

ANTIMICROBIOLOGICAL EFFECTS OF NEW NATURAL ANTISEPTIC FORMULATION ON NON-INFECTED VENOUS LEG ULCER: PILOT STUDY

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Venous leg ulcers represent a significant public health problem that will increase as the population ages. Numerous herbs and their extracts are potentially conducive to wound healing, including the ability to serve as antimicrobial, antifungal, astringent etc. The aim of the study was to establish the in-vivo antimicrobial effects of herbal hydrogel formulation DermaplantG. The major components of the DermaplantG were the extracts of *Allii bulbosus*, *Hyperici herba* and extract of *Calendulae flos*. A total of 12 patients with non-infected venous leg ulcers were treated twice daily, for 5 weeks, with new hydrogel formulation. All ulcers showed clinical signs of contamination or colonization without signs of systemic infection. Premoistening the swab with sterile saline was considered when the surface of the wound was dry. The tip of the swab was rolled on its side in a zigzag pattern for at least one full rotation. Standard methods for isolation and identification of aerobic and anaerobic bacteria were used.

On baseline assessment, a large number of different types of bacteria were detected in all venous leg ulcers. *S. aureus* and *P. aeruginosa* were isolated from almost all controls.

On baseline, mixed bacterial flora (50%) was isolated in six venous leg ulcers (five ulcers with *S. aureus*-*P. aeruginosa* and one ulcer with *E.coli*-*Enterobacter spp.*-*P.aeruginosa*). At the end of the treatment in DermaplantG group in 8 venous ulcers were detected *S. aureus* (66.66%) and *P. aeruginosa* (16.66%), and one venous leg ulcer was detected as sterile (8.33%). The number of different types of isolated bacterial species decreased significantly ($P < 0.05$) after the use of DermaplantG herbal preparations. Therapy in DermaplantG group was administered without any side effects.

The preliminary results of this pilot study demonstrate potential antimicrobial effects of herbal therapy on non-infected venous leg ulcers. *Acta Medica Medianae* 2011; 50(3):40-44.

Key words: venous leg ulcer, microbiological flora, DermaplantG hydrogel

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Introduction

Venous ulcers are common chronic disease requiring continuous therapeutic surveillance, thus significantly influencing the quality of life (1,2). A wide variety of agents are available for treatment of venous leg ulcers, including ointments and dressings (3). The microbiological profile of chronic ulcers of the lower limbs has application to general principles of treatment as well as institution-specific guidelines for management (4,5). Numerous scientific debates suggest the

importance of bacterial colonization and its role in ulcer infections and their treatment. The role of bacteria in the pathogenesis of chronic venous ulcers is still unresolved. Generally, infections of the skin, soft tissues and ulcers are most commonly induced by gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* (1,2). Within ulcer microflora there are other numerous aerobic, anaerobic and mixed bacteria: *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *enterococcus faecalis*, *Enterobacter cloacae*, *Peptococcus magnus*, *Escherichia coli*, *Streptococci* group D, *Peptostreptococcus spp.*, *Bacteroides fragilis* and others (2,4,6). Clinical signs of infections are related to the type and amount of ulcer microflora. Within the same ulcer, after the administered treatment and a period of time, alterations of type and number of bacteria in all their relationships are possible.

Herbal therapy is becoming increasingly popular among patients. Numerous herbs and their extracts are potentially conducive to wound healing, including the ability to serve as an

antimicrobial, antifungal, astringent and etc. (7-11). DermaplantG contain extracts of *Allii bulbosus*, *Hyperici herba* and extract of *Calendulae flos*.

This study presents as support of abilities on DermaplantG herbal treatment of venous leg ulcers and their antimicrobiological effects in clinical practice because there is no enough clinical investigations in this area.

Aim

The aim of the study was to establish the healing and microbiological effects of DermaplantG herbal therapy on non-infected venous leg ulcers.

Materials and methods

The study was opened, randomized and controlled at the Clinic of Dermatology and Venerology, Clinical Centre Niš, Serbia. There were in total 12 included patients with non-infected venous leg ulcers. To be defined as having venous ulcers, all patients needed to have varicose veins with chronic venous insufficiency and clinical diagnosis of venous leg ulcer with the value of ankle brachial index (ABI) > 0.85.

Antimicrobial Activity In Vivo

All ulcers showed clinical signs of contamination or colonization, such as increased exudation, friable granulation tissue, discoloration or augmentation of slough, without signs of systemic infection. The wound bed was first cleaned with sterile saline solution, and superficial slough was debrided using tweezers and scissors. Premoistening the swab with sterile saline was considered when the surface of the wound was dry. The tip of the swab was rolled on its side in a zigzag pattern for at least one full rotation.

The swabs were stored in transport medium and were sent to the laboratory, where they were processed immediately. Standard methods for isolation and identification of aerobic and anaerobic bacteria were used (12).

DermaplantG ointment was applied two times on the venous leg ulcers and on ulcers surrounding. Appropriate care, washing and bandaging of ulcerations were performed every day.

Patients in our study were not previously treated at least 2 weeks with similar herbal therapy and any other supportive physical therapy.

Descriptive statistics are reported as means \pm SD. The Wilcoxon signed-rank test was done to compare the number of isolated species before and after the treatment of the venous leg ulcers. Data were analyzed using the analysis of variance for multiple comparisons (SPSS 14 for Windows), with significance set at $p < 0.05$. In this study, the used protocol was recommended by manufacturer and guidelines of good clinical practice. Written informed consent was obtained from all patients prior to enrolment.

Results

Detailed characteristics of the demographic characteristics of sex, age, duration of venous leg ulcers and ABPI index of patients are provided in Table 1.

Table 1. Baseline patient characteristics (mean \pm SD)

Patients characteristic		DermaplantG1
Sex	M	8 (66.66%)
	F	4 (33.33%)
Age (years)		66.82 \pm 8.20
Duration (weeks)		7.43 \pm 1.75
ABPI		0.97 \pm 0.06

Table 2. Microbiologic isolates in DermaplantG group

Wound No	No	Baseline	I week		III week		V week	
		Description	No	Description	No	Description	No	Description
1	2	<i>S. aureus</i> <i>P. aeruginosa</i>	1	<i>P. aeruginosa</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>
2	2	<i>S. aureus</i> <i>P. aeruginosa</i>	1	<i>P. aeruginosa</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>
3	1	<i>P. aeruginosa</i>	1	<i>P. aeruginosa</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>
4	1	<i>S. aureus</i>	1	<i>S. aureus</i>	2	<i>S. aureus</i> <i>P. aeruginosa</i>	1	<i>S. aureus</i>
5	2	<i>S. aureus</i> <i>P. aeruginosa</i>	1	<i>S. aureus</i>	2	<i>S. aureus</i> <i>P. aeruginosa</i>	1	<i>S. aureus</i>
6	1	<i>S. aureus</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>
7	1	<i>S. aureus</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>
8	1	<i>E. coli</i>	1	<i>P. aeruginosa</i>	1	<i>P. aeruginosa</i>	0	-
9	1	<i>S. aureus</i>	1	<i>S. aureus</i>	1	<i>P. aeruginosa</i>	1	<i>S. aureus</i>
10	2	<i>S. aureus</i> <i>P. aeruginosa</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>	1	<i>P. aeruginosa</i>
11	2	<i>S. aureus</i> <i>P. aeruginosa</i>	1	<i>S. aureus</i>	1	<i>S. aureus</i>	1	<i>P. aeruginosa</i>
12	3	<i>E. coli</i> <i>Enterobacter spp.</i> <i>P. aeruginosa</i>	2	<i>E. coli</i> <i>S. aureus</i>	2	<i>S. aureus</i> <i>P. aeruginosa</i>	2	<i>S. aureus</i> <i>P. aeruginosa</i>

The results of microbiologic isolates during DermaplantG1 treatments and control are presented in Table 2.

On baseline assessment, a large number of different types of bacteria were detected in all venous leg ulcers in both treatment groups. *S. aureus* and *P. aeruginosa* were isolated from almost all controls. The predominant flora in DermaplantG group was *S. aureus* and *P. aeruginosa*. On baseline, mixed bacterial flora (50%) was isolated in 6 venous leg ulcers (5 ulcers with *S. aureus*-*P. aeruginosa* and 1 ulcer with *E. coli*-*Enterobacter spp.*-*P. aeruginosa*). At the end of the treatment in DermaplantG group in 8 venous ulcers were detected *S. aureus* (66.66%) and *P. aeruginosa* (16.66%), and 1 venous leg ulcer was detected as sterile (8.33%). The number of different types of isolated bacterial species decreased significantly ($P < 0.05$) after the use of DermaplantG herbal preparations. Herbal therapy was administered without any side effects.

Discussion

Our study evaluated the microbiological efficacy of herbal based ointment Dermoplant G in the therapy of venous ulcers. Dermoplant G contained dry water extract of *Allii bulbosus*, dry ethanol extract of *Hyperici herba* and oily extract of *Calendulae flos*. The selected extracts possessed antimicrobial (antiseptic), anti-inflammatory and regenerative properties. The antimicrobial efficacy of this herbal therapy was demonstrated in patients with non-infected venous leg ulcerations. No side effects or adverse reactions were detected during five weeks of two-daily application of the extract-containing herbal preparations which support the safety and good tolerability of this product. Favorable action of herbal preparations on healing parameters and antibacterial effects are explained by the medical properties of DermaplantG plants and their extracts from the view-point of pharmacognosy, pharmacological and phytochemical studies (9-14). The antimicrobial properties of garlic (*Allium sativum*) have been shown in many studies using different tests even against highly resistant pathogens. The antibacterial and antifungal activity was attributed to the major compounds allicin and thiosulphonates. A recent paper of Ejaz et al. (15) have shown the beneficial effects of "aged garlic solution" (AGS) on wound closure, epithelization, dermal matrix regeneration and angiogenesis on chicken dorsum skin excisional wound-healing assay. Aqueous solution of garlic (1%) have enhanced burn wound healing in dogs after 21 days of exposure (16). Methanol extracts of herb, stems, flower and leaves *H. perforatum* possessed significant antimicrobial activity. Bioguided fractionation led to flavonoids quercetin and biapigenin, responsible for weak antimicrobial activity, while recent research has shown antimicrobial activity of hypericin comparable to standard antibiotics (17).

The more important for our study are wound healing properties of *Hypericum perforatum*. Recent papers have strongly suggested the effectiveness of hypericum ethanol and oil extracts (17) on wound-healing partly due to the antibacterial activity (18) and anti-inflammatory effect (19). *H. perforatum* extracts stimulate fibroblast collagen production and activate the fibroblast cells into polygonal shapes, which play an important role in repair and closing the wounds. Constituents identified in *Calendula* such as saponins, micro-nutrients, flavonoids and poly-saccharides may be responsible for the anti-edematous, anti-inflammatory, antiseptic, anti-oxidant and wound healing effects of the plant (20). The activity of tincture made from flowering tops and fresh leaves was more effective after oral administration than the topical administration of tincture of *Calendula* flowers and leaves (21).

From microbiological perspective, successful herbal venous leg ulcer therapy is dependent on the hosts' ability to maintain control over the microorganisms on venous leg ulcer tissue. Results of found aerobic and anaerobic bacteria in our study in both groups are similar with those in the literature.

The published reports indicate that bacterial colonization of leg ulcers is universal and that many colonizing microbes are potentially pathogenic in causing local or systemic infection. The infection is often polymicrobial, where isolates range from two to six per specimen. Although the exact pathogenic role of all bacterial isolates may be vague, the synergistic interaction in polymicrobial infections has been extensively studied (6,22-25). Organisms such as *Staphylococci* or *Streptococci* are difficult to eradicate without systemic antibiotics (21,22). The results from our study showed that in patients those species could not be adequately removed with the DermaplantG treatments.

Topical antimicrobial agents are essential in wound care management. However, their use has also been associated with cytotoxicity, delayed healing, the emergence of bacterial strains resistant to common antimicrobial agents, and the appearance of contact dermatitis (21,25).

Conclusion

The results of this pilot study demonstrate antimicrobiologic effects of DermaplantG herbal therapy on non-infected venous leg ulcer. These are preliminary observations raise the need for appropriately designed clinical studies which should demonstrate and evaluate the inherent properties of herbal preparations desirable to achieve the venous leg ulcers healing.

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ANTIMIKROBNI EFEKTI NOVIJIH ANTISEPTIČNIH PREPARATA NA BAZI LEKOVITOG BILJA NA VENSKE ULKUSE NOGU

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Venske ulceracije nogu predstavljaju značajan problem zdravstvene zaštite koji se stalno uvećava sa povećanjem broja starijih bolesnika. Za lečenje venskih ulkusa nogu dostupan je veliki broj terapijskih modaliteta kao što su masti i sredstva za previjanje. Brojni ekstrakti lekovitih biljaka i preparati na njihovoj bazi potencijalno indukuju epitelizaciju rana, koja se bazira na osobinama da deluju antimikrobno, antifungicidno i adstringentno.

Cilj studije bio je da se utvrde in-vivo antimikrobni efekti DermpalantG hidrogela na bazi lekovitog bilja, u čiji sastav ulazi Allii bulbosus, Hyperici herba e ekstrakt Calendulae flos. Ukupno 12 bolesnika sa neinficiranim venskim ulkusima potkolenice tretirani su dva puta dnevno, pet nedelja, novom formulacijom u obliku hidrogela. Sve ulceracije su pokazivale kliničke znake bakterijske kontaminacije ili kolonizacije ali bez znakova sistemske infekcije. Površina ulkusa prethodno je očišćena i prebrisana standardnim štapićem koji je natopljen fiziološkim rastvorom i ostavljena da bude vlažna. Vrh brisa je okretan ka unutrašnjoj strani sa zig-zag kratkim pomeranjem, pri čemu je načinjena najmanje jedna puna rotacija oko ose brisa. Korišćene su standardne metode za identifikaciju aerobnih i anaerobnih bakterija.

Na početku ispitivanja identifikovan je veliki broj različitih tipova bakterija na svim venskim ulkusima. *S. aureus* i *P. aeruginosa* izolovani su na svim kontrolnim pregledima. Na početnom ispitivanju, mešovita bakterijska flora (50%) izolovana je kod 6 venskih ulkusa (5 ulkusa sa *S. aureus*-*P. aeruginosa* i 1 ulkus sa *E.coli*-*Enterobacter spp.*-*P.aeruginosa*). Na kraju tretmana DermaplantG grupe kod 8 venskih ulkusa determinisan je *S. aureus* (66.66%) i *P. aeruginosa* (16.66%), a jedan venski ulkus je determinisan sterilnim (8.33%). Broj različitih tipova izolovanih bakterija značajno je smanjen ($P < 0.05$) posle korišćenja DermaplantG biljnog preparata. Terapija je sprovedena bez neželjenih efekata.

Preliminarni rezultati ove studije pokazuju potencijalne antimikrobne efekte biljnog preparata kod venskih ulceracija bez znakova infekcije. *Acta Medica Medianae* 2011; 50(3):40-44.

Ključne reči: venski ulkus potkolenice, mikrobiološka flora, DermaplantG hidrogel