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Exploration of antimicrobial and wound healing activities using ethanolic leaf extract gel of *Nyctanthes arbor-tristis* on rabbits

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ABSTRACT

The present study aimed to investigate the anti-microbial and wound healing activities of *Nyctanthes arbor-titris* leaves extract gel on rabbits. The leaf extracts of the plant were tested for their *in vitro* antimicrobial activity by agar well diffusion method with different concentrations. Animals in group 1 were without treatment and served as control (Carbapol gel base). Animals in group 2 received the 5% w/v *N. arbor-tristis* leaf gel extract base. Animals in group 3 received 10% w/v *N. arbor-tristis* gel extract base. Animals in group 4 received gel containing betadine Gel was applied on the wound once daily up to 14-15 days starting from the second day of wounding. The prepared gel was characterized for its physicochemical constants, preliminary phytochemical analysis, spreadability, pH. Carbopol 934 was tried and finally, the gel which showed good spreadability and pH was selected for wound healing property of herbal gel of *N. arbor-tristis.* Gel formulation prepared with a 10% *N. arbor-tristis* exhibited a response in terms of wound epithelialization, angiogenesis and number of hair follicles at wound area better than the gel base on the 15th day post-wound day. Application of gel base produced further advantages by increasing hydroxyproline content and collagen fiber thickness. This study explored the wound healing potential of a gel containing 10% *N. arbor-tristis* through topical application of total extract in a model of excisional wound healing in rabbits.

Keywords: *Nyctanthes arbor-tristis*, anti-microbial, wound healing, extract gel, excision, agar well diffusion.

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INTRODUCTION

Medicinal plants have been used since time immemorial for the treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns (Govindrajan et al., 2007).

A wound is defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues. Wound healing is the natural process of the body for regenerating dermal and epidermal tissue, it involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in three overlapping phases viz. inflammation, cellular proliferation and remodeling, respectively. Inflammation is a phase of 0 to 3 days which involves migration of neutrophils around the incision. Proliferation is of 3 to 12 days in which incisional space is filled with granulation tissue. The remodeling phase is of 3 to 6 months which involves a synthesis of collagen fibers leading to an increase in tensile strength of the skin (Gupta et al., 2006).

"*Nyctanthes* is a Greek word that means "night flowering." The taste is in the flock of medicine in India, as the leaves used are bitter and pungent" (Deodhar and Rana, 1997).

Parijata (*Nyctanthes arbor-tristis*), commonly known as night jasmine, is a wonderful plant that is described in Ayurveda with its enormous medicinal value. Other than Parijata, it is popularly known as Har-shinghar. Different parts of this plant are used for various medical purposes. Its leaves have broad-spectrum medicinal use such as anti-bacterial, anti-inflammatory, anti-pyretic and antihelminthics effects (Salim et al., 2008).

The leaves of *N. arbor-tristis*, besides being used in the treatment of sciatica and arthritis, are advocated for various kinds of fevers and painful conditions by Ayurvedic physicians (Enoch et al., 2005).

N. arbor-tristis (family: Oleaceae) demonstrate diverse pharmacological and biological activities like antihelminthic, anti-inflammatory, analgesic, antipyretic and ulcerogenic activities (Tyrell et al., 2017). The plant also possesses anti-malarial, leishmanicidal, amoebicidal, antiallergic, tranquilizing, antihistaminic, purgative activities and recently reported hepatoprotective, antispermatogenic and anti-oxidant activities also (Drew et al., 1972).

N. arbor-tristis is mainly characterized by the presence of phenylethanoid derivatives and iridoid glycosides. It is used in traditional medicine as a stomachic, carminative, intestinal astringent and expectorant in biliousness, piles, hair tonic and various skin diseases. The present study was undertaken to investigate the wound healing activity of *N. arbor-tristis* in view of its diverse pharmacological application in ancient and modern systems of medicine. Leaves of *Nyctanthes arbor-tristis* as ethanolic extract investigated for the wound healing action for the first time (Al Bari et al., 2006).

"Microbes are microscopic living organisms that can be found all over the world, but are too little to see with the unaided eye. They can be found in the water, dirt, and atmosphere" (Hara et al., 1979).

MATERIALS AND METHODS

Plant collection

The leaves of *N. arbor-tristis* were collected from the local surroundings of Peddapuram (A.p) and kept to dry in a shady area. The plant was identified and authenticated by Dr. T. Raghuram Taxnomist, Maharani College Peddapuram, Andhra Pradesh India, where a voucher specimen No (22420) of the plant was kept in the herbarium.

Preparation of ethanolic extract of *Nyctanthes arbor-tristis*

Plants that had recently been harvested were included. Then washed from the soil, dried for about 20 days in the shade, and coarsely ground in something like a conventional grinding machine. The concentrate was allowed to blanch in ethanol for three days before being subjected to three hours of hot percolation (Rout et al., 2007). After that, the solution was filtered and concentrated. Distillation was conducted at 80°C on the condensed material. The arrival of solvent signalled the end of the extraction process.

To make an ethanolic extract, the extract was moved to a blank shallow tray that had already been calculated then dissipated through a thick paste on a water bath at 50°C. Desiccators with anhydrous calcium chloride were used to dry the concentrated product (Singh et al., 2006).

The extract's yield was calculated as a percentage (Ali, 2008).

Preparation of *Nyctanthes arbor-tristis* gel

Topical herbal gels containing either plant extract 1% were prepared using carbopol as a gelling agent. Carbopol gel was prepared by dispersing carbopol powder (2.5 g of gel are precisely measured and distributed in 25 ml quantity of deionized water with the aid of a magnetic stirrer (1500 rpm). The drug or the plant extract were added to make a 10% concentration, then the pH was adjusted to pH 7-7.5 NaOH 10% with continuous stirring till gel was formed. The povidone-iodine gel was prepared by a similar method. The obtained gel preparations were tested before application to animals. The tested parameters included color, consistency, washability, pH, spreadability (Ramawat and Mérillon, 2008).

Evaluation of extract gel

Physical evaluation

Physical properties including appearance and grittiness were examined.

Measurement of pH

An automated pH meter was used to calculate the pH of different gel formulations. 2.5 g of gel is precisely measured and distributed in 25 ml of distilled water before being processed for two hours (Metzger et al., 2004).

Determination of spreadability

At room temperature, the gel's spreadability was assessed. After 1 min, the spreading diameter of 20.01 g of gel was determined between two horizontal glass plates. A 0.5 g of gel was placed within a 1 cm diameter circle pre-marked on a 20 × 20 cm glass plate, which was then covered with a second glass plate. For 5 minutes, a 500-g weight was permitted to lie on the upper glass plate. The diameter of the gel increased as it spread (Olowa and Nuneza, 2013).

Centrifugal test

All of the other specific formulations were collected by centrifugation individually in something like a tube 10 cm and 1 cm broad (Hettich Standard Centrifuge) for 5, 15, 30 and 60 min at 2000 rpm, and afterward, agglomeration and gel stabilization were tested (Nadkarrni et al., 1982).

Skin irritation test

The herbal gel 8 g was prepared and immediately applied on the shaved skin and left for an hour.

Preliminary phytochemical investigation

The ethanolic extract was subjected to chemical tests to determine the phytochemical constituents qualitatively.

Determination of antibacterial activity

Media preparation

11.5 g of nutrient agar powder was dissolved in 500 ml of distilled water. To completely dissolve all elements, heat was applied to this mixture while stirring and sterilising. The nutrient agar was enabled to cool but not solidify after autoclaving. Each plate was filled with nutrient agar and set aside on a sterile surface until the agar solidified (Badam et al., 1988).

Test bacterial strains

The clinical bacteria isolate includes gram-positive bacteria and

gram-negative bacteria.

Determination of zone of inhibition by agar diffusion

Crude ethanolic extract can be tested for antimicrobial activity through this method. Agar plates were prepared separately for this, and test bacterial strains were seeded individually over the surface of the agar plates after overnight culture. 6 mm wells were punctured over the agar plates. The ethanolic extract was then prepared in DMSO at concentrations of 100 and 200 µg/ml. After that, 50 L of ethanolic extract was poured into the wells. As a positive regulation, gentamycin (25 g/ml) was used. Plates are then incubated for 24 h at 37°C. The experiment was done three times. The diameter of the inhibition zone around the well filled with ethanolic extract was utilised to determine the effectiveness of the treatment. Antimicrobial characteristics can be found in this extract. The antibacterial effect is indicated by the presence of a significant zone of inhibition (Hiremath et al., 2016).

Wound healing activity

Experimental animals

The study used rabbits of either sex that weighed between 1.8 and 2.5 kg. Specific polypropylene cages were used to hold the livestock. They are given *ad libitum* diet of retail pellets combined with veggies and water. The livestock was kept in a holding space with a ventilation system for 12-hour intervals of light and shadow. The temperature in the room was adjusted to 232°C, with a humidity of 45 percent.

Group I: A test group of rabbits was given a basic gel base treatment.
Group II: Ethanolic extracts of *Nyctanthes arbor-tristis* at a low dose (5% w/w) gel were given to rabbits
Group III: Rabbits were given a high-dose gel of ethanolic extract of *Nyctanthes arbor-tristis* (10% w/w).
Group IV: Betadine, a standard curing agent, was applied topically to rabbits.

Excision wound model

Lignocaine was used to anesthetize the rabbits, and a 500 mm^2 wound was developed. The outlined skin was then gently sliced to its maximum thickness. On 1 mm^2 graph paper, wounds were drawn on the day of the wounding, followed by a four-day delay until the 12th day. Then, on alternating days until the healing process was over. The size of the wound was assessed daily. The contraction of the wound was determined using the formula below, and changes in wound area were evaluated regularly. The research groups' wound healing significance is determined by adding the injured region days specifically to control groups. Epithelialization time was reported (Kiew and Baas,1984).

$$\% \text{ wound contraction} = \frac{\text{Final diameter (cm)}}{\text{Initial diameter (cm)}} \times 100$$

Epithelisation period

It was assessed by counting how many days it took for the Escher to slip off the wound surface despite having left a festering wound.

Histopathological studies

After full healing of excision, incision, and dead burn wounds, wound tissue specimens from the monitor, examination, and normal groups were taken, and after standard processing, 6-mm thick parts were cut and stained with hematoxylin and eosin**.**

Statistical analysis

The evidence is seen as a calculated standard deviation of the mean (SEM). Dunnett's several contrast test is used after a one-way study of variance (ANOVA). The t-statistics range varied from p-value towards 0.001.

RESULTS

Phytochemical analysis

Preliminary phytochemical research identified alkaloids, tannins, saponins, and carbohydrates, as well as terpenoids.

The agar well diffusion method was used to assess antimicrobial activity (Table 1).

Table 1. Zone of inhibition of ethanolic extract of *Nyctanthes arbor-tristis.*

Antibacterial activity		Zone of inhibition (mm)		
Type of bacteria	**Name of organism**	**N. arbor- tristis (100 µg/ml)**	***N. arbor- tristis* (200 µg/ml)**	**Gentamycin (25 µg/ml)**
Gram-positive bacteria	*Staphylococcus aureus*	15.1 ± 0.08	17.1 ± 0.35	19.0 ± 0.58
	Bacillus substillis	15.4 ± 0.57	16.9 ± 0.57	21.0 ± 0.33
Gram-negative bacteria	*Eschericheria coli*	17.1 ± 0.33	19.1 ± 0.41	20.0 ± 0.45
	Pseudomonas aerugenosa	14.1 ± 0.67	16.3 ± 0.67	19.0 ± 0.57
	Pseudomonas putida	14.2 ± 0.35	18.1 ± 0.54	19.0 ± 0.58

Values are expressed as mean ± SEM; n = 3.

Antimicrobial activity of ethanolic extract of *Nyctanthes arbor-tristis*

The antibacterial activity of ethanolic extracts of *Nyctanthes arbor-tristis* (100 and 200 mg/ml) at different concentrations was tested using the agar diffusion method against gram-positive and gram-negative bacteria. *Staphylococcus aureus* (17.1 mm), *Bacillus subtilis* (16.9 mm), *Escherichia coli* (19.1 mm), *Pseudomonas aeruginosa* (16.3 mm), and *Pseudomonas putida* (17.6 mm) were among the species with zones of inhibition (Figure 1). As compared to a regular prescription, the ethanolic extract showed strong antibacterial activity.

Using the gram-negative organism *E. coli*, the ethanolic extract of *N. arbor-tristis* showed a minimum inhibitory concentration of 125 mg/ml.

Ethanolic extract of *Nyctanthes arbor-tristis* healing of excision wound model

The wound healing contracting capacity of ethanolic extract of *N. arbor-tristis* in different concentrations was significantly greater than that of the control group using a gel base on an excision wound model. From the third day onwards, the extract-gel treated groups demonstrated considerable wound healing, which was equivalent to the standard medication, betadine, treated group in the excision wound model. The wound closing period was shortened because the percentage of wound contraction was strong with the high dose of *Nyctanthes arbor-tristis*, as well as the 10 percent w/w extract group showed 100 percent contraction in excision wound healing in (16.230.21) days, which was nearly equal to the betadine-treated group (15.910.12) days. Beginning on the 6th day, the animals in the 10% w/w extract population demonstrated significant wound contraction, reaching 100% wound closure in (20.70.21) days. In an excision model, the latest work discovered that while both concentrations of leaf extracts of *N. arbor-tristis* (10% extract gel versus 5% extract gel) have significant wound healing activity (Kumar et al., 2007). (Tables 2 and 3)

Effect of different doses of *Nyctanthes arbor-tritis* extract on wound healing activity

Effect of Nyctanthes arbor-tristis extract (10%w/w gel) on wound

This is shown in Figures 2 and 3.

Standard: Betadine on wound

This is shown in Figures 4 to 6.

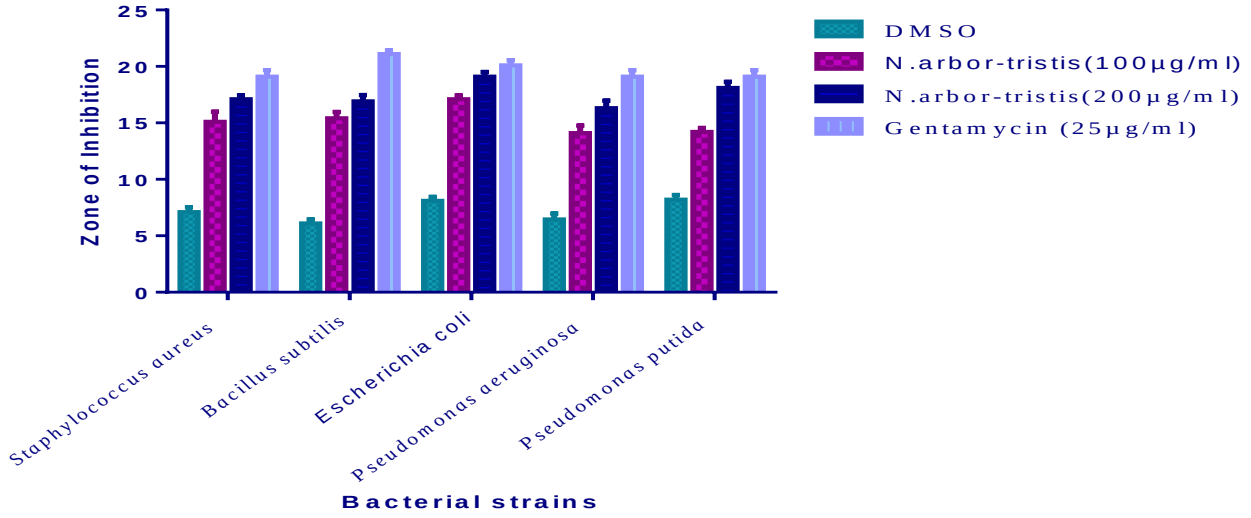

Figure 1. Zone of inhibition of ethanolic extract of *Nyctanthes arbor-tristis* (anti-microbial activity).

DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical analysis of *N. arbor-tristis* ethanolic extract showed the existence of alkaloids, tannins, saponins, carbohydrates, cardiac glycosides, terpenoids, phenols, proteins, and amino acids. As a result, preliminary phytochemical tests are important and useful in identifying chemical constituents in plant material, which can lead to quantitative estimation (Manisha et al., 2009).

Table 2. Effect of topical application of gel containing ethanolic extract of *Nyctanthes arbor-tristis* leaves on wound healing contraction of excision wound in rabbits.

Group	Post wound healing days								Epithelization
	0 day	3rd day	6th day	9th day	12th day	15th day	18th day	21st day	
Control	2.54 ± 0.09	2.40 ± 0.03	2.36 ± 0.08	2.30 ± 0.04	2.13 ± 0.04	1.86 ± 0.03	1.53 ± 0.03	1.22 ± 0.02	24.68 ± 0.17
Ethanolic extract of *N. arbor-tristis* 5% (w/w)	2.53 ± 0.08	2.31 ± 0.09	2.01 ± 0.06	1.32 ± 0.09	0.91 ± 0.07	0.61 ± 0.07	0.03 ± 0.09		20.7 ± 0.21
Ethanolic extract of *N. arbor-tristis* 10% (w/w)	2.54 ± 0.11	2.23 ± 0.08	1.75 ± 0.11	1.09 ± 0.12	0.33 ± 0.99	0.12 ± 0.33			16.23 ± 0.21
Standard	2.58 ± 0.01	2.11 ± 0.33	1.51 ± 0.33	0.91 ± 0.06	0.21 ± 0.05	0.09 ± 0.02			15.91 ± 0.12

Table 3. Percentages of wound healing shown by an ethanolic extract of *Nyctanthes arbor-tristis* in the excision wound model.

Group	Treatment	Percentage (%) of wound healing on the day						
		3rd day	6th day	9th day	12th day	15th day	18th day	21st day
I	Control	5.5 ± 0.02	7.0 ± 0.02	9.4 ± 0.04	16.14 ± 0.06	26.77 ± 0.05	39.7 ± 0.03	51.9 ± 0.05
II	Ethanolic extract of *N. arbor-tristis* 5% (w/w)	8.69 ± 0.17	20.5 ± 0.10	47.8 ± 0.01	64.03 ± 0.05	75.88 ± 0.03	98.8 ± 0.01	
II	Ethanolic extract of *N. arbor-tristis* 10% (w/w)	12.2 ± 0.13	31.1 ± 0.10	57 ± 0.30	88.6 ± 0.29	95.2 ± 0.16		
IV	Standard (betadine)	18.2 ± 0.05	41.4 ± 0.12	64.7 ± 0.07	91.8 ± 0.25	96.5 ± 0.04		

Values are expressed as mean ± SEM; n = 2 animals in each group; $p < 0.001$ When compared to control.

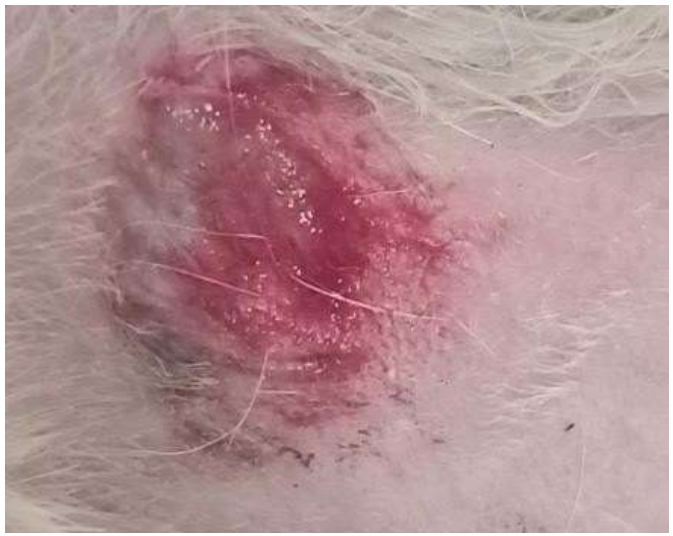

Figure 2. An excision wound on day 0.

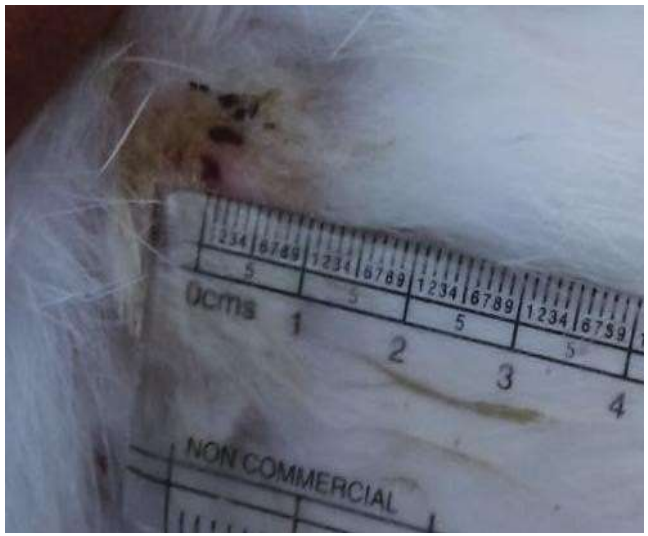

Figure 3. An excision wound on day 15 (test).

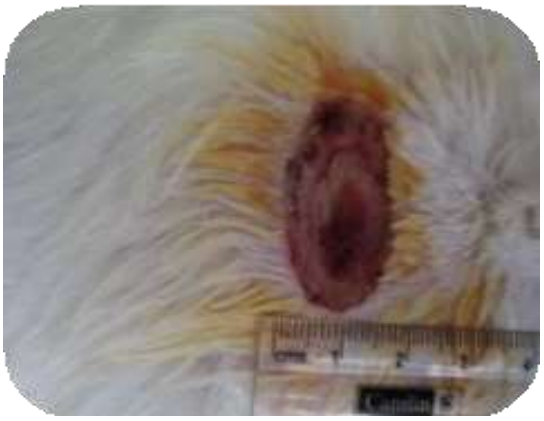

Figure 4. An excision wound day 0 std.

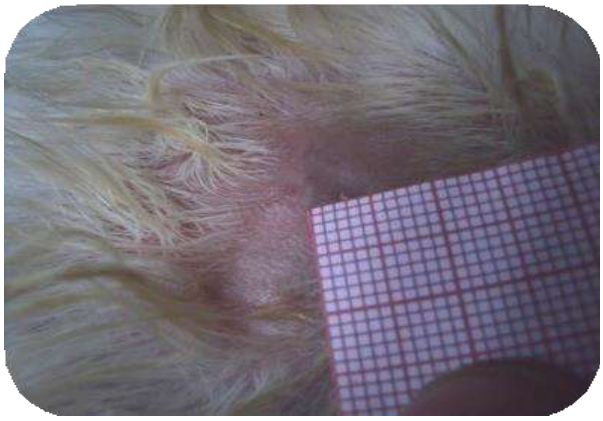

Figure 5. An excision wound day 15 std.

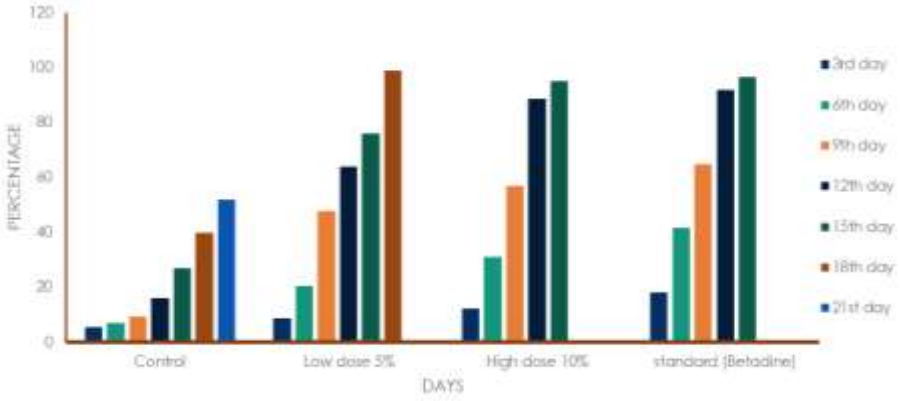

Figure 6. Measurement of percentage of wound contraction in excision wound model.

Antimicrobial activity

The chemical composition of *N. arbor-tristis* ethanolic extracts was linked to their antimicrobial activity. The inhibition zones' diameters were measured in millimetres. Alkaloids, tannins, saponins, and other phytochemical components of *N. arbor-tristis* were identified as preliminary phytochemical components. The existence of these bioactive compounds in plant materials has been linked to antimicrobial activity in several studies. The presence of these secondary metabolites in plants causes biological activity in humans and animals, which is why they are used as herbs. These compounds also defend the plant from microorganism infection, insect predation, and herbivore predation (Chadwik et al., 2012).

Wound healing activity

To assess the *N. arbor-tristis* wound healing capacity, wound contraction and epithelisation time parameters were calculated. Glycosides, tannins and saponins' free radical scavenging activity are thought to be responsible for wound healing. Glycosides, tannins, saponins have been shown to minimise lipid peroxidation by increasing vascularity as well as preventing or delaying the onset of cell necrosis (Shrivastava et al., 2018). Lipid peroxidation plays a role in a variety of injuries, including burns, infected wounds, and skin ulcers. As a result, to improve collagen fibre strength, glycosides, saponins and tannins are vital for astringent properties that aid wound healing. The greater the reduction, the more effective the drug. In other words, if the drug is more effective, the wound can close faster. Higher rates of wound healing are seen in the treatment community could attribute increased fibroblast and macrophage activity. The involvement of microbial metabolites, slower wound healing in control. In animals, hydroxyproline is a marker of better wound healing conditions. Hydroxyproline is an amino acid contained in granular tissue's collagen fibres. Its quantitative calculation is directly related to the production of collagen, and its estimate aids in understanding the pace at which the wound's connective tissue heals (Das et al., 2012). In relation to the control group, the hydroxyproline was significantly higher,

suggesting increased collagen content. The number of fibroblasts, well-formed hair follicles with no macrophages, and inflammatory cells in rabbit skin treated with the compound were all comparable to the normal (Siddique et al., 2006).

Conclusion

The current review was expected to limit tissue harm and give satisfactory tissue perfusion and oxygenation, legitimate nourishment and clammy injury mending climate to re-establish the physical coherence and capacity of the influenced part. In the current review, rabbits were treated with *N. arbor-tristis* ethanolic extract 10% w/w gel for 15 days timespan, taking perception in each third day. It was seen that total epithelization of both the injury models takes about 15 days. This is the period of complete healing of a wound. Thus, it can be concluded that the plant extract at 300 mg dose/kg b.w. can be a good solution for the healing of both wounds. Thus, the folklore claim for the use of *N. arbor-tristis* leaves in the healing of the wounds can be justified by the present study.

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