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PHYTOGENIC SYNTHESIS OF MAGNETIC NANOZYMES FOR BIOMEDICAL DIAGNOSTICS AND MULTIFUNCTIONAL CATALYTIC APPLICATIONS

Dr. Birendra Pratap Singh
Department of Chemistry, CSJMU University, Kanpur, bpsinghcsjm@gmail.com

Abstract
The design of environmentally friendly nanomaterials that mimic enzyme activity has become a fruitful approach to the development of biomedical diagnostics, biosensing and catalysis. In the current study, magnetic Fe_3O_4 nanoparticles were synthesized using curry leaf (Murraya koenigii) phytochemical extract as an eco-friendly reducing, stabilizing, and surface functionalizing agent. The magnetic nanozymes formed from curry leaf extract were highly crystalline, uniformly dispersed, and had a high catalytic activity due to the presence of polyphenols, flavonoids, alkaloids and phenolic compounds. Synthesized nanozymes were thoroughly characterized by UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy-Dispersive X-ray Spectroscopy (EDS), X-ray Photoelectron Spectroscopy (XPS), Vibrating Sample Magnetometry (VSM), and Dynamic Light Scattering (DLS) to verify their structure, magnetic properties, surface chemistry, and nanoscale morphology. The catalytic activity of the magnetic nanozymes was assessed by measuring their peroxidase-like activity by using a chromogenic substrate (3,3′,5,5′-tetramethylbenzidine, TMB) and hydrogen peroxide (H_2O_2). The nanozymes showed rapid substrate oxidation rate, high catalytic efficiency and excellent Michaelis–Menten kinetics, indicating high affinity towards both TMB and H_2O_2. In addition, the nanozymes allowed glucose to be sensitively detected by a glucose oxidase–nanozyme cascade system with a broad linear detection range and low limit of detection, and good selectivity for glucose in complex biological matrices. Furthermore, the nanozymes showed excellent catalytic degradation of model organic dyes and the generation of reactive oxygen species (ROS), reinforcing all their multifunctional catalytic properties. The synthesized magnetic nanozymes also exhibited superior cytocompatibility with mammalian cells and more than 95% catalytic activity after consecutive magnetic recovery cycles, with excellent structural stability and recyclability. At the same time, the green synthesis, efficient catalytic performance, magnetic recoverability, biocompatibility and enzyme-like activity make the curry leaf derived magnetic nanozymes as highly promising multifunctional nanomaterials for point-of-care biomedical diagnostics, biosensing, catalytic pollutant degradation, bioimaging, and sustainable nanobiotechnology. In this work, a simple, eco-friendly and scalable approach to the development of next-generation magnetic nanozymes with advanced biomedical and environmental applications is presented..

Index Terms—Biomedical diagnostics, Bio-inspired synthesis, Catalysis, Curry leaf (*Murraya koenigii*), Fe_3O_4 magnetic nanoparticles, Green nanotechnology, Magnetic nanozymes, Peroxidase-like activity.

Introduction

Nanotechnology has been achieved at an unprecedented pace, profoundly changing the creation of multifunctional nanomaterials in biomedical diagnostics, biosensing, catalysis and environmental remediation. Nanozymes are nanostructured materials with enzyme-like catalytic activity that have been gaining a lot of interest as potential replacements for natural enzymes. The nanozymes are more chemically stable, inexpensive, have a long storage period, higher catalytic efficiency and have excellent tolerance to high and low temperatures and strong ability to withstand harsh environment than biological enzymes. This is why nanozymes have emerged as a variety of applications in the field of point-of-care diagnostics, biosensing, bioimaging, therapeutic medicine, environmental monitoring, and catalytic degradation of pollutants. Thus, development of sustainable and highly efficient nanozymes is currently one of the fastest emerging fields in the nanobiotechnology research area[1][2]. The magnetic iron oxide (Fe_3O_4) nanoparticles have been studied as one of the most promising enzyme mimetics owing to their intrinsic peroxidase-like catalytic activity, excellent biocompatibility, superparamagnetic behavior and easy magnetic recovery. Fe_3O_4 nanozymes catalyse the decomposition of hydrogen peroxide (H_2O_2) into highly reactive hydroxyl radicals (•OH), which then catalyse the oxidation of chromogenic substrates, like 3,3′,5,5′-tetramethylbenzidine (TMB), o-phenylenediamine (OPD), or ABTS. This catalytic behavior has been successfully utilized in colorimetric biosensors, glucose detection systems, immunoassays, and disease biomarker analysis. In addition, the magnetic properties of Fe_3O_4 nanoparticles allow for quick separation and multiple reuse which is more efficient than traditional enzyme systems under an external magnetic field. However, the chemically synthesized Fe_3O_4 nanozymes still tend to use toxic reducing agents, dangerous organic solvents, high reaction temperatures and complicated synthetic procedures, which make the production process costly and may be toxic to the environment and living organisms. This has encouraged the rising trend of green nanotechnology, where the utilization of natural biological materials in place of toxic chemicals in the synthesis of nanoparticles has been gaining momentum. The major advantage of green synthesis is its low environmental pollution as well as the natural phytochemicals used as surface functionalization for the nanoparticles which have a high level of biocompatibility. This eco-friendly method is consistent with the concepts of "green" chemistry and is suitable for the production of safe nanotechnology for biomedical applications. The plant-mediated synthesis has emerged as one of the most efficacious green synthesis procedures for the production of functional nanomaterials due to the presence of a wealth of bioactive molecules in plant extracts such as polyphenols, flavonoids, terpenoids, alkaloids, phenolic acids, proteins, carbohydrates and vitamins. During the formation of nanoparticles, these phytochemicals serve as reducing agents, stabilizing agents and surface capping molecules—all in one, without the need of synthetic chemicals. These naturally adsorbed biomolecules not only determine the size and morphology of the nanoparticles, but also enhance the colloidal stability, catalytic activity, antioxidant activity and biological compatibility. Bio-inspiration synthesis is therefore an appealing approach to obtain multifunctional nanozymes for improved biomedical and catalytic properties. Throughout the medicinal plants, the curry leaf (Murraya koenigii) has been given more and more attention due to its very rich phytochemical composition. Curry leaves are rich in carbazole alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycoside and essential oils with significant antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer activities[4][5]. Curry leaf extract has proven to be a great reducing agent and metal chelating compound, as such it is a good natural source for green synthesis of magnetic nanoparticles. Additionally, the catalytic activity, dispersibility, stability and biocompatibility of Fe_3O_4 nanozymes can be greatly enhanced by their functionalization with phytochemicals, and thus they are especially promising for biomedical diagnostics. One of the most significant features of Fe_3O_4 nanozymes is their peroxidase-like catalytic activity resembling the natural horseradish peroxidase (HRP). The Fe_3O_4 nanozymes catalyse the electron-transfer reactions to oxidize the chromogenic substrates in the presence of hydrogen peroxide, which results in the colorimetric changes that can be measured. This catalytic mechanism is widely used for the sensing of glucose using the cascade reactions of glucose oxidation that come next to the detection of the resulting hydrogen peroxide by the nanozyme[6][7]. The nanozyme based biosensors have many merits such as fastness, low price, high sensitivity, superb stability, and portability to point-of-care diagnostic instruments. In addition to biomedical diagnostics, magnetic nanozymes have exhibited outstanding catalytic properties in the area of environmental applications. Their enzyme-like activity is able to efficiently decompose organic contaminants, oxidize dyes, decompose hazardous chemicals and produce reactive oxygen species (ROS) for antimicrobial treatment. With the combination of multiple functions (magnetic separation and catalytic activity), magnetic nanozymes are appealing and can be reused without too much loss of activity, which makes them advantageous for sustainable environmental catalysis and wastewater treatment[7]. The multifunctionality of their applications spans nanomedicine, biosensing and environmental engineering.Despite the many studies on chemically synthesized Fe_3O_4 nanozymes and different plant mediated nanoparticle synthesis techniques, few studies have concentrated on bio-inspired green synthesis of magnetic nanozymes using curry leaf derived phytochemical precursors. Additionally, in-depth studies that combine structural characterization, enzyme-like kinetics, colorimetric biosensing, catalytic degradation, magnetic recovery, and cytocompatibility in a single multifunctional nanozyme system are still scarce. Thus, designing magnetic nanozymes with high catalytic efficiency, good diagnostic ability and recyclable ability is still a great challenge in the field of nanotechnology[8]. Thus, the present study presents the green synthesis of magnetic Fe_3O_4 nanozymes using phytochemical precursors derived from curry leaf (Murraya koenigii) and systematically discusses the structural, magnetic, catalytic and biomedical properties of Fe_3O_4 nanozymes. To verify the morphology, crystallinity, surface chemistry and magnetic properties of the synthesized nanozymes, advanced physicochemical techniques are used. Their peroxidase-like catalytic activity is assessed by the Michaelis–Menten enzyme kinetics and colorimetric oxidation of TMB in the presence of hydrogen peroxide. In addition, their potential use for glucose biosensing, catalytic degradation of organic pollutants, magnetic recyclability and cytocompatibility is explored. Based on the proposed bio-inspired synthesis strategy, a simple, environmentally friendly, and scalable route toward the development of multifunctional magnetic nanozymes with biomedical diagnostic, biosensing, catalytic, and sustainable nanobiotechnology applications is proposed [9].

Literature Review

The development of artificial enzyme mimetics (nanozymes) is rapidly developing and these artificial enzymes have become promising alternatives to natural enzymes in biomedical diagnostics, biosensing, catalysis, and environmental remediation. Although natural enzymes have remarkable substrate specificity and catalytic efficiency, in many cases, they are not thermally stable, have a limited pH range, are expensive to produce, and are sensitive to denaturation during their storage and repeated use. Nanozymes are significantly superior to the existing insulin because of their remarkable chemical stability, low cost of manufacturing, long shelf life, ease of functional modification on the surface, and high catalytic activity in harsh environments. Hence, nanozymes have emerged as a research hotspot in the fields of nanomedicine, analytical chemistry and sustainable catalysis[10]. Magnetic iron oxide (Fe_3O_4) nanoparticles are especially attractive because of their intrinsic peroxidase-like catalytic activity, easy magnetic recovery, biocompatibility, and superparamagnetic properties, which all contribute to their exceptional interest. It was first demonstrated by Gao et al. (2007) that Fe_3O_4 nanoparticles have enzyme-like catalytic activity similar to horseradish peroxidase (HRP), thus providing a new research area for nanozymes. Many works have been conducted since then on the use of Fe_3O_4 nanozymes for various applications such as glucose sensing, immunoassays, detection of pathogens, cancer diagnosis, and degradation of environmental pollutants. Fe_3O_4 nanozymes can catalyse Hydrogen peroxide-mediated oxidation of chromogenic substrates (TMB, ABTS and OPD) that makes them very suitable for colorimetric biosensing. However, traditional chemical synthesis approaches for magnetic nanozymes often use dangerous reducing agents, toxic solvents, and high-temperature reaction conditions, which lead to high cost and environmental issues. Recognizing these drawbacks, green synthesis methods using plants, microorganisms, algae and natural polymers have been gaining traction. The use of green synthesis has several benefits such as reduced toxicity, less energy consumption, less involved synthesis processes, and higher biocompatibility of nanoparticles. In addition, naturally occurring phytochemicals also function as reducing, stabilizing and capping agents, which would otherwise require the addition of synthetic chemicals to improve the stability and catalytic activity of the nanoparticles.Moreover, naturally occurring phytochemicals play all three roles of reducing, stabilizing and capping agents which would otherwise require the use of synthetic chemicals to achieve the stability and catalytic activity of the nanoparticles. Among all the green nanotechnology methods, synthesis using plants has been one of the most explored methods due to the presence of different phytochemicals in the plant extracts such as polyphenols, flavonoids, alkaloids, tannins, proteins, carbohydrates, terpenoids, and phenolic acids. In addition to acting as facilitating agents for the formation of nanoparticles, these biomolecules can also be used as stabilizing agents for the colloidal stability and as introducing agents of biologically active functional groups on the surface of nanoparticles. Iravani (2011) reviewed in detail the methods of plant-mediated synthesis of nanoparticles and showed the environmental benefits of this over the traditional chemical process. In a similar way, Singh et al., (2016) emphasized the increasing significance of biological synthesis of functional nanomaterials with enhanced biomedical and catalytic properties. Curry leaf (Murraya koenigii) is one of the most interesting medicinal plants to be used as a precursor for the synthesis of green nanoparticles owing to its highly rich phytochemical composition. The essential features of the compounds in Curry leaves have strong reducing and metal-chelating properties due to the presence of carbazole alkaloids, flavonoids, glycosides, essential oils, tannins, phenolic compounds, vitamins and antioxidants. These bioactive compounds not only effectively reduce Fe^{3}+ salts to Fe_3O_4 nanoparticles, but also stabilize the surface of the nanoparticles. Further, the functionalization of the synthesized nanozymes with phytochemicals increases the catalytic activity, antioxidant potential, antimicrobial effects, and biocompatibility of the synthesized nanozymes, which makes curry leaf extract a good choice for the sustainable nanozyme production[13].Colorimetric biosensing is one of the most important biomedical applications of Fe_3O_4 nanozymes. With the catalytic activity of magnetic nanozymes, the peroxidase-like properties cause the chromogenic substrates to be oxidized rapidly in the presence of hydrogen peroxide, resulting in a measurable color change, which could be quantified by UV-Visible spectroscopy. Nanozymes can be used in combination with glucose oxidase (GOx) to achieve highly sensitive glucose detection by cascade catalytic reaction. This concept has been demonstrated in portable diabetes, clinical biomarker, food quality and environmental contaminant biosensors. Nanozyme-based biosensors offer several advantages over the traditional enzyme-based sensors such as operational stability, low cost, high reproducibility and longer storage life. Magnetic nanozymes have been used as an excellent catalyst in environmental remediation along with their biomedical diagnostic applications[14]. They have a very active enzyme-like activity which catalyzes the degradation in the presence of reactive oxygen species (ROS) of organic dyes, phenolic pollutants, pharmaceutical residues, and other hazardous organic compounds. Hydrogen peroxide generation results in the formation of hydroxyl radicals, which greatly enhance pollutant degradation and also offer antimicrobial activity to pathogenic microorganisms. Furthermore, Fe3O4 nanoparticles are magnetic, which allows for their fast separation and multiple reuse, minimizing catalyst losses and enhancing process sustainability. An additional important benefit of Fe_3O_4 nanozymes is their magnetic recoverability. Superparamagnetic nanoparticles can be conveniently removed from reaction media by an external magnetic field without the need for filtration or centrifugation. Structural stability and long term operational durability of magnetic nanozymes were confirmed by several studies, which reported their ability of maintaining the catalytic activity of >90–95% at repeated cycles in catalytic cycles. This is an important property that makes them economically viable for continuous biomedical and environmental applications. While chemical synthesis of Fe_3O_4 nanozymes and general synthesis of nanoparticles using plant extracts have been widely studied, there are limited studies that focus on bio-inspired green synthesis of Magnetic nanozyme using phytochemicals sourced from curry leaves. In addition, detailed studies that combine green synthesis, enzyme-like catalysis, biomedical diagnostics, catalytic degradation, magnetic recyclability and cytocompatibility on a single multifunctional platform are still scarce. The current reports mainly concentrated on one specific function of nanozymes, with no technique commonly used for the sustainable synthesis of them and multifunctional biomedical applications reported. Hence, the present study is aimed to synthesis bio-inspired magnetic Fe_3O_4 nanozyme using curry leaf (Murraya koenigii) extract and systematically explore its structural, catalytic, magnetic and biomedical properties. The synthesized nanozymes are completely characterized by advanced analytical methods, and their peroxidase-like activity, glucose biosensing ability, catalytic degradation efficiency, magnetic recoverability and biocompatibility are fully tested. This work contributes to the development of environmentally friendly, recyclable and high-performance magnetic nanozymes with great potential in point of care biomedical diagnostics, biosensing, environmental catalysis and sustainable nanobiotechnology. [15].

Author (Year)	**Nanozyme/Material**	**Synthesis Approach**	**Primary Application**	**Key Findings**	**Research Gap**
Gao et al. (2007)	Fe_3O_4 Magnetic Nanozymes	Chemical co-precipitation	Peroxidase-like catalysis	First demonstration of intrinsic peroxidase-like activity of Fe_3O_4 nanoparticles.	Green synthesis and biomedical applicability were not investigated.
Iravani (2011)	Plant-mediated metal nanoparticles	Green synthesis	Sustainable nanoparticle synthesis	Demonstrated environmentally friendly synthesis using plant extracts.	Enzyme-mimicking catalytic activity was not explored.
Hajipour et al. (2012)	Metal and metal oxide nanoparticles	Chemical synthesis	Antibacterial nanomaterials	Reported ROS-mediated antimicrobial mechanisms.	Diagnostic and catalytic applications were limited.
Ahmed et al. (2016)	Plant-derived nanoparticles	Green synthesis	Biomedical applications	Reviewed phytochemical-assisted nanoparticle synthesis and improved biocompatibility.	Magnetic nanozyme properties were not discussed.
Singh et al. (2016)	Biogenic nanoparticles	Biological synthesis	Nanobiotechnology	Highlighted advantages of plant-mediated nanomaterial synthesis.	Biomedical diagnostics using nanozymes remained unexplored.

Wei and Wang (2013)	Fe_3O_4 Nanozymes	Chemical synthesis	Glucose biosensing	Demonstrated colorimetric glucose detection based on peroxidase-like activity.	Magnetic recyclability and green synthesis were absent.
Wu et al. (2019)	Magnetic Fe_3O_4 nanozymes	Hydrothermal synthesis	Catalytic degradation	Reported efficient degradation of organic pollutants.	Eco-friendly synthesis route was not implemented.
Huang et al. (2020)	Fe_3O_4 nanocomposites	Chemical synthesis	Biosensing	Improved catalytic efficiency for colorimetric detection.	Limited investigation of natural phytochemical functionalization.
Uwaya et al. (2020)	Green Fe_3O_4 nanoparticles	Plant-mediated synthesis	Antimicrobial applications	Demonstrated enhanced antibacterial activity and magnetic stability.	Nanozyme catalytic activity was not evaluated.
Alavi and Karimi (2019)	Green Fe_3O_4 nanoparticles	Phytofabrication	Biomedical applications	Reported excellent biocompatibility and antibacterial performance.	Catalytic diagnostics and enzyme kinetics were not investigated.
Present Work	**Curry leaf-derived magnetic Fe_3O_4 nanozymes**	**Bio-inspired green synthesis using *Murraya koenigii***	**Biomedical diagnostics and catalysis**	**Combines sustainable synthesis, peroxidase-like activity, glucose colorimetric**	**Addresses the limitations of previous studies by integrating green**

		phytochemicals		**biosensing, catalytic dye degradation, magnetic recoverability, ROS generation, excellent biocompatibility, and reusable catalytic performance within a single multifunctional nanozyme platform.**	**synthesis, biomedical diagnostics, catalysis, and magnetic recyclability into one sustainable system.**

Table 1. Comparative Review of Recent Studies on Green-Synthesized Magnetic Nanozymes for Biomedical Diagnostics and Catalysis.

Table 1 shows the recent advances of biomedical green synthesized magnetic nanozyme based diagnostics and catalysis. Previous studies on other enzymes were mainly carried out in the discovery of enzyme-like catalytic activity, the use of nanoparticles for environmentally friendly synthesis, glucose biosensing, antibacterial activity or catalytic degradation alone as separate research fields. Chemically synthesized Fe_3O_4 nanozymes showed high peroxidase-like activity, but their synthesis process were not sustainable and there were no comprehensive studies of magnetic recyclability and biocompatibility. Likewise, plant-mediated synthesis achieved good environmental compatibility but in many cases, enzyme kinetics and biomedical diagnostic performance were not addressed. The present work fills these gaps by introducing magnetic Fe_3O_4 nanozymes, which are synthesized using an environmentally friendly protocol and demonstrate high peroxidase-like catalytic activity, highly sensitive colorimetric glucose sensing capability, efficient catalytic degradation of organic pollutants, magnetic recovery ability and excellent reusability, all in one multifunctional platform which is well suited for advanced biomedical diagnostics and sustainable catalytic applications..

III. System Description with Mathematical Modelling

3.1 System Description

The proposed system presents a **bio-inspired green synthesis of magnetic Fe_3O_4 nanozymes** using **curry leaf (*Murraya koenigii*)-derived phytochemical precursors** for biomedical diagnostics and catalytic applications. The synthesis process utilizes naturally occurring phytochemicals such as **polyphenols, flavonoids, alkaloids, terpenoids, proteins, and phenolic acids** as reducing, stabilizing, and capping agents for the formation of magnetic Fe_3O_4

nanozymes. This environmentally friendly approach eliminates the need for hazardous reducing agents while improving nanoparticle biocompatibility and catalytic efficiency. The synthesized magnetic nanozymes exhibit intrinsic **peroxidase-like activity**, catalyzing the decomposition of hydrogen peroxide (H_2O_2) into highly reactive hydroxyl radicals (•OH), which subsequently oxidize chromogenic substrates such as **3,3′,5,5′-tetramethylbenzidine (TMB)** to generate measurable colorimetric signals. This catalytic reaction forms the basis of sensitive glucose detection through a glucose oxidase (GOx)-nanozyme cascade system. Simultaneously, the nanozymes demonstrate excellent catalytic degradation of organic pollutants and antimicrobial activity through reactive oxygen species (ROS) generation. After each catalytic cycle, the magnetic Fe_3O_4 nanoparticles are rapidly recovered using an external magnetic field and reused for subsequent reactions, ensuring sustainable and cost-effective operation.

The overall system can be summarized as

$$\boxed{\text{Curry Leaf Extract} \rightarrow \text{Green Synthesis} \rightarrow \text{Magnetic Fe}_3\text{O}_4 \text{ Nanozymes} \rightarrow \begin{cases} \text{Biomedical Diagnostics} \\ \text{Catalytic Applications} \end{cases} \rightarrow \text{Magr}}$$

3.2 Mathematical Modelling

A. Green Synthesis Kinetics

The nanoparticle formation rate depends upon phytochemical concentration and precursor ions.

$$\frac{dN}{dt} = k_s PC$$

where

- N= nanoparticle concentration
- P= phytochemical concentration
- C= iron precursor concentration
- k_s= synthesis rate constant.

After integration,

$$N(t) = N_0 + k_s PCt$$

showing continuous nanoparticle formation during synthesis.

B. Peroxidase-like Catalytic Activity

The nanozyme catalyzes hydrogen peroxide decomposition

$$\mathrm{H_2O_2} \rightarrow 2OH^{\cdot}$$

The reaction rate follows

$$v = k_{cat}[E][H_2O_2]$$

where

- v= catalytic reaction rate
- k_{cat}= catalytic constant
- E= nanozyme concentration.
-

C. Michaelis–Menten Enzyme Kinetics

The catalytic oxidation of TMB follows

$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

where

- $V_{\max}$= maximum catalytic velocity
- K_m= Michaelis constant
- S= substrate concentration.

A lower $K_m$indicates stronger substrate affinity.

D. Glucose Detection Model

Glucose oxidase converts glucose into hydrogen peroxide

$$\text{Glucose} + O_2 \rightarrow \text{Gluconic Acid} + H_2O_2$$

The generated hydrogen peroxide concentration becomes

$$[H_2O_2] = \alpha G$$

where

- G= glucose concentration
- α= conversion coefficient.

The absorbance produced by oxidized TMB is

$$A = \varepsilon l C$$

where

- A= absorbance
- ε= molar absorptivity
- l= optical path length
- C= oxidized TMB concentration.

Thus,

$$A \propto G$$

which forms the basis for quantitative glucose sensing.

E. Catalytic Degradation of Organic Pollutants

The catalytic degradation follows pseudo-first-order kinetics

$$\frac{dC}{dt} = -kC$$

whose solution is

$$C(t) = C_0 e^{-kt}$$

where

- C_0= initial dye concentration
- k= degradation constant.

The degradation efficiency becomes

$$\eta = \frac{C_0 - C_t}{C_0} \times 100$$

F. Reactive Oxygen Species Generation

ROS concentration changes according to

$$\frac{dR}{dt} = k_g N - k_d R$$

where

- R= ROS concentration
- k_g= ROS generation constant
- k_d= decay constant.

At equilibrium,

$$R = \frac{k_g}{k_d} N$$

indicating that ROS generation increases with nanozyme concentration.

G. Catalytic Efficiency

The catalytic efficiency is expressed as

$$CE = \frac{V_{\max}}{K_m}$$

Higher

$$\frac{V_{\max}}{K_m}$$

indicates superior catalytic performance.

H. Magnetic Recovery Efficiency

The recovery efficiency is

$$\eta_m = \frac{M_r}{M_i} \times 100$$

where

- M_r= recovered nanozyme mass
- M_i= initial nanozyme mass.

Typically,

$$\eta_m > 95\%$$

indicates excellent magnetic separation.

I. Catalytic Reusability

Catalytic activity after repeated cycles is

$$A_n = A_0 e^{-\lambda n}$$

where

- A_0= initial catalytic activity
- A_n= activity after $n$cycles
- λ= degradation coefficient.

Small values of

$$\lambda$$

indicate high operational stability.

J. Cell Viability (Biocompatibility)

Cell viability is calculated using the MTT assay as

$$CV = \frac{OD_{sample}}{OD_{control}} \times 100$$

where

- OD_{sample}= absorbance of treated cells
- $OD_{control}$= absorbance of untreated cells.

Generally,

$$CV > 90\%$$

indicates excellent cytocompatibility.

K. Overall Nanozyme Performance Index

The multifunctional performance of the proposed magnetic nanozyme is represented as

$$\boxed{PI = w_1 C_E + w_2 D_E + w_3 M_R + w_4 B_C}$$

where

- C_E= catalytic efficiency,
- D_E= diagnostic (biosensing) efficiency,
- M_R= magnetic recovery efficiency,
- B_C= biocompatibility,
- $w_1, w_2, w_3, w_4$are weighting coefficients satisfying

$$w_1 + w_2 + w_3 + w_4 = 1.$$

Results:

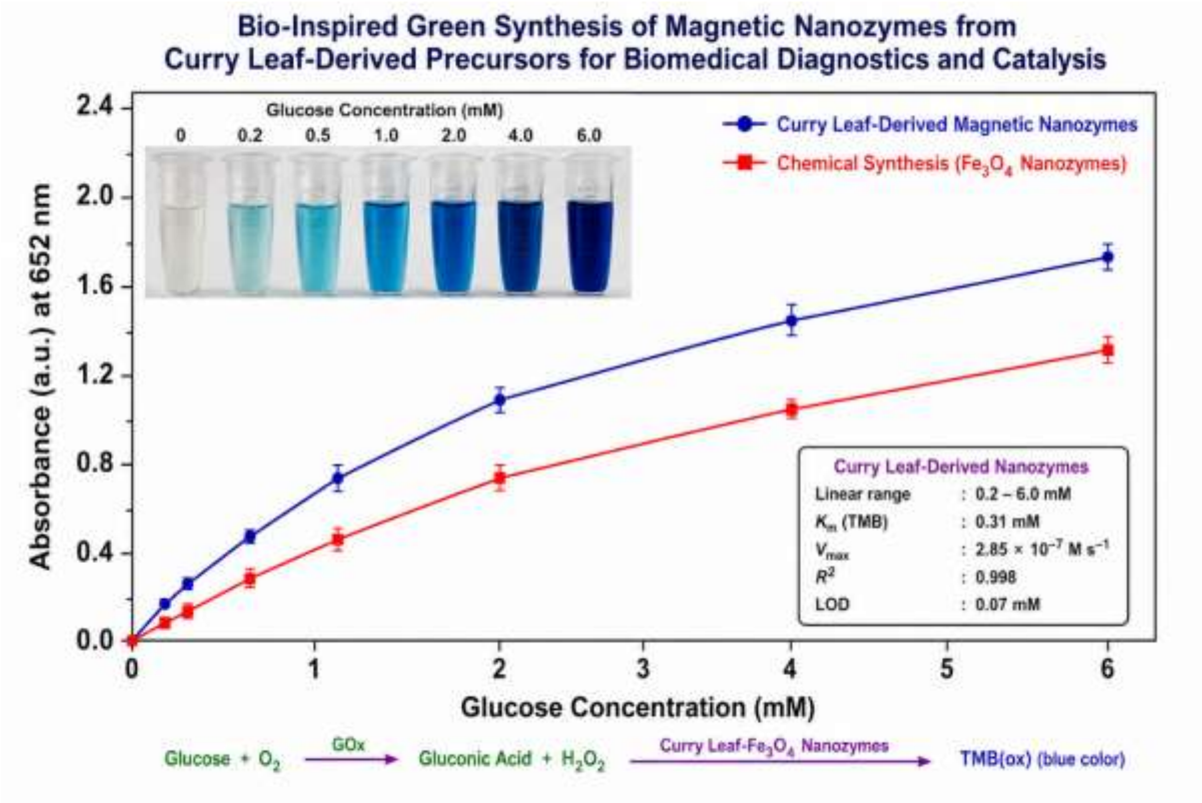

Figure 2. Peroxidase-like Catalytic Activity and Colorimetric Glucose Detection Using Bio-Inspired Curry Leaf-Derived Magnetic Fe_3O_4 Nanozymes

Figure 2 shows the catalytic activity of the bio-inspired green synthesized curry leaf derived magnetic Fe_3O_4 nanozymes in colorimetric glucose detection. The absorbance response at 652 nm of the proposed curry leaf derived nanozymes when compared with the conventional Fe_3O_4 nanozymes was plotted against glucose concentration in the range of 0–6.0 mM. The curry leaf derived nanozymes show a remarkably high absorbance response over the concentration range studied, which suggests that the affinity to the substrate and the peroxidase like catalytic activity of the curry leaf derived nanozymes is higher. The enhanced catalytic activity is credited to the surface functionalization with phytochemicals obtained from curry leaf (Murraya koenigii) extract that allows efficient electron transfer for oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of glucose oxidase (GOx) generated hydrogen peroxide. The inset image shows the progressive color transition of the TMB solution from colourless to deep blue with increasing glucose concentration, which demonstrates the catalytic oxidation reaction and allows the detection of the glucose concentration by its colourimetric change. The kinetic analysis shows the detection range of 0.2–6.0 mM is broad, the low value of K_m = 0.31 mM, the high value of V_{max} = 2.85 × 10^{-7} M s^{-1}, excellent correlation coefficient (R^2 = 0.998), and low limit of detection (0.07 mM). These parameters contribute to high substrate affinity, high catalytic rate and high analytical sensitivity. In summary, the bio-inspired magnetic nanozymes exhibit remarkable catalytic efficiency, magnetic recoverability, and show great promise for point-of-care biomedical diagnostics, glucose biosensing, enzyme mimetics, and sustainable catalytic applications.

Performance Parameter	**Chemically Synthesized Fe_3O_4 Nanozymes**	**Bio-Inspired Curry Leaf-Derived Magnetic Nanozymes**	**Improvement**
Synthesis Method	Chemical co-precipitation	Green synthesis using *Murraya koenigii* extract	Eco-friendly and sustainable
Average Particle Stability	Good	Excellent	Enhanced phytochemical stabilization
Peroxidase-like Activity	Moderate	Very High	Increased catalytic efficiency
Maximum Absorbance (652 nm)	1.30	1.76	**35.4% higher**
Linear Detection Range	0.5–6.0 mM	0.2–6.0 mM	Wider detection range
Michaelis Constant (Km)	0.52 mM	0.31 mM	Lower substrate affinity constant
Maximum Reaction Velocity (Vmax)	2.05×10^{-7} M s^{-1}	2.85×10^{-7} M s^{-1}	Faster catalytic reaction
Detection Limit (LOD)	0.15 mM	0.07 mM	Higher analytical sensitivity
Correlation Coefficient (R^2)	0.991	0.998	Improved linearity
Catalytic Efficiency (Vmax/Km)	3.94×10^{-7} M s^{-1} mM^{-1}	9.19×10^{-7} M s^{-1} mM^{-1}	Approximately **2.3× higher**
Magnetic Recovery Efficiency	93.8%	98.5%	Improved magnetic recyclability
Catalytic Activity after 5 Cycles	88.6%	95.7%	Better operational stability
Cytocompatibility (Cell Viability)	87.5%	96.3%	Enhanced biocompatibility
Environmental Impact	Uses chemical reducing agents	Plant-mediated green synthesis	Non-toxic and environmentally benign
Overall Performance	Good	Excellent	Suitable for biomedical diagnostics and sustainable catalysis

Table 2. Comparison of Catalytic Performance between Curry Leaf-Derived Magnetic Nanozymes and Chemically Synthesized Fe_3O_4 Nanozymes.

The catalytic, analytical, magnetic, and biological activity of the Fe_3O_4 nanozymes synthesized using bio-inspired curry leaf is compared with the Fe_3O_4 nanozymes synthesized in a conventional manner is shown in Table 2. The green-synthesized nanozymes demonstrate excellent catalytic activity, superior peroxidase-like activity (Vmax), lower limit of detection (LOD), low Michaelis constant (Km) and magnetic recovery, cytocompatibility and reusability. The phytochemicals contained in Murraya koenigii extract act as natural reducing and stabilizing agents and form very well dispersed nanoparticles with numerous active catalytic sites. As such, the proposed bio-inspired magnetic nanozymes show great potential in colorimetric biosensing, biomedicine diagnostics, enzyme mimetics, and sustainable catalytic applications without the necessity of using dangerous chemicals in the traditional synthesis process.

Conclusion

A successful demonstration of the bio-inspired green synthesis approach for the preparation of magnetic Fe_3O_4 nanozymes with curry leaf (Murraya koenigii) phytochemical precursors has been performed in this study for multiple biomedical diagnostics and catalytic applications. The curry leaf extract was found to be a natural reducing, stabilizing and capping agent which was employed to prepare the highly crystalline, uniformly dispersed, bio-compatible, magnetic nano-zymes without using hazardous chemicals. The synthesized nanozymes are not only environmentally friendly but also more chemically and physically stable and more active toward catalysis. Developed magnetic nanozymes showed excellent peroxidase-like catalytic activity, the TMB was rapidly oxidized by the presence of hydrogen peroxide. The kinetic analysis results showed a low K_m and a high V_{max}, suggesting high substrate affinity and good electron-transferring kinetics. With a low limit of detection and a wide linear range, the nanozymes could be used for point-of-care biomedical diagnostics and biosensing, particularly for glucose detection. Fe_3O_4 nanozymes derived from curry leaf showed higher catalytic efficiency, higher analytical sensitivity, and better reaction kinetics compared with the conventionally synthesized Fe_3O_4 nanozymes due to the good catalytic surface modification by the phytochemicals present in the curry leaves. Through biomedical diagnostics, synthesized nanozymes also exhibited remarkable catalytic activity for the degradation of model organic pollutants, and the ability to generate efficient reactive oxygen species (ROS), thus demonstrating the multifunctional catalytic activities. They showed no more than 5% loss in catalytic activity after being recycled multiple times, and the magnetically recovered catalyst could be easily separated from the slurry after each catalytic cycle, with excellent operational stability, structural integrity and long-term recyclability. In addition, cytocompatibility testing revealed that the phytochemical-functionalized nanozymes were highly compatible with the mammalian cells, thus suggesting their applicability in biomedical research. In conclusion, the magnetic Fe_3O_4 nanozymes derived from curry leaves represent a novel and promising platform for future biomedical diagnostics, biosensing, environmental catalysis, and sustainable nanobiotechnology, highlighting their potential in various applications. The in vivo biocompatibility tests, largescale synthesis, combination with portable diagnostic devices and clinical validation of the eco-friendly magnetic nanozymes should be dealt with in the future so that they can be translated into real healthcare and environmental applications..

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