Biosynthesis of ZnO nanoparticles using *Hagenia abyssinica* leaf extracts; their photocatalytic and antibacterial activities

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**ABSTRACT**

This study investigates the green synthesis of ZnO NPs using an aqueous solution of *hagenia abyssinica* leaf extract, which acts as a reduction of zinc acetate dihydrate as a reducing agent and stabilizing agent. The synthesized ZnO NPs were characterized by various techniques such as XRD, SEM, FTIR, and UV–Vis spectroscopy. The XRD pattern confirmed that the hexagonal crystalline phase structure and average crystal size of the synthesized nanoparticle was 27.833 nm. Several functional groups were detected using the Fourier transform infrared (FTIR) method. Within 120 minutes of sunlight irradiation, 83.17% photocatalytic degradation of MO was recorded, with an initial concentration of 15 ppm and a catalyst dosage of 40 mg. The present study shows a novel, eco-friendly method to synthesize zinc oxide nanoparticles that have potential applications in water treatment and dye degradation. The disc diffusion method was used to determine antibacterial activity against gram-positive (*S. aureus* and *S. epidermidis*) and gram-negative (*E. coli* and *K. pneumoniae*) bacterial strains. The biosynthesized ZnO nanoparticles were highly effective against *S. epidermidis* with inhibition zones of 21 ± 1.0 mm at 30 mg/mL and less effective against *E. coli* with inhibition zones of 16 ± 1.0 mm at 10 mg/mL.

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1. Introduction

Waste-water contaminants from fabric, printing, and manufacturing, as well as other industrial dye contaminants, harm the ecosystem, posing environmental and human health problems [1]. Organic dyes are widespread contaminants in wastewater and are particularly hazardous to the environment. Organic dyes are the main source of contamination in the textile and food industries. Pollutants are also extremely hazardous, as they cause cancer in both humans and natural sources [2].

Nowadays, biomedical nanomaterials have received more concerns because of their notable biological properties and biomedical applications. Metal oxide nanoparticles have a wide range of applications in the biomedical area, including antibacterial, anticancer, drug transport, cell imaging, and biosensing [3]. ZnO nanoparticles have strong antibacterial activities, due to their strong surface volume ratio, allowing germs to disperse and penetrate more easily [4]. Metal oxide nanoparticles may cause harm by releasing reactive oxygen species (ROS) and causing oxidative stress [5]. Zinc oxide nanoparticles are widely used in a variety of industries due to their unique properties, which include anti-corrosion, anti-bacterial, photo-catalytic, antioxidant, low electron conductivity, and high heat resistance [6].

Photocatalysis has been successfully used to remove pollutants from water and wastewater in large quantities [7,8]. Zinc oxide nanoparticles have been synthesized by physical and chemical methods, however, these approaches are toxic and environmentally dangerous compounds [9]. To overcome this problem, several technologies have been developed to remove dye from wastewater such as adsorption [10–12], photocatalysis [13–15], and advanced oxidation process [16]. Among all these treatments, photocatalysis has received attention due to its high efficiency, environmentally friendly, and easy operation [17].

Among the plants that have been a green synthesis of ZnO NPs studied by various plant extracts such as *passiflora foetida* fruit [18], *Averrhoa carambola* fruits [19], *cannabis sativa* leave [20], *peltophorum pterocarpum* leaf [21] and *Carissa edulis* fruits [22]. In comparison to chemical methods, the green synthesis method has several advantages, such as less time consumption, low-cost precursors, and high-purity product; the handling procedure is very simple and does not require any expensive equipment.

*Hagenia abyssinica* is a flowering plant that is native to the high-elevation Afarmontane regions of central and eastern Africa, ranging from Sudan to Ethiopia. It is called African redwood and East African rosewood in...
English, and kosso in Amharic [23]. Hagenia abyssinica is a monotypic genus in the Rosaceae family. It was once common in Ethiopia’s semi-humid highland forests between 2450 and 3250 m above sea level. It is mainly found in a mountainous area of Ethiopia’s central, south-western, and south-eastern regions [24]. In Ethiopia, the society used the *Hagenia abyssinica* leaves to treat diarrhea, typhoid, cough, livestock diseases, healing injured, tapeworm and wound [25].

This paper describes a novel, simple and cost-effective technique for the biosynthesis of ZnO nanoparticles. Because the synthesis method is based on the green *Hagenia abyssinica* extract, it avoids the use of hazardous chemicals, making the end product more biocompatible and safe. The biosynthesized ZnO NPs are more effective in photocatalytic and antibacterial activities against methyl orange dye compared to chemical synthesized methods. To the best of our knowledge, this will be the first study to demonstrate the biosynthesis, photocatalytic and antibacterial activities of ZnO NPs produced from *Hagenia abyssinica* leaf extract.

### 2. Material and method

#### 2.1. Materials

*Hagenia abyssinica* leaves were collected from Debre Markos University, Ethiopia. Zinc acetate dihydrate, sodium hydroxide, methyl orange, ethanol, and deionized water. All of the chemicals used were analytical grade and were used without further purification.

#### 2.2. Preparation of extract

The leaves of the *hagenia abyssinica* were washed several times with distilled water used to remove dust particles, and the remaining moisture was sun-dried. The dried leaves were ground into powder and sieved using 355 μm sieves. 20 g powdered leaves were mixed with 100 ml double distilled water and heated to 75°C for 60 minutes and stirred in the solution using a magnetic stirrer at 700 rpm. After cooling at room temperature, the extract was filtered with Whatman No. 1 filter paper and the supernatant was taken for further experimentation, as described in Figure 1.

#### 2.3. Green synthesis of ZnO nanoparticles

The leaves of *Hagenia abyssinica* were collected and washed using distilled water, then dried in the shade to remove any remaining moisture. 90 ml of *Hagenia abyssinica* plant leaf extract, 60 ml of 5.9265 g of zinc acetate dihydrate, and 60 ml of 1.08 g sodium hydroxide were mixed with a (3:2:2) ratio. The mixture was still forming yellow-colored precipitate after 2 h of stirring at 800 rpm. The precipitates were washed in ethanol and distilled water to eliminate contaminants before being dried in an oven at 100°C for 8 h. The green synthesized ZnO NPs powder was calcined in a furnace at 400°C for 2 h [26].

#### 2.4. Characterization

The crystalline structure of ZnO NPs was determined by XRD and evaluated by (XRD-7000, Shimadzu Corporation, Japan). An x-ray diffractometer with Cu Kα radiation (1.5406 Å) was used to accomplish the diffraction within a 2θ range of 20° to 80°. Operating voltage 40kv and current is 30 mA with a divergence slit of 1-degree continuous scanning mode. The optical properties of the UV–Vis spectrometer were done using (Shimadzu-UV 1800 spectrometer) from the range of 200–800 nm. The FTIR was used to determine different functional groups in the KBr phase using a (JASCO FTIR-6800) instrument between 4000 and 400 cm⁻¹. The surface morphology was analyzed by scanning electron microscopy (SEM) using the instrument (JCM-6000 PLUS Benchtop SEM, JEDL, Japan).

![Diagram of the preparation of the hagenia abyssinica extracts.](image)
2.5. Photocatalytic activity

In this investigation, we analyzed the photodegradation of organic dyes, one of the dyes is MO, and this dye is a toxic azo dye mostly present in textile, leather, and paper industries. The photocatalytic tests were done by sunlight irradiation. The degradation of the MO was followed in a 50 ml solution of six different dye concentrations (5, 10, 15, 20, 25 and 30 mg/L) and the ZnO catalyst dose (10, 20, 30, 40, 50 and 60 mg) were used. The decolorization efficiency of MO dye was evaluated using a UV–Vis spectrophotometer at $\lambda_{\text{max}}$ = 464 nm. In this experiment, 50 ml of MO dye solution of 15 ppm was taken in a quartz tube with different doses of ZnO (10, 20, 30, 40, 50 and 60 mg) nanoparticles was added as a catalyst to the investigation of photocatalytic activity and was stirred for 30 min in the dark before irradiation. Then, the solution mixture was exposed to sunlight for 2 h. 10 ml of the samples were withdrawn at 30 minutes at a regular time interval. Before measuring the absorbance, the suspension was centrifuged at 800 rpm for 5 minutes and filtered to remove the catalyst particles. The absorbance at $\lambda_{\text{max}}$ = 464 nm was recorded using the UV–Vis spectrophotometer. The % of degradation of dyes was determined using equation (1) [27].

$$\% \text{ degradation} = \left( \frac{C_0 - C_t}{C_0} \right) \times 100\%$$  \hspace{1cm} (1)

Where $C_0$- the initial concentration of MO at time $t = 0$
$C_t$- the concentration of MO at different time $t$

2.6. Antibacterial activity

In a solution of biosynthesized ZnO nanoparticles, the discs were immersed in distilled water. The disc diffusion method was used to evaluate the antibacterial activity of biosynthesized ZnO nanoparticles against two gram-positive ($S.\ \text{aureus}$ and $S.\ \text{epidermidis}$) and two gram-negative ($E.\ \text{coli}$ and $K.\ \text{pneumoniae}$) bacteria. As a control, the TTC standard was used. After that, the biosynthesized ZnO nanoparticles (10, 20 and 30 mg/ml) and TTC standard were placed on a disc and incubated at $37^\circ\text{C}$ for a day. To estimate the antibacterial activity, the diameter (mm) of the inhibitory zone around the wells was measured [28].

3. Results and discussion

3.1. XRD analysis

The XRD patterns show that the green synthesized ZnO nanoparticles were free of impurities as shown in Figure 2. The XRD pattern had the characteristics of diffraction peaks seen at 2θ values of 31.78°, 34.46°, 36.28°, 47.56°, 56.62°, 62.90°, 66.46°, 67.98°, 69.14°, 72.54° and 77.60° along with their Miller index plane of (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202), respectively. The results confirm that the hexagonal crystal structure of zinc oxide and the peaks exhibited in the XRD pattern spectrum were similar to other reports studied [29]. There was no additional peak in the XRD pattern, indicating that the synthesized NPs were of high purity [30,31].

The measured diffraction reflections from the XRD pattern are completely in accordance with the standard of (JCPDS: 36–1451) ZnO NPs [26]. The crystal particle size can be calculated from the FWHM of the highest intensity and sharp peak, which corresponds to (101) using the Debye Scherrer formula [31]. By using equation (2) the calculated value of the ZnO particle size from the

![Figure 2. XRD of ZnO nanoparticles.](image-url)
XRD pattern was found to be 27.833 nm. As shown in Table 1, there are different structural parameters from XRD.

\[
D = \frac{0.89\lambda}{\beta \cos \theta}
\]

(2)

Where 0.89- Scherrer’s constant
\(\lambda\) - X-ray wavelength (1.5406A)
\(\beta\) - FWHM (full width at half maximum)
\(\theta\) - Bragg’s angle of diffraction

### 3.2. FTIR analysis

Peaks at 3437.49, 2345.01, 2077.92, 1623.76, 1384.63, 1104.04, 617.10, 485.97, and 428.11 cm\(^{-1}\) were observed in the FT-IR study of biosynthesized ZnO nanoparticles (Figure 3). The broad peak around 3437.49 cm\(^{-1}\) corresponds to hydroxyl O-H stretching of alcohols and phenols. The peak around 2345.01 cm\(^{-1}\) represented the triple bond of C and N stretching of nitrile groups and the peak at 2077.92 cm\(^{-1}\) assigned to the C and C triple bond of stretching. The bands at 1623.76 cm\(^{-1}\) indicate the presence of primary amines and the peaks at 1384.63 cm\(^{-1}\) was assigned to C-O bond stretching [32]. The peaks observed at 428.11, 485.97 and 617.10 cm\(^{-1}\) could correspond to stretching of Zn-O vibrations [33].

#### 3.3. Photocatalytic Activity

Figure 4 shows the effect of varying ZnO catalyst dosages, with varied photo-catalyst concentrations ranging from 10 to 60 mg while keeping the initial methyl orange dye concentration constant (10 ppm). To understand the effect of catalyst dosage, the other conditions were kept constant. When the catalyst dosage increased from 10 mg to 40 mg the photodegradation of methyl orange increased from 36.82% to 83.17% within 120 minutes, but if we further increased the photo-catalyst dosage from 40 mg to 60 mg, the degradation of methyl orange decreased from 83.17% to 80.99% at 2 h. Other researchers also reported the effect of catalyst dosage on the degradation of MO using Eucalyptus Globulus and Calotropis Procera leaves with catalyst doses of 30 mg and 1.5 g/dm\(^3\) and its degradation efficiency of 97.3% and 81% under UV-light, respectively [34,35].

Under sun-light irradiation, the effect of ZnO catalyst dosage on MO degrading activity was studied. Figure 4 shows that the amount of catalyst was changed from 10 to 60 mg, with the highest photodegradation activity of methyl orange observed at 40 mg of catalyst dose. When the catalyst dose is increased from 20 mg to 40 mg the degradation increases rapidly up to 83.17% within 2 h, and the
degradation efficiency of methyl orange decreases when increased the dose above 40 mg. Increasing the amount of ZnO catalysts above the optimum value may result in the aggregation of ZnO catalysts. The first reason is that the active sites in the catalyst are rendered inaccessible to light absorption [36]. The other reason is that when the solution becomes turbid, the activity of ZnO activation during the sunlight irradiation decreases [37].

Figure 5 shows the photo-degradation activity of MO at different catalyst doses of ZnO (10–60 mg), while the initial dye concentration of MO remains constant (15 mg/L). When the catalyst dose of ZnO was increased from 10 to 40 mg, the decolonization performance of MO enhanced from 36.15% to 82.83%, but the degradation slightly decreased from 82.83% to 79.64% within a period of 120 minutes.

As shown in Figure 6, the optimum amount of initial concentration is described. The initial dye concentration affects the degradation activity, with dye concentrations varying from 5 to 30 ppm at a constant ZnO catalyst dose of 40 mg. The degradation of MO rose to 15 ppm when the dye concentration increased but by increasing the initial concentration to more than 15 ppm the degradation efficiency decreased, this is because the photon cannot reach the catalyst surface.

Figure 4. The effect of catalyst dose on the photocatalytic degradation of MO dye (pH = neutral, MO = 15 mg/L).

Figure 5. The effect of ZnO catalyst dosage on the degradation of MO dye under sunlight at the initial concentration of 15 ppm.
As the initial dye concentration increases, the number of active sites lowers the formation of radicals and which leads to decrease degradation \[38\]. Other investigations also studied the effect of initial concentration on degradation of MO using *Eucalyptus Globulus* and *Calotropis Procerca* leaves with its initial dose of 10 and 20 ppm, its degradation efficiency of 97.3% and 81% under UV-light, respectively \[34,35\]. In Table 2, there are comparisons of earlier work done on MO dye degradation, with a recent report using zinc oxide nanoparticles. There are also several pieces of researches in the literature that report on the degradation efficiency of organic dyes using chemically synthesized methods \[39–43\].

**Figure 6.** The effect of the initial concentration of MO dye on the photocatalytic degradation.

**Table 2.** The comparison of previous work done on MO dye degradation, with a recent report using zinc oxide nanoparticles.

| S.No | Plant extract                  | Catalyst dose | Dye pollutant | Dye conc. | Irradiation source | D%  | Time (min) | Ref  |
|------|--------------------------------|---------------|--------------|-----------|--------------------|-----|------------|------|
| 1.   | *Chlamydomonas Reinhardtii*    | 50 mg         | MO           | 10 ppm    | Sun-light          | 90  | 120        | [44] |
| 2.   | *Eucalyptus Globulus*          | 30 mg         | MO           | 10 ppm    | UV-light           | 97.3| 60         | [34] |
| 3.   | *Pullulan*                     | 150 mg        | MO           | 10 ppm    | UV-light           | 97  | 300        | [45] |
| 4.   | *Calotropis Procerca*          | 1.5 g/dm³     | MO           | 20 ppm    | UV-light           | 81  | 100        | [35] |
| 5.   | *Nephelium Lappaceum*          | 100 mg        | MO           | 10 ppm    | UV-light           | 84  | 120        | [42] |
| 6.   | *Abelmoschus Esculentus*       | 175 mg        | MB           | 32 ppm    | UV-light           | 100 | 60         | [46] |
| 7.   | *Artocarpus Gomezianus*        | 50 mg         | MB           | 5 ppm     | Sunlight           | 60  | 100        | [47] |
| 8.   | *Hagenia Abyssinica*           | 40 mg         | MO           | 15 ppm    | Sun-light          | 83.17| 120        | Present work |

**Figure 7.** The bandgap using tauc’s plot.
3.3.1. UV-Vis diffuse absorption spectrophotometer

As shown in Figure 8, the UV–Vis region was used to determine the optical absorption properties of green synthesized ZnO nanoparticles; Figure 8 shows the optical absorption spectra of ZnO NPs in the region of 200–800 nm at room temperature. The UV–Vis absorption spectra of MO degradation using Sunlight with 40 mg of ZnO NPs catalyst. The highest intensity of the organic pollutant (dye) of MO is 464 nm; however, as the time of degradation rises, this peak decreases. Equation 3, was used to compute the bandgap of the NPs.

\[(\alpha h\nu)^2 = A(\nu - E_g)^n\]  

In this equation, \(n\)-direct/indirect transition semiconductor, \(\alpha\)-absorption coefficient, \(h\)-Plank’s constant, \(\nu\)-Light frequency, \(E_g\)-bandgap energy and \(A\) is Constant.

As described in Figure 7, it can be determined the bandgap energy by extra-plotting of Tauc’s plot \(h\nu\) in the x-axis vs \((\alpha h\nu)^2\) in the y-axis [30]. From Tauc’s plot, the bandgap energy of the synthesized Zinc Oxide NPs was 3.21 eV.

3.3.2. Mechanism of photocatalytic degradation

Scheme 1 shows the proposed MO degradation mechanism. Many researchers have described the photo-degradation mechanism of ZnO nanoparticles. Organic dye was decolorized using ZnO nanoparticles in the following way [48]: First, the organic pollutants diffuse to the surface of ZnO NPs from the liquid phase, and second organic pollutants adsorbed on the surface of ZnO NPs, followed by oxidation and reduction in the adsorbed phase.

Finally, the products were removed from the interface region. The photocatalytic activity of reactive species (\(\text{OH}^-\) and \(\text{O}_2^-\)) can be formed by the interaction between catalysis and sunlight irradiation, and these reactive species interact with organic dyes resulting degradation of organic pollutants [49]. The photocatalytic efficiency of nanoparticle catalysts is higher than that of the normal photocatalysts; this could be due to a higher surface-to-volume ratio [50].

The \(e^-\) reacts with oxygen at the conduction band to generate \(^{\cdot}\text{O}_2^-\) and these species degrade the organic dye methyl orange. The dye methyl orange can react with \(h^+\) (an oxidant) to degrade this dye [51]. At the valence band \(h^+\) reacts with methyl orange, and some of it reacts with \(\text{H}_2\text{O}\) and \(\text{OH}^-\) and forms \(\cdot\text{OH}\). All these radicals decolorized the dye methyl orange to \(\text{CO}_2\) and \(\text{H}_2\text{O}\) [52]. The equation describes the photo-degradation reactions, which give \(\text{CO}_2\) and \(\text{H}_2\text{O}\) as the products of the decolorization as studied by various scholars [53].

\[
\begin{align*}
\text{ZnO} + h\nu &\rightarrow \text{ZnO} (e^-_{\text{CB}} + h^+_{\text{VB}}) \\
h^+_{\text{VB}} + \text{H}_2\text{O} &\rightarrow \cdot\text{OH} + \text{H}^+ \\
e^-_{\text{CB}} + \text{O}_2 &\rightarrow \text{O}_2 \\
\cdot\text{OH} + h^+_{\text{VB}} &\rightarrow \cdot\text{OH}
\end{align*}
\]

\(\cdot\text{OH} + \text{Organic pollutants (MO)} + \text{O}_2 \rightarrow \text{Organic ions} + \text{CO}_2 + \text{H}_2\text{O}\)

3.4. Scanning electron microscopy (SEM) analysis

ZnO synthesized NPs with hagenia abyssinica leaf extract were recorded for SEM images using drop coated. The morphology characteristic of the synthesized ZnO NPs was studied using SEM analysis. Figure 9 is a SEM image of ZnO nanoparticles. The reports indicate that the SEM image of green synthesized ZnO has a rod shape. The SEM image observed that there was more agglomeration with a large area; it may be because of the high extract concentration.

3.5. Antibacterial activity

The antibacterial activity of biosynthesized ZnO nanoparticles against gram-positive (\(S.\) \text{aureus} and \(S.\) \text{epidermidis}) and gram-negative bacteria (\(E.\) \text{coli} and \(K.\) \text{pneumoniae}) was evaluated using the disc diffusion method at three different concentrations (10, 20, and 30 mg/mL). As indicated in (Figure 10 and Table 3), biosynthesized ZnO nanoparticles illustrated effective antibacterial activity in both gram-positive (\(S.\) \text{aureus} and \(S.\) \text{epidermidis}) and gram-negative (\(E.\) \text{coli} and \(K.\) \text{pneumoniae}) bacterial strains. The highest zone of inhibition (21 ± 1.0) was obtained against \(S.\) \text{epidermidis} at 30 mg/mL, while the lowest antibacterial activity was obtained against \(E.\) \text{coli} at 10 mg/mL of biosynthesized ZnO nanoparticles.
3.6. Kinetic studies of photocatalytic degradation of MO

As shown in Figure 11, the plot of ln \( \frac{C_0}{C_t} \) on the y-axis with time on the x-axis is a straight line followed by pseudo-first-order kinetics described by a modified Langmuir-Hinshelwood model using equation (4) [54].

\[
\ln \left( \frac{C_0}{C_t} \right) = kt \tag{4}
\]

Where Co- the initial concentration of MO before irradiation
Ct- the concentration of MO at any time t after irradiation
k- rate constant \( \text{min}^{-1} \)
t- irradiation time (min)

Scheme 1. Proposed MO degradation mechanism.

Figure 9. SEM image of Zinc Oxide nanoparticles with different resolution.
According to the graph in Figure 11, the rate constants for degradation of methyl orange dye at 15 ppm with ZnO catalyst doses of (10, 20, 30, 40, 50 and 60 mg) were 4.1x10^{-3}, 6.4 x 10^{-3}, 1.16 x 10^{-2}, 1.54 x 10^{-2}, 1.144 x 10^{-2} and 1.4 x 10^{-2} min^{-1} respectively. The correlation coefficient of the doses of (10, 20, 30, 40, 50 and 60 mg) of MO dye was recorded $R^2 = 0.9631, 0.9421, 0.9511, 0.963, 0.967$ and 0.9649, respectively.

The total catalyst dose of the correlation coefficient ($R^2$) is greater than 0.91 and it fits with the standard of the pseudo first-order kinetic model.

4. Conclusion

In summary, the green synthesized ZnO nanoparticles using Hagenia abyssinica leaves are simple, cost-effective, and environmentally friendly. The particle size and surface morphology of the green synthesized nanoparticles of ZnO were characterized using XRD, FTIR, and SEM. The XRD pattern indicates the formation of Zinc Oxide nanoparticles and the crystal size of the ZnO nanoparticles was 27.833 nm. The presence of functional groups in biosynthesized ZnO nanoparticles was identified using FTIR. The photocatalytic degradation of the MO dye after 120 minutes of sunlight
irradiation was 83.17% for the catalyst dose of 40 mg biosynthesized ZnO NPs. It is also reported that the degradation of methyl orange dye meets the pseudo-first-order kinetics. Antibacterial tests revealed that biosynthesized ZnO nanoparticles inhibited the growth of both Gram-positive and Gram-negative bacteria.

**List of Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| FTIR         | Fourier transform infrared |
| FWHM         | Full Width at Half Maximum |
| MO           | Methyl Orange |
| MB           | Methylene Blue |
| NPs          | Nanoparticles |
| SEM          | Scanning electron microscopy |
| TTC          | 2,3,5-triphenyltetrazolium chloride |
| UV-light     | Ultra violet light |
| UV-Vis       | Ultra violet visible |
| XRD          | X-ray diffraction |

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**Author’s Contributions**

Both the authors designed this study, developed the proposal, interpreted the results and statistical analysis, drafted and revised the paper before submitting the final manuscript, read and approved it.

**Conflict interests**

The authors confirm that there are no competing interests in this research work.

**Data Availability Statement**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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