

Original Research Article

Evaluation of anti-inflammatory and antioxidant activities of *Sedum sediforme* extracts

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Abstract

Purpose: To evaluate the *in vivo* anti-inflammatory effect and *in vitro* antioxidant activity of the areal part of *Sedum sediforme* (Jacq.) extracts.

Methods: The plant was extracted with solvents of varying polarity (Methanol, chloroform and ethyl acetate, respectively) allowed its separation into three sub-fractions: crude extract, chloroform extract and ethyl acetate extract (CrE, ChE and EaE, respectively). Total polyphenol contents of the extracts were determined. The phorbolmyristate acetate (PMA) induced mice ear edema method was using to evaluate the anti-inflammatory activity. The *in vitro* scavenging activity was evaluated using enzymatic and non-enzymatic methods. Chelating ability of extracts was assessed using Fe²⁺-ferrozine complex.

Results: The highest content of phenolics compound was in EaE. The administration of CrE (12.5 and 25 mg/kg) reduced ear edema induced by PMA (%I = 35.81 ± 3.18 % and 38.57 ± 2.80 %, respectively), the effect was comparable with that of diclofenac used as a reference drug (%I = 38.84 ± 1.87 %). The *in vitro* scavenging activity of *S. sediforme* extracts confirmed that the CrE has the highest enzymatic and non-enzymatic activity with a half maximal inhibitory concentration (IC₅₀) = 0.063 ± 0.005 mg/mL and 0.178 ± 0.006 mg/mL, respectively. However, the ChE present an excellent chelating activity with a half-maximal effective concentration (EC₅₀) of 0.397 ± 0.001 mg/mL.

Conclusion: The results show that *S. sediforme* extracts have a strong antioxidant and anti-inflammatory activities which lend some support their use in the traditional medicine.

Keywords: *Sedum sediforme*, Anti-inflammatory, Superoxide scavenger, Cytochrome C, Metal chelating

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INTRODUCTION

Inflammation is the primary reaction of the immune system to infection or tissue injury, main to safety of the human body in opposition to those insults. Prolonged or chronic inflammation

is harmful and has a very important role within the development of diseases such as Alzheimer's, arthritis, cardiovascular disease and diabetes [1]. Acute inflammation, which is described by pain, heat, redness and swelling includes many series of events including

increased permeability, vasodilatation, fluid exudation and migration of leukocytes. In an inflammatory environment, a massive quantities of superoxide radical and different free radicals was produced by activated neutrophils and macrophages via the NAD(P)H oxidase. Similarly, free radicals created in inflammation might also lead to toxic effects once produced at high levels during oxidative burst [2]. The offered anti-inflammatory medicaments (steroidal and non-steroidal) present wide side effects. Consequently, many researches were devoted to the look for a new anti-inflammatory agent from herbal sources. Medicinal components from plants play important role in conventional traditional medicine, *Sedum* species are the home of traditional remedies that are mostly used as hypotensive, laxative and emetic and anti-inflammatory agents [3]. *Sedum sidiforme* is among the medicinal plant used in Algeria. The aim of this study is to evaluate the anti-inflammatory, superoxide scavenger and metal chelating activity of *Sedum sediforme* extracts.

EXPERIMENTAL

Materials

Sedum sediforme was collected from N'Gaous, Batna (Algeria) Algeria, at the end of March 2012 (between 12.00- 14.00 h), and identified by Professor Oudjih Bachir, Department of Agronomy, Batna University, Batna, Algeria. A voucher specimen was deposited in an official *herbarium* of the same department (no. I.A.B./990) for future reference. All chemical reagents were purchased from Fluka, Sigma Chemicals, and Prolab (Germany).

Adult male mice were obtained from the Pasteur Institute of Algeria (weighting twenty-five – thirty g). Animals were kept on a 12-hour light/dark cycle and housed in cages (polycarbonate). The animals were transferred to the laboratory at least one week before the experiments. The animal experiments were performed in accordance with international guidelines for the use and care of laboratory animals [4]. Ethical approval for the animal experimentation was obtained from Ethics Review Committee of Faculty of Exact Sciences and Nature and Life Sciences, University of Biskra, Biskra, Algeria (approval ref no. 307/V.D.P.G.).

Extraction of phenolic compounds

The extraction was carried out using various polar and non-polar solvents, according to the method of Markham [5]. *S. sediforme* powder was soaked in methanol with a ratio of 1:10 w/v,

under agitation overnight at 4 °C. The extract was filtered to obtain the first filtrate, the solvent of this filtrate was removed under reduced pressure (Rotavapor: Germany, bÜchi461) below 45 °C to obtain the methanol extract and it was coded as CrE. In order to obtain a general view of the plant compounds it can be use a non-polar to polar solvent scale liquid-liquid extraction of the CrE : hexane (defatting), chloroform (aglycone flavonoids extraction), and ethyl acetate (glycoside flavonoids extraction). The percolates were *evaporated* to give chloroform and ethyl acetate (ChE and EaE, respectively) extracts. All of these fractions were stored prior to use at -20 °C.

Determination of total phenolic content

The Folin–Ciocalteu reagent was using to determine total phenolic content [6]. After incubation for 4 min of 200 µL of diluted sample and 1 mL of diluted Folin–Ciocalteu reagent 1:10 (v/v), 800 mL of NaCO₃ aqueous solution (75 g/L) was added. The absorbance measured at 760 nm after incubation (1 h at 25 °C). Gallic acid (0.2 – 1 mg/mL; Sigma) was used as the standard for the calibration curve, and total phenolic content was expressed as microgram gallic acid equivalent (µg GAE)/mg extract.

Evaluation of anti-inflammatory activity of CrE

The method of Garrido *et al* [7] with slight modification, PMA was used to induce ear edema on the right ear of each mouse (4 µg/ear of, in 20 µL of acetone). The left ear (control) of the same mouse received 20 µL of the vehicle (acetone). The CrE of *S. sediforme* was injected 12.5 and 25 mg/kg body wt. in normal saline (D₁ and D₂, respectively) 1h before PMA application. With a reference group treated with diclofenac (10 mg/kg body wt.) and an untreated group (control group). Mice were euthanized by cervical dislocation, 6 hours after PMA application, and a disc (6-mm diameter) was removed with a metal punch from each ear and weighed. Ear edema was calculated by according to the following equation:

$$\text{Edema weight} = \text{the weight of right disc (treatment)} - \text{the weight of the left disc (vehicle)}$$

Inhibition percentage was expressed as the reduction in weight.

Superoxide scavenging activity

Enzymatic assay: Superoxide radical (O₂⁻) which generated by the xanthine/xanthine

oxidase (X/XO) are able to reduce cytochrome c. The effects of *S. sedifforme* extracts on the generation of O_2^- were determined according to the method of Selloum *et al* [8] by using a purified XO from bovine milk with specific activity of 2055.05 nmole/min/mg of enzyme. Purification of bovine milk XO was carried out according to Baghiani *et al* [9]. The reaction mixture, in air-saturated phosphate buffer (50 mM, pH 7.4) supplemented with EDTA (0.1 mM), contained 100 μ M of xanthine (substrate) and cytochrome c (25 μ M), and corresponding concentration of each extract (CrE, ChE and EaE) diluted in phosphate buffer. One min after the addition of enzyme, reduced cytochrome c was spectrophotometrically determined at 550 nm against blanc (enzyme-free mixture). The amount of generated superoxide was calculated using an extinction coefficient $\epsilon_{550} = 21100 \text{ M}^{-1} \cdot \text{cm}^{-1}$. The results are expressed as extract concentration that inhibits the reduction of the moiety amount of cytochrome c (IC_{50}).

Non-enzymatic Assay (NBT test): Superoxide radicals were generated by the PMS/NADH (phenazinemethosulfate/nicotinamide adenine dinucleotide) system according to the method described by Ani *et al* [10]. The reaction mixture consisted of 3 mM of β -NADH, varying concentrations of each *S. sedifforme* extracts, 1 mM of nitrotetrazolium blue chloride (NBT) and 0.3 mM of PMS. All samples were dissolved in phosphate buffer 0.1 M, pH 7.8. The reaction was conducted at 25 °C (room temperature) for 2 min and initiated by the addition of PMS for 3 min. The variation of *the absorbance* at wavelengths of 560 nm was measured. Decrease in absorbance in the presence of various plants extracts indicated the superoxide scavenging activity. Superoxide quenching activity (%) of was calculated as in Eq 1.

$$\text{Superoxide quenching (\%)} = \{100(A_c - A_s)/A_c\} \dots\dots\dots (1)$$

where A_c is the control absorbance, and A_s is the absorbance in the presence of extracts. Evaluating the superoxide scavenging activity of extracts was based on IC_{50} (concentration required to cause a 50 % inhibition).

Evaluation of metal chelating activity

The chelating of Fe^{2+} (ferrous ions) by plant extracts was estimated as described by Le *et al* [11]. Briefly, different concentrations of *S. sedifforme* extracts were added to a 0.05 mL $FeCl_2$ solution (0.6 mM) and left for incubation at 25 °C for 5 min. The reaction, left standing at 25 °C for 10 min, after adding of ferrozine (0.1 mL, 5

mM). Absorbance was then measured spectrophotometrically at 562 nm and the percentage of ferrous iron-chelating effect was calculated using Eq 2.

$$\text{Ferrous iron-chelating effect (\%)} = \{100(A_c - A_s)/A_c\} \dots\dots\dots (2)$$

where A_c is the control absorbance of all samples without extracts, and A_s is the absorbance in the presence of extracts or EDTA as reference chelators. The effective concentration which produce 50 % of Fe^{2+} ions chelating (EC_{50}) was calculated.

Statistical analysis

The results are expressed as mean \pm SD or SEM. Wherever applicable, the results were subjected to ANOVA (one-way analysis of variance), Dunnett's and multiple comparison test used to compare between extracts and standards, where the variations between extracts to each other were determined by Tukey's multiple comparison test, using GraphPad program, p value ≤ 0.05 was considered as significant.

RESULTS

Total phenolic content

The content of polyphenols decreased in the following rank order: EaE > ChE > CrE (Table 1).

Table 1: Total polyphenol content of *S. sedifforme* extracts

Extract	Total phenols content (mg AG-Eq/g)
CrE	78.24 \pm 1.15
ChE	100.54 \pm 1.78
EaE	107.43 \pm 1.41

Anti-inflammatory effect of *S. sedifforme* extract

The results showed that the edema weight of the control group was 7.26 \pm 0.15 mg, it was reduced by the diclofenac to 4.44 \pm 0.13 mg and reduced by the CrE D₁ (12.5 mg/Kg) and D₂ (25 mg/kg) to 4.66 \pm 0.23 mg and 4.46 \pm 0.20 mg, respectively. The CrE of *S. sedifforme* (D₁ and D₂) present an inhibition percentage of inflammation (%I) equal 35.81 \pm 3.18 % and 38.57 \pm 2.80 %, respectively. The effect of the CrE was comparable to that of diclofenac used as a reference drug (%I = 38.84 \pm 1.87 %). However, the inhibition activity of *S. sedifforme* extract is not significant ($p \leq 0.05$) compared to the group treated with diclofenac.

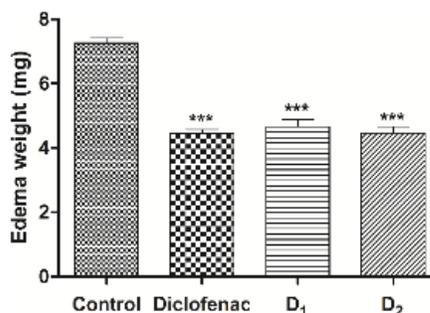


Figure 1: Anti-inflammatory effect of *S. sediforme* CrE; D₁ and D₂ (12.5 and 25 mg/kg dose, respectively) reduced ear edema induced by PMA in mice. Values are mean \pm SEM (n = 8). Comparisons of groups are made with respect to control group; *** $p \leq 0.001$

Superoxide scavenging activity

Enzymatic assay (Cytochrome c test): Under the experimental conditions, the amount of $O_2^{\cdot-}$ generated by XO per minute in the absence of extracts was 10.86 ± 0.02 mM. The results showed that the scavenging effect of *S. sediforme* extracts was in a dose dependent manner (Figure 2 A), this effect was decrease in the following order: CrE ($IC_{50} = 0.063 \pm 0.005$ mg/ml), ChE (0.173 ± 0.014 mg/ml) and EaE (0.425 ± 0.002) (Figure 2 B).

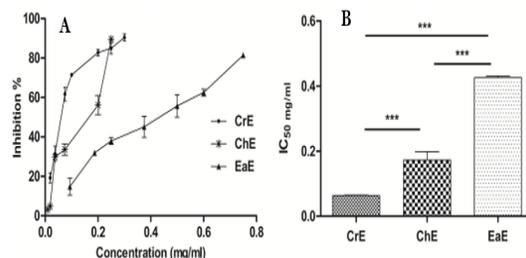


Figure 2: Superoxide scavenging activity of *S. sediforme* extracts using Cytochrome c test. Kinetic competition between the extracts and $O_2^{\cdot-}$ generated by the xanthine/xanthine oxidase system (A), and the half maximal inhibition of cytochrome c activity concentration (IC_{50}) (B). Values are expressed as mean \pm SD (n=3); *** $p \leq 0.001$

Non-enzymatic activity

The results showed that *S. sediforme* extracts had exhibited a scavenging activities in a dose-dependent manner (Figure 3 A). The lower IC_{50} value indicated the greater scavenging activity (Figure 3 B), CrE had the strongest superoxide scavenging activity with IC_{50} equal 0.178 ± 0.006 mg/mL, followed by ChE and EaE (0.505 ± 0.003 and 0.515 ± 0.002 mg/mL, respectively).

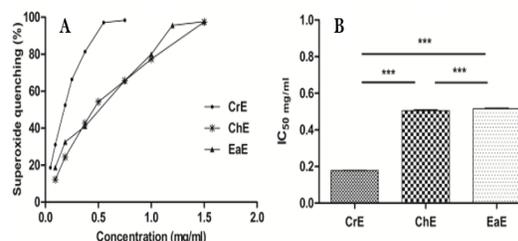


Figure 3: Superoxide quenching activity of *S. sediforme* extracts using NBT test. The kinetic of superoxide-scavenging effect (A) and the inhibitory concentration of 50% (IC_{50}) (B), where $O_2^{\cdot-}$ was generated by NADH/PMS system. Values are expressed as mean \pm SD (n=3); *** $p \leq 0.001$

Metal chelating activity

The effect of *S. sediforme* extracts on Fe^{2+} and ferrozine complex formation is shown in figure 4. The chelating ability of extracts increased with concentrations (Figure 4 A). The inhibition of the formation of ferrous and ferrozine complex by *S. sediforme* extracts, indicate a chelating activity of ferrous ion before ferrozine. EDTA exhibited strong chelating ability of ferrous ion ($EC_{50} = 5.887 \pm 0.004$ μ g/mL) compared with those of phenolics extracts (Figure 4 B). ChE has an excellent chelating ability ($EC_{50} = 0.397 \pm 0.001$ mg/mL), followed by EaE and CrE (0.526 ± 0.047 mg/mL and 0.985 ± 0.007 mg/mL, respectively).

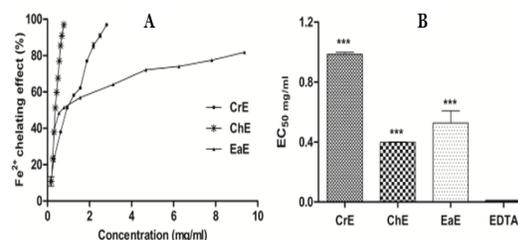


Figure 4: Ferrous iron-chelating ability of *S. sediforme* extracts. The kinetic of chelating effect of extracts (A) and EC_{50} , effective concentration of extracts and EDTA for 50% of chelating activity (B). Results are expressed as mean \pm SD (n = 3). Comparison of extracts was realized against EDTA; *** $p \leq 0.001$

DISCUSSION

High phenolic content of plants have been popularly used for the treatment of many diseases. Phenolic compounds such as phenolic acid and flavonoids possess diverse biological activities (anti-inflammatory and anti-atherosclerotic activities) [12].

Application of PMA induced an inflammatory response and caused the activation of protein kinase C (PKC) with a transient increase in

prostanoid production associated [13]. This reaction reaches a maximum after few hours (6h) and subsides after 24h, multiple applications of PMA produce a more prolonged inflammatory response characterized by inflammatory cell influx, ear edema and epidermal cell hyperplasia [14]. In the present study, crude extract of *S. sediforme* showed an anti-inflammatory effect and reduced ear edema induced by PMA in mice. *S. sediforme* extract (D₁ and D₂) achieved a high degree of anti-inflammatory activity, the two doses of CrE presented approximately the same degree of inhibition. However, the effect was comparable with that of diclofenac.

The superoxide anion is formed by the univalent reduction of triplet-state molecular oxygen (³O₂). This process is mediated by redox-reactive compounds; enzymatically (NADPH oxidases and XO) or non-enzymatically. A number of substances such as nitrate and oxygen can act as hydrogen acceptors for XO; it has been mentioned that cytochrome c is also reduced by XO/cytochrome c system [15].

The superoxide scavenging activity of *S. sediforme* extracts was measured using the ability of these extracts to scavenge superoxide anion radicals (O₂^{•2}) generated by the xanthine/xanthine oxidase system, the amount of generated superoxide was determined by measuring the reduction of cytochromec⁺³. CrE presents a strong superoxide scavenging capacity nearly, 3-folds and 7-folds higher than that of ChE and EaE, respectively. Other researchers showed that EaE of *Tamus comminus* was the most potent scavenger of superoxide anion radical [16].

The inhibition of cytochrome c reduction is due to dual effects of *S. sediforme* extracts. First, these compounds inhibit the XO activity and secondly, some compounds can scavenge superoxide radicals. In order to determine whether inhibition of cytochrome c reduction was due to the inhibition of xanthine oxidase or to the superoxide scavenger activity, a non-enzymatic system of superoxide generation was used.

The non-enzymatic assay (NBT test) was based on the capacity of *S. sediforme* extracts to inhibit the reduction of NBT in the NADH–NBT–PMS system. In this system, the PMS–NADH coupling reaction reduces NBT and produce the superoxide anion from dissolved oxygen from [10]. The capacity of CrE to inhibit the reduction of NBT was approximately, 3-folds higher than that of ChE and EaE. Boumarfeg *et al* [17] have tested the extracts of *Teucrium polium* by the same test and the results demonstrated that this

plant had a concentration-dependent manner scavenging activity by neutralizing superoxide radicals in the same order as shown in the results obtained by enzymatic method (Cyt C). Synergic effect could be responsible for the improvement of these effects. This theory could explain the strong activity of CrE despite its low polyphenols content.

The important role of ferrous ions as catalysts of oxidative processes, leading to the formation of many free radicals via Fenton reaction. It is reported that the generated free radicals can cause the production of oxyradicals, lipid peroxidation and DNA damage. Ferrous ions (Fe²⁺) chelating molecules may cause important antioxidative effects by retarding metal-catalyzed oxidation [18]. The formation of the colored complex, Ferrous with Ferrozine, was disrupted by chelating agent leading to a decrease of color [11].

Ethylenediaminetetraacetic acid (EDTA) can chelate ferrous ion in the center and block the Fe²⁺–ferrozine complex formation [19]. Polyphenolic compounds should be able to chelate transition metals because of the high charge density of the phenoxide group generated on deprotonation [20]. ChE of *S. sediforme* contains a high content of polyphenols and its strong activity could be explained by the aglycone nature of its flavonoids.

CONCLUSION

The CrE of *S. sediforme* present a powerful *in vivo* anti-inflammatory effect. The treatment of mouse skin with a PKC activator, such as PMA, induces the formation of free radicals and for that reason the *in vitro* antioxidant effect of *S. sediforme* extracts was evaluated. The examined extracts have a high scavenging activity for the superoxide anion *in vitro* using xanthine/xanthine oxidase system. However, this activity of *S. sediforme* extracts may be due to the enzymatic inhibition and/or superoxide scavenger effect, for this reason an NBT assay was carried out to prove the scavenging effect of this plant. All extracts showed a moderate ferrous ion chelating ability. These results could represent an important contribution for the understanding of the anti-inflammatory effects of this plant and its use in the traditionally medicine.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities (Laboratory of Applied Biochemistry, Faculty of Nature and Life Sciences, Univ. Setif) pertaining to claims relating to the content of this article will be borne by the authors.

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