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Assessment of *in vitro* anti-inflammatory, hemostatic, antimicrobial, photoprotective and antioxidant activities of the Algerian species *Suaeda monodiana*

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The efficient cutaneous wound healing process constitutes a critical challenge for clinical and fundamental research. Indeed, agents that prevent bacterial infections, the excessive production of free radicals, and inflammation may enhance wound healing. In this context, the biological activities of the methanolic extract prepared from the species *Suaeda monodiana* Maire were assessed. The antioxidant activity was tested by five different methods, and the sun protection factor was measured. The hemostatic activity was evaluated by determining plasma re-calcification time, and the anti-inflammatory effect was carried out by heat-inducing hemolysis and albumin denaturation tests. The antimicrobial activity was evaluated by the agar disk diffusion assay against seven strains. As a result, the tested extract has a rich chemical composition and possesses interesting photoprotective (SPF at 46.49±0.05) and antioxidant activities. This extract showed the ability to inhibit protein denaturation (IC_{50} at 1.22±0.8 mg/mL) and to protect the erythrocytes membrane (IC_{50} at 2.39±0.3 mg/mL). Moreover, the Methanol extract significantly shortens the clotting time and inhibits the growth of all the tested strains with minimum inhibitory concentrations ranging between 31.25 to 250 µg/mL. Furthermore, due to its pharmacological properties, *S. monodiana* species could be used in pharmaceutical formulations for the treatment of skin diseases.

Keywords: Anti-inflammatory, Antimicrobial, Antioxidant, Hemostatic, Photoprotective, *Suaeda monodiana*

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Introduction

Wound healing is an interactive, complex and dynamic process important for tissue regeneration and repair after cutaneous damage caused by physical, environmental, or biological insults such as traumas, abrasions, burns, pressure sores, and surgical interventions[1]. This process involves several consecutive and overlapping phases, including hemostasis, inflammatory response, cell proliferation, and tissue remodeling[2]. Homeostasis is the shortest stage of wound healing that starts immediately after the skin injury to reduce blood loss at the wound site[3]. Following the inflammatory phase, which is initiated by the recruitment of pro-inflammatory cells, it liberates several proteolytic enzymes and pro-inflammatory cytokines to remove all the foreign particles and tissue debris in the wound site. Also, these immune cells generate reactive oxygen species (ROS) to preserve the damaged tissue site from microbial infections. However, the production of reactive oxygen species at high concentrations could induce lipid peroxidation and oxidative stress in the inflamed site, which contributes to the persistence of the wound and chronic pathogenesis. At this stage, the microbial infection of the wound could aggravate the inflammatory response, leading to non-healing wounds and several severe syndromes, such as septicemia. The third step of the wound-healing process is the epithelialization phase; cell proliferation is accelerated during this stage, and a new extracellular matrix is generated in the wound area. Finally, the newly formed tissue occurs its tensile strength after the remodeling stage, where collagen type III is replaced with collagen type I[2,3].

Many conditions may delay the wound healing process and induce scar formation, including the excessive production of reactive oxygen species, the

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prolonged and exaggerated inflammatory reactions and the possible microbial infections associated with the multidrug-resistant strains. Thus, developing new wound healing agents that exhibit fast hemostatic abilities and efficient anti-inflammatory, antimicrobial, regenerative and antioxidant activities is necessary.

Halophytes are plants that survive in saline habitats and under extreme climatic conditions. These characteristics give halophyte species the ability to produce a variety of primary and secondary metabolites such as vitamins, essential oils, polyphenols, carotenoids, steroids and terpenoids associated with many biological and pharmacological activities[4]. Among the halophytes, we are interested in the species *Suaeda monodiana* Maire from the genus *Suaeda* of the family Chenopodiaceae. This genus includes about 100 species, distributed on coasts, deserts, lakeshores, saline and alkaline lands around the world[5]. The species *S. monodiana* is an annual shrub with erect branches and very swollen leaves, oval in shape and very obtuse at the top[6]. It is widely used in popular medicine to treat infected wounds, burns and dermatic affections. However, studies on the phytochemical and pharmacological properties of *S. monodiana* species have not been reported. The present study reports the first investigation of *in vitro* anti-inflammatory, hemostatic, photoprotective, antimicrobial, and antioxidant activities of *S. monodiana* growing in Algeria.

Material and Methods

Apparatus

All spectrophotometric measurements were carried out using a UV-Vis 7220G spectrophotometer (Beijing Beifen-Ruili Analytical Instrument, China). For potentiometric calculations, Inolab series WTW720 pH meter (Hellmuth Walter, Germany) with a potential measurement function using a redox potentiometric cell (Platinum/ silver–silver chloride electrode; Pt/Ag-AgCl; 3M KCl) (Gomel' SGH-185, Germany).

Plant material

The plant material *S. monodiana* was collected in June 2019 from Touffana, Batna (Longitude: 6.6271 and Latitude: 35.4832, Aures region, Algeria) and was identified by Professor Bachir Oudjehih, Agronomic Institute of the University of Batna-1, under the reference number 977/ LCCE.

Preparation of plant extract

200 g of the whole plant *S. monodiana* were macerated twice with 2 l of methanol for 48 h. The solvent was then removed using a rotary evaporator to obtain 4.5 g of the methanolic extract (MSM). To prepare the aqueous extract (ASM), 5 g of the whole plant was infused in 100 mL of double-distilled water. After filtration, the obtained extract was diluted ten times with double-distilled water.

Phytochemical screening

Different classes of secondary metabolites present in *S. monodiana* methanolic extract were detected according to different colourimetric methods[7].

Determination of total bioactive contents

The total phenolic and flavonoid contents of the methanolic extract were assessed spectrophotometrically using the Folin-Ciocalteu and trichloroaluminum technics, respectively[7].

Antioxidant activities

The ability of MSM extract from the species *S. monodiana* to scavenge free radicals and/or to reduce transition metals was evaluated by five different methods, namely ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC), hydrogen peroxide and DPPH radical scavenging activities and antioxidant activity by potentiometric method[7,8].

In vitro anti-inflammatory activities

The anti-inflammatory effect of MSM extract and diclofenac sodium as the standard drug was tested using two methods: egg albumin denaturation[8] and heat-induced lysis tests[9].

Egg albumin denaturation assay

Two millilitres of samples (extract or diclofenac sodium) prepared at different concentrations were mixed with 200 µL of egg albumin and 2.8 mL of phosphate-buffered saline (PBS, pH 6.4). The obtained solutions were agitated and incubated at 37°C for 15 min and then heated at 70°C for 5 min. After cooling at ambient temperature, the absorbance was recorded at 660 nm. The percentage of protein denaturation inhibition was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times [V_S / V_C - 1]$$

where V_S = absorbance of the test sample and Vc = absorbance of the control (sample was replaced with double-distilled water).

Heat-induced lysis inhibition

Blood was collected in Na-Oxalate tubes from a healthy adult subject who did not consume any non-steroid anti-inflammatory drugs for at least two weeks before the experiment. Blood samples were stored at 4°C for 24 h before the use, then centrifuged (5 min/2500 rpm), and the supernatant was removed. The aliquot was washed using a sterile saline solution followed by centrifugation (2500 rpm for 5 min). The process of clearing the supernatant was repeated three times. 40% suspension (v/v) was prepared using phosphate-buffered saline (10 mM, pH 7.4). 5 mL of the isotonic buffer containing 50, 100, 200, 400, and 800 µg/mL of the MSM extract were put into two duplicate sets of centrifuge tubes. The control treatment was prepared with isosaline instead of the extract, 50 µL of RBC suspension was added to each tube and stride gently. One pair of tubes was incubated at 54°C temperature for 20 min in a water bath. The other pair was preserved at 0-5°C temperature in an ice bath. The centrifugation of the mixture was done for 5 min at 5000 rpm, and the absorbance of free haemoglobin was taken at 560 nm. Diclofenac (200 µg/mL) was used as a reference standard. The per cent inhibition of hemolysis was calculated according to the following equation:

$$\text{Inhibition of hemolysis (\%)} = 100 \times (1 - [(OD_2 - OD_1)/(OD_3 - OD_1)])$$

where OD_1 = test sample unheated; OD_2 = test sample heated and OD_3 = control sample

Antimicrobial activity

The antibacterial activity of the MSM extract was evaluated by the agar disk diffusion assay[10], against six bacterial strains and one fungus strain. Indeed, strains from the American Type Culture Collection (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 1117, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 90029), as well as the pathogenic bacteria (*Klebsilla pneumonae*, *Escherichia coli* and *Staphylococcus aureus*) were supplied by the Institute of Pasteur Algiers, Algeria and the Laboratory of Bacteriology, University Hospital Center-Batna (CHU), respectively.

***In vitro* hemostatic activity**

The ability of *S. monodiana* crude extract to assess hemostasis was determined based on the measurement of plasma re-calcification time[8]. Firstly, 400 µL of platelet-poor plasma was added to 400 µL of the tested extract prepared at different concentrations (2 to 6 mg/mL). The re-calcification was initiated by adding 400 µL of calcium chloride solution (0.025 M), and the mixture was incubated at 37°C, where the re-calcification process was monitored with a stopwatch. The period for fibrin appearance was recorded as plasma re-calcification time, and the shortening rate of clotting time was measured by the following equation:

$$R\ \% = [(T_1 - T_2)/ T_1] \times 100$$

where R = shortening rate of clotting time; T_1 = clotting time of control group; and T_2 = clotting time of sample administration time.

***In vitro* sun protection factor assay**

The efficacy of methanolic extract to absorb UV radiations (UVA and UVB) was assessed spectrophotometrically. The absorbance values of the tested extract were determined at 290 to 320 nm with 5 nm intervals, and the sun protection factor was calculated by the following equation[8].

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where CF = 10 (correction factor); EE: erythemal efficiency spectrum; and I: solar simulator intensity spectrum.

Statistical analysis

The results were given as the means ± SD (P <0.05) for three replicates for each sample and values were calculated by linear regression analysis and expressed as µg EAA/mg extract for total antioxidant capacity and ferric reducing antioxidant power, IC_{50} for DPPH free radical-scavenging assay and a percentage of inhibition (%) for the anti-inflammatory and hydrogen peroxide scavenging assays, plasma re-calcification time for hemostatic test and mol-eq/L for antioxidant activity by potentiometric method. Furthermore, the sun protection factor was statically analyzed using the ANOVA test. Significant differences between means were determined by one-way ANOVA, followed by the Duncan test and all the statistics were carried out using Graph Pad Prism 5.04.

Results

Phytochemical screening

The methanolic extract obtained from the species *S. monodiana* was screened for its phytochemicals

(Table 1). Many secondary metabolites, including phenols, cardiac glycosides, tannins, flavone aglycones, glycosides, and flavonoids, were detected in this extract. However, triterpenes, alkaloids, proteins, and saponins were absent.

Total bioactive contents

The total phenolic and flavonoid contents of the methanolic extract prepared from the halophytic species *S. monodiana* were performed spectrophotometrically using the calibration curves established by gallic acid and quercetin, respectively (Table 2). According to the results, high levels of phenolic compounds and flavonoids were found in this extract.

Table 1 — Phytochemical constituents of the methanolic extract from *Suaeda monodiana*

Phytochemicals	MSM extract
Tannins	+++
Glycosides	+++
Flavonoids	++
Cardiac glycosides	+
Flavone aglycones	+
Terpenoids	-
Alkaloids	-
Cholesterol	-
Proteins	-
Phenols	+++
Saponins	-

(+) presence of phytochemicals, (++) moderate presence of phytochemicals, **(+++)** indicating that phytochemicals are strongly present, (-) absence of phytochemicals

Table 2 — Total phenolic and flavonoid contents of *Suaeda monodiana*

Extract	Total phenolic content* (µg AGE/mg)	Total flavonoid content** (µg QE/mg)
MSM	464.63±0.09	182.67±0.26

*Total phenolic content was expressed as µg equivalents of gallic acid per mg of dry extract. **Total flavonoid content was expressed as µg equivalents of quercetin per mg of dry extract.

Antioxidant activity

The antioxidant activity of methanolic extract obtained from the species *S. monodiana* was estimated by five different tests. All the tested samples (MSM extract and reference standards) showed antioxidant potential in all the tested systems (Table 3). At the 100 µg/mL concentration, the tested extract showed a potent capacity to scavenge hydrogen peroxide free radicals better than ascorbic acid used as a reference. Moreover, a strong ability to reduce iron and molybdenum transition metals was found in FRAP and TAC assays. In the potentiometric test, the aqueous extract (ASM) exhibited a potent antioxidant ability to reduce Fe^{3+} to iron Fe^{2+}. However, in the DPPH assay, the methanolic extract exhibited moderate antioxidant activity compared to all reference molecules (BHA, BHT, α-Tocopherol, tannic and ascorbic acids).

Anti-inflammatory activities

The *in vitro* anti-inflammatory effect of the species *S. monodiana* was assessed by two methods (Table 4). According to the results, the tested extract was able to prevent protein denaturation (IC_{50} at 1.22±0.8 mg/mL) and to protect the human erythrocyte membrane against lysis induced by heat (IC_{50} at 2.39±0.3 mg/mL) in a dose-dependent manner. The observed anti-inflammatory effect of the crude extract was better than that of diclofenac used as a reference molecule in both tests.

Antimicrobial activity

According to the findings, *S. monodiana* crude extract indicated a broad spectrum of antimicrobial effects (Table 5). Indeed, this extract inhibited the growth of all the tested bacterial and fungi strains in a dose-dependent manner. However, the observed antimicrobial effect was weak compared to reference antibiotics (gentamicin and penicillin). This possessed

Table 3 — Antioxidant properties of the methanolic extract from the species *Suaeda monodiana*

Extract and standards	DPPH assay[a] IC_{50} (µg/mL)	H_2O_2 assay[a] % of inhibition at 100 µg/mL	FRAP assay[a] µg EAA/mg ex	TAC assay[a] µg EAA/mg ex	AOA assay[a] mol-eq/L
MSM	244.9±0.006[e]	73.32±0.67[a]	170.38±0.83	44.36±0.003	1.96±0.35
BHA[b]	6.82±0.49[b]	NT	NT	NT	/
BHT[b]	22.32±0.02[d]	NT	NT	NT	/
Tannic acid[b]	7.74±0.19[b]	NT	NT	NT	/
Ascorbic acid[b]	3.1±0.002[a]	62.32±0.26[b]	NT	NT	/
α-Tocopherol[b]	13.02±0.17[c]	NT	NT	NT	/

[a]Values expressed are mean ± SD of three measurements (P <0.05); [b]Reference compounds; NT: not tested.
/: indicates the absence of antibacterial activity

Table 4 — Anti-inflammatory activities of the methanolic extract from the plant *Suaeda monodiana*

Extract and standards	Concentration (mg/mL)	% of inhibition of RBC hemolysis[a]	% of inhibition of egg albumin denaturation[a]
MSM	4	87.43±1.55	74.89±1.71
	2	43.80±1.02	56.95±1.13
	1	14.53±0.86	48.02±1.53
	0.5	5.32±1.24	25.57±0.43
Diclofenac[b]	5	73.51±1.09	43.36±1.21

[a]Values expressed are means ± SD of three measurements (P <0.05); [b]Reference compound.

Table 5 — Results of the antimicrobial activity of the methanolic extract from *Suaeda monodiana*

	Inhibition zone (mm)						
Dilution (mg/mL)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*P. aeruginosa* ATCC 1117	*S. aureus*	*K. pneumonae*	*E. coli*	*C. albicans* ATCC 90029
1	9±0.3	8±0.6	8±0.1	8±0.1	9±0.3	11±0.6	8±0.1
0.5	8±0.2	8±0.3	7±0.3	7±0.2	8±0.1	8.5±0.2	8±0.4
0.25	7±0.5	-	-	-	-	8±0.3	7±0.3
0.125	-	-	-	-	-	8±0.5	7±0.6
0.0625	-	-	-	-	-	8±0.3	-
0.03125	-	-	-	-	-	-	-
MIC (µg/mL)	125	250	250	250	250	31.25	62.5
Penicillin (10 µg/mL)	40±0.1	32±0.3	32±0.3	40±0.4	23±0.4	32±0.3	20±0.4
Gentamicin (10 µg/mL)	38±0.5	34±0.2	39±0.1	42±0.2	27±0.5	36±0.1	19±0.2

Values expressed are means ± SD of three measurements (P <0.05). (-) No zones of inhibition around the discs. MIC: the minimum inhibitory concentration.

strong antimicrobial activities with inhibition zones ranging between 20 to 40 mm for penicillin and 19 to 42 mm for gentamicin.

In vitro hemostatic activity

The hemostatic effect of *S. monodiana* extract was assessed through the measurement of plasma re-calcification time (Fig. 1). According to the obtained findings, the addition of the methanolic extract at different concentrations significantly shortened the clotting time in a dose-dependent manner compared to the control group.

In vitro sun protection factor assay

The photoprotective effect of crude extract from the species *S. monodiana* was determined *in vitro* by measuring its sun protection factor (Table 6). According to the results, the crude MSM extract from *S. monodiana* constitutes an excellent UVA and UVB filter. Indeed, high absorbance values ranged between 5.196 and 4.467 at the waves of 290 to 320 nm were recorded, and a very strong sun protection factor was found (46.49 ± 0.05).

Discussion

The present study reported the *in vitro* assessment of the pharmacological activities of the species

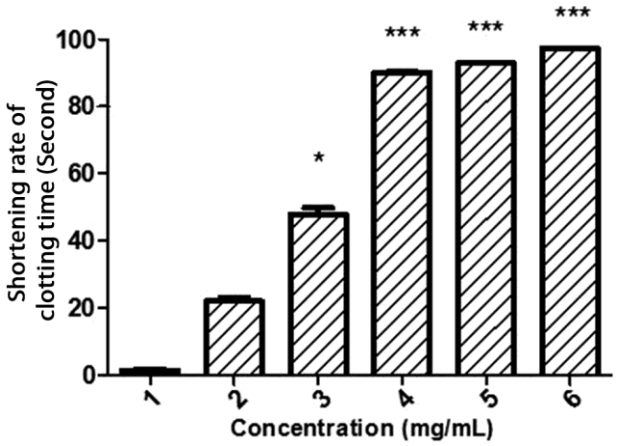

Fig. 1 — Shortening rate of clotting time after the addition of methanolic extract from *S. monodiana.* Significance was determined by one-way ANOVA followed by post hoc Tukey test; * and ***P <0.05 and 0.01 respectively, compared between the samples.

S. monodiana. The phytochemical screening of the MSM extract reveals the presence of many classes of secondary metabolites. Indeed, several studies on the chemical composition of species from the genus *Suaeda* displayed their richness in secondary metabolites with interesting biological properties. The same chemical profile detected in *S. monodiana* was found in *S. prostrata* and *S. microphylla* plants[11] .

Table 6 — Photoprotective effect of MSM extract obtained from *Suaeda monodiana*

λ (nm)	EE×*I* (normalized)	MSM extract	
		absorbance	SPF
290	0.015	5.196±0.010	0.78±0.01
295	0.0817	4.890±0.015	3.98±0.02
300	0.2874	4.718±0.012	13.52±0.03
305	0.3278	4.615±0.018	15.12±0.05
310	0.1864	4.567±0.049	8.51±0.04
315	0.0837	4.510±0.015	3.78±0.04
320	0.018	4.467±0.009	0.80±0.02
Total	1	/	46.49±0.05

Nevertheless, many other classes of compounds were identified in other *Suaeda* taxa: both species *S. vera* and *S. spicata* showed the presence of amino acids, polysaccharides, coumarins, saponins and alkaloids[12]. Also, various phytochemicals, including phenolics, flavonoids, terpenoids, tannins, saponins, steroids, alkaloids, glycosides and quinones have been detected in the plants *S. monoica*, *S. peruinosa* and *S. fruticosa*[13-15]. The GC-MS analysis of *S. maritima* extract revealed the presence of fatty acids, essential oils, steroids and phenolic compounds[16]. The phytochemical analysis of *S. aegyptiaca* and *S. vermiculata* indicated the presence of steroids, alkaloids, coumarins, flavonoids, tannins, saponins, cardiac glycosides, phenols, terpenoids, while the anthraquinones were absent[17,18]. Furthermore, many flavonoids and phenolic acids were identified in *S. fruticosa*, *S. pruinosa*, *S. mollis* and *S. maritime*[19-21].

S. monodiana extract contains high levels of phenolic and flavonoid compounds. According to previous studies, lower levels of polyphenols were reported in several plants from *Suaeda* taxa compared to the results obtained in the present study[11,21]. The observed variances in the types and amounts of phenolic compounds between plants belonging to the same genus could be related to environmental factors. Indeed, phenols and flavonoids biosynthesis by halophyte species is improved under salinity conditions to reduce oxidative stress damages[22]. Furthermore, the harvesting period of plant material could influence the quantities of total bioactive compounds. Indeed, the species *S. japonica* collected in different periods exhibited variations in total phenolic and flavonoid contents depending on the amount of sunlight and the growth time[23]. Also, the type of solvents used in the extraction procedure could affect the phytochemical content of the extracts. Previous reports on different plants from *Suaeda* genus indicated that polar solvents are the most suitable for phenolic compound extraction[15,17,18,20].

Also, the results showed that *S. monodiana* extract has interesting antioxidant activities in various tested methods. This result was expected since various fractions prepared from *Suaeda* species have the potential to inhibit lipid peroxidation and exhibit high reducing potentials, scavenging and chelating antioxidant activities[16,19,20,24-26]. The observed antioxidant effect of *S. monodiana* could be associated with the ability of this species to grow under many stressful conditions, including high salinity and other hostile conditions. Indeed, it is well-known that high concentrations of NaCl cause oxidative stress in plant cells by the perturbation of electron transport in chloroplasts and mitochondria, which induces the formation of reactive oxygen species (ROS) such as singlet oxygen, hydrogen peroxide, superoxide and hydroxyl radicals[27]. However, according to many studies, *Suaeda* plants as halophytes could withstand salt-triggered oxidative stress due to their powerful enzymatic and non-enzymatic antioxidant systems and their capacity to biosynthesize high levels of phenolic compounds with strong antioxidant activities[18-20]. Indeed, it was proven that *S. salsa* had a strong ability to produce, under salinity conditions, certain exogenous antioxidants, particularly flavonoids[28].

Inflammation is a complex physiologic response that protects the human body against physical, chemical, and infectious aggressions. However, a prolonged and non-controlled inflammatory process can cause extensive tissue damage and many chronic disorders. Indeed, during the inflammatory process, immune cells release lysosomal enzymes that act as mediators of inflammation. These enzymes react non-specifically and cause cell membrane lysis of all the exposed cells in the inflamed site due to their capacity to degrade various components of the connective tissue, such as collagen, elastin, and protein-mucopolysaccharide complexes, which contribute to tissue injury. Also, the denaturation of tissue proteins during this pathogenesis could induce auto-antigen production, which increases the degree of inflammation. Thus, products capable of preventing protein denaturation and the release of deleterious lysosomal enzymes could constitute effective anti-inflammatory alternatives to non-steroidal anti-inflammatory drugs known for their wide range of adverse effects. The methanolic extract from

S. monodiana exhibited a strong anti-inflammatory effect and prevented protein denaturation and the lysis of red blood cell membranes. The anti-inflammatory properties of species belonging to the genus *Suaeda* are well-documented. *S. japonica* and *S. asparagoides* extracts decreased the expression of many inflammatory mediators and pro-inflammatory cytokines, including cyclooxygenase-2 and interleukin 6, and induced the suppression of inducible nitric oxide synthase (iNOS) expression and nitric oxide production[24,29]. *S. salsa* extracts decreased vascular permeability and inhibited the formation of granuloma by the suppression of inflammatory mediators, the decrease of MDA content in inflammatory exudates, and the increase of catalase enzymatic activity[28]. *S. fruticosa* extract had a strong anti-inflammatory effect and reduced paw oedema induced by carrageenan[26]. Extracts prepared from *S. vermiculata* possessed remarkable *in vivo* anti-inflammatory effects associated with their richness in tannins and flavonoids[30].

However, *S. monodiana* extract displayed moderate antimicrobial effects. These results agree with the findings of many previous studies on species from *Suaeda* genus, which reported moderate to strong antimicrobial activities against the strains *S. aureus, E. coli, K. pneumonia, P. aeruginosa* and *C. albicans*[15,25,30]. The antimicrobial activity of the species *S. monodiana* may have a great impact on cutaneous wound healing and skin reconstruction processes. Since most of the tested strains in this study are highly associated with wounds and burn infections, their inhibition may prevent prolonged inflammation and the excessive production of reactive oxygen species in the wound site, accelerating the wound healing process. An interesting antibacterial effect was observed against *E. coli*. These strains are resistant to antibiotics and have virulence factors that are considered the principal cause of wound infections of surgical incisions, traumatic injuries, and foot ulcers[31]. Also, the MSM extract showed a strong inhibitory effect on *C. albicans*, a ubiquitous fungal organism that exists as a commensal and opportunistic pathogen. In the cases of immune-compromised individuals with genetic deficiencies or malignancy, *C. albicans* is responsible for many diseases. It has been documented that the opportunistic colonization of burn wounds with *C. albicans* leads to invasive candidiasis and contributes to non-healing and chronic wounds. Indeed, infected wounds with *C. albicans* were inflamed and represented differential tissue architecture; their healing process was notably delayed[32]. In addition, this extract possesses an inhibitory effect on *P. aeruginosa*. This strain is a common cause of nosocomial infections that extends the hospitalization periods after surgeries and increases the risk of septicemia. This causes a massive immune response in the burn wound microenvironment by increasing the levels of many pro-inflammatory cytokines and chemokines[33]. *S. monodiana* extract also exhibits moderate antibacterial activity against *S. aureus*. This opportunistic pathogen, the most common microorganism responsible for post-operative wound infections, is the main cause of septicemia and skin and soft-tissue infections[34]. The chronicity of the wound is associated with the co-infection and the symbiosis between several microbial strains. Co-infections with *S. aureus* and *P. aeruginosa* have been reported to have late wound healing rate. Since *S. aureus* cannot produce a strong biofilm, the co-infection with *P. aeruginosa*, facilitates the creation of a pathogenic biofilm that impairs the wound healing process and increases antibiotic resistance, resulting in chronic infection[35]. Thus, the MSM extract of *S. monodiana* may constitute a valuable source of antimicrobial agents that alleviate the possible co-infection due to the sensitivity of both strains (*S. aureus* and *P. aeruginosa*) and its richness in polyphenols known for their antimicrobial and wound-healing activities[36].

S. monodiana extract showed significant hemostatic activity. This effect could be explained by the richness of *S. monodiana* in minerals, especially calcium. Calcium ions possess a critical role in the primary and secondary hemostasis process, and many of the component reactions in blood coagulation are either Ca^{2+}-dependent or require calcium for the interaction of proteins with membrane surfaces[37]. This hemostatic activity suggests the ability of the species *S. monodiana* to prevent abnormal bleeding and to maintain intravascular blood in a fluid state. Indeed, the tested extract may interfere with intrinsic, extrinsic, and common coagulation pathways, which indicates its possible ability to favour the hemostatic process by the induction of possible vasoconstriction, platelet plug installation, and the formation of a fibrin clot.

In addition, the tested extract had a very strong ability to absorb UVB radiations, demonstrating its possible ability to prevent skin photo-damage,

sunburn and the harmful effects of both short and long-wavelength UV radiation. Indeed, the strong photoprotective effect of *S. monodiana* MSM extract could be associated with the presence of a high level of phenolic compounds known for their photoprotective activity[38] and for their ability to prevent lipid peroxidation and the generation of UV-induced oxygen free radicals[39]. However, species belonging to the same genus, including *S. monoica* and *S. maritima* showed moderate photoprotective effects compared to the results of the present study[40]. The differences in the chemical profiles between species from the same genus could explain this variation.

Conclusion

The present study reported the first investigation of *in vitro* pharmacological activities of the medicinal plant *S. monodiana* to confirm its traditional use for treating skin diseases. The phytochemical screening of the MSM extract revealed the presence of several classes of secondary metabolites, namely phenols, cardiac glycosides, tannins, flavone aglycones, glycosides, and flavonoids. This extract contains high levels of phenolic and flavonoid compounds and has important photoprotective and antioxidant activities. The MSM extract has strong *in vitro* anti-inflammatory effects better than diclofenac significantly shortens the clotting time, and shows a broad spectrum of antimicrobial effects. Furthermore, *S. monodiana* extract could be used in pharmaceutical and cosmetics formulations to treat many skin affections due to its strong hemostatic, photoprotective, antimicrobial, anti-inflammatory and antioxidant properties. However, more detailed *in vivo* studies and phytochemical investigations are required to confirm its safety and to identify the bioactive compounds responsible for the observed pharmacological properties.

Conflict of interest

The authors declare no conflict of interest.

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