



Original research article

## Wound healing effect of silver nanoparticles from *Tridax procumbens* leaf extracts on *Pangasius hypophthalmus*



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## ARTICLE INFO

## Keywords:

Wound healing

*Pangasius hypophthalmus*

Silver nanoparticles

*Tridax procumbens*

## ABSTRACT

Wound healing is a complex process and has been the subject of intense research for a long time. Plants are natural sources which have been widely used for wound healing. The recent emergence of nanotechnology has provided a new pharmacological modality for silver nanoparticles to be used in wound healing process. Silver nanoparticles are a good source for drug targeting due to its high chemical stability, catalytic activity, wound healing activity and antimicrobial nature.

In this study Silver nanoparticles were synthesized using *Tridax procumbens* leaf extract. Nanoparticles were characterized using UV–vis absorption spectroscopy, and Scanning Electron Microscopy (SEM). The efficacy of the synthesized silver nanoparticles on wound healing was investigated in *Pangasius hypophthalmus*.

Biosynthesis of silver nanoparticles from the leaf extract of *Tridax procumbens* was found to be cost effective and time conserving. We compared the effectiveness of the synthesized silver nanoparticles from *Tridax procumbens* on wound healing with silver nitrate and leaf extract. Collagen deposition and fibrosis formation occurred much earlier on wounds treated with silver nanoparticles from *Tridax procumbens*.

The synthesized Silver nanoparticles from *Tridax procumbens* showed enhanced wound healing activity in fish, also improving the epithelialisation and appearance of the wound when compared to that of silver nitrate and leaf extracts of *Tridax procumbens*.

### 1. Introduction

Wounds are the consequence of injuries to the skin that disrupts other soft tissues. Healing of wound is an intricate and persistent process of tissue repair and remodelling in response to injury [1]. In the past few decades synthesis of nanoparticles from plant sources have proved to be an effective and alternate method for the novel production of nanoparticles [2]. Silver nanoparticles have received attention due to their physical, chemical, and biological properties that attributed to the catalytic activity [3]. Synthesis of nanoparticles through biological method is rapid, eco-friendly and economical [4]. Moreover, the biological synthesis is of great success due to metal tolerance of these entities. Reports are there on the extra cellular biosynthesis of silver nanoparticles using microbes, plants and also pure compounds from plants [5].

*Tridax procumbens* is one of the most common plants used by rural and tribal communities to cure various health ailments including dermal wounds and injury. The results show that *T. Procumbens* leaf juice possesses dose-dependent prohealing property [6]. All the available reports of *Tridax procumbens* and its pharmacological properties

make this plant more acceptable for the present study. *Tridax procumbens* is traditionally known for its antiviral effect, antioxidant efficacies, wound healing activity, insecticidal and anti-inflammatory activity. The extracts of the leaves of *Tridax procumbens* have been proved to decrease the time taken for blood clotting and thus helps in haemostasis. In adult albino rats, of either sex, wounds were created by subcutaneous implantation of sterilised plastic tubes. This wound was healed by *Tridax procumbens* leaf extracts [7].

The primary objective of this study was to biosynthesize silver nanoparticles using the extract of *T. procumbens* leaves. The synthesized silver nanoparticles were tested for wound healing efficacy.

*Pangasius hypophthalmus* is a fresh water catfish used for consumption [8]. Investigations on the bacterial infection in *Pangasius hypophthalmus* were reported. In this study, the impact of silver nanoparticles on the wound healing process in *Pangasius hypophthalmus* was carried out.

Wound healing is a complex process and has been the subject of intense research for a long time. We investigated the wound-healing properties of silver nanoparticles in fish model and found that rapid healing and improved architecture occur in a dose-dependent manner

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Received 20 November 2018; Received in revised form 13 July 2019; Accepted 25 July 2019

Available online 26 July 2019

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[9]. The purpose of the present study is to assess the wound healing efficiency of silver nanoparticles of the leaf extract of *Tridax procumbens* on *Pangasius hypophthalmus*.

## 2. Materials and methods

*Tridax procumbens* leaves were collected from the outskirts of Chennai and their identification was authenticated. The leaves were washed with distilled water and shade dried for 15 days. The dried leaves were powdered. 1 g of powder was mixed with 10 ml distilled water with constant mixing for 20 min. After incubation the mixture was filtered using Whatman filter paper. The extract was stored at 4 °C for further use.

### 2.1. Preparation of nanoparticles

To 100 ml of 1 mM AgNO<sub>3</sub> solution, 10 ml of leaf extract was added separately to two conical flasks, stirred at 200 rpm using magnetic stirrer for 3 h and kept in dark at room temperature. By mixing both solutions Ag<sup>+</sup> ions were reduced and it clustered together to form monodispersed nanoparticles as a transparent solution in aqueous medium. The colour change was observed from light yellowish green to brown colour within 15 min.

### 2.2. Experimental design

Healthy living specimens of *Pangasius hypophthalmus* weighing 15 to 25 gm and 8 to 13 cm in length were purchased from Jeevan aquarium, Porur, Chennai. Consent for performing experiments on fish was approved by the Institutional Ethical Committee and the experiment was conducted in accordance with National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). They were brought to the laboratory in well-aerated containers, to avoid hyperactivity, physical injuries and stress to the fish. The healthy specimen was then transferred to glass aquaria (50 × 25 × 25 cm) containing tap water. The fish were acclimatized to the laboratory conditions for 20 days prior to experimentation. Fish were fed with artificial pellet diet. Water was replaced with clean water whenever necessary [10]. The fish were divided into 4 groups (C, T1, T2, T3) of 6 fish each. Six fish were taken in four tanks each containing 10 litres of water. An artificial wound was made using punch biopsy needle in the belly region above the lateral line near tail in all the fish [11]. Six fish in a tank served as control (C) and the other 3 tanks containing 6 fish each were treated with AgNO<sub>3</sub> (T1), *Tridax procumbens* extract (T2) and silver nanoparticles of *Tridax procumbens* extract (T3) respectively. The wounded *Pangasius hypophthalmus* were allowed to rear in the experimental trough. The area of wound in the test fish were measured once in 2 days. The experiment was continued for a period of 10 days. The tissue from the wound was collected every 5 days from all the experimental tanks for histological studies. The tissues were quickly washed with saline solution (0.75% NaCl) and immediately fixed in 10% formalin solution [12].

## 3. Results

Formation of silver nanoparticles of *T. procumbens* can be easily monitored by the change in colour of the reaction mixture from greenish brown to dark brown. Reduction of silver ion to silver nanoparticles results in the change in colour [13].

### 3.1. Characterization of silver nanoparticles of *T. procumbens*

UV-vis spectroscopic analysis of silver nanoparticles synthesized from aqueous extracts of *T. procumbens* leaves was done. Fig. 1(a) shows the UV-vis absorption spectra recorded for the silver nanoparticle solutions after 24 h of reaction time. The UV visible spectra showed that

the maximum absorbance of the solution is at 445 nm.

The shape, size distribution and morphology of the synthesized nanoparticles were studied using Scanning Electron Microscopy (SEM) at Anna University, Chennai.

Fig. 1(b) shows the SEM image of the synthesized silver nanoparticles. The image showed that the silver nanoparticles obtained are spherical in shape and are of 70 nm in size.

### 3.2. Healing response

The areas of wound of all experimental fish were measured once in 2 days and recorded (Tables 1–4). The healing responses of the fish were recorded. The current study revealed the impact of silver nanoparticles on the wounded skin. The study reported that the fish treated with silver nanoparticles of *T. procumbens* showed highest healing response at the end of the 10th day.

### 3.3. Histological analysis

The histology of wounded tissues of control and test groups was studied and depicted in Fig. 2(a) to (j). On zeroth day when the wound was induced the epithelial cells were totally damaged. From fifth day onwards, the epithelial cells architecture improved in the group treated with herbal extract, silver nitrate and silver nanoparticles of *T. procumbens* whereas in control, there was a slow progress in healing. On the 10th day, a complete recovery of the architecture of epithelial layers in the group treated with silver nanoparticles of *T. procumbens* was seen when compared to control. Whereas the process was relatively slow in fishes treated with plant extract and silver nitrate.

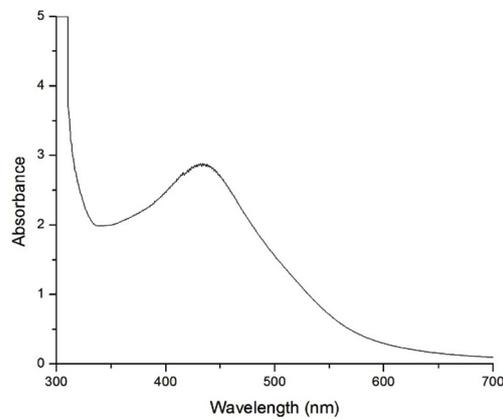
## 4. Discussion

The experimental fish *Pangasius hypophthalmus* showed erratic movements, found restless and also showed avoidance reactions because of the wound irritation initially. Histological investigation appears to be a very sensitive parameter and is crucial in determining the cellular changes that may occur in target organs, such as epithelial layers. The histological reports showed partial loss of stratified squamous cell layer due to induced injury on the initial day.

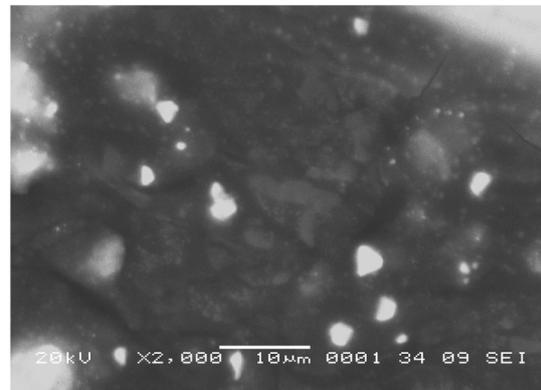
On the 5<sup>th</sup> day control group showed infiltration of inflammatory cells, necrosis of epidermis and dermis and also granulomas formation. On the 5<sup>th</sup> day T1 group shows haemorrhage and necrosis in epidermis and dermis due to the damage of blood vessels. On the 5<sup>th</sup> day T2 group showed the formation of haemorrhage and infiltration of inflammatory cells due to a faster response of immunological cells and recovery chance and may be due to the healing effect of *Tridax procumbens* extract. On the 5<sup>th</sup> day T3 group shows the formation of haemorrhage and deposition of collagen on the surface of the skin because of the silver nanoparticles of *Tridax procumbens*. Hence at the end of 5<sup>th</sup> day T3 group showed a better result when compared to the other groups.

On the 10<sup>th</sup> day control group depicts formation of necrosis, inflammation and no epithelialization. On the 10<sup>th</sup> day T1 group showed formation of fibrosis on the skin surface. On the 10<sup>th</sup> day T2 group showed formation of epithelialisation and deposition of collagen on the surface of the skin and it could be due to the healing effect of *Tridax procumbens*. On the 10<sup>th</sup> day T3 showed formation of well laid collagen and epithelialisation.

Previous studies have shown the pro-healing properties of silver that not only act as antimicrobial agent but also quicken closure and healing time of wounds [14,15]. Silver nanoparticles have potentially decreased the side effects of silver as a compound. Furthermore, surface exposure is larger on using silver nanoparticles and they exhibit unique physicochemical and biological properties [16–18]. Studies prove the ability of silver nanoparticles in reducing inflammation, scar appearance and promoting wound healing [17,19]. Silver nanoparticle treated skin is found to resemble that of normal skin and their mechanical



(a). UV-Vis absorption spectral analysis : Silver nanoparticles synthesized using *Tridax procumbens* leaf extract shows an absorbance peak at 445nm.



(b). SEM Image of Silver nanoparticles of *Tridax procumbens*

**Fig. 1.** a). UV-vis absorption spectral analysis: Silver nanoparticles synthesized using *Tridax procumbens* leaf extract shows an absorbance peak at 445 nm. b). SEM Image of Silver nanoparticles of *Tridax procumbens*.

**Table 1**

Healing response of wound in the control group.

Days	Wound size(cm)	Healing response (%)
Initial	1.77	0
2 <sup>nd</sup> day	1.53	13.56
4 <sup>th</sup> day	1.26	28.81
6 <sup>th</sup> day	0.93	47.46
8 <sup>th</sup> day	0.78	55.93
10 <sup>th</sup> day	0.61	65.54

**Table 2**

Healing response of wound in the group treated with silver nitrate (T1).

Days	Wound size(cm)	Healing response (%)
Initial	1.77	0
2 <sup>nd</sup> day	1.42	19.77
4 <sup>th</sup> day	1.13	36.16
6 <sup>th</sup> day	0.84	52.54
8 <sup>th</sup> day	0.66	62.71
10 <sup>th</sup> day	0.53	70.05

**Table 3**

Healing response of wound in the group treated with *T. procumbens* leaf extract (T2).

Days	Wound size(cm)	Healing response (%)
Initial	1.77	0
2 <sup>nd</sup> day	1.36	23.16
4 <sup>th</sup> day	1.05	40.68
6 <sup>th</sup> day	0.76	57.06
8 <sup>th</sup> day	0.59	66.67
10 <sup>th</sup> day	0.39	77.96

**Table 4**

Healing response of wound in the group treated with silver nanoparticles of *T. procumbens* (T3).

Days	Wound size(cm)	Healing response (%)
Initial	1.77	0
2 <sup>nd</sup> day	1.23	30.51
4 <sup>th</sup> day	0.95	46.33
6 <sup>th</sup> day	0.71	59.89
8 <sup>th</sup> day	0.49	72.32
10 <sup>th</sup> day	0.14	92.09

function [20]. The present study shows the ability of silver nanoparticles in aiding epithelialisation. In addition it also shows that the healing was considerably slower in wounds not treated with silver nanoparticles. The re-epithelialization on the wounded surface of fish was similar to that of re-epithelialisation of human skin [21].

**5. Limitations of the study**

The current investigation revealed the effectiveness of silver nanoparticles as a wound healer of *Pangasius hypophthalmus* in intensive conditions. Considering *Pangasius hypophthalmus* as an ornamental fish, they are susceptible to attack by predators. Wounds created by such incidents needs to be attended and the current study can be extended to study its impact. Local sequence alignment results show 50% similarity between *Pangasius hypophthalmus* and humans. Further clinical study is to be performed to evaluate the potential of this wound healing process for mammalian use.

**6. Conclusion**

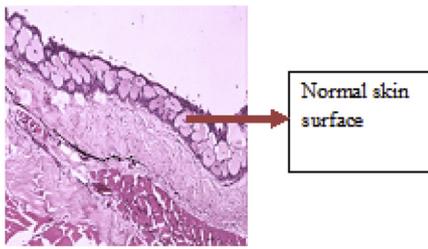
Healing of wound is quite a complicated process involved in epidermal regeneration, fibroblast proliferation and neovascularization. In the present investigation, an attempt has been made to study the dynamic process of regeneration of superficial tissues and healing process in fish skin. The aqueous leaf extract of the plant *Tridax procumbens* was found to be most suitable for the biosynthesis of silver nanoparticles. Silver nanoparticles of *Tridax procumbens* were found to be an effective remedial measure for wound healing. In view of the fact that *Pangasius hypophthalmus* and human genotype possess 50% of clinical relevance, this study might be used a base for developing wound healing formulations for the need of mankind. Wound healing experiments using *Pangasius hypophthalmus* model showed that the application of silver nanoparticles onto an open wound reduces inflammation, induces significant wound contraction by epithelialisation, and promotes healing.

**Ethics statement**

The experiment was conducted in accordance with National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

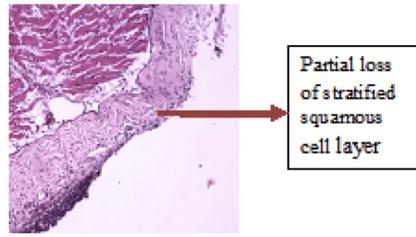
**Funding**

This research did not receive any specific grant from funding



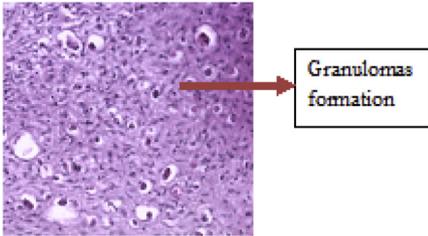
Normal skin surface

(a). Microscopic image of normal epithelial tissue of *Pangasius hypophthalmus*.



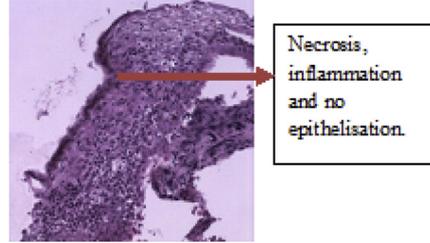
Partial loss of stratified squamous cell layer

(b). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* on the initial day.



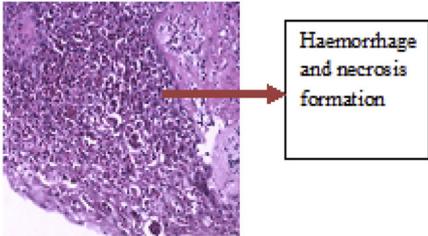
Granulomas formation

(c). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* on the 5th day (C)



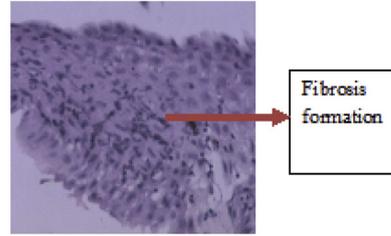
Necrosis, inflammation and no epithelisation.

(g). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* on the 10<sup>th</sup> day (C).



Haemorrhage and necrosis formation

(d). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with AgNO<sub>3</sub> on the 5th day (T1).



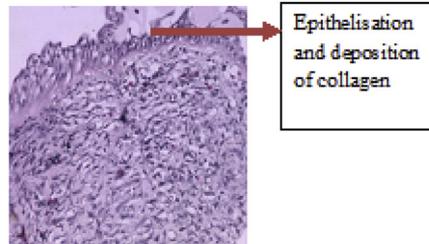
Fibrosis formation

(h). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with AgNO<sub>3</sub> on the 10<sup>th</sup> day (T1).



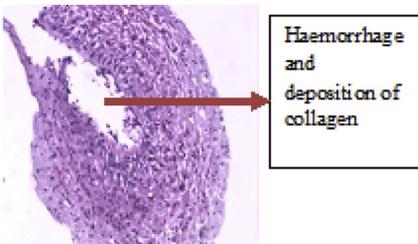
Haemorrhage and infiltration of inflammatory cells

(e). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with plant extract on the 5th day (T2).



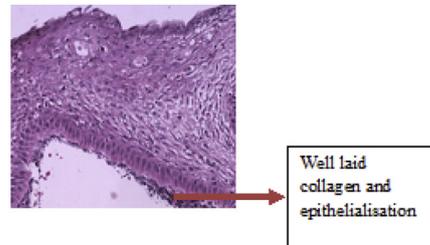
Epithelisation and deposition of collagen

(i). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with plant extract on the 10<sup>th</sup> day (T2).



Haemorrhage and deposition of collagen

(f). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with silver nanoparticles of plant extract on the 5<sup>th</sup> day (T3).



Well laid collagen and epithelisation

(j). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with silver nanoparticles of plant extract on the 10<sup>th</sup> day (T3).

**Fig. 2.** a). Microscopic image of normal epithelial tissue of *Pangasius hypophthalmus*. b). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* on the initial day. c). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* on the 5th day (C). d). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with AgNO<sub>3</sub> on the 5th day (T1). e). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with plant extract on the 5th day (T2). f). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with silver nanoparticles of plant extract on the 5<sup>th</sup> day (T3). g). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* on the 10<sup>th</sup> day (C). h). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with AgNO<sub>3</sub> on the 10<sup>th</sup> day (T1). i). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with plant extract on the 10<sup>th</sup> day (T2). j). Microscopic image of wounded epithelial tissue of *Pangasius hypophthalmus* treated with silver nanoparticles of plant extract on the 10<sup>th</sup> day (T3).

agencies in the public, commercial, or not-for-profit sectors.

### Declaration of Competing Interest

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

### Acknowledgment

The authors would like to thank the Histopathology department of Madras Veterinary College, Vepery for assisting in histological analysis.

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