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Antioxidant and Antibacterial Activities of Bio-synthesized Al-Hanaa Nanoparticles

This study examined the antibacterial and antioxidant properties of natural nanoparticles. Henna was employed as a free radical scavenger material at concentrations of 0.00030, 0.00035, 0.00040, 0.00045, and 0.00050 g/l, resulting from exposure to nuclear radiation in humans. Disk diffusion and the DPPH (2, 2-diphenyl-1-picrylhydrazyl) test were used to evaluate the bactericidal and antioxidant capabilities of the nanoparticles, respectively. According to the study's findings, gram-negative Escherichia coli (E. coli) bacteria was susceptible to the antibacterial action of the biosynthesized nanoparticles. The findings of the antioxidant test demonstrated that these nanoparticles have a concentration-dependent ability to eliminate DPPH radicals, resulting in a more powerful antioxidant activity at higher nanoparticle concentrations. Our findings concluded that Al-Hannaa nanoparticles have potential use in the food and pharmaceutical industries as a major source of antioxidants and antibacterial agents.

Keywords: Al-Hannaa nanoparticles; Antioxidants; Antibacterial agents; Biosynthesis
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1. Introduction

As evidenced by recent research, nanomaterials have vital roles in the development of nanoscience and related technologies [1-3]. There is a lot of study to get rid of free radicals, nanoscopic minerals like gold and silver were employed as antioxidants [4].

Because of their high surface areas, nanoparticles have a particular intrinsic reactivity that makes them attractive candidates for the development of therapies based on nanoparticles [5]. The dimensions, content, and mass of nanoparticles have a significant impact on their surface functions [6].

Oxidative stress, a phenomenon caused by reactive oxygen species (ROS) such as superoxide and hydroxyl radicals, can harm live cells in biological systems. Free radicals are often scavenged by antioxidant molecules, preserving the equilibrium between oxidant and antioxidant processes [7]. Numerous naturally occurring antioxidants found in plants are known to exist. Free radicals are already being scavenged by it like a sweeping oxygen. Natural antioxidants are receiving a lot more attention these days in an effort to replace synthetic antioxidants. Because of its adverse effects, such as cancer, it was restricted. On the other hand, exposure to pollutants or toxins can cause an excess of reactive oxygen species, which can upset the balance between antioxidant and oxidant programs and result in more severe infections [8].

Herbal remedies include high concentrations of antioxidant molecules [9, 10]. Anthocyanins, phenolic compounds, flavonoids, and carbohydrates are the main components of natural herbal antioxidants [11-13], which have a wide variety of biological activities, including anti-inflammatory, anti-microbial, and anti-cancer actions.

The aim of the current study was to look into the Al-Hannaa nanoparticles' antibacterial and antioxidant properties.

Antioxidant molecules are found in significant amounts in herbal treatments [9, 10]. Natural herbal antioxidants are mostly composed of anthocyanins, phenolic compounds, flavonoids, and carbohydrates [11-13]. These compounds have a variety of biological properties, such as anti-inflammatory, anti-microbial, and anti-cancer effects.

The goal of the current study was to look into the Al-Hannaa nanoparticles' antibacterial and antioxidant properties.

Hanna is a plant of the hedic family that follows the rank Humanities are ethnic or perennial for age around Three years and may extend to ten permanent.

The primary component known as (Lawson) and its molecule are the most significant of the many glycosidic compounds found in henna leaves. The red-orange dye, chemical type 2-hydroxy-1,4-naphthoquinone, or hennotannic acid, is found in the leaves of the henna plant (Lawsonia inermis), as well as in water hyacinth (Pontederia crassipes) [15], common walnut (Juglans regia) [14], and other plants. For almost 5,000 years, people have colored their hair and skin with henna extracts that contain lawsone. Lawsone produces a strong, long-lasting stain that persists until the skin or hair is removed when it chemically combines with the keratin protein in skin and hair through a Michael addition reaction. More lawsone–keratin interactions result in darker pigmented stains.

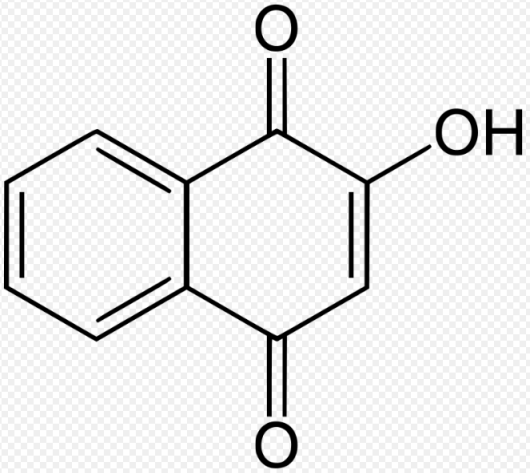


Fig. (1) 2-Hydroxy-1,4-naphtoquinone (HNQ), the dye substance in Hanna (*Lawsonia inermis*). hennotannic acid: lawsone Created with bkchem+inkscape [14]

2. Experimental Part

In order to monitor the surface roughness and topography of the precipitated thin films, four grams of henna powder and 100 milliliters of distilled water (DW) solvent were combined, stirred, and left at 45°C for 60 minutes. The mixture was then filtered through filter paper, which served as a stock extract. Atomic force microscopy was used to measure the prepared fairy's dimensions. The average diameter was 21 nm. Figures (2) and (3) provide atomic force microscopy (AFM) images along with particle size distribution histograms, indicating that the particles are almost spherical in form and ultrafine.

The following methodology was used to investigate the antibacterial activity of henna nanoparticles: bacterial samples were procured from the Biotechnology Center at Alnahrain University. To cultivate bacteria, cultures were first disseminated out on agar plates. The extract, biosynthesized nanoparticles, and deionized water were added to sterilized discs that had a thickness of 5 mm. The discs were then stored at 37°C. In this experiment, the positive controls were streptomycin and gentamicin [16]. Based on the inhibitory zone close to the disc, the antibacterial action was made clear.

Prokaryotic bacteria are primitive, unicellular organisms devoid of organelles and a nucleus. The bacteria are typically one micron in size and come in a variety of forms, including spheres, rods, and spirals [17]. Bacteria have a single strand of DNA known as a nucleoid. The cytoplasm of the bacterium may have a tiny circular (plasmid) containing certain genes, including those resistant to antibiotics. The bacterial cell membrane is made up of two lipid layers. Food and water molecules may flow through the membrane, which acts as a barrier separating the cytoplasm from the outside world [18]. Additionally, the membrane shields the cell from the pressure outside that is brought on by the osmotic pressure. The importance of the gut microbiota to human health has been the subject of recent research [19]. Bacteria are living things that consist of one cell that is not animals or plants. It usually is measured by micrometers in length. One gram of soil contains 40 million bacteria. One milliliter of freshwater contains one million bacteria. The whole earth is estimated to contain about five nano million bacteria [20].

The purpose of the bacterial cell wall is to provide the cell structure, strength, and rigidity while shielding it from mechanical harm and osmotic rupture [21]. Bacteria cell walls may be classified into two primary groups based on their composition, features, and roles: Gram-positive (+) and Gram-negative (–). Teichoic acids that are exclusive to the wall of Gram-positive cells are linked to a thick layer of peptidoglycan (PG) that ranges in thickness from 20 to 50 nm [22]. Gram-negative cell walls, on the other hand, have more intricate structural and chemical makeup.

To be more precise, the cell wall of Gram-negative bacteria is made up of a thin layer of PG and an outer membrane that covers the surface membrane. Gram-negative bacteria have an outer membrane that is often resistant to hydrophobic substances, such as detergents. One of their special components is lipopolysaccharides, which raise the negative charge of cell membranes and are crucial to the structural integrity and survival of the bacteria [23]. The way that bacteria are susceptible to nanoparticles (NPs) in the presence of them is largely determined by the structure of their cell walls. The components of a bacterial cell are as follows: (a) the thick, multilayered peptidoglycan (PG) sheath that surrounds the cytoplasmic membrane makes up the cell wall of a Gram-positive bacterium. As may be observed, the teichoic acids are embedded in and linked to the PG and lipoteichoic acids extend into the cytoplasmic membrane. (b) A Gram-negative bacterial cell wall is composed of an outer membrane linked by lipoproteins to thin and single-layered PG. The PG is placed within the periplasmic space that is formed between the outer and inner membranes. The outer membrane includes porins and lipopolysaccharide molecules [24]. Thus, Gram-positive bacteria look purple under the microscope, and Gram-negative bacteria look red.

Escherichia coli, also known as E. coli, is a rod-shaped, coliform bacterium of the genus Escherichia that is typically found in the lower intestine of warm-blooded organisms. It is a Gram-negative, facultative anaerobic (it can produce adenosine triphosphate by aerobic respiration if oxygen is present, but can also switch to fermentation or anaerobic respiration if oxygen is lacking) coliform bacteria [25], as illustrated in Fig. (2). One of the most prevalent strains in the human digestive system, Escherichia coli grows best at 37°C. Typically, cells have a rod-like morphology and measure between 0.25 to 1.0 μm in diameter and 2.0 μm in length [26,27].

The antioxidant capacity of Al-Hannaa nanoparticles was evaluated by the utilization of the DPPH (2, 2-diphenyl-1-picrylhydrazyl) test. To put it briefly, 30 mg/ml of DPPH solution was made, and 524 nm was used to measure its absorbance. Water exposed to gamma rays served as the control. Every sample underwent an experiment conducted at the Department of Physics, College of Science at University of Baghdad.

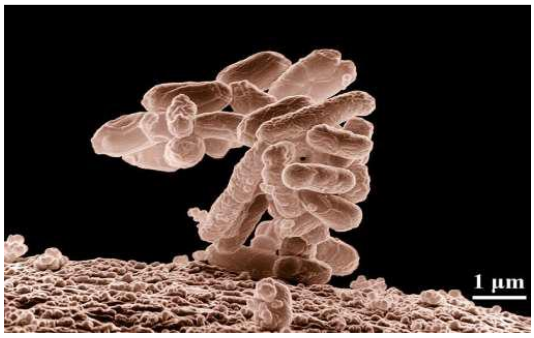
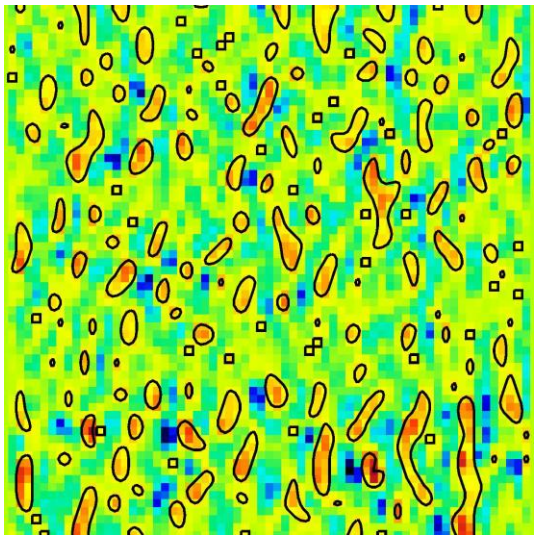


Fig. (2) The image of E.coli bacteria [25]

3. Results and Discussion

The AFM micrographs were collected utilizing Nanoscope III 3100 digital instrument to evaluate the surface roughness and topography of deposited thin films. The roughness and grain size of AFM images are measured using the root mean square (RMS) method. AFM was utilized to determine the morphology and size of the prepared Al-Hannaa. The prepared nanoparticles from Al-Hannaa have average diameter of 28 nm. Figures (3) and (4) show AFM images along with particle size distribution histograms. The particles are nanosized and somewhat spherical in shape, according to the researchers.



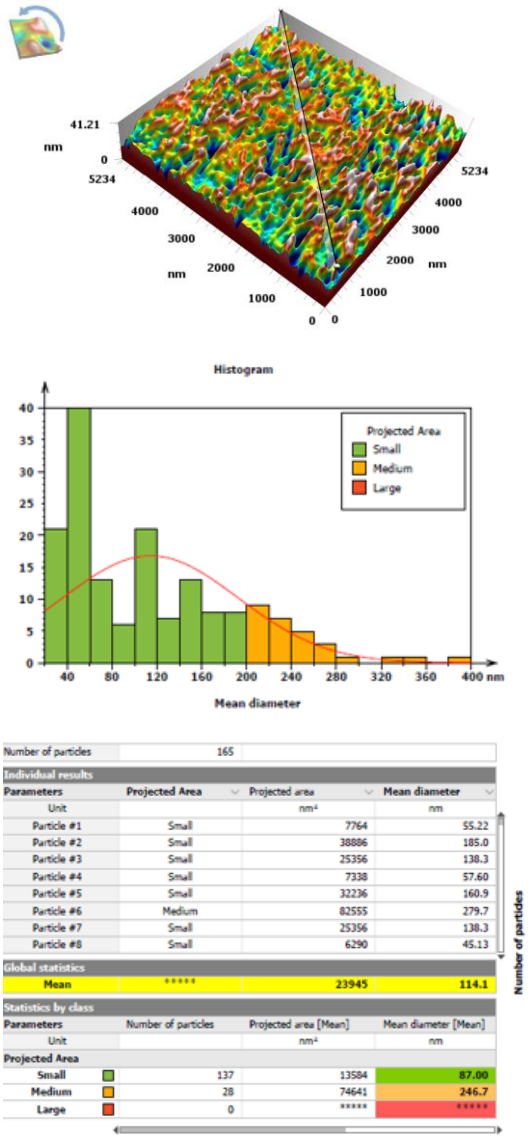
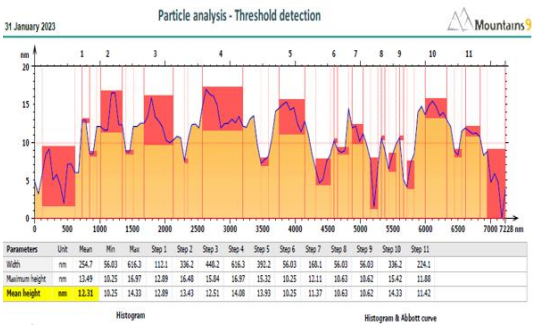


Fig. (3) The AFM images of the Al-Hannaa nanoparticles prepared in this work

The UV-visible absorption spectra of prepared Al-Hanna with different concentrations were recorded in the range 200-800 nm as shown in Fig. (5). For all concentrations, these spectra revealed a surface plasmon resonance absorption peak centered about 524 nm.



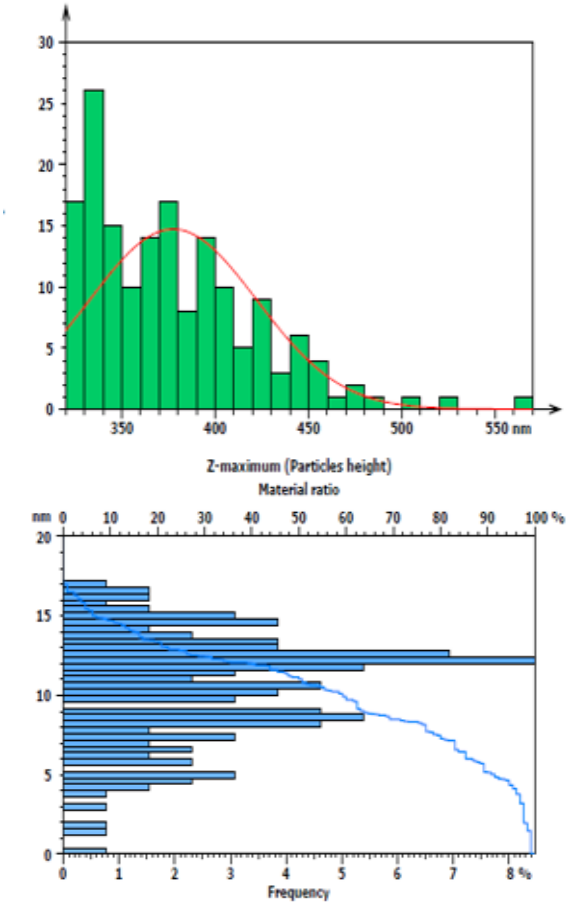
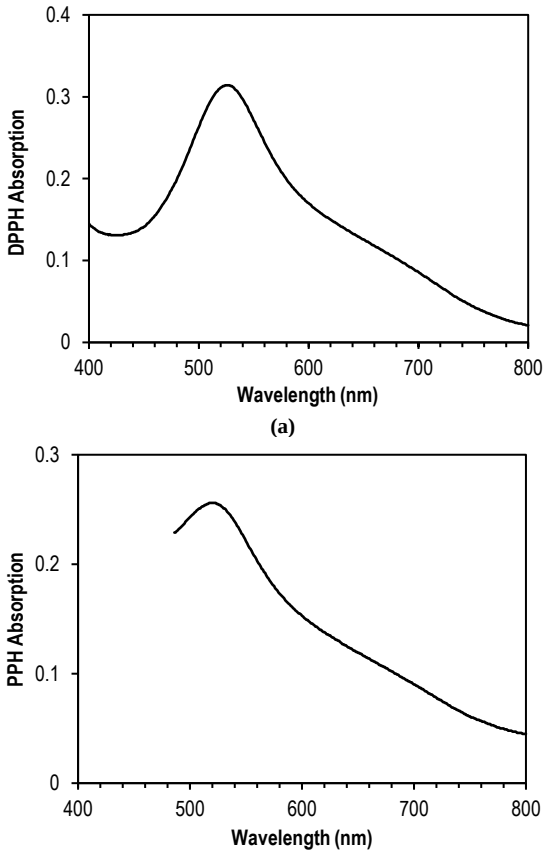


Fig. (4) The AFM histograms of the particle size distribution for Al-Hannaa nanoparticles prepared in this work



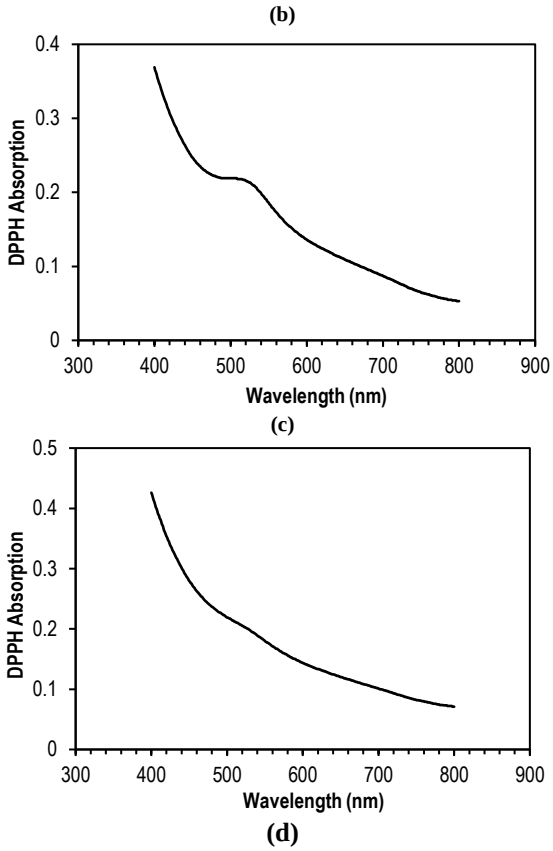


Fig. (5) Values of DPPH absorption and inhibition% for samples added with various concentrations of Al-Hannaa nanoparticles after irradiation, (a) irradiated water, (b) 35×10^{-5} g/l concentration on Al-Hannaa nanoparticles, (c) 40×10^{-5} g/l concentration on Al-Hannaa nanoparticles, (d) 45×10^{-5} g/l concentration on Al-Hannaa nanoparticles

The findings of this experiment are presented in table (1) when different Al-Hannaa concentrations are added to a fixed volume of deionized water samples following the irradiation procedure. The samples were made at the time of the measurements, differing only in the content of Al-Hannaa and having the same ratio of DPPH solution to irradiated deionized water samples. All samples' DPPH absorbance at 524 nm was determined using a UV-visible spectrophotometer.

The antioxidant or free radical inhibition activity of Al-Hannaa was measured by lowering DPPH, which lowers absorbance at 524 nm. Figure (5) illustrates the absorption of DPPH radical in the presence of antioxidant Al-Hannaa nanoparticles added subsequent to irradiation.

From the results, DPPH radical absorption has a specific decrease with increasing Al-Hannaa concentrations until $45x10^{-5}$ g/l concentration is reaching the concentration value as shown in Fig. (6). The maximum inhibition% of free radicals was found with $45x10^{-5}$ g/l concentration as shown in Fig. (7).

Table (1) The percentage absorption, and the inhibition of free radicals for different concentration of Al-Hannaa nanoparticles

Water samples	DPPH absorption	Inhibition %
irradiated	0.314	------
Al-Hanna nanoparticles concentration ×10^{-5} g\l		
35	0.255	18.8
40	0.214	31.8
45	0.203	35.4
50	0.203	35.4

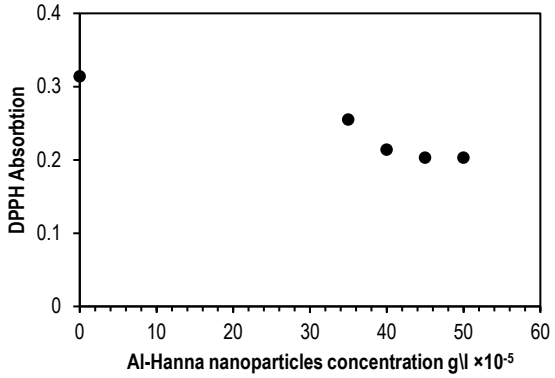


Fig. (6) DPPH absorption for all samples with Al-Hannaa added after irradiation as a function of concentration

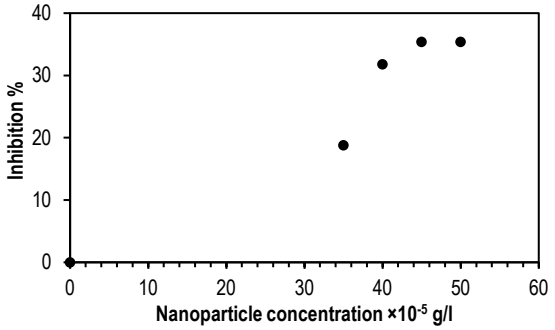


Fig. (7) Free radical inhibition % for all samples with Al-Hannaa nanoparticles added after irradiation as a function of concentration

Using the agar diffusion technique, the antibacterial properties of Al-Hannaa were investigated. The inhibitory zones that were seen surrounding the disks that contained Al-Hannaa demonstrated how effective Al-Hannaa's antibacterial qualities were (Fig. 8). Al-Hannaa's ability to inhibit bacterial cell development may be partially attributed to their vast surface area and compact size, which provide them an appropriate contact interface with the bacteria [27]. It has been suggested that the large-scale leaking of bacteria's macromolecules may be the cause of their demise. The inhibition zones found in this investigation are displayed in Fig. (8).

The results presented in table (2) showed that the concentration 30×10^{-5} g\l of nanoparticles from Al-Hannaa has a greater (IZD=17 mm) ability to inhibit the *E.coli* than the concentration 3.7×10^{-5} g\l extract (IZD=10 mm), as shown in Fig. (8). However, the inhibition potential of both extracts against this type of bacteria could be considered identical as the difference in the inhibition zone diameter was not more than 7 mm.

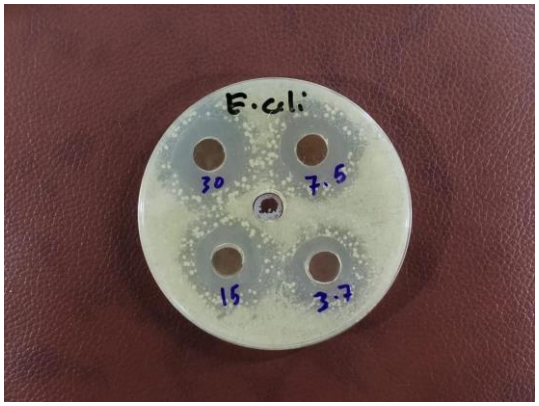


Fig. (8) The inhibit of *E.Coli* using 3.7, 7.5 , 15, 30×10^{-5} g\l concentration of nanoparticles extracted from Al-Hanaa

Table (2) The diameter of E. coli bacterial killing for Al-Hannaa nanoparticles concentration

Al-Hannaa concentration	The diameter of E. coli bacterial killing
0.000037	10
0.000075	15
0.000150	15
0.000300	17

4. Conclusions

The results of this paper confirmed that the Al-Hannaa nanoparticles used in this work have a high ability to scavenge free radicals which are caused when human exposed to nuclear radiation. With a 17mm inhibitory zone, the produced Al-Hannaa demonstrated strong antibacterial activity against the pathogenic bacterium E. coli. Al-Hannaa can be used as suitable materials to manage infectious diseases and maintain human health because bacterial contamination can occur upon the proliferation of these organisms in the body and environment, causing many forms of infections and subsequently serious health threats and even death. In light of our findings, the Al-Hannaa biosynthesized in this work can be used to eliminate microbial contamination, especially that caused by bacteria.

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