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Acaricidal activity of synthesized titanium dioxide nanoparticles using *Calotropis gigantea* against *Rhipicephalus microplus* and *Haemaphysalis bispinosa*

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ABSTRACT

Objective: To assess the acaricidal activity of titanium dioxide nanoparticles (TiO_2 NPs) synthesized from flower aqueous extract of *Calotropis gigantea* (*C. gigantea*) against the larvae of *Rhipicephalus* (*Boophilus*) *microplus* [*R. (B.) microplus*] and the adult of *Haemaphysalis bispinosa* (*H. bispinosa*). **Methods:** The lyophilized *C. gigantea* flower aqueous extract of 50 mg was added with 100 mL of $TiO(OH)_2$ (10 mM) and magnetically stirred for 6 h. Synthesized TiO_2 NPs were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), and Energy dispersive X-ray spectroscopy (EDX). The synthesised TiO_2 NPs were tested against the larvae of *R. (B.) microplus* and adult of *H. bispinosa* were exposed to filter paper impregnated method. **Results:** XRD confirmed the crystalline nature of the nanoparticles with the mean size of 10.52 nm. The functional groups for synthesized TiO_2 NPs were 1 405.19, and 1 053.45 cm^{-1} for $-NH_2$ bending, primary amines and amides and 1 053.84 and 1 078.45 cm^{-1} for C-O. SEM micrographs of the synthesized TiO_2 NPs showed the aggregated and spherical in shape. The maximum efficacy was observed in the aqueous flower extract of *C. gigantea* and synthesized TiO_2 NPs against *R. (B.) microplus* (LC_{50}=24.63 and 5.43 mg/L and r^2=0.960 and 0.988) and against *H. bispinosa* (LC_{50}= 35.22 and 9.15 mg/L and r^2 = 0.969 and 0.969), respectively. **Conclusions:** The synthesized TiO_2 NPs were highly stable and had significant acaricidal activity against the larvae of *R. (B.) microplus* and adult of *H. bispinosa*. This study provides the first report of synthesized TiO_2 NPs and possessed excellent anti-parasitic activity.

1. Introduction

Ticks parasitism is a major obstruction for cattle production in many parts of the world. *Rhipicephalus* (*Boophilus*) *microplus* [*R. (B.) microplus*] is an obligate hematophagous parasite of domestic and wild animals that serves as vector of infectious agents lethal to cattle[1]. The data showed that *R. (B.) microplus* occurred predominantly on cattle (42.4%), buffaloes (12.5%), goats (25.5%) and pigs (8.2%) and *Haemaphysalis bispinosa* (*H. bispinosa*) mostly parasitized goats (31.5%) rather than cattle (12.0%) and buffaloes (10.8%)[2].

Titanium dioxide naoparticles (TiO_2 NPs) possessed interesting optical, antimicrobial, chemical stability and catalytic properties which lead to industrial applications such as pigment, fillers, catalyst supports and photo catalyst[3-5]. Rajakumar *et al*[6] reported the TiO_2 NPs were biosynthesized and characterized using *Eclipta prostrata* (*E.*

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prostrata) leaf extract. Green synthesized TiO_2 NPs using the leaf extract of *Nyctanthes arbor-tristis (N. arbor-tristis)* and used for anti-inflammatory, anti-fungal, anti-microbial and anti-leishmanial activities were reported[7]. TiO_2 also showed antibacterial activity against *Escherichia coli (E. coli)*[8] and *Bacillus megaterium (B. megaterium)* using environmental light[9].

Calotropis gigantea (*C. gigantea*), a common medicinal plant in Indian subcontinent, has purgative, anthelmintic, anti-convulsant, sedative and anti-pyretic effect[10,11]. Several phyto constituents have been isolated and identified from different parts of the *C. gigantea* belonging to the category of alkaloids, glycosides, flavanols, tannins, saponins, sterols and triterpenoids[12]. The leaf extract of *C. gigantea* was reported as a potential pest control inhibiting activity against *Sitophilus zeamais (S. zeamais)*[13]. The anthelmintic activity of crude aqueous and methanolic extracts of *Calotropis procera (C. procera)* flowers were evaluated through *in vitro* and *in vivo* studies as evident from their mortality and possessed good activity against nematodes, *Haemonchus contortus (H. contortus)*[14] and the latex showed a concentration dependent larvicidal activity within 20 min of application against the larvae of *H. contortus*[15]. The anti-tick efficacy of combined aqueous leaves extracts of *Azadirachta indica (A. indica)*, *Nicotiana tabacum (N. tabacum)*, flowers of *C. procera* and seeds of *Trachyspermum ammi (T. ammi)* were evaluated using adult immersion test, larval packet test, ear bag method and exerted dose and time dependent response against all the developmental stages of *R. (B.) microplus*[16].

Yadav *et al*[17] reported that the adulticidal activities of leaf methanol extract of *C. gigantea* was investigated against *H. contortus*. The maximum efficacy was observed in the leaf methanol extracts of *Nelumbo nucifera (N. nucifera)*, *Acalypha indica (A. indica)*, and hexane extract of *Manilkara zapota (M. zapota)* against the adults of *H. bispinosa*[18]. Acaricidal activity of essential oils extracted from the seeds of *Cuminum cyminum (C. cyminum)*, berries of *Pimenta dioica (P. dioica)* and leaves of *Ocimum basilicum (O. basilicum)* were tested against *R. (B.) microplus*[19].

In the present investigation, biologically synthesized TiO_2 NPs using aqueous flower extract of *C. gigantea* were found to produce a high acaricidal activity and provides first report on the antiparasitic activity of synthesized TiO_2 NPs against *R. (B.) microplus* and *H. bispinosa*.

2. Materials and methods

2.1. Materials

The flowers of *C. gigantea* L. (Asclepiadaceae) was collected from Melvisharam, Tamil Nadu, India. Taxonomic identification was done by Dr. C. Hema, Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference. Titanium oxyhydrate [$TiO(OH)_2$, purity 99.0%] was purchased from Himedia, Mumbai, India.

2.2. Collection of parasites

R. (B.) microplus (Acari: Ixodidae) engorged female ticks (20-50 individuals) were collected directly from infested animals, placed in identified cardboard boxes and transported to the laboratory. In the laboratory, the engorged females were adhered by the dorsal surface in a glass plate (120 mm×80 mm), with double sided adhesive tape. This glass plate was put inversely on a glass petridish (150 mm diameter), aiming at to collect the eggs lay. The engorged females were maintained in a biological oxygen demand incubator at (27±1) ℃, RH 680% and a 12:12 h photoperiod. In order to obtain larvae of the same age cohort, egg batches were collected daily in separate hatchings tubes of polyethylene with screw caps. Larvae used for the bioassays were 14-21 d old[20,21]. The adults of *H. bispinosa* (Acarina: Ixodidae) fly were collected mainly inside the ears, and occasionally also from other body parts of cattle. The parasites were identified in the Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu.

2.3. Filter paper impregnated bioassay test

The aqueous leaf extract of *C. gigantea*, $TiO(OH)_2$ solution and synthesized TiO_2 NPs were used in filter paper impregnated tests in sealed glass jars. Different concentrations of aqueous extract and $TiO(OH)_2$ solution in 10 to 50 mg/L, respectively and synthesized TiO_2 NPs in 4 to 20 mg/L were prepared. Ten pairs of larvae of *R. (B.) microplus* and adults of *H. bispinosa* were used for bioassay test in each concentration. A series of filter paper envelopes (Whatman filter paper No.1, 16 cm×20 cm) with micropores treated with each concentration of extracts. Each envelope was treated with 3 mL solution uniformly distributed with a pipette on internal surfaces. Five envelopes were impregnated with each tested solution. The control papers were impregnated with distilled water, with three replicates for each individual concentration. The impregnated paper was air dried for 10 mins. The dose response data were subjected to probit analysis to determine the LC_{50} value for 24 h exposure under constant climatic conditions (25 ℃; 12 h: 12 h, L: D)[22].

2.4. Synthesis of TiO_2 NPs

The fresh flower of *C. gigantea* aqueous extract was

prepared by taking 5 g of thoroughly washed and finely cut flowers in 250 mL Erlenmeyer flask along with 100 mL of distilled water and then boiling (60℃) the mixture for 20 min. The extract was filtered with Whatman filter paper No.1 and lyophilized. 100 mL of distilled water was made into 10 mM $TiO(OH)_2$. 50 mg of the lyophilized plant aqueous extract was combined and magnetically stirred for 6 h. The mixture was subjected to ultra sonication for 30 mins to separate out the agglomerates formed. The powder was filtered and then boiled at 90℃ for 2 h to get the synthesized TiO_2 NPs. For the purification of synthesized TiO_2 NPs, 5 mL of 1 mM sucrose and 5 mL of 95% ethanol were added into the solution as a linking conjugate between $TiO(OH)_2$ and aqueous flower extract.

2.5. Characterization of TiO_2 NPs

The resulting pellet was dissolved in deionized water and filtered through millipore filter (0.45 μm). The synthesized nanoparticles were characterized by X-ray diffraction (XRD) spectroscopy (Diffractometer with Philips® PW 1830 X-ray generator). Fourier transform infrared (FTIR) spectra were measured using Perkin elmer spectrum one instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. Powder samples for the FTIR were prepared similar to powder diffraction measurements. The FTIR spectra of synthesized TiO_2 NPs were analysed, which exposed the possible functional groups for the formation of nanoparticles. For the Scanning electron microscopic (SEM) studies, 25 μL of sample was sputter-coated on copper stub, and the images of nanoparticles were studied using SEM (JEOL, ModelJFC-1600). Energy dispersive X-ray spectroscopy (EDX) was carried out to determine the chemical composition of the synthesized TiO_2 NPs.

2.6. Data analysis

The dose dependent mortality data were analysed using the SPSS statistical program[23]. LC_{50} and their associated confidence intervals were estimated after 24 h at different concentrations using probit analysis[24]. Lethal concentrations at the 50% and slope levels were considered significantly different if their associated confidence intervals did not overlap. All differences were considered significant if $P<0.05$.

3. Results

3.1. Impregnated method

In the present study, the percent mortality of plant extract and $TiO(OH)_2$ solution were 94, 73, 52, 41 and 33; 88, 69, 59, 45 and 17 against the larvae of *R. (B.) microplus* and 76, 53, 39, 23 and 16; 79, 59, 33, 26 and 16 against the adults of *H. bispinosa* at 50, 40, 30, 20 and 10 mg/L, respectively. The synthesized TiO_2 NPs showed the percent mortality of 100, 100, 86, 51 and 39 against the larvae of *R. (B.) microplus* and 100, 89, 69, 33 and 17 against the adults of *H. bispinosa* at 20, 16, 12, 8 and 4 mg/L, respectively.

The LC_{50} values of plant extract, $TiO(OH)_2$ solution and the synthesized TiO_2 NPs against the larvae of *R. (B.) microplus* were 24.63, 23.09 and 5.43 mg/L (r^2=0.960, 0.972 and 0.988) and against *H. bispinosa* were 35.22, 34.09 and 9.15 mg/L, (r^2=0.969, 0.947 and 0.969), respectively (Table 1). This proves that concentration plays an important role in acaricidal activity. The control showed nil mortality in the concurrent assay.

3.2. Characterization of synthesized TiO_2 NPs

XRD pattern of the synthesized TiO_2 NPs obtained in the present study is shown in Figure 1A and 1B. The synthesized TiO_2 NPs diffraction peaks at 2 θ values at 27.33°, 35.83°, 43.87°, 54.02°, 56.39°, 66.64° and 74.07° assigned to the (110), (101), (210), (211), (220), (301) and (320), respectively and the planes of a faced center cubic lattice of titanium were obtained. These peaks were having the combined characteristics of $TiO(OH)_2$ and the plant aqueous extract. The XRD pattern shows seven intense peaks in the whole spectrum of 2 θ values as the mean size of 10.52 nm. A few unassigned peaks were also noticed in the vicinity of the characteristic peaks (JCPDS File No.89.6975). XRD results suggested that crystallization of the bio organic phase occurs on the surface of the TiO_2 NPs.

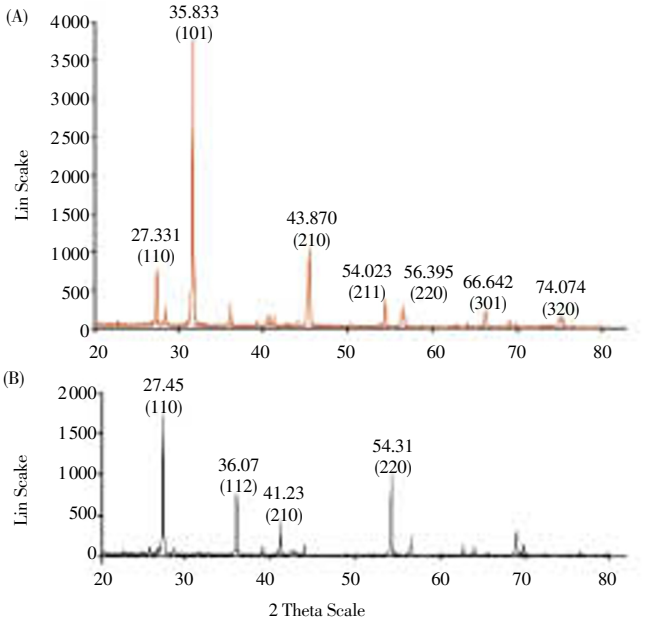

Figure 1. XRD pattern of (A) TiO_2 NPs synthesized from aqueous leaf extracts of *C. gigantea* and (B) $TiO(OH)_2$.

Table 1

Acaricidal activity of flower aqueous extract of *C. gigantea*, $TiO(OH)_2$ solution and synthesized TiO_2 NPs against the larvae of *R. (B.) microplus* and the adults of *H. bispinosa*.

Species	Extract	Concentrations (mg/L)	Percent mortality[a] (mg/L) ±SD	LC_{50}(LCL -UCL) (mg/L)	Slope	r^2
R. microplus	Aqueous extract	50	94.00±1.73			
		40	73.00±2.16			
		30	52.00±1.00	24.63(19.95-30.41)	73	0.960
		20	41.00±1.98			
		10	33.00±0.57			
	$TiO(OH)_2$ solution	50	88.00±1.15			
		40	69.00±1.00			
		30	59.00±0.57	23.09(20.66-25.80)	59	0.972
		20	45.00±1.89			
		10	17.00±1.73			
	Synthesized TiO_2 NPs	20	100.00±0.00			
		16	100.00±0.00			
		12	86.00±1.89	5.43(4.48-6.57)	51	0.988
		8	51.00±0.57			
		4	39.00±1.00			
H. bispinosa	Aqueous extract	50	76.00±1.52			
		40	53.00±1.15			
		30	39.00±1.00	35.22(32.25-38.47)	39	0.969
		20	23.00±0.57			
		10	16.00±0.57			
	$TiO(OH)_2$ solution	50	79.00±1.00			
		40	59.00±1.73			
		30	33.00±1.52	34.09(30.80-37.73)	26	0.947
		20	26.00±0.57			
		10	16.00±1.15			
	Synthesized TiO_2 NPs	20	100.00±0.00			
		16	89.00±1.00			
		12	69.00±1.73	9.15(8.28-10.10)	38	0.969
		8	33.00±1.51			
		4	17.00±0.57			

Control -Nil mortality; LC_{50}- lethal concentration; UCL: upper confidence limit; LCL: lower confidence limit; r^2 regression coefficient. [a] Mean value of three replicates.

Fourier transforms infrared (FTIR) peaks for the $TiO(OH)_2$, *C. gigantea* flower aqueous extract and synthesized TiO_2 NPs are showed in Figures 2A, 2B and 2C, respectively. The functional groups for *C. gigantea* flower aqueous extract and synthesized TiO_2 NPs were 3 416.33 and 3 395.81 cm^{-1} for O-H alcohol, 2 926.82 and 2 921.32 cm^{-1} for alkyl C-H stretch, 1 742.39 for C=O saturated aldehyde and 1 404.39 cm^{-1} for amide C-N stretch. Hence, it proves that synthesized TiO_2 NPs have been synthesized with plants compounds involved in the biological reduction of the TiO_2. The functional groups for the $TiO(OH)_2$ and synthesized TiO_2 NPs were 1405.19 and 1053.45 cm^{-1} for $-NH_2$ bending, primary amines and amides and 1053.84 and 1078.45 cm^{-1} for C-O. This proves that the nanoparticles and the plant derived chemicals were involved in the capping of the synthesized TiO_2 NPs.

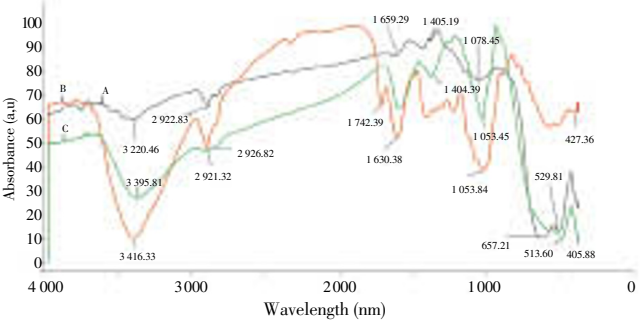

Figure 2. FTIR spectra of (A) $TiO(OH)_2$, (B) dried *C. gigantea* flower powder and (C) TiO_2 NPs synthesized from aqueous leaf extracts of *C. gigantea*n.

The SEM studies provided the informations on the morphology, particle size, aspect ratio, *etc*. SEM micrographs of the synthesized TiO_2 NPs showed the aggregated, spherical in shape and with an average of size of 160-220 nm (Figure 3A, 3B and 3C). EDX proves the chemical compositions and the purity of synthesized TiO_2 NPs (Figure 3D).

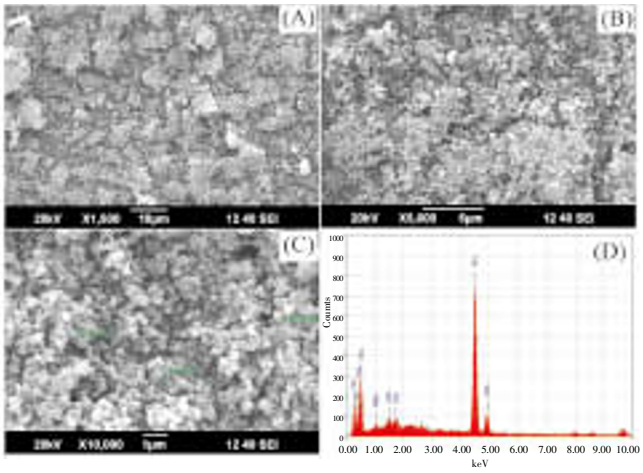

Figure 3. (A) SEM micrograph of synthesized TiO_2 NPs with magnification at 1 500×, (B) SEM micrograph of synthesized TiO_2 NPs with magnification at 5 000×, (C) SEM micrograph of synthesized TiO_2 NPs with magnification at 10 000 and (D) Energy-dispersive X-ray spectroscopy (EDX) exhibiting the chemical components of the synthesized TiO_2 NPs.

4. Discussion

The nanoparticle synthesis is important as the instability or aggregation of nanoparticles would lead to decrease in their biological activities[25]. The toxicity of alcoholic extract of *C. procera* against *Anopheles stephensi (A. stephensi)* and *Culex quinquefasciatus (C. quinquefasciatus)* larvae showed the LC_{50} values of 109.71 and 387.93 mg/L, respectively[26].

Marimuthu *et al*[27] reported the acaricidal activity of aqueous crude leaf extracts and synthesized Ag NPs using *Mimosa pudica (M. pudica)* against *R. (B.) microplus* showed the LC_{50} values of 52.01 and 8.98 mg/L, respectively. Acaricidal activity of aqueous leaf extract of *M. zapota* and synthesized Ag NPs were carried out against *R. (B.) microplus* with LC_{50} values of 16.72 and 3.44 mg/L; r^2= 0.856 and 0.783, respectively[21]. The synthesized ZnO NPs prepared by wet chemical method using zinc nitrate and sodium hydroxide as precursors and soluble starch as stabilizing agent showed the LC_{50} values of 13.41 mg/L and r^2= 0.982 against the *R. (B.) microplus*[28]. The highest efficacy was reported in 5 mM $TiO(OH)_2$ solution and synthesized TiO_2 NPs against *Hippobosca maculata (H. maculata)* and *Bovicola ovis (B. ovis)* with LD_{50} values of 33.40, 34.74 mg/L and 7.09 and 6.56 mg/L, respectively[29].

Zahir and Rahuman[30] reported that the highest mortality was found in the aqueous leaf extracts of *Euphorbia prostrata* (*E. prostrata*) and synthesized Ag NPs against the adult of *H. bispinosa* (LC_{50}=10.16 and 2.30 ppm and LC_{90}=70.27 and 8.28 ppm), respectively. The maximum efficacy was observed in the aqueous extract of *Musa paradisiaca (M. paradisiaca)* and synthesized Ag NPs against *H. bispinosa* with LC_{50} values of 28.96 and 1.87 mg/L; r^2=0.990 and 0.963, respectively[31]. Elango and Rahuman[32] have reported that the parasitic activity was found in leaf ethyl acetate extract of *Andrographis lineata (A. lineata)*, methanol extract of *Aegle marmelos (A. marmelos), A. paniculata*, and *Cocculus hirsutus (C. hirsutus)* against *H. bispinosa* (LC_{50}=395.27, 358.45, 327.21 and 420.50 ppm), respectively. The acute toxicity of engineered NPs in aquatic environments at high concentrations considered being safe in the environment, nano-TiO_2 remarkably enhanced the toxicity of copper to *Daphnia magna (D. magna)* by increasing the copper bioaccumulation[33].

The XRD pattern of synthesized TiO_2 NPs obtained in the present study was showed the diffraction of 2 θ and the peaks at 27.33°, 35.83°, 43.87°, 54.02°, 56.39°, 66.64° and 74.07° assigned to the (110), (101), (210), (211), (220), (301) and (320) planes of a faced center cubic lattice of titanium were obtained. A comparison of these results with earlier reported by Velayutham *et al*[29] that the XRD analysis of synthesized TiO_2 NPs using *Catharanthus roseus (C. roseus)* leaf extract showed three distinct diffraction peaks at 27.43°, 36.03°, and 54.32° which indexed the planes 110, 101, and 211, respectively. The pattern reflects the shape of the wave functions of the electronic eigenstates of the Ti-O-Ti-O chain on the TiO_2 (110)/H_2O interface[34].

The functional groups for the synthesized TiO_2 NPs from *C. gigantea* flower aqueous extract were 3395.81 cm^{-1} for O-H alcohol, 2921.32 cm^{-1} for alkyl C-H stretch, 1742.39 for C=O saturated aldehyde and 1404.39cm^{-1} for amide C-N stretch. Pawar *et al*[35] reported the origin of the vibrational bands at 3 225-3 451 cm^{-1} due to the NH stretching of aromatic amines, at 2 845-2 914 cm^{-1} due to aromatic CH-stretching, at 504 cm^{-1} due to CH out of plane bending vibration. The CH out of plane bending mode has been used as a key to identify the type of substituted benzene. The bands at 1572 and 1489 cm^{-1} were attributed to C=N and C=C stretching mode of vibration for the quinonoid and benzenoid units of polyaniline. The peaks at 1296 and 1239 cm^{-1} were assigned to C-N stretching mode of benzenoid ring. Monodispersed nanostructured TiO_2 spheres were obtained by the Sol-gel method modified with ethylene glycol from the particles had a shape close to spherical and a smooth surface[36].

In the present study SEM micrographs of the synthesized TiO_2 NPs showed aggregated, spherical in shape and with an average of size of 160-220 nm. The SEM images of the *B. subtilis* synthesized TiO_2 NPs were spherical, oval in shape, individual as well as a few aggregates having the size of 66-77 nm[37].

In conclusion, this is an eco-friendly and inexpensive approach for green synthesis of TiO_2 NPs using aqueous flower extract of *C. gigantea* against the larvae of *R. (B.) microplus* and adults of *H. bispinosa*. The present green synthesis shows that the renewable source of *C. gigantea* is used as an effective reducing agent for the synthesis of TiO_2 NPs. Our results suggest that the *C. gigantea* extract and synthesized TiO_2 NPs have the potential to be used to control blood-sucking parasites. The results reported here open the possibility of further investigations of efficacy on the parasitic properties of natural product extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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