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ORIGINAL ARTICLE

Wound healing activity and phytochemical screening of latex extract of *Dorstenia foetida* plant

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ABSTRACT
The present study aimed to evaluate the effect of latex of Dorstenia foetida on the healing of cutaneous wounds of experimental male rabbits and to screen the latex bioactive phytochemicals by qualitative phytochemical tests. Fifteen male rabbits were equally and randomly divided into five groups; negative control (without treatment), positive control (treated with povidone iodine), high dose %100, middle dose %50 and low dose %25 of latex extract. Area of wound was measured every day from the first day to 12th day, and data were recorded. Results showed that, the latex of D. foetida at two concentrations used 50% and 100% showed wound healing activity in experimental rabbits better than that of povidone iodine. The wounds treated with 50% and 100% were completely recovering on the 11th and 12th day of treatment respectively compare to that treated with standard drug which were not completely recovering till after 12th day of treatment. The optimum concentration used of latex was 50%, which showed better effect and faster wound healing recovering compare to high and low levels used. Phytochemical screening of D. foetida's latex revealed the presence of bioactive secondary metabolites such as alkaloids, tannins, phenols, coumarins, steroids and terpenoids. In conclusion, the latex extract of D. foetida documented beneficial and significant effects to accelerate the rate of wound healing enclosure in the experimentally-induced wounds in rabbits. Therefore, it could be used as alternative natural source for treating cutaneous wounds especially 50% of it. Further biochemical and pharmacological studies are recommended.
Keywords; *Wound healing, Latex, Extract, Phytochemical screening, Dorstenia foetida*

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INTRODUCTION

Natural products especially that derived from medicinal plants represents the safest, effective and natural alternative for treating many ailments instead chemical drugs. Wounds is one of the common healthy problems mainly caused by physical, chemical and microbial insults which may be associated with loss function of organ or physical disabilities [1]. In developing countries wounds are still a major problem, often having severe complications due to the delay of treatment in the most cases with the high costs for therapy by chemical drugs [2]. Therefore, there is an urgent need to search for alternative and safe agents to solve these problems. Using of medicinal plants in the traditional remedies to cure wounds is potential to improve the healing and at the same time to reduce the financial burden. Several plants and herbs are used in traditional medicine to treat skin disorders including wound injuries [3].

Dorstenia foetida is a glabrous herb of the family Moraceae, distributed in some countries including Yemen. It is often growing in deciduous and succulent bushland on open places and rocks outcrops up to an altitude of 2100 m [4]. The latex of this plant is used in Yemeni traditional medicine for treating skin diseases and in particular leishmaniasis [5]. Also, in Ethiopia *D. foetida* roots are used to cure leprosy, liver diseases and intestinal worms [6]. Furthermore, in Oman the cooked tuberous roots are used as food [7].

Previous studies revealed that *D. foetida* have a variety of biological activities as antimicrobial, antifungal [8], antioxidant, anti-inflammatory [9] and estrogenic or anti-estrogenic properties [10]. To the best of our knowledge, there are no report were conducted on the wound healing activity of *D. foetida*, therefore the current study was designed to investigate the wound healing activity and phytochemical screening of latex extract of *D. Foetida*.

MATERIAL AND METHODS

This investigation was carried out in Modern specialization college, Sana'a, Republic of Yemen during the period of May to August 2021.

Plant Materials

The aerial part (stems and leaves) of *D. foetida* were collected from Hajjah region, Yemen. Plant was authenticated at Department of Biology, Hajjah University, Yemen.

Scientific name: Dorstenia foetida

Local name in Yemen: Conser or Sonker.

Collection the plant latex extract

The stems and leaves of *D. foetida* were collected from Hajjah region and the collected samples were cleaned by washing with tap water for five minutes then dried at room temperature for one hour, after that the dried parts were cut to obtain their latex which was collected in sterile test tubes. Finally, the tubes containing the latex were stored at refrigerator at 4 °C.

Preparation different concentrations of latex: Three concentrations of plant latex (100%,50% and 25%) were prepared by adding the following:

1- Zero mL of sterile distilled water to 1 mL of latex to get 100%.

2-One mL of sterile distilled water to 1 mL of latex to get 50%.

3-Three mL of sterile distilled water to 1 mL of latex to get 25%.

Phytochemical screening

Phytochemical examinations were carried out for plant latex using different standard methods as described by Harborne [11].

Wound healing assay

Male rabbits weighing 450 to 500 g were housed under normal conditions of light, room temperature and humidity. The dorsal skin was shaved and cleaned with betadine under anesthesia, and one open full-thickness wound approximately 1.5 × 1.5 cm long was incised up to the level of subcutaneous adipose tissue by means of a surgical blade (the wounding process steps are showing in Fig.1). After the wounding process, each rabbit was housed in a sterilized cage and given autoclaved food with redistilled water in order to prevent bacterial infection. After 24 h following the wounds in the control and experimental groups were treated topically one daily. Fifteen rabbits were randomly divided into five groups each group contain three animals. The first group was left without treatment (control group), the second was treated with Povidone iodine (standard drug), the third group received %100 of extract and the forth group received %50 of extract while the fifth one received %25 of extract. Area of wounds was measured every day, from the first day to 12th day. The data were analyzed via SPSS software (ver.16) at the significant level of $P<0.05$.

Figure1: Wounding process; **A)** Shaving and sterilizing the dorsal skin of rabbit; **B)** Measure the area of wound; **C)** Drawing the diameter of wound; **D)** Incise the skin by surgical blade and scalpel

RESULTS

Wound healing activity

The wound healing activity of *D. foetida* was evaluated by assessing the effect of its latex extract on the cutaneous wounds made on the dorsal skin of male rabbits. The area of wound was measured (by cm) from the first day of treatment to 12th day compared to the effect of standard drug povidone iodine and control. Results are depicted in Fig.2.

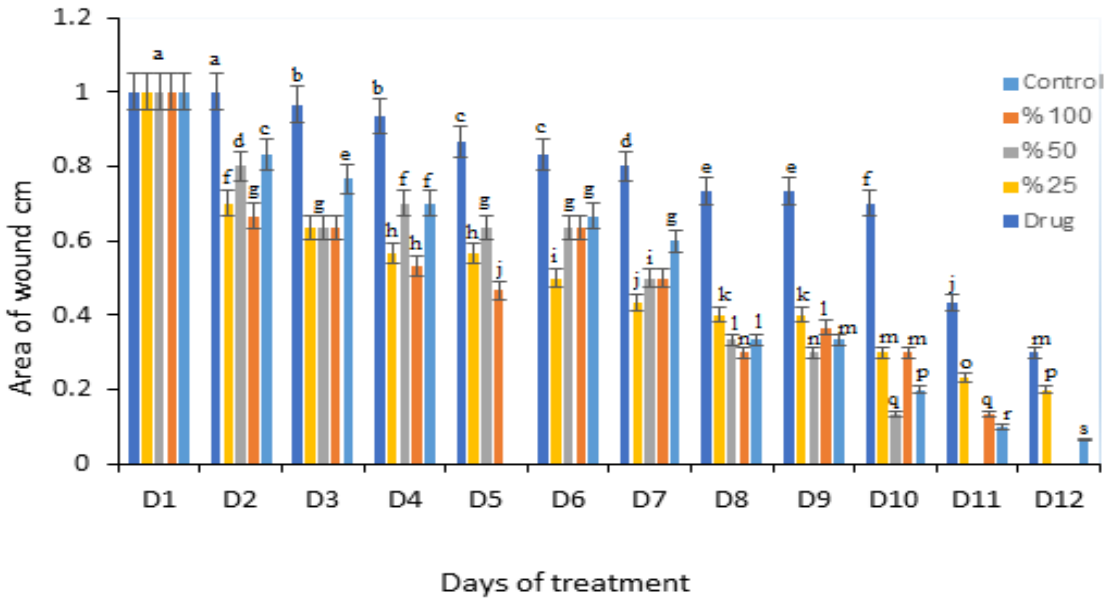

Figure 2: Effect of latex of *Dorstenia foetida* on cutaneous wounds of male rabbits during 12 day of treatment. Different letters indicate groups that differ statistically $P<0.05$.

It was observed that, the area of wounds was decreased with the daily treatment and reached to be zero after 11th and 12th day of treatment in concentrations 50% and 100% of latex respectively, that means a completely healing for wounds treated with these concentrations of latex extract compare to the wounds treated with low concentration used 25% and standard drug (povidone iodine) which were not completely recovering in the most rabbits till after 12th day of treatment (Fig.3).

Figure 3: Cutaneous wounds in the experimental male rabbits after 12th day of treatment with 25%, 50%, 100% of latex extract of *Dorstenia foetida* compared to Standard drug (povidone iodine) and control (no treatment).

Also, the results in table 1 shows the day of completely healing of wounds in all treated animals, it was observed that, the all wounds in all animals treated with 50% of latex were faster healing and returned to be normal on the 11th day of treatment (Fig.4) compare to the wounds treated with other treatments.

Table 1: Day of completely healing of wounds with different treatments.

Group	No. Rabbit	Day of completely healing
Control	1	After 12th
	2	12th
	3	After 12th
100%	1	11th
	2	12th
	3	12th
50%	1	11th
	2	11th
	3	11th
25%	1	After 12th
	2	11th
	3	After 12th
Standard drug	1	After 12th
	2	12th
	3	After 12th

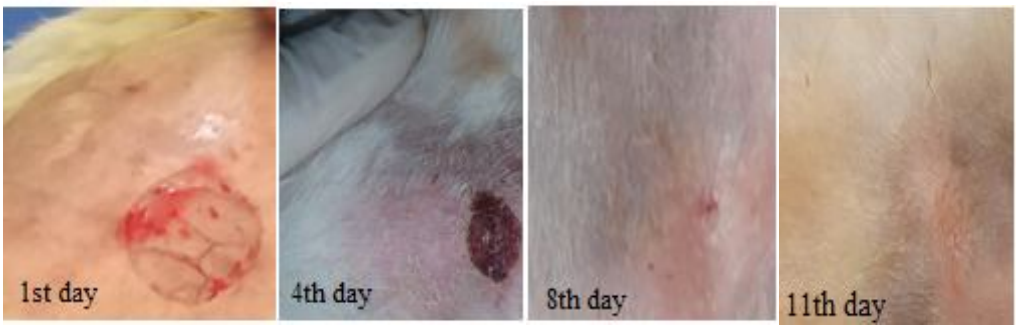

Figure 4: Shows phases of healing for wound in rabbits treated with 50% of latex extract of *Dorstenia foetida* during 11 day of treatment.

Interestingly, during this study we observed that, the zone of wounds in rabbits treated with plant latex returned to be normal and the hair of rabbit at the site of wound was naturally growing better than that of positive and negative control.

Phytochemical screening

Phytochemical screening of latex of *D. foetida* revealed the presence of various secondary metabolites by a positive reaction with the respective test reagent. The extract showed presence of alkaloids, tannins, coumarins, phenols, steroids and terpenoids, results are summarized in table 2.

Table 2: Screening of secondary metabolites in latex extract of *Dorstenia foetida*.

Test	Reagent	Result	Observation
Alkaloids	Dragendorff's	+	Yellow precipitate
Phenolic compounds	$Fecl_3$ (5%)	+	Bluish black color
Tannins	$Fecl_3$ (1%)	+	Bluish black color
Steroids and Terpenoids	Salkowski's test	+	Reddish brown color ring
Coumarins	NaOH (10 %)	++	Yellow color

Key: (+) Positive Test

DISCUSSION

The latex extract of *D. foetida* at two concentrations 50% and 100% showed wound healing activity in experimental rabbit's model better than that of povidone iodine.

The remarkable wound healing activity of *D. foetida*'s latex is mainly attributed to the presence of bioactive secondary metabolites which were detected by phytochemical screening as alkaloids, tannins, phenolic and coumarins compounds, these phytochemicals were previously reported to promote wound healing process due to their astringent and antimicrobial property which seems to be responsible for wound contraction and increased the rate of epithelialization. Begashaw et al. [12] reported that, alkaloids could promote the early stages of wound recovering by stimulating the growth of colonies from fibroblast precursors. Also, phenolic components especially flavonoids are well known as potent antioxidant and free radical scavenging agents, and they are believed to be one of the most important components of wound healing, they have ability to reduce lipid peroxidation by preventing or slowing onset of cell necrosis and improving vascularity, hence increasing the collagen fibers strength by increasing circulation or by preventing cell damage and by promoting DNA synthesis [12, 13]. Furthermore, tannins as astringent factors are responsible of wound contraction and could increase the rate of epithelialization at the granulation formation and scar remolding phases [14]. Beside these bioactive phytochemicals, *D. foetida* is rich in coumarins as a major bioactive class of secondary metabolites in its latex. Results of previous study indicated to that, *D. foetida* is rich in furanocoumarins, as plant phototoxins, with a variety of pharmacological properties including antimicrobial, antifungal [8], antioxidative and anti-inflammatory [9]. The correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well [15].

The present study is the first work has evaluated the wound healing activity of *D. foetida* with a potent wound healing results were recorded for its latex extract in the experimental animals. Similar results were reported from other plants which revealed a good wound healing activity and their activity were also imputed to some active components, et al. [16] investigated the wound healing activity of four extracts of *Sambucus ebulus* leaves *in vivo*. The wound healing activity of extracts was evaluated by means of pharmacological methods in mice and rats. Their results revealed faster re-modeling was observed in the extract treated groups in comparison to the vehicle and negative control ones and a flavonoid 3-O-glucoside was isolated and reported as an active component of the methanolic extract. Also,

Ajand and Roshanai [17] study showed a significant reduction ($p<0.05$) in incisional wounds area in the all treated groups with root extract of *Withania somnifera* ointment which was significantly higher on the 8th day of treatment.

CONCLUSION

Based on the findings obtained of this work, the latex of *D. foetida* could be suggested as promising natural source to cure the cutaneous wounds as indicated by its potent wound healing activity in the experimental rabbits especially 50% concentration of it, which was the optimal dose with faster wound recovering ability was evaluated, and this estimated activity may have imputed to its content of alkaloids, tannins, phenolic and coumarins.

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