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Eco-Friendly Synthesis of Iron Oxide Nanoparticles using *Abrus Precatorius* Seed and Evaluation of their Antibacterial and Antioxidant Activities

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Abstract

The current study uses Abrus precatorius (AP) seed to synthesize iron oxide nanoparticles (AP-FeONPs) in a green way. Utilizing SEM, XRD, IR, and UV-DRS, the generated nanoparticles were characterized. Using the DPPH assay, the antioxidant activity of AP-FeONPs was evaluated using ascorbic acid as the standard and determined their IC50 values as 22.40 µg/ml (AP-FeONPs) and 10.3112 µg/ml (ascorbic acid). The bio fabricated iron oxide nanoparticles (AP-FeONPs) demonstrated strong antioxidant activity in comparison to ordinary vitamin C. The antibacterial properties of AP-FeONPs were assessed against Bacillus subtilis and Escherichia coli at concentrations as high as 100µl. The results showed that, at high concentrations of 100µl, AP-FeONPs possesses significant bacterial inhibition against Bacillus Subtilis and Escherichia coli.

Keywords: Abrusprecatorius, UV-DRS, SEM, AP-FeONPs, XRD, DPPH Assay

Introduction

The terms "nanotechnologies" and "nanoscale technologies" are frequently used to describe studies and applications of matter have the size between 1 and 100 nanometers (nm). Particle size and their unique properties play a significant role in nanotechnology. [1]. Molecular nanotechnology, focused on the particular technological goal of precisely modifying atoms and molecules to create macroscale objects [2]. One of the main goals of nanotechnology is the development of novel instruments and creation of new nanotools frequently involves improving the uses of existing ones. The imaging of native biomolecules, biological membranes, and tissues is a crucial field of research for nanobiology researchers. The use of cantilever array sensors and the use of nanophotonics to control molecular processes in living cells are two more areas related to nanobiology [3]. Molecular self-assembly and extensions of traditional device physics are among the related studies and applications [4].

In the nanotechnology, all interconnected scientific disciplines creating novel materials with nanoscale dimensions to controlling matter at the atomic level [5]. Foods can be loosely divided into

classes such as proteins, lipids, carbohydrates, vitamins, and minerals based on their nutritional properties. Foodborne infections lead to food poisoning or rotting. Food borne infections and many of them lead to food poisoning or rotting. Silver and gold have been utilized in various ratios with other materials, including milk, ghee, honey and leaf juice to cure certain illnesses in Siddha medicine [6]. A macroscopic substance or group of materials is the starting point for "top-down" techniques [7]. Silver nanoparticles have applied in the field of wastewater treatment, sustainable energy production, drug delivery and catalysis [8].

Organic chemical contamination of groundwater systems is a major environmental hazard. The severity of this risk is explained by their toxicity to both people and animals. Several volatile organic compounds are recognized as pollutants in groundwater by the United States Environmental Protection Agency [9]. The top-down method places a strong emphasis on organization and planning when it comes to problem-solving or decision-making [10]. Both top-down and bottom-up approaches are frequently used to create nanoparticles, which are typically 0.1 to 1000 nm in each spatial dimension [11]. The top-down method is frequently applied in organizational planning, policymaking, and software system development. This method ensures coherence and consistency throughout the system or structure since all of its parts strive towards a common goal set by the higher levels of the hierarchy [12]. The top-down strategy has certain disadvantages despite its advantages. One of the primary objections is that it may result in a lack of feedback from stakeholders or lower-level personnel, possibly ignoring pragmatic issues or realities on the ground. Because choices are made higher up the chain of command, it could also lead to a slower rate of change and adaptability. Furthermore, if individuals at the top lack a thorough understanding of the specific sectors under supervision, their instructions may be less effective or helpful. Therefore, for best outcomes, the top-down method may need to be tempered with bottom-up iterative modifications [13].

Herbal preparations made by extraction, fractionation, purification, concentration, or other processes that may create nutritional or medicinal constituents for immediate consumption or as a basis for herbal drugs are referred to as medicines from plants by the World Health Organisation [14]. Some authors defined medicinal plants as those that have active *constituents* that are used to treat illness or lessen suffering [15]. Therapeutic herbs have utilized as antibacterial experts and as a source of secondary metabolites with organic activity in addition to curing human ailments [16]. India is the most well-known nation in the world for its flavours and traditional medicines, which have a wider range of physiological and pharmacological characteristics [17]. Medicinal herbs play a **consequential** role in preventing disease and therapeutic plants used in herbalism, some of which have therapeutic properties [18, 19]. In the traditional drug arrangement, medicinal plants are the most expensive bioresource of treatments. They also serve as a form of therapy [20]. People have more aware of the importance of medicinal herbs in recent years [21].

The identification and isolation of typically occurring compounds is the main goals of therapeutic plant research [22]. Because they contain phytochemical components, therapeutic plants are used for the treatment of human illnesses [23]. Therapeutic plants are currently in a remarkable vital viewpoint due to their exceptional features as a vast reservoir of medicinal phytochemicals that could stimulate the development of new treatments. Most plant-based phytochemicals, including phenolics and flavonoids, have a considerable effect in preventing disease and promoting good health [24]. Alkaloids, steroids, tannins, glycosides, pitches, phenols, alkaloids, and fixed and unpredictable oils are among the dynamic combinations found in plants. These mixtures are concentrated in specific areas, such as leaves, blooms, bark, seeds, natural products, roots, and so on [25].

Several phytochemicals that are members of specific compound classes can inhibit a broad range of bacteria in vitro [26]. Since ancient times, plant materials have been crucial to phytomedicines. [27]. Primary chemicals include proteins, common sugars, and chlorophyll, whereas secondary ingredients

include phenolic compounds, alkaloids, and terpenoids. Both primary and secondary ingredients are phytochemicals [28]. Phenolic compounds, also known as polyphenols, are active phytochemicals that are produced by auxiliary digestion and comprise one of the most abundant and widely distributed groups of plant elements [29]. In order to treat a variety of illnesses, enormous medicinal plants were used [30]. It is acknowledged that primary sources of novel compounds with substantial therapeutic potential is medicinal plants [31].

Abrus precatorius belongs to fabaceae family. The composition of AP-seed is carbohydrate (42.42%), protein (39.2%), fiber (9.1%) ash (5.3%) and (5.0%. Traditional uses of AP-seed include treating tetanus, animal scrapes, sores, and wounds, as well as preventing rabies. It has anti-inflammatory and antibacterial properties and also applied in wound treatment [32-37].

The green fabrication of iron oxide nanoparticles (AP-FeONPs) from an aqueous extract of *Abrus precatorius* seed (AP- seed) is the main focus of this study. The synthesised AP-FeONPs is characterised using UV-VIS spectroscopy, FT-IR spectroscopy, SEM and XRD. The antibacterial activity of synthesised AP-FeONPs against *E. Coli* and *Bacillus subtilis* was evaluated using the well plate method. Using the DPPH test technique, the antioxidant activity of AP-FeONPswas assessed.

Experimental Method

Preparation of AP- seed Extract

After being cleansed with deionised water, the AP-seeds were dried to remove any remaining water. About 25 g of fine powder of AP- seeds were mixed with 500 mL of deionised water in a 1000 mL beaker. After 1 hour of heating, the mixture was filtered. For later usage, the filtrate was kept in the refrigerator.

Fig 1: *Abrusprecatorius* seed (AP- seed)

Fabrication of Iron oxide nanoparticles (AP-FeONPs)

50 ml of 0.5 M iron chloride solution (aqueous) was mixed with 450 ml of AP- seed extract to create iron nanoparticles. In two to three minutes, the plant extract turned black and the colour became more and more intense. After that, the AP-FeONPswere centrifuged and repeatedly washed with deionised water. The colour shift is shown in Figure 2.

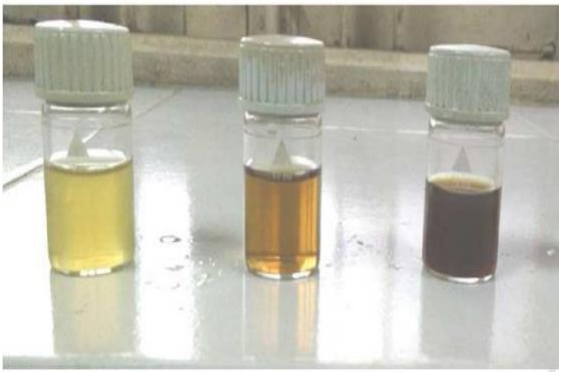

Fig 2: Fabrication of AP-FeONPs

Anti-bacterial Screening

Test organisms

Gram-positive bacteria of *Bacillus substilis* and gram-negative bacteria of *E. coli* were used for the antibacterial screening. The Sri Kaliswari College in Sivakasi, Tamil Nadu, India, served as the origin of all the stock cultures.

Anti-bacterial Screening method

The well diffusion method was used to assess the antibacterial activity [38,39]. Mueller Hinton Agar (MHA) was first added to the plates. To create an even inoculum, the test culture was progressively pushed

out in three directions across the plate using a sterile cotton swab. The plate was allowed to air dry for three to five minutes. Wells measuring 5 mm in diameter were created on the surface of the agar. The plates were incubated at 37 ºC for 24 hours. The zone of inhibition was measured using a scale that included the well diameter.

Antioxidant activity

Free radicals are reactive molecules that play a variety of physiological roles and have been connected to a number of diseases, such as cancer, arthritis, and liver damage. Therefore, it is necessary to investigate substances that have antioxidant or free radical scavenging properties. Antioxidants are compounds that, at low concentrations relative to the substrate, considerably slow down or prevent the oxidation of an oxidisable substrate.

DPPH, or 1,1-diphenyl-2-picrylhydrazyl radical, has been the main compound used to evaluate antioxidants' capacity to scavenge free radicals. The corresponding hydrazine is produced when the DPPH free radical reacts with hydrogen donors. Stable free radicals can be produced by DPPH in methanol or water solutions. This approach made it plausible to estimate the antiradical power of an antioxidant activity by measuring the drop in DPPH absorbance at 517 nm **[**40, 41]. When an antioxidant scavenges the DPPH by donating hydrogen to form a stable DPPH molecule, the absorbance drops, causing the colour to change from purple to yellow. The molecule's absorbance at 517 nm disappeared when an electron or hydrogen radical from an antioxidant material was accepted.

Results and Discussion

GC-MS chromatogram of aqueous AP-seed extract

GC-MS chromatogram of the AP-seed extract is shown in figure 3. Fourteensecondary metabolites having peak retention times of 9.23, 10, 14.12, 14.87, 16.02, 16.6, 17.6, 18.25, 19.05, 20.28, 21.28, 23.58, 26.13, and 29.7 were identified in this chromatogram. Phenol, 2-propyl-3-phenyl pyrazol-5-ol, 1-(1-naphthalene-1-yl) but-3en-1-ol, bis (4-methoxy phenyl) methane, methy13-methyl pentadecanoate, n-decanoic acid, (E)-methyl octadic-10-enoate, and ester elaidic acid isopropyl were detected in this GC-MS chromatogram. Among them, a peak corresponding to the retention time 17.6 minutes is the strongest one and this indicates the corresponding secondary metabolite is present as maximum in the AP-seed extract.

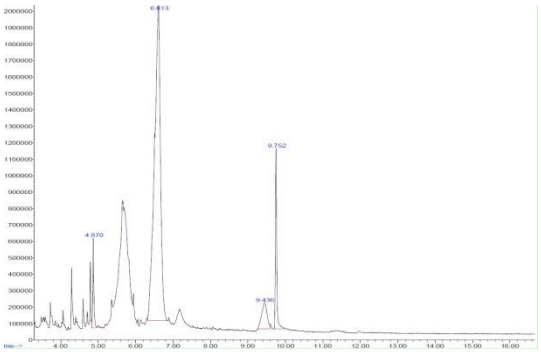

Fig 3: The GC-MS chromatogram of the AP-seed extract

FT-IR Analysis of AP-FeONPs

FT-IR spectroscopy data were utalized for the identification of the functional groups of biomolecules and also predict the reducing & stabilizing agent for the formation of AP-FeONPs. The presence of Fe-O in AP-FeONPs were confirmed by the absorption bands at about 516 cm^{-1}. Additionally, the O–H and C=C ring stretching also observed at 3379 and 1651 cm^{-1} in the fabricated AP-FeONPs. Biochemical compounds from AP-seed extract also adsorbed on the surface of AP-FeONPs which stabilize the colloids [36,37]. The FT-IR spectra of the AP-FeONPs is shown in Figure 4.

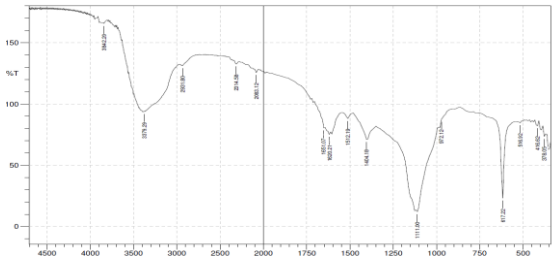

Fig4:FT-IR spectra of AP-FeONPs

UV – DRS analysis of AP-FeONPs

AP-FeONPs produced using environmentally friendly techniques have unique absorption characteristic peak at in the UV-Vis range is shown in Figure 5. AP-FeONPs biosynthesized using AP-seed extract displayed an excitation peak at approximately 320 nm, corresponding to a 1.5 eV bandgap energy [42].

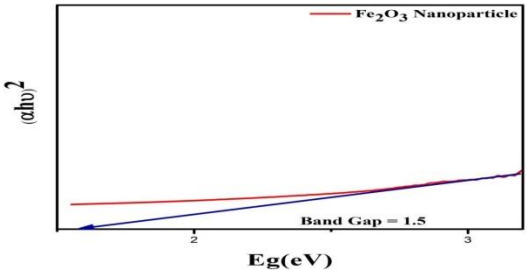

Fig 5: UV – DRS spectra of AP-FeONPs

XRD Examination of AP-FeONPs

The XRD pattern of the AP-FeONPs produced by AP-seed extract is shown in Figure 6. The absence of distinctive diffraction peaks in the iron oxide XRD pattern demonstrated the amorphous nature of nanoscale particles [43].

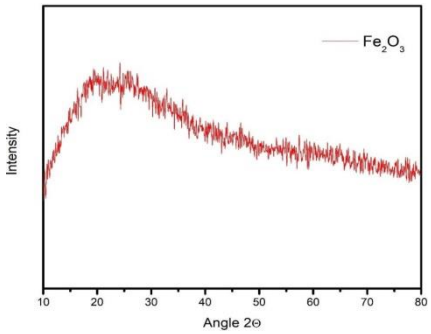

Fig 6: XRD pattern of AP-FeONPs

SEM Analysis of AP-FeONPs

The bio-synthesized AP-FeONPs are not always uniform and might aggregate, as shown in the Figure 7 [(a-b) & c]. The average diameter of the spherical shape AP-FeONPs was 21.59 nm. The formation of large agglomerated AP-FeONPsmay have been caused by lower capping capability of AP-seed.

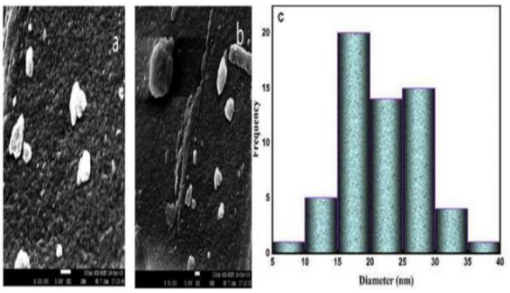

Fig 7: (a–b) SEM image morphology;(c) Size distribution histogram of AP-FeONPs

Antibacterial activity of AP- Seed extract & AP-FeONPs

The antibacterial properties of AP- Seed extract &AP-FeONPs were evaluated in the current study against *Bacillus subtilis and Escherichia coli* at concentrations as high as 100µl. The results showed that, at high concentrations of 100µl, both AP- Seed extract &AP-FeONPs possesses significant potential antibacterial activity against *Bacillus Subtilis* and *Escherichia coli*. **Table 1** and Figure 8 present the findings.

Fig 8: Antibacterial screening of AP- Seed extract & AP-FeONPs

Table 1: Zone of inhibition of AP- Seed extract & AP-FeONPs

Types of bacteria	Name of bacterial species	Zoneof inhibition (mm)	
		AP- Seed extract (100µl)	AP-FeONPs (100µl)
Gram Positive	*Bacillus subtiles*	0.7	0.6
Gram Negative	*E.Coli*	0.3	0.5

Antioxidant activity of AP-FeONPs by DPPH Assay

The % inhibition (I) of DPPH free radical scavenging potential is used to evaluate the antioxidant activity of bio-formed AP-FeONPs. With increasing concentrations (500, 400, 300, 200, 100, 50, 25, 10µg /mL), the results indicate that the inhibitory percentage value for ascorbic acid and AP-FeONPs increases. The bio-fabricated AP-FeONPs exhibit a maximum efficiency of 92% at this high concentration. This is caused by AP-FeONPs coated with strong bioactive compounds. The surface coating's biospecies are strong DPPH radical scavengers. **Tables 2 & 3** and Figures 9 and 10 display the antioxidant activity results of both standard and AP-FeONPs respectively. Figure 11 shows the comparison of the IC_{50} values for the standard and AP-FeONPs.

Table 2: IC_{50} value of Ascorbic acid (Standard)

Absorbance of control	Conc (µg/ml)	Absorbance of Ascorbicacid	Inhibition (%)	IC50 (µg/ml)
0.435	0.98	0.401	7.81	
	1.95	0.342	16.78	10.3112
	3.9	0.315	27.59	
	7.81	0.252	42.07	
	15.63	0.196	54.94	
	31.25	0.129	70.34	
	62.5	0.073	83.21	
	125	0.048	88.99	
	250	0.039	91.03	
	500	0.034	92.18	

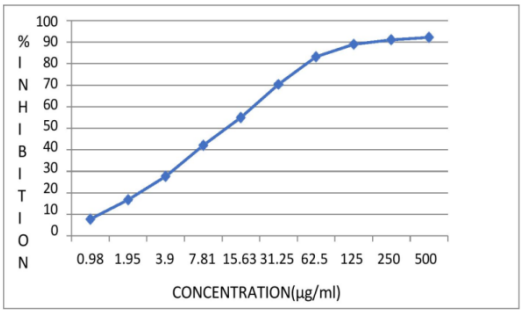

Figure 9: Free radical scavenging activity of ascorbicacid

Table 3: IC_{50} value of AP-FeONPs

Absorbance of control	Conc(µg/ml)	Absorbance of sample	Inhibition (%)	IC50 (µg/ml)
0.435	0.98	0.430	1.15	22.40
	1.95	0.4046	6.98	
	3.9	0.381	12.41	
	7.81	0.348	20.02	
	15.63	0.311	27.70	
	31.25	0.267	38.62	
	62.5	0.202	53.56	
	125	0.164	62.30	
	250	0.152	65.06	
	500	0.149	65.74	

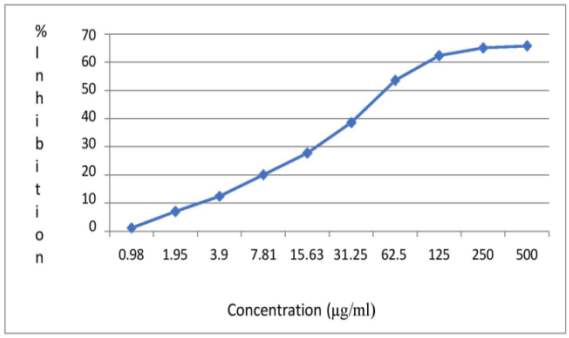

Figure 10: Free radical scavenging activity of AP-FeONPs

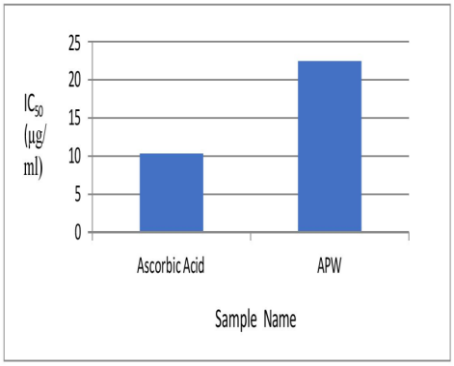

Figure 11: Comparative IC50 value of DPPH free radical scavenging activity of ascorbic acid & (APW-AP-FeONPs)

Conclusion

Spherical-shaped amorphous AP-FeONPs with an average size of 21.59 nm were harvested from AP-Seed extract by Eco-friendly green route. Utilising SEM, XRD, IR, and UV-VIS spectroscopy, the bio fabricated AP-FeONPs were characterized. Using the DPPH assay, the antioxidant activity of the AP-FeONPs was assessed. Both AP-FeONPs and vitamin C have IC_{50} values of 22.40µg/ml and 10.3112µg/ml respectively. When compared to standard vitamin C, AP-FeONPs exhibited good antioxidant activity. The antibacterial properties of both AP- Seed extract & AP-FeONPs were evaluated in the current study against *Bacillus subtilis and Escherichia coli* at concentrations as high as 100µl. The results showed that, at high concentrations of 100µl, AP-FeONPs possesses significant potential antibacterial activity against *Bacillus Subtilis and Escherichia coli*.

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