



Green Synthesis: An Eco-friendly Route for the Synthesis of Iron Oxide Nanoparticles

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Green approach has received major attention for the synthesis of metal oxide nanoparticles. One such metal oxide nanoparticles are iron oxide nanoparticles (IONPs). IONPs have fetched a great deal of interest in recent era because of their magnetic nature, as they can be easily recovered from the reaction mixture by applying an external magnetic field. Although, a variety of chemical and physical methods of synthesis are known, green synthesis is safer, sustainable and biologically acceptable. Plants and microbes are the main biological materials used for the green synthesis. In present review, the synthesis of IONPs by using plants, bacteria, fungi and algae have been highlighted. IONPs produced by plants, fungi, bacteria and algae usually falls in 1–100 nm range and are of distinct shapes like cubic, tetragonal crystalline, spherical, cylindrical, elliptical, octahedral, orthorhombic, hexagonal rods, nanosphere and quasi spherical. Furthermore, these biomaterials play role of reducing, capping, stabilizing and fabricating agents in green synthesis of nanoparticles. The review put forward a comprehensive report of various routes used for synthesizing IONP, biologically. Intuition into the procedures for synthesis of nanoparticles will help to nourish our learning in the area of nanotechnology.

Keywords: antimicrobial, biological synthesis, characterization, green approach, iron oxide, nanoparticles

INTRODUCTION

Nanotechnology is propitious and widely flourishing branch of science that has received major success in the era of modern technology. Nanoparticles are materials that shows uniqueness in size (generally varies from 1 to 100 nm), structure, physio-chemical, electric, magnetic, thermal, mechanical, catalytic, optical scattering properties and shape (Chen et al., 2013; Rastar et al., 2013; Pantidos and Horsfall, 2014; Arya et al., 2019; Hussain et al., 2019; Saleh et al., 2019; Turan et al., 2019; Vasantharaj et al., 2019). Properties and reactivity of the nanoparticles is largely defined by their ultra-small size and large surface area (Campos et al., 2015; Srikar et al., 2016; Mourdikoudis et al., 2018). Nanoparticles are highly intriguing as they exist as transition between bulk matters and atomic or molecular structures. A bulk material has constant physical and chemical properties regardless of its size, but at the nano-level this scenario is often different. Several bulk materials have demonstrated astonishing features when studied at nano scale (Thakkar et al., 2010). As far as structure of the nanoparticles is concerned, a greet degree of complexity is observed. They consist of three layers i.e., the surface layer, the shell and the core. Each layer differs from other in functional groups like metal ions, surfactants, small molecules and polymers. Core is generally represented as the nanoparticles (Khan et al., 2019). The properties like size, shape, composition and structural framework are specific to nanoparticles and need to be optimized during the synthesis process (Revati et al., 2011). Based upon their distinct characteristics, NPs are categorized into different

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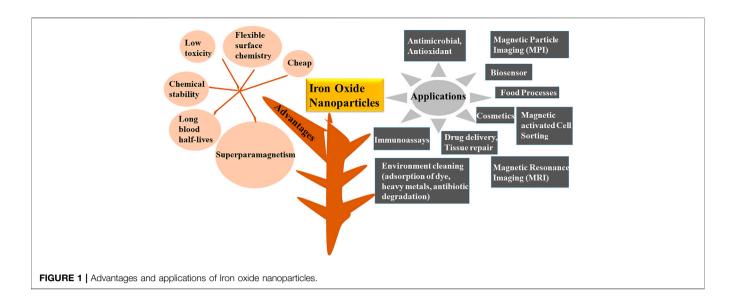
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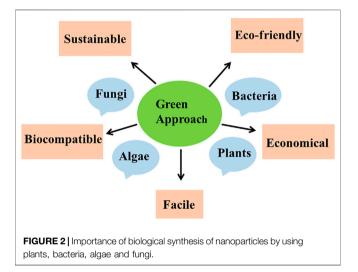
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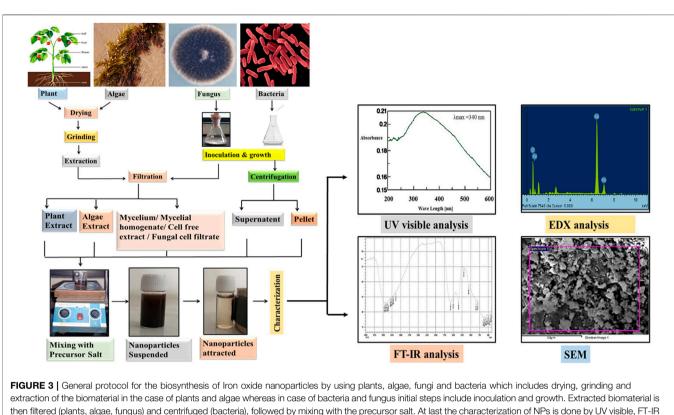
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classes including-carbon and lipid based, metal, ceramics, semiconductor and polymeric (Bhatia, 2016; Ealias and Saravanakumar, 2017; Amoabediny et al., 2018; Khan et al., 2019). Different metal oxides like iron oxide, nickel oxide, zinc oxide, copper oxide, silver oxide, titanium dioxide, tin oxide, tungsten (di, tri) oxide, magnesium oxide, silicon and gold oxides have broad applications in the environment (detection of toxins and pollutants, remediation, photo degradation, water treatment), catalysis, textile industry, electronics (batteries, optical limiting devices, gas sensor), mechanical industries, pharmaceutical sector (cancer therapy, drug delivery, tissue repair) and energy scavenging (nanogenerators) (Oskam, 2006; Laurent et al., 2008; Kulkarni and Muddapur, 2014; Kumar et al.,2018; Mei and Wu, 2018; Odularu, 2018; Aminabad et al., 2019; Khan et al., 2019; Khan et al., 2019; Malakootian et al., 2019; Massironi et al., 2019; Saleh et al., 2019; Shnoudeh et al., 2019; Vahed et al., 2019; Vasantharaj et al., 2019; Vinci and Rapa, 2019; Yusof et al., 2019; Borah et al., 2020; Hernández et al., 2020; Vahidi et al., 2020; Mughal et al., 2021). Out of these metal oxides nanoparticles, one of the preeminent biocompatible nanoparticles are iron oxides as they have magnificent minuscule physical characteristics like superparamagnetism, firmness in liquid solution, low suseptibility to oxidation, long blood half-lives, flexible surface chemistry with wide range of applications in environmental regulation like antibiotic degradation, adsorption of dyes, food related processes, biomedical (drug delivery, magnetic cell sorting, magnetic resonance imaging (MRI), magnetic particle imaging (MPI), immunoassays, tissue engineering, stem cell tracking, hyperthermia treatment of cancer), bioengineering, cosmetics and bio sensing along with antimicrobial activity against various pathogens like fungus, bacteria and ROS (Figure 1) (Khalil et al., 2017; Beheshtkhoo et al., 2018; Sruthi et al., 2019; Vasantharaj et al., 2019; Bhuiyan et al., 2020; Gao et al., 2020; Malhotra et al., 2020; Mondal et al., 2020; Sharma et al., 2021; Tong et al., 2021). Iron oxide nanoparticles (IONPs) also demonstrates typical core and shell structure. Owing to this core-



shell structure IONPs possess characteristics of both hydrous iron oxides and metallic iron (Li and Zhang, 2006). Recently, these are also defined as "Nanozyme" for their native enzyme like activities. They posess catalytic properties similar to various oxidases like peroxidases, catalase, sulfite oxidase and superoxide dismutase which could make them a new enzyme mimics (Zhang et al., 2019; Gao et al., 2020). One another astonishing feature of these particles is their potential to join together with different biological molecules like peptides, enzymes, nucleic acids, lipids, fatty acids and various metabolites. Molecules immobilized on the nanoparticles demonstrate superior properties and high reusuability over the unimmobilized counterpart (Singh et al., 2016; Jubran et al., 2020). Different chemical, physical and biological methods have been employed for the synthesis of IONPs. Chemical synthesis generally comprises of coprecipitation, micro-emulsion techniques, flow-injection, reverse micelles, sol-gel synthesis and hydrothermal reactions (Laurent et al., 2008; Wu et al., 2008). In these kinds of processes,



then filtered (plants, algae, fungus) and centrifugues spectroscopy, EDX and SEM techniques.

particle dispersal, clumps and size equalization is the main obstacle. Besides, chemical based methods also includes the utilization of solvents like sodium borohydride, hydrazine, potassium bitartrate and sodium dodecyl sulfate, these all are harmful to the environment due to the generation of noxious waste flows. Furthermore, physical genesis includes milling, grinding and thermal ablation; all these methods are hourly costly due to the thorough energy requirement. Another big disadvantage of this approach is its extremely low output yield (Rauwel et al., 2015; Moghaddam et al., 2014; Khalil et al., 2017; Gahlawat and Choudhury, 2019; Borah et al., 2020). It has been cleared that there is a need to shift toward green, safer, biologically acceptable, stable, cost effective, fast and ecofriendly method of synthesis (Figure 2) (Salam et al., 2012; Senthilkumar and Sivakumar, 2014; Aziz et al., 2015; Adelere and Lateef, 2016; Ovais et al., 2016). Biological biosynthesis of iron oxide nanoparticles is based upon the usage of living biota like plants, bacteria, algae, actinomycetes, viruses and fungi (Thakkar et al., 2010; Schröfelet al., 2014; Vijayaraghavan and Ashokkumar, 2017; Mirza et al., 2018; Latif et al., 2019; Gahlawat and Choudhury, 2019; Sharma et al., 2019; Vasantharaj et al., 2019). This organic synthesis depends on the usage of universal solvent i.e., water and generates nanoparticles that are free from venomous chemical impurities and made them widely accepted in biomedical field (Pantidos and Horsfall, 2014; Gholampoor et al., 2015). Bioreduction and biosorption are the two basic steps followed by natural synthesis. Bioreduction is the process in

which metal ions are reduced chemically into their stable forms and biosorption refers to the process of binding of metal ions on the surface of organism like cell wall, peptides to form more stabilized complexes (Pantidos and Horsfall, 2014). Moreover, biobased synthesis excludes subsidiary steps like capping or adhering of the biologically active compounds on their surface for producing stable particles. In addition, this type of synthesis processes are less time consuming as compared to physicochemical methods (Gahlawat and Choudhury, 2019). In this review we mainly emphasize on the biological synthesis of iron oxide nanoparticles by bacteria, fungi, algae and plants (**Figure 3**), along with their merits and demerits.

BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES USING PLANTS

Plants are generally considered as freely available, easy to handle, harmless and cheap material for synthesis of various types of nanoparticles (Noruzi, 2015). The biosynthesis process utilized distinct parts like roots, leaves, seeds, flowers, fruits, peels, petals, whole plant and seed husk as these are rich in various biomolecules like carbohydrates, amino acids, flavonoids, proteins, saponins, terpenoids, nitrogenous compounds that function as a reducer, stabilizer, redox mediators and capping agents in the synthesis of nanoparticles (Mittal et al., 2013; Royand Das, 2015; Rajesh Kumar and Bharath et al., 2017;

Mirza et al., 2018; Nadeem et al., 2018; Vasantharaj et al., 2019; Mondal et al., 2020). In current era, an extensive research is carried out on the synthesis of IONP by plants. One principal reason behind this can be the fact that bacteria and fungi demands lengthy incubations whereas phytochemicals present in plants are capable of reducing the metal ions in comparatively less time (Singh et al., 2018). Recent studies clarifies that plant extracts of Solanum trilobatum, Ziziphora tenuior, Persia Americana, Abutilon indicum, Azadirachta indica, Camellia sinensis and green tea leaves were utilized for the synthesis of IONPs of different size range (Khalil et al., 2017; Arularasu et al., 2018). Current reports also speculate that nanoparticles which are synthesized by using plant extracts are much more stabilized than the conventionally synthesized nanoparticles (Bibi et al., 2019) and are usually of distinct shapes like spherical, cubical, cylindrical, needles, stems, prisms and dendrites (Abdullah et al., 2020). The features of synthesized nanoparticles mainly depends on the discrete criterions like type of the plant extract, volume ratio of the extract and metallic salt solutions and reaction conditions (pH, temperature and incubation time) (Abdullah et al., 2020; Singh et al., 2018). So, on the basis of these highlighted parameters, different methods of synthesizing iron-oxide nanoparticles have been employed by researchers which vary slightly from one another in choice of starting material, method of plant extract preparation, selection of iron salt, presence or absence of NaOH, variable reaction condition and the method of collecting synthesized nanoparticles.

A captive work on the synthesis of Iron oxide NPs from leaves, fruits, seeds, seed coat, flowers, peels, petals or whole plant generally begins with the washing of starting material followed by extraction. As elaborated in Figure 3 the most common procedure involves, collection of plant parts either fresh, dried form from the market or from waste (in case of banana peels and pruned tea) and rinsing with tap water followed by their washing either with distilled or deionized water. Nextly, these washed plant parts are generally kept for drying at room temperature (Khalil et al., 2017; Beheshtkhoo et al., 2018; Lohrasbi et al., 2019) or in oven at 40–60°C for 1–4 h, (Majumder et al., 2019; Bhuiyan et al., 2020; Izadiyan et al., 2020; Ruiz et al., 2020), under shade (Razack et al., 2020), in sunlight (Arularasu et al., 2018) or air dried (Kanagasubbulakshmi and Kadirvelu, 2017; Karade et al., 2019; Jamzad and Bidkorpeh, 2020) succeeded by weighing. After this, the sample is grounded into fine powder by using pestle mortar/blender/willy mill/electric grinder (Khalil et al., 2017; Bibi et al., 2019; Hooda and Sharma, 2020; Izadiyan et al., 2020; Razack et al., 2020; Ting and Chin, 2020). Distilled water, double distilled water, methanol or ethanol: water (1:1) is added to the powder and the content is boiled under continuous stirring by using magnetic stirrer hot plate, water bath or heater mantle under reflux (Beheshtkhoo et al., 2018; Lohrasbi et al., 2019). An optional step of filtration using a cheese cloth was performed (before boiling) by some researchers (Ting and Chin, 2020). Boiling step was replaced by shaking for 120 min in the protocol used by Bibi et al. (2019). But in case of Gardenia resinifera and Kappaphycus alvarezii extraction, protocol shows slight variation as powdered form of leaves was soaked in methanol solution for two days at room temperature followed

by centrifugation at 1,000 rpm for 10 min whereas, in case of Kappaphycus alvarezii, powder was soaked in distilled water for 1 day before use. In other studies on Borassus flabellifer and Terminalia belerica extraction was carried out using soxhlet apparatus employing ethanol or distilled water (Akhter et al., 2019) as a solvent. The extraction mixture is finally filtered using Whatman Filter Paper, filter membrane (0.45 µm), nylon mesh, Millipore hydrophillic filter (0.22 µm) or under vacuum conditions. In some studies by Beheshtkhoo et al. (2018), Chauhan and Upadhyay (2019), Jamzad and Bidkorpeh(2020), Lohrasbi et al. (2019) and Razack et al. (2020) green synthesis of NPs by plants shows a supplementary step of centrifugation (2000 rpm for 5 min and 7,000 rpm for 15 min) of filtrate next to filtration. In Carica papaya (Bhuiyan et al., 2020) extract preparation, the filtrate obtained was concentrated using rotary evaporator. The clear solution obtained either after filtration or centrifugation was considered as a sample for synthesis of IONPs. Some volume of the extract is then mixed with precursor salt solutions (Iron Sulfate heptahydrate, FeCl₃.6H₂O (Fe NO₃)₃.9H₂O, FeSO₄, FeCl₃, FeCl₂.4H₂O, FeCl₂, FeSO₄.5H₂O) of different molarity as mentioned in Table 1. and finally mixture is heated (60-85°C range) or vigorously stirred for preferred time (30-60 min) and temperatures as in the protocol. Incubation of plant extract with the precursor salts changes the color of solution depending upon the type of iron salts used that visually intensifies with the production of nanoparticles. After the synthesis, some methods further proceed by continuous stirring over magnetic stirrer (Kanagasubbulakshmi and Kadirvelu, 2017; Arularasu et al., 2018; Beheshtkhoo et al., 2018; Akhter et al., 2019; Devi et al., 2019; Karpagavinayagam andVedhi, 2019; Lohrasbi et al., 2019; Majumder et al., 2019; Asghar et al., 2020; Bhuiyan et al., 2020; Hooda and Sharma, 2020; Izadivan et al., 2020; Jamzad and Bidkorpeh, 2020; Madubuonu et al., 2020; Periakaruppan et al., 2020; Razack et al., 2020; Rong et al., 2020; Ting and Chin, 2020), centrifugation step (Kanagasubbulakshmi and Kadirvelu, 2017; Kanagasubbulakshmi and Kadirvelu, 2017; Khalil et al., 2017; Arularasu et al., 2018; Beheshtkhoo et al., 2018; Bibi et al., 2019; Chauhan and Upadhyay, 2019; Devi et al., 2019; Karpagavinayagam andVedhi, 2019; Lohrasbi et al., 2019; Vasantharaj et al., 2019; Asghar et al., 2020; Bhuiyan et al., 2020; Hooda and Sharma, 2020; Izadiyan et al., 2020; Jamzad and Bidkorpeh, 2020; Periakaruppan et al., 2020; Razack et al., 2020; Rong et al., 2020; Ting and Chin, 2020) or magnetic decantation (Madubuonu et al., 2020; Ruiz et al., 2020) at preferred time, temperature and rpm. In Karade et al. (2019) method after mixing of extract with the precursor salt, 2 M sodium acetate salt was added at room temperature followed by heating at 80°C for 2 h. All the stirring and centrifugation steps are excluded in this protocol. The pellets of IONPs obtained are subjected for final washing with distilled water, ethanol, deionized water, methanol or double distilled water. The synthesis finally concludes with drying of the pellet (NPs) either in hot air oven, vacuum drying (Kanagasubbulakshmi and Kadirvelu, 2017), air dried (Karade et al., 2019), furnace (Bibi et al., 2019), vacuum hot air oven (Karpagavinayagam andVedhi, 2019) or in the room

TABLE 1 | Biosynthesis of Iron oxide nanoparticles by using plants.

Sr.No	Name of the plant	Bio-material used	Quantity	Iron precursor used	Size (nm)	Shape	Magnetization	Applications	References
1	Sageretia thea	Fine powdered fresh leaves	30 g in 200 ml distilled water	Iron sulfate heptahydrate	~29 nm	Tetragonal crystalline	-	Anticancer, antimicrobial, antileishmanias, cytotoxicity assay	Khalil et al. (2017)
2	Lagenaria siceraria	Air dried leaves	5 g in 100 ml double distilled water (boiling)	FeCl ₃ .6H ₂ O (0.01 M)	30–100 nm	Cube	-	Antimicrobial	Kanagasubbulakshmi and Kadirvelu (2017)
3	Daphne mezereum	Dried leaves	5% (w/v) i.e., 5 g in 100 ml deionized water	FeCl ₃ .6H ₂ O (0.1 M)	6.5–14.9 nm	Spherical	Paramagnetic	Dye removal	Beheshtkhoo et al. (2018)
4	Ruellia tuberose	Leaf extract	5 g in 100 ml distilled water	FeSO ₄ (1 M), 1 g NaOH	20–80 nm	Hexagonal rods	-	Photocatalytic, antibacterial	Vasantharaj et al. (2019)
5	Kappaphycus alvarezii	Dried whole plant	1 g in 25 ml of distilled water	FeCl ₂ .4 H ₂ O ,FeCl ₃ .6H ₂ O, glycine (amino acid) (C ₂ H ₅ NH ₂)	10–30 nm	Irregular or hexagonal	Ferromagnetic (M _s 18.89 emu/g,M _r 1.54 emu/g)	Photocatalytic, antibacterial	Arularasu et al. (2018)
6	Platanus orientalis	Dried leaves	5 g grinded dry leaves in 50 ml DDW	Fe(NO ₃) ₃ .9H ₂ O (99.8%)	38 nm	Spherical	-	Antifungal	Devi et al. (2019)
7	Avecinnia marina	Flowers	5 g in 100 ml millipore distilled water	Aqueous ferric chloride (1 m mol)	10–40 nm	Honeycomb like, smooth surface, non uniform	-	Methanol oxidation	Karpagavinayagam and Vedhi (2019)
8	Punica granatum	Seeds	20 g in 250 ml of distilled water	Iron chloride (1 M)	25–55 nm	Variable, semi- spherical (aggregated)	-	Photocatalytic	Bibi et al. (2019)
9	Plantago major	Aqueous leaf extract	5 g dried leaves in 100 ml deionized water	FeCl ₃ .6H ₂ O (0.1 M)	4.6–30.6 nm	Spherical	Paramagnetic	Dye removal	Lohrasbi et al. (2019)
10	Lawsonia inermis	Powdered plant	5 g powder in 50 ml deionized water	FeSO4·7H20 (0.02 M), NaOH (1 mol/L)	150–200 nm	Spherical	-	Antimicrobial	Chauhan and Upadhyay (2019)
11	Terminalia belerica	Fruits	10.5 g herb powder in 300 ml water	Ferrous chloride (0.1 M)	15–23 nm	Spherical	-	Antibacterial	Akhter et al. (2019)
12	Musa paradisiaca	Powdered waste banana peels (dried)	-	FeCl ₃ (0.1 M)	60 nm	Cylindrical	-	Arsenic remediation	Majumder et al. (2019)
13	Gardenia resinifera	Powdered fresh leaves	10 g powder in methanol	FeCl ₃ .6H ₂ O (0.4 mM), sodium acetate (2 M)	3–8 nm	Spherical	Superparamagnetic	Cytotoxic assay	Karade et al. (2019)
14	Borassus flabellifer	Seed coat	30 g crushed seeds in 250 ml ethanol	Ferric chloride (0.2 M) and ferrous sulfate (0.1 M) (2:1), NaOH (0.1 M)	30–200 nm	Hexagonal		Antimicrobial, antioxidant, cytotoxic assay	
15	Hibiscus rosa- sinensis	Dried petals	2 g petals powder in 100 ml distilled water	Ferric choride (25 mM) and ferrous chloride (25 mM) (2:1)	65 nm	Spinel	-	Biscuits fortification	Razack et al. (2020)
16	Carica papaya	Dried leaves	20 g fine powder in 1 L deionized water (boiling)	FeCl ₃ .6H ₂ O (0.1 M), NaOH (1 M)	2.159 nm	Not uniform (agglomerated particles)	-	Antibacterial	Bhuiyan et al. (2020)
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TABLE 1 | (Continued) Biosynthesis of Iron oxide nanoparticles by using plants.

Sr.No	Name of the plant	Bio-material used	Quantity	Iron precursor used	Size (nm)	Shape	Magnetization	Applications	References
17	Psidium guajava	Fresh dried leaves	10 g crushed leaves in 100 ml distilled water	FeCl ₃ .6H ₂ O	1–5 nm	Spherical	Superparamagnetic	Antibacterial	Madubuonu et al. (2020)
18	Citrus	Fresh leaves	25 g leaves in 250 ml DW	Iron chloride (0.1 mM)	15–80 nm	Spherical	-	Antibacterial	Hooda and Sharma (2020)
19	<i>Malus pumila</i> (apple)	Peels	75 g peels in 300 ml mili- Q H ₂ O	FeCl ₂ .4H ₂ O (20 mM) FeCl ₃ .6 H ₂ O (40 mM), NaOH (1 M)	50–100 nm	Elliptical and spherical	-	Decolorization of dye	Ting and Chin (2020)
20	Camellia sinensis L	Grinded waste of pruned teas	10 g of pruned tea in 100 ml deionized water	FeSO ₄ .5H ₂ O (0.1 M), NaOH (0.5 M)	28.5 nm	Regular spherical	Superparamagnetic	Antioxidant	Periakaruppan et al. (2020)
21	Syzygium cumini	Leaves	10 g chopped leaves in 100 ml deionized water	FeCl ₃ (0.010 mol/L)	40–52 nm	Spherical	-	Antibacterial, antifungal, aflatoxin B ₁ adsorption	Asghar et al. (2020)
22	Juglans regia	Dried green husk	1 g green husk in 100 ml boiling water	FeCl ₃ .6H ₂ O (97%), FeCl ₂ .4H ₂ O (99%), NaOH (2 M)	12.6 nm	Cubic	-	Cytotoxic assay	Izadiyan et al. (2020)
23	Pyrus sinkiangensis Yu	Peels	27 g peels in 360 ml ethanol and water mixture (1:1)	FeSO ₄ .7H ₂ O (0.1 M)	10–90 nm	Irregulary shaped	-	Cr (IV) removal	Rong et al. (2020)
24	Cymbopogon citratus	Powdered leaves	100 g powder in 800 ml DW	FeCl ₃ .6H ₂ O (0.26 M and 0.52), Na ₂ CO ₃	9 ± 4 nm	Irregular cubic	Superparamagnetic (M _s = 28emu/g)	Nanotoxicological	Ruiz et al. (2020)
25	Laurus nobilis L	Leaves	20 g leaves in 200 ml DW	FeCl ₃ .6H ₂ O (0.1 M)	8.03 ± 8.99 nm	Spherical	-	Antimicrobial	Jamzad and Bidkorpeh (2020)

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(Devi et al., 2019) at desired temperature conditions and time. Extra step of annealing at 500°C for 2 h was done after drying of NPs (Khalil et al., 2017; Arularasu et al., 2018) but in Razack et al. (2020) biosynthesis method dried iron oxide nanoparticles are further treated for calcination in muffle furnace at 400°C for 3 h. In the protocol followed by Chauhan and Upadhyay (2019) for the synthesis of IONPs from Lawsonia inermis (whole plant extract), nanoparticles obtained were capped with tyrosine by dispersing the NPs, in the tyrosine solution for 2 h at 4°C with continuous stirring followed by washing with deionized water and methanol before the final drying of the NPs. The synthesized nanoparticles are functionally characterized by using different techniques. Preliminary characterization is done by using UV-VIS spectrophotometry. Further analysis of NPs is performed by Fourier Transform Infrared Spectroscopy (FTIR) (surface functional groups detection), X ray diffractometer (for crystal size determination based on Debye-Scherrer's equation), Energy-dispersive X ray spectrometer (EDS) (for determining elemental composition), High-resolution Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (HRTEM) (for size and shape determination), Thermal—gravimetric differential thermal analysis (for estimating the incorporation of bioactive components of the plants extract by studying the % weight loss of the nanoparticles by giving heat treatment (30-800°C), Zeta Potential analysis (helps in elucidating surface properties) and most important characteristics i.e., magnetization (Ferromagnetic, Paramagnetic and Superparamagnetic) is studied by using Vibrating Sample Magnetometer (VSM) by providing magnetic field. The brief information gathered from various recent reports is summarized in the tabular form (Table 1) below.

BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES USING FUNGI

Extracellular synthesis of iron oxide nanoparticles by using fungal species is considered to be advantageous bearing in mind the ease of scale up, use of economical raw materials for growth, high biomass forming capacity, simple downstreaming steps, low toxicity of residue and economic feasibility (Tarafdar and Ralia, 2013; Guilger-Casagrande and Lima, 2019; Chatterjee et al., 2020). In addition to this, fungal species exhibit superior tolerance and bioaccumulation property which aid the synthesis of metal nanoparticles (Agarwal et al., 2017). The interactions between microorganisms and metals have been well studied (Beveridge et al., 1996) and this capability of microbes has been employed to accumulate and/or extract metals in various biological processes such as bioleaching, heavy metal removal and bioremediation (Gericke and Pinches, 2006). Fungi are known to secrete large amount of extracellular enzymes which are competent of hydrolyzing metals. This enzymatic reduction of metal ions is predicted to be responsible for the synthesis of various extracellularly synthesized nanoparticles. One such enzyme found in fungi is nitrate reductase (Abdeen et al., 2016). Although, the overall mechanisms of Metal

nanoparticles (MNPs) synthesis using fungi are not completely understood. The MNPs synthesis by fungi is considered to be relied on the biological material used and the applied reaction conditions. Biological material includes the strain of microorganism used, the growth parameters and method of sample preparation whereas the reaction conditions stress on metal precursor used, concentration of metal precursor, pH, temperature of reaction (Silva et al., 2015). While reviewing the literature one would come across a great degree of variability with regard to the starting bio-material used for biosynthesis. Some methods used fungal biomass to synthesize the nanoparticles, others used the cell free filtrate of the fungal culture or fungal biomass homogenate whereas some have employed fungal cell filtrate (Figure 3). The most widely employed method involved growing the fungi in appropriate growth medium followed by the separation of fungal mycelia by centrifugation. The mycelia thus obtained is mixed with a metal precursor salt and incubated at 28°C for 4-5 days. The further filtration of this solution gives a suspension containing synthesized nanoparticles (Kaul et al., 2012; Bhargava et al., 2013). In another method the fungi are cultured in a suitable media and the mycelia is separated by filtration. After thorough washing with sterile distilled water the fungal biomass is dispensed in sterile distilled water and incubated on rotary shaker for 1-5 days. Upon incubation, cell free filtrate (CFF) is collected by filtering out mycelium with the aid of Whatman filter paper 1. and obtained cell free filtrate is treated with iron precursor salt mixture comprising ferric chloride (FeCl₃.6H₂O) and ferrous sulfate (FeSO₄. 7H₂O) in 2:1 M ratio (Chatterjee et al., 2020), FeCl₃(10⁻³ M) (Tarafdar and Ralia, 2013) and incubated again on the rotary shaker for few h to achieve the synthesis of IONPs. Another method used by Abdeen et al. (2016) represents a blend of microbial and physical process for the synthesis of iron oxide nanoparticles. In this method a fine homogenate of fungal mycelium was prepared in distilled water using a cyclomixer. To this homogenate metal precursor salt FeCl₃ or FeSO₄ at the concentration of 2000 ppm was added. The resulting mixture is incubated for 6 days under static condition. The synthesized IONP's were collected by centrifugation and were washed thoroughly with ethanol. The further drying of the nanoparticles was achieved with the help of a supercritical pressure reactor at temperature of 300°C and 850 psi pressure. In one more dramatic variation, fungal cell filtrate (FCF) was used for the synthesis of iron oxide nanoparticles. In this method the selected fungal strain was cultured in the appropriate medium and on the completion of incubation period the culture medium is filtered through Whatman filter paper 1. using a vacuum pump. The filtrate thus obtained is centrifuged and re-filtered to obtain the final fungal cell filtrate (FCF). Equal volume of FCF and solution containing ferric trichloride (FeCl₃) and ferric dichloride (FeCl₂) salt solution (2:1 mM final concentration) are agitated at room temperature for just 5 min. An immediate visual change in color demonstrates the synthesis of IONP's (Mahanty et al., 2019). This seems to be the simplest method for the synthesis of IONPs by fungus, surpassing the need of tedious filtration procedures and expensive equipments. In one such study, Iron oxide nanoparticles were synthesized from the cell free extract of

Sr.No	Fungal strain	Bio- material used	Iron precursor used	Size (nm)	Shape	Magnetization	Applications	References
1	Fusarium oxysporum	Fungal biomass	K_3 [Fe(CN) ₆] and K_4 [Fe(CN) ₆] (2:1 mM)	20–50 nm	Quasi- spherical	Ferrimagnetic	-	Bharde et al. (2006)
2	Verticillium sp	Fungal biomass	K ₃ [Fe(CN) ₆] and K ₄ [Fe(CN) ₆] (2:1 mM)	100–400 nm	Cubo- octahedrally	Ferrimagnetic	-	Bharde et al. (2006)
3	Curvularia lunata	Fungal biomass	Fe ₂ O ₃ (1,000 ppm)	20.7 nm	-	-	-	Kaul et al. (2012)
4	Chaetomium globosum	Fungal biomass	Fe ₂ O ₃ (1,000 ppm)	25 nm	-	-	-	Kaul et al. (2012)
5	A. fumigatus	Fungal biomass	Fe ₂ O ₃ (1,000 ppm)	42.4 nm	-	-	-	Kaul et al. (2012)
6	A. wentii	Fungal biomass	Fe ₂ O ₃ (1,000 ppm)	46 nm	-	-	-	Kaul et al. (2012)
7	<i>Aspergillus</i> oryzae TFR9	Cell free filtrate	FeCl ₃ (1 mM)	10–24.6 nm	Spherical	-	-	Tarafdar and Ralia (2013)
8	Aspergillus japonicus	Fungal biomass	Potassium ferricyanide K_3 [Fe(CN) ₆] and potassium ferrocyanide K_4 [Fe(CN) ₆] (1 mM: 0.5 mM)	60–70 nm	Cubic	-	-	Bhargava et al. (2013)
9	Aspergillus sp	Fungal biomass	FeSO ₄ (3.0 mM)	5–200 nm	Spherical	-	-	Pavani and Kumar (2013)
10	Alterneria alternata	Fungal biomass	Iron (III) nitrate (1 mM)	9 nm	Spherical	-	Antibacterial	Mohamed et al. (2015)
11	Aspergillus niger YESM	Fungal homogenate	FeCl _{3,} FeSO ₄ (2000ppm each)	18 nm for Fe and 50 nm for Fe ₃ O₄	Spherical	Superparamagnetic for Fe and ferromagnetic-like for Fe ₃ O ₄	-	Abdeen et al. (2016)
12	Trichoderma asperellum	Fungal cell filtrate	FeCl ₂ :FeCl ₃ (1:2 mM)	18–32 nm	Spherical	-	-	Mahanty et al. (2019)
13	Phialemoniopsis ocularis	Fungal cell filtrate	FeCl ₂ :FeCl ₃ (1:2 mM)	6–22 nm	Spherical	-	-	Mahanty et al. (2019)
14	Fusarium incarnatum	Fungal cell filtrate	FeCl ₂ :FeCl ₃ (1:2 mM)	15–55 nm	Spherical	-	-	Mahanty et al. (2019)
15	Aspergillus niger BSC-1	Cell free filtrate	FeCl ₃ . 6H ₂ O) and ferrous sulfate (FeSO ₄ . 7H ₂ O) in 2 mM:1 Mm	20–40 nm	Orthorhombic	Superparamagnetic	Cr (VI) removal	Chatterjee et al. (2020)

TABLE 2 | Biosynthesis of Iron oxide nanoparticles by using fungi.

A. niger BSC-1. XRD analysis indicates crystalline orthorhombic structure of magnetite nanoparticles. The width of the IONPs determined using TEM was found to be in between 20-40 nm and the average size of 17.29 nm was calculated Debye-Scherrer equation. The particles exhibited superparamagnetic behavior with 55.19 emu/g of saturation magnetization, 0.0045 Oe of coercivity and 1.61 emu/g of retentivity (Chatterjee et al., 2020). Nanoparticles synthesized by A. oryzae TFR9 had a sized range between 10 and 24.6 nm from DLS analysis. A TEM micrograph depicts the formation of spherical IONPs (Tarafdar and Ralia, 2013). The nanoparticles synthesized by mycelia homogenate of Aspergillus niger YESM under supercritical condition of ethanol yielded pure iron and major proportion of Fe₃O₄ spherical nanoparticles. The average size determined from the SEM analysis is 18 and 50 nm for iron and Fe₃O₄ nanoparticles, respectively. The measurement of magnetic properties reveals superparamagnetic and ferromagnetic-like behaviors for Fe and Fe₃O₄ nanoparticles, respectively (Abdeen et al., 2016). Concise information about the different reports encountered for the synthesis of IONP's by fungus till date can be obtained from the following Table 2.

BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES USING BACTERIA

Prokaryotic system has also been studied extensively as a key model in the field of nanotechnology due to their ubiquitous presence, fast doubling time, potential to grow under adverse conditions, inexpensive and simple media for cultivation (Pantidos and Horsfall, 2014; Fariq et al., 2017). This system has been considered as a best way for synthesis of nanoparticles of various shapes, size distribution, structural framework, physical and chemical properties by reducing metal ions (Gahlawat and Choudhury, 2019). Microorganisms cumulates and detoxicate metals by the activity of reductase enzyme, so this property has been exploited for synthesizing metallic nanoparticles by using salts as a reaction precursors (Singh et al., 2016; Alam et al., 2020). Reduction of metal ions is a crucial step in the synthesis process. It is under the control of numerous factors like functional groups on the cell wall (required for bio mineralization), strain type, environmental conditions including pH, temperature, growth medium, concentration of salts. All the above discussed factors directly or indirectly affect the size, shape and composition and

yield of nanoparticles (Saratale et al., 2018; Gahlawat and Choudhury, 2019; Iravani and Varma, 2020). Among them, temperature, pH and incubation time for the microbial culture are the paramount factors, as this green approach of synthesis is regulated by the enzymatic system for reduction of metal ions (NADH reductase), so biosynthesis depends upon optimum conditions. High temperature and pH completely inactivates the enzymes and halts their action, in turn, slows down the nanoparticles formation (Sundaram et al., 2012; Garg et al., 2020; Jubran et al., 2020). Besides enzymes, proteins, peptides, cofactors and organic molecules also contributes in the synthesis process as stabilizer, capping and reducing agents (Singh et al., 2016). A sequence of reports on the biosynthesis of nanoparticles indicates the deployment of cytoplasmic extracts, supernatant, extracellular polysaccharides as a reduction activator molecules (Singh et al., 2016). Generally, two distinct mechanisms i.e., intracellular and extracellular has been explored by bacteria for NPs synthesis (Javaid et al., 2018; Gahlawatand Choudhury, 2019; Grasso et al., 2020; Tsekhmistrenko et al., 2020). Intracellular synthesis is based on the ion transportation in the microbial cells. The cell wall is the main player in this process as the enzymes present in the cell wall are involved in the metal reduction whereas extracellular process is based on the aggregation of metal ions on the surface of microbial cells via electrostatic interaction between the ions and negatively charged cell wall enzymes and soluble enzymes secreted by microbes (Narayanan and Sakthivel, 2010; Hulkoti and Taranath, 2014; Punjabi et al., 2018; Singh et al., 2018; Mughal et al., 2021). Extracellular methods has achieved major success than intracellular protocols as former synthesis excludes downstream processing, centrifugation, sonication and washing steps for the recovery of product and make the process fairly cost effective (Singh et al., 2016). Thus, high—efficiency and lucrative extracellular method has more industrial applications due to easy extraction process (Srivastava and Constanti, 2012). Bacterial system generally consists of distinct defense mechanisms like extracellular precipitation, intracellular segregation, and efflux pumps to withstand with all environmental conditions. This property helps in the production of NPs with wide applications like antimicrobial, anti biofilm, anticancer and environment cleaning (dye degradation, pollutants (Cd) removal (Fang et al., 2019; Gahlawat and Choudhury, 2019).

Manv reports indicates that Actinobacter sp., Thermoanaerobacter Bacillus subtilis, Thiobacillus sp., thioparus, Aquaspirillum magnetotacticum, Magnetotactic bacterium MV-1, Magnetospirillum magnetotacticum, M. magnetotacticum (MS-1), M. gryphiswaldense, Geobacter metallireducens GS-15, Thermophilicbacteria TOR-39, Microbacterium hominis, Bacillus licheniformis, Lactobacillus casei and Lactobacillus fermentum are the bacterial strains that has been used for both intracellular and extracellular synthesis of iron oxide nanoparticles (Gholampoor et al., 2015; Saif et al., 2016; Singh et al., 2016). Many extracellular enzymes perform the function of electron shuttle system for reducing metal ions. Research shows that some bacteria like Geothrix fermentans, Mycobacterium paratuberculosis and Shewanella oneidensi reduces Fe³⁺ ions in a diffusible manner by secreting some

redox compounds that act as an electron shuttle between the host and substrate (Sundaram et al., 2012). Hence, this route of synthesis is clean and economically acceptable. Current review will focus mainly on the extracellular synthesis of iron oxide nanoparticles by bacterial system.

Iron oxide nanoparticles synthesis generally begins with the selection of bacteria (from soil, lyophilized culture). Isolation step includes growing the sample on the media like BH1 agar (Sundaram et al., 2012), Blood agar (Jubran et al., 2020) or nutrient agar (Fatemi et al., 2018) followed by their incubation under controlled conditions at 35-37°C for 24-48 h and in case of blood agar medium incubation is carried out under controlled conditions of carbon dioxide. After the achievement of sufficient growth single colony is transferred to the nutrient medium, such as Luria broth, Nutrient broth or MRS broth (Sundaram et al., 2012; Fani et al., 2018; Fatemi et al., 2018; Torabian et al., 2018; Jubran et al., 2020) followed by incubation and centrifugation to pellet out the bacterial biomass. After centrifugation most critical step of IONPs synthesis initiates by taking either supernatant (Sundaram et al., 2012; Fatemi et al., 2018; Jubran et al., 2020) or cell pellet (Fani et al., 2018; Torabian et al., 2018). The protocol after using pellet (dissolved in Phosphate Buffer Saline) required additional step of centrifugation at 3000 g for 10 min and washing including short incubation in liquid nitrogen (-196°C) for 5 min, followed by subjecting the cells to a brief heat shock by steam bathing the cell pellet at 37°C for 15 min. Nextly, centrifugation is done at 12000g for 30 min and the supernatant is collected as cytoplasmic extract (Fani et al., 2018; Torabian et al., 2018). In Kaul et al. (2012), protocol, pellets are dissolved in sterile distilled water. The supernatant and suspension of pellet is mixed with the precursor salts (Fe₂O₃, FeSO₄, FeCl₃.6H₂O) in the desired volume. The optimization of the reaction conditions is done by adjusting the pH in the preferred range (5-9). Lastly incubation of the mixture solution is done at different temperature conditions depending upon the strain of bacteria used. After the incubation initial indication of the nanoparticles is observed from the color change i.e., brick red to dark brown (Sundaram et al., 2012), colorless to black (Fani et al., 2018; Torabian et al., 2018), golden yellow to turbid brown (Fatemi et al., 2018) and dark green to blackish brown (Jubran et al., 2020) and the synthesized IONPs are dried and characterized. The synthesis of nanoparticles by Lactobacillus casei and Lactobacillus fermentum extract is little bit modified as there is inclusion of 10 min centrifugation at 2500 g to separate the synthesized nanoparticles followed by drying the pellet of NPs in the oven at 40°C for 24 h (Fani et al., 2018; Torabian et al., 2018). According to the protocol followed by Fatemi et al. (2018), the synthesized IONPs were subjected to the filtration using Whatman Filter paper No.1 and 0.2 µm syringe filters. The filtrate consisting NPs was then concentrated by using rotary balloon at 20-30 rpm having steam bath temperature of 60°C and circulator temperature of 15-10°C. Finally, the powder of nanoparticles were dried inside the laminar air flow under sterile conditions. In general, Lactic acid bacteria (LAB) are considered as an attractive microbial source of various nanoparticles as they are non-pathogenic and produce wide array of enzymes (Yusof et al., 2019). Moreover, the LAB are

Sr.No	Bacteria	Salt	Size (nm)	Shape	Applications	References
1	Bacillus subtilis	Fe ₂ O ₃ (2 mM)	60–80 nm	Spherical	-	Sundaram et al. (2012)
2	Alcaligens faecalis	a. Fe ₂ O ₃	a. 12 nm			Kaul et al. (2012)
		b. FeSO ₄ (1,000 ppm)	b. 43.6 nm			
3	Lactobacillus casei	Iron sulfate (10 ⁻³ M)	10–15 nm	Spherical	-	Torabian et al. (2018)
4	Lactobacillus fermentum	Iron sulfate (10 ⁻³ M)	10–15 nm	Spherical	-	Fani et al. (2018)
5	Bacillus cereus (HMH1)	FeCl ₃ .6H ₂ O	18.8–28.3 nm	Spherical	Cytotoxicity assay	Fatemi et al. (2018)
6	Bacillus subtilis	FeCl ₃ (2 mM)	12–32 nm	Spherical	-	Jubran et al. (2020)

TABLE 3 | Biosynthesis of Iron oxide nanoparticles by using bacteria.

facultative anaerobic bacteria and possess high negative electro kinetic potential. Due to this, the LAB got attracted toward various metal ions under both oxidizing and reducing conditions which aids the synthesis of metal and metal oxide nanoparticles (Prasad and Jha, 2009; Królet al., 2018). In addition to this, being a Gram-positive bacteria, the cell wall consists of peptidoglycan, proteins, polysaccharides, teichoic and lipoteichoic acid which can serve as a site for biosorption and bioreduction of metal ions (Chapot-Chartier and Kulakauskas, 2014).

The most crucial step is now the characterization of NPs by different techniques like X-ray diffraction (determine the particle size of the particles by using Sherrer's equation i.e., $D = k\lambda/\beta \cos \theta$), FT-IR spectroscopy (functional group analysis) and SEM, TEM analysis for depicting the morphology of the particles. Scanning and Transmission electron microscopy highlights that nanoparticles are spherical in shape and size falls in the 10–80 nm range. **Table 3** illustrates the characteristics of iron oxide nanoparticles synthesized by different bacterial strains.

BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES USING ALGAE

Algae is defined as photosynthetic organisms without the structure of roots and leaves. Microalgae (unicellular) and macroalgae or seaweeds (multicellular) both are extensively used in the nanotechnology field for the synthesis of distinct types of metallic (gold, silver, palladium, iron and copper) nanoparticles (Subramaniyam et al., 2016; Agarwal et al., 2017). Like plants and bacteria algal bodies are also the factory of different biomolecules like proteins, fat, carbohydrates, cell peptides, alkaloids, terpenes, macrolides, wall polysaccharides, glycoproteins (comprised of various functional groups like carbonyl, hydroxyl, carboxyl, sulfonate) and enzymes that plays a pivotal role in reduction, capping, fabrication and stabilization of nanoparticles (Siddiqi and Husen, 2016; Subramaniyam et al., 2016). Hence this approach is considered as safe, simple, cost effective and pollution free for nanoparticles production.

A significant work for the synthesis of IONPs initiates by the collection of sample. Similarly in case of algae the seaweeds are firstly washed with distilled water, followed by their drying (Salem et al., 2019; Siji et al., 2018). But in Yew et al. (2016) protocol the bleaching step is performed immediately after washing and then sample is dried under sunlight for 72 h.

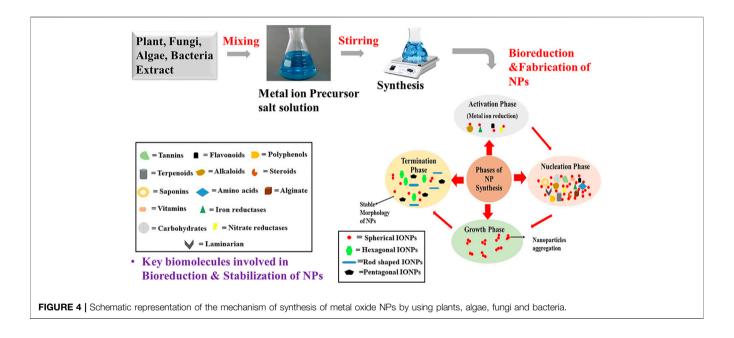
Secondly, the samples are grinded, freeze dried (Mahdavi et al., 2013), weighed, mixed with desired volume of distilled water and boiled (Mahdavi et al., 2013; Salem et al., 2019; Siji et al., 2018). In Yew et al. (2016) protocol boiling step is replaced by soaking of dried biomaterial in deionized water for 1day. The extract obtained after boiling is subjected to filtration step and filtrate is labeled as bioextract for further synthesis steps. The extract is then mixed with precursor salts (FeCl₃.6H₂O, FeCl₂.4H₂O or FeCl₃) of different molarity as mentioned in Table 4. The mixture is then continuously stirred (Mahdavi et al., 2013; Yew et al., 2016; Salem et al., 2019) followed by centrifugation (Mahdavi et al., 2013; Salem et al., 2019), washing and drying. Finally the NPs are characterized by using FT-IR, XRD, SEM, TEM and VSM. All these techniques helps in elucidating the functional groups, crystal structure, size, shape and the magnetic characteristics of synthesized nanoparticles. The information about the nanoparticles obtained from various algal extracts are highlighted in Table 4.

MECHANISM OF SYNTHESIS OF IONPS BY PLANTS, FUNGI, BACTERIA AND ALGAE

Biological entities like plants, bacteria, algae and fungi have been extensively used in the synthesis of metal oxide nanoparticles (Ishak et al., 2019). These biological sources contains diverse biomolecules like alkaloids, saponins, tanins, flavonoids, proteins, carbohydrates, steroids, enzymes (NADPH dependent nitrate reductases, iron reductases), amino acids, cell wall components (alginate, laminarian), exopolysaccharides (EPS) that helps in the bioreduction, stabilization and capping of the nanoparticles (Kumar et al., 2007; Ishak et al., 2019; Jacinto et al., 2020; AlKhattaf, 2021). Underlying mechanism of bioreduction of metal ions by these key biomolecules is still not confirmed but from scientific reports it has been assumed that surface chemistry (functional groups like -CHO, -C=O,-COOH, -OH, -C-O-C, -C=C) of these reducing agents plays an important role in the reduction of metal ions. Bioreduction generally comprises of four phases-activation phase, nucleation phase, growth phase and termination phase (Figure 4). The first phase, activation phase is the slow growth phase of nanoparticles as it includes the reduction of metal ions from the precursor salt solution with the help of biomolecules present in the extract. Secondly, in the nucleation phase there is growth of the crystal on the metal nuclei with the simultaneous reduction by biometabolites. Reducing agents (like flavonoid (quercetin) adsorbed on the surface of

TABLE 4 | Biosynthesis of IronOxide nanoparticles by using algae.

Sr No	Algae	Bio-material used	Iron precursor salt	Size (nm)	Shape	Magnetization	Applications	References
1	Sargassum muticum (brown seaweed)	Freeze-dried powder	FeCl ₃ .6H ₂ O (0.1 M)	18 ± 4 nm	Cubic	Superparamagnetic (M _s 22.1 emu/g)	-	Mahdavi et al. (2013)
2	<i>Kappaphycus alvarezii</i> (red seaweed)	Dried, grinded powder	FeCl ₂ .4H ₂ O, FeCl ₃ .6H ₂ O, NaOH (1 M)	14.7 nm	Spherical	-	-	Yew et al. (2016)
3	Chaetomorphaantennina (green algae)	Cell free extract	FeCl ₂ .4H ₂ O, NaOH	9–10 nm	-	Ferromagnetic	-	Siji et al. (2018)
4	<i>Turbinriaturbinata</i> (brown algae)	Cell free extract	FeCl ₂ .4H ₂ O, NaOH	8–14 nm	-	Ferromagnetic	-	Siji et al. (2018)
5	Pterocladia capillacea (red seaweed)	Dried powder	FeCl ₃ (0.1 M)	16.85–22.47 nm	Nanosphere	-	Antimicrobial, antifungal	Salem et al. (2019)
6	<i>Colpomenia sinuosa</i> (brown seaweed)	Dried powder	FeCl ₃ (0.1 M)	11.24–33.71 nm	Nanosphere	-	Antimicrobial, antifungal	Salem et al. (2019)



metal nanoparticles, work as chelating and capping agents. Nextly in the growth phase complete reduction of metal ion occur from monovalent or divalent oxidation state to zero valent oxidation states. In this growth progression stage, metallic nanoparticles aggregate among themselves to attain different morphology. Finally, in the termination phase final stable morphology of nanoparticles are obtained and are capped by biomolecules so that steric repulsion among them increases which is thought to reduces the problem of agglomeration (Gan et al., 2012; Akhtar et al., 2013; Jeevanandam et al., 2016; Ishak et al., 2019). Typically, Iron oxide nanoparticles synthesized by chemical and physical routes are extremely reactive in nature and can easily undergo oxidation by atmospheric oxygen to agglomerate together. In addition to this, in order to minimize surface energy arising because of the high surface-area-to volume ratio, nanoparticles usually tend to aggregate. Varity of stabilizers are added in the

chemical and physical synthesis procedure to prevent agglomeration of the synthesized nanoparticles. No such stabilizing agents are required in biological synthesis methods as biomolecules present in the extract act as capping agents and stabilized the synthesized nanoparticles (Gudikandula and Maringanti, 2016). From the biomedical application point of view, the size of iron oxide nanoparticles should be less than 20nm, they must be uncapped, should posses good magnetic properties and have critical transition temperature (Tc). In the green synthesis approach, different reaction parameters like temperature, pH, stirring, metal salt concentration and volume of extract are very crucial to obtained nanoparticles of proper shape, size, crystallinity, purity, stability and morphology. In plants mediated nanoparticle synthesis, choice of the starting material is very important. Each part of the plant varies in the concentration of reducing agents like flavones, terpenoids, sugars,

ketones, amides, anthracenes, alkaloids. Leaf extract is considered as the best source for the synthesis of metal oxide nanoparticles as it contains high concentration of phytochemicals that helps in bioreduction of metal ions. Out of bacteria and fungi, fungi is considered as an excellent source for the synthesis of metal oxide nanoparticles. The main reason for its wide usage is its capability to secrete large amount of enzymes and withstand flow pressure and agitation (Gudikandulaand Maringanti, 2016; Singh et al., 2018; Ajinkya et al., 2020).

DISCUSSION

Biocompatible synthesis of nanoparticles is the centralized area of the study in the field of nanotechnology. Green sources are the main players in the biological synthesis of iron oxide nanoparticles due to the presence of wide variety of biomolecules showing strong reduction of metal ions to form nanoparticles from the bulk metal. There are number of reasons which make biological synthesis of nanoparticles very lucrative. First of all, this emerges as an inexpensive approach as simple physical conditions are required for the synthesis, no requirement of sophisticated instruments, and because of the wide availability of the biological agents employed. Next point which is worthy to consider is the absence of toxic chemicals required for the synthesis which make these particles biocompatible and environment friendly. Moreover, water is the key solvent employed in most of the green synthesis approaches adding additional meaning to the green technology. Lastly, no separate capping agents are required

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for the stabilization of the biologically synthesized nanoparticles as the biomolecules present in the extract are self -sufficient to carry out this action, so no agglomeration is encountered. However, the biggest challenge for this approach is the reproducibility of this method. Such method presents a passive approach toward the synthesis of the nanoparticles where the reactants are utilized passively and partially. The unreacted components can lead to the formation of undesired products with varied properties making the method nonreproducible. The immediate recovery of the product can solve the problem to certain extent but it is still a challenge for the researchers working with this green synthesis. Researchers are required to focus on the size controlled synthesis of the nanoparticles as size is the key factor deciding the properties and thus utility of the nanoparticles. Further, compilation of current work proves that so far numerous queries about the parameters controlling size, shape, crystallinity, yield of the nanoparticles produced and precise magnetic properties of the nanoparticles synthesized using green approach are still not explained. Hence, further research in these directions is exceedingly necessary to fill these lacunas and come up with more controlled studies.

AUTHOR CONTRIBUTIONS

P, N, and AS designed, wrote the manuscript, revised and finalized the draft. KK supervised the whole project and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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