



OPEN Potential biological applications of environment friendly synthesized iron oxide nanoparticles using *Sageretia thea* root extract

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The green synthesis of Iron oxide nanoparticles (IONPs) has shown numerous advantages over conventional physical and chemical synthesis methods as these methods non-ecofriendly and uses toxic chemicals and complicated equipments. In present study, Iron oxide nanoparticles (IONPs) were created using simple, sustainable, eco-friendly and green chemistry protocol. The roots of novel medicinal plant *Sageretia thea* was used as bio-template for the preparation of IONPs. Further, the synthesis of IONPs was confirmed using different analytical tools like UV-Vis, FT-IR, XRD, EDX, and SEM. The average sizes of (IONPs) were found to be 16.04 nm. Further, asynthesized IONPs were evaluated for several biological potentials including antibacterial, antifungal Anti-radical potentials (DPPH) and cytotoxicity assay. Antibacterial potencies were investigated using bacterial strains (in the concentration range of 1000–31.25 µg/mL) revealing significant antibacterial potentials. ABA and SAU was reported to be least susceptible while KPN was observed to be most susceptible strain in bactericidal studies. Further different fungal strains were used to investigate the antifungal potentials of IONPs (in the concentration range of 1000–31.25 µg/mL) and revealed strong antifungal potencies against different pathogenic strains. Furthermore, MRA, FA and ANI were most susceptible and ABA was least susceptible in fungicidal examination. Significant cytotoxicity potential was examined using brine shrimp cytotoxicity assay, thus revealing the cytotoxic potential of asynthesized IONPs. The IC₅₀ for *S. thea* based IONPs was recorded as 33.85 µg/mL. Strong anti-radical potentials (DPPH) assay was performed to evaluate the ROS scavenging potential of S.T@IONPs. The highest scavenging potential was noted as 78.06%, TRP as 81.92% and TAC as 84% on maximum concentration of 100 µg/mL. In summary, our experimental results concluded, that asynthesized IONPs have strong antibacterial, antifungal, DPPH scavenging and cytotoxic potentials and can be used in different biological applications. In nutshell, our as-prepared nanoparticles have shown potential bioactivities and we recommend, different other in vitro and in vivo biological and bioactivities to further analyze the biological potentials.

Keywords *Sageretia thea*, Phytochemicals, Iron oxide nanoparticles, Antibacterial, Antifungal, DPPH

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Nanotechnology (NT) is one of the most important and rapidly emerging field in engineering and material science, which deals with synthesis, characterization, and formation of nanoparticles (NPs)¹. The word “nano” is borrowed from Greek language which means extremely particles, small from size range 1–100 nm and possess fascinating properties like electrical, magnetic, high surface area, durable, physiochemical, biological, and mechanical properties². There are various NPs based on their shape, structure and size such as (nanocube, nanowire, nanowire, cluster, core shell, and bimetallic) etc³. Nanoparticle and their derived nanomaterial have wide range of application in different sectors including medicine delivery, food, agriculture, cosmetics, cancer detection/ diagnostics, cancer therapy, and numerous more^{3,4}. Up till now many metals and metal oxide NPs have been synthesized thus far including nickel, zinc, gold, silver, cobalt, magnesium, and platinum etc⁵. Among these synthesized nanoparticles the more adjustable and multifunctional iron oxide (IONPs) have attracted the scientists from various fields⁶, due to easy synthesis and their significant biological properties⁷. IONPs have also been shown as a strong antibacterial, antifungal, antioxidant, anti-inflammatory, anti-cancer, and enzyme inhibition potentials⁸. Many researchers have been drawn to IONPs nanoparticles due to the intriguing characteristics and wide range of uses, including (bioremediation, cosmetics, diagnostics, and biomedicine)⁹. Their application in magnetic resonance imaging (MRI) therapy has recently increased¹⁰. The magnetic characteristics, cost-effectiveness, considerable changeable oxidation state, and crystal structure of IONPs have attracted many researcher¹¹. One of the most common forms of iron is iron oxide, which has a very broad range of uses¹². It is typically employed as an anode in batteries, a photo-catalyst in the photo-degradation of phenolic compounds, a sensor for CO and other hazardous gases, and in the pharmaceutical industry¹³. Three methods are used for the preparation of nanoparticles such as physical, chemical, and biological. There are different sized (NPs) that are often created using both chemical and physical methods¹⁴. The physical methods typically include pyrolysis, thermal evaporation, ball milling, laser ablation, and spark discharge, whereas the chemical approach prepares NPs using a variety of chemicals¹⁵. These synthesis methods face several quite challenging, when it comes to expanding precursors, hazardous reducing, stabilizing, and capping agents, organic toxic solvents etc^{16,17}. Moreover, these techniques are quite costly due to utilization of many costly equipment. So the scientific community solved these problem with development of safe, cleaner, and sustainable approach via green synthesis¹⁸. These alternative approach of nanoparticles utilized as green, environmentally benign, and cost efficient platform as a strong stabilizing, reducing and capping agents from medicinal plants, algae, fungi, bacteria and other natural sources¹⁹. Further, the plant based mediated synthesis of NPs have gaining significant attention of researcher in interdisciplinary field due to easy obtainability of various biomolecules, simple plant extract, and rapid reaction²⁰. However, among these different natural sources, medicinal plants and their parts such as roots, stem, leaves, flower, seeds, peels etc. are considered as the most valuable candidates for the production of nanoparticles (NPs)²¹. Recently, the scientific community is mainly focusing on plant extracts and presence of associated bioactive compounds in order to determine their pharmacological effects^{22,23}. Additionally, plants extract make easy synthesis of nanoparticles and also control the proper size and shape. Thus plant extract is a rich source of different medicinal phytochemicals such as terpenoids, alkaloid, flavonoids, saponins, phenols, minerals, and vitamins as considered strong reducing and stabilizing agent during the process of phytofabrication²⁴. Green nanotechnology has thus undergone a significant development as a substitute for the production of stable and safe products employing various medicinal plants²⁵. Previously, different medicinal plants were utilized for the synthesis of iron oxide nanoparticles²⁶. The medicinal and fruit edible plant *Sageretia thea* (Osbeck) is an evergreen shrub, locally known as Chinese sweet plum or sweet plum^{27,28}, is widely distributed in Eritrea to northern India, South China, Malaysia, Arabian country, Korea, Japan and also present in temperate region of southern and eastern Asia. Pakistan is hosting 4 species mainly distributed in different region including districts Mohmand, Bajaur, Kurram, Khyber, Waziristan, Malakand, Islamabad, Kashmir etc²⁹. Genus *Sageretia* has about 35 species with 15 endemic species from this region³⁰. *S. thea* contain various medicinal phytochemicals which are used to cure colds, fever, and inflammatory diseases³¹. The leaves and branches have a wide range of pharmacological effects such as anti-inflammatory, anti-cancer, and antioxidant activity through scientific research^{32,33}. Although *S. thea* is used to create bonsais, in traditional herbal medicine for the treatment of hepatitis³⁴. The roots of this plant exhibited a protective effect on lipoprotein in low density against the oxidative modification³⁵. The goal of the present study was to investigate the biological potential of medicinal plant *Sageretia thea* roots extract as a strong reducing, stabilizing and capping agents for the synthesis of IONPs. Further, the synthesis of IONPs was rectified using various analytical tools including UV-Visible spectroscopy, XRD, FTIR, EDX, SEM and finally different in vitro bioactivities were done.

Materials and methods

Plant collection and Processing

Fresh and healthy roots of medicinal plant *Sageretia thea* (family: Rhamnaceae) were collected from the hilly areas of Mohmand, Tamanzai, KPK, Pakistan following established protocol and permission was obtained during month of June. Our plant study complies with relevant institutional, national and international guidelines and legislation. Further, the plant material was identified and taxonomically validated by well-known taxonomist Dr. Syed Afzal Shah, Assistant Professor at Department of Biological Sciences, National University of Medical Sciences Rawalpindi, Pakistan. The voucher specimens were submitted to gene bank of the National University of Medical Sciences Rawalpindi, Department of Biological Sciences, for allotment of voucher number (SAS-567), multiplication and to ensure availability for future use. In the next step, the plant specimen was identified by well-known taxonomist. The plant roots were thoroughly washed by tap water to remove all kinds of surface impurities and as shade dried for about 2 to 3 weeks. After complete drying, the plant roots material was crushed into pure fine powder and the plant powder was used to prepare plant extract.

Preparation of plant extract

For the preparation of extract 30 gram dried plant roots powder of *Sageretia thea* was added to 500 mL flask containing 300 mL of distilled water. For shaking the solution, the flask was placed on magnetic stirrer and was continuously heated at 80 °C for 2 h. Next, the extract was allowed to cool and was then three times filtered using Whatman filter papers to achieve pure aqueous plant extract. Finally, pH of extract was recorded as 6.27 at room temperature and was stored at 4 °C for future purpose.

Green synthesis of iron oxide NPs

The synthesis of Iron oxide NPs (IONPs) was performed by reducing Iron acetate precursor salt using *Sageretia thea* plant extract. In order to achieve this purpose, 2 g Iron acetate salt was steadily mixed with 200 mL filtered *S. thea* plant extract, which gave reddish brown color solution. The pH of the solution was measured and was recorded as 7.0. Next, the mixture was continuously heated at 60 °C and constant stirring at 500 rpm for 2 h. Further, the obtained solution was centrifuged at 4000 rpm/15 minutes. After centrifugation, the supernatant was discarded and pellet assumed as Iron oxide NPs were carefully washed 3 times with distilled water to remove all kinds of uncoordinated materials and impurities. The achieved powder assumed as Iron oxide NPs was incubated at ~100 °C until the water evaporated and the particles were fully dried. After successful incubation, the synthesized NPs were annealed at 400 °C to obtain pure crystalline IONPs. After accomplishing room temperature the obtained sample was stored in air tight vials (15 mL falcon tubes parafilm) and were stored in cool, dry and dark place. Finally, the synthesized IONPs were characterized using various characterization tools.

Characterization of IONPs

The synthesis of IONPs was confirmed using different standard characterization techniques namely; SEM, EDX, FT-IR, XRD, UV-visible spectroscopy.

Ultraviolet–visible spectroscopy

Using a UV-VIS spectrophotometer, the bio-reduction of metal ions into metal nanoparticles was observed. A straightforward color shift of the plant extract from greenish brown to reddish brown allowed observers to see the reduction in iron ions. A stock solution of iron oxide nanoparticles was diluted to 5 mg/5 mL of deionized water and adequately sonicated using a Sonicator machine in order to get the UV-Vis absorption spectra. To achieve this purpose, the IONPs were scanned and absorbance was measured in the range of 200 nm to 600 nm using UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan). An adsorption band about 329 nm was visible in the UV-Vis absorption of IONPs, indicating the successful production of IONPs. Using a quartz cuvette with a 1 cm light path length, light absorption spectra were obtained and shown relative to distilled water.

X-ray diffraction (XRD) analysis

The phase purity, structural analysis and crystalline nature of biogenic IONPs was determined by (PANalytical XRD (Netherlands) analysis in the range of 2θ from 10° to 90° using Cu/K radiation of wavelength 1.5406 Å. operated at 40 kV with 30 mA fixed at room temperature. The Debye-Scherrer equation ($D = K\lambda / \beta \cos \theta$) formula was utilized to determine the corresponding particle size. The formula takes into account the following factors: λ is the X-ray wave-length (1.5406 Å), K is constant (0.9), D is the crystal size perpendicular to the reflecting planes, β is the angular full width at half maximum in radians, and θ is the Bragg's angle.

Fourier transformed infrared (FTIR) analysis

To determine the different bioactive functional groups involved in reduction and stabilization of IONPs, FT-IR analysis was performed. The sample measurement range was set in FT-IR spectroscope. To achieve this purpose, the synthesized IONPs were scanned in the range of 550 to 3850 cm^{-1} at a resolution of 4 cm^{-1} and different spectra were obtained to determine the different functional groups involved in the reduction, stabilization and capping of IONPs which enhance their biological and biomedical potentials.

Characterization by SEM-EDX analysis

The prepared IONPs actual size and surface morphology was determined using SEM (EM (NOVA FEISEM-450 applied with Energy Dispersive X-ray detector). To secure the IONPs and prevent their spread, many drops of the solution were carefully placed on the stub that was covered with carbon tape. The sample was placed into SEM machine and was subjected to SEM at accelerating voltage of 10 KV and images at 50 kx magnification were taken. Further, the (EDX) spectroscopy analysis was performed to investigate the elemental analysis of synthesized IONPs and to confirm its synthesis.

Biological evaluation

Synthesis of nanoparticles was confirmed through different characterization techniques. In the next step, different biological activities were performed to investigate their different biological potentials.

Antibacterial activity

Anti-bacterial potential of *Sageretia thea* formulated IONPs was determined using the disc diffusion method against gram-negative strains of *Pseudomonas aeruginosa*, *pneumonia* and *Escherichia coli*, while *Bacillus subtilis* and *Staphylococcus aureus* are used as gram positive strains. In order to reactivate the frozen cultures for later usage, several bacterial strains were inoculated on nutrient agar medium. We created and autoclaved nutrient agar medium to support the development of several bacterial strains. After allowing the medium to cool, it was placed to petri dishes, so it could harden. A homogeneous bacterial lawn was created on petri plates by

spreading 100 μL of bacterial cultures there in order to assess the antibacterial potential. Further, the petri plates underwent para-filming and incubation was done at $37^\circ\text{C}/24\text{ h}$. The bactericidal potential of nanoparticles was assessed at several doses (1000–31.25 $\mu\text{g}/\text{mL}$). Filter discs (6 mm) filled with (10 μL) test samples (IONPs) were meticulously positioned on even lawns, with DMSO serving as the negative control and 10 μg streptomycin disc serving as the positive control for IONPs orchestrated by *Sageretia thea*. Following a 24-hour incubation period at 37°C , the bacterial strains' corresponding MIC values were computed within the different concentration range of 1000–32 $\mu\text{g}/\text{mL}$, and the inhibition zones were measured in millimeters.

Antifungal activity

The *Sageretia thea* formulated IONPs was examined using five different fungal strains such as *Candida albicans*, *Fusarium solani*, *Mucor racemosus*, *Aspergillus flavus*, and *Aspergillus niger*. The media used for Sabouraud dextrose agar was prepared and autoclaved. After allowing the medium to cool, it was placed onto petri dishes to solidify. To create homogenous lawns of various fungal strains, several fungal strains were then spread out on petri plates in the next phase. Further, the petri plates were para-filmed and placed in an incubator set to 28°C for 48 h in order to grow. 10 μL of the sample solution was poured onto filter discs, which were then put on media plates. DMSO served as the negative control and 10 μg of nystatin as the positive control. Additionally, the plates were incubated at 28°C for 48 h overnight. The zones of inhibition were measured via Vernier caliper. Various dependent doses of IONPs in the range of 1000–31.25 $\mu\text{g}/\text{mL}$ were used. Finally, the MICs values were calculated at lowest concentration of IONPs.

Brine shrimps cytotoxicity assay (BSCA)

BSCA assay was performed to determine the cytotoxicity potential of *Sageretia thea* mediated iron oxide NPs. The cytotoxic impact of green IONPs was obtained by using larvae of *Artemia salina* (Ocean Star, UT, and USA). The *Artemia salina* eggs, which were incubated for 48 h at 30°C in sea salt solution (3.8 g/1 mL) in the presence of light, gave rise to the brine shrimp larvae. Around about 10 fully mature nauplii were put using a micropipette and placed into glass vials with IONPs and seawater for a 24-hour incubation period. Different doses of IONPs (500–3.91 $\mu\text{g}/\text{mL}$) were prepared and were used to determine their dose dependent response. The mature nauplii, vincristine sulphate, and seawater in the vials were used as the positive control, while the nauplii, DMSO, and seawater in the vials were utilized as the negative control. Additionally, after a 24-hour incubation period, the number of dead shrimp in each vial was counted, and GraphPad software was used to calculate the percentage (%) of inhibition.

Antioxidant assay of IONPs

Using the DPPH free radical scavenging test, the antioxidant activity of the iron NPs produced by *Sageretia thea* was investigated. The IONPs' antioxidant activity (AA) was assessed by utilizing a micro-plate reader to evaluate dilutions of the compound at different concentrations (200–5 $\mu\text{g}/\text{mL}$) for its ability to scavenge free radicals. The reaction mixture was prepared in order to assess the antioxidant capacity of IONPs. To prepare the reagent solution, 0.1 g DPPH (2.4 mg and 25 mL) of methanol were combined. To evaluate the antioxidant effectiveness of IONPs, ascorbic acid (AA) was used as the positive control and dimethyl sulfoxide (DMSO) as the negative control. To create 100 μL of reaction solution, the protocol calls for combining 20 μL of test sample (S.T@IONPs) with 180 μL of reagent solution. The reaction mixture was then loaded into different wells of 96-well plate and was transferred into incubator for 1 h. Finally, the reaction mixture was scanned at 571 nm using microplate reader to determine the % scavenging potential of DPPH applying the formula below: Detailed study layout is provided in Fig. 1.

Results and discussion

Emerging innovative nano-crystals technology that ensures simple, eco-sustainable approach and uses nontoxic green and natural chemicals replaced the approaches that utilized challenging tasks, costly and toxic chemicals and machinery. The present work provides facile green fabrication approach exploiting the green chemicals from aqua leaves broth of potentially medicinal plant; *Sageretia thea* for reducing iron acetate salt. Previously^{36–38}, etc. have synthesized nanosized particles using *Hibiscus rosa-sinensis*, *Avicennia marina* and *Lagenaria siceraria* respectively. The green compounds of plants have vast range of active molecules including polyphenolics, kaempferols, flavonoids, alkaloids, saponins etc.^{39,40}. The green compounds being rich source of electrons have potential reducing and stabilizing capabilities. Therefore, green compounds of plants are being utilized for the fabrication of different metal and metal-oxides nanocrystals^{41,42}. Herein, we have reported the synthesis, characterization and various bio-applications of *Sageretia thea* based IONPs.

The fabrication of IONPs was confirmed when aqua extract of *S. thea* changed its color from greenish brown to reddish brown solution upon the addition of precursor salt (Ferric acetate basic). After the successful synthesis, *S. thea* based IONPs were characterized by observing maximum absorption (SPR spectral analysis) utilizing Ultra Violet visible photometry. The SPR spectra revealed highest absorption of 329 nm which is the standardized range of fabricated IONPs. The stability, size and shape of any orchestrated biomaterial can directly be encountered by studying SPR spectral peak Fig. 2.

The transmission infrared bands/spectra were analyzed to find out the presence of bioactive functional elements, biomolecules/functional groups adsorbed upon the surface of our green synthesized IONPs. Figure 3 showed transmission infrared spectra. The FT-IR spectra of *S. thea*-IONPs revealed significant transmissions peaks at 3110.06 cm^{-1} , 2108.98 cm^{-1} , 1966.75 cm^{-1} , 1856.13 cm^{-1} , 1660.05 cm^{-1} and 1111.12 cm^{-1} , which are ascribed to stretching vibrations of -OH, C=C, C=C, aromatic compounds, C=O, and C-O bonds. Also, the transmission peak observed at 537.42 cm^{-1} is attributed to strong bond vibrations of Fe-O from $\alpha\text{-Fe}_2\text{O}_3$ (hematite phase). In addition, the FT-IR pattern showed the presence of -OH, C-H, C=O, C $\equiv$ N, C=C, and C-O

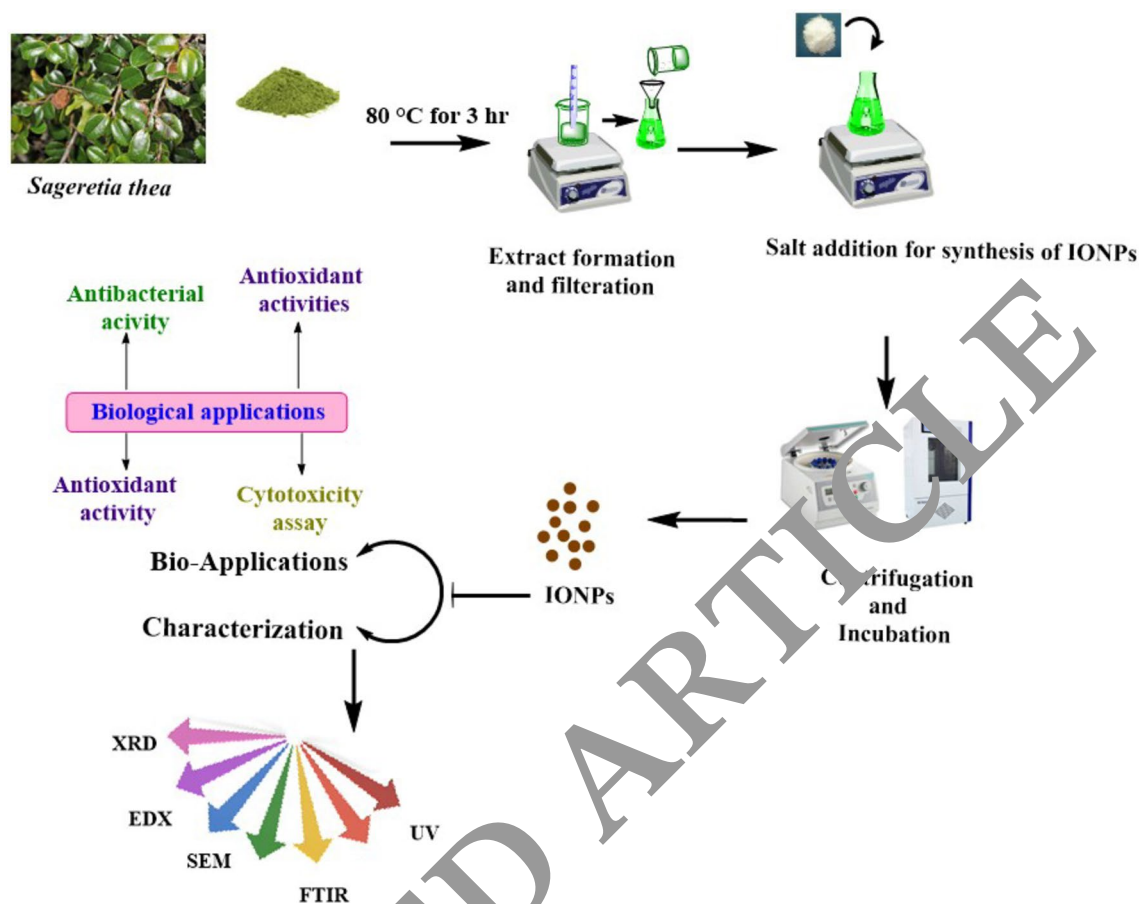


Fig. 1. Detailed study layout showing green synthesis of *Sageretia thea* mediated IONPs. The study describes each and every step and instruments used in current study.

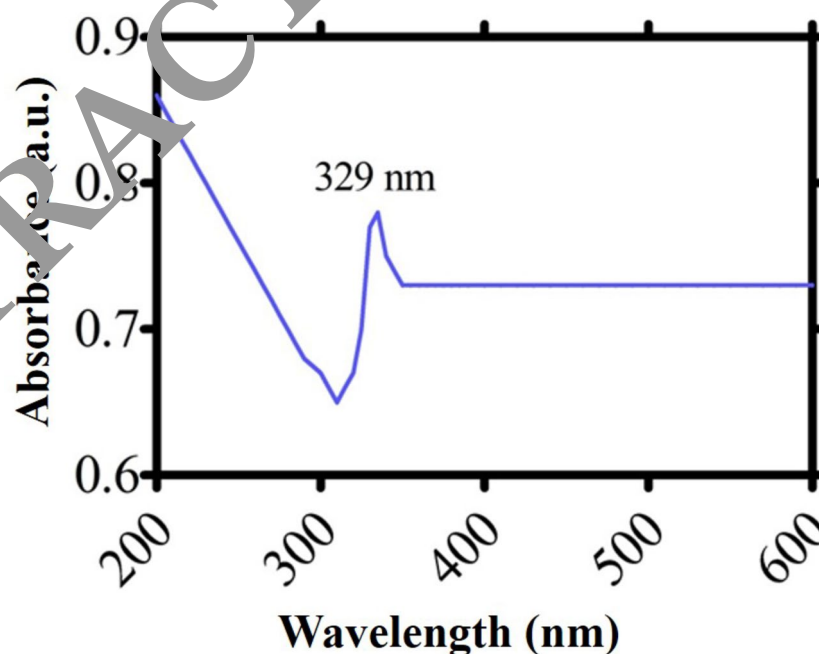


Fig. 2. UV-VIS spectroscopy for *Sageretia thea* root mediated IONPs.

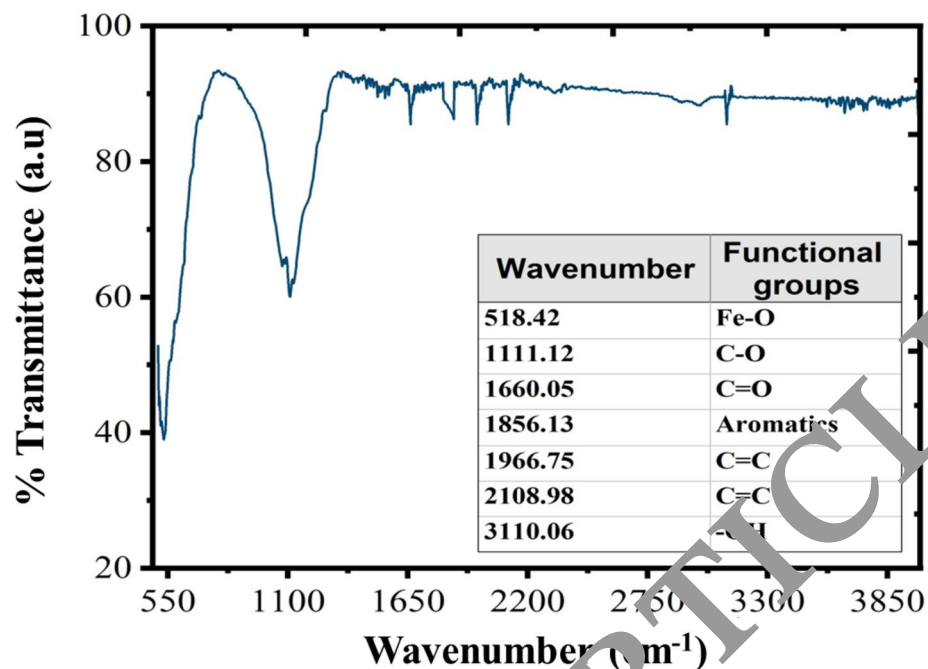


Fig. 3. FT-IR pattern of *Sageretia thea* mediated IONPs.

bonds which might have active role in reducing salt, assembly of nano-crystals, tuning the shape and stabilization of nanosized crystals. According to some previous reports it is considered that phenolic and flavonoids presents in the green extract plays very important role in performing aforementioned tasks. Therefore, it can be assumed that green extract obtained from *Sageretia thea* might have considerable amount of phenolic and flavonoids so are capable of having dual role in reduction and stabilization based on previous data^{43–45}.

The X-ray crystallography was employed for proper phase identification, geometry and purity of S.T-IONPs. Figure 4. Showed the XRD crystallographic analysis of ST-IONPs. The crystallographic spectra revealed diffraction bands observed at 23.56, 25.63, 41.11, 46.12, 56.87 and 63.51 illustrating different crystallographic planes like 012, 110, 113, 021, 018 and 214 respectively. In addition, the Bragg peaks observed for S.T-IONPs are in accordance with the diffraction standards of pure phase α -Fe₂O₃ nano sized particles (JCPD: 079–1741). The sharp peaks of XRD crystallographic studies have shown the purity and crystalline nature of greenly orchestrated IONPs. The average size 16.04 nm was calculated from peak width, by utilizing quantitative approximation devised by Karl⁴⁶. The absence of any extra peak clearly indicated the purity of greenly orchestrated nanoparticles. The XRD data of current findings is in accordance to previous literature^{46,47}.

Furthermore, the presence or absence of different elements were revealed utilizing EDX. The sharp peak at 0.29 KeV indicated the existence of oxygen, iron and carbon. The existence of carbon can be ascribed to the grid support. No impurity (absence of any additional metal) was found which further confirmed the purity of *S. thea* mediated IONPs. Further, the EDX data indicated the presence of signal for elemental iron at 0.30 which is in agreement to the previous research reports^{48–50}. The EDXA spectrum is illustrated in Fig. 5.

The SEM image of *S. thea* mediated IONPs are shown in Fig. 6. The SEM images confirmed that the greenly synthesized nano sized particles are almost spherical in shape and are agglomerated.

Antimicrobial capabilities

The bactericidal and fungicidal activities of greenly orchestrated IONPs were investigated utilizing various fungus and bacterial strains^{51,52}. Among them, *Escherichia coli* (ECO), *Bacillus subtilis* (BSU), *Klebsiella pneumonia* (KPN), *Pseudomonas aeruginosa* (PA), and *Staphylococcus aureus* (SAU) was exploited for potential bactericidal effect. However, *Fusarium solani* (FSO), *Candida albicans* (CAL), *Aspergillus flavus* (AFL), *Mucor racemosus* (MRA) and *Aspergillus niger* (ANI) was utilized for the investigation of potential fungicidal effects. Results are illustrated in Figs. 7 and 8. *Bacillus subtilis* (BSU), *Escherichia coli* (ECO) and *Staphylococcus aureus* (SAU) were inhibited on all the concentration used in this study. ABA and SAU was reported to be least susceptible while KPN was observed to be most susceptible strain in bactericidal studies. The MICs values are 31.25 μ g/mL, 31.25 μ g/mL, 31.25 μ g/mL, 125 μ g/mL and 62.5 μ g/mL for BSU, SAU, ECO, KPN and SAU respectively. Furthermore, MRA, FA and ANI were most susceptible and ABA was least susceptible in fungicidal examination. The MICs values are 31.5 μ g/mL, 31.5 μ g/mL, 31.5 μ g/mL 62.5 μ g/mL and 125 μ g/mL for FSO, ANI, MRA, CAL and AFL respectively. All the concentrations used in antimicrobial study showed dose-dependent responses. The positive control exhibited highest inhibitions in both cases (fungus P.C (positive control): Nystatin) and bacterial PC: Streptomycin) inhibitory activities). Different concentrations of IONPs 1000–31.25 μ g/mL were employed on different aforementioned strains (fungus and bacterial). From the Figs. 7 and 8, we can conclude that our nanoparticles exhibited excellent anti-fungus and antibacterial potential. Our results are in accordance to the

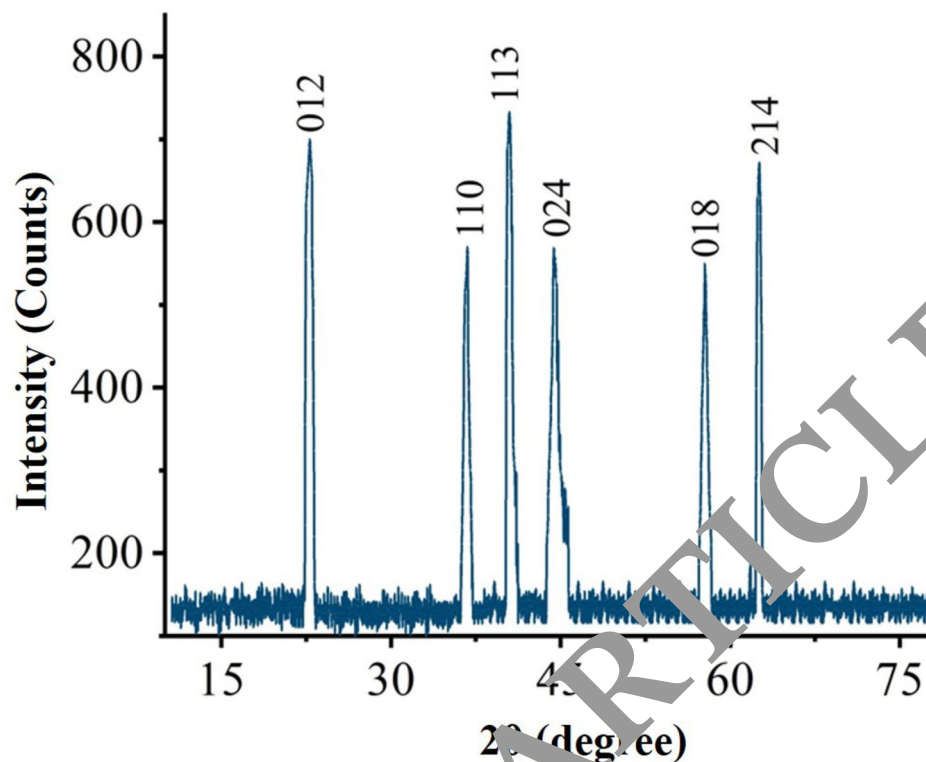


Fig. 4. XRD pattern of *Sageretia thea* mediated IONPs.

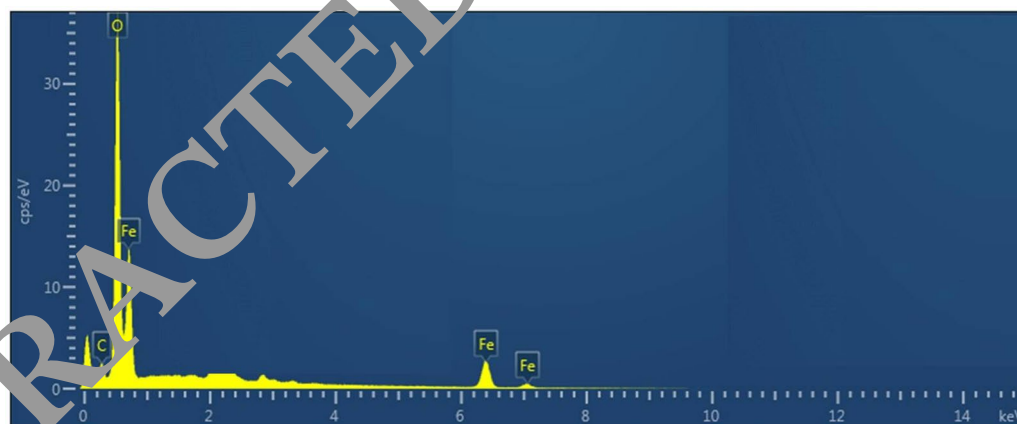


Fig. 5. EDX analysis of *Sageretia thea* based IONPs.

previous research studies using different pathogenic microbial strains^{53,54}. According to available literature, these antimicrobial potentials of greenly fabricated nanoparticles are due to different factors; surface deficits of NPs^{55,56}, production of ROS (reactive oxygen species.)^{57,58}, adsorbed surface biomolecules^{59,60}. Also, the NPs when enter into bacterial cells, results into disorganization of the peptidoglycan layer, membrane injury and cell death⁶¹.

Cytotoxicity assay

The cytotoxicity assay is high throughput test to analyze the cytotoxicity ability of asynthesized nanoparticles against newly hatched nauplii of brine shrimps^{62,63}. The ASLA test was performed to investigate the toxicity potentials of *S. thea* mediated IONPs. Different concentration ranges were used ranging from 3.91 to 500 µg/mL. The vincristine sulfate was used as positive control. The % mortality is shown in Fig. 9. The IC₅₀ for *S. thea* based IONPs was noted at 33.85 µg/mL while the IC₅₀ values was calculated as 4.991 µg/mL for vincristine sulphate. The final results confirmed the category of general cytotoxic for greenly created nano-sized particles. The results of the current study are in alignment with previously published cytotoxicity assays reports^{64,65}.

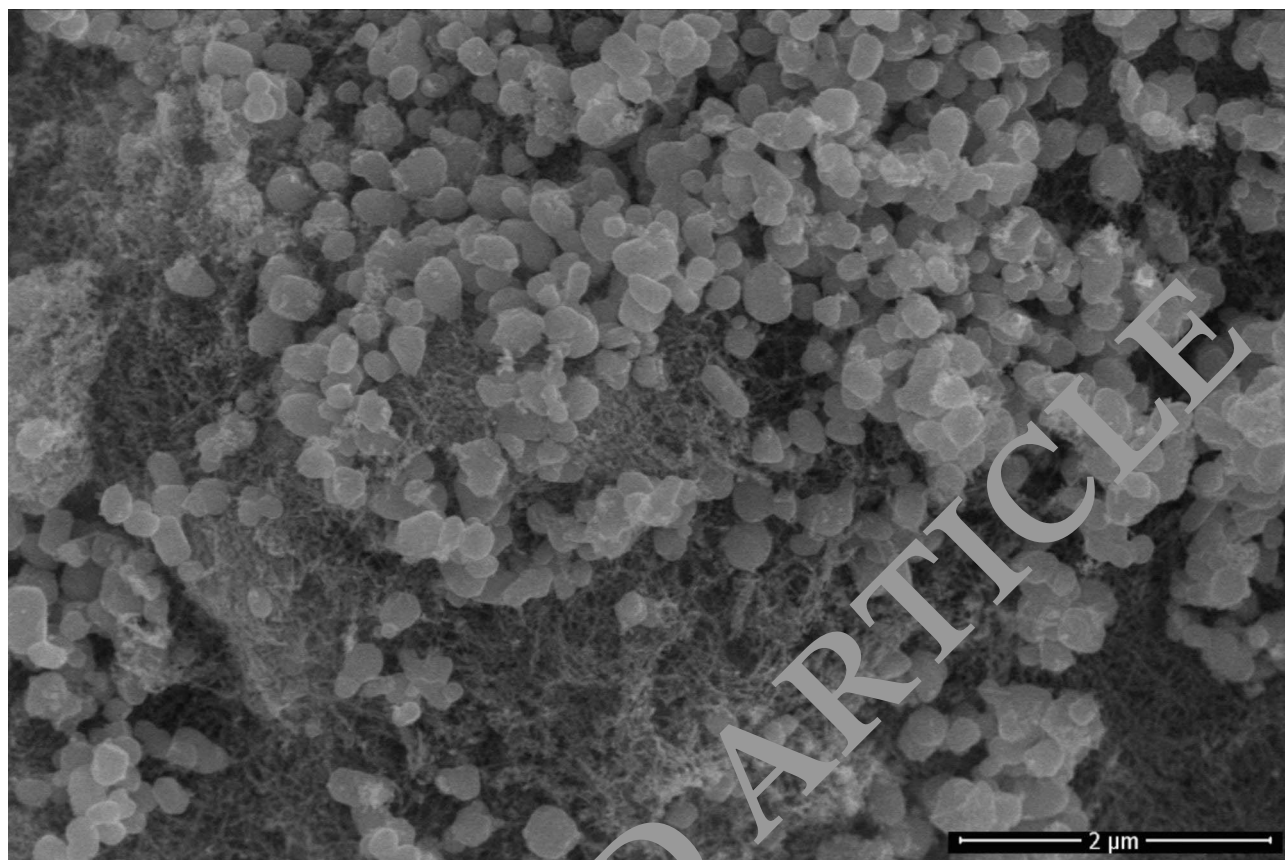


Fig. 6. SEM image of *Sageretia thea*-mediated IONPs.

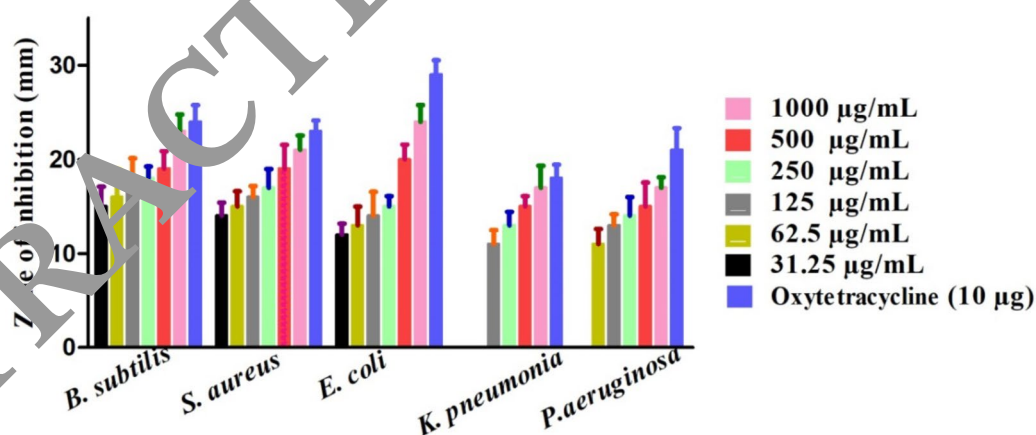


Fig. 7. Anti-bacterial potentials of *Sageretia thea*-IONPs.

Antioxidant potentials of IONPs

The greenly orchestrated nano sized particles (5–200 $\mu\text{g/mL}$) were used for the investigation of anti-radicle potentials^{39,66}. To achieve this aim, DPPH-radical scavenging, TAC and TRP test were exploited to record the adsorbed reductones upon the surface of ST@IONPs. The highest scavenging potential was noted as 78.06%, TRP as 81.92% and TAC as 84% on maximum concentration of 200 $\mu\text{g/mL}$ which determined the strong cytotoxicity potentials of ST@IONPs. The detailed DPPH, TRP and TAC activities are illustrated in Fig. 10. This scavenging activity, TRP and TAC assays might be due to different constituents of active green chemicals in plants; carotenoids, phenolics, terpenes, saponins etc^{67–69}. The results of our study concluded excellent anti-radicle potency of greenly orchestrated IONPs which are in agreement to earlier literature reports^{70–72}.

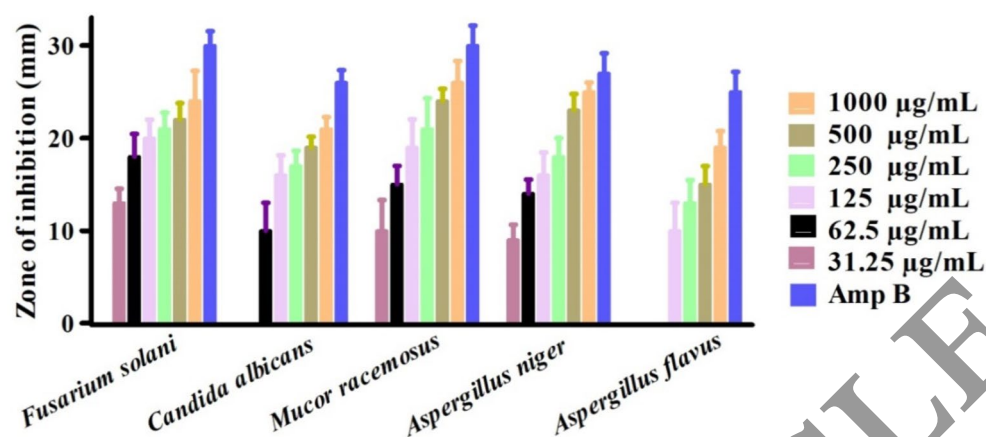


Fig. 8. Anti-fungal potentials of *Sageretia thea*-IONPs.

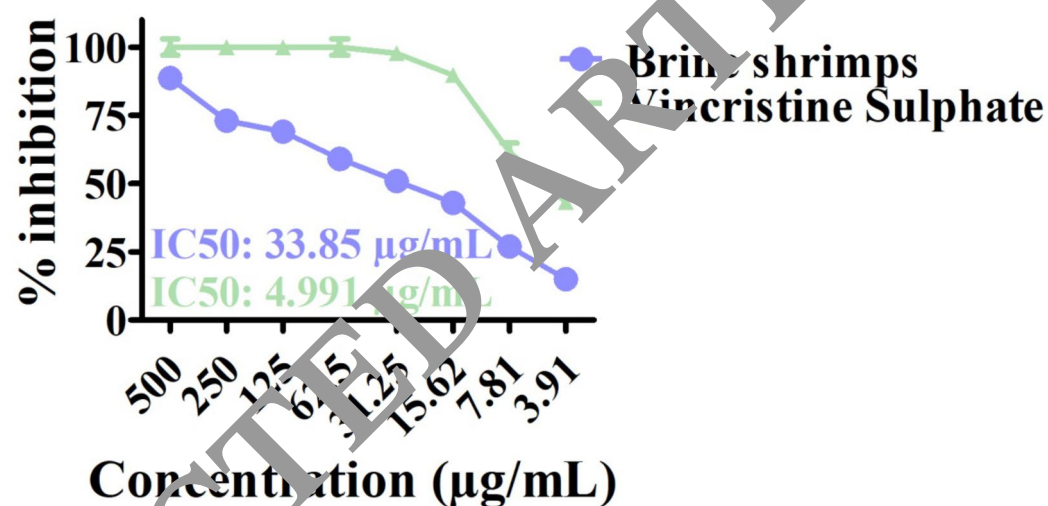


Fig. 9. Brine shrimps activity of *Sageretia thea*-FeONPs.

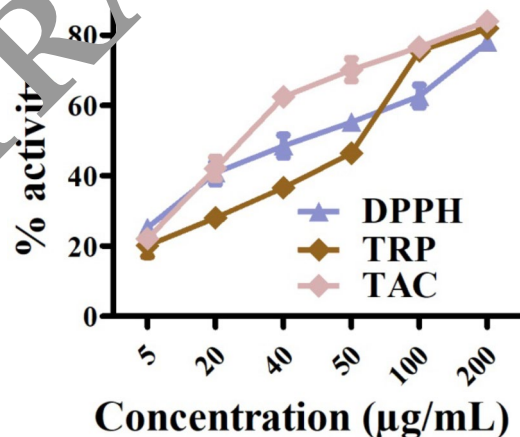


Fig. 10. Antioxidant potential of *Sageretia thea* mediated FeONPs.

Conclusion

Metal and metal oxide nanoparticles (MONPs) present a bright area of research for the development of novel treatment strategies in Nano-medicine. However, new methods was continuously investigated for tunable, ecofriendly, and effective synthesis of NPs. Herein, a complete green synthesis method has been adopted to successfully produce MNPs like Fe_2O_3 using leaves broth of medicinal plant; *Sageretia thea*. Different biological activities such as enzyme inhibition cytotoxicity (brine shrimps), antimicrobial (using fungal and bacterial strains), and antiradical assays have determined significant role of S.T-IONPs in treatment of human ailments. Significant bactericidal and fungicidal activities are reported for green S.T-IONPs. Greenly orchestrated IONPs have shown significant results against *A. salina* (Sea monkeys). The present study revealed excellent antioxidant potential for *Sageretia thea* based IONPs. The bio-inspired IONPs effectively scavenged radicles of DPPH and exhibited excellent antiradical capability. In future, we suggest different other in vitro and in vivo bioactivities to further analyze the biomedical potencies of the asynthesized NPs. It is also recommended to perform dye degradation assays to combat different environmental issues.

Data availability

All the raw data in this research can be obtained from the corresponding authors upon reasonable request.

Received: 16 May 2024; Accepted: 13 November 2024

Published online: 16 November 2024

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Acknowledgements

The authors extend their appreciation to the Researchers supporting project number (RSP2024R185), King Saud University, Riyadh, Saudi Arabia.

Author contributions

M.I, J.I, R.U. and F.Z designed and conceived the study idea. M.I completed the experiments. J.I, F.Z, B.A.A, T.Y, and S.I, analysed the data and performed visualizations and statistical data analysis. M.I and J.I wrote the original draft. G.K, G.M, I.A, S.K., K.M.A, M.S.E, M.R, S.K, and R.I reviewed and edited the manuscript. R.I and M.R, reviewed the manuscript and provided funds. J.I provided the resources and supervision. All authors made valuable revisions and edited the manuscript and approved the last version.

Funding

Open Access funding enabled and organized by Projekt DEAL. Researchers supporting project number (RSP2024R185), King Saud University, Riyadh, Saudi Arabia.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

This study does not include human or animal subjects.

Statement on guidelines

All experimental studies and experimental materials involved in this research are in full compliance with relevant institutional, national and international guidelines and legislation posing a conflict or bias.

Additional information

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