

EFFECTS OF A TOPICAL PREPARATION CONTAINING *SAMBUCI FOLIUM* EXTRACT IN EXPERIMENTAL MODEL OF THERMAL SKIN BURNS ON RATS

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Abstract

The astringent, anti-inflammatory, antiseptic and cicatrizing effect of a cold-cream containing *Sambuci folium* soft extract was established in experimental model of thermal skin burns, in Wistar rats. Animals in the first group, treated with cold-cream containing 10% vegetal extract, had a favourable evolution, the wound healing being almost complete after 21 days. The reference group, treated with 1% silver sulfadiazine cream, and the control group, treated with cold-cream base, showed a delay of wound healing, the epithelisation after 21 days being incomplete.

Rezumat

Studiul a evidențiat efectul astringent, antiinflamator, antiseptic și cicatrizant al unui preparat de tip *cold-cremă* cu extract moale din *Sambuci folium*, asupra regenerării tisulare într-un model experimental de arsură tegumentară, la șobolani rasa Wistar. Animalele din primul lot, tratate cu *cold-cremă* cu 10% extract vegetal, au avut o evoluție favorabilă a plăgii, vindecându-se aproape complet după 21 de zile. Loturile tratate cu cremă cu 1% sulfadiazină argentică (lotul martor) și, respectiv, cu bază de unguent (lotul control) au prezentat o întârziere în vindecarea plăgii arse, după 21 de zile epitelizarea fiind incompletă.

Keywords: *Sambuci folium*; cold-cream; skin burns.

Introduction

Sambucus nigra L. species, Elder, *Caprifoliaceae* family, is native to most of Europe, northwest Africa and southwest Asia, in wet and dry fertile soils, in sunny locations, forests, riversides [1, 6].

In the specialty papers, there are incomplete data on the chemical composition of Elder leaves. From a phytochemical point of view, *S. nigra* species contain several compounds: cyanide glycosides (sambunigrin), flavonosides (rutoside), anthocyanins (in fruits), saponins, catechic tannin, mucilages, volatile oil (in flowers), polyphenolic acids, sterols, lectins, sugars, organic acids, vitamins, lipids, amino acids, mineral salts [3].

In the Romanian ethno pharmacology, for the treatment of abscesses and furunculosis, Elder leaves are used externally in the fresh state or in infusions [4].

Millions of cases of skin burns are recorded around the world and it is estimated that burns cause approximately 265 000 deaths per year. Taking into account the extent and depth of the lesions, the suffering is more or less severe but the complications of burns represent one of the most difficult pathologies [7, 9].

The aim of this study was to assess the astringent, anti-inflammatory, antiseptic and cicatrizing effects of a topical preparation containing 10% Elder leaves soft extract on the tissue regeneration in an experimental model of thermal skin burns, in Wistar rats.

Materials and Methods

Plant material

The leaves of *S. nigra* species were collected in May 2011 from the Botanical Garden of the University of Craiova, Dolj County, Romania. Voucher specimens are deposited in the Herbarium of the University of Medicine and Pharmacy of Craiova.

Reagents and solvents

All of the analytical grade reagents and solvents were Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland) products.

Preparation of tincture and soft extract

Sambuci folium tincture was obtained by percolation, according to the Romanian Pharmacopoeia Xth edition [16], in a 1:5 ratio of vegetal product–extraction solvent (70° ethanol). Until use, 20% tincture was filtered and stored in dark bottles, at 10–12°C. Then, the tincture was evaporated slowly until the consistency of a soft extract, at 50–60°C, under reduced pressure, using a Heidolph rotary evaporator.

Physico-chemical analysis

The tincture is a colourful clear liquid. The soft extract is viscous, semi-solid, coloured, with uniform appearance, soluble in 70° ethanol (solvent used for extraction) [16]. Relative density and refractive index of tincture were determined with a pycnometer and Abbé refractometer respectively, on the fourth decimal precision.

The quality conditions were established according to Romanian Pharmacopoeia Xth edition [16]: iron (up to 0.001%), heavy metals (up to 0.001%), and alcohol concentration and evaporation residue, for tincture; iron (up to 0.04%), heavy metals (up to 0.04%) and loss on drying, for the soft extract.

Using appropriate standards, flavonosides and their aglycones were separated and identified by thin-layer chromatography (TLC), according to specialty literature [15, 17].

According to Romanian Pharmacopoeia Xth edition [16], through the reaction with aluminium chloride and using rutoside as reference, the quantitative determination of flavonosides was made by spectrophotometric method.

Preparation of the cold-cream with 10% soft extract

After weighing, the soft extract was embedded in a cold-cream H/L-type ointment base, obtained according to United States Pharmacopoeia [17]. Initially solubilized in a small amount of 70° ethanol, the vegetal extract was emulsified in the ointment base, by continuous mixing, at room temperature.

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Experimental model

The study was performed on three groups of adult male Wistar rats, each of ten animals, weighing between 290 and 330 g. Both before and after the burns, the rats were kept in the animal facility of the University of Medicine and Pharmacy of Craiova, under standard conditions of light, temperature, food and water (*ad libitum*).

Tests were performed according to the European Council Directive No. 86/609/EEC, the European Convention on the Protection of Vertebrate Animals (2005), and the Government Ordinance No. 37/2.02.2002. The

Ethics Committee of the University of Medicine and Pharmacy of Craiova approved the experimental protocol.

General anaesthesia was induced by intramuscular injection of 85 mg/kgbw ketamine hydrochloride (Ketalar[®], Parke-Davis) and 6 mg/kgbw xylazine hydrochloride (Rompun[®], Bayer).

The hair was removed from the higher dorsal region of the Wistar rats and burns were inflicted on an area of about 1.5 cm², using a special stainless steel device, cone-shaped, weighing 350 g, with 1 cm diameter, and equipped with a control thermometer. The metallic device, heated in boiling water, to 100°C, was applied to each rat locally for five seconds [5, 10, 11, 13].

The creams were applied daily in the form of thin films and skin burns from the dorsal region were treated as follows: cold-cream with 10% *Sambuci folium* soft extract (SfE) for the first group; 1% silver sulfadiazine cream (SDA) for the second group (reference); cold-cream base (CCB) for the third group, considered as control.

The evolution of the skin burns was monitored daily, for three weeks. All animals had initially third degree skin burns, considering the macroscopic appearance of the lesions: epithelial and underneath connective tissue necrosis, until the muscle layer; about 4 mm perilesional oedema; intense erythema.

Histological study

In order to dynamically track the evolution of the wound healing process, burnt skin was sampled from each group of animals, under general anaesthesia, at intervals of 7, 14 and 21 days, with approximately 3 mm of perilesional area. Immediately after sampling, the burnt skin fragments were fixed in 10% buffered neutral formalin, for 72 hours, at room temperature, and then included in paraffin.

For the histological study, 4-µm thick serial sections were cut on a Microm HM350 rotary microtome equipped with a waterfall based section transfer system (STS, Microm). Routinely stained with Hematoxylin–Eosin (HE) and Masson's trichromic, sections were assessed under the light microscope and photographed with a Nikon Eclipse 90i microscope (Apidrag, Romania), equipped with a QImaging Rolera cooled CCD camera. Images were captured and archived using the Image ProPlus 7 AMS software (Media Cybernetics Inc, Buckinghamshire, UK).

Statistical analysis

Statistical evaluation of the experimental results was performed using Student's *t*-test. Differences were considered statistically significant for $p < 0.05$.

Results and Discussion

The results of the physico-chemical analysis of *Sambuci folium* tincture and soft extract are shown below (Table I).

TLC analysis performed on *Sambuci folium* tincture and soft extract has revealed the presence of rutoside, luteol-7-glucoside, polyphenolic acids and coumarins.

Table I
Physico-chemical analysis of *Sambuci folium* tincture and soft extract, according to Romanian Pharmacopoeia Xth edition

Physico-chemical characteristics	<i>Sambuci folium</i> tincture	<i>Sambuci folium</i> soft extract
Aspect	clear liquid	semi-solid, viscous
Colour	yellowish green	dark brown
Smell	typically, slightly aromatic	typically, slightly aromatic
Taste	slightly bitter, burning	slightly bitter
Relative density	0.9547	–
Refractive index	1.3652	–
Iron [%]	<0.001	<0.04
Heavy metals [%]	<0.001	<0.04
Alcohol content [%]	68.5	–
Dry residue [%]	5.65	–
Loss on drying [%]	–	12.35
Qualitative analysis (TLC)	flavonosides	flavonosides
Quantitative spectrophotometric analysis	5.15 mg rutoside / 100 mL tincture	21.85 mg rutoside / 100 g soft extract

Macroscopic assessment

An immediately noticeable white-gray area of coagulation necrosis occurs due to application on the rats' skin of a metallic device heated to 100°C, for five seconds. In the following days, the light brown-blackish wound has raised edges surrounded by a reddish zone of hyperaemia and oedema.

After the application of creams, the evolution of burnt skin wounds was variable, as follows (Figure 1):

- rats treated with cold-cream containing 10% *Sambuci folium* soft extract (SfE group) had a favourable evolution, with a good epithelisation, the wound healing being almost complete for 21 days;

- compared with SfE group, the second group (SDA), treated with 1% silver sulfadiazine cream, and the control group (CCB), treated with cold-cream base, showed a delay of wound healing, having an incomplete epithelisation after 21 days.

Microscopic assessment

Seven days after the burn, a thick coagulation necrotic area was evidenced at the surface of skin wound, composed mainly of deformed collagen fibres with variable tinctoriality. An inflammatory infiltrate zone of polymorphonuclear neutrophils separates the collagen fibres from the viable area. Remnants of pilosebaceous follicles with marked signs of degeneration were also highlighted.

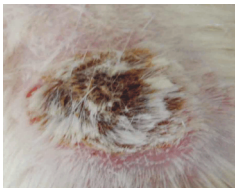
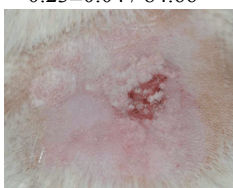
Day	SfE	SDA	CCB
	<i>Burnt skin wound area (mean±SD) [cm²] / Wound healing rate [%]</i>		
	1.34±0.16 / 10.66**	1.42±0.15 / 5.33**	1.45±0.17 / 3.33**
7			
	0.65±0.09 / 56.66**	0.74±0.09 / 50.66**	0.81±0.11 / 46**
14			
	0.23±0.04 / 84.66*	0.35±0.03 / 76.66*	0.48±0.05 / 68*
21			

Figure 1

Evolution of burnt skin wounds, after the application of creams: SfE – cold-cream with 10% *Sambuci folium* soft extract; SDA – 1% silver sulfadiazine cream (reference group); CCB – cold-cream base (control group); SD – standard deviation; * – $p < 0.01$; ** – $p < 0.05$

The inflammatory reaction was much lower and likewise the post-combustion oedema was much reduced for SfE group (Figure 2, a and b).

The presence of vacuoles of oedema placed between necrotic area and the muscular layer predominated in the microscopic lesions for SDA group (Figure 2, c and d).

Both in extent and in terms of number of polymorphonuclear cells, the inflammatory reaction was much stronger for CCB group compared with SfE or SDA groups (Figure 2, e and f).

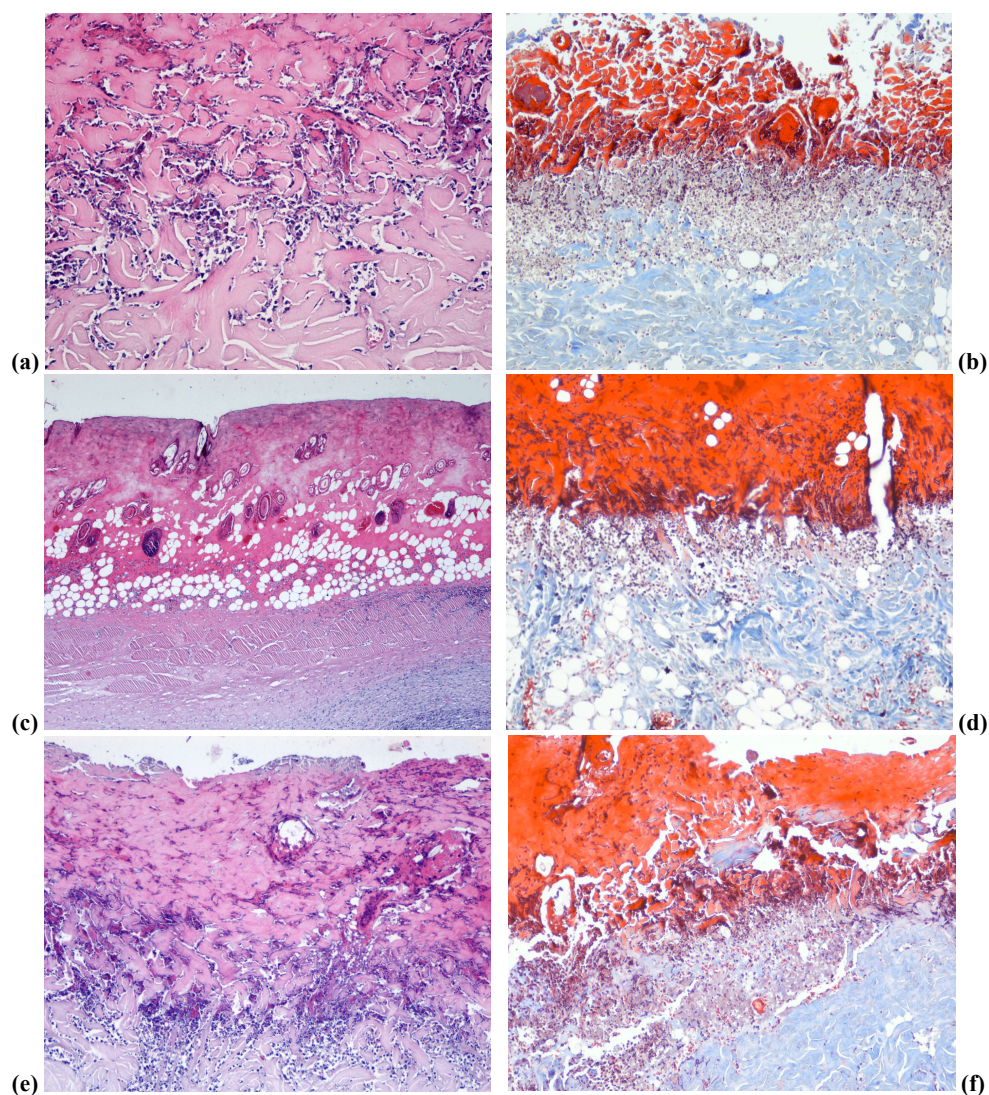


Figure 2

Microscopic evolution of burnt skin wounds, seven days after the application of creams: (a, b) cold-cream with 10% *Sambuci folium* soft extract; (c, d) 1% silver sulfadiazine cream – reference group; (e, f) cold-cream base – control group. Hematoxylin–Eosin (HE) stain: (a) and (e) $\times 100$; (c) $\times 40$. Masson's trichromic stain: (b), (d) and (f) $\times 100$

Fourteen days after the injury, both at the site of necrosis and into the underlying viable conjunctive tissue, the skin wounds for SfE group showed a thinned coagulation necrotic area with many cells of the immune system.

The boundary between necrotic area and viable tissue was marked by a thin band of polymorphonuclear neutrophils and lymphocytes. A rich vascular network of angiogenesis was found into the depth of the wound (Figure 3, a and b).

The coagulation necrotic area remained fairly thick for SDA group. At the level of coagulation area and into the underlying conjunctive tissue, the vacuoles of oedema were revealed. The limit of necrotic area and viable conjunctive tissue was highlighted by a thick belt of polymorphonuclear neutrophils and lymphocytes (Figure 3, c and d).

For CCB group, the area of coagulation necrosis has more abundant and extensive inflammatory infiltrate. Comparing with SfE or SDA groups, small numbers of neoformation vessels were observed (Figure 3, e and f).

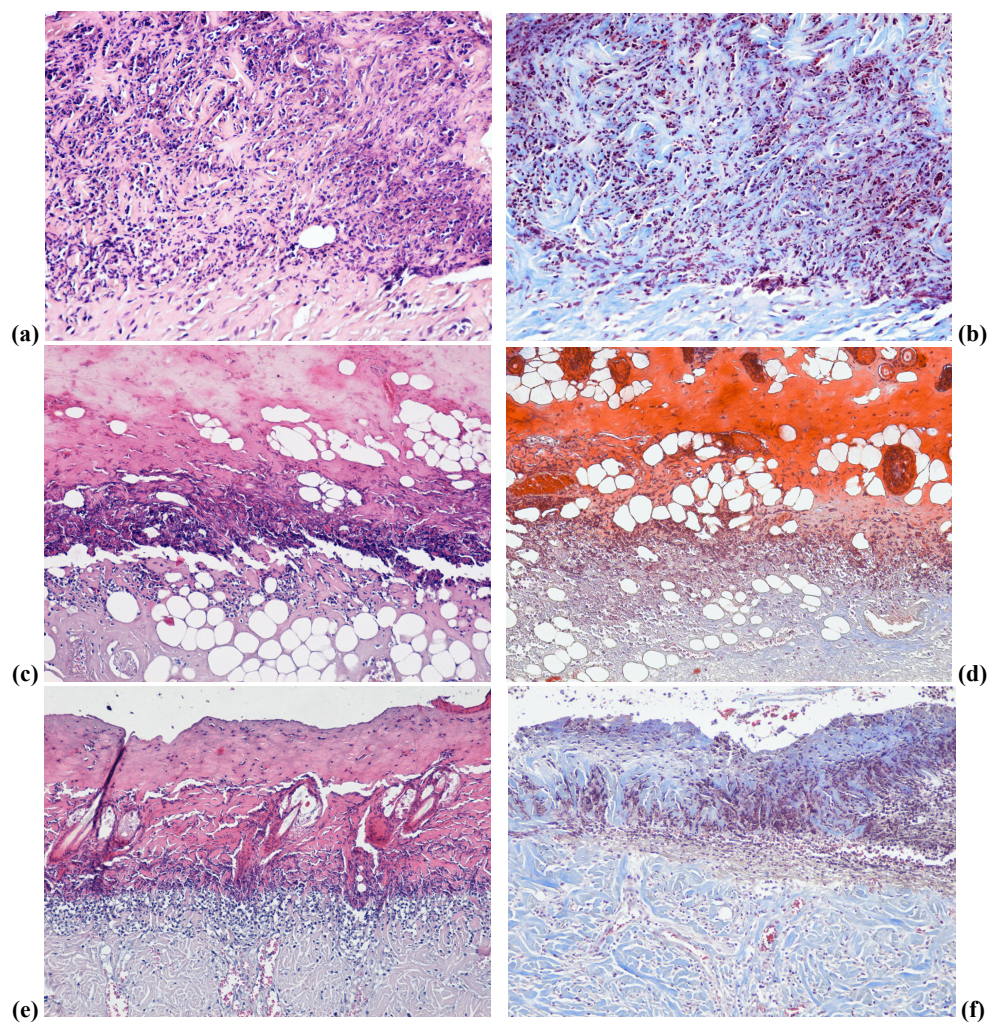
For SfE group, at *21 days* after the burn, the inflammatory infiltrate was much reduced, the granulation tissue was largely well recovered, and the re-epithelization of skin wound was well expressed. The neoformation vascular network showed a remodelling process highlighted by mature-type blood vessels (Figure 4, a and b).

An abundant inflammatory infiltrate, under the coagulation necrotic area, was observed for SDA group. The granulation tissue is not sufficiently developed. Compared with SfE group, the re-epithelization process of the burnt skin wound was reduced (Figure 4, c and d).

For CCB group it was noted the persistence of coagulation necrotic area and of abundant inflammatory infiltrate with difficult re-epithelization (Figure 4, e and f).

The content of flavonoids and tannins of the cold-cream with 10% *Sambuci folium* soft extract has a favourable effect on burnt skin wounds. Bioflavonoids have several main pharmacological actions: antioxidant, anti-inflammatory, epithelizing (wound healing) in burns, atonic and superficial wounds, vasculotropic and capillaroprotective (decreasing the permeability and increasing the resistance of the capillaries) [2, 8, 11, 12, 14].

Astringent, antiseptic, anti-inflammatory and epithelizing actions of tannins based on their ability to precipitate proteins, forming a film or membrane which tends to tighten. In addition, the bees wax from the composition of cold-cream has emollient, cicatrizing and biostimulative effect [11, 14].

**Figure 3**

Microscopic evolution of burnt skin wounds, 14 days after the application of creams: (a, b) cold-cream with 10% *Sambuci folium* soft extract; (c, d) 1% silver sulfadiazine cream – reference group; (e, f) cold-cream base – control group. Hematoxylin–Eosin (HE) stain: (a) $\times 200$; (c) and (e) $\times 100$. Masson's trichromic stain: (b) $\times 200$; (d) and (f) $\times 100$

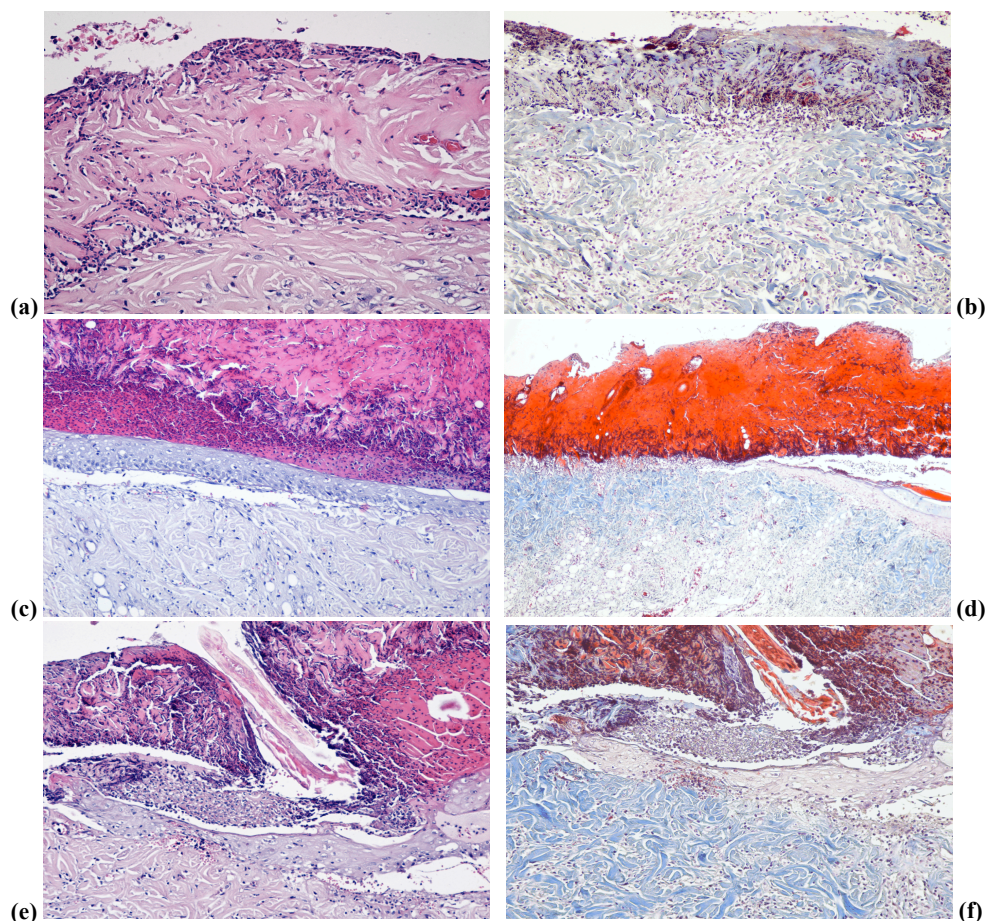


Figure 4

Microscopic evolution of burnt skin wounds, 21 days after the application of creams: (a, b) cold-cream with 10% *Sambuci folium* soft extract; (c, d) 1% silver sulfadiazine cream – reference group; (e, f) cold-cream base – control group. Hematoxylin–Eosin (HE) stain: (a), (c) and (e) $\times 100$. Masson's trichromic stain: (b) and (f) $\times 100$; (d) $\times 40$

Conclusions

Sambuci folium tincture and soft extract were obtained and physico-chemically characterized according to Romanian Pharmacopoeia. The 10% soft extract was embedded in a cold-cream type ointment base, prepared according to United States Pharmacopoeia.

Astringent, anti-inflammatory, antiseptic and cicatrizing action of the cold-cream containing 10% *Sambuci folium* soft extract, in experimental model of thermal skin burns, is mainly due to the content of flavonoids and

tannins of the natural product. In addition, cold-cream with vegetal extract promotes more rapid the appearance of neoangiogenesis capillaries.

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References

1. Atkinson M.D., Atkinson E., *Sambucus nigra* L. *J. Ecology*, 2002; 90(5): 895–923.
2. Becić F., Mulabegović N., Mornjaković Z., Kapić E., Prasović S., Becić E., Kusturica J., Topical treatment of standardised burns with herbal remedies in model rats. *Bosn. J. Basic Med. Sci.*, 2005; 5(4): 50–57.
3. Bruneton J., Pharmacognosie, Phytochimie, Plantes médicinales, 4e édition, revue et augmentée, Lavoisier Tec & Doc, Paris, 2009; 436–438.
4. Butură V., Enciclopedie de etnobotanică românească, Ed. Științifică și Enciclopedică, București, 1979; 219.
5. Busuioc C.J., Popescu F.C., Mogoșanu G.D., Lascăr I., Pirici I., Pop O.T., Mogoantă L., Angiogenesis assessment in experimental third degree skin burns: a histological and immunohistochemical study. *Rom. J. Morphol. Embryol.*, 2011; 52(3): 887–895.
6. Ciocârlan V., Flora ilustrată a României. *Pteridophyta et Spermatophyta*, Ed. Ceres, București, 2000; 748.
7. Forjuoh S.N., Burns in low- and middle-income countries: a review of available literature on descriptive epidemiology. Risk factors, treatment, and prevention. *Burns*, 2006; 32(5): 529–537.
8. Han M.C., Durmus A.S., Karabulut E., Yaman I., Effects of Turkish propolis and silver sulfadiazine on burn wound healing in rats. *Revue Méd. Vét.*, 2005, 156(12): 624–627.
9. Jeschke M.G., Finnerty C.C., Suman O.E., Kulp G., Mlcak R.P., Herndon D.N., The effect of oxandrolone on the endocrinologic, inflammatory, and hypermetabolic responses during the acute phase postburn, *Ann. Surg.*, 2007; 246(3): 351–360; discussion 360–362.
10. Mihele D., Manolescu B., Mladin P., Mihele D., Oprea E., The cicatrizing action of a new topical product based on *Ribes nigrum* essential oil. *Farmacia*, 2010; 58(4): 465–470.
11. Mogoșanu G.D., Popescu F.C., Busuioc C.J., Pop O.T., Pârvănescu H., Lascăr I., Mogoantă L., The effect of a topical treatment based on *Sambuci flos* extract in experimental thermal third degree skin burns. *Studia Universitatis „Vasile Goldiș”, Seria Științele Vieții (Life Sciences Series)*, 2011; 21(4): 701–708.
12. Moldovan M., Ciorrea L., Efficacy evaluation of different cream formulations on healthy skin properties. *Farmacia*, 2010; 58(6): 787–794.
13. Popescu F.C., Busuioc C.J., Mogoșanu G.D., Pop O.T., Pârvănescu H., Lascăr I., Nicolae C.I., Mogoantă L., Pericytes and myofibroblasts reaction in experimental thermal third degree skin burns. *Rom. J. Morphol. Embryol.*, 2011; 52(3 Suppl): 1011–1017.
14. Sântar I.P., Akkol E.K., Yalçın F.N., Koca U., Keleş H., Yesilada E., Wound healing potential of *Sambucus ebulus* L. leaves and isolation of an active component, quercetin 3-O-glucoside. *J. Ethnopharmacol.*, 2010; 129(1): 106–114.
15. Wagner H., Bladt S., Plant drug analysis. A thin layer chromatography atlas, 2nd edition, Springer Verlag, Berlin–Heidelberg, 1996; 125–146, 195–236.
16. ***, Farmacopeea Română ediția a X-a. Ed. Medicală, București, 1993; 419–421, 921, 922.
17. ***, United States Pharmacopeia–National Formulary: USP 34–NF 29, U.S. Pharmacopeial Convention, <http://www.usp.org/usp-nf/development-process/policies-guidelines/usp-nf-general-notice>, 2011.