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Green synthesis of iron nanoparticles via *Rhizoclonum riparium* (Roth) Harvey: A promising nanotherapy against the promastigote forms of *Leishmania donovani* parasites

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Original Research

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Abstract:

Visceral leishmaniasis, a life-threatening parasitic disease caused by *Leishmania donovani* (Roth) Harvey and transmitted by *Phlebotomus* sandflies, continues to pose a significant global health concern. Conventional therapies like amphotericin B and miltefosine are often limited by severe side effects and the emergence of resistance, necessitating the development of safer and more effective alternatives. In this study, iron-nanoparticles (INPs) were biosynthesized using green macroalga *Rhizoclonium riparium* using $FeCl_3$ (0.005 M) at pH 6, and the synthesized INPs were characterized using UV-Vis spectroscopy, transmission electron microscopy (TEM), energy-dispersive X-ray (EDX) spectroscopy, and dynamic light scattering (DLS), confirming their spindle-shaped morphology with average particle size of 82 nm. *In vitro* anti-leishmanial activity of the INPs was assessed against *L. donovani* using MTT assay, demonstrating significant efficacy with an IC_{50} value of 43.36 μg/mL. These results suggest that algal-mediated INPs represent a promising eco-friendly nanotherapeutic strategy for the treatment of visceral leishmaniasis.

Keywords: Green nanosynthesis; Iron nanoparticles (INPs); *Leishmania donovani*; MTT assay; Nanobiotechnology; *Rhizoclonium riparium* (Roth) Harvey; Visceral leishmaniasis

1. Introduction

Visceral leishmaniasis is a life-threatening, dangerous parasite illness that attacks the internal organs and impacts the most impoverished people (Boelaert et al., 2009). It is the second biggest parasitic killer disease in the world (Desjeux, 2004) and is commonly recognised as 'Kala-Azar' ('Black Fever') in India, because it causes hyperpigmentation of the skin during the development of the disease (Stauch et al., 2011). The disease is caused by a protozoan parasite, *Leishmania donovani*, transmitted by *Phlebotomus argentipes* sandflies (Akhoundi et al., 2016). The parasites consist of two different forms, one is promastigote, the flagellar stage mainly found in the blood of the vector, and another is amastigote, the aflagellar stage of the parasite mainly found in the bloodstream of the human body. Both forms of these parasites are infectious (de Souza et al., 2018).

The types of leishmaniasis include cutaneous leishmaniasis, mucocutaneous leishmaniasis, visceral leishmaniasis, and Post-Kala-Azar dermal leishmaniasis (Stauch et al., 2011; WHO, 2020). Among the four types of leishmaniasis, Visceral leishmaniasis is the only type that shows a threat to life, and is closely associated with underdeveloped countries (Boelaert et al., 2009; Bhattacharya et al., 2024b). It reveals irregular fever, weight loss, cachexia, splenomegaly, anaemia, pancytopenia, and hypergammaglobulinemia (Brahmachari, 1922; Karimi et al., 2016). The major therapeutic drugs, available in the market, are amphotericin B, miltefosine, paromomycin, pentavalent antimonial, etc. but they possess serious side effects, such as

cardiotoxicity, reversible kidney failure, pancreatitis, anemia, heartbeat, muscle cramps or pain, nausea, vomiting, blurred or double vision, seizures, numbness, tingling, pain, or weakness in hands or feet, shortness of breath, skin rash or itching, sore throat, fever, unusual bleeding or bruising, etc. (Murray, 1999; Croft et al., 2006; Hurissa et al., 2010; Van Griensven et al., 2010).

The prevalence and geographic range of visceral leishmaniasis are increasing yearly, making it a global health concern. In 2022, the World Health Organization received 12,842 new cases of visceral leishmaniasis throughout the world, and 80 countries declared it endemic (WHO, 2023). There are six major nations that account for more than 90% of cases of visceral leishmaniasis, including Bangladesh, India, Nepal, Sudan, Ethiopia, and Brazil, where the disease is mainly caused by *L. donovani* (Chappuis et al., 2007; Bhattacharya et al., 2024a). In the Indian subcontinent, over 200 million people are estimated to be at risk of developing visceral leishmaniasis (Lukes et al., 2007). Most of the cases of leishmaniasis are reported from only 4 states of India- West Bengal, Jharkhand, Bihar, and Uttar Pradesh (Thakur et al., 2018; Dhamnetiya et al., 2021; Gill et al., 2021; Pyne et al., 2022). Therefore, India is working harder to overcome these obstacles and standing in the way of eliminate visceral leishmaniasis (Bora, 1999; Kaur et al., 2024).

Even though parasite infections are spreading quickly across the globe, relatively little research has been done on how to cure them. The key to solving this problem is the development of alternative drugs from natural sources. Nanotechnology, a modern area of science, has proved its effectiveness by decreasing the toxic side effects of conventional drugs with increasing specificity.

To overcome this obstacle, scientists in the new era have used metallic nano-bio-technologies as an alternative form of natural medicine due to their small size and unique properties. As connected to our research, the scientists have observed the uses of this nanobiotechnology against *Leishmania* parasites (*in vitro*), and they have found that these metallic nanoparticles have potential anti-leishmanial activity. The selenium nanoparticles (SeNPs) can prevent the growth of both amastigote and promastigote forms of *Leishmania major*, which is biosynthesized via *Bacillus* sp. (Shakibaie et al., 2010). The silver nanoparticles (AgNPs) are highly effective against the infection of *Leishmania donovani* (Mohebali et al., 2009; Baiocco et al., 2011). The iron oxide nanoparticles (IONPs) show potential effects against the promastigote form of both *Leishmania major* and the anterior flagella of *Leishmania tropica* (Khatami et al., 2017; Ahmed et al., 2023). Moreover, it has also been reported that the AgNPs, AuNPs, TiO_2NPs, ZnONPs, and MgONPs have potent anti-leishmanial activity against *Leishmania major* parasites (Jebali et al., 2013; de Souza et al., 2018).

Apart from their anti-parasitic potential, recent studies have broadened the scope of green nanobiotechnology by utilizing plant-based synthesis to create metal nanoparticles with a wide range of biological applications. Zhang et al. (2021) demonstrated the green synthesis of NiO nanoparticles using *Calendula officinalis*, revealing significant antioxidant and anti-cancer activities. Kazemi et al. (2023) reported the involvement of secondary metabolites from *Dracocephalum kotschyi* in copper nanoparticle formation. In the same year, Kota et al. (2023) employed *Chromolaena odorata* leaf extract to synthesize CuO/Cu nanoparticles, which showed effective wound healing potential. Younas et al. (2024) showed that selenium nanoparticles synthesized via a green route significantly enhanced biochemical traits and metabolite content in sesame seed oil. Most recently, Mohammadhosseini et al. (2025) used *Cannabis sativa* methanolic extract to fabricate silver nanoparticles, underscoring its rich phytochemical profile and biological efficacy. Banerjee et al. (2022) also utilized silver nanoparticles from *Baccaurea ramiflora* fruit as a potent anti-cancer agent. These examples reflect the growing importance of green synthesis in creating multifunctional nanomaterials from biological sources, paving the way for eco-friendly solutions in medicine and agriculture.

In this study, we have used iron nanoparticles (INPs) against the promastigote form of *Leishmania donovani* (*in vitro*), and to the best of our knowledge this application of INPs against visceral leishmaniasis is the first time documented. We have synthesized these INPs via green nanobiotechnology, specifically through the utilization of *Rhizoclonium riparium*. This marks the first reported instance of such synthesis. *Rhizoclonium* is a genus of filamentous green algae that belongs to the Cladophoraceae family. It can spontaneously synthesize INPs through both in situ and ex situ processes. The synthesized INPs were characterized using light microscopy, UV-Visible (UV-Vis.) spectroscopy, transmission electron microscopy (TEM), energy dispersive X-ray (EDX) analysis, and dynamic light scattering (DLS) for particle size measurement. Furthermore, we applied these INPs to the promastigote form of *L. donovani* parasitic cells in an in vitro assay over a 48-hour period, utilizing the MTT assay to investigate the impact of these nanoparticles (Fig. 1).

2. Experimental

2.1 Reagents and materials

This experiment is divided into two phases. The first phase involves the synthesis of INPs using *Rhizoclonium* biomass and its characterization, while the second phase focuses on the application of these INPs against *L. donovani* promastigotes.

2.2 Algal strain maintenance

The pure culture of *R. riparium* was obtained from the culture collection of Calcutta University (AL/CCCU/FW-17), and was maintained in a 3N-BBM culture medium ($NaNO_3$75 g/L, $CaCl_2 \cdot 2H_2O$ 2.5 g/L, $MgSO_4 \cdot 7H_2O$ 7.5 g/L, $K_2HPO_4 \cdot 3H_2O$ 7.5 g/L, KH_2PO_4 17.5 g/L, NaCl 2.5 g/L, and microelements $FeCl_3 \cdot 3H_2O$ 97 mg/L, $MnCl_2 \cdot 4H_2O$ 41 mg/L, $ZnCl_2$ 5 mg/L, $CoCl_2 \cdot 3H_2O$ 2 mg/L, $Na_2MoO_4 \cdot 2H_2O$ 4 mg/L), supplemented with antibiotics at 20 °C undercool fluorescent lights at 20-30 µmol photons m^{-2} s^{-1} with 16:8 h light: dark cycle.

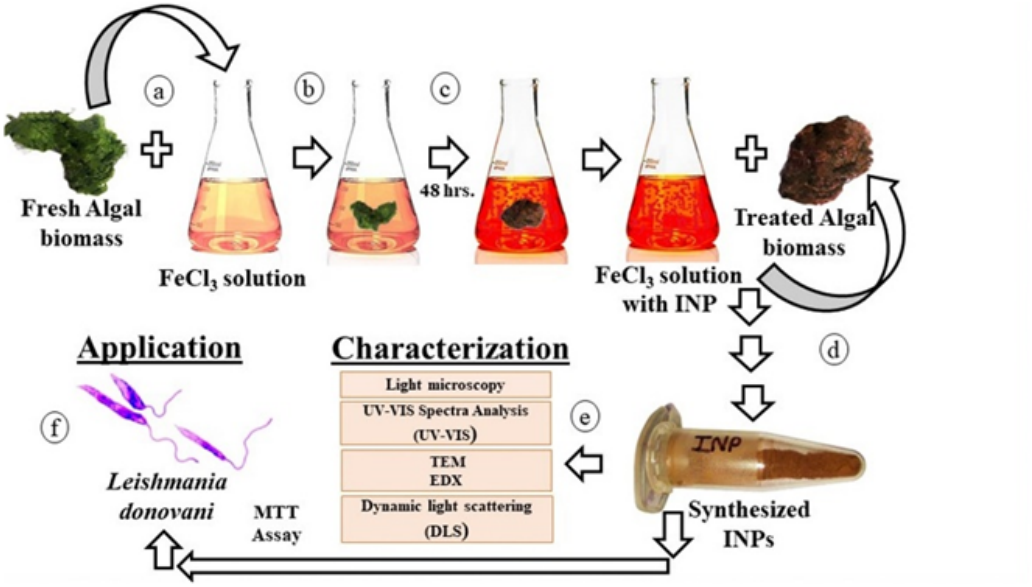

Figure 1. The overview of the production of INPs by thallus of *Rhizoclonium riparium*: a and b. Inoculation of fresh algal biomass in $FeCl_3$ solution (0.005 M); c. Formation of *in-situ* and *ex-situ* INPs after 48 hours; d. Extraction of INPs; e. Characterization of INPs; f. Application of INPs on the promastigote form of *Leishmania donovani* (MHOM/IN83/AG83) parasite.

2.3 Production of iron nanoparticles

Around 250 mg (fresh weight) of healthy growing biomass of *R. riparium* in the exponential phase was collected from the culture media and carefully washed multiple times with sterile double-distilled water to remove the excess media components. The biomass was added to 50 mL of $FeCl_3$ solution (0.005 M, pH 6) and the experimental flasks were kept in an orbital shaker (Eyela LTR 700, China) at 70 rpm for successive time intervals (24 h, 48 h, and 96 h) at 25 °C in darkness. The production of INPs can be identified by a distinct color shift from fresh green to brown biomass and from light yellow to brown experimental solution.

2.4 Extraction of the iron nanoparticles

After 24-48 hours, the brown biomass was taken out from the experimental solution and washed 2-3 times using sterile double-distilled water, followed by the extraction of INPs according to the standard protocol (Roychoudhury et al., 2016). Then, the nanoparticles were pelleted by using a C-24 BL Remi cooling centrifuge (Maharashtra, India) at 14500 rpm for 20 minutes, followed by complete air drying under laminar air flow.

2.5 Microscopic observation

The images of iron-exposed algal filaments were captured with 10x and 40x magnification using a Carl Zeiss 18 (EDS 8100) microscope with Zeiss Inca Penta FETX 3 (Oxford Instruments). The morphology of the nanoparticles was observed using a JEOL JEM 2100 high-resolution transmission electron microscope operating at an accelerating voltage of 200 kV, equipped with an EDX detector at an accelerating voltage of 80 kV.

2.6 UV-Vis. spectral analysis

UV-Vis. spectroscopy was performed to verify the creation of INPs. The sample was diluted into distilled water using a small aliquot, and the absorption of the nanoparticle suspensions was recorded at wavelengths of 200-800 nm using a Hitachi U-2900, UV-Visible spectrophotometer (Tokyo, Japan).

2.7 Dynamic light scattering (DLS)

The hydrodynamic size of the INPs was measured using the dynamic light scattering (DLS) setup with a Malvern Zetasizer Nano ZS Analyzer (UK). The sample was prepared by dilution with water, which was followed by ultrasonication and syringe filtration.

2.8 Extract preparation of iron nanoparticles for application

To prepare the stock solution of INP for the application, at first, 1 mg portions of dried INP dust particles were mixed with 1 mL of ultra-pure Milli-Q water, and then sonicated for 10 min, at 5 min intervals at 60% amplitude in a Hielscher UP100H ultrasonic processor (Teltow, Germany).

2.9 Parasite culture

The promastigotes of *Leishmania donovani* (MHOM/IN/83/AG83) were kept at 24 °C in M199 medium with 10% FBS and 1.0% penicillin-streptomycin. Then, for maintenance of culture, at every 48-72 hours, the promastigotes were sub-cultured using an inoculum of 1×10^6 cells.

2.10 Cell viability assay

The *in vitro* cell viability was determined by the MTT colorimetric assay developed by Mosmann. The principle of the MTT assay is to measure the viability of cells by determining the activity of mitochondrial enzyme succinate-dehydrogenase within viable cells that can cleave the MTT salt into formazan, a blue-colored product. The amount of formazan produced is directly proportional to the viability

of cells and the intensity of color is measured in the ELISA Plate Reader at 560 nm.

In our experiment, to check the effect of INPs on *Leishmania donovani* promastigotes, each 100 µL of the promastigotes (1×10^5 cells/mL/well) harvested from the logarithmic growth phase was added to a total of twelve wells with increasing concentration, in a 96-well microtiter plate. The different concentrations of 15.125, 31.25, 62.5, 125, 250, and 500 µg/mL were prepared in a cell growth medium from a 1 mg/mL stock solution of INP. Further, the cells were incubated for 48 hours in a Biochemical Oxygen Demand (BOD) incubator with INP treatment, with a control set. After the 48-hour incubation period, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated at 36 °C for 6 hours. Then, the 96-well plate was centrifuged at 2000 rpm for 4 minutes, and the supernatant was discarded. Then, 50 µL of methanol was added to the pellet. Finally, the absorbance was measured in an ELISA plate reader at 560 nm, where the intensity of formazan color indicates the viability of promastigotes. Pentamidine was used as a positive control, and a stock solution was prepared at a concentration of 1 mg/mL.

The value was therefore calculated by Eq. (1):

$$\text{Promastigote viability} = (\text{Absorbance of treated promastigotes} / \text{Absorbance of untreated promastigotes}) \times 100\% \quad (1)$$

2.11 Determination of cytotoxicity

The INPs' cytotoxicity against normal and healthy cells was assessed using the MTT test. BEAS-2B cells, a bronchial epithelial cell line, were cultured in DMEM supplemented with fetal bovine serum (FBS, 10%), 100 IU/mL penicillin, and 100 µg/mL streptomycin. The cells were seeded in a 96-well plate with INPs and cultured for 24 hours in a CO_2 incubator. After incubation, 20 µL of MTT solution (10% v/v of 5 mg/mL) was added to each well. The resulting formazan crystals were dissolved in DMSO for absorbance measurements at 560 nm.

The cell viability was calculated by Eq. (2):

$$\text{Cell viability} = (\text{Absorbance of treated BEAS 2B cells} / \text{Absorbance of untreated BEAS 2B cells}) \times 100\% \quad (2)$$

The selectivity index (SI) of the INPs was then computed by dividing the CC_{50} (concentration at which 50% of the BEAS-2B cells were viable) by the IC_{50} value observed against the *L. donovani* promastigotes.

2.12 Determination of promastigote viability by cell visualisation

To validate the MTT results, both untreated and INP-treated promastigotes were observed under a bright field microscope. Cells were incubated in a 96-well plate for 48 hours, followed by centrifugation and the supernatant was removed. Then, the precipitate was equally distributed on a slide and visualised under a bright light. The number of cells was counted, and cellular morphological alterations were examined.

3. Results and discussion

3.1 Biosynthesis of iron nanoparticles

The green color of fresh algal biomass was turned into a brownish-red color (Fig. 2 b, e) after 24-48-hour time intervals. The color of the $FeCl_3$ solution turned into an excessive brownish-red color, which indicates the formation of INP (Fig. 2 a, d). Therefore, it can be concluded that *R. riparium* can be utilized in the spontaneous synthesis of INP through both *in situ* and *ex situ* processes. It can be synthesized correctly by using specific parameters like $FeCl_3$ solution (0.005 M at pH-6), with a 1:5 ratio of $FeCl_3$ and biomass concentration. This is the first report of *Rhizoclonium*-mediated *in situ* and *ex-situ* INP production using the whole algal thallus instead of algal extract.

3.2 Characterization of iron nanoparticles

Finally, the INPs were obtained under specific parameters. The reddish-brown-colored biomass and experimental sus-

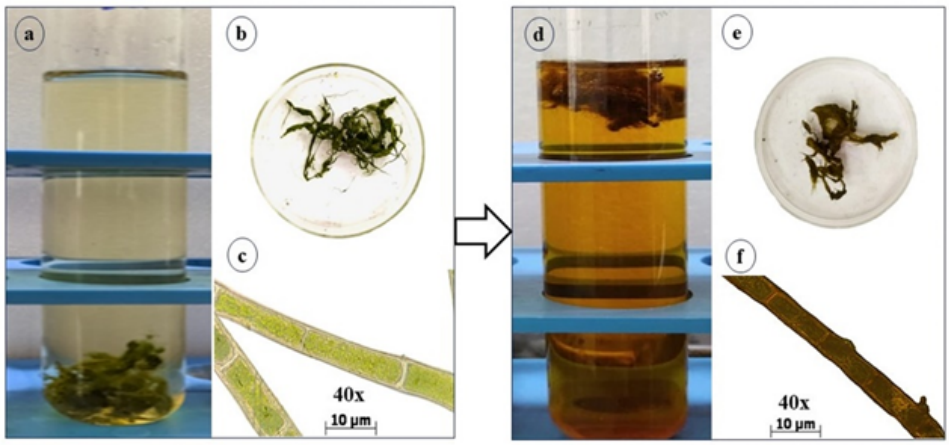

Figure 2. Color of the algal biomass: a. The color of $FeCl_3$ solution before production of INPs; b. The color of biomass before being treated with INPs; c. The light microscopy shows the fresh cells of *R. riparium*; c. The color of FeCl3 solution after production of INPs after 48 hours; e. The color of biomass after being treated with INPs; f. The light microscopy shows iron deposition inside the cells of *R. riparium*.

pension were obtained after INP production. Under a light microscope, the color change of the thallus was observed due to the deposition of *in situ* INPs (Fig. 2: c, f). UV-Visible spectroscopic analysis shows that the characteristic peaks of INP were observed at 380 nm, which is due to charge transfer spectra (Fig. 3 a). The TEM picture of INP clearly shows that the product is entirely composed of crystals with a relatively uniform, spindle morphology, and the images indicate the average size distribution of INPs was $18.31 \pm 8.32 \times 3.33 \pm 1.11$ nm (Fig. 3 b). The DLS results indicated that the distribution of INP particles ranges from 68.6 to 105.7 nm, with a mean particle size of 82 nm. Most of the particles are approximately 78.82 nm (46.4%), followed by those measuring 91.28 nm (Fig. 3 c). The energy dispersive X-ray analysis (EDX) spectra showed the presence of iron in INPs Fig. 3 d).

3.3 Cell viability assay

The screening of INP through MTT assay on the *Leishmania donovani* parasite revealed the anti-leishmanial activity with increasing concentration of INP, followed by a subsequent reduction of formazan color intensity after 48 hours. The IC_{50} value for the INP against *L. donovani* promastigotes was 43.36 µg/mL, which is better than the control drug pentamidine (Fig. 4).

3.4 Determination of cytotoxicity

Drug specificity is related to cytotoxicity. As this INP has been found to have potent anti-leishmanial activity, it is essential to determine if it has any cytotoxic effect against normal cells. The cytotoxicity assays aim to ascertain whether the possible drug candidate has any toxic impact on healthy and normal cells. A viability percentage of 84% was observed at the 100 µg/mL concentration of the extract, suggesting its parasite-specific activity (Fig. 4) with a CC_{50} value of 551.9 µg/mL. The value of the selectivity index (SI) represents a drug's selectivity for the target cells over normal healthy cells. On this account, the SI value of the INPs was calculated to be 12.72, a value much higher than the minimum standard SI value of 2.

3.5 Determination of promastigote viability by cell visualisation

As visualisation is one of the most important aspects of recognizing any drug action, the INP-treated promastigotes were examined under a bright field microscope after MTT to validate the results. Fig. 5 shows a consistent decrease in promastigotes' growth as well as significant morphological alterations following INP treatment. With increasing concentration of INPs, the promastigotes have lost their typical spindle-shaped structure and become roundish in shape. They have also lost their characteristic flagella at higher concentrations. Thus, the outcomes of the study confirm the anti-leishmanial efficacy of INPs.

So, *R. riparium*-mediated iron nanoparticles, a novel discovery in science, have been found to effectively reduce parasite burden in culture after 48 hours, as demonstrated by the cell viability assay. The IC_{50} value of 43.36 µg/mL indicates the leishmanicidal potentiality of these algae-mediated INPs against the parasite, suggesting it is a novel and good alternative to conventional drug treatment. The experiment demonstrates a continuous reduction in the proliferation of promastigotes, along with notable morphological changes subsequent to INP therapy. Thus, this study revealed another new direction in the elimination of visceral leishmaniasis via the application of algae-mediated INPs.

3.6 Comparison the finding of this work with the previously reports

In the last two decades, scientists have utilized various types of algae to synthesize INPs for a wide range of applications. The INP serves as a contrast agent for MRI, a therapeutic agent for cancer treatments based on hyperthermia, a drug carrier for target-specific drug delivery, a gene carrier for gene therapy, and is utilized abundantly due to the presence of active metabolites (Chan et al., 1993; Matheson

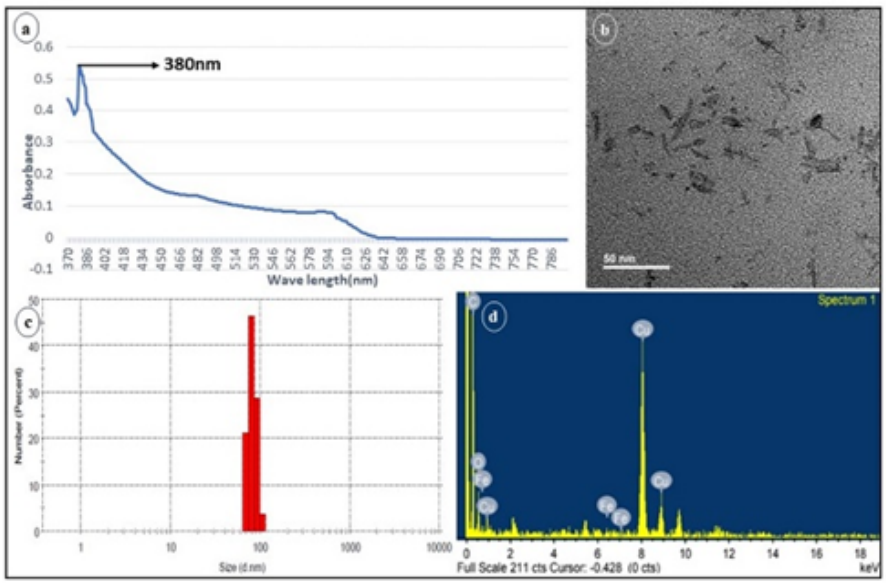

Figure 3. Characterization of the synthesized nanoparticles: a. UV-Vis. spectroscopy analysis; b. TEM analysis; c. DLS analysis; d. EDX analysis.

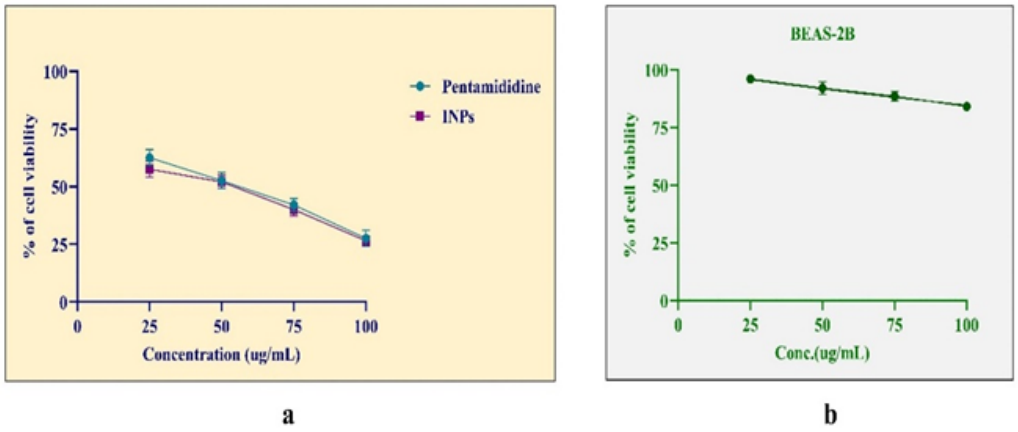

Figure 4. a. Graphical representation of cell viability percentage of *Leishmania donovani* cells after INPs and control drug pentamidine treatment. **b**. Graphical representation of the cell viability percentage of BEAS-2B cells after INP treatment.

et al., 1994; Beets-Tan et al., 1998; Babes et al., 1999; Gupta et al., 2005; Iida et al., 2007; Tiwari et al., 2008; Mohapatra et al., 2011). Till now, green algae are mostly utilized to synthesize INP (33%), followed by brown algae (30%) and blue-green algae (24%) (Mondal et al., 2024b). More than 35 species of macro and microalgae have been reported to be capable of synthesizing INPs among which *Chlorella*, *Chlorococcum*, *Spirogyra*, *Scenedesmus*, *Ulva*, *Enteromorpha*, and *Aegagropila* are notable green algae that scientists have utilized for INP synthesis (Kassi et al., 2008; Subramaniyam et al., 2015; Subramaniyam et al., 2016; Fraga-García et al., 2018; Mashjoor et al., 2018; Ghanbariasad et al., 2019; Sadiq Hawezy et al., 2020; Ahmed et al., 2021; Bensy et al., 2022; Yu et al., 2022; Mondal et al., 2024c).

Till now, some unicellular algae and cyanobacteria, e.g., *Anabaena*, *Calothrix*, *Arthrospira*, *Leptolyngbya*, *Spirulina*, and *Oscillatoria* have been reported to synthesize INP as to whole-cell conditions without extract formation (Brayner et al., 2009; Sadiq Hawezy et al., 2020; Banerjee et al., 2021a; Banerjee et al., 2021b; Haris et al., 2023; Mondal et al., 2024a). On the other hand, macro algae, like e.g. *Sargassum*, *Dictyota*, *Petalonia*, *Padina*, *Colpomenia*, *Spatoglossum*, *Turbinaria*, etc., formed INPs in extract form (Mahdavi et al., 2013; Chandran et al., 2016; El-Kassas et al., 2016; Khaleelullah et al., 2017; Salem et al., 2019; Salem et al., 2020; El-Sheekh et al., 2021; Palaniyandi et al., 2023). Our experiment is a novel work to produce INPs via macro algae with the whole thallus without extraction. In this study, we have synthesized INP via *R. riparium*, a green alga, using $FeCl_3$ solution (0.005 M, pH 6). After 24 to 48 hours, the spindle-shaped INPs were produced within a range of $68-91$ nm. Previously, *Arthrospira platensis*, *Leptolyngbya alborus*, and *Pseudostaurosira trainorii* have also been reported to synthesize spindle-shaped INPs along with 24.26×5.45 nm, 47.42×7.7 nm, and $50-70$ nm in size (Roychoudhury et al., 2016; Banerjee et al., 2021a; Banerjee et al., 2021b). The UV-Vis. spectrum peak of INP in the current experiment was observed at 380 nm; however, additional UV absorbance peaks of INP were identified

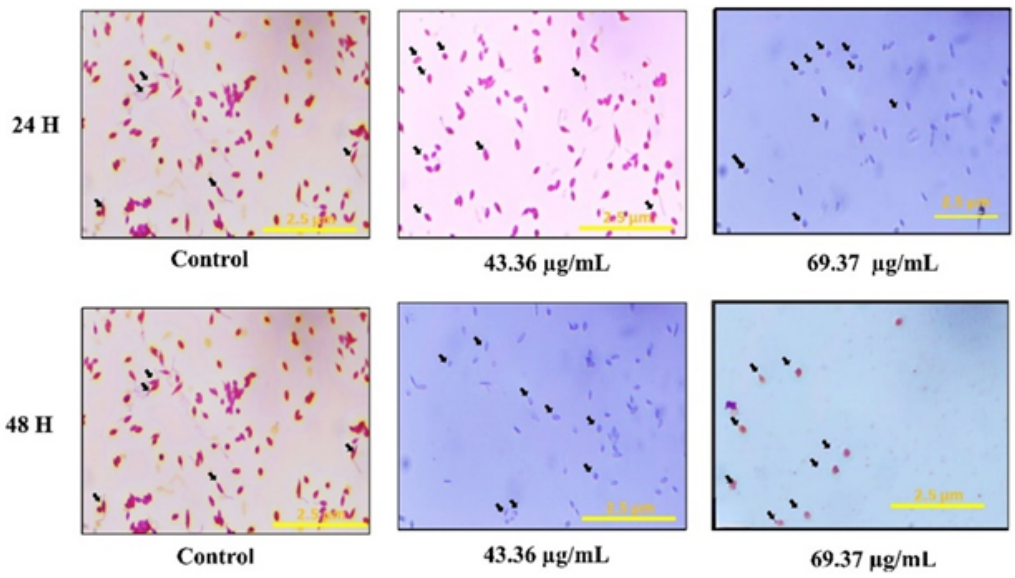

Figure 5. Cellular morphology study by Leishman stain.

within the range of 230 to 480 nm (Mahdavi et al., 2013; Subramaniyam et al., 2015; El-Kassas et al., 2016; Salem et al., 2020; Banerjee et al., 2021b; Roychoudhury et al., 2022; Mondal et al., 2024c; Mondal et al., 2024a). As far as our knowledge goes, this is the first time *R. riparium* has been involved in synthesizing INP without any extract preparation as a whole, uninterrupted thallus.

Several studies have explored the promising role of algae-mediated nanoparticles in anti-leishmanial therapy due to their natural bioactivity, eco-friendly synthesis, and reduced cytotoxicity. For instance, Atef et al. (2025) reported that zinc selenide nanoparticles coated with *Ulva fasciata* hydroalcoholic extract significantly inhibited *Leishmania major* growth, indicating the therapeutic potential of seaweed-derived nanomaterials. Similarly, Mohamed Abdoul-Latif et al. (2023) demonstrated the green synthesis of silver oxide nanoparticles using *Ericaria amentacea* algal extracts, which exhibited potent anti-leishmanial effects. These findings align with the growing evidence that seaweed-derived compounds possess direct anti-parasitic properties, further supporting algae as both reducing agents and active contributors in nanoparticle-based parasitic control (Cheng et al., 2025).

Among various metallic nanoparticles, INPs have attracted particular interest due to their redox properties, catalytic activity, and biocompatibility. Previous studies have reported the successful application of chemically or bacterially synthesized INPs against *Leishmania* spp., including *L. major* and *L. tropica*, where they disrupted membrane integrity and generated reactive oxygen species, leading to parasite death (Rastogi et al., 2025). However, the synthesis of INPs using algae and their application against *Leishmania donovani* remains largely unexplored. In this context, the present study is the first to report the biosynthesis of INPs using the filamentous green alga *R. riparium* and to evaluate their efficacy against the promastigote form of *Leishmania donovani*. This dual novelty using *Rhizoclonium* as a bio-reductant and targeting *L. donovani* with algal-derived INPs represents a significant advancement in green nanotherapeutics for parasitic diseases and opens new avenues for marine algal nanotechnology in neglected tropical disease management.

4. Concluding remarks

In this study, an environmentally sustainable approach was investigated to mitigate the adverse effects associated with conventional treatments for visceral leishmaniasis. INPs were biosynthesized using the green macroalga *R. riparium*, in accordance with the principles of green chemistry and ecological sustainability. The entire thallus of *R. riparium* was found to mediate the rapid, fluent, and spontaneous synthesis of INPs through both *in situ* and *ex-situ* processes. These nanoparticles were thoroughly characterized, and their bioactivity was evaluated against the promastigote form of *Leishmania donovani*, where significant anti-leishmanial efficacy was observed.

This study presents a novel and eco-friendly approach for combating visceral leishmaniasis through the biosynthesis and application of INPs. To the best of our knowledge, this is the first report demonstrating the green synthesis of INPs using the filamentous green alga *R. riparium*. Furthermore, it is also the first study to evaluate the anti-leishmanial activity of algal-derived INPs against the promastigote form of *Leishmania donovani*, highlighting their significant inhibitory potential. The synthesis process was rapid, spontaneous, and followed green chemistry principles, with thorough physicochemical characterization validating the successful formation of nanoparticles.

Looking ahead, these promising results pave the way for further research into the mechanisms underlying the anti-parasitic action of nanoparticles. Future investigations should focus on *in vivo* validation, toxicity profiling in mammalian models, formulation development, and targeted delivery strategies to enhance therapeutic efficacy. Moreover, the scalability of algal-based nanoparticle synthesis and its integration into combinatorial therapies or topical applications present exciting prospects in both pharmaceutical and public health contexts. These findings underscore the potential of marine algal resources in the development of sustainable nanomedicines.

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Abbreviations

3N-BBM: Triple Nitrate Bold Basal Medium; **AgNPs:** Silver Nanoparticles; **AuNPs:** Gold nanoparticles; **DLS:** Diffraction Light Scattering; **EDX:** Energy Dispersive X-ray; **FBS:** Fetal Bovine Serum; **INPs:** Iron Nanoparticles; **MgONPs:** Magnesium Oxide Nanoparticles; **MTT:** 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; **SeNPs:** Selenium Nanoparticles; **TEM:** Transmission Electron Microscopy; **TiO_2NPs:** Titanium oxide nanoparticles; **UV-Vis.:** UV-Visible Spectroscopy; **WHO:** World Health Organization; **ZnONPs:** Zinc Oxide Nanoparticles.

Authors contributions

SD: Writing-Original draft preparation and editing. **AM:** Conceptualisation, Methodology, Writing-Reviewing and Editing. **IB:** Writing-Original draft preparation, Methodology, Writing-Reviewing and Editing. **NP:** Methodology. **SP:** Overall supervision, Writing-Reviewing, and Editing.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ahmed, M.B., Neama, A., Ahmed, F.M.N.A. (2023) Effect of biosynthesized iron oxide nanoparticles against anterior flagella of *Leishmania tropica*. *HIV Nurs.* 23(1):731–735.
DOI: https://doi.org/10.31838/hiv23.01.123.

Ahmed, N., Vione, D., Rivoira, L., Carena, L., Castiglioni, M., Bruzzoniti, M.C. (2021) A review on the degradation of pollutants by Fenton-like systems based on zero-valent iron and persulfate: Effects of reduction potentials, pH, and anions occurring in wastewaters. *Molecules* 26(15):4584.
DOI: https://doi.org/10.3390/molecules26154584.

Akhoundi, M., Kuhls, K., Cannet, A., Votýpka, J., Marty, P., Delaunay, P., Sereno, D. (2016) A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoSNegl. Trop. Dis.* 10(2):e0004349.
DOI: https://doi.org/10.1371/journal.pntd.0004349.

Atef, Z., Livani, F., Koohsar, F., Faridnia, R., Yadagiri, G., Kalani, H. (2025) Zinc selenide (ZnSe) nanoparticle coated with green seaweed (*Ulva fasciata*) hydroalcoholic extract as an anti-leishmanial compound on leishmania major. *PLOS ONE* 20(4):e0321219.
DOI: https://doi.org/10.1371/journal.pone.0321219.

Babes, L., Denizot, B., Tanguy, G., Jeune Le, J.J., Jallet, P. (1999) Synthesis of iron oxide nanoparticles used as MRI contrast agents: A parametric study. *J. Colloid Interface Sci.* 212(2):474–482.
DOI: https://doi.org/10.1006/jcis.1998.6053.

Baiocco, P., Ilari, A., Ceci, P., Orsini, S., Gramiccia, M., Muccio Di, T., Colotti, G. (2011) Inhibitory effect of silver nanoparticles on trypanothione reductase activity and *Leishmania infantum* proliferation. *ACS Med. Chem. Lett.* 2(3):230–233.
DOI: https://doi.org/10.1021/ml1002629.

Banerjee, S., Banerjee, I., Dutta, M., Pal, R. (2021a) Fabrication of iron nanoparticles using *Leptolyngbya valderiana* and investigation of its Cr (VI) removal potential in the free and biomass-associated forms. *Algal Res.* 58:102373.
DOI: https://doi.org/10.1016/j.algal.2021.102373.

Banerjee, S., Bhattacharya, A., Roychoudhury, P., Dasgupta, A.K., Dutta, M., Pal, R. (2021b) *Arthrospira platensis* (Cyanobacteria) a potential biofactory for fluoromagnetic nanoiron production. *Phycologia* 60(1):62–72.
DOI: https://doi.org/10.1080/00318884.2020.1851010.

Banerjee, S., Islam, S., Chattopadhyay, A., Sen, A., Kar, P. (2022) Synthesis of silver nanoparticles using underutilized fruit *Baccaurea ramiflora* (Latka) juice and its biological and cytotoxic efficacy against MCF-7 and MDA-MB 231 cancer cell lines. *S. Afr. J. Bot.* 145:228–235.
DOI: https://doi.org/10.1016/j.sajb.2021.09.016.

Beets-Tan, R.G.H., Engelshoven Van, J.M.A., Greve, J.W.M. (1998) Hepatic adenoma and focal nodular hyperplasia: MR findings with superparamagnetic iron oxide-enhanced MRI. *Clin. Imaging* 22(3):211–215.
DOI: https://doi.org/10.1016/S0899-7071(97)00117-4.

Bensy, A.D.V., Christobel, G.J., Muthusamy, K., Alfarhan, A., Anantharaman, P. (2022) Green synthesis of iron nanoparticles from *Ulva lactuca* and bactericidal activity against enteropathogens. *J. King Saud Univ. Sci.* 34(10):101888.
DOI: https://doi.org/10.1016/j.jksus.2022.101888.

Bhattacharya, I., Paul, S. (2024a) Mushroom and mushroom-derived compounds in the management of leishmaniasis. *Res. J. Biotechnol.* 20(1):250–259.
DOI: https://doi.org/10.25303/201rjbt2500259.

Bhattacharya, I., Pyne, N., Paul, S. (2024b) *In vitro* and in silico approaches manifest the anti-leishmanial activity of wild edible mushroom *Amanita princeps*. *In Silico Pharmacol* 13(6):1–22.
DOI: https://doi.org/10.1007/s40203-024-00287-0.

Boelaert, M., Meheus, F., Sanchez, A., Singh, S.P., Vanlerberghe, V., Picado, A., Meessen, B., Sundar, S. (2009) The poorest of the poor: A poverty appraisal of households affected by visceral leishmaniasis in Bihar, India. *Trop. Med. Int. Health* 14(6):639–644.
DOI: https://doi.org/10.1111/j.1365-3156.2009.02279.x.

Bora ,D. (1999) Epidemiology of visceral leishmaniasis in India. *Natl. Med. J. India* 12(2):62–68.

Brahmachari ,U.N. (1922) A new form of cutaneous leishmaniasis-dermal leishmanoid. *Ind. Med. Gaz.* 57(4):125–127.

Brayner, R., Yéprémian, C., Djediat, C., Coradin, T., Herbst, F., Livage, J., Fiévet, F., Couté, A. (2009) Photosynthetic microorganism-mediated synthesis of akaganeite (β-FeOOH) nanorods. *Langmuir* 25(17):10062–10067.
DOI: https://doi.org/10.1021/la9010345.

Chan, C.F., Kirpotin, D.B., Jr Bunn, P.A. (1993) Synthesis and evaluation of colloidal magnetic iron oxides for the site-specific radiofrequency-induced hyperthermia of cancer. *J. Magn. Magn. Mater.* 122(1-3):374–378.
DOI: https://doi.org/10.1016/0304-8853(93)91113-L.

Chandran, M., Yuvaraj, D., Christudhas, L., Kv, R. (2016) Bio-synthesis of iron nanoparticles using the brown seaweed, *Dictyota dicotoma*. *Biotechnol* 12(12):112.

Chappuis, F., Sundar, S., Hailu, A., Ghalib, H., Rijal, S., Peeling, R.W., Alvar, J., Boelaert, M. (2007) Visceral leishmaniasis: What are the needs for diagnosis, treatment and control? *Nat. Rev. Microbiol* 5(11):873–882.
DOI: https://doi.org/10.1038/nrmicro1748.

Cheng, W., Yang, X., Yang, D., Zhang, T., Tian, L., Dao, J., Feng, Z., Hu, W. (2025) Recent advances in research on inhibitory effects of seaweed extracts against parasites. *Mar. Drugs* 21(4):171.
DOI: https://doi.org/10.3390/md23040171.

Croft, S.L., Sundar, S., Fairlamb, A.H. (2006) Drug resistance in leishmaniasis. *Clin. Microbiol. Rev.* 19(1):111–126.
DOI: https://doi.org/10.1128/CMR.19.1.111-126.2006.

Desjeux ,P. (2004) Leishmaniasis: Current situation and new perspectives. *Comp. Immunol. Microbiol. Infect. Dis.* 27(5):305–318.
DOI: https://doi.org/10.1016/j.cimid.2004.03.004.

de Souza, A., Marins, D.S.S., Mathias, S.L., Monteiro, L.M., Yukuyama, M.N., Scarim, C.B., Löbenberg, R., Bou-Chacra, N.A. (2018) Promising nanotherapy in treating leishmaniasis. *Int. J. Pharm.* 547:421–431.
DOI: https://doi.org/10.1016/j.ijpharm.2018.06.018.

Dhamnetiya, D., Jha, R.P., Shalini Bhattacharyya, K. (2021) India's performance in controlling visceral leishmaniasis as compared to Brazil over past three decades: Findings from the global burden of disease study. *J. Parasit. Dis.* 45(4):877–886.
DOI: https://doi.org/10.1007/s12639-021-01375-0.

El-Kassas, H.Y., Aly-Eldeen, M.A., Gharib, S.M. (2016) Green synthesis of iron oxide (Fe_3O_4) nanoparticles using two selected brown seaweeds: Characterization and application for lead bioremediation. *Acta Oceanol. Sin.* 35:89–98.
DOI: https://doi.org/10.1007/s13131-016-0880-3.

El-Sheekh, M.M., El-Kassas, H.Y., Shams El-Din, N.G., Eissa, D.I., El-Sherbiny, B.A. (2021) Green synthesis, characterization applications of iron oxide nanoparticles for antialgal and wastewater bioremediation using three brown algae. *Int. J. Phytoremediation* 23(14):1538–1552.
DOI: https://doi.org/10.1080/15226514.2021.1915957.

Fraga-García, P., Kubbutat, P., Brammen, M., Schwaminger, S., Berensmeier, S. (2018) Bare iron oxide nanoparticles for magnetic harvesting of microalgae: From interaction behavior to process realization. *Nanomaterials* 8(5):292.
DOI: https://doi.org/10.3390/nano8050292.

Ghanbariasad, A., Taghizadeh, S.M., Show, P.L., Nomanbhay, S., Berenjian, A., Ghasemi, Y., Ebrahiminezhad, A. (2019) Controlled synthesis of iron oxyhydroxide (FeOOH) nanoparticles using secretory compounds from *Chlorella vulgaris* microalgae. *Bioengineered* 10(1):390–396.
DOI: https://doi.org/10.1080/21655979.2019.1661692.

Gill, N., Pandey, D., Roy, N. (2021) Kala-azar in India- progress and challenges towards its elimination as a public health problem. *Wkly. Epidemiol. Rec.* 26:267–279.

Gupta, A.K., Gupta, M. (2005) Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials* 26(18):3995–4021.
DOI: https://doi.org/10.1016/j.biomaterials.2004.10.012.

Haris, M., Fatima, N., Iqbal, J., Chalgham, W., Mumtaz, A.S., El-Sheikh, M.A., Tavafoghi, M. (2023) *Oscillatoria limnetica* mediated green synthesis of iron oxide (Fe_2O_3) nanoparticles and their diverse *in vitro* bioactivities. *Molecules* 28(5):2091.
DOI: https://doi.org/10.3390/molecules28052091.

Hurissa, Z., Gebre-Silassie, S., Hailu, W., Tefera, T., Lalloo, D.G., Cuevas, L.E., Hailu, A. (2010) Clinical characteristics and treatment outcome of patients with visceral leishmaniasis and HIV co-infection in northwest Ethiopia. *Trop. Med. Int. Health* 15(7):848–855.
DOI: https://doi.org/10.1111/j.1365-3156.2010.02550.x.

Iida, H., Takayanagi, K., Nakanishi, T., Osaka, T. (2007) Synthesis of Fe_3O_4 nanoparticles with various sizes and magnetic properties by controlled hydrolysis. *J. Colloid Interface Sci.* 314(1):274–280.
DOI: https://doi.org/10.1016/j.jcis.2007.05.047.

Jebali, A., Kazemi, B. (2013) Nano-based antileishmanial agents: A toxicological study on nanoparticles for future treatment of cutaneous leishmaniasis. *Toxicol. In Vitro* 27(6):1896–1904.
DOI: https://doi.org/10.1016/j.tiv.2013.06.002.

Karimi, A., Alborzi, A., Amanati, A. (2016) Visceral leishmaniasis: An update and literature review. *Arch. Pediatr. Infect. Dis.* 4(3):e31612.
DOI: https://doi.org/10.5812/pedinfect.31612.

Kassi, M., Afghan, A.K., Rehman, R., Kasi, P.M. (2008) Marring leishmaniasis: The stigmatization and the impact of cutaneous leishmaniasis in Pakistan and Afghanistan. *PLoSNegl. Trop. Dis.* 2(10):e259.
DOI: https://doi.org/10.1371/journal.pntd.0000259.

Kaur, R., Kumar, R., Chaudhary, V., Devi, V., Dhir, D., Kumari, S., Yanadaiah, P., Pandey, K., Murti, K., Pal, B. (2024) Prevalence of HIV infection among visceral leishmaniasis patients in India: A systematic review and meta-analysis. *Clin. Epidemiol. Glob. Health* 25:101504.
DOI: https://doi.org/10.1016/j.cegh.2023.101504.

Kazemi, M.S., Imani, Z. (2023) Effect of secondary metabolite compounds of *Dracocephalum kotschyi* Boiss plant on synthesis of Cu nanoparticles. *Trends Phytochem. Res.* 7(4):311–321.
DOI: https://doi.org/10.71596/tpr.2023.3061356.

Khaleelullah, M.M.S.I., Murugan, M., Radha, K.V., Thiyagarajan, D., Shimura, Y., Hayakawa, Y. (2017) Synthesis of super-paramagnetic iron oxide nanoparticles assisted by brown seaweed *Turbinaria decurrens* for removal of reactive navy-blue dye. *Mater. Res. Express* 4(10):105038.
DOI: https://doi.org/10.1088/2053-1591/aa9131.

Khatami, M., Alijani, H., Sharifi, I., Sharifi, F., Pourseyedi, S., Kharazi, S., Nobre, M.A.L., Khatami, M. (2017) Leishmanicidal activity of biogenic Fe_3O_4 nanoparticles. *Sci. Pharm.* 85(4):36.
DOI: https://doi.org/10.3390/scipharm85040036.

Kota, S., Dumpala, P., Sajja, R., Anantha, R. (2023) Phytoconstituents of *Chromolaena odorata* (L.) leaf extract for the synthesis of copper oxide/copper nanoparticles and evaluation of their biological potential in wound healing. *Trends Phytochem. Res.* 7(3):186–206.
DOI: https://doi.org/10.30495/tpr.2023.1990359.1363.

Lukes, J., Mauricio, I.L., Schonian, G., Dujardin, J.C., Soteriadou, K., Dedet, J.P., Kuhls, K., Tintaya, K.W.Q., Jirku, M., Chocholova, E., Haralambous, C., Pratlong, F., Oborník, M., Horak, A., Ayala, F.J., Miles, M.A. (2007) Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. *Proc. Natl. Acad. Sci. U.S.A.* 104(22):9375–9380.
DOI: https://doi.org/10.1073/pnas.0703678104.

Mahdavi, M., Namvar, F., Ahmad, M.B., Mohamad, R. (2013) Green biosynthesis and characterization of magnetic iron oxide (Fe_3O_4) nanoparticles using seaweed (*Sargassum muticum*) aqueous extract. *Molecules* 18(5):5954–5964.
DOI: https://doi.org/10.3390/molecules18055954.

Mashjoor, S., Yousefzadi, M., Zolgharnain, H., Kamrani, E., Alishahi, M. (2018) Organic and inorganic nano-Fe_3O_4: *Alga Ulva* flexuosa-based synthesis, antimicrobial effects and acute toxicity to briny water rotifer *Brachionus rotundiformis*. *Environ. Pollut.* 237:50–64.
DOI: https://doi.org/10.1016/j.envpol.2018.02.036.

Matheson, L.J., Tratnyek, P.G. (1994) Reductive dehalogenation of chlorinated methanes by iron metal. *Environ. Sci. Technol.* 28(12):2045–2053.
DOI: https://doi.org/10.1021/es00061a012.

Mohamed Abdoul-Latif, F., Ainane, A., Aboubaker, I.H., Houssein Kidar, B., Mohamed, J., Lemrani, M., Abourriche, A., Ainane, T. (2023) *Ericaria amentacea* algae extracts: A sustainable approach for the green synthesis of silver oxide nanoparticles and their effectiveness against leishmaniasis. *Processes* 11(11):3227.
DOI: https://doi.org/10.3390/md23040172.

Mohammadhosseini, M., Mahdavi, B., Kianasab, M.R. (2025) From plant to nanoparticle: Evaluating the biological efficacy of *Cannabis sativa* L. methanol extract, unlocking the biopotential of *Cannabis sativa* L. in the green fabrication of silver nanoparticles and their characterization. *Trends Phytochem. Res.* 9(1):78–94.
DOI: https://doi.org/10.71596/tpr.2025.1203169.

Mohapatra, M., Anand, S. (2011) Synthesis and applications of nanostructured iron oxides/hydroxides-A review. *Int. J. Eng. Sci. Technol.* 2(8):127–146.
DOI: https://doi.org/10.4314/ijest.v2i8.63846.

Mohebali, M., Rezayat, M.M., Gilani, K., Sarkar, S., Akhoundi, B., Esmaeili, J., Satvat, T., Elikaee, S., Charehdar, S., Hooshyar, H. (2009) Nanosilver in the treatment of localized cutaneous leishmaniasis caused by *Leishmania major* (MRHO/IR/75/ER): An *in vitro* and *in vivo* study. *Daru* 17(4):285–289.

Mondal, A., Dey, I., Mukherjee, A., Ismail, A., Satpati, G.G., Banerjee, S., Paul, S., Paul, S., Pal, R. (2024a) *Spirulina* biomass loaded with iron nanoparticles: A novel biofertilizer for the growth and enrichment of iron content in rice plants. *Biocatal. Agric. Biotechnol.* 61:103387.
DOI: https://doi.org/10.1016/j.bcab.2024.103387.

Mondal, A., Mukherjee, A., Pal, R. (2024b) Psychosynthesis of nanoiron particles and their applications-A review. *Biocatal. Agric. Biotechnol.* 55:102986.
DOI: https://doi.org/10.1016/j.bcab.2023.102986.

Mondal, A., Paul, S., Pal, R., Paul, S. (2024c) Nano iron loaded algal biomass: For better yield, amino acid and iron content in rice-A 'nano-phycofertilizer'. *Algal Res.* 81:103573.
DOI: https://doi.org/10.1016/j.algal.2024.103573.

Murray ,H.W. (1999) Kala-azar as an AIDS-related opportunistic infection. *AIDS Patient Care STDS* 13(8):459–465.
DOI: https://doi.org/10.1089/108729199318183.

Palaniyandi, T., Baskar, G., Bhagyalakshmi, V., Viswanathan, S., Wahab Abdul, M.R., Govindaraj, M.K., Sivaji, A., Rajendran, B.K., Kaliamoorthy, S. (2023) Biosynthesis of iron nanoparticles using brown algae *Spatoglossum* asperum and its antioxidant and anticancer activities through *in vitro* and *in silico* studies. *Part. Sci. Technol.* 41(7):916–929.
DOI: https://doi.org/10.1080/02726351.2022.2159900.

Pyne, N., Paul, S. (2022) Screening of medicinal plants unraveled the leishmanicidal credibility of *Garcinia cowa*; highlighting Norcowanin, a novel anti-leishmanial phytochemical through *in-silico* study. *J. Parasit. Dis.* 46(1):202–214.
DOI: https://doi.org/10.1007/s12639-021-01441-7.

Rastogi, V., Singh, B., Saxena, U., Singh, P., Jain, A., Yadav, P. (2025) Nanotherapeutics in Leishmaniasis. In Applications of Nanotherapeutics and Nanotheranostics in Managing Infectious Diseases. *Academic Press,* 523–550.
DOI: https://doi.org/10.1016/B978-0-443-28836-4.00022-6.

Roychoudhury, P., Ghosh, S., Pal, R. (2016) Cyanobacteria mediated green synthesis of gold-silver nanoalloy. *J. Plant Biochem. Biotechnol.* 23:73–78.
DOI: https://doi.org/10.1007/s13562-015-0311-0.

Roychoudhury, P., Golubeva, A., Dąbek, P., Pryshchepa, O., Sagandykova, G., Pomastowski, P., Gloc, M., Dobrucka, R., Kurzydłowski, K., Buszewski, B., Witkowski, A. (2022) Study on biogenic spindle-shaped iron-oxide nanoparticles by *Pseudostaurosira trainorii* in field of laser desorption/ionization applications. *Int. J. Mol. Sci.* 23(19):11713.
DOI: https://doi.org/10.3390/ijms231911713.

Sadiq Hawezy, H.J., Sdiq, K.H., Qadr, V.A., Anwer, S.S., Salih, S.J. (2020) Biosynthesis of magnetite-nanoparticles using microalgae (*Spirulina* SP and *Spirogyra* SP). *Plant Arch.* 11(7):1222.
DOI: https://doi.org/10.37506/ijphrd.v11i7.10263.

Salem, D.M.S.A., Ismail, M.M., Aly-Eldeen, M.A. (2019) Biogenic synthesis and antimicrobial potency of iron oxide (Fe_3O_4) nanoparticles using algae harvested from the Mediterranean Sea, Egypt. *Egypt. J. Aquat. Res.* 45(3):197–204.
DOI: https://doi.org/10.1016/j.ejar.2019.07.002.

Salem, D.M.S.A., Ismail, M.M., Tadros, H.R.Z. (2020) Evaluation of the antibiofilm activity of three seaweed species and their biosynthesized iron oxide nanoparticles (Fe_3O_4-NPs). *Egypt. J. Aquat. Res.* 46(4):333–339.
DOI: https://doi.org/10.1016/j.ejar.2020.09.001.

Shakibaie, M., Khorramizadeh, M.R., Faramarzi, M.A., Sabzevari, O., Shahverdi, A.R. (2010) Biosynthesis and recovery of selenium nanoparticles and the effects on matrix metalloproteinase-2 expression. *Biotechnol. Appl. Biochem.* 56(1):7–15.
DOI: https://doi.org/10.1042/BA20100042.

Stauch, A., Sarkar, R.R., Picado, A., Ostyn, B., Sundar, S., Rijal, S., Boelaert, M., Dujardin, J.C., Duerr, H.P. (2011) Visceral leishmaniasis in the Indian subcontinent: Modelling epidemiology and control. *PLoSNegl. Trop. Dis.* 5(11):e1405.
DOI: https://doi.org/10.1371/journal.pntd.0001405.

Subramaniyam, V., Subashchandrabose, S.R., Ganeshkumar, V., Thavamani, P., Chen, Z., Naidu, R., Megharaj, M. (2016) Cultivation of Chlorella on brewery wastewater and nano-particle biosynthesis by its biomass. *Bioresour. Technol.* 211:698–703.
DOI: https://doi.org/10.1016/j.biortech.2016.03.154.

Subramaniyam, V., Subashchandrabose, S.R., Thavamani, P., Megharaj, M., Chen, Z., Naidu, R. (2015) *Chlorococcum* sp. MM11-A novel phyco-nanofactory for the synthesis of iron nanoparticles. *J. Appl. Phycol.* 27:1861–1869.
DOI: https://doi.org/10.1007/s10811-014-0492-2.

Thakur, L., Singh, K.K., Shanker, V., Negi, A., Jain, A., Matlashewski, G., Jain, M. (2018) Atypical leishmaniasis: A global perspective with emphasis on the Indian subcontinent. *PLoSNegl. Trop. Dis.* 12(9):e0006659.
DOI: https://doi.org/10.1371/journal.pntd.0006659.

Tiwari, D.K., Behari, J., Sen, P. (2008) Time and dose-dependent antimicrobial potential of Ag nanoparticles synthesized by a top-down approach. *Curr. Sci.* 95:647–655.

Van Griensven, J., Balasegaram, M., Meheus, F., Alvar, J., Lynen, L., Boelaert, M. (2010) Combination therapy for visceral leishmaniasis. *Lancet Infect. Dis.* 10(3):184–194.
DOI: https://doi.org/10.1016/S1473-3099(10)70011-6.

WHO (2023) Global leishmaniasis surveillance, 2022: Assessing trends over the past 10 years. *World Health Organ.,* 471–487.

——— (2020) Leishmaniasis WHO fact sheet.

Younas, Z., Ahmad, I., Yousaf, T., Raja, N., Mashwani, Z.U.R. (2024) Selenium nanoparticle-mediated enhancement of plant biochemistry and metabolite enrichment: GC-MS profiling of selenium-enriched sesame seed oil. *Trends Phytochem. Res.* 8(4):234–247.
DOI: https://doi.org/10.71596/tpr.2024.936631.

Yu, C., Tang, J., Su, H., Huang, J., Liu, F., Wang, L., Sun, H. (2022) Development of a novel biochar/iron oxide composite from green algae for bisphenol-A removal: Adsorption and Fenton-like reaction. *Environ. Technol. Innov.* 28
DOI: https://doi.org/10.1016/j.eti.2022.102647.

Zhang, Y., Mahdavi, B., Mohammadhosseini, M., Rezaei-Seresht, E., Paydarfard, S., Qorbani, M., Karimian, M., Abbasi, N., Ghaneialvar, H., Karimi, E. (2021) Green synthesis of NiO nanoparticles using *Calendula officinalis* extract: Chemical charactrization, antioxidant, cytotoxicity, and anti-esophageal carcinoma properties. *Arabian J. Chem.* 14(5):103105.
DOI: https://doi.org/10.1016/j.arabjc.2021.103105.