % of HE ointment showed a very good effect in both doses (99.4 % and 98.9 % degree of healing, respectively). Moreover, both the high and the low doses showed a positive effect compared to the control group (97.1 %), the petrolatum (98.1 %), and Madecassol (99.6 %) treatments but also to the treatments with HP and HT ointments.

▶ **Fig. 2** shows the overall degree of healing of each group. In treatment with 0.05 % HP ointment, there were wounds of greater initial area, but this ointment resulted in 99.9 % healing on day 15. It is noteworthy that HE ointments had the best healing rates, due to the faster reduction in wound area compared to other treatments. The photo documentation in ▶ **Fig. 3** shows representative images of the wound areas of the various mice groups.

▶ **Table 1** Qualitative and quantitative composition (% v/v) of EOs.

	Compounds	RI aver.	HE	HP	HT
1.	(3E)-2,3-dimethylhepta-1,3-diene	902	0.2	–	–
2.	α-thujene	913	1.0	0.1	0.4
3.	α-pinene	928	**19.0**	6.4	**13.9**
4.	α-fenchene	938	0.5	0.2	tr
5.	camphene	940	0.3	0.6	tr
6.	dehydrosabinene = thuja-2,4(10)-diene	943	–	tr	–
8.	3-methyl-nonane	962	3.5	–	**10.2**
8.	β-pinene	972	**8.7**	6.1	1.5
9.	6-methyl-5-hepten-2-one	979	–	tr	–
10.	myrcene	984	1.8	0.9	1.4
11.	hexenyl acetate	985	–	–	–
12.	n-decane	1000	0.2	–	0.4
13.	α-phellandrene	1001	tr	0.1	tr
14.	α-terpinene	1011	0.1	0.3	1.2
15.	p-cymene	1018	0.8	0.2	0.9
16.	limonene	1022	1.6	2.2	0.6
17.	cis-ocimene	1030	0.7	0.3	tr
18.	trans-ocimene	1040	1.9	0.2	tr
19.	γ-terpinene	1050	0.3	0.5	2
20.	2-methyl-decane	1056	1.8	0.8	4
21.	terpinolene	1080	0.2	0.8	0.5
22.	n-undecane	1094	1.0	0.7	1.8
23.	n-nonanal	1097	–	tr	tr
24.	endo-fenchol	1108	0.2	0.2	–
25.	α-campholenal	1119	0.1	0.1	tr
26.	allo-ocimene	1128	0.1	–	–
27.	trans-pinocarveol	1129	0.2	0.2	–
28.	trans-verbenol	1135	0.2	–	–
29.	camphor	1137	tr	0.1	–
30.	camphene hydrate	1141	tr	0.1	–
31.	isoborneol	1152	0.1	0.6	–
32.	pinocarvone	1153	-	–	tr
33.	borneol	1158	0.3	0.3	–
34.	3-methyl-undecane	1162	–	1.0	–
35.	terpinen-4-ol	1167	0.1	0.2	tr
36.	α-terpineol	1181	0.2	0.9	–
37.	myrtenol	1186	0.2	0.2	–
38.	verbenone	1198	0.1	-	–
39.	citronellol	1219	tr	0.1	–
40.	geraniol	1245	–	tr	–
41.	linalool acetate	1246	0.4	–	–
42.	2-undecanone	1285	0.1	0.1	–
43.	tridecane	1300	0.1	–	–
44.	α-longipinene	1337	2.1	0.2	–
45.	α-cubebene	1338	–	–	tr
46.	α-ylangene	1360	0.3	0.5	tr
47.	α-copaene	1365	0.4	0.2	1.2
48.	α-duprezianene	1367	–	0.1	–
49.	β-bourbonene	1370	0.4	–	tr
50.	geranyl acetate	1373	0.1	–	–
51.	β-cubebene	1376	0.1	–	–

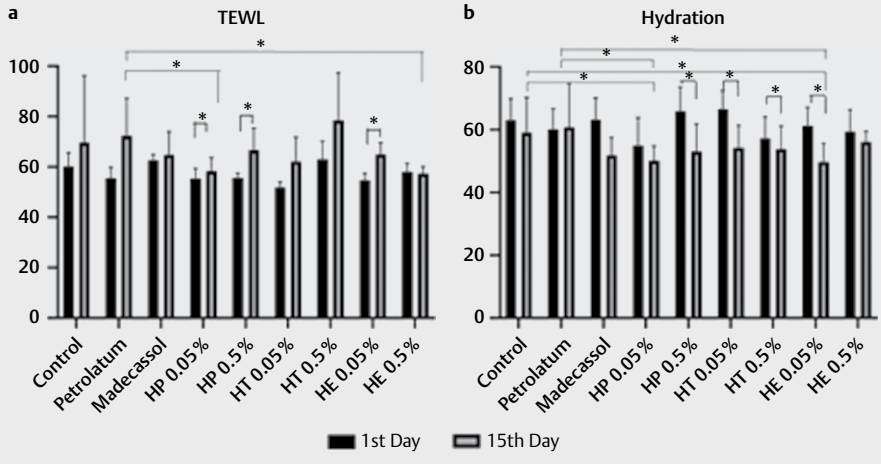
▶ **Table 1** Continued.

	Compounds	RI aver.	HE	HP	HT
52.	italicene	1379	–	0.1	–
53.	β-elemene	1380	0.2	–	–
54.	β-longipinene	1384	0.3	–	–
55.	longifolene	1389	–	0.4	–
56.	α-cedrene	1389	0.1	–	–
57.	2-epi-β- funebrene	1397	–	0.1	tr
58.	E-caryophyllene	1406	**5.3**	2.6	**14.0**
59.	β-cedrene	1409	0.9	–	–
60.	β-duprezianene	1413	–	0.6	–
61.	β-copaene	1413	–	–	0.5
62.	β-gurjunene	1424	0.5	–	–
63.	aromadendrene	1430	0.6	–	–
64.	α-himachalene	1433	0.4	**6.9**	–
65.	α-humulene	1437	0.6	–	1.8
66.	E-β-farnesene	1445	1.6	–	–
67.	allo-aromadendrene	1450	0.4	–	–
68.	ishwarane	1453	2.0	**22.0**	–
69.	γ-muurolene	1461	0.7	3.4	3.3
70.	germacrene D	1466	**12.5**	1.0	**8.2**
71.	γ-himachalene	1467	–	1.2	1.2
72.	β-selinene	1473	1.0	2.2	–
73.	valencene	1476	–	1.4	1.3
74.	α-selinene	1480	1.0	1.9	1.2
75.	4-epi-cis-dihydroagaro-furan	1482	–	0.6	–
76.	α-muurolene	1483	0.8	–	0.9
77.	β-himachalene	1485	–	3.5	–
78.	epizonarene	1488	–	0.2	–
79.	trans-β-guaiene	1492	–	0.2	–
80.	E, E-α-farnesene	1492	0.8	–	–
81.	δ-amorphene	1494	–	–	tr
82.	γ-cadinene	1496	1.5	0.8	2.2
83.	7-epi-α-selinene	1499	–	0.2	–
84.	δ-cadinene	1506	3.1	1.4	4
85.	γ-dehydro-ar-himachelene	1514	–	0.4	–
86.	cadina-1,4-diene	1514	0.2	–	tr
87.	γ-vetivenene	1518	–	0.4	–
88.	α-cadinene	1519	0.4	–	tr
89.	α-calacorene	1524	0.1	0.1	0.5
90.	β-calacorene	1544	tr	0.2	–
91.	E-nerolidol	1547	0.5	–	–
92.	caryophyllenol	1550	–	0.6	–
93.	3Z-hexenyl-benzoate	1553	0.1	–	–
94.	himachalene epoxide	1556	–	0.2	–
95.	spathulenol	1558	1.5	–	0.9
96.	caryophyllene oxide	1563	1.8	0.9	**9.7**
97.	cubeban-11-ol	1573	0.1	–	–
98.	salvial-4(14)-en-1-one	1574	–	–	1.1
99.	viridiflorol	1578	0.2	–	–
100.	rosifoliol	1581	0.3	–	–
101.	humulene epoxide II	1589	tr	–	0.5
102.	junenol	1597	0.3	–	–
103.	1-epi-cubenol	1608	0.1	–	tr
104.	epi-α-cadinol	1609	–	tr	–
105.	cubenol	1620	0.5	–	–

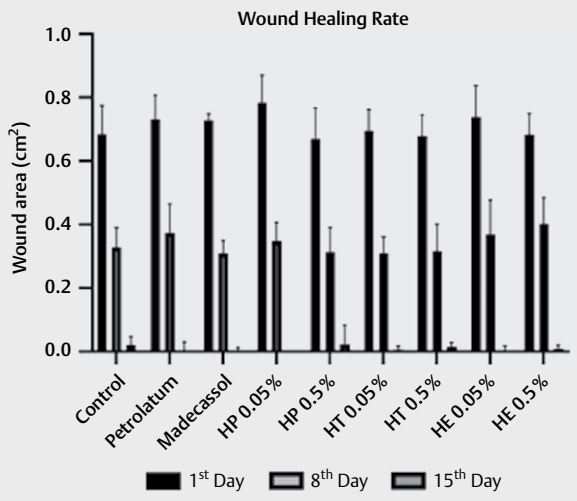
▶ **Table 1** Continued.

	Compounds	RI aver.	HE	HP	HT
106.	τ-muurolol	1626	2.3	–	0.9
107.	torreyol = α-muurolol	1632	0.5	–	–
108.	allo-aromadendrene epoxide	1637	–	–	0.9
109.	α-cadinol	1640	0.8	–	0.9
110.	himachalol	1640	–	3.1	–
111.	selin-11-en-4-α-ol	1643	–	2.6	–
112.	allohimachalol	1650	–	0.5	–
113.	intermedeol	1652	–	1.7	–
114.	trans-calamenen-10-ol	1656	0.2	–	–
115.	cadalene	1664	tr	0.9	–
116.	germacra-4(15), 10(14)-trien-1-α-ol	1665	–	–	1.0
117.	eudesma-4(15),7-dien-1β-ol	1676	–	–	1.8
118.	cyclocolorenone	1743	–	0.1	–
119.	benzyl benzoate	1757	0.1	0.1	–
120.	n-hexadecanol	1877	0.3	–	–
121.	nonadecane	1898	0.1	–	–
122.	heneicosane		2097	0.1	0.1
Total identification			94.2	88.1	96.8
[α]D20			- 14.89	− 0.25	− 12.58
			(c 0.10)	(c 1.61)	(c 0.103)
EO yield (% v/dry weight)			0.9	–	0.6
Grouped components			**HE**	**HP**	**HT**
Monoterpene hydrocarbons			37.1	18.9	22.4
Oxygenated monoterpenes			2.1	3.0	0.0
Sesquiterpene hydrocarbons			38.3	53.1	40.3
Oxygenated sesquiterpenes			9.1	10.3	17.7
Others			7.6	2.8	16.4

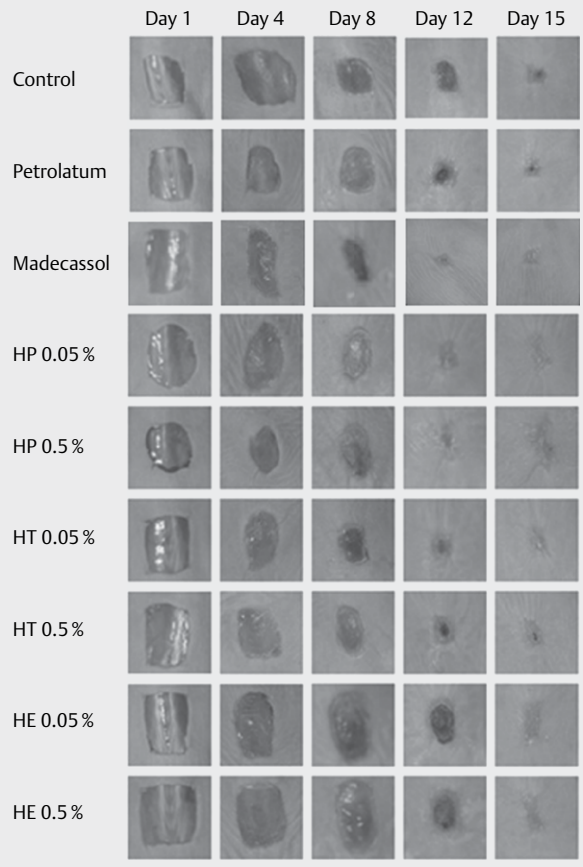
Components listed in order of elution from a HP 5MS column. RI aver. Retention indices calculated against C9-C25 n-alkanes on the HP 5MS column; average value from three samples. tr: traces. Concentrations below 0.01 % are marked as -; **main compounds** >5 %.



▶ **Fig. 1** (**a**) Transepidermal water loss (TEWL) values for the various mice groups (control; petrolatum; Madecassol; HP 0.05 %; HP 0.5 %; HT 0.05 %; HT 0.5 %; HE 0.05 %; HE 0.5 %) on Day 1 and Day 15 of the experiment. (**b**) Hydration values for the various mice groups on Day 1 and Day 15 of the experiment. Values are presented as mean ± SD of 3-–4 independent experiments (n = 6 mice per group). Statistical analysis was performed using Student's t-test or One-way ANOVA (in comparison to the control group and the group treated with petrolatum); * $p < 0.05$.



▶ **Fig. 2** The effect of the different treatments (control; petrolatum; Madecassol; HP 0.05 %; HP 0.5 %; HT 0.05 %; HT 0.5 %; HE 0.05 %; HE 0.5 %) on the wound healing process on young mice. The wounds from each group were photographed at time 0 and on Day 1, Day 8, and Day 15. A Nikon Nikkor AF-S Micro 60 mm f/2.8 G ED, SWMED IF camera was used, located at a distance of 30 cm from the animals. The photographs were digitized, and the wound area (cm2) was measured using Adobe Photoshop C5.



▶ **Fig. 3** Representative images of the wound areas of the various mice groups (control; petrolatum; Madecassol; HP 0.05 %; HP 0.5 %; HT 0.05 %; HT 0.5 %; HE 0.05 %; HE 0.5 %), as recorded over a 15-days period.

After histopathological examination of skins from each group, no total healing was observed in any treatments, and their degree of inflammation was significantly different.

The lowest inflammation was observed in the group treated with the low dose of HE ointment, 0.05 %. This is illustrated by the following images (▶ **Fig. 4**), which belong to the mice treated with HE 0.05 %. Interestingly, the skin's normal structure was maintained, and it is also worth noting that some elements of regenerated hair follicles existed in the wounded skins. HE showed significant healing properties also at the highest dose (0.5 %), similar to that of the lower dose (0.05 %).

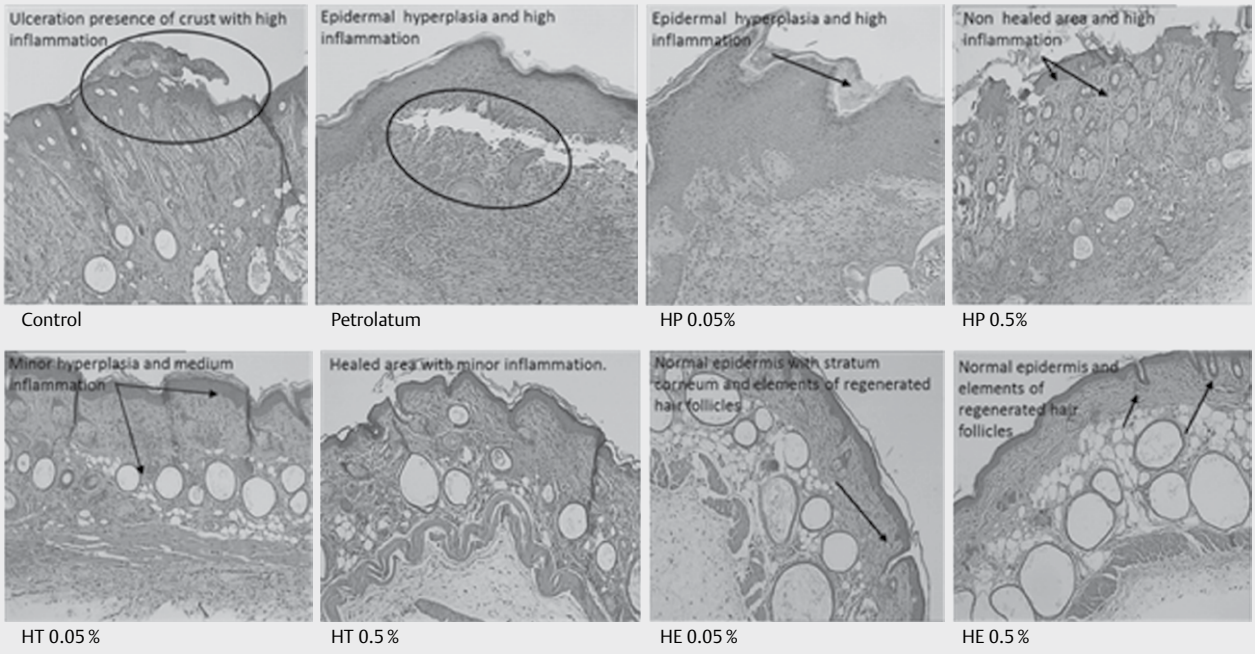
On the contrary, we observed that there was still acute inflammation in treatment with the control that had not been administered any formulation. More specifically, the image even shows ulceration in the presence of "inflammatory overgrowth." But also, treatment with petrolatum had strong inflammation, as the nuclei of the polymorphonuclear cells are visible, characteristic of acute inflammation, shown as black dots under microscope observation. The structure of the skin with the characteristic layers has changed.

Treatment with a 0.5 % high dose of HT ointment was also effective, presenting a healed area with minor inflammation, whereas treatment with the lower concentration of 0.05 % ointment of the same plant had weaker results, with minor hyperplasia and with medium inflammation. These are evident in some photos from the microscope (▶ **Fig. 4**).

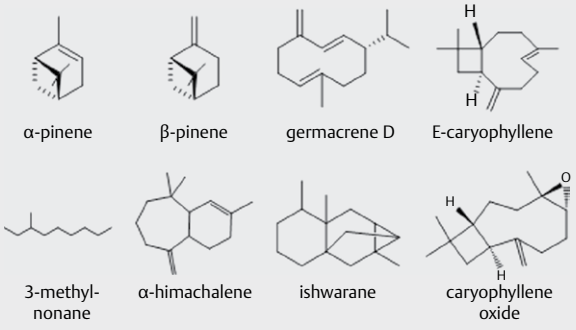
The effect of HP ointment at either low (HP 0.05 %) or high concentration (HP 0.5 %) was not shown to be effective during the experiment. Especially in the mice with HP 0.05 % treatment, the inflammation (in the presence of polymorphonuclear cells) is predominant, mainly in the dermis, similar to that of treatment with petrolatum.

To the best of our knowledge, the present work is the first study revealing the wound healing properties of the EOs from *Hypericum* spp., which are likely to contribute to the wound healing efficacy of the *Hypericum* preparations. Taking everything into account, it appeared that the control group and the group treated with petrolatum had normal healing progress due to the skin's ability to self-heal but also had most of the elements of inflammation and alteration of the skin structure. Low-dose HP and HT had a good clinical picture, but it was not the same for the degree of inflammation. Moreover, higher concentrations resulted in wound healing delay, indicating dose-dependent toxicity related to the EOs, except for *H. empetrifolium*, which showed significant wound healing and anti-inflammatory effects in both EO doses. In comparison with the other 2 species under investigation, HE EO yields a high concentration of monoterpenes hydrocarbons (37.1 %, *vs.* 18.9 % and 22.4 % for HP and HT, respectively). Furthermore, α-pinene (19.0 % in HE) (▶ **Fig. 5**) has been previously reported to possess anti-inflammatory activities [17].

In conclusion, the significant wound healing properties of HE confirm the traditional use of this plant in Greece for wounds and skin inflammations [18]. It is worth mentioning that in the quote by Dioscorides, *hypericon* could be attributed to HE since the pre-



▶ **Fig. 4** Representative histopathological images of the back skin of SKH-hr1 hairless mice (magnification 100×) on Day 15 of treatment with petrolatum, HP 0.05 %, HP 0.5 %, HT 0.05 %, HT 0.5 %, HE 0.05 %, HE 0.5 %, and without treatment (control) (magnification 100×). Samples were stained with hematoxylin and eosin. Arrows and circles pointing the events of wound healing (*i. e.* ulceration presence of crust with high inflammation [control]; epidermal hyperplasia and high inflammation [petrolatum and HP 0.05 %]; non-healed area and high inflammation [HP 0.5 %]; minor hyperplasia and medium inflammation [HT 0.05 %]; normal epidermis with stratum corneum and elements of regenerated hair follicles [HE 0.05 %]; normal epidermis with elements of regenerated hair follicles [HE 0.05 %].



▶ **Fig. 5** Chemical structures of the most abundant compounds.

viously reported *H. coris*[4] is not growing wild in Greece, in contrast to its closely related species, HE [19].

Material and Methods

Plant material

Aerial parts from HE and HT were collected from natural populations in Greece (in Crete and Thessaloniki, GPS position 35.25463477968957, 25.37766337130051 and 40.636971, 22.976209, respectively), during the flowering stage. The collected plant materials were recognized and authenticated by Prof. Z. Kypriotakis and Dr. E. Antaloudaki for HE (Department of Agriculture, TEI of Crete, and Department of Biology, University of Crete) and by Assoc. Prof. Th. Constantinidis for HT (Biology Department, NKUA). Voucher specimens were deposited in the Herbarium of Natural History Museum, University of Crete (15981) and in the Laboratory of Pharmacognosy and Chemistry of Natural Products (Skaltsa & Grafakou 03), respectively. The EO of HP was purchased from Florihana (France, LOT FLEO59-B120917F) and further subjected to GC-MS analysis. The plant names have been checked according to http://www.theplantlist.org [20].

Hydro-distillation of essential oils, GC-MS spectrometry analysis, identification of compounds

To obtain the EOs from HE and HT, the air-dried plant materials were subjected to hydro-distillation, according to the procedure described before [14]. The 3 EOs were subsequently analyzed by GC-MS and finally stored at −20 °C before being used for the *in vivo* experiments. GC-MS analyses and identification of the chemical compounds were carried out as described previously [14].

Animals

Animal care was performed according to the guidelines established by the European Council Directive 2010/63/EU. Fifty-four female SKH-hr1 hairless mice (3–12 weeks old, 17–40 g, n = 6) were used in this study. All mice originated from the breeding stock of

the Small Animal Laboratory of the Section of Pharmaceutical Technology, Department of Pharmacy (EL 25 BIO 07). The animal room was kept at 23 ± 1 °C and 25–55 % humidity and was illuminated by yellow fluorescent tubes in a 12 h light and dark cycle. The mice had unrestricted continuous access to standard chow diet (Nuevo SA-Farma-Efyra Industrial and Commercial SA, Greece) and fresh water. The experimental procedure was approved by the National Peripheral Veterinary Authority (Protocol Number: 1064/20-02-2019) after the affirmative opinion of the Animal Protocols Evaluation Committee.

Experimental design for in vivo wound healing effect in a mouse model

The experimental protocol for the evaluation of wound healing has been used for years from the Laboratory of Dermatopharmacology, and has been recently described by Sofrona and colleagues [21]. Briefly, full-thickness (*i. e.*, epidermis, dermis, and subcutis) wounds of 1 cm^2 (1.0 cm × 1.0 cm) were induced on the dorsal skin of anesthetized mice by intraperitoneal administration of a cocktail of ketamine (100 mg/kg) and xylazine (7 mg/kg).

Mice (n = 54) were randomly divided into 9 treatment groups of 6 animals per group. The first group was the control (untreated mice); the second group received the petrolatum vehicle (100 % petroleum jelly); and the third group received the Madecassol cream (*Centella asiatica* extract used as positive control), the rest of the groups received 0.05 and 0.5 % w/w petrolatum ointment of each EO (▶ **Table 2**). The different treatments were applied once per day for 14 days, where complete (99.9 %) healing was clinically observed in one of the groups (HP 0.05 %).

Evaluation of TEWL, hydration, erythema, thickness, and elasticity

Skin parameters, including TEWL, hydration, erythema, skin thickness, and elasticity were evaluated with noninvasive biophysical methods. TEWL and skin hydration were measured by using the Tewameter TM 210 (Courage + Khazaka Electronic GmbH) and the Corneometer CM820 (Courage + Khazaka Electronic GmbH), respectively. Erythema was measured by using the spectrophotometer Mexameter MX 18 (Courage-Khazaka). Skin thickness was scored in mm using an electronic digital caliper (Powerfix Prof Milomex Ltd), 3 cm above the tail. Elasticity was measured by using CUTOMETER MPA 580 (Courage-Khazaka). All of the above-mentioned measurements were conducted on the first (just before the induction of the wounds) and last day in healed areas, as described before [19]. This is particularly important, as they are sensitive measurements, which evaluate the skin barrier restoration; as a result, they must be cautiously measured the last day only in areas fully healed to provide reliable results.

▶ **Table 2** Treatments.

Control	Control group
Petrolatum	Petrolatum
Madecassol	Madecassol cream
HP 0.05 %	*H. perforatum* 0.05 % ointment
HP 0.5 %	*H. perforatum* 0.5 % ointment
HT 0.05 %	*H. triquetrifolium* 0.05 % ointment
HT 0.5 %	*H. triquetrifolium* 0.5 % ointment
HE 0.05 %	*H. empetrifolium* 0.05 % ointment
HE 0.5 %	*H. empetrifolium* 0.5 % ointment

Photodocumentation, percentage of wound healing, and histological analysis

The wounds from each group were photographed at time zero and then on the 4th, 8th, 12^{th,} and 15th day. A Nikon Nikkor AF-S Micro 60 mm f/2.8 G ED, SWMED IF camera was used, located at a distance of 30 cm from the animals. The photographs were digitized, and the wound area was measured using Adobe Photoshop C5. Wound closure was defined as a reduction in the wound area and the results were expressed as a percentage (%) of the original wound area. After the mice were sacrificed, the back skin was removed, fixed in formaldehyde, and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin (H&E). The extent of inflammatory cell infiltration, parakeratosis, and hyperkeratosis, epidermal thickness, and Munro abscess were blindly evaluated by an experienced anatomopathologist.

Statistical analysis

All results are presented as means ± SEM of 3 different experiments for each sample. The data were tested for normality and distribution. Data were evaluated by Student's t-test and 1-way analysis of variance (ANOVA) using the SPSS v 18.0 statistical analysis software (IBM SPSS software package, Inc.). The p-value of ≤ 0.05 was set as a significance level for all data. Graphs were generated using GraphPad Prism 8.4.2 (GraphPad Software, Inc.).

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Conflict of Interest

The authors declare no conflicts of interest regarding the current research work.

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