

DESIGN, SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING TRIDAX PROCUMBENS FLOWER EXTRACT

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ABSTRACT

An eco-friendly ethanolic extract of the *Tridax procumbens* plant is used in the manufacture of silver nanoparticle (AgNps), which are then examined using UV and other analytical methods. Silver nanoparticle are prepared and tested for biological activity by measuring the invitro anti-oxidant activity using ascorbic acid (vitamin C) and DPPH as standards, as well as the invitro anti-inflammatory activity using protein denaturation assay. The colour shift from colourless to reddish brown indicates the creation of silver nanoparticles during biosynthesis and peak absorption at 400-490 nm indicates AgNps generation. They showed significant anti-oxidant and anti-inflammatory properties when compared to standard under comparable condition. Thus in-vitro data are suitable for animal studies.

KEYWORDS: *Tridax procumbens*, antioxidant, DPPH assay, anti-inflammatory, Sliver nanoparticle.

INTRODUCTION

Tridax procumbens Linn. is a wild plant, found as weed throughout India. The plant is native to tropical America and naturalized in tropical Africa, Asia, and Australia. Local people knew it as "Ghamara," in English popularly called 'coat buttons' and is dispensed for "Bhringraj" by some of the practitioners for hair growth in Ayurveda. The pharmacognostic studies give pharmacopoeial standards like physical constants and leaf constants. The phytochemical screening revealed the presence of alkaloids, carotenoids, flavonoids, fumaric acid, β -itosterol, saponins, and tannins. It is richly endowed with carotenoids, saponins, oleanolic acid, and ions like sodium, potassium, and calcium. Luteolin, glucoluteolin, quercetin, and

isoquercetin have been reported from its flowers.

It is known for its number of pharmacological activities like hepatoprotective activity, anti-inflammatory, wound healing, antidiabetic activity, hypotensive effect, immunomodulating property, anticancer activity, antioxidant activity, bronchial catarrh, dysentery, diarrhea, preventing falling of hair, promoting the growth of hair, and antimicrobial activity against both gram-positive and gram-negative bacteria (Dattaray, D., 2022). The leaf juice possesses antiseptic, insecticidal, and parasiticidal properties as a remedy against conjunctivitis and is used also to check hemorrhage from cuts, bruises, and wounds with insect repellent (Kumar, S., Prasad, A., Iyer, S.V., and Vaidya, S., 2012).

T. procumbens has been used since ancient times to treat wounds and skin diseases and to stop blood clotting in folk medicine. It possesses anticoagulant, antileishmanial, antioxidants, anticancer, immunomodulatory agent, insecticidal, anthelmintic cardiovascular, antiseptic, antimicrobial, and insecticidal properties (Ingole, V.V., Mhaske, P.C., and Katade, S.R. 2022). This is rich in alkaloids, steroids, carotenoids, flavonoids (such as catechins, centaurein, and bergenins), fatty acids, phytosterols, tannins, and minerals. Concoctions of extracts from *T. procumbens* leaves, stems, flowers, and roots are used to treat patients suffering from diabetes, arthritis, inflammatory reactions, and even applied to open wounds (Gubbiveeranna, V., and Nagaraju, S., 2016).

Green silver nanoparticles of *T. procumbens* could be safe, as they are endowed with potential antimicrobial activity against multi-drug resistant (MDR) clinical isolates and human lung carcinoma cells (Pungle, R., et al., 2022). Histopathological study further confirmed the almost normal skin structure of treated animal tissue compared to standard and negative control. Thus, green synthesized AgNP-loaded chitosan-based topical gel can potentially be used for wound healing applications (Fatima, F., ET AL 2021). It shows a number of pharmacological activities like hypotensive, insecticidal, leishmanicidal, hair growth-promoting, wound healing, anti-inflammatory, hepatoprotective, and immunomodulatory, due to the presence of phenolics, tannins, saponins, and glycosides. Hence, efforts were taken to evaluate fractions of methanolic extract for antioxidant activity by the DPPH method.

The ethyl acetate and n-butanol fractions have shown significant activity, which is comparable to the activity of standard antioxidant ascorbic acid (Agrawal, S.S., Talele, G.S., and Surana, S.J., 2009).

The anti-inflammatory activities of the extracts of *Calotropis gigantea* R.Br. and *Tridax procumbens* Linn. were assessed on carrageenin-induced paw edema along with the standard drug, ibuprofen. The ibuprofen significantly reduced paw edema at the dose of 200 mg/kg body weight, orally. (Das, S., Das, S., Das, M.K., and Basu, S.P., 2009). The aqueous extract of *Tridax procumbens* leaves was lyophilized and studied on the excision wound model, rat skin fibroblast, and rat paw oedema. *Tridax procumbens* did not significantly increase the fibroblast count compared with ibuprofen (Prabhu, V.V., Nalini, G., Chidambaranathan, N., and Kisan, S.S., 2011). Based on the above facts, the present work investigates the biosynthesis of silver nanoparticles from the flower extract of *Tridax procumbens* by ecofriendly method, explore in vitro antioxidant activity by DPPH assay, and examine the anti-inflammatory activity by protein denaturation.

MATERIAL AND METHODS

Collection of samples

The flowers were collected around the Aravindh Herbals Labs, Rajapalayam, Tamilnadu for this investigation.

Method of preparation of samples

The flowers were grinded using mortar and pestle. Grinded rhizomes were stored for 24 hours and concentrated the extract.

Pharmacological evaluation

Preparation of Silver Nanoparticle

The fresh *Tridax procumbens* (TPET) extract solution was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300 mL Erlenmeyer flask along with 100 mL of, sterilized double distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered through Whatman filter paper no. 1 and stored at -15°C and could be used within 1 week. The filtrate was treated with aqueous 1 mM AgNO₃ solution in an Erlenmeyer flask and incubated at room temperature. As a result, a brown-yellow solution was formed, indicating the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by aqueous extract of plant parts to generate extremely stable silver nanoparticles in water. After read the absorbance with different nanometer 400 – 600 nm.

In vitro TPET AgNps Antioxidant activity

Tridax procumbens (TPET AgNps) aqueous extract investigated for in vitro antioxidant

activity by DPPH, ABTS, FRAP and NO for the estimation of anti-oxidant potential of *Tridax procumbens* (TPET AgNps) aqueous extract. *Tridax procumbens* (TPET AgNps) also tested by DPPH assay.

Determination of DPPH radical scavenging activity

Antioxidant activity in the sample *Tridax procumbens* were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (Brand-illiams et al., 1995). About 100 μ L of SC extract was taken in the microtiter plate, 100 μ L of 0.1% methanolic DPPH was added to the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration from purple to yellow and pale pink were considered as strong and weak positive respectively. Read the plate on Elisa plate reader at 490nm. Standard ascorbic acid was used as reference. All the analysis was performed in triplicates and the average values were taken.

Radical scavenging activity was calculated by the following equation

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100.

In-vitro TPET AgNps anti-inflammatory activity - Inhibition of albumin denaturation

The reaction mixture was prepared separately by mixing 0.5 ml aqueous extract of TPET AgNps and its compounds A, B, and C (1mg/ml) with 0.45 ml aqueous solution of bovine albumin fraction (5%). The pH (6.3) of the solution was adjusted using a small amount of 0.1N HCl at 37 °C for 20 min, then heat to 57 °C for 30 min. Cool the solution and transfer it to the 96 well plates and measure the absorbance at 660 nm. Standard was used as diclofenac sodium (1000 μ g/ml) and the control contain 0.05ml distilled water. The percentage of inhibition of albumin denaturation was calculated by the following formula, Percentage of inhibition (%) = [(A control – A sample) / A control] x 100 Where A control – Absorbance of reaction mixture except drug. A sample – absorbance of the reaction mixture with the Sample.

RESULT AND DISCUSSION

Flower extract of *Tridax procumbens* powder has shown anti-inflammatory, antioxidant properties, and biosynthesis of silver nanoparticles. Bioreduction of AgNO₃ by chemical constituents of extract was observed by color change from colorless to reddish brown in

color. *Tridax procumbens* synthesized AgNPs were initially confirmed by UV-visible spectrophotometer absorbance (SPR band) at 471 nm (1.015 OD). The SEM image (morphology) of the AgNPs shows spherical in shape. TPET AgNPs size range from 20 to 100 nm (Table 1) (Figs. 1). *Tridax procumbens* TPET flower extract has shown in vitro antioxidant activity by DPPH assay in the current investigation. At TPET AgNPs, the inhibition percentages are 70% (Table 2), compared to the standard ascorbic acid. As a result, even a low concentration of antioxidant activity is good compared to the standard ascorbic acid vitamin C. A previous paper reported the green synthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of *Tridax procumbens* (TNP), which acts as the source of the reducing and capping agent. The distinctive absorption at 370 nm suggested synthesis of TNPs, which was confirmed by TEM (Pungle, R., et al., 2022). A previous paper suggested that *Tridax procumbens* was analyzed for reducing power ability as an antioxidant using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and for total phenolics using the Folin-iocalteu method. The results of this analysis revealed the fact that plants are rich sources of natural antioxidants (Habila, J.D., et al. 2010).

The inhibition of the albumin denaturation method was used to measure the anti-inflammatory activity in vitro. In comparison to standard diclofenac sodium, neither the crude extract TPET nor its separated constituents exhibit any appreciable anti-inflammatory efficacy. When compared to standard diclofenac sodium, the aqueous extracts of TPET exhibit moderate anti-inflammatory efficacy. TPET had a 51.9%.

(Table 3). While we were carrying out different concentrations in a dose-dependent way and comparing them with the standard, many of them showed the antioxidant and anti-inflammatory properties of TPET. A previous paper reported that the anti-inflammatory activity of *T. procumbens* aerial parts could be at least in part due to COX-1, COX-2 enzyme inhibition, and free radical-scavenging activities, which may be attributed to the presence of flavonoids and other polyphenols in the extracts (Jachak, S.M., et al. 2011). Overall investigation results, such as TPET AgNPs, had significant antioxidant and moderate anti-inflammatory activity. The silver nanoparticle was prepared from an ethanolic extract of *Tridax procumbens*, and it was verified by UV analysis and color observation. The biosynthesis of TPET AgNPs was characterized by instrumental analysis. The ethanolic extract TP ET biosynthesized silver nanoparticle had significant anti-oxidant activity by DPPH assay and good anti-inflammatory activity.

Table 1: UV analysis.

S.No	nm	Maximum absorbance (n=3)
1	420	0.45
2	480	0.43
3	540	0.42
4	620	0.38
5	680	0.42
		Max 400-490 nm

Table 2: Invitro Antioxidant Activity.

TPET AgNPs				
S.NO	COD	SOD	% inhibition	Average
1	1.52	0.67	67	70%
2	1.52	0.69	69	
3	1.52	0.75	74	
Standard Vit C - Ascorbic acid				87.64%
	0.34	0.04	88.24	
	0.34	0.04	88.24	
	0.34	0.05	85.29	

Table 3: Invitro Anti-inflammatory activity by protein denaturation.

TP ET AgNPs				
S.NO	COD	SOD	% inhibition	Average
1	1.54	0.72	53.2	51.9%
2	1.54	0.74	51.9	
3	1.54	0.76	50.6	
Standard Diclofenac				87.96%
1	0.36	0.04	88.89	
2	0.36	0.04	88.89	
3	0.36	0.05	86.11	

**Fig 1: UV analysis.**

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