Research Article

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Withania frutescens: Chemical characterization, analgesic, anti-inflammatory, and healing activities

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Abstract: *Withania frutescens* (*W. frutescens*) is a medicinal plant that is largely used in the Morrocan pharmacopeia for disease treatment. This work was conducted to investigate the chemical characterization, analgesic, anti-inflammatory, and healing activities of *W. frutescens*. The chemical characterization of *W. frutescens* extract was done using HPLC; the anti-inflammatory test was performed with doses 300, 400 and 450 mg/kg, and the healing activity was assessed using two creams (extract 5% and extract 10%). Phytochemical analysis revealed the presence of phenolic compounds. The results of the anti-inflammatory test were more pronounced when compared with the reference drug with a maximum inhibition percentage of 82.20% ± 8.69 obtained at the dose of 450 mg/kg. Local application of 10% plant cream induced 80.17% ± 7.89 of inflammation inhibition when compared with the indomethacin drug 92.33% ± 11.27. The studied plant extract showed a promising healing activity with the following percentage: 99.03% ± 0.76 (extract 10%), 98.61% ± 1.91 (extract 5%), and 57.43% ± 2.97 (control); meanwhile, the value reached to 100% ± 0.02 for the drug that was used as a reference within the first 2 weeks. The plant studied in this work would be a promising source for conceptualizing effective drugs against inflammatory diseases.

Keywords: healing activity, anti-inflammatory activity, HPLC, creams, extract

1 Introduction

Morocco is a very old nation with a very rich civilization and culture and related to herbal medicine [1]. Traditional Moroccan medicine was born long before the arrival of the Arabs. Berbers communities used therapies that are still practiced today. The empirical use of different traditional herbal preparations is therefore extremely important for effective candidate plants for further ethnobotanical and pharmacological studies [2]. In addition, some active ingredients derived from medicinal plants are applied in many sectors, such as cosmetics, perfumery, aromatherapy, pharmaceuticals, and food processing as well as in the hygiene and sanitary industries [3]. Other industries (agri-food, pharmaceutical, perfumery, and aromatherapy) are looking for alternative natural and ecological bioactive sources. The therapeutic effects of medicinal plants pave the way for their use in medicine and the pharmaceutical industries in the prevention or treatment of certain infectious, cardiovascular, inflammatory, neurodegenerative, cancerous, and other diseases. The use of alternative medicine practice is explained by several reasons including the high cost of modern medicine, socio-cultural practices of populations, and the need for treatment options for resistant pathogens using alternative agents derived from plants [4].

Withania frutescens is frequently used by the indigenous population to treat different diseases and clinical symptoms using different preparation methods.

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To look for a potential scientific basis that may enable to support or to criticize the traditional use of *W. frutescens*, the current work was conducted to screen some pharmacological activities of *W. frutescens* collected from the Northern Morocco.

2 Materials and methods

2.1 Plant material

The plant material used in this study consists of foliar parts of *W. frutescens* (Figure 1) collected on April 2018 (the season when development and flowering are at their peak) – 34.01300500°N and 4.75206833°W. The botanical identification was carried out by Professor Amina BARI (Faculty of Science – Fez, Morocco), and the specimens were deposited in the herbarium of the faculty under voucher number BPRN69. The harvested leaves were then rinsed and dried out at a temperature of 35°C. The powder obtained was extracted by hydro-ethanolic maceration consisting of 70% ethanol and 30% distilled water with a proportion (g/mL) of 1/10 for 24 h [5]. The preparation of creams 10% (extract 10%) and 5% (extract 5%) was based on neutral petroleum jelly to make a local application of the crude extract of the plant studied.

Figure 1: The biological form of the plant *Withania frutescens* L.

2.2 Animals used

Male rats obtained from the ECWP (Emirates Wildlife Propagation Center) of Missour (Eastern Morocco), weighing from 100 to 150 g; animals were housed in cages (five rats/cage) in a controlled environment with a temperature maintained at 22 ± 2°C and a light-dark cycle (12 h) for 2 weeks as the acclimatization period. The procedures used in the current research work were in accordance with the internationally accepted Guide for the Care and Use of Laboratory Animals. The Animal Ethics Review Committee of the Faculty of Sciences of Fez, Morocco, reviewed and approved this study.

2.3 Determinations of total polyphenols

The plant extract (100 µL) was properly diluted in a test tube initially containing 6 mL of distilled water, and 500 µL of Folin reagent was then added and stirred. After 5 min, 20% Na_2CO_3 solution (1.5 mL) was added. The solution was completed to 10 mL with distilled water. After a 2 h incubation at room temperature, the absorbance was measured with a blank made from distilled water using a Selecta UV-Visible spectrophotometer. A calibration curve at different concentrations of gallic acid was plotted. The total phenol contents in the extracts were expressed in milligrams (mg) gallic acid equivalent per gram (g) of the dry matter weight (mg EAG/g MS) [6].

2.4 Leaf extract analysis by high-performance liquid chromatography (HPLC)

The *W. frutescens* foliar extract was analyzed by reverse-phase liquid chromatography using the HPLC chain, equipped with a UV-visible detector, a quaternary pump type LC A20, and a manual injector-type Ryodine. A Wakosil II type C18 column was used. The flow rate of the mobile phase was 1 mL/min in a ternary gradient mode composed of ACN-MeOH/water acidified to 0.2% orthophosphate. A volume of 20 µL was injected at room temperature. The standards were injected at concentrations of 1 mg/mL to determine whether or not they were present in the extract. Both the extract and the standards (ferulic acid, gallic acid, apeginin, epicatechin, epicatechin gallate, *p*-coumaric acid, quercetin, rosmarinic acid, rutin, vitamin C, caffeic acid, luteolin, tannic acid, and syrinic acid) are filtered through the 0.45 µm porosity syringe filter. The UV detector was switched on at least 1 h before analysis. The chromatographic

column must be conditioned for at least 15 min with the elution solvent of initial composition (water 0.2% H_3PO_4 (V/V)/methanol/acetonitrile 96/2/2 (V/V/V)). A first empty gradient chromatographic test should always be carried out beforehand (to ensure that there are no co-elution interference peaks), by injecting 20 µL methanol/water 80/20 (V/V) into the HPLC system.

2.5 Anti-inflammatory activity

The evaluation of anti-inflammatory activity of our ethanol extract was conducted using the Winter method [7]. Groups of five rats were formed. The extract was administered orally at doses of 300, 400, and 450 mg/kg; the same extract was administered locally (local application) at doses of 5% and 10%, 30 min and 1 h, respectively, before the injection of carrageenan 1% (NaCl 0.9%) under the plantar fascia of the right hind leg; the circumference of the paste was measured before the injection of carrageenan, then after each hour from the third hour to the sixth hour after administration of the subcutaneous carrageenan. Two inflammatory references were used: one oral (Diclofenac 1%) and the other dermal (Indomethacin/ointment), and the percentage of inflammation inhibition was then calculated according to the following formula:

$$\%\ \text{inhibition} = \frac{(S_t - S_0)\ \text{Control} - (S_t - S_0)\ \text{treated})}{(S_t - S_0)\ \text{Control}} \times 100$$

S_0 is the circumference before carrageenan injection and S_t is the circumference at a given time after administration of carrageenan.

2.6 Analgesic activity

The analgesic test was determined by estimating the number of abdominal contortions induced by intraperitoneal (I.P.) injection of acetic acid (0.7%) (torsion test). Groups of five rats were formed. One and a half hours after oral administration of the extracts (450 mg/kg), rats were dosed with 10 mg/mL of 0.7% dilute acetic acid by the I.P. route. The painful syndrome was characterized by stretching movements of the hind legs and twisting of the dorsal-abdominal muscles. After 5 min (latency time) of the injection of the acetic acid saline solution, we counted for each rat, the number of twists for the next 30 min [8].

2.7 Healing activity

The healing effect was evaluated using the thermal burn method. The experimental protocol used in this study was consistent with the standard recommendations for the care of animals used in research and teaching [9]. The animals were randomly divided into five lots of five rats each, the different lots received locally either neutral cream (excipient), or extract 5%, or extract 10% and the reference healing agent sulfadiazine silver 1% as follows:

- A control group does not suffer thermal burns and does not receive any treatment.
- A positive control group of rats receiving locally 1% sulfadiazine silver (standard healing).
- A negative control group of rats treated with neutral cream (contains no anti-healing molecules).
- An experimental group of six rats each receiving locally the 5% extract mixed with the neutral cream.
- An experimental group of six rats each receiving locally 10% extract mixed with neutral cream.
- The burns were carried out on the back (the dorsal region of the rat) previously shaved, by a metal heated in boiling water (100°C) until thermal equilibrium (reached at 5 min, Figure 2). After heating, it was removed from the water, quickly wiped off, then applied without pressure for 20 s [8–10]. After the induction of burns, the rats in the treated groups were each given a topical application of the product intended for their batch once a day for 20 days.

The photographs taken were processed by the image processing software ImageJ® (National Institute of Mental Health, Bethesda, Maryland, USA), which we adapted for our study. This program can provide accurate measurements of microscopic lengths and widths in any unit system. To deduce the percentage of wound contraction, the average surface area of the four wounds in a batch was calculated and compared with the surface area of the burn on the first day, using the following mathematical equation [11]:

$$\%\ \text{contraction} = \frac{\text{size of the initial wound } J_0 - \text{wound size at } J_n}{\text{size of the initial wound}} \times 100$$

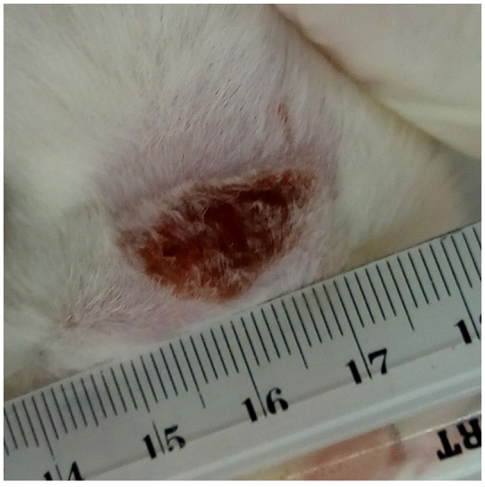

Figure 2: The burned area in first-day rats.

The re-epithelialization period was estimated by the number of days required for total wound closure without any residual sharp injury [12].

2.8 Statistical analysis

Statistical analysis was performed using one-way ANOVA using GraphPad Prism with 95% confidence limits ($p < 0.05$).

3 Results and discussion

3.1 Determination of total polyphenols

After adding the Folin-Ciocalteu reagent to the solution to be studied, a blue color was obtained; the intensity of which depends on the concentration of total polyphenols (TPC) present in the solution. The determination of these polyphenols was based on a standard range using gallic acid as the standard. The quantification of polyphenols was done according to a linear calibration curve ($Y = 0.0425X + 0.2218$; $R^2 = 0.985$) performed by gallic acid at different concentrations under the same sample conditions, and the content of the total polyphenols obtained was 21.704 ± 0.138 mg GAE/g; this concentration was important when compared with the found data [5].

3.2 Leaf extract analysis by high-performance liquid chromatography (HPLC)

The analysis of the hydro-ethanol extract of *W. frutescens* was carried out to identify different phenolic compounds of the plant (Figure 3), which confirmed the presence of polyphenols and phenolic acids that

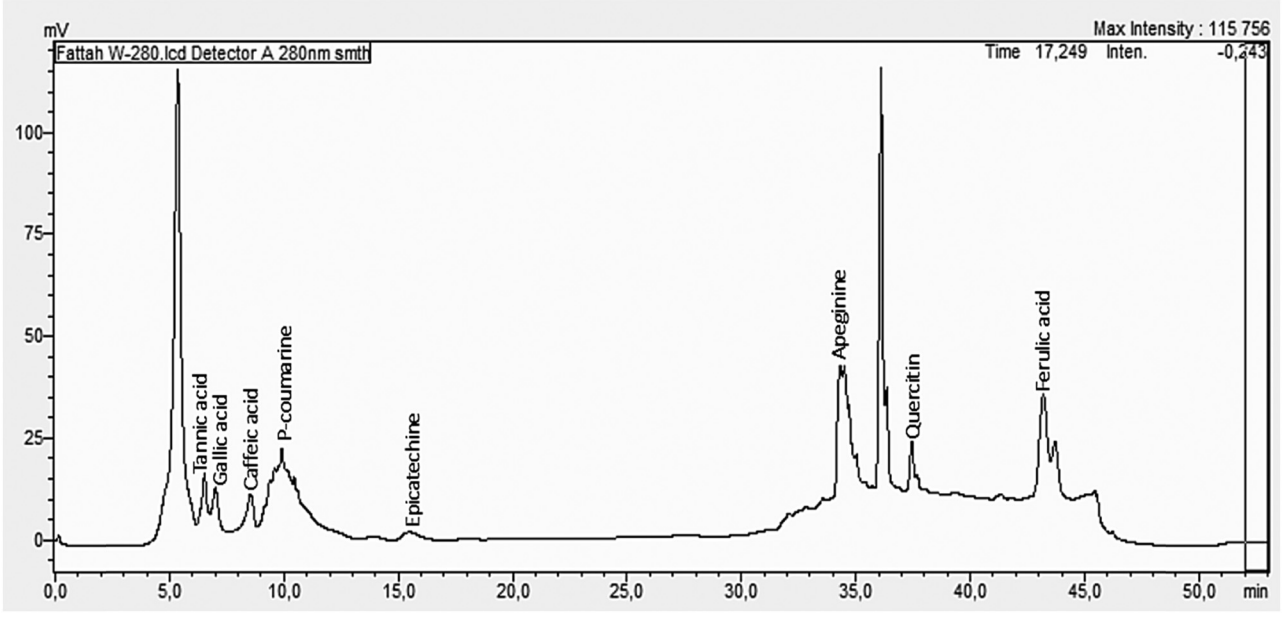

Figure 3: HPLC chromatographic profile of *W. frutescens* extract.

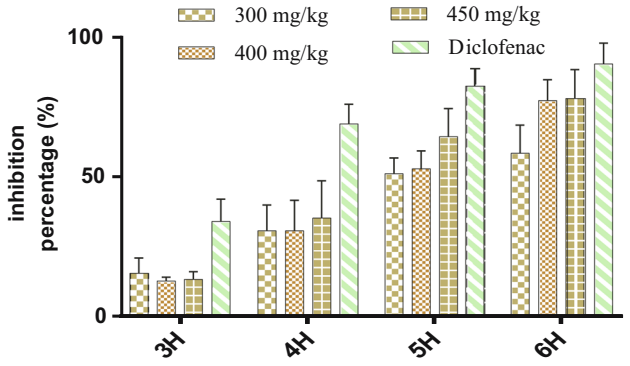

Figure 4: Effect of *W. frutescens* extract administered orally at different doses and 1% Diclofenac on carrageenan-induced edema in rats. Values are expressed as average ± SEM.

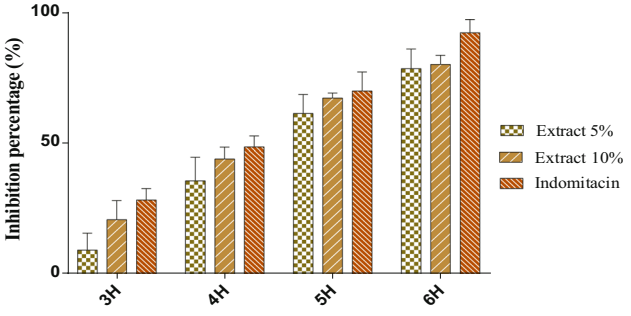

Figure 5: Effect of *W. frutescens* ethanolic extract applied dermally to 5% and 10% creams and Indomethacin on carrageenan-induced edema in rats. Values are expressed as average ± SEM.

were previously revealed by phytochemical screening. It should be noted that during our analyses, not all compounds were identified due to the lack of adequate standards. According to the results of phytochemical analyses, we note the presence of flavonoid compounds (epicatechin and apigenin) and phenolic acids (caffeic acid, ferulic acid, and *p*-coumaric acid) identified by HPLC. Thus, polyphenols and flavonoids have attracted considerable interest because of their broad-spectrum and the diversity of their biological effects (antimicrobial and anti-inflammatory) [13,14]. The phenolic acids were also revealed as hydroxycinnamic compounds and their derivatives that possess antioxidant activities, anti-collagenase, anti-inflammatory, and anti-tyrosinase, as well as protective effects against ultraviolet (UV) rays. HPLC analysis of this extract showed the presence of two hydroxycinnamic acids: *p*-coumaric acid and caffeic acid [15]. *p*-Coumaric acid is considered to be a phenolic acid of cinnamic acid origin which is synthesized from tyrosine and phenylalanine. It is a major precursor that has a role in the synthesis of other phenolic compounds, such as caffeine and ferulic acids as reported in the earlier literature as *p*-coumaric acid and its conjugated exhibiting antimicrobial, antioxidant, and anti-inflammatory properties [16]. On the other hand, caffeic acid is one of the most common phenolic acids that possess medicinal properties such as antioxidant, antimicrobial, anti-inflammatory, anti-tumor, and anti-diabetic drugs [17,18].

3.3 Anti-inflammatory activity

Inflammation induced by carrageenan injection is widely used as a test to evaluate the peripheral anti-inflammatory effects. Subcutaneous injection of carrageenan induces the secretion of pro-inflammatory tissue chemical mediators such as prostaglandins, histamine, serotonin, and bradykinin [21]. The results obtained showed that the extract of *W. frutescens* administered orally has an anti-inflammatory effect, with a maximum inhibition of 82.20% ± 8.69 at 450 mg/kg followed by 78.20% ± 10.53 at 400 mg/kg mean while Diclofenac 1% inhibited edema by 90.28% ± 7.50. These findings suggest that the ethanol extract of *W. frutescens* possesses a lower anti-inflammatory activity when compared with the reference (Figure 4).

The ethanol extract of *W. frutescens* administered locally through the skin at doses of 5% and 10% appears to have a greater anti-inflammatory effect than Indomethacin; the 10% cream more significantly reduced the swelling of the legs injected by carrageenan, with a maximum inhibition reaching 80.17% ± 7.89. In the 5% cream, the anti-inflammatory activity was less pronounced and the maximum inhibition reached only 78.57% ± 16.83 (Figure 5). At the same time, the drug (Indomethacin ointment) used as a maximum positive control of inhibition reached 92.33% ± 11.27.

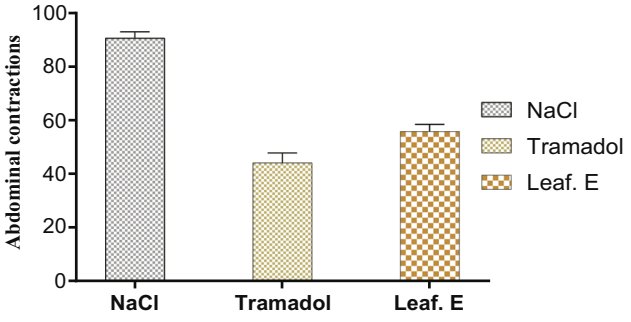

Figure 6: Analgesic activity of the studied extract compared to the tramadol and control group $n = 5$; ***$p < 0.001$.

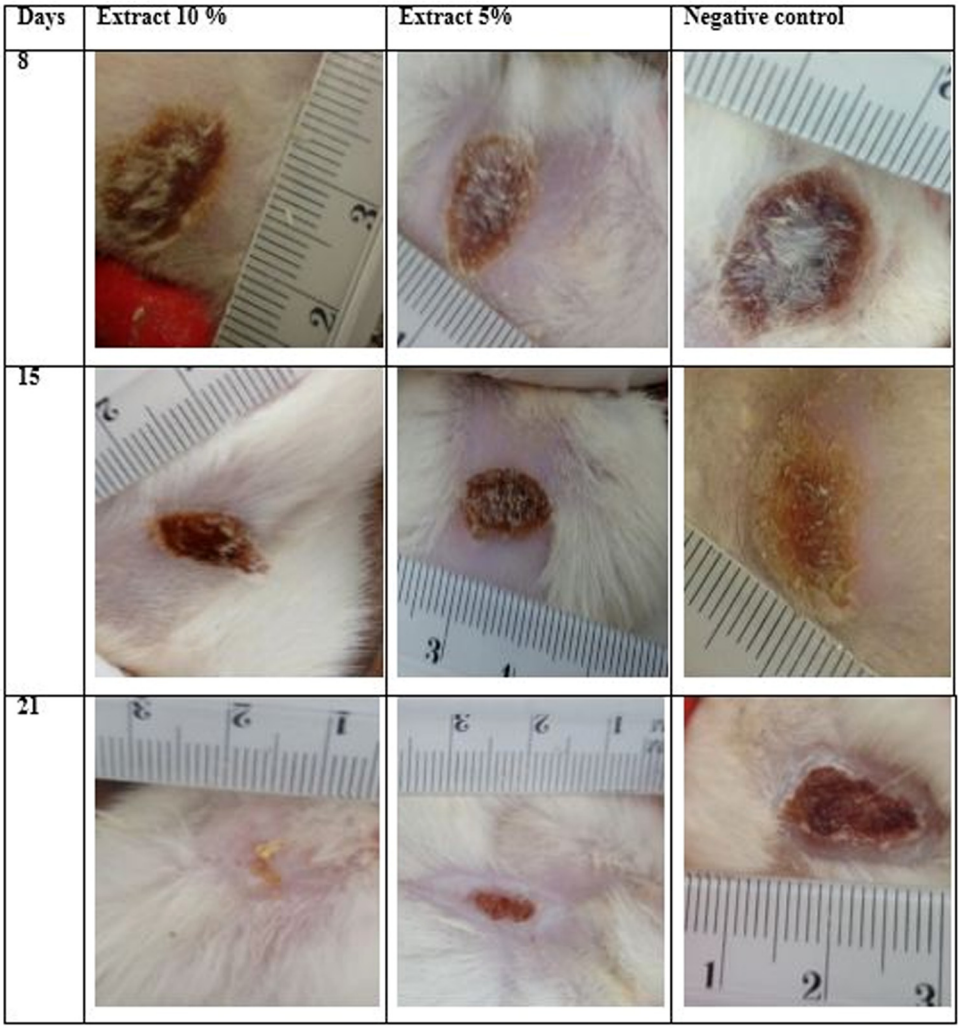

Figure 7: Aspects of group burns: positive control, extract 10%, extract 5%, and negative control during treatment days.

This anti-inflammatory activity of the ethanol extract, administered either orally or locally, can be explained on the one hand by the difference in chemical composition and on the other hand by the likely existence of polar phenolic compounds. In addition, phenolic compounds represented mainly by flavonoids and tannins are currently of great scientific interest as they are considered to be powerful antioxidant, antimicrobial, and anti-inflammatory agents [20–22]. In addition, this plant has a wealth of phytochemical compounds (tannins, mucilage, alkaloids, coumarins, and free quinone) [5]. The 10% cream showed significant inhibition of induced rat leg edema following carrageenan injection with maximum efficacy at 5 and 6 h. Indeed, the injection and induction of carrageenan caused the secretion of several chemical mediators which were responsible for the inflammatory process. This inflammatory response was biphasic, with the initial phase lasting about 1 h due to the release of histamine and serotonin, while bradykinin was released in the second phase (1.5–3 h), and prostaglandin biosynthesis occurs after the third hour [21]. These chemical mediators increase the permeability of the blood capillaries in the parts that are inflamed. As a result, exudate escapes from the bloodstream into the interstitial space. This exudate is the cause of localized edema, which in turn compresses nerve endings and thus determines a sensation of pain [23,24]. Taking into account these indications, it was proposed that the observed effect may be due to the ability of the *W. frutescens* extract to inhibit prostaglandin synthesis by the cyclooxygenase route.

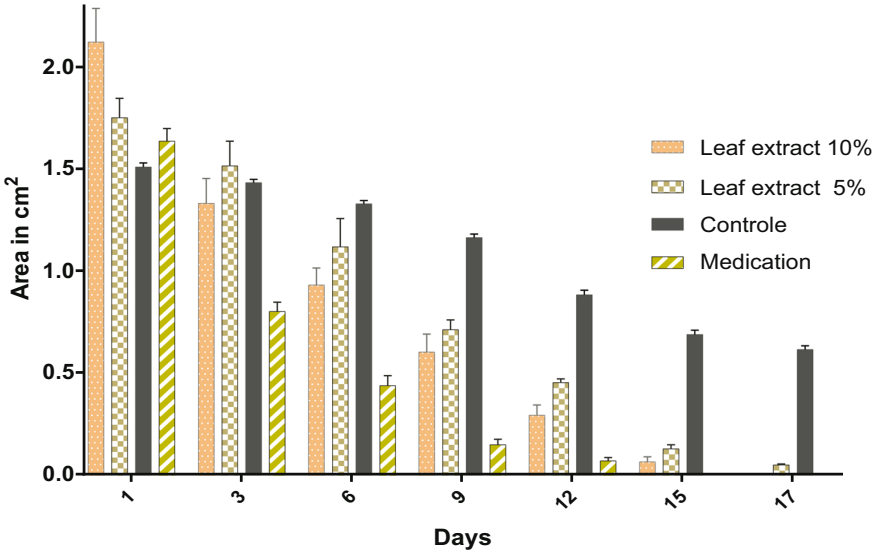

Figure 8: Healing effect of ethanolic extract 5% and 10% *W. frutescens* applied dermally and sulfadiazine silver. Values are expressed as average ± SEM, $p < 0.05$ is considered to be significant.

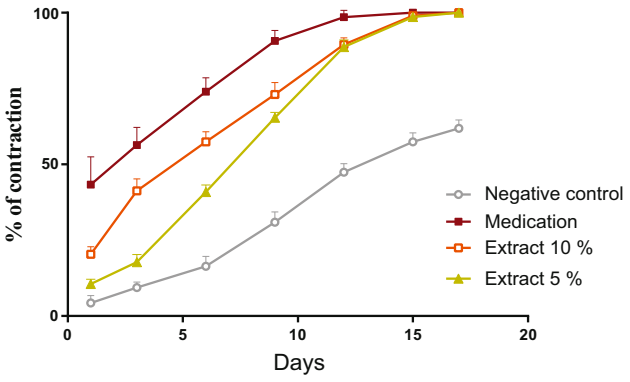

Figure 9: Evolution of the average percentage of animal burns: medication, extract 10%, extract 5%, and negative control.

3.4 Analgesic activity

The evaluation of the analgesic activity of the studied extract of *W. frutescens* was carried out using the acetic acid method. Only the 450 mg/kg, considered to be an effective anti-inflammatory, was studied for its possible analgesic activity, and this dose was the same as the one used of the *Withania somnifera* extract, which belongs to the same family of *W. frutescens* [25]. The results of this test are shown in Figure 6, and it is noted that the abdominal contractions of the rats treated with the extract are significantly lower than those of the control group that received only physiological NaCl solution with 55.60 ± 5.94 and 90.4 ± 5.27, respectively. Rats treated with the reference analgesic have a slightly greater effect than rats treated with the extract with 44 ± 8.09 abdominal contractions, and the percentage of inhibition of the test and reference extract was 51.40 ± 7.71% and 38.58 ± 3.91%, respectively.

Contractions induced by intraperitoneal injection of acetic acid is a method used to study the peripheral analgesic effect of a substance. The pain caused by the injection of the latter is due to the release of serotonin, histamine, bradykinin, and prostaglandins (PGEβα, PGFβα). These mediators induce stimulation of peripheral neurons and then induce an increase in vascular permeability [19,26]. The foliar extract of the studied plant significantly inhibited abdominal torsion in a dose-dependent manner. This analgesic effect can be attributed to the inhibition of the release of chemical mediators that are responsible for abdominal pain.

3.5 Healing activity

One of the objectives of this work was to determine the healing activity of the ethanol extract of *W. frutescens*; to achieve this goal, two types of ointments were formulated with the tested extracts: the first was based on the 10% ethanol extract and the second was based on the 5% extract (Figure 7). The difference in wound surface sizes between the group of *W. frutescens* extract at 5% and 10% on the one hand and those of the negative control groups and silver sulfadiazine is shown in Figure 8.

The evolution of wound size treated with the 10% and 5% extract when compared with the untreated one showed that during the first days after burns, rats from the two groups treated with 5% and 10% extract did not show any significant reduction in the size of the wounds induced by the skin burn compared with the untreated rats. After six days of the first treatment, the lot of the 10% extract recorded a surface area 0.93 ± 0.18 cm^2 smaller than that of the 5% extract (1.11 ± 0.31), matching, respectively, to contraction percentages of 57.43% ± 7.47 and 40.89% ± 5.17, against 16.36% ± 3.26 of the control lot and 73.96% ± 4.59 of the reference drug. After 15 days, the wounds of the 10% and 5% extract batch healed completely, while the control batch still had average surfaces of 0.68 ± 0.04 cm^2 matching to a contraction percentage of 57.43% ± 2.97 (Figure 9).

The healing effect of *Withania* extract at a dose of 10% when compared with the standard (Sulfadiazine silver) showed that wounds treated with sulfadiazine recorded their first size reductions with a percentage of contraction on day 3 of 41.25% ± 8.81; hence, this percentage remains lower than those recorded in rats treated with the 10% extract during the same medication period and that it is about 56.37% ± 5.81. Based on the results obtained, we could confirm that the ointment of *W. frutescens* extract exhibited a dose-dependent healing activity since the surface reductions and retraction percentages recorded in wounds treated by the 10% extract exceed those treated by the 5% extract. Like any natural product, the healing effect of the extract was due to the various phytochemical components of its composition. Ainsiles as natural healing products showed their effects through one of the following mechanisms: antimicrobial effect, anti-inflammatory, antioxidant, stimulation of collagen synthesis, and cell proliferation [27].

4 Conclusion

According to the results obtained in this work, we could confirm that *W. frutescens* growing in Morrocan soil possesses interesting pharmacological properties such as anti-healing, anti-inflammatory, and analgesic, and finally, hoping this study contributes to society that prophylactic agents act against inflammatory diseases.

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Conflict of interest: Authors declare no conflict of interest.

References

[1] Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 2005;27(1):1–93. doi: 10.1016/j.mam.2005.07.008.

[2] Jaz HA. Characterization and biological activities of essential oils of some species of Lamiaceae. Pakistan: University of Agriculture; 2009.

[3] Elmoussaoui A, Jawhari FZ, Bousta D, Bari A. Phytochemical characterization and antioxidant activity of the northern Moroccan species: *Withania frutescens* (L.). Asian J Pharm Clin Res. 2019;12:276–9. doi: 10.22159/ajpcr.2019.v12i6.32119.

[4] Chahmi N, Anissi J, Jennan S, Farah A, Sendide K, ElHassouni M. Antioxidant activities and total phenol content of *Inula viscosa* extracts selected from three regions of Morocco. Asian Pac J Trop Biomed. 2015;5(3):228–33. doi: 10.1016/S2221-1691(15)30010-1.

[5] Winter CA, Risley EA, Nuss GW. Carrageenin-induced Edema in Hind Paw of the rat as an assay for antiinflammatory drugs. Exp Biol Med. 1962;111(3):544–7. doi: 10.3181/00379727-111-27849.

[6] Hernández-Pérez M, Rabanal RM. Evaluation of the anti-nflammatory and analgesic activity of *Sideritis canariensis* var. pannosa in mice. J Ethnopharmacol. 2002;81(1):43–7.

[7] Olfert CBM, William AA. CCPA, Le Conseil canadien de protection des animaux (1993) Manuel sur le soin et l'utilisation des animaux d'expérimentation. Québec: Université de laval; 2011.

[8] Hoşnuter M, Gürel A, Babucçu O, Armutcu F, Kargi E, Işikdemir A. The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury. Burns. 2003;30(2):121–25. doi: 10.1016/j.burns.2003.09.022.

[9] Hamdi Pacha Y, Belkhiri A, Benazzouz M, Benhamza L, Bensegueni L. Evaluation de l'activité cicatrisante suite à des br û lures expérimentales de quelques plantes algériennes. Revue Méd Pharm Afr. 2002;16:1–7.

[10] Cai EZ. Creation of consistent burn wounds: a rat model. Arch Plast Surg. 2014;41(4):317. doi: 10.5999/aps.2014.41.4.317.

[11] Gopinath D, Ahmed MR, Gomathi K, Chitra K, Sehgal PK, Jayakumar R. Dermal wound healing processes with curcumin incorporated collagen films. Biomaterials. 2004;25(10):1911–7.

[12] Thakur R, Jain N, Pathak R, Sandhu SS. Practices in wound healing studies of plants. Evid Based Complement Altern Med. 2011;2011:17. doi: 10.1155/2011/438056.

[13] Guo T, Wei L, Sun J, Hou CL, Fan L. Antioxidant activities of extract and fractions from Tuber indicum Cooke & Massee. Food Chem. 2011;127(4):1634–40. doi: 10.1016/j.foodchem.2011.02.030.

[14] Maimoona A, Naeem I, Saddiqe Z, Jameel K. A review on biological, nutraceutical and clinical aspects of French maritime pine bark extract. J Ethnopharmacol. 2011;133(2):261–77. doi: 10.1016/j.jep.2010.10.041.

[15] Taofiq O, González-Paramás AM, Barreiro MF, Ferreira IC, McPhee DJ. Hydroxycinnamic acids and their derivatives: cosmeceutical significance, challenges and future perspectives, a review. Molecules. 2017;22(2):281. doi: 10.3390/molecules22020281.
[16] Pei K, Ou J, Huang J, Ou S. p-Coumaric acid and its conjugates: dietary sources, pharmacokinetic properties and biological activities. J Sci Food Agric. 2016;96(9):2952–62. doi: 10.1002/jsfa.7578.
[17] Heleno SA, Martins A, Queiroz MJ, Ferreira IC. Bioactivity of phenolic acids: Metabolites versus parent compounds: a review. Food Chem. 2015;173:501–13. doi: 10.1016/j.foodchem.2014.10.057.
[18] Yang WS. IRAK1/4-targeted anti-inflammatory action of caffeic acid. Mediat Inflamm. 2013;2013:2013. doi: 10.1155/2013/518183.
[19] Reanmongkol W, Noppapan T. Antinociceptive, antipyretic, and anti-inflammatory activities of *Putranjiva roxburghii* Wall. leaf extract in experimental animals. J Nat Med. 2009;63:290–96. doi: 10.1007/s11418-009-0336-6.
[20] Chen HQ, Jin ZY, Wang XJ, Xu XM, Deng L, Zhao JW. Luteolin protects dopaminergic neurons from inflammation-induced injury through inhibition of microglial activation. Neurosci Lett. 2008;448(2):175–9. doi: 10.1016/j.neulet.2008.10.046.
[21] Bose A, Mondal S, Gupta JK, Ghosh T, Dash GK, Si S. Analgesic, anti-inflammatory and antipyretic activities of the ethanolic extract and its fractions of Cleome rutidosperma. Fitoterapia. 2007;78:15–520. doi: 10.1016/j.fitote.2007.05.002.
[22] Akindele AJ, Adeyemi OO. Antiinflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus*. Fitoterapia. 2007;78(1):25–28. doi: 10.1016/j.fitote.2006.09.002.
[23] Rousselet MC, Vignaud JM, Hofman P. Inflammation et pathologie inflammatoire. Paris: People's Medical Publishing House; 2005.
[24] Devulder B, Hatron PY. Physiologie de l'Inflammation. Physiologie de l'Inflammation. Cedex, Paris: Masson; 2002. p. 15.
[25] Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. Altern Med Rev. 2000;5(4):334–46.
[26] Vanderlinde FA. Evaluation of the antinociceptive and anti-inflammatory effects of the acetone extract from *Anacardium occidentale* L. Braz. J Pharm Sci. 2009;45(3):437–42. doi: 10.1590/S1984-82502009000300008.
[27] Bahramsoltani R, Farzaei MH, Rahimi R. Medicinal plants and their natural components as future drugs for the treatment of burn wounds: an integrative review. Arch Dermatol Res. 2014;306(7):601–17. doi: 10.1007/s00403-014-1474-6.