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Research Article

WOUND HEALING POTENTIAL OF ETHYL ACETATE OF SEEDS EXTRACT FROM *CELTIS AUSTRALIS*

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ABSTRACT

The present study was aimed to evaluate the wound healing activity of the ethyl acetate extract of seeds of *C. australis* using incision wound model in Sprague-Dawley rats. The healing effect produced by the plant extract, was assessed by measurement of wound sizes every 2 days until total recovery. Wounds were induced in rats divided into three groups of 5 animals each (Control, group treated with plant extract, group treated with standard ointment (Madécassol®)).

Our results showed a general decrease of wound area upon application of the treatment and with time. The ethyl acetate extract of *C. australis* significantly promoted very significantly ($P<0.01$) the wound healing activity. The wound healing rate is accelerated (41%, 63% and 94% at day 2, 4 and 6 respectively) when compared to that of control group (23%, 38% and 47%). The wound healing rate of plant extract is similar to that exhibited by standard ointment (40%, 64% and 97%). To our knowledge it is the first time that this activity is reported with Celtis australis extract. The plant extracts yielded higher polyphenolic compounds content followed by polyphenols (367,96mg/Kg of seeds), flavaonoids (11,11mg/Kg of seeds) and condensed tannins (516,8mg/Kg of seeds). A positive linear correlation was established between these compounds and the wound healing property. The results obtained from this study revealed promising wound healing property of ethyl acetate extract of seeds from *C. australis* and provide a scientific rational for the traditional uses of this plant.

Keywords: *C. australis;in vitro* study; Polyphenols; Rats; Wound healing.

INTRODUCTION

Woundis defined as disrupt of anatomical integrity from violence or trauma. Wound healing is a natural and complex phenomenon that induces an important biological process involving tissue repair and regeneration.[1, 2]The process involves three different phases including haemostasis, inflammation and regeneration.[3]Thus, when an injury occurs, the vascular integrity of the injured area is disrupted leading to extravasationsof blood into the surrounding tissue. The haemostasis stage leads to the formation of thrombus by blood coagulation thanks to platelets adhesion/accumulation. The inflammatory step involves the appearance of granulocytes to clear the wound area by destroyinginvading microorganisms. The regeneration or repair stage is a complex process characterized by endothelial buddingin the nearby blood vessels forming new capillaries (neovascularization) that penetrate and nourish the injured tissues (re-epithelization).[4]

The use of medicinal plants is getting increasingly popular as they arebelieved to be beneficial and free from side effects. Therefore, traditional knowledge of medicinal plants and their use by populations are not only useful for conservation of traditional knowledge and biodiversity but also for primary health care and drug development. In Morocco, the use of traditional medicine for the treatment of various disorders and illnesses has been practiced for generations and a large part of the population in the country uses medicinal plants in its day to day healthcare needs.[5-7]However, the rationale for theutilization of medicinal plants has rested largely on long-term clinical experience

withlittle or no scientific data on their properties, safety and efficiency

Cannabaceae is a large family, containing about 15 genera and 200 species. The largest genus, Celtis, includes about 60 species. Among these species is *Celtis Australis* (known as the European nettle tree, Mediterranean Hackberry or honeyberry) a deciduous tree endemic to southern Europe, North Africa, and southwestern Asia.[8, 9]In Moroccan traditional medicine, *C. australis* commonly called "Taghzaz" is used to treat gastro-intestinal ailments.[7]In Indian traditional medicine, the paste obtained from the bark of *C. australis* is considered as an important remedy for bone fracture and also applied to pimples, contusions, sprains and joint pains. The decoction of both leaves and fruits is used in the treatment of amenorrhea, heavy menstrual and inter-menstrual bleeding, diarrhea, dysentery and peptic ulcers.[10]

The objective of the present study is to evaluate the wound healing activity of ethyl acetate extract obtained from seeds of *C. australis*.

MATERIAL AND METHODS

Plant material

C. Australis was collected during spring 2015 in the El Jadida city (Morocco). Plant material was authenticated by a specialist. A voucher specimen (reference CA1/15) is kept on file in our laboratory.

Preparation of plant extract

The air-dried seeds (500g) of *C. australis* were powdered mechanically and sieved using a fine muslin cloth. The obtained powder is exhaustively extracted by soxhlet usingethyl acetate solvent at 35°C, giving150g of the crude preparation. It was then filtered and concentrated using a rotary evaporator under reduced pressure at 35°C to prevent thermal decomposition of labile compounds. The crude extract (45g) is stored at +4°C until use.

Animals

Male Sprague-Dawley rats (weighing 160-180 g) were used for all experiments. They were maintained under standard laboratory conditions (ventilated cage system, temperature 25 ± 2°C, relative humidity 60 ± 10 %, 12 h light/12 h dark cycle, food and water *ad libitum*).

All protocols performed in this study were conducted in accordance with internationally accepted principles for use and care of laboratory animals.

Phytochemical screening

Total polyphenols

Quantification of total polyphenols was made using a modified method.[11] A volume of 20µl of each extract (0.5g/ml) was mixed with 1.6ml of Folin-Ciocalteu's reagent (previously diluted with distilled water (1:10 v/v) and 0.4ml of 20% sodium carbonate (Na_2CO_3). The mixture was vortexed and allowed to stand for 1h at room temperature for color development. Absorbance was then measured at 765nm. Total phenols content was expressed as mg of Gallic acid equivalent/g of extract (mgGAE/gE) using the following equation based on the calibration curve: y=0.036x – 0.0196, R^2=0.9878, where x was the absorbance and y was the Gallic acid equivalent (mg/g). The data are presented as mean of triplicate.

Total flavonoids

Total flavonoids content of both extracts was quantified using the modified method.[12]A volume of 20µl of each extract was added to 0.4ml of distilled water and 30µl of sodium nitrite (5%). The mixture is vortexed and incubated during 5min at room temperature. After then, 20µl of $AlCl_3$ (10% in methanol) and 200µl of sodium carbonate (M) were added. The absorbance was readied at 510nm. Total flavonoids content was expressed as mg of Quercetin equivalent/g of extract (mgQE/gE) using the following equation based on the calibration curve: y=0.018x, R^2=0.9899, where x was the absorbance and y was the Quertecin equivalent (mg/g). The data are presented as mean of triplicate.

Total condensed tannins

The condensed tannins content was performed according to the modified method.[13]To a volume of 50µl of each extract (0.5g/ml), 1.5 ml of a solution of vanillin (4% in methanol) and 750µl of concentrated hydrochloric acid were added. The mixture was vortexed and incubated for 20 min at room temperature. The absorbance was readied at 550 nm. Condensed tannins content was expressed as mg of Catechin equivalent/g of extract (mgCE/gE) using the following equation based on the calibration curve: y=0.01x, R^2=0.9838, where x was the absorbance and y was the Catechin equivalent (mg/g). The data are presented as mean of triplicate.

Wound healing activity

The rats were divided randomly into three groups of 5 animals each. They were anesthetized with ether. A square area (about 2cm²) was delimited along the dorso-lumbarregion. It was depilated with razor,

disinfected and wound (15mm of long and 5mm of depth) was inflicted on each rat using a surgical blade.

Test samples (1g/wound area) were applied on the wounded area every 2 days starting from the day of wound induction to the respective animals. Group 1 received plant extract; Group 2 received standard ointment (Madécassol®) while group 3 receives any treatment.

Wound healing evaluation was made by measurement of wound sizes every 2 days during 12 days (D_2, D_4, D_6, D_8, D_{10} and D_{12}).

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA). P Values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

Despite the advances achieved by modern pharmacology and medicine, 80% of the worldwide population benefitsfrom the contributions of traditional medicine in termsof health care, especially in developing countries, inthe absence of a modern medical system.[14]Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1-3 % of modern drugs.[15]A lot of the folk remedies treating wounds and skin disorders come from medicinal plants as is the case of*Celtis australis*described to treat various disorders among which pimples and contusions.[10]

The ethyl acetate extract of seeds from *C. australis* showed a significant (P<0.01) wound healing activity when topically applied in rats. As shown in figure 1, the wound area measurement showed that wound sizesof plant treated group were significantly reduced (18mm at $D_0$to 1mmat D_6)as compared tocontrol group (18mm at D_0 to 9,6mm at D_6).

The results presented in term of percentage wound size reduction are shown in figure 2.The percentages of healing rate of plant extract treated groupwere significant (P<0.01) since the second day (41%) and remove almost completely to the sixth day (94%). In the same conditions, the healing rates of control group werenearly half weaker than test groups (23% atD_2 and 47% at D_6). It is to note that the effect produced by standard ointment (Madécassol®) was comparable to the one of plant extract (40% at D_2, 97% at D_6).

The preliminary phytochemical analysis of theplant extract showed thepresence of polyphenol compounds.The contents of total phenols, flavonoids and condensed tannins of ethyl acetate extract of seeds from*C. australis*revealed a high level of these compounds (40,02mg/g of extract). This levelis higher than some other plants reported in the literature.[16-18]As shown in table 1, the condensed tannins are the most important group (58.1%) followed by phenols (41.4%) and flavonoids (0.5%).This phytochemical profile is similar to that of methanolic extract from jujube fruits.[18]However, it is well known that the recovery of polyphenol contents from plant extracts is influenced by different parameters, notably the nature of extractive solvent, the degree of polymerization of compounds and the part of plant used.[19, 20]

Wound healing is a process by which damaged tissue recovered its normal state as rapidly as possible and wound contraction is the process of shrinkage of the wound area. Various studies have reported that the process of wound healing can be promoted by several plant extracts rich in active compounds, such as flavonoids, triterpenes, alkaloids and tannins.[21-25]

Our results showed a positive linear correlation between the polyphenolic compounds and the wound healing property (data not shown).

The exactmechanism by which *C. australis* extract exerts its effect in wound healing cannot be proposed from the present findings. However, recent studies have shown that polyphenolic compounds play an important role in wound healing due to their antimicrobial and antioxidant activity, metal chelating ability, enzyme inhibition properties and angiogenesis.[26, 27]

Our previous studies have reported the antioxidant and antimicrobial properties of *C. australis* extracts.[28, 29]These properties might prevent the inflammatory process, by preventing the oxidative stress by scavenging the highly reactive free radicals, in one hand and infectious process by killing or inhibiting invading microorganisms in the other hand.Thus, topical treatment with *C. australis* extract accelerate wound healing in rats, most likely through shortening the inflammatory stage.

CONCLUSION

The current study demonstrated that wounds dressed with ethyl acetate of seeds extract of *C. australis*, as topical application of wounds significantly promoted healing activity by increasing the rate of wound contraction comparable to that of Madécassol®, a standard ointment used in wound healing. To our knowledge it is the first time that this activity is

reported with *C. australis* extract.The plant extract showed a high amount of polyphenolic compounds which might be responsible of the wound healing property. However, further phytochemical studies are needed to investigate the active compound(s) responsible for this pharmacological activity.

Table 1: Chemical composition of polyphenolic compounds in ethyl acetate extract of seed from *Celtis Australis* (n=3, M±SD)

Compounds	Content mg/g of extract	Percentage	Content mg/kg of seeds	Yield (%)
Total condensed tannins[a]	23,26 ± 0,01	58,1	516,8	0.051
Total polyphenols[b]	16,56 ± 0,04	41,4	367,96	0.036
Total flavonoides[c]	0,20 ± 0,02	0,5	11,11	0.001

[a] Expressed as mg Catechin equivalent/g of extract
[b] Expressed as mg gallic acid equivalent/g of extract
[c] Expressed as mg Quercetin equivalent/g of extract

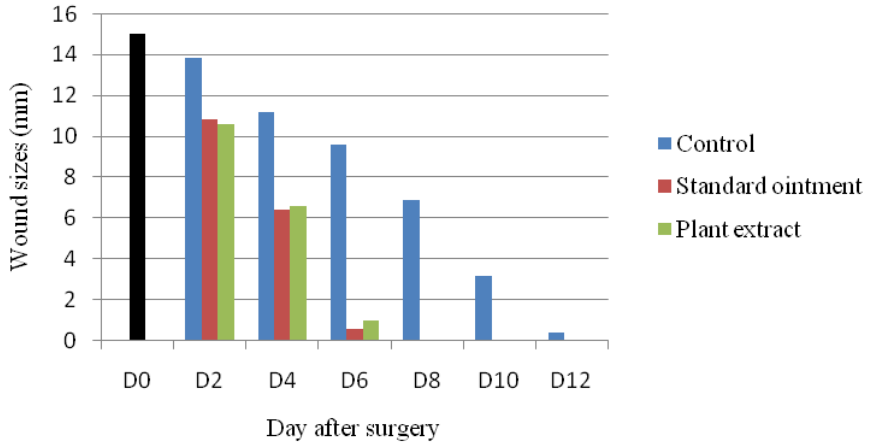

Fig. 1: Effect of ethyl acetate extract of seeds from *C. australis* on wound sizes (n=5, M±SD)

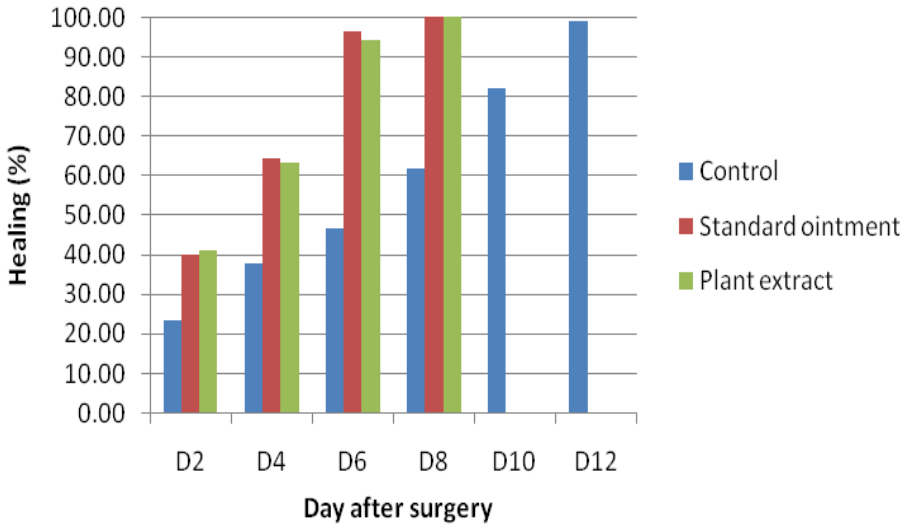

Fig. 2: Effect of ethyl acetate extract of seeds from *C. australis* on wound healing rate (n=5, M±SD)

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