Phytochemical mediated synthesis and characterization of Iron Nanoparticles using *Saraca asoca* leaves extract

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ABSTRACT

Iron is said to be very ubiquitous among all transition metals and also available profusely in the earth's crust. Using nanotechnology, several types of iron oxide nanoparticles have been synthesized. As previously reported Iron in the nanoscale (1-100nm) can show very potent catalytic, magnetic and antibacterial properties. This study shows that iron oxide nanoparticles are synthesized using a green, eco-friendly and economical route method synthesized particles are more stable and biocompatible due to the use of biological entities. The leaves extract of *'Saraca asoca'* was used for the bio fabrication of Iron Oxide Nanoparticles. Phytoconstituents such as tannins, flavonoids, etc. may act as reducing and capping agents which also enhanced the functional properties of the synthesized nanoparticles. The characterization of the nanoparticles were investigated by FTIR and PXRD techniques. The FITR interprets that these particles were synthesized by the stabilizing functional groups. Powder XRD patterns interpret the crystallites to be cubic and face-centered with less than 100 nm in size. This study highlights the biosynthesis and possible potential application of these nanoparticles that can be further explored in the field of biomedicine, bioengineering, bioremediation, and biosorption.

Key Words: Iron oxide, nanotechnology, phytochemical mediated synthesis, magnetite.

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INTRODUCTION

Nanotechnology and nanoparticles are the two most important topics' of recent researches. [1-5] Nanoscience has been steadily advancing in recent years. Several novel new techniques evolved for the synthesis of nanoparticles are less toxic and harmful to nature [6, 7]. Over the last decade, researchers have found that nanoparticles have great applications that can be exploited for various therapeutic aspects in bioengineering and biomedicines [8, 9]. Several particles that have great properties are easily synthesized from various inorganic and organic compounds [10], but are very important for the simultaneous therapy and diagnosis of inorganic materials nowadays because they can be easily modifying [11], high loading capacity for the drug and stability [12]. Various metals have been studied for the synthesis of nanoparticle, of which Iron is also of great interest but least studied for its self-degrading nature though iron at the nanoscale can show very potent catalytic, magnetic and antibacterial properties, various types of iron oxide nanoparticles have been synthesized in last 40 years, such as SPION (superparamagnetic iron nanoparticles), nZVI (nano zero-valent iron Fe^0), Iron oxides such as maghemite (g-Fe2O3), magnetite (Fe3O4). Among all the magnetite iron nanoparticles, one is a promising candidate that has been proven biocompatible in nature. The form of Magnetite (Fe3O4) usually has a cubic inverse spinel structure with oxygen which results in the closed packing of the FCC (face-centered cube) and iron cations occupy of interstitial tetrahedral and octahedral sites [13, 14].

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Phytochemical mediated synthesis is opted for the synthesis of iron oxide nanoparticles which should have high magnetization, non-toxic, and biocompatible for delivery of the target drug, their size must be less than 100 nm, and comprise a special phytochemical surface coating to achieve antimicrobial properties.

'*Ashokbriksh*' is botanically known as *Saraca asoca (Roxb.)* has been reported as a great panacea in Indian Ayurveda and in use since ancient times. It's well known medicinal properties such as antimennorhagic, oxytocic and uterine tonic, antimicrobial, anticancer, anti-inflammatory, antiarthritic, anti-nephrolithiatic, cardioprotective effect, etc. The Ashok plant is geographically distributed mainly in the regions of Asia and some parts of North America. This tree is abundantly found throughout India, especially in moderate climatic regions. Many plants are said to be medicinally important due to their biologically active compounds such as alkaloids, flavonoids, terpenoids, steroids, phenolics, anthraquinones, tannins, glycosides, saponins and other phytochemicals [15].

MATERIALS AND METHODS

Plant Collection: Fresh leaves of the selected *Saraca asoca* plant were collected from a local botanical garden and identified authentically.

Plant extract preparation: Green Leaves of the selected plant were washed and air-dried and weighed 20 grams and crushed with the use of mortar and pester adding the required amount of distilled water to make a fine slurry. The mixture was kept in 100 ml of 50% ethanol for 12-16 hours with constant agitation at 30 minutes intervals. The extract was filtered twice using a muslin cloth and the Whatman No.1 filter paper. The filtrate was stored at 4°C.

Phytochemical analysis:

The prepared leaves extract of *Saraca asoca* were subjected to different qualitative tests to confirm the presence of different phytochemicals which are confirmed by the given tests [16].

Tannins: 0.5 g of dried leaves sample powder boiled in 20 ml of water and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Alkaloids: 1% aqueous hydrochloric acid added in 1 milliliter of aqueous extract and placed on a steam bath. 1 ml of the filtrate taken and treated using Dragendorff's and Mayer's reagent and observed for Turbidity or precipitation which is considered as an evidence for the presence of alkaloids.

Glycosides: 0.5 g sample extract was dissolved in 1 ml water followed by the addition of aqueous sodium hydroxide and observed yellow color formation which indicates the presence of glycosides.

Saponins: 5ml of distilled water was added to the extract and shaken well in the test tube for 10 minutes and observed for foam formation that confirms the presence of saponins.

Terpenoid (Salkowski's test): Add 200µl of sample extract in 1 ml chloroform followed by a few drops of concentrate H_2SO_4. and observed for an immediate reaction resulting in a reddish-brown precipitate which indicates the presence of terpenoids.

Flavonoids: Few drops of lead acetate solution were added to 1 ml of the sample extract and observed for yellow precipitate formation which indicates the presence of flavonoids.

Phenols: A few drops of ferric chloride solution were added to the sample extract and observed for a bluish-green or reddish color which indicates the presence of phenol.

Steroids (Libermann- Burchard Test): Two ml of sample extract was mixed with ten volumes of chloroform and conc. H_2SO_4 dropped slowly along the sides of the test tubes and observed a low sulfuric acid fraction to turn yellow with greenish fluorescence which confirms the presence of steroids.

Catechins: Few drops of concentrated hydrochloric acid and Echrlich reagent were added to 1ml of the sample extract and observed for the appearance of pink color which indicates the presence of catechin.

Coumarins: A few drops of alcoholic sodium hydroxide were added to the sample extract and observed for the appearance of yellow color which indicates the presence of coumarin.

Quinones: A few drops of conc. H_2SO_4 or aqueous sodium hydroxide solution was added to 1 ml of sample extract and observed for color formation which indicates the presence of the quinoid compound.

Xanthoproteins: A few drops of concentrated nitric acid and a few ml of ammonia were added to 1 ml of the sample extract and observed for the appearance of red precipitate which indicates the presence of xanthoprotein.

High-Performance Liquid Chromatography (HPLC): HPLC is a versatile, robust, and novel method used

commonly in analytical chemistry and phytochemistry to identification, quantification, and purification with individual components of various test mixture. This peculiar analysis shows, the similarity index of the results calculated based on the relative value, the retention time of the selected marker compounds as reference standards [17].

Phytochemical mediated synthesis of nanoparticles: The synthesis of Nanoparticles is done by the green route method also known as Biological synthesis. It is one of the simplest single step bioreduction methods using the leaves extracts of *Saraca asoca* which are eco-friendly resources. 0.1M solution of Iron III Chloride (100mL Distilled water) was taken in Erlenmeyer flask and kept for continuous stirring on a magnetic stirrer for 2-3 hours. For the reduction purpose, the leaf extract of *Saraca asoca* was added dropwise at an interval of 30 minutes. After 2-3 hours the solution was kept for drying. The dried particles were washed 3-5 times using 100% ethanol and dried [18].

Bioreduction mechanism of plant extracts in the synthesis of metallic nanoparticles:

$$\text{Iron III Chloride} + \text{Plant extract} \rightarrow \text{Iron oxide NPs} + \text{By} - \text{product}$$

Nanoparticles Characterization:

The following techniques were used characterization the synthesized nanoparticles :

1. Powder X-ray diffraction: XRD is remarked as a powerful method for studying nanoparticles. X-ray diffractograms of nanoparticles provide more information such as phase composition as crystallite size, lattice structure, strain for crystallographic orientation. Powder diffraction often uses a use search/match procedure to identify components in a sample. In addition, the areas under the peak are related to the amount of each phase present in the sample. To make accurate quantifications of experimental results by using the Bragg's law and for the determination of crystal structures and the average size of the crystallites can be calculated by using the Scherrer formula [19, 20].

2. Fourier Transform Infrared Spectroscopy: This is a consequent technique used to identify organic, polymeric, and, few inorganic materials. In this technique, Infrared light is used to scan the test samples to observe the chemical properties. Infrared radiation ranging from 10,000 to 100 cm^{-1} is passed through a sample, some of the radiation gets absorbed and some of it passed through. The radiation absorbed by the sample molecules is converted into rotational/ vibrational energy which is imparted as a spectrum, resulting in signals ranging from 4000 cm^{-1} to 400 cm^{-1}, which is detected by the sample fingerprinting molecule of the test sample [21, 22].

RESULTS AND DISCUSSION:

Phytochemical analysis: The biologically active Phytocompounds analyzed in the leaves extract of *Saraca asoca* includes alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, steroids, catechins, coumarins, quinones, xanthoproteins. Their presence is shown in Table 1 where '+++' represents strong presence, '++' moderate, '+' poor and '-' not present.

Table 1: Status of bioactive compounds present in *Ficus religiosa.*

Sr. No.	Biologically active Phytocompounds	*Saraca asoca*
1.	*Alkaloids*	+++
2.	*Glycosides*	-
3.	*Flavonoids*	++
4.	*Tannins*	++
5.	*Phenolics*	+++
6.	*Saponin*	++
7.	*Terpenoids*	+++
8.	*Steroids*	+++
9.	*Catechins*	-
10.	*Coumarins*	+
11.	*Quinones*	-
12.	*Xanthoproteins*	-

High-Performance Liquid Chromatography (HPLC) Analysis:

The results of HPLC analysis of the leaves extract of *Saraca asoca* are indicating a peak of 1,2,3 and 4 which may be derived from quercetin, ellagic acid, eugenol, and Gallic acid respectively (Figure 1). The chromatogram peak was identified based on the retention time of sample analyte injected separately and by the addition of a standard analyte [23]. The area and quantity results are shown in Figure 1.

Figure 1 shows the content percent of phytochemicals present in the analyte:

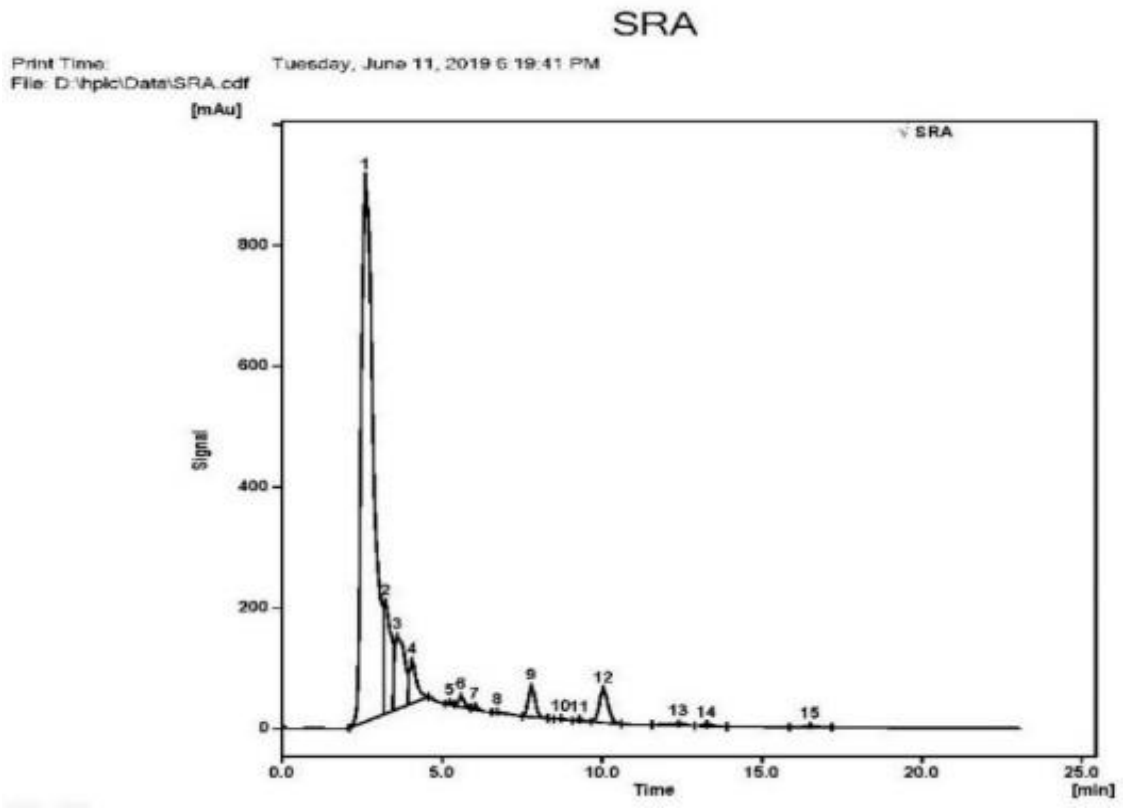

Figure 1. HPLC Chromatogram of Saraca asoca a leaf extract.

Phytochemical Mediated Synthesis of Nanoparticles using Saraca asoca:

This study has shown that Iron Nanoparticles could be synthesized using the leaf extract of *Saraca asoca*. Likewise, extracts of various plants such as *Moringa oleifera* [24], *Lagenaria siceraria* [25], *Mangifera indica*, *Azadiracta indica*, *Murraya Koenigii*, *Magnolia champaca* [26], *Lawsonia inermis*, *Gardenia jasminoides* [27], *Hordeum vulgare*, *Rumex acetosa* [28] and *Glycosmis mauritiana* [29] have been reported for the synthesis of iron oxide nanoparticles. In this process, a reddish-brown suspension/ precipitate leaves extract was observed that dried at 80^{o} C after completion of the synthesis process.

Characterization of Iron nanoparticles:

1) PXRD Analysis: The XRD pattern obtained by iron oxide nanoparticles is shown in Figure 2. Strong peaks with 2-theta values of 35.271, 26.970, and 11.956 which are correspondent to the *hkl* 311, 220, 111 reported in the JCPDS file No. 19-0629 [30].

The average crystallite sizes of iron oxide nanoparticles can be assessed using the "Debye-Scherrer's' equation" [31, 32] which is commonly used to calculate the relationship between the peak broadening in XRD and the average particle size by the given equation:

$$d = \frac{k\lambda}{(\beta * cos\theta)}$$

Where,

- *'d' is the 'particle size' of the crystal.*
- *k is the Scherrer's constant (0.9).*
- *λ is the X-ray wavelength (0.15406 nm).*
- *β is the peak width at half-height.*
- *θ is the Bragg's angle.*

The given equation is used to determine the crystallite size of the synthesized iron nanoparticles. The average crystalline size of the sample is in range of 9.44 nm and the crystalline lattice is magnetite, with a face-centered cubic structure.

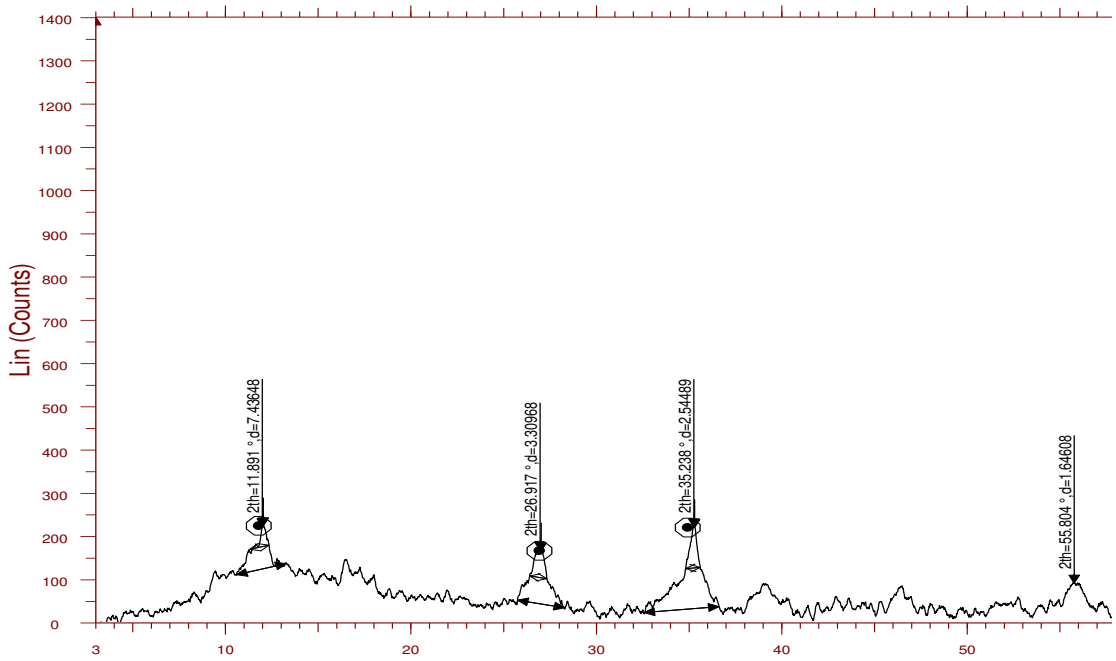

Figure 2. XRD plot of synthesized Iron Nanoparticles.

2) FTIR analysis:

FTIR spectroscopy is used to identify the presence of functional groups on nanoparticles loaded with *Saraca asoca* active components based on peak values in the region of infrared radiation. Figure 3 shows the FTIR spectra of the functional groups attached to the synthesized Iron nanoparticles according to the given wavenumbers 3426.15, 3193.12, 1631.41, 1400.07, 646.70 the functional groups reported are: Alcohol (Intramolecular), Primary Amine, Amine Salt, Carboxylic Acid (Usually Centred), Conjugated Alkene, Cyclic Alkene, Phenol, Sulphate, Halo Compounds which are found to be attached. These groups have been depicted as both downgrading and confining agents in the process of green synthesis.

Table 2: Wavenumbers showing corresponding groups.

Sr. No.	Wavenumber Or Group Frequency (Cm^{-1})	Bond Type	Bond Origin	Functional Group
1.	3426.15	Strong, Medium	O-H Stretching, N-H Stretching	Alcohol (Intramolecular), Primary Amine
2.	3193.12	Strong, Weak, Strong	O-H Stretching, Stretching, N-H Stretching	Carboxylic Acid (Usually Centred), Alcohol (Intramolecular), Amine Salt
3.	1631.41	Medium	C=C Stretching, N-H Stretching, C=C Stretching	Conjugated Alkene, Amine, Cyclic Alkene
4.	1400.07	Medium, Medium, Strong	O-H Bending, S =O Stretching	Phenol, Sulphate
5.	646.70	Strong	C-Br Stretching	Halo Compound

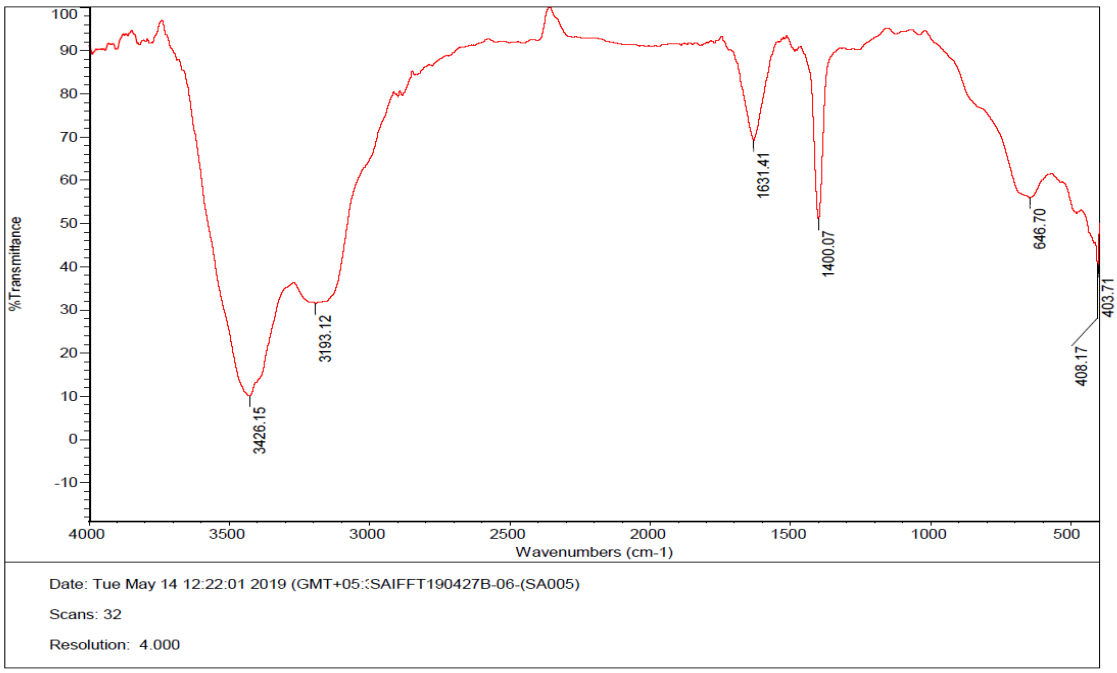

Figure 3. FTIR Plots showing wavenumbers of functional groups.

Loaded phytochemical nanoparticles can have various applications such as MRI contrast enhancement, immunoassay, Tissue repairment, hyperthermia, detoxification of biological fluids, cell separation, drug delivery, and others, [33]. These particles can be further investigated for their efficacy because the iron nanoparticles synthesized through the green route have high magnetization values, some of which can be tested for their cytotoxic effects and can be proven as good antibacterial agents because very good in biomedicine. To be biocompatible, it is used as a forum for ferrofluids, biosensors, targeted delivery agents [20, 21, 34, 35].

CONCLUSION:

This study shows that the green route method of nanoparticle synthesis using the leaves extract of *Saraca asoca* plant is one of the most rapid and efficient means and is highly benign, cost-effective, and sustainable. This study reveals that iron oxide nanoparticles synthesized through the biological route are more stable, crystalline, least impure, and may possess efficient, active, and unique properties such as excellent adsorption capacity, ferrofluidic, high magnetization, biocompatible, catalysts, bio sensitive, drug carrier, etc. according to recent researches. So, the synthesized iron nanoparticles can be further explored in various fields of biomedicine, bioremediation, biosorption, and bioengineering.

NOTE: SC and VS has equally contributed in research work so both are regarded as equal first author.

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