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Antioxidant Efficacy of Iron Nanoparticles from Aqueous Seed Extract of *Cuminum Cyminum*

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Abstract: Nanotechnology apportions with the appliances, materials, and machines at nanometer scale with the irreplaceable properties that the structure endows miniature dimensionality. The nanoparticles have far-reaching applications because it is swifter, lighter and portable. Amidst metal nanoparticles, iron nanoparticles are consumed to scour exterminates of groundwater and heavy metals from the dirt. Therapeutic plants are singled out as they cater outspread phytochemicals that are managed to accomplish biotic functions. In the present investigation, the seed extract of *Cuminum cyminum* was prepared for the synthesis process and the fetched iron nanoparticles were thereupon characterized by UV, XRD and FTIR. The UV-Visible spectra indited two peaks at 220 nm and 272 nm for the orchestrated particles. The XRD spectra affirmed that they were crystalline in nature. The FTIR spectra emblazoned the occurrence of phenolic compounds in the extract that might have accounted for their production. After ABTS assay, the nanoparticles set out elevated percentage of scavenging activity than in DPPH assay. In this fashion, the particle has been made evident to own antioxidant efficacy.
Keywords: Cuminum cyminum, Biogenesis, Iron nanoparticles, Antioxidant efficacy.

Introduction

Nanotechnology is one of the pullulating zones in biotechnology with neoteric properties. Nanosized particles of metals and metal oxides have captured foremost recognition because of the stupendous characteristics acquired by them[1]. They could be manufactured by physical, alchemical and organic shortcuts. The physical method is high-priced and entails knacked labors, whereas the rigorous use of chemicals is noxious to the environment. From this time forth, biological method is preferred to synthesize the nanoparticles as they are economical, terrain-friendly; innocuous and barely pernicious[2]. Cosmos has graced us with magnificent botanical wealth and bountiful species of plants. In the prehistoric period, plant based drugs had been broadly used for treating and curing dissimilar illnesses along with inconsistent systems of medication. 80% of globe's denizen still hinges on medicinal plants for their primitive healthcare requirements[3]. The extensive usage of synthetic drugs ensues in antagonistic drug reactions piled with adverse side effects[4], whereas medicinal plants are frequently opted for their outstrip compatibility with the human body as well they pose reduced amount of side effects.

Spices are the architectonic chunks of flavour and taste. They are normally comprised of fibre, carbohydrate, fat, sugar, protein, gum, ash, which are trounced in assisting the process of digestion. When the spices are minced or trampled, the cell matrix ruptures thereby they liberate the volatile compounds. Among the spices, *C. cyminum* is well-known for its aroma and antioxidant properties. It is commonly identified as zeera or jeera, which belongs to the family, Apiaceae. The key phytochemical of the cumin fruit is essential oil, which

constitutes around 2.5% to 4.5% of the entire fruit[5]. Some of the diseases without doubt can be healed by day-to-day intake of their seeds that include: piles, common cold, cancer, insomnia, disorders in skin, respiratory tract, kidney and liver etc. Along with, it regulates the menstrual cycle, promotes the memory power and completes the mental function[6]. Aforesaid impressive effectiveness of cumin forwarded the current study to contemplate the seeds of *C. cyminum* as a prime source to develop iron nanoparticles.

Materials and Methods

Formulation of aqueous seed extract of *C. cyminum*

The fresh seeds of *C. cyminum* were amassed from the Koyambedu market, Chennai. It was progressively suffused in running tap water, latterly in sterile distilled water and was blotted to remove the moisture. Happening after, the seeds were dehumidified for one week at room temperature. 1 g of the sample was decoctioned in sterile distilled water at 70^{o}C for 5 min in hot water bath. The lucid extract was trickled through whatmann filter paper no.1. The bright, yellow hued filtrate, hence hoarded was customized to curtail ferric chloride solution.

Extrication, refinement and characterization of iron nanoparticles

The seed extract of *C. cyminum* was adjoined to 0.1 M ferric chloride solution in the ratio of 1:2. A pre-eminent change of colour was spotted. The as synthesized iron nanoparticle solution (CcFeNPs) was foster enkindled at 70^{o}C and the intensity of color was noted. The CcFeNPs solution was centrifuged at 8,000 rpm for 15 min. The pellets were rinsed for few times in sterile distilled water and drained at 50^{o}C for 2 h in hot air oven. In a while, they were granulated using mortar and pestle to well-formed powder, which were then focused to characterization studies by enlisting Uv Spectroscopy, XRD and FTIR.

Free radical scavenging activity of CcFeNPs

Antioxidant Assay

Antioxidants are the biologically dynamic augments that are used to safeguard our health from cell damage by abolishing the free radicals. The indicated radicals can emanate from chemicals by both synthetic and natural ones that immigrates the body from foods, drinks and adulterated air. They pare the metabolism, which ultimately batter the cells[7]. Thusly, affluent source of antioxidants in everyday life aids us to retard from multitudinal diseases. Sources of antioxidant subsume fruits, vegetables and green leafy vegetables. Predominantly, antioxidant assays benefit to categorize the antioxidant potency of any molecule. At this view, the antioxidant property of CcFeNPs was verified by exhibiting DPPH and ABTS assays.

DPPH assay:

DPPH (2,2 diphenyl-1-picryl-hydarzyl) usually represents as a free radical. Its usefulness as a scavenger is positioned based on the transmission of electrons from one molecule to other molecule. Its crystals are unsolvable in water. When dispersed in ethanol, it comes into view as a violet tinted solution[8]. The use of DPPH proffers a simple and speedy mode to assess antioxidants by spectrophotometry[9]. The rule in the background of the assay is the decrement of DPPH by virtue of the appearance of hydrogen devoting antioxidant[10].

The fundamental protocol for DPPH assay was performed as follows: the solution of DPPH was prepared by using methanol and the OD value of solution was set at 0.630 ± 0.2. Then 50 µl of sample was added to 2 ml of DPPH and incubated for 20 min. The absorbance obtained was checked at 517 nm.

The percentage of inhibition of DPPH activity[11] = [(Abs of blank - Abs of sample) / Abs of blank] x 100.
Where,
Abs of blank = optical density of control,
Abs of sample = optical density of CcFeNPs solution.

ABTS assay

ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] is a cationic complex molecule, which in addition serves as radical scavenger[12]. It is a protonated radical and decreases the proton radical. ABTS is regularly needed in the food industry for determining the antioxidant capabilities of the food. With the summation of sodium per sulphate, the ABTS is altered into a cationic radical[13]. It is a chemical compound, which is valued to discern the reaction kinetics and precise enzymes. The ABTS solution was dissolved in water and diluted with ethanol.

The standard protocol for ABTS assay was performed as follows: the solution OD value was set at 0.700 ± 0.2. Then 20 µl of sample was added to 2 ml of ABTS and incubated for 6 min. The absorbance obtained was checked at 734 nm.

The decrease in abs[11] = [(Abs of blank - Abs of sample) / Abs of blank] *100
Where,
Abs of blank = optical density of control,
Abs of sample = optical density of CcFeNPs solution.

Results and Discussion

Due to the matchless attribute of the nanoparticles, its corporeal status offers us a clue to accord with them[14]. When the seed extract of *C. cyminum* was reckoned to the ferric chloride solution, the production of CcFeNPs can be perceptibly marked-out with the change in colour from light brown to blackish brown associated with the decrease in pH. The sudden transformation was owing to the excitation of Surface Plasmon vibrations of the CcFeNPs in solution (Fig. 1, Table 1). The acidic pH of the extract upholds the evolution of CcFeNPs. It is apparent that it necessitates acidic environment to dwindle to ferric ions.

Table 1: pH analysis of CcFeNPs

S.No.	Sample	Colour of the extract	pH of the seed extract	Colour of the CcFeNPs solution	pH of the CcFeNPs solution
1.	Aqueous seed extract of *C. cyminum*	Light yellow	4.94	Blackish brown	1.48

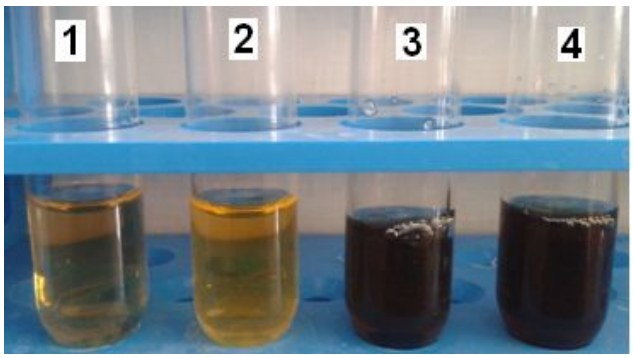

Fig. 1: Synthesis of CcFeNPs from aqueous seed extract of *C. cyminum*

Tube 1 - Aqueous seed extract of *C. cyminum*
Tube 2 - 0.1 M Ferric chloride solution
Tube 3 - Ferric chloride solution + seed extract
Tube 4 - Heating at 70°C, dark brown turns to blackish brown

UV- Vis analysis

The analysis is executed to figure out whether there is a conglomeration of CcFeNPs has arisen or not. UV-Vis analysis is a stage receptive skill, which was implemented to keep an eye on the biopauperization of metallic ferric ions in the solution. A diminutive volume was clutched from the reaction admixture and a spectrum was browsed at a wavelength from 300 nm to 500 nm[15]. The absorption peaks were gained supportively at 220 nm and 272 nm for CcFeNPs (Fig. 2).

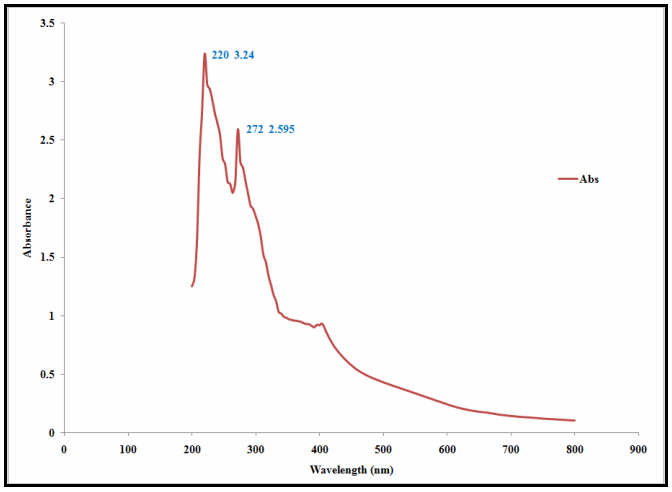

Fig. 2: UV- Vis spectra of CcFeNPs

XRD

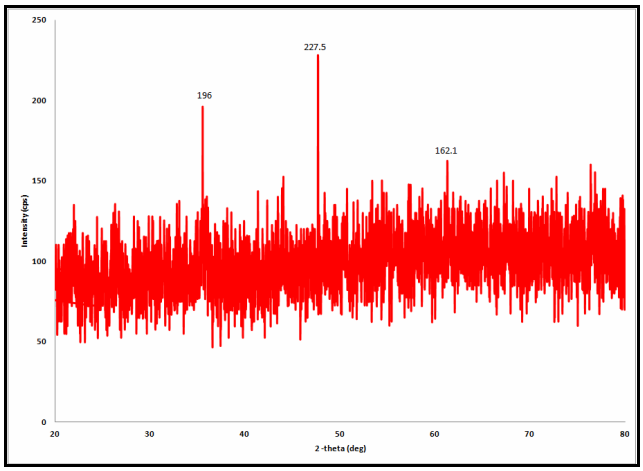

Fig. 3: XRD array of CcFeNPs

The phase detection and crystalline architecture of the CcFeNPs were characterized through the medium of x-ray powder diffraction. The XRD prototype of CcFeNPs was pinpointed to hold strong peaks with 2θ values of 35.2°, 48.5° and 62.3°. Already stated, conform to the planes of (196), (227.5), (162.1), which chronicles the establishment of CcFeNPs as defined crystals (Fig. 3).

FTIR

An infrared spectrum of the CcFeNPs was matriculated using Fourier Transform Infrared Spectroscopy. The atomized sample was packed on the matrices and examined for the incidence of purposeful groups. CcFeNPs were interspersed with impermeable KBr and compressed to a plate in order to identify the interacted functional groups in the biomolecules that are liable for the bioreduction of CcFeNPs. The chief absorption bands formed were attained as follows: The peaks 3420 cm^{-1} and 1052 cm^{-1} signify the characteristic band of O-H group and sulphate groups respectively. The band 665 cm^{-1} and 592 cm^{-1} stand in for the Fe-O stretching. The N-H amide bending group was obtained at the peak 1619 cm^{-1}. The band 1412 cm^{-1} is assigned to the C-C stretching. The band at 801 cm^{-1} indicates the growth of CcFeNPs (Fig. 4). Based on the above mentioned

outcomes, the existence of phenolic compounds and proteins might have conquered opportunity to appear as well to counterbalance the CcFeNPs.

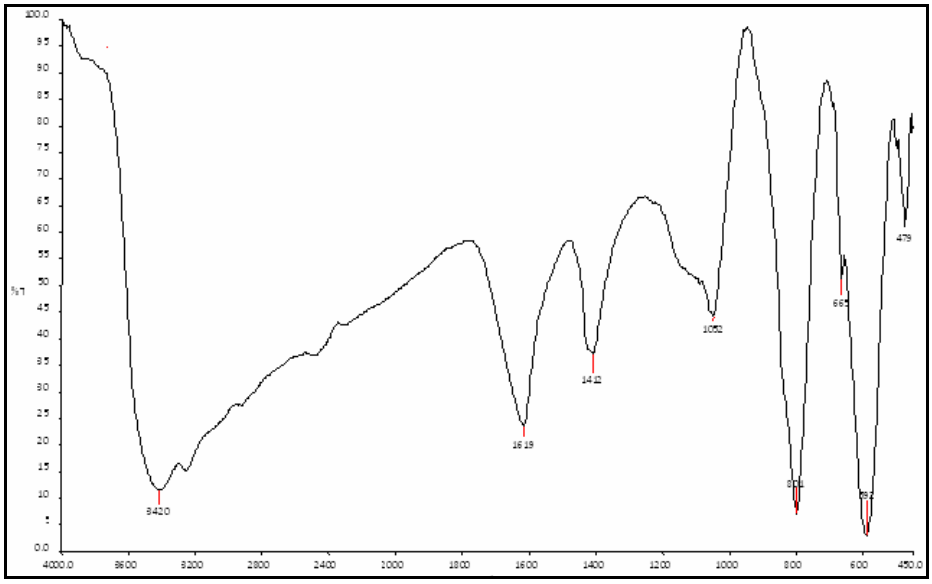

Fig. 4: FTIR scale of CcFeNPs solution

Antioxidant Assay

The antioxidant assays, for instance, DPPH and ABTS were acquired for the CcFeNPs. In the DPPH, the optical density of the reaction mixture obtained at 517 nm was 0.243, which corresponds to 61.4% of scavenging activity, whereas in the ABTS, the optical density of the reaction mixture wangled at 734 nm was 0.132, which resembles to 81.4% of scavenging activity. Both the assays announce that the particle retains antioxidant property. ABTS assay had proclaimed extra scavenging activity than DPPH assay for the treated CcFeNPs (Fig. 5, Table 2).

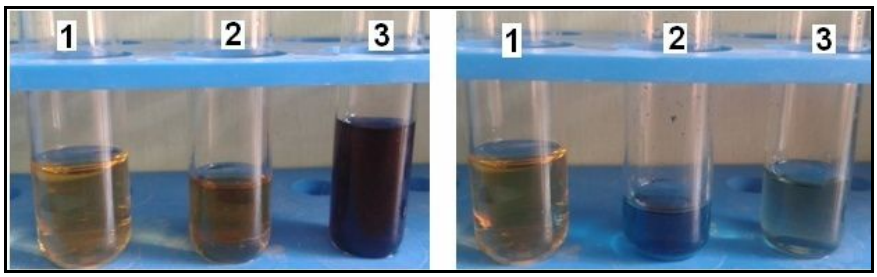

Fig. 5(a): DPPH assay **Fig. 5(b): ABTS assay**

Fig. 5: Antioxidant assays

DPPH assay

Tube 1 - Aqueous seed extract of *C. cyminum*
Tube 2 - DPPH sample (CcFeNPs solution + DPPH solution)
Tube 3 - DPPH solution

ABTS assay

Tube 1 - Aqueous seed extract of *C. cyminum*
Tube 2 - ABTS sample (CcFeNPs solution + ABTS solution)
Tube 3 - ABTS solution

Table 2: Antioxidant assays of CcFeNPs

S.No.	Antioxidant assays	Wavelength (nm)	OD	% of Scavenging
1.	DPPH assay	517	0.243	61.4
2.	ABTS assay	734	0.132	81.4

Conclusion

Iron nanoparticles were fruitfully fabricated by practicing the aqueous, seed extract of *C. cyminum*. Organic solvents were not utilized in the contemporary protocol. Henceforth, it is uncomplicated to synthesize them. The study will be further prolongated to ascertain the surface topology and size of the CcFeNPs.

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