

Tridax Procumbens -Silver Boost: Green-Synthesized Agnps Infuse Collagen Membranes For Periodontal Regeneration

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Abstract

The aim of the study is to synthesize silver nanoparticles from aqueous extract of *Tridax procumbens* to investigate the wound healing property with the histopathological studies. Silver nanoparticles (AgNPs) were prepared by combining silver nitrate (0.1 mM) with the plant extract and the synthesized nanoparticles was characterized by UV, FT-IR, SEM, TEM and XRD analysis. The results revealed that nanoparticles were sized ranging from 20 – 90 nm, stabilized, crystalline and spherical in shape. A histopathological study was further showed that the synthesized nanoparticles have wound healing property against zebra fish by caudal fin regeneration compared with that of positive and negative control. Hence, the green synthesized AgNPs has the potential against wound healing mechanism and can be used for periodontitis is closely associated with systemic inflammatory conditions.

Keywords: green synthesis; histopathology; silver nanoparticles; *Tridax procumbens*; wound healing, periodontitis.

Introduction

Traditional treatments have originated from botanical sources, and contemporary research has identified new compounds that can serve both preventive and therapeutic purposes. In response to adverse conditions, plants undergo physiological adjustments and alterations in their biochemical traits, leading to the production of various secondary metabolites. Numerous studies have shown that secondary metabolites, such as alkaloids, terpenes, and polyphenols, possess antimutagenic and medicinal properties [1,2,3].

Nanotechnology has received a lot of attention from researchers in modern material science and technology. The greater surface area to volume ratio of nanoparticles is their most important and distinctive feature [4]. The main advantage of using plant extracts to make silver nanoparticles (AgNPs) is that they are easily accessible, nontoxic, and usually nontoxic plants that can help reduce silver ions. AgNPs have numerous medical uses, including antibacterial, antifungal and anticancer therapies. The primary advantages of using plant extracts to make metal nanoparticles are their accessibility, safety, and non toxicity, which help to stabilize and reduce the metal ions. Among the several phytoproducts present in plants that are directly involved in the reduction of silver ions are terpenes, flavones, ketones, aldehydes, amides, and carboxylic acids. Phytochemicals can synthesize AgNPs more quickly than microbes [5,6].

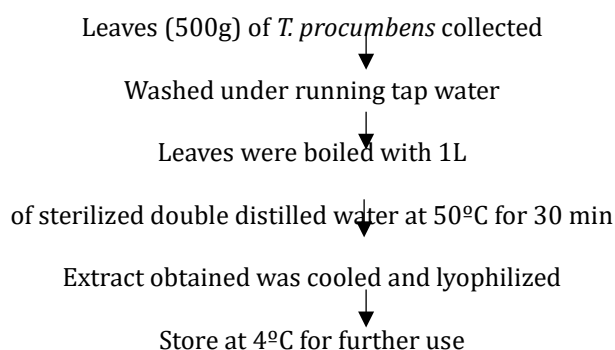
The disruption of normal biological tissues and organs is known as wound healing. It promotes healing in the injured areas through dynamic, controlled, and molecular cell mechanisms. Among the advancements in the field of wound healing, emerging trends in the healthy economy are expanding quickly. Natural ingredients are frequently used by researchers to create therapeutic medications that have the ability to cure wounds [7]. Medicinal plants with diabetic properties are mostly found in the leaves. Many plants are rich in bio-active constituents which have diabetic property and the use of medicinal herbs are cheaper when compared with that of drugs [8-12].

In tropical and subtropical regions of tropical America, the perennial herb *Tridax procumbens* L. thrives as a weed. It belongs to the family Asteraceae, a procumbent herb commonly used by traditional healers and tribal groups to treat a range of ailments and skin issues. β sitosterol, carotenoids, luteolin and linolenic acid that promotes epithelialization and wound healing [13]. This present study is focused on green synthesis of AgNPs from fresh leaves of *T. procumbens* and evaluation of antidiabetic activity of both *T. procumbens* AgNPs nanoconjugates.

Methodology

Preparation of plant extract

Fresh leaves of *T. procumbens* L. were collected from Chrompet, Chennai, India (Fig.1a), washed under running tap water to remove the debris and was finally rinsed with deionized water. One liter of sterilized deionized distilled water (DDW) was added and heated in a water bath at 50°C for 30 min. The extract obtained was cooled and double filtered and the filtrate was lyophilized and the powder obtained was stored in an amber bottle. Analytical grade chemicals supplied by S.D. Fine Chemicals and Hi-Media chemicals were used for the study.



Synthesis of silver nanoparticles

5 mL of aqueous leaf extract of *T. procumbens* L. was added to 50 mL of aq. AgNO₃ (1 mM). The reaction mixtures were stored under dark condition at room temperature and the color change of the reaction mixtures was monitored to determine nanoparticle formation which was indicated by appearance of dark brown color. Once the solutions reached to a maximum intensity, the vials were stored at room temperature to prevent more agglomeration of the AgNPs. 50 mL aliquot of AgNO₃ solution containing 5 mL of DDW was used as a negative control and DDW as blank for measuring UV-Visible absorption spectra. The reduction of Ag⁺ to Ag⁰ was monitored by UV-Visible spectral analysis. Synthesized AgNPs and control samples were scanned from 190–900 nm using a UV-Visible absorption spectrophotometer [14]. The morphology, size and elemental composition of the synthesized AgNPs have been identified by using scanning electron microscope (SEM) (Hitachi SU6600). One drop of colloidal AgNPs were loaded on a carbon coated copper grid and dried under vacuum. Then, the transmission electron microscopic (TEM) images and selected area electron diffraction (SAED) pattern of synthesized AgNPs were captured on a FEI-TECNAI G2 (T-30) transmission electron microscope at an accelerating voltage of 250 kV.

Phytochemical Analysis

Phytochemical analysis of the aqueous extract of *T. procumbens* was carried out using standard protocols [15-19].

Total Phenol Content

Twenty μ L of extracts was prepared using the supernatant and made up to 3 mL of distilled water. Then, 0.5 mL of Folin-Ciocalteu's phenol reagent was added to all the tubes. The tubes were then placed in the incubator for 3 min at 45°C. After 3 min, 2 mL of 20% Na₂CO₃ was added to all the tubes and kept for incubation after which, its absorbance was measured at 650 nm [20].

Total Flavonoid Content

Flavonoid contents were determined by slightly modified spectrophotometry method of Karadeniz *et al.* (2005). The aliquot of the sample (0.5 mL) was taken in a test tube add 3 mL of distilled water and 0.3 mL of 5% sodium nitrite were added. The solution was vortexed and allowed to stand at room temperature for 5 min. and 0.6 mL of 10% aluminium chloride was added to the solution. After 6 min, 2 mL of 1 M sodium hydroxide was added to the test tube. The solution was made up to 10 mL with distilled water. The absorbance was read at 510 nm [21].

DPPH assay

The percentage of antioxidant activity of each substance was assessed by DPPH free radical assay. The reaction mixture consisted of adding different concentrations of sample (10-100 µg/mL), 1 mL of water and 1 mL of DPPH radical solution. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 30 min of reaction using a UV-VIS spectrophotometer. The control solution was prepared by mixing methanol (1.0 mL) and DPPH radical solution (1.0 mL) and 1 mL of methanol serves as blank [22].

The scavenging activity percentage was determined according to

% of inhibition = $\frac{\text{Control O.D} - \text{Sample O.D}}{\text{Control O.D}} \times 100$.

Anti-inflammatory Activity: - HRBC Membrane Stabilization Method

The anti-inflammatory activity of *T. procumbens* was assessed by *in vitro* HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with isosaline. To 1 mL of HRBC suspension, equal volume of test drug in different concentrations (10-100 µg/mL) was added. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm [23].

UV-Visible Absorbance Spectroscopy

UV-Visible spectroscopy analysis was carried out on a Jasco UV absorption spectrophotometer V-650 with a resolution of ± 1 nm between 200 and 800 nm processing a scanning speed of 200 nm/min. Equal amounts of the suspension (0.5 mL) were taken and analyzed at room temperature. The progress of the reaction between metal ions and the plant extract was monitored by UV-Visible spectra of silver nanoparticles in aqueous solution with different wavelength in nanometers from 200 to 800 nm [24]. The reduction of silver ions and formation of silver nanoparticles occurred after 24hrs of reaction. Control was maintained by using AgNO₃.

Fourier Transforms Infrared Spectroscopy (FTIR)

For FTIR measurements, the synthesized silver nanoparticles solution was centrifuged at 10000 rpm for 30 min. The pellet was washed thrice with 5 mL of deionized water to get rid of the free proteins or enzymes that are not capping the silver nanoparticles. The pellet was dried by using vacuum drier. It was analyzed by FTIR [24].

Scanning Electron Microscope

The morphology, size and elemental composition of the synthesized AgNPs have been identified by using scanning electron microscope (SEM) and transmission electron microscope (TEM) (Hitachi SU6600). One drop of colloidal AgNPs were loaded on a carbon coated copper grid and dried under vacuum and selected area electron diffraction (SAED) pattern of synthesized AgNPs were captured on a FEI-TECNAI G2 (T-30) transmission electron microscope at an accelerating voltage of 250 kV [24].

Wound Healing Property

Zebra fish were anesthetized by 0.02% tricaine (Tokyo Chemical Industry) in 0.03% artificial seawater. Caudal fins were amputated with a razor. The amputation site was as follows. Straight amputation: parallel to the dorsal-ventral axis at the tip of a non-segmented fin ray that was connected to the uroneural. Stepwise amputation: the dorsal and ventral fin rays were amputated proximally or distally, respectively. proximal amputation was at the same site as the straight

amputation, and distal amputation was at three to four segments of lepidotrichia distal to the proximal amputation. The amputated caudal fins were preserved in 10% formaldehyde solution for the cells to be in contact and later carried for histopathology analysis [25]. The fins were then incubated four times in staining buffer (100 mM Tris HCl (pH 9.5), 50 mM MgCl₂, 100 mM NaCl, 0.1% Tween 20, 1 mM levamisole) for 5 minutes at RT. After four incubations with staining buffer, the fins were incubated with substrate in staining buffer (substrate: 4.5 µl/ml NBT, 3.5 µl/ml BCIP) for over an hour at RT and washed twice in PBS for 10 minutes each. Samples were observed and photographed in the same solution with a camera (Pixera Penguin 600CL) connected to a stereoscopic microscope (Leica M165 FC).

Results

Phytochemical Analysis

500g of plant samples were collected, washed, shade dried and extracted by soxhlet method (Fig. 1). Phytochemical analysis was carried using standard procedure and was evaluated for the phytoconstituents. The extract showed the presence of alkaloids, carbohydrates, proteins, phenols and flavonoids and absence of glycosides (Table 1). The results were interpreted with the research data showed cited [26-30]. Quantitative analysis report also showed the total phenol content of 92mg/g/GAE and 119mg/g/QE of total flavonoid content (Table 2). According to the authors reported had significant data of the analysis with that of the research data observed [31,32].

Synthesis of nanoparticles

Nanoparticles were synthesized from *T. procumbens* extract using 1mM silver nitrate solution. Among the concentrations of the samples used, 8mg of sample showed maximum synthesis of nanoparticles (Fig. 5).

Characterization of silver nanoparticles

The UV result showed that the nanoparticles has been synthesized at 456 nm (Fig. 2,3). On addition of different concentrations of the samples with reaction to silver nitrate solution the colour of the samples changed from pale green to red brownish coloured solution showing the formation of silver nanoparticles having similar results reported in the literature studies [33-36]. FT-IR analysis of the nanoparticles synthesized showed the functional groups such as phenols from capping agents stabilizes the nanoparticles formed (Fig. 4). The functional groups capping due to plant extract showed that 3439 cm⁻¹ due to N-H stretching and O-H over lapping. A peaks at 2918 cm⁻¹ and 2362 cm⁻¹ can be assigned to alkyne group present in phytoconstituents of extract. The peak at 1636 cm⁻¹ corresponds to amide C=O stretching. From the results of FT-IR obtained it showed that the extract showed maximum strong capping agent of the nanoparticles synthesized which adhere to the research literatures [37-38].

SEM analysis of the nanoparticles showed spherical shaped particles, agglomerated and the particle size ranges from 20 - 90µm. The HRTEM analysis showed the particles are spherical in shape with particle size of 20nm and the existence of crystal-like pattern confirms the Ag nanoparticles crystalline formation (Fig. 5,6). The TEM analysis shows that the silver nanoparticles are well dispersed and predominantly spherical in shape, while some of the NPs were found to be having structures of irregular shape, the particle size showing at 50 nm [3940]. X-ray diffraction method was carried out to investigate the crystalline structure along with lattice characteristics of the synthesized AgNPs. The angle 2θ values ranging from 20° to 100° using Cu Kα whose wavelength range 2.997 Å. Three peaks can be seen clearly in the fig. 9 that appeared at 2θ values 30.0°, 49.5° and 57.8°. These 2θ values are the representation of the following diffraction planes (29.09), (48.55) and (57.8) of the structure of the synthesized silver nanoparticles (Fig. 7). The maximum intensity peak found at (29.09) plane that is the ideal orientation of the structure. The study shows that the nanoparticles formed resembles with the results obtained by the literature studies [40].

Antioxidant activity

The dose-dependent activity of the sample with different concentrations from 10 – 100 µg/mL showed the chemical shift of the electrons produced to change the colour from purple to yellow

which is shown in the table 3, Fig. 8. Sample concentration at 10 µg/mL showed 27.08 % of inhibition and at 100 µg/mL showed 58.33 % of inhibition. According to the existing results obtained, the work showed good anti-oxidant potential from the nanoparticles synthesized [41].

Anti-inflammatory activity

Plant extract consists of various phytoconstituents which helps to inflammatory mediators like prostaglandins and reactive oxygen species (ROS) showing potential antiinflammatory drugs (Table. 4, Fig. 9). The generation of silver nanoparticles by the capping agents of phytoconstituents of the extract showed alkaloids which helps to cover the silver nanoparticles produced by preventing them from aggregating and stabilizing them promoting anti-inflammatory activity [41].

Caudal fin regeneration

AgNPs synthesized from *T. procumbens* showed caudal fin regeneration of zebra fish which shows the cell proliferation of the tissue repair at lower dosage while, the dosage was increased, the tissue regeneration was observed to be very low and mortality of the zebra fish was observed which is been quoted with research articles published [42-43]. Group I and II showed the caudal fin regeneration and as the concentration of the samples was increased, death of the zebra fish was observed (Table 5-7).

Zebra fishes showed some of the significant features like two to three cell layers thick with compact keratinocytes, underlying dermis intact, with well-organized collagen fibers and capillary networks and no inflammatory infiltrate observed before treatment. Treatment at 100µg concentration showed the morphological changes as complete epithelial closure, restoration of epidermal layers, and reduced inflammatory cells, reformation of basement membrane beneath the new epidermis and remodeling phase; tissue architecture approaches normal, with maturation of collagen fibers and reduced cellularity. Treatment at 150 and 200µg concentration showed the significant morphological changes as acute inflammatory phase - cellular debris and leukocyte infiltration indicate the initiation of immune-mediated clearance and epidermal discontinuity at the wound margin; infiltration of neutrophils and macrophages; edema and tissue disorganization (Fig. 10,11,12).

Conclusion

Plants-mediated (*T. procumbens*) synthesis of silver nanoparticles is an eco-friendly, cost-effective method where phytochemicals in plant extracts act as reducing and stabilizing agents for Ag⁺ ions. Generally, AgNPs from plant extracts show antibacterial activity against periodontal pathogens and may help prevent biofilms implicated in periodontal disease. Such AgNPs also have strong antioxidant activities that could help modulate inflammation. Plantsynthesized AgNPs combine antimicrobial and anti-inflammatory potential with eco-friendly synthesis — desirable features for periodontal therapy delivery systems (e.g., gels, membranes, coatings) that aim to control bacterial infection and support tissue healing.

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Table. 1. Phytochemical analysis of aqueous extract obtained from *Tridax procumbens*

S.No.	Phytochemical	Inference
1	Alkaloids	Present
2	Carbohydrate	Present
3	Glycosides	Absent

4	Saponins	Present
5	Protein	Present
6	Phenolic compound	Present
7	Flavonoids	Present

Table. 2. Quantitative analysis of aqueous extract obtained from *Tridax procumbens*

Phytoconstituents	Absorbance
Total phenol content	92 mg/g/GAE
Total flavonoid content	119 mg/g/QE

Table. 3. Antioxidant activity of *Tridax procumbens* extract

sample conc. µg/mL	% of inhibition
10	27.08
20	31.25
30	35.41
40	39.58
50	41.66
60	43.75
70	47.91
80	52.08
90	54.16
100	58.33

Table. 4. Anti-inflammatory activity of *Tridax procumbens* extract

sample conc. µg/mL	% of inhibition
10	15
20	21.25
30	26.25
40	28.75
50	35
60	38.75
70	41.25
80	43.75
90	47.5
100	51.25

Table. 5. Grouping of zebra fish using AgNps of *Tridax procumbens* extract

Grouping	Feed Type	Concentrations Used
Group I	Fish Feed + sample	50µg
Group II	Fish Feed + sample	100µg
Group III	Fish Feed + sample	150µg

Group IV	Fish Feed + sample	200µg
Control	Fish Feed Only	-

Table. 6. Measurement of caudal fins before treatment

S.No.	Group	Measurement (in cm)
1.	Group I	2.8
2.	Group II	3.1
3.	Group III	3.4
4.	Group IV	3.5
5.	Control	3.2

Table. 7. Measurement of caudal fins after treatment

S.No.	Group	Measurement (in cm)
1.	Group I	3.4
2.	Group II	3.6
3.	Group III	3.4
4.	Group IV	3.5
5.	Control	3.2

Fig. 1
T.



procumbens extraction

Soxhlet Extraction

Condensed Extract

Fig. 2
Nanoparticles Synthesis

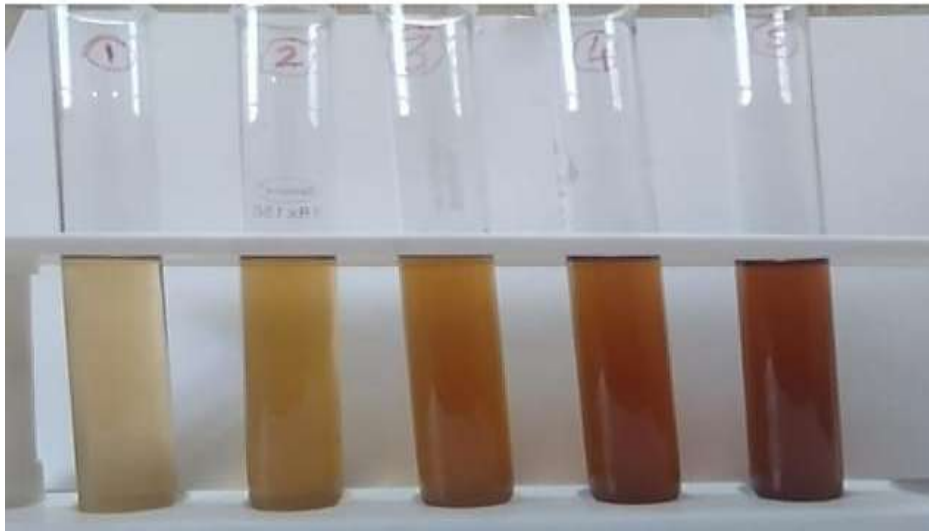


Fig. 3

UV

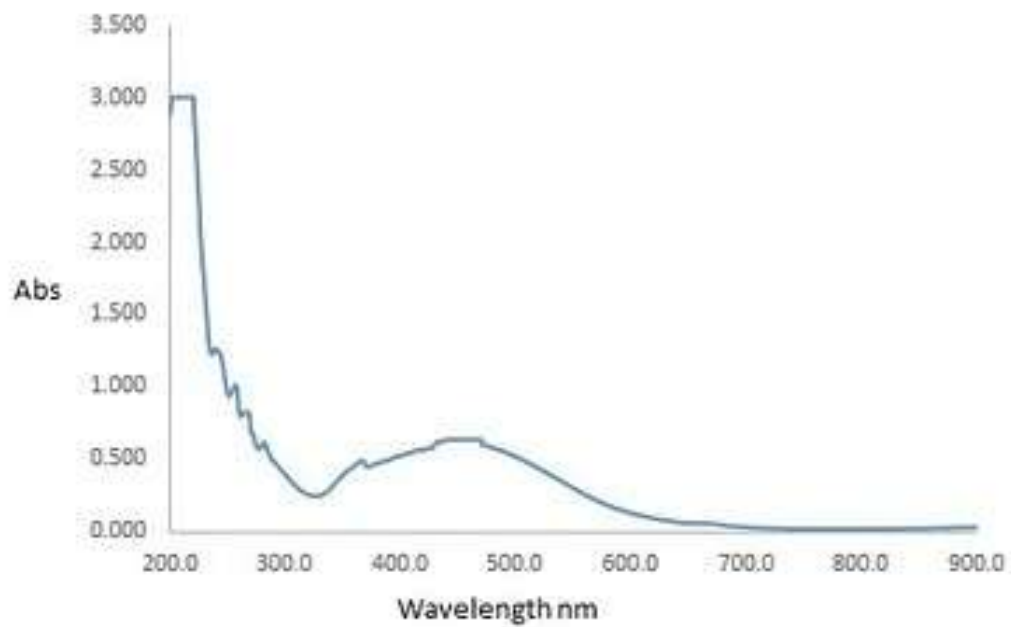


Fig. 4 FT-IR

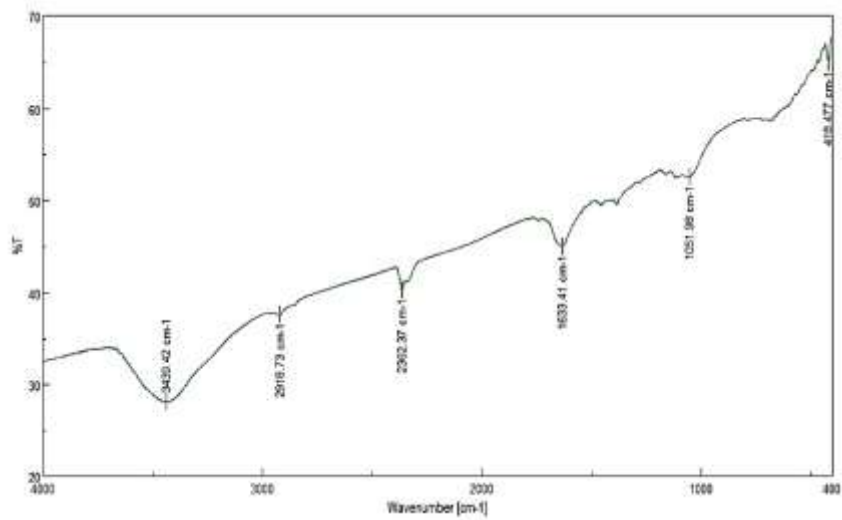


Fig. 5
SEM

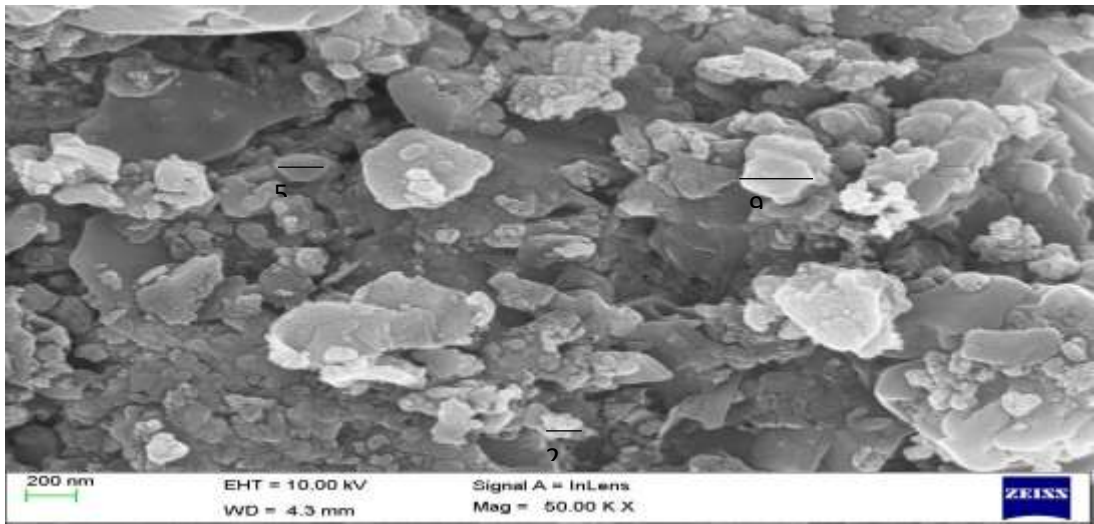


Fig. 6
HRTEM

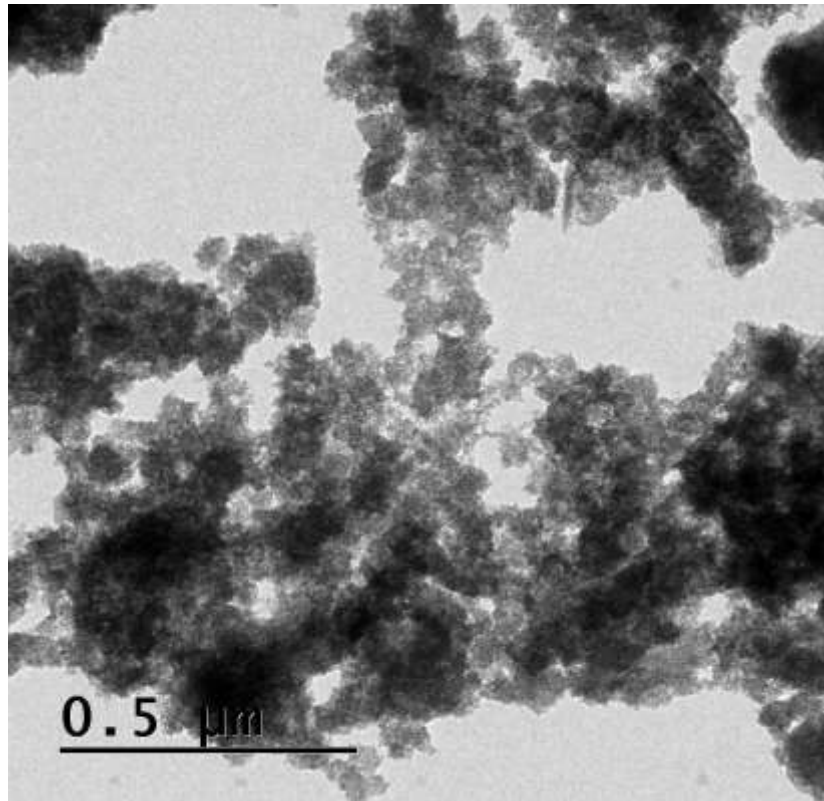


Fig
XRD

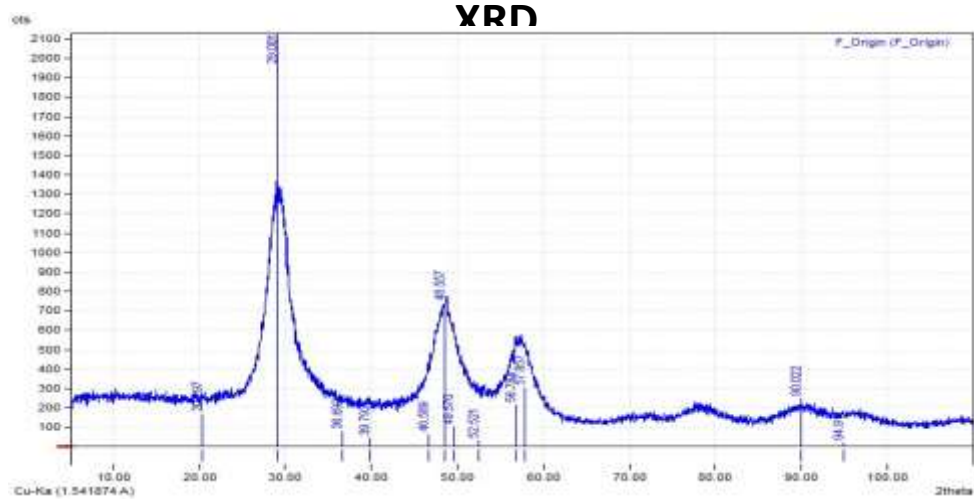


Fig. 8
Antioxidant activity of *f. procumbens*

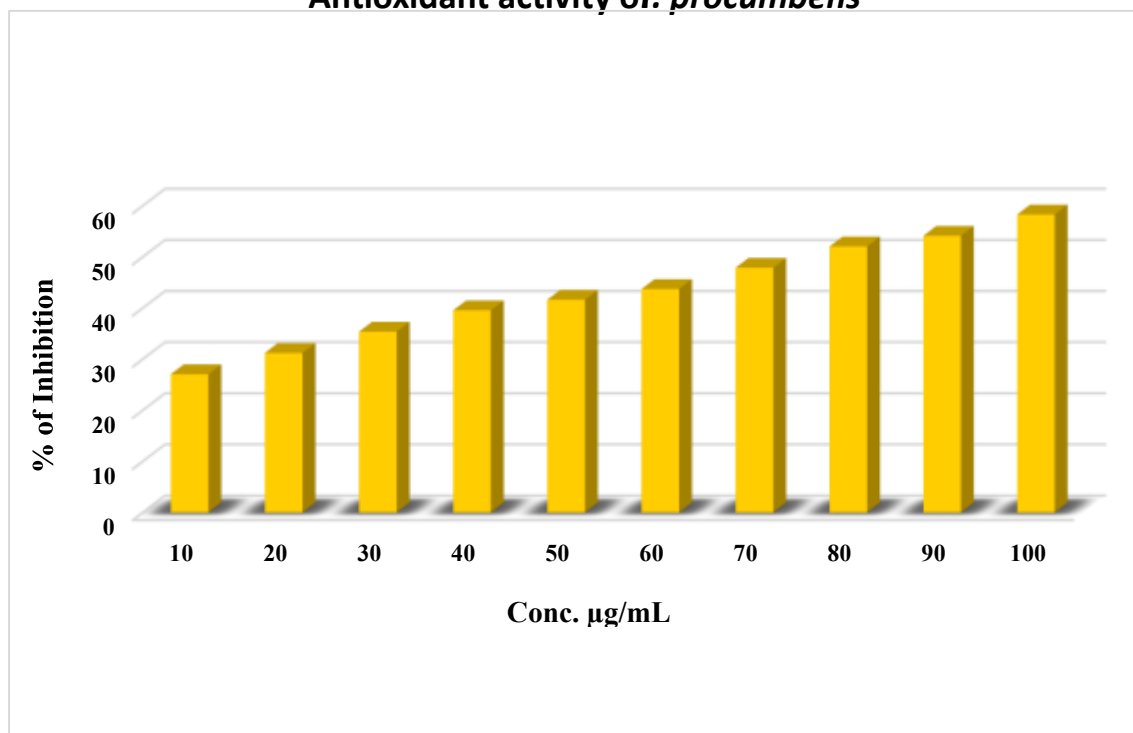


Fig.9Anti-inflammatory Activity

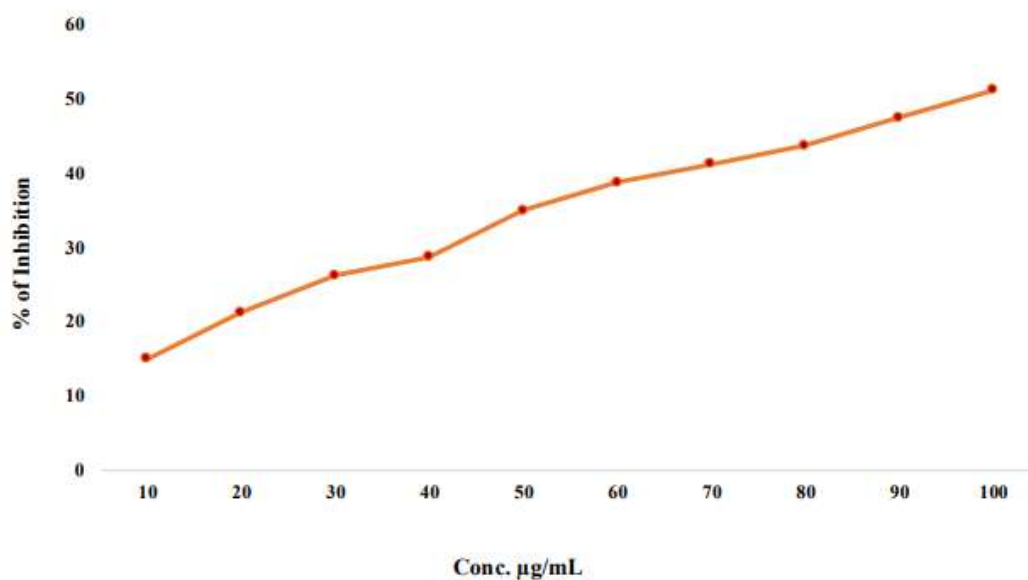


Fig. 10 Found healing activity of zebra fish treated with silver nanoparticles synthesized from extract
Before Treatment

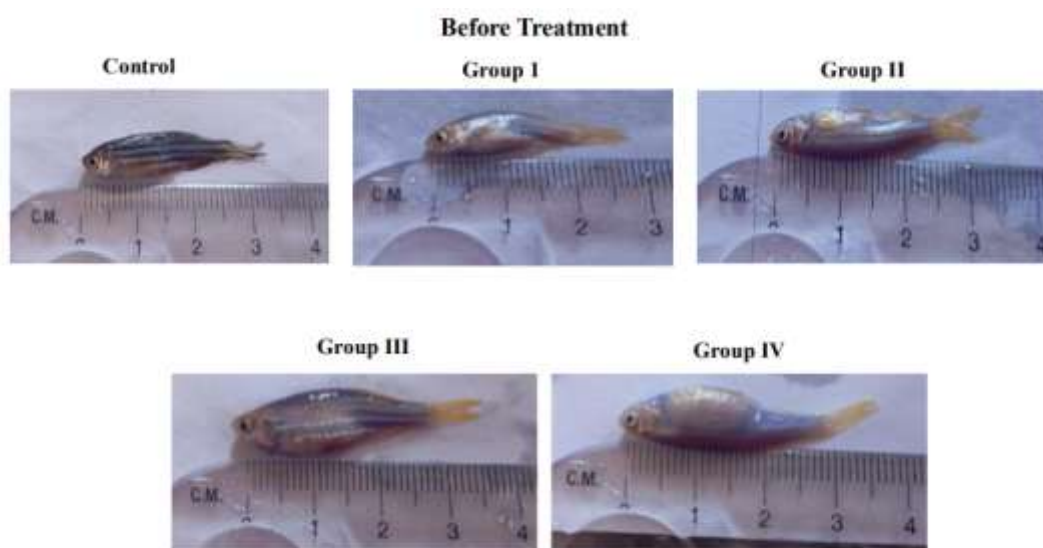


Fig. 11
After Treatment

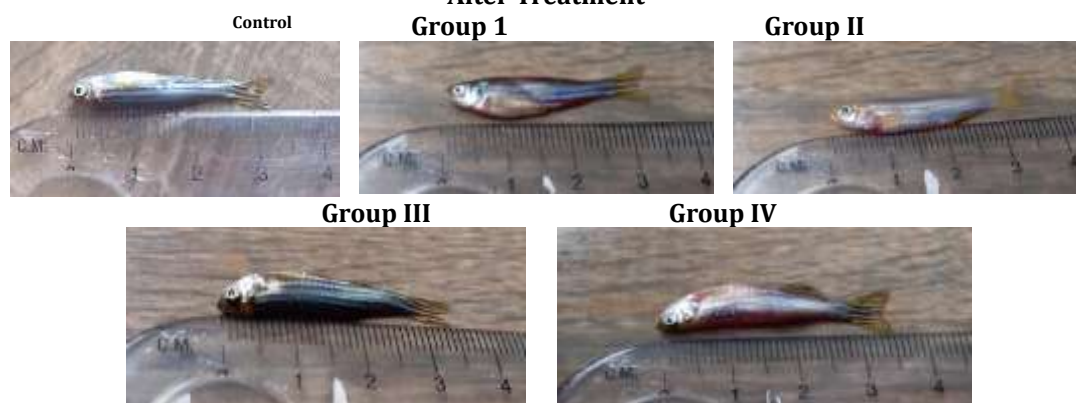
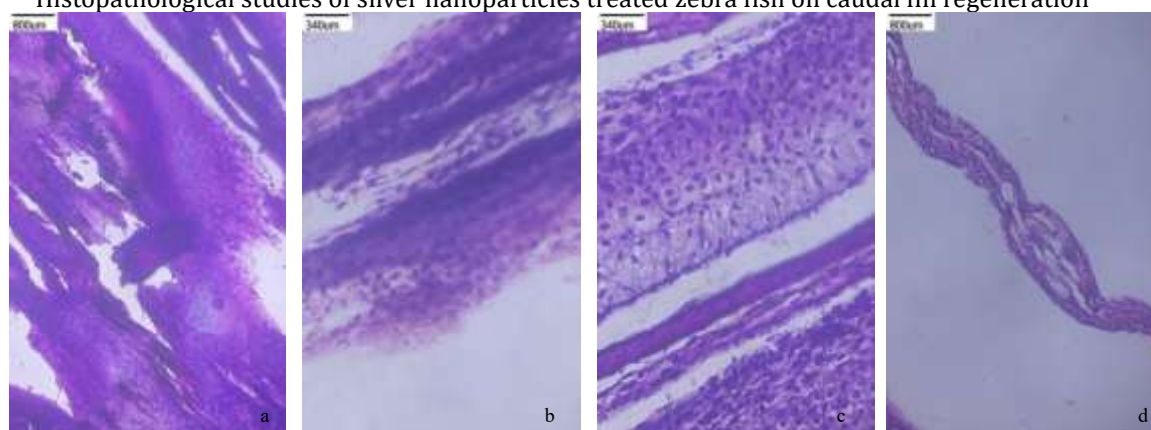


Fig. 12

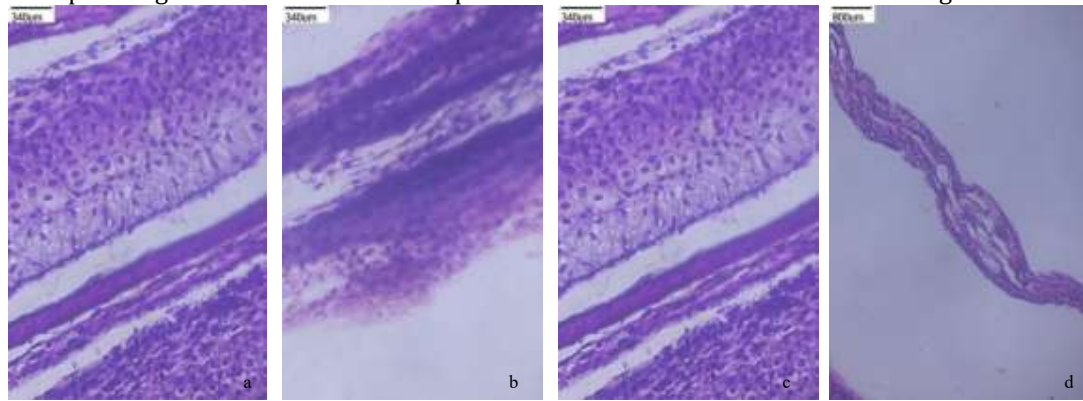
Histopathological studies of silver nanoparticles treated zebra fish on caudal fin regeneration



- a. Before treatment
- b. After treatment at 50µg conc.
- c. After treatment at 100µg conc.
- d. After treatment at 200µg conc.

Fig. 12

Histopathological studies of silver nanoparticles treated zebra fish on caudal fin regeneration



- a. Before treatment
- b. After treatment at 50µg conc.
- c. After treatment at 100µg conc.
- d. After treatment at 200µg conc.