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Comparative Studies on the Antioxidant, Antifungal, and Wound Healing Activities of *Solenostemma arghel* Ethyl Acetate and Methanolic Extracts

Fatma F. Abdel-Motaal [1,2,*], Zainab M. Maher [3], Samah F. Ibrahim [4], Amany El-Mleeh [5], Maged Behery [6] and Asmaa A. Metwally [7,*]

1 Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt
2 Unit of Environmental Studies and Development, Aswan University, Aswan 81528, Egypt
3 Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt; zainab.maher@vet.svu.edu.eg
4 Department of Clinical Sciences, College of Medicine, Princess Nourah Bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; sfibrahim@pnu.edu.sa
5 Department of Pharmacology, Faculty of Veterinary Medicine, Menoufia University, Shebin Elkoum 32514, Egypt; amany.ahmed1074@gmail.com
6 Department of Anatomy and Embryology, Faculty of Medicine, Benha University, Benha 13518, Egypt; maged.behery@fmed.bu.edu.eg
7 Department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, Egypt
* Correspondence: fatma.fakhry@aswu.edu.eg (F.F.A.-M.); asmaaabdelsalam104@yahoo.com (A.A.M.)

Abstract: Various herbal compounds are used for medical purposes due to their safety, as there are no or minimal side effects. This study was performed to assess the wound healing and antioxidant activities of ethyl acetate (EtOAc) and methanolic extract (MeoH) of *Solenostemma arghel* (*S. arghel*). Their antifungal activities were also evaluated against isolated swabs of equine wounds. They underwent GC-MS analysis for the characterization of both extracts. For wound healing evaluation, forty-five male albino rats were divided into three groups; the control group was treated with normal saline, and the other two groups were treated with *S. arghel* EtOAc and MeoH extract gels, respectively. The wounds were examined clinicopathologically and immunohistochemistry on the 3rd, 7th, and 14th days post-wounding. GC-Ms analysis of *S. arghel* recorded fifty-one volatile organic compounds (VOCs) within EtOAc extraction and thirty VOCs in MeoH extract. VOCs represented in EtOAc extract showed higher antioxidant activity and better and faster wound healing than VOCs of MeOH extract. The treated groups showed improved wound healing clinically and pathologically in comparison with the control group as they decreased the wound surface area (WSA) and percent (WSA%) and increased the wound contraction percent (WC%), epithelization, fibroblast proliferation with neovascularization, and reduced the inflammatory reaction. Moreover, the treated groups showed higher expression of vascular endothelial growth factor (VEGF) compared with the control. The EtOAc extract showed higher antifungal activity against *Penicillium funiculosum*, *P. jensenii*, *M. cinctum*, and *Candida albicans*, which were isolated from infected clinical equine wounds, than MeOH extract. The treated groups showed improved wound healing clinically and pathologically in comparison with the control group as they decreased the wound surface area (WSA) and percent (WSA%) and increased the wound contraction percent (WC%), epithelization, fibroblast proliferation with neovascularization, and reduced the inflammatory reaction. Moreover, the treated groups showed higher expression of vascular endothelial growth factor (VEGF) compared with the control. Additionally, the two extract gels showed promising healing of equine wounds. In conclusion, the study recommended the use of *S. arghel* EtOAc extract as it was proven to promote wound healing compared with MeoH extract.

Keywords: wound healing; *Solenostemma arghel*; antifungal; secondary metabolites; medical fungi

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1. Introduction

The wound is the disruption of cellular and anatomical continuity of living tissue produced by physical, chemical, electrical, or microbial insults to the tissue [1]. The subcutaneous tissue loses its integrity when wounded and colonized by microbes. These microorganisms involve potential pathogens, which interrupt the wound healing process in the injured tissues [2,3]. Wound infection is a major factor in the progression of chronicity, delaying wound healing [4].

The development of wound infection is an ongoing problem for many patients. Infected wounds can cause great distress in terms of associated morbidity and mortality, increased length of hospital admission, delayed wound healing, and increased discomfort, and have long been known to increase healthcare costs significantly, as the exudate levels are relatively higher in infected wounds. Pus and odor may indicate that the patient is pyrexial or has an increased neutrophil count [5].

Most chronic wound microbiome research is focused on bacteria, but contamination of the wound by fungi needs more elucidation. Ref. [6] classified seventeen fungal species from different wounds, which obstruct the healing of these wounds.

The yeasts of the genus *Candida* were the most numerous fungus. In particular, the high relative abundance of fungus within the polymicrobial ecology of wound infections, when present, shows that the fungus can be a major contributor to the biofilm of wounds. In addition, the diversity of fungal genera and species found in and among such wounds is more than previously thought or recorded [7]. The vulnerability to fungal wound infection is also increasing in geographical locations with a warm and humid climates [8].

The link between microbial aggregates and wound healing has been studied using a number of animal models. In order to imitate wound healing, most research used small rodents. However, the architecture of these species' skin and healing patterns differ significantly from those of humans [9]. Horses have thick, tight skin, similar to humans [10]. In humans and horses, wound edge contraction has a minor role (20–25%) in extremity/limb healing, in contrast to the situation in loose-skinned animals such as rodents [11].

Horses appear to be valid model animals for wound healing in humans because of their similar skin architecture and the relative role of contraction and epithelialization in healing [12,13]. Because of their surroundings, horses' wounds have a significant risk of infection, as the infected wounds house a variety of microorganisms. However, these germs can be challenging to identify and not react to antibiotic treatment, resulting in chronic non-healing wounds [14]. Therefore, in the study, we selected some cases of equine wounds for clinical application of the gel and to find a resolution for the problem of equine wounds through the investigation of new wound-healing agents.

Traditional medicine is widespread throughout the world, using natural medicinal plants. *Solenostemma arghel* is a desert medicinal plant indigenous to African countries (Egypt, Sudan, Algeria, Libya, and Chad) [15], which is considered folk medicine. African people use this plant as an anti-inflammatory, anti-rheumatic, antispasmodic, anti-diabetic, anti-nutrition, antimicrobial, and anticancer agent [16–19]. The medical effect of different parts of this plant comes especially from leaves because of phytochemicals richness, including various phenolic acids, flavones, glycosylated flavonoids, polyphenols, b-carotene, b-sitosterol, monoterpenes, pregnenes, and pregnane [17,20].

The present study was designed to compare the antifungal, antioxidant, and wound healing activities of EtOAc and MeoH *S. arghel* extracts in albino rats besides the clinical application of the extracts for investigation of wound healing in equines.

2. Materials and Methods

2.1. Chemical and Reagents

Methanol was purchased from (Lab-Scan Analytical Sciences, Gliwice, Poland). Ethyl acetate was purchased from ADWIC (Cairo, Egypt). XylaMed® (xylazine HCL, 100 mg, Bimeda, Inc., Oakbrook Terrace, IL, USA). Ketamax® (ketamine HCL, 50 mg) was obtained

from (Troikaa Pharmaceuticals Ltd., Gujarat, India). Carbopol 940® was purchased from (Loba Chemie Company, Mumbai, India). All chemicals were of analytical grades.

2.2. Plant Materials

The vegetative part of *S. arghel* was collected from the extremely arid desert region of Southern Egypt, Aswan University campus, Aswan. The plant was washed with tap water, leaves and flowers were separated from the stem, air-dried at room temperature, followed by grinding into a powder with a grinder (mortar and pestle).

2.3. Preparation of Plant Extract

Powdered leaves and flowers (100 gm) were extracted with two organic solvents (EtOAc and MeoH (0.1 gm/mL)). Extraction was incubated overnight at room temperature and repeated using an ultrasonic homogenizer. The extracts were decanted, filtered, and the residue was re-extracted again under the same experimental conditions to ensure full extraction. The clear filtrates were air-dried using a rotary evaporator at 45 °C [21].

2.4. Collection and Preservation of Material from Injured Parts for Investigation of Yeast and Fungal Infections

By using sterile swabs, samples were collected from 12 equine species (a horse, a mare, 5 donkeys, and 5 female donkeys), which were admitted to the Brooke Hospital in Aswan, Egypt, suffered from traumatized wounds. Swabs were placed in a sterile cryotube and stored at −40 °C. These samples were soon cultured on Sabroud media for isolation of fungi and yeast, which were morphologically identified using a light microscope, and the isolated fungi were purified and kept in slants.

2.5. Antifungal Activity Assay

For screening of antifungal activity of Arghel plant extracts, a food technique was followed [22]. Sabroud medium was prepared and sterilized; then, the medium was supplemented with EtOAc and MeoH plant extracts at two concentrations (5 mg/mL and 10 mg/mL), mixed well, and kept at 4 °C overnight. The mycelial disc (0.5 cm in diameter) was deposited in the center of the plate (5.0 cm in diameter) according to the poisoned food method, which was used to evaluate the antifungal effect against pathogens [23]. After further incubation for 7 days at 28 ± 2 °C for the fungal strains tested, the diameters of fungal growth for control (fungus in media without any extraction and containing only solvent) and treated plates were measured with a calculation of the inhibition percentage [24].

2.6. DPPH Antioxidant Activity

The DPPH solution (0.5 mmol/L) was prepared in 95% methanol, and different concentrations (50, 100, and 150 µg) of Ethyl acetate and Methanol plant extracts were used. A total of 2 mL of DPPH solution (0.5 mnol/L) was added to (1 mL) of the test sample and incubated for 30 min at room temperature in darkness. After 30 min, the absorbance was measured at 517 nm. Ascorbic acid (AA) served as a positive control at a concentration of 10 µg. The percentage of the DPPH radical scavenging was calculated as:

$$\text{Inhibition of DPPH radical (\%)} = [(\text{control absorbance (Ai)} - \text{extract absorbance (At)})/(\text{control absorbance (Ai)})] \times 100$$

$$\text{Inhibition (\%)} = (\text{Ai} - \text{At})/\text{Ai} \times 100\%$$

2.7. GC-MS Analysis of Released VOCs from S. arghel EtOAc and MeoH Extracts

The extracted VOCs from the *S. arghel* were analyzed and identified by gas chromatography–mass spectrometry (GC/MS). The GC-MS analysis was carried out by using a TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp, Carlsbad, CA, USA), which connected with a Thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5 MS column (30 m × 0.32 mm

i.d., 0.25 µm film thickness). Analysis was achieved using helium gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 60 °C for 1 min; increasing 4.0 °C/min to 240 °C and held for 1 min. Both injector and detector were held at 210 °C, and diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450. The chemical constituents of the essential oil (Eos) identification was de-convoluted using (AMDIS version 2.70, http://www.amdis.net/, accessed on 11 April 2022) and identified by its retention indices (relative to n-alkanes C8–C22) mass spectrum matching to authentic standards (when available) or Wiley spectral library collection, and NSIT library database.

2.8. Preparation of S. arghel EtOAc and MeoH Extracts 2% Gels

The two gels were prepared according to [25]. In a mixture containing 100 mL D. W, 1.5 g of Carbopol 940® was added slowly and mixed well at a higher speed. Followed by the addition of extract until gel formation with 2% concentration.

2.9. Evaluation of In Vivo Wound Healing Activity

2.9.1. The Ethical Approval

All experimental procedures in this study were approved by the ethical committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt (VM-2022-0030). All animals were euthanized at the experimental end. The clinical study on equine was carried out according to the ethics of the Brooke Hospital in Aswan, Egypt.

2.9.2. Animals

Forty-five (45) male albino rats aged 2–3 months and with 150–200 g body weight were purchased from Abou-Rawah farm, Egypt. Rats were housed in plastic cages with balanced diet ad libitum, with clean water readily accessible to the caged rats in clean bottles under constant environmental conditions (temperature of 25 °C, normal daylight, and relative humidity of 45–55%). The rats were kept a week before the experiment for Acclimatization.

2.9.3. Excisional Wound Model

After the adaptive period, the rats were anesthetized with an intraperitoneal (IP) injection of ketamine (70 mg/kg) and xylazine (7 mg/kg) combination, and the hair on the back was shaved and disinfected with ethyl alcohol (70%). A 1.5 cm wound was created with the aid of toothed tissue forceps, surgical blades, and pointed scissors to keep the skin tension constant during wounding. The hemostasis was achieved by compression with sterile gauze, and wounds were left untreated for 24 h. After wound creation, the rats were housed individually, each rat in a separate cage. The wound site was cleaned with a tampon soaked in alcohol. The rats were randomized into 3 groups (15 rats each). The excisional cutaneous wounds of the control group (Group I) were treated topically with 0.9% normal saline once daily, and the excisional wounds of the other two groups (Group II, Group III) were treated topically with *S. arghel* EtOAc and MeoH extracts 2% gels, respectively. The two herbal formulations were applied to the wounds once daily for 14 days starting from the second day of wounding.

2.9.4. Assessment of Wound Diameter

The wound size was measured and photographed on 0, 3, 7, and 14 days post-treatment for visual comparison before and after clearance of the wound area. The wound surface area (WSA), wound surface area percent (WSA%), and wound contraction percent (WC%) were calculated according to the following formulae:

$$WSA = \pi r^2 \ (cm^2).$$

$$WSA\% = \frac{WSA \times 100}{1.8}\%$$

$$WC\% = 100\% - WSA\%$$

2.9.5. Histopathology

On the 3rd, 7th, and 14th days: 5 rats from each group were euthanized, and the wounds with part of normal skin were preserved in 10% neutral buffered formalin. The tissues were subjected to different concentrations of ethyl alcohol (70–80–90–100%), embedded in paraffin wax at 40–60 °C, sectioned into 5 µm thickness, and stained with hematoxylin–eosin stain [26].

2.9.6. Immunohistochemistry

Immunohistochemistry assessment was performed according to [27]. Retrieval of antigen was performed by de-waxing and immersion of skin sections in a citrate buffer solution (0.05 M and pH 6.8). These sections were then treated with H_2O_2 (0.3%) and protein block. They were then incubated with polyclonal rabbit VEGF (BioGenix, 49026 Milmont Drive, Fremont, CA 94538 Emergo Europe, Molenstraat 15, NL-2513 BH The Hague, The Netherlands, cat. No. AR483-5R, dilution 1/100). The slides were washed up with PBS and then incubated with a goat anti-rabbit secondary antibody (cat. no. K4003, EnVision+™ System Horseradish Peroxidase Labelled Polymer; Dako, Glostrup, Denmark) for 30 min at room temperature. Eventually, slides were visualized with a DAB kit and stained with Mayer's hematoxylin as a counterstain was evaluated under the light microscope. The staining intensity was assessed and reported as a percentage of positive cells in approximately 8 high power fields.

2.9.7. Clinical Study

The two prepared gels were topically applied on 5 equines (3 horses and 2 donkeys) that suffered from recent and old, traumatized contaminated wounds; some wounds were treated before without any tendency to wound healing.

2.9.8. Treatment Procedures

Before the gel application, the wound area was prepared according to the type of wound (old or recent); in a recent wound, hemorrhages were controlled by packing, and the wound area was aseptically prepared. In an old wound, unhealthy tissues were surgically debrided and freshened, and hemorrhages were controlled by packing.

2.10. Statistical Analysis

All statistical analyses were carried out by computer software (SPSS version 16.0, Chicago, IL, USA) using One-Way Analysis of Variance (ANOVA), and the data are presented as mean ± standard deviation (SD) values. The differences between mean values were considered significant at $p < 0.05$. The graphs were performed using Microsoft Excel 2010.3.

3. Results

3.1. GC-Ms Analysis of VOCs Produced by S. arghel in EtOAc and MeoH Extractions

Fifty-one VOCs were recorded in GC-Ms analysis of *S. arghel* EtOAc extraction (Table 1 and Figure 1a) while only thirty VOCs were recognized in plant MeoH extract (Table 2 and Figure 1b). The most abundant compounds in EtOAc extract (Table 1) were represented by Vitamin E (alpha-tocopherol) (9.99%); Diisooctyl phthalate (9.32%); Methyl hexadecadienoate and 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9-(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b,9a-trihydroxy-1,1,6,8-tetramethyl-[1ar-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-(7.36% each). Among the eight minor components of EtOAc extract, the most notable were 8-Dodecen-1-ol-acetate (Z)-; Undec-10-ynoic acid-heptadecylester; Undec-10-ynoic acid-octadecyl ester (0.62% each); Linoleoyl chloride; Digitoxin and Oxiranepentanoic acid-3-undecyl-, methyl ester, trans, (0.85% each) (Table 1).

Table 1. GC-MS analysis of VOCs produced by *S. arghel* leaves extracted by Ethyl acetate.

NO	VOCs Compounds	Molecular Formula	Rt	MW	Area%
1	17-Octadecynoic acid	$C_{18}H_{32}O_2$	4.44	280	3.11
2	2,2-dimethyl-5-(3-methyloxiranyl)-cyclohexanone	$C_{11}H_{18}O_2$	4.44	182	3.11
3	Linoleoyl chloride	$C_{18}H_{31}ClO$	4.60	298	0.85
4	2,2,3,3,4,4 Exadeutero,octadecanal	$C_{18}H_{30}D_6O$	4.80	274	1.13
5	1-Dodecanol, 3,7,11-trimethyl	$C_{15}H_{32}O$	4.80	228	1.13
6	1,3,5-Triazine-2,4-diamine,6-chloro-n-ethyl-	$C_5H_8ClN_5$	9.31	173	1.49
7	1-Chlorooctadecane	$C_{18}H_{37}Cl$	9.31	288	1.49
8	2-Aminoethanethiol,hydrogen sulfate (Ester)	$C_2H_7NO_3S_2$	10.30	157	1.28
9	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	10.30	238	1.28
10	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	11.74	282	1.53
11	1-Dodecene	$C_{12}H_{24}$	11.74	168	1.53
12	3-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	11.74	282	1.53
13	Docosane	$C_{22}H_{46}$	13.87	310	1.60
14	Farnesene epoxide, E	$C_{15}H_{24}O$	14.26	220	1.49
15	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	14.26	290	1.49
16	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	14.36	206	2.91
17	9H-Fluorene	$C_{13}H_{10}$	15.65	166	4.94
18	(E,E)-1,3,5-Tridecatriene-7,9,11-triyne	$C_{13}H_{10}$	15.65	166	4.94
19	8-Dodecen-1-ol, acetate, (Z)-	$C_{14}H_{26}O_2$	15.81	226	0.62
20	Undec-10-ynoic acid, heptadecylester	$C_{28}H_{52}O_2$	15.81	420	0.62
21	Undec-10-ynoic acid, octadecyl ester	$C_{29}H_{54}O_2$	15.81	434	0.62
22	Bisabolol oxide B (2-Furanmethanol, tetrahydro-à,à,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl),[2S-[2à,5á(R)]]-	$C_{15}H_{26}O_2$	17.08	238	2.40
23	Acetic acid, 10,11-dihydroxy-3,7,11 trimethyl-dodeca-2,6-dienyl ester	$C_{17}H_{30}O_4$	17.08	298	2.40
24	2-Monooleoylglycerol trimethylsilyl ether	$C_{27}H_{56}O_4Si_2$	17.25	500	0.99
25	2,3-Bis[(trimethylsilyl)oxy]propyl (9z,12z)-9,12-octadecadienoate	$C_{27}H_{54}O_4Si_2$	17.25	498	0.99
26	Benzoic acid, 2,4-bis(trimethylsiloxy)-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	17.25	370	0.99
27	1-Tetradecanol	$C_{14}H_{30}O$	18.02	214	1.27
28	Loliolide,2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl	$C_{11}H_{16}O_3$	19.20	196	2.17
29	3′,4′,7-Trimethylquercetin	$C_{18}H_{16}O_7$	20.14	344	1.27
30	Neophytadiene	$C_{20}H_{38}$	20.36	278	5.88
31	3-Eicosyne	$C_{20}H_{38}$	20.36	278	5.88
32	Digitoxin	$C_{41}H_{64}O_{13}$	20.58	764	0.85
33	Oxiranepentanoic acid, 3-undecyl-, methyl ester, trans	$C_{19}H_{36}O_3$	20.58	312	0.85
34	01297107001 Tetraneurin—A-diol	$C_{15}H_{20}O_5$	20.62	280	0.89
35	1-Heptatriacotanol	$C_{37}H_{76}O$	20.62	538	0.89
36	Phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl	$C_9H_9F_3O_2$	20.87	206	1.34

Table 1. *Cont.*

NO	VOCs Compounds	Molecular Formula	Rt	MW	Area%
37	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	21.09	312	1.83
38	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	21.09	252	1.83
39	7,9-Di-tert-butyl-1-oxaspiro(4,5)dec,a-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	21.77	276	4.97
40	Isochiapin B	$C_{19}H_{22}O_6$	22.46	346	1.45
41	Isochiapin B %2<	$C_{19}H_{26}O_6$	22.46	350	1.45
42	6,8-Di-c-á-glucosylluteolin	$C_{27}H_{30}O_{16}$	22.73	610	1.37
43	2,2-Dideutero octadecanal	$C_{18}H_{34}D_2O$	24.39	270	1.31
44	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	24.39	254	1.31
45	Phytol	$C_{20}H_{40}O$	24.85	296	5.16
46	9,12,15-Octadecatrienoic acid, 2,3-bis [(trimethylsilyl) oxy]propyl ester, (Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	25.10	498	1.13
47	Ethyl iso-allocholate or Ethyl 3,7,12-trihydroxycholan-24-oate	$C_{26}H_{44}O_5$	27.26	436	1.21
48	Diisooctyl phthalate	$C_{24}H_{38}O_4$	30.97	390	9.32
49	Vitamin E (alpha-tocopherol)	$C_{29}H_{50}O_2$	32.02	430	9.99
50	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9-(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b,9a-trihydroxy-1,1,6,8-tetramethyl-,[1ar-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-	$C_{24}H_{32}O_8$	34.94	448	7.36
51	Methyl hexadecadienoate	$C_{17}H_{30}O2$	34.94	266	7.36

Table 2. GC-MS analysis of VOCs produced by *S. arghel* leaves extracted by methanol.

No.	VOCs Compounds	Formula	RT	MW	Area%
1	Heptane, 2,2,4,6,6-pentamethyl-	$C_{12}H_{26}$	4.43	170	4.083
2	Tetradecane, 2,2-dimethyl-	$C_{16}H_{34}$	4.43	226	4.083
3	Decane, 3,6-dimethyl-	$C_{12}H_{26}$	5.22	170	1.81
4	3-Ethyl-3-methylheptane	$C_{10}H_{22}$	5.22	142	1.81
5	Eicosane	$C_{20}H_{42}$	7.38	282	2.53
6	2-Bromotetradecane	$C_{14}H_{29}Br$	7.38	276	2.53
7	Hexadecane	$C_{16}H_{34}$	7.38	226	2.53
8	Dodecane	$C_{12}H_{26}$	7.733	170	1.883
9	Tetradecane	$C_{14}H_{30}$	7.733	198	1.883
10	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	7.733	268	1.883
11	Heneicosane	$C_{21}H_{44}$	9.090	296	10.310
12	Heptadecane	$C_{17}H_{36}$	9.090	240	10.310
13	Octadecane, 1-iodo-	$C_{18}H_{37}I$	9.090	380	10.310
14	Octadecane, 2-methyl-	$C_{19}H_{40}$	9.170	268	1.835
15	Hexacosane	$C_{26}H_{54}$	9.496	366	8.846
16	Heptadecane, 2-methyl-	$C_{18}H_{38}$	9.496	254	8.846
17	Heptadecane, 8-methyl-	$C_{18}H_{38}$	9.496	254	8.846
18	Pentacosane	$C_{25}H_{52}$	9.587	352	2.645

Table 2. *Cont.*

No.	VOCs Compounds	Formula	RT	MW	Area%
19	Triacontane	$C_{30}H_{62}$	9.587	422	2.645
20	Heptadecane, 9-octyl-	$C_{25}H_{52}$	9.685	352	2.395
21	Hentriacontane	$C_{31}H_{64}$	11.378	436	11.344
22	Sulfurous acid, hexyl octyl ester	$C_{14}H_{30}O_3S$	11.378	278	11.344
23	Heptacosane	$C_{27}H_{56}$	11.487	380	2.169
24	Octadecane	$C_{18}H_{38}$	11.939	254	8.357
25	Sulfurous acid, butyl heptadecyl ester	$C_{21}H_{44}O_3S$	12.271	376	2.020
26	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9diene-2,8-dione	$C_{17}H_{24}O_3$	14.571	276	5.491
27	1,3-Pentadiene, 1,1-diphenyl-, (Z)	$C_{17}H_{16}$	14.571	220	5.491
28	Dibutyl phthalate	$C_{16}H_2O_4$	15.241	278	10.14
29	Heneicosane, 3-methyl-	$C_{22}H_{46}$	19.092	310	2.024
30	2-methyloctacosane	$C_{29}H_{60}$	19.092	408	2.024

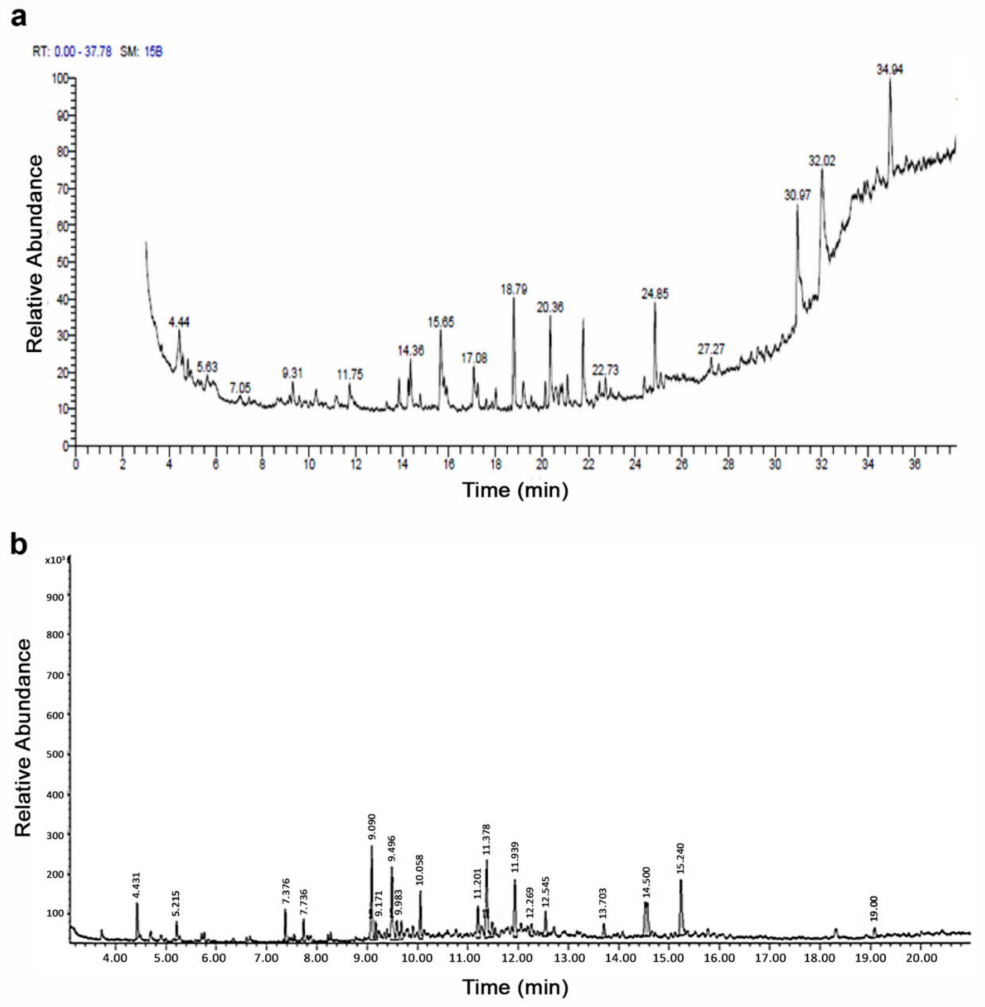

Figure 1. Gas chromatography-mass spectrometry (GC-MS) analysis charts of released volatile organic compounds emitted from *S. arghel* ethyl acetate (**a**) and methanol (**b**) extracts.

The GC–MS analysis of MeoH extract predominantly revealed the presence of the main constituents as Hentriacontane; Sulfurous acid-hexyl octyl ester (11.34% each);

Heneicosane; Heptadecane; Octadecane-1-iodo- (10.31% each) and Dibutyl phthalate (10.14%). While low VOCs percentage was recorded with Decane, 3,6-dimethyl-; 3-Ethyl-3-methylheptane (1.81% each); Octadecane, 2-methyl- (1.85%) and Dodecane; Tetradecane; 1-Iodo-2-methylnonane (1.88% each) (Table 2).

3.2. Antioxidant Activity of S. arghel

Both EtOAc and MeoH extracts showed recognized antioxidant activity. This activity increased with increasing the concentration of both extracts from 50 µg to 150 µg. While EtOAc extract showed higher antioxidant activity (19.21–58.23%) than methanol extract (13.71–44.82%) (Figure 2).

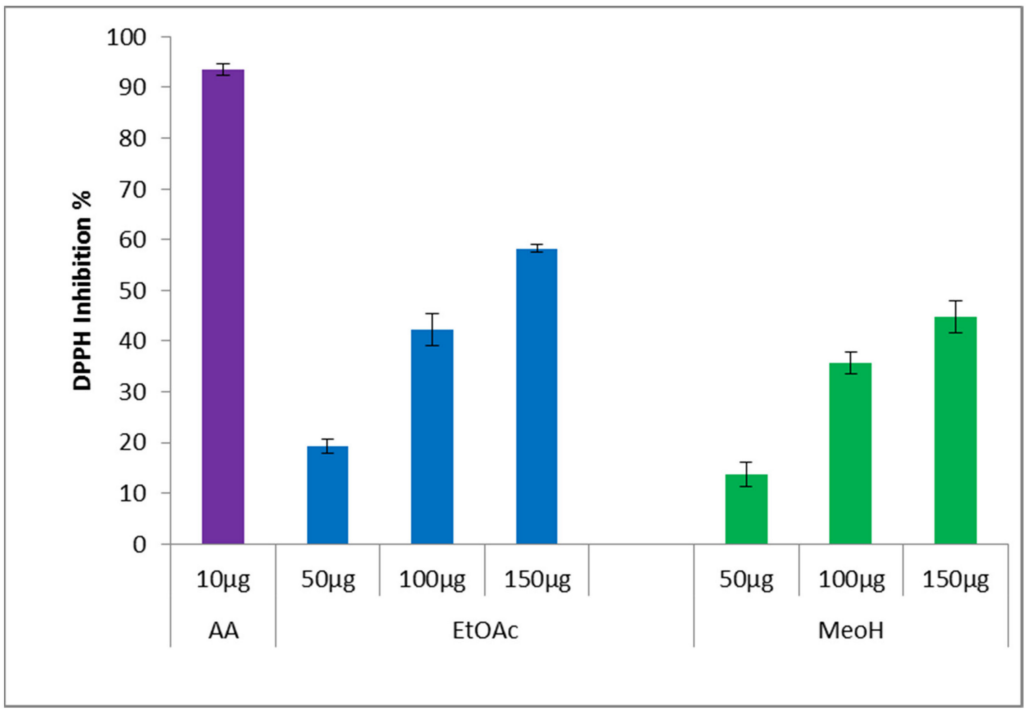

Figure 2. Antioxidant activity calculated by DPPH inhibition percentage of *S. arghel* ethyl acetate and methanol extracts at 50,100 and 150 µg compared with control (Asorbic acid, AA).

3.3. Contaminated Wounds by Fungi and Yeast

Exposed subcutaneous tissue provides a favorable substratum for fungi to contaminate and colonize the wounds. In this study, variable animals' wounds were contaminated by three fungal species and one species of yeast. These contaminants were identified as *P. funiculosum*, *P. jensenii*, *M. cinctum*, and *C. albicans*. The most abundant species in wounds was *C. albicans* (18.0 colonies), which was isolated from all wounds of horses (7.0 colonies), mare (2.0 colonies), donkeys (3.0 colonies), and female donkeys (6.0 colonies). *Myrothecium cinctum* (12.0 colonies) and *P. funiculosum* (8.0 colonies) colonized horse (5.0 colonies and 3.0 colonies), mare (one colony and 2.0 colonies), three donkeys (3.0 colonies and 1.0 colony) and four female donkeys (3.0 colonies and 2.0 colonies), respectively. While, *P. jensenii* (5.0 colonies) was isolated from a horse (one colony), two female donkeys, and two donkeys (two colonies each).

3.4. Antifungal Activity of S. arghel VOCs

Generally, VOCs represented in *S. arghel* EtOAc extract showed higher antifungal activity than MeOH extract (Figure 3a,b). All fungal species were inhibited by *S. arghel* secondary metabolites extracted by MeoH and EtOAc in both concentrations (5 mg and 10 mg/mL). *Penicillium funiculosum* was the most sensitive to both extracts, with concentrations 5 mg and 10 mg/mL, where the inhibition percentage was high and ranged between

54.05 and 61.35%. Interestingly, the reaction of *M. cinctum* to *S. arghel* differed from other studied fungi, where this fungus recorded a higher inhibition percentage with MeoH extract (44.54% and 52.23%) than EtOAc (29.15% and 48.18%) with 5 mg and 10 mg/mL, respectively. *Pencillium jensenii* and *C. albicans* showed clear inhibition with 10 mg EtOAc (37.14% and 33.57%, respectively), while MeoH extract in both concentrations showed less inhibition percentage.

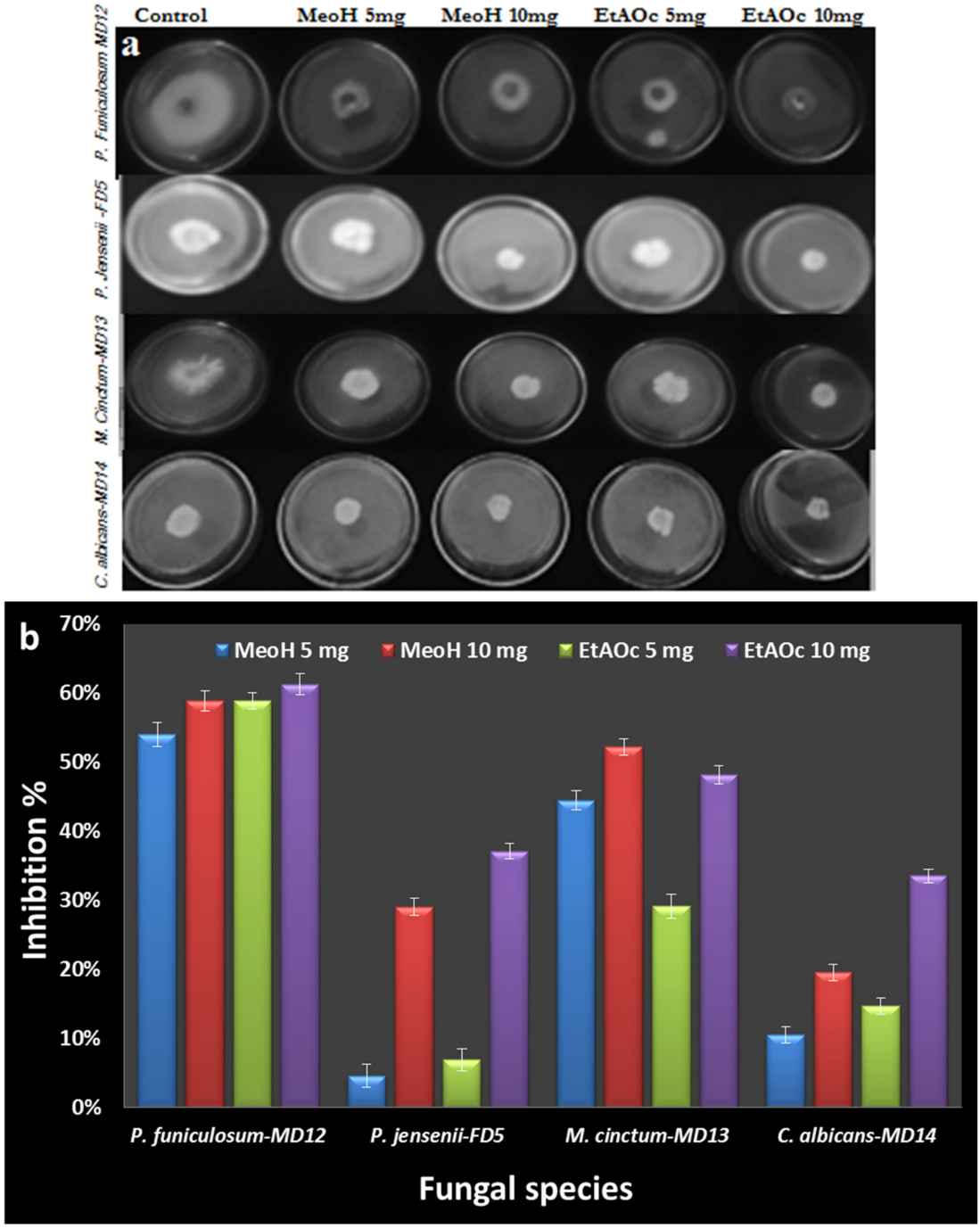

Figure 3. Screening of antifungal activity of Arghel plant extracts using food technique method. Inhibition of fungal growth in Sabroud medium supplemented with EtOAc and MeoH plant extracts at two concentrations (5 mg/mL and 10 mg/mL) compared with control (**a**). Histogram showed the inhibition percentage of pathogenic fungi isolated from contaminated wounds at 5 mg/mL and 10 mg/mL of both EtOAc and MeoH plant extracts (**b**).

3.5. Clinical Observations

On the clinical assessment of wounds (Figure 4), it was observed that on the day of wounding, the wound edges showed hyperemia, edema, and swelling of the tissues around the wound margins. On the 3rd day post wounding, wounds of GI showed yellowish exudate with shrinkage of the wounds, which was accompanied by hyperemia and swelling of the wound edges. While in GII, the wounds were covered with brownish scabs with shrinkage and significantly decreased wounds size compared with the control. Additionally, the wounds of GIII were non-significantly narrower than the wounds size of GI, with brownish scab covering the wound surface, which sloughed from some of them. On the 7th day post-treatment, the wounds of GI were covered with thick moist scabs with significantly less wound contraction in comparison with GII and GIII. While the wounds size of GII was non-significantly narrower than that of GIII. On the 14th day post-treatment, the wounds contraction of GII and GIII were significantly higher than GI. The wounds of GII showed complete healing with scab formation in comparison with the wound of GIII. Meanwhile, the wounds of GI showed incomplete healing.

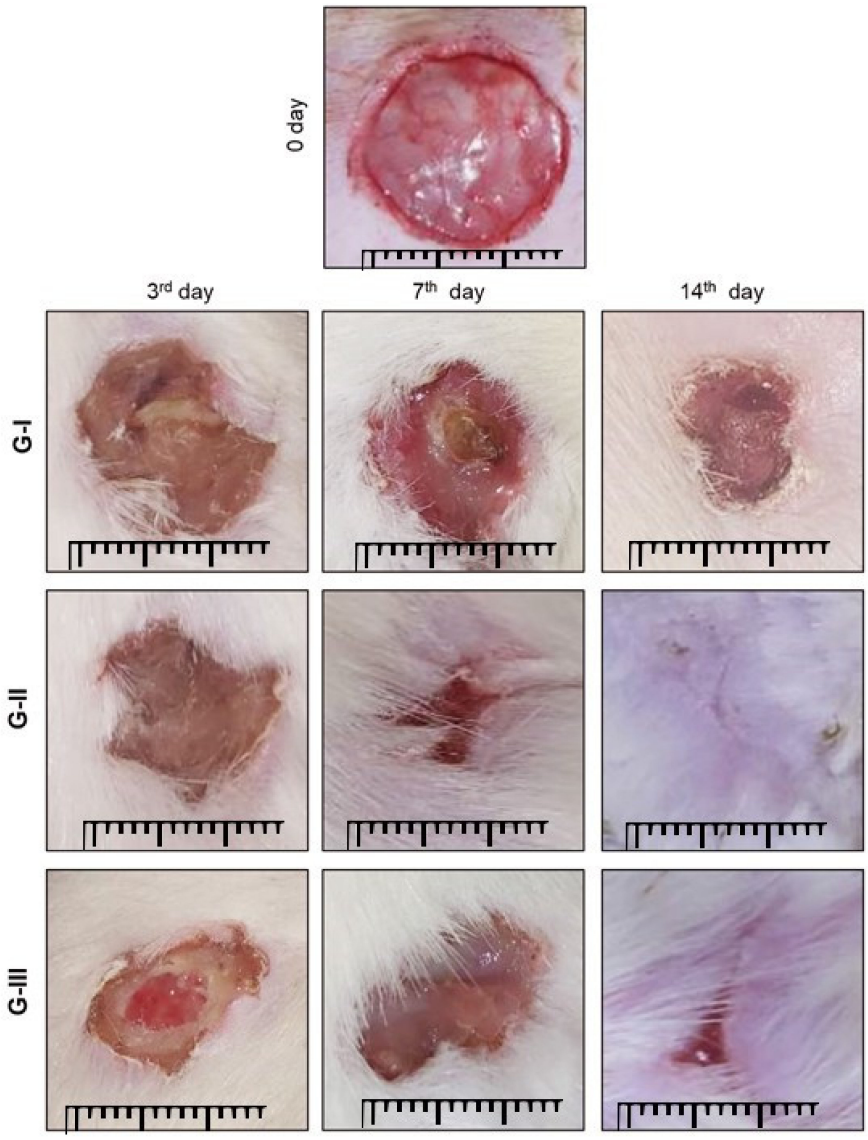

Figure 4. Photograph showing wound healing in GI, GII, and GIII at 3rd, 7th, and 14th days post-treatment, where the healing process accelerated in wounds treated with GI in comparison with GI and GIII groups.

The WSA and WSA% (Tables 3 and 4) on the 3rd and 7th day post-treatment significantly decreased in GII compared with GI, while the WC% (Table 5) significantly increased in GII compared with GI when p was $\leq$0.05. On the 14th day post-treatment, the WSA and WSA% significantly decreased in GII and GIII compared with GI; furthermore, the WC% significantly increased in GII and GIII compared with GI when p was $\leq$0.05.

Table 3. Mean + SD of WSA in full-thickness skin wounds in control and treated groups.

Time in Days	GI	GII	GIII
3	1.56 ± 0.336	0. 80 ± 0.27 [a]	0.97 ± 0.53
7	1.2 ± 0.35	0.47 ± 0.18 [a]	0.7 ± 0.3
14	0.12 ± 0.6	0.002 ± 0.004 [a]	0.02 ± 0.03 [a]

([a]): Significant changes when compared with control group when $p \leq 0.05$.

Table 4. Mean ± SD of WSA% in full-thickness skin wounds in control and treated groups.

Time in Days	GI	GII	GIII
3	86.7±18.7	44.6 ± 14.94 [a]	54 ± 29.4
7	66 ± 19.7	25.96 ± 9.8 [a]	39.4 ± 16.7
14	6.4 ± 3.2	0.08 ± 0.2 [a]	0.86 ± 1.7 [a]

([a]): Significant changes when compared with control group when $p \leq 0.05$.

Table 5. Mean ± SD of WCP in full-thickness skin wounds in control and treated groups.

Time in Days	GI	GII	GIII
3	13.3 ± 18.7	55.4 ± 14.94 [a]	30.3 ± 28.4
7	33.9 ± 19.7	74.04 ± 9.8 [a]	60.4 ± 16.7
14	93.6 ± 3.2	99.9 ± 0.2 [a]	99.14 ± 1.7 [a]

([a]): Significant changes when compared with control group when $p \leq 0.05$.

3.6. Histopathological Findings

As shown in (Figure 5), on the 3rd day post-treatment, the microscopic examination of wounds in the GI revealed ulcerated and desquamated necrotic mass of tissue, inflammatory reaction with intense polymorph nuclear cells infiltration and dilated blood vessels with hemorrhage and edema of the underlying dermis. While in wounds of GII, the dermal blood vessels were congested in addition to fibroblast cells proliferation and with the presence of mononuclear cells infiltration. GIII showed congested blood vessels, hemorrhage, and increased inflammatory reaction. On the 7th day, GI showed fewer polymorph-nuclear cells in the dermis but more macrophages infiltration, less edema, and hemorrhages compared with the 3rd day of the experiment. While, GII showed moderate fibroblast proliferation, mild collagen deposition, and increased angiogenesis in the upper layer of the granulation tissue. The pathological examination of wounds in GIII revealed little fibroblast proliferation with increased inflammatory cells proliferation. On the 14th day, there was enhanced repair and remodeling of the wound area. The wounds of GI showed complete epithelization but with increased necrotic tissues and inflammatory cells. Moreover, GII revealed complete epithelization with few polymorph-nuclear cells in the dermal tissue with full skin thickness and healing near to the normal skin. Wounds of GIII were characterized by complete epithelization with intense polymorph-nuclear cells infiltration in the dermal tissue and with increased necrotic tissue.

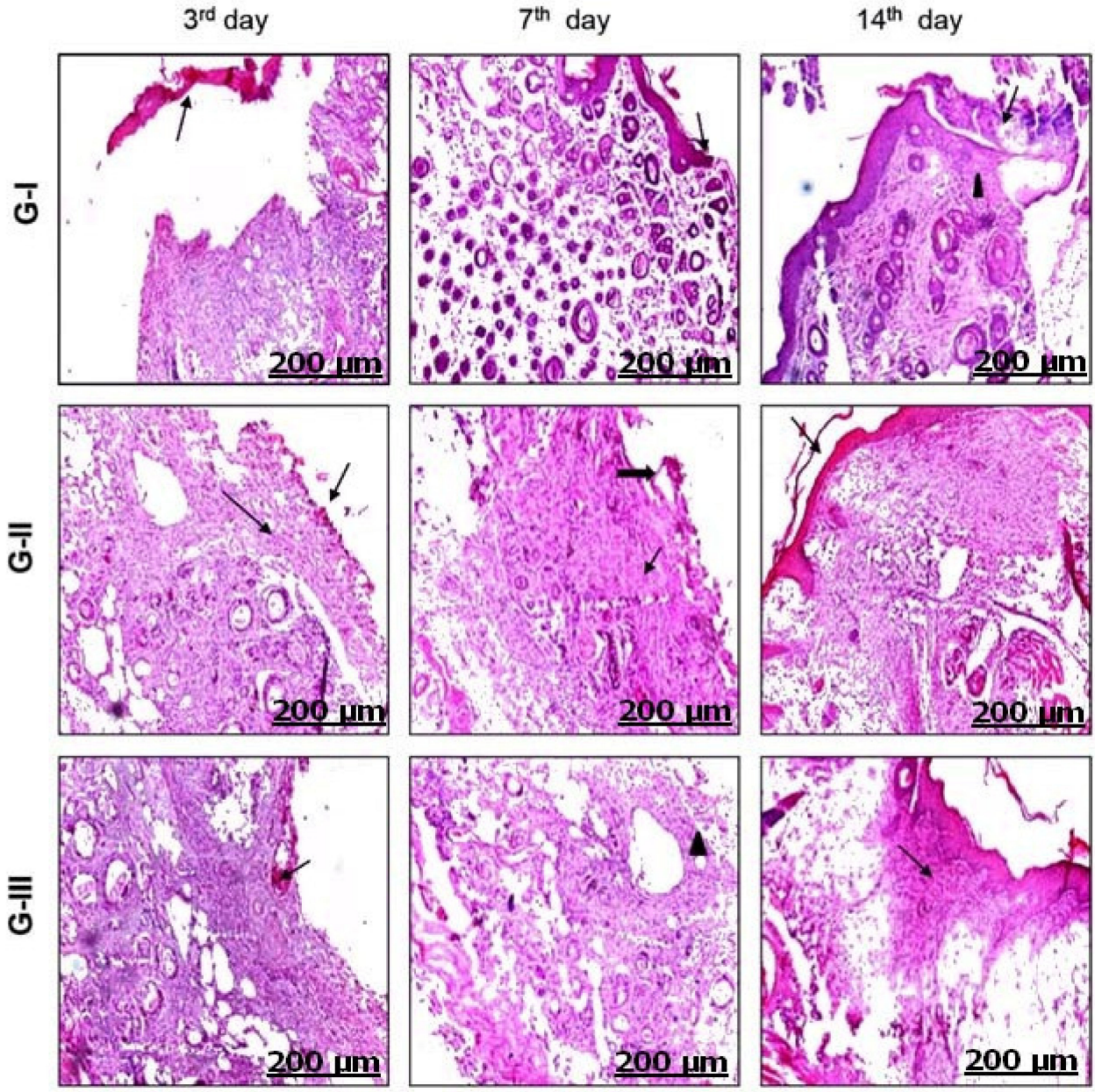

Figure 5. Photomicrograph of the control and treated groups on the 3rd, 7th, and 14th days post-treatment where GI at 3rd day showed ulcerated and desquamated necrotic tissue with inflammatory cells infiltration (arrows), while at 7th day, there were dilated blood vessels with epithelization. On 14th day, increased mononuclear inflammatory cells infiltration with necrotic tissue mass at the wound area was shown. While GII on the 3rd day showed inflammatory cells infiltration and mild angiogenesis, and on the 7th day, fibroblast proliferation with neovascularization and granulation tissue formation was shown. Moreover, on the 14th day, full skin thickness and healing near to normal skin were shown. GIII showed congested blood vessels with mild fibroblast proliferation on the 3rd day, while on the 7th day, increased fibroblast proliferation and neovascularization were shown. On the 14th day post-treatment, complete epithelization of wounds with mononuclear inflammatory cells infiltration was shown.

3.7. Immunohistochemistry Findings

Microscopic examination of the wounds stained with vascular endothelial growth factor immune-staining on the 3rd, 7th, and 14th days post-treatment, where GI showed mild expression of VEGF within the endothelial cells of blood vessels and fibroblast at 3rd,

7th, and 14th days. While, GII showed mild VEGF on the 3rd day within endothelial cells of blood vessels, which increased on the 7th and 14th days within the fibroblast cells and endothelial cells of blood vessels besides epithelial cells of granulation tissue. In GIII, on the 3rd day of the experiment, VEGF was expressed mild reaction within the endothelial cells of blood vessels, and increased on the 7th and 14th days and appeared within the endothelial cells of blood vessels, fibroblast cells, and epithelial cells (Figure 6).

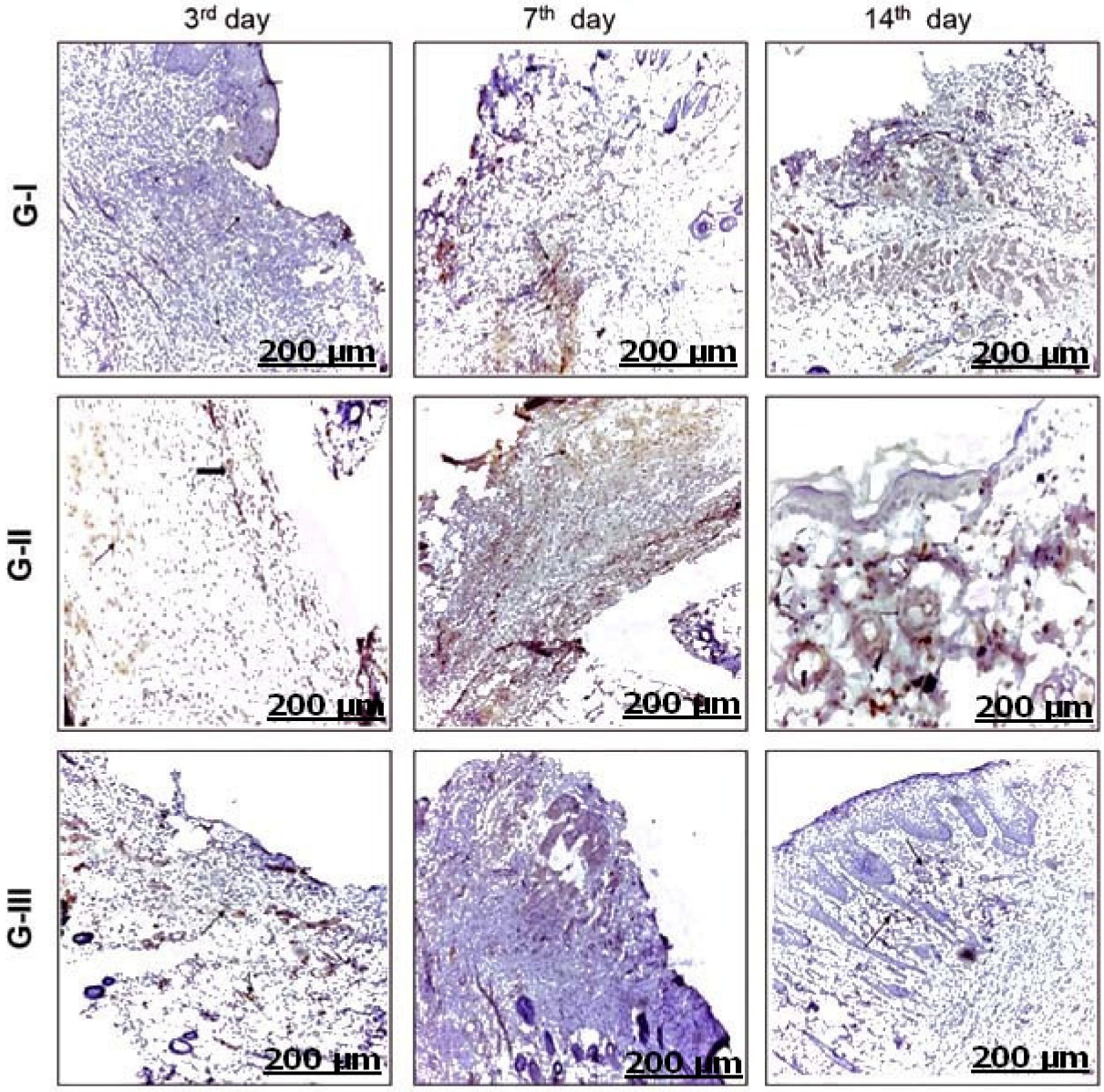

Figure 6. Immunohistochemical expression of VEGF in control and treated wounds. GI showed mild expression of VEGF within the endothelial cells of blood vessels and fibroblast on the 3rd, 7th, and 14th days. GII showed mild VEGF on the 3rd day within endothelial cells of blood vessels, which then increased on the 7th and 14th days within the fibroblast cells and endothelial cells of blood vessels besides epithelial cells of granulation tissue. In GIII, on the 3rd day, VEGF was mild within the endothelial cells of blood vessels, and increased on the 7th and 14th days and appeared within the endothelial cells of blood vessels, fibroblast cells, and epithelial cells.

3.8. Clinical Study

Wound management strategies applied, such as the surgical debridement in the case of the old wounds, which were considered very important for the removal of dead and

necrotized tissues that provide a suitable environment for the action of the gel for good and clean healing. Any hemorrhage that could have resulted from surgical debridement was arrested immediately and controlled by packing after clearance of the wound.

By application of the herbal formulations to the equines wounds, it was observed that wounds treated with *S. arghel* EtOAc gel showed rapid wound epithelization compared with *S. arghel* MeoH extract gel, and the *S. arghel* EtOAc gel was easily applied on wound surface compared with *S. arghel* MeoH extract gel (Figures 7–9).

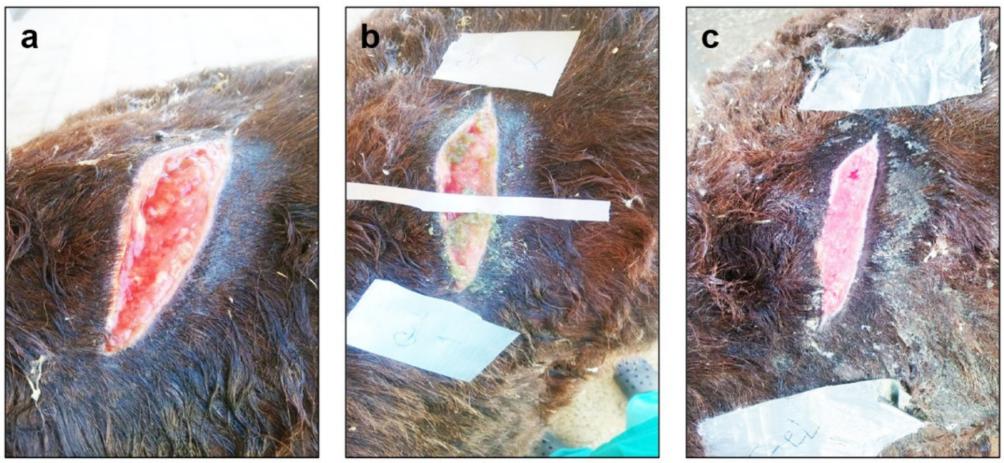

Figure 7. (**a**) Four-year-old male donkey with an old lacerated accidental wound on the back. (**b**) The wound was divided into two parts and treated with gel (1) *S. arghel* EtOAc gel and gel (2) *S. arghel* MeoH extract gel. (**c**) The same animal, after 9 days of treatment, showed decreased wound size and complete epithelization of the wound.

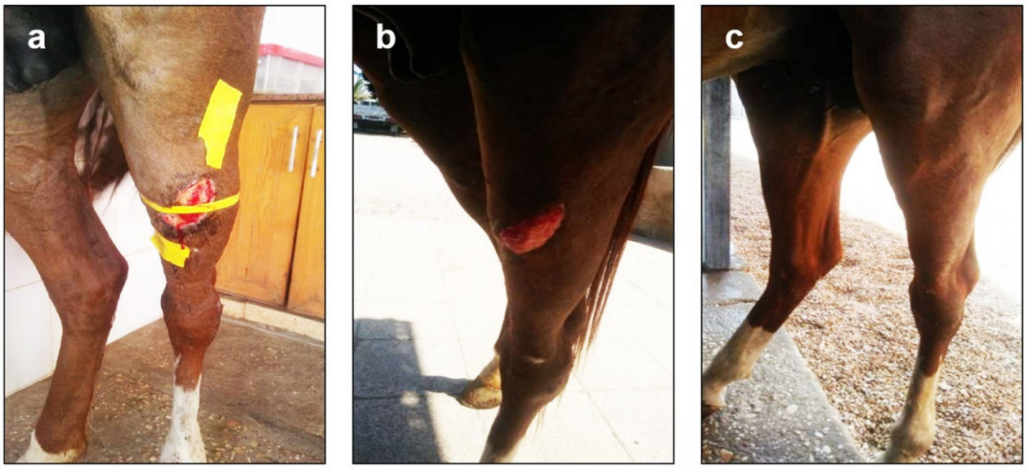

Figure 8. (**a**) Nine-year-old horse with recent wound at the left hind limb, the wound was divided into two parts and treated with and treated with gel (1) *S. arghel* EtOAc gel and gel (2) *S. arghel* MeoH extract *gel.* (**b**) The same animal, after 18 days of treatment, showed decreased wound size and complete epithelization with hypergranulation tissue due to the wound being near a movable part. (**c**) The same animal, after a month and a half, showed complete wound healing.

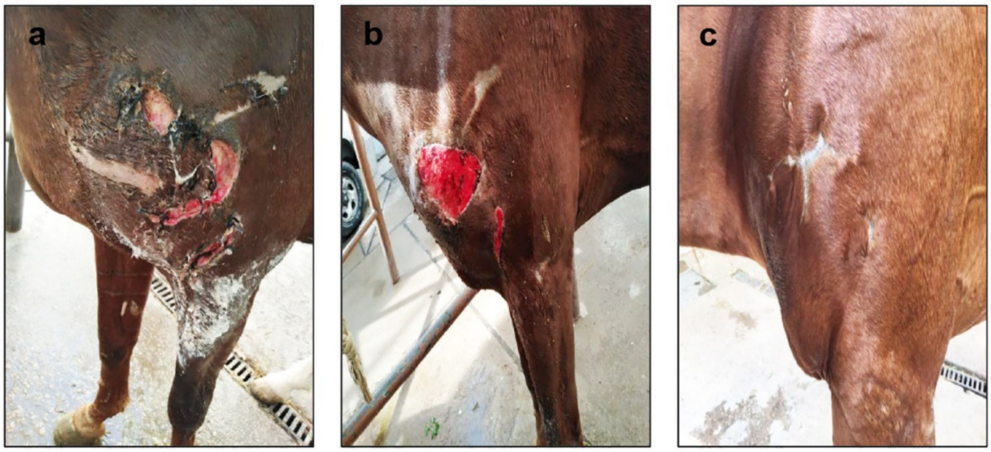

Figure 9. (**a**) Eleven-year-old horse with an old accidental wound nearly at the level of elbow joint of the left forelimb (movable area) after different trials of treatment and suturing showed no tendency for healing with wound dehiscence. (**b**) The same wound after one week of treatment with *S. arghel* EtOAc gel showed complete epithelization with decreased wound size. (**c**) The wound, after 2 months of treatment, showed complete healing.

4. Discussion

The wound induces loss of the integrity of the skin resulting in functional imbalance, possibly accompanied by disability or even death [28]. Therefore, the wound healing process is one of the utmost medical issues in human and veterinary medicine, which explains the urgent need for developing new agents that possess wound healing activities [25].

Recently medicinal plants' antioxidant and antimicrobial activity attracted many researchers. Almost all these phytochemicals activities are emulated with artificial antimicrobials and antioxidants [29]. The current study was conducted to evaluate the antifungal, antioxidant, and efficiency of topical *S. arghel* EtOAc and MeoH extracts for the cutaneous wounds treatment in experimentally induced wounds and equines suffered from traumatized wounds. Biomembrane-forming yeasts and cutaneous opportunistic pathogens were most strongly associated with wound necrosis and poor outcome [6]. VOCs extracted from *S. arghel* by EtOAc and MeoH in this study match VOCs identified by many previous studies with qualifications of biological and medical activities (Table 6). These activities were confirmed in this study by limiting the growth of fungal isolates from clinical cases of equines wounds due to active compounds of arghel EtOAc and MeoH extracts (Tables 1 and 2) (Figure 3a,b). Both extracts inhibited the growth of the filamentous fungi (*P. funiculosum*, *P. jensenii,* and *M. cinctum*) and yeast (*C. albicans*) isolated from wounds. *C. albicans* is the most common human pathogen responsible for painful mucosal infection [30]. Therefore, inhibition of yeast cells is needed to limit the rapid reproduction of cells and reduce the pain. Interestingly, *C. albicans* colonized all studied wounds while filamentous fungi (*P. funiculosum*, *P. jensenii,* and *M. cinctum*) were irregularly distributed in the studied animals' wounds. These results confirmed the hypothesis that infected skins serve as a portable for filamentous fungi to enter the host body [31]. Both extracts inhibited the sporulation of these fungi, which supports wound recovery. Fungal spores supported fungi in wound colonization and prolonged the wound's inflammation [6]. Thus, limiting fungal contamination is an important step for wound treatment. Moreover, the antioxidant characteristics of *S. arghel* (Figure 2) encouraged the inhibition of fungi and wound healing.

Table 6. Activities of some VOCs emitted from *S. arghel* extractions (EtAoH and MeoH).

Compound Name	Activity
17-Octadecynoic acid	Increased the efficacy of angiotensin II (17-ODYA-effect) as well as simultaneous incubation with miconazole (epoxygenase-inhibitor) and CAY 10434 (hydroxylaseinhibitor) [32]
1-Chlorooctadecane	Hepatitis, bronchitis, tight chest, lung diseases and asthma treatment, anti-diabetic, anticancer, anti-ulcer, antioxidant, antimalarial, anti-diarrheal, prophylactic, antimicrobial, anti-inflammatory, antipyretic potentials, wound healing [33].
9-Octadecenoic acid (z)-	Antimicrobial, anti-inflammatory [34], antioxidant activity, anticancer, anemiagenic, insectifuge, antiandrogenic, dermatitigenic [35]
Docosane	Antibacterial activity [36]
2,4-Di-tert-butylphenol	Fungicidal, antioxidant activity, anticancer [37]
8-Dodecen-1-ol, acetate, (Z)-	Pesticide [38]
1-Tetradecanol	Anti-inflammatory effect and initiation of the reformation of the soft tissues [39]
Neophytadiene	Anti-inflammatory, analgesic, antipyretic, antimicrobial, and antioxidant compound [40,41]; Carminative, Gastrin inhibitor Antiulcerative, Histamine release inhibitor, Antiprotozoal (Leishmania) Antiparasitic [42]
Digitoxin	Anesthetic, proliferative diseases treatment, dementia treatment, Cardiotonic, Diuretic [43]
Oxiranepentanoic acid, 3-undecyl-, methyl ester, trans	Anti-inflammatory [40,44] antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic,5-Alpha reductase inhibitor, potent mosquito larvicide [44]
1-Heptatriacotanol	Antibacterial, anticancer, antiprotozoal, chemo-preventive, anti-inflammatory, antimalarial, anti-flu, antiviral, enzyme inhibitor, anti-hyper-cholesterolemic [45].
13-Heptadecyn-1-ol	Anti-inflammatory, antifungal [34].
2,2-Dideutero Octadecanal	Antimicrobial activity [46]
9-Hexadecenoic acid	Anti-inflammatory [34]
Phytol	Anti-inflammatory, anticancer, diuretic [47], antimicrobial and antioxidant, [48], [40]; Lipid metabolism regulator, antiparasitic, antihelmintic, antiprotozoal (Leishmania) Histamine release inhibitor, spasmolytic [42]
9,12,15-Octadecatrienoic acid, 2,3-Bis[(Trimethylsilyl) Oxy]propyl ester, (Z,Z,Z)	Anti-inflammatory, hypocholesterolemic, anticancer, hepatoprotective, nematicide, antihistaminic, antieczemic, antiacne, antiarthritic, and antiandrogenic activities [49]
Diisooctyl phthalate	Antimicrobial [50].
Vitamin E (alpha-tocopherol)	Analgesic, neuropathic pain [51], lipid peroxidase inhibitor, anti-inflammatory, free radical scavenger, spasmolytic, histamine release inhibitor anti-infective [42]
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9-(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b,9a-trihydroxy-1,1,6,8-tetramethyl-,[1ar-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-	Of a therapeutic value in the treatment of abscesses, boils, abdominal pain, acne, colic, fatigue, gout, headache, renal disorders, impotence, measles, mental disorders, wounds, and bleeding [52–54]
Methyl Hexadecadienoate	Antifungal activity [55].
Heneicosane	Antimicrobial activity [56].
Heptadecane	Anti-inflammatory and antioxidant [57].

Table 6. *Cont.*

Compound Name	Activity
Octadecane, 1-iodo-	Antiviral [58] antioxidant [59], anticonvulsant [60], anti-inflammatory [61] and hepatoprotective [62], treatment of constipation, wounds, and stress [63].
Hentriacontane	Antitumor activity [64]. Its cytotoxic effect on lymphoma cells was also reported [65]. Antimicrobial and anticancer activity [66]. Anti-inflammatory activity as it inhibits the inflammatory mediators [67] through the blockage of the NF-kB pathway via down-regulating pro-inflammatory mediators (NO, PGE2 and LTB4) and cytokines (TNF-a, IL-6, IL-1b and IL-10) [68].
Heptacosane	Anti-corrosive and antioxidant [69]
Sulfurous acid, butyl heptadecyl ester	Antimicrobial [70]
Dibutyl phthalate	Antimicrobial [71]

It is known that *S. arghel* is used in traditional medicine as an antispasmodic, anti-inflammatory, and anti-rheumatic herbal [72]. The obtained results revealed that the surgically induced cutaneous rats' wounds showed accelerated wound closure with minimal scare formation after treatment with *S. arghel* EtOAc extract gel in comparison with that treated with *S. arghel* MeoH extract gel. Ref. [73] reported that there are chemical compounds that occur naturally in plants and possess important wide applications in medicine, and so the wound healing activity of *S. arghel* is attributed to the phytochemical components of EtOAc extract, which have anti-inflammatory and antioxidant effects (Table 6).

The *S. arghel* EtOAc extract has good analgesic action due to its phytochemical ingredients as Neophytadiene, Digitoxin, and Vitamin E (alpha-tocopherol), which were recorded for its analgesic action (Table 6). As a result, topical use of *S. arghel* has a good effect in reducing the pain.

Equines are known for their tendency to wounding, probably due to their inquisitive nature, large size, and confining in areas with potential obstacles such as metal or wire and their known difficulties with healing. The present study showed the excellent beneficial use of *S. arghel* in the treatment of recent and old traumatized wounds in equines. Extract application resulted in relieving edema and inflammation around the wounds, disappearance of infection, and observable decreased wound surface. After extract application, the wounds were not covered with bandages due to the authors of [74] reporting that the bandaged wound took a longer time to heel than unbandaged wounds. The application of the extracts absorbing exudate, keeping the wound moist, which helps epithelization, prevent contamination of wound from flies due to presence of Oxiranepent Hexadecanoic Anoic Acid, 3-UndecyL-, Methyl Ester, Trans, which has pesticide activity as recorded by [44]. Ref. [75] reported that delayed healing associated with distal limb wounds is a particular problem in equine clinical practice. In this study, the topical extract of *S. arghel*, especially EtOAc extract, showed accelerated the wound healing of the distal limb wound.

5. Conclusions

From the aforementioned results, it can be concluded that *S. argel* possesses antifungal, antioxidant, and wound healing activities. These findings were well appreciated, with histopathological studies suggesting that *S. argel* can serve as a good therapeutic agent for the treatment of cutaneous wounds as well as beneficial effects for the treatment of equine wounds, especially those related to the distal limbs.

Author Contributions: F.F.A.-M., Z.M.M., A.E.-M., M.B. and A.A.M. conceived and designed the study. F.F.A.-M., isolated and identified the wound-associated fungi and screened the antifungal activity. F.F.A.-M. prepared the extracts, GCMS analysis, and identification of extract components. Z.M.M. and A.A.M. conducted the experiment and collected the data. Z.M.M. performed histopathological and immunohistochemistry studies. F.F.A.-M., S.F.I., Z.M.M., A.E.-M., M.B. and A.A.M. organized and analyzed the data. S.F.I. funded the acquisition. F.F.A.-M., Z.M.M., S.F.I., A.E.-M., M.B. and A.A.M. interpreted the data, wrote the paper, and revised the final draft. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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