International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 04 Issue 02 February 2024

Page No: 59-63

DOI: https://doi.org/10.47191/ijpbms/v4-i2-04, Impact Factor: 7.79

Synthesis of Phenylethanoid Nanoparticle and Study of Its Antibacterial Effect

Nadya J. Ibraheem[1*], Mays U. Hashim[2], Ahmed F. Farhood[3], Zainab A. Radhi[4]

[1,2,4] College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq

[3] Ministry of Agriculture, The Education Veterinary Hospital, Babylon, Iraq

ABSTRACT

Phenylethanoid nanoparticles has been hypothesized to exert wide range of biological effects, one of them antimicrobial. phenylethanoid acts as free radical-scavenger and metal-chelator. Today, there is a considerable interest in it because it is safe, nontoxic. The generation of Phenylethanoid nanoparticles was confirmed by change of Phenylethanoid extract color from brown. Bovine fecal samples were collected by direct rectal retrieval using disposable gloves, isolation and identification of *E. coli* , Nanoparticles of phenylethanoid of were prepared by the nanoprecipitation method according to Gaonkar,Jasim *et al*., evaluation was done by agar well diffusion method. Highly antibacterial activity was recorded in comparison to plant extract. The antibacterial activity of Phenylethanoid was evaluated by agar well diffusion method. Six isolates of *E. coli* were identified and all of them were showed inhibition zones from 16-20 mm. From this study, the antibacterial activity of Phenylethanoidwas more efficient to eradicate. The goal of current study is to eradicate pathogenic bacteria (*Escherichia coli*) that isolated from bovis feces infected with diarrhea by alternative treatment Phenylethanoid nanoparticles.

ARTICLE DETAILS

Published On:
17 February 2024

Available on:
https://ijpbms.com/

INTRODUCTION

Escherichia coli, commonly known as E. coli, is a Gram-negative, rod-shaped bacterium belonging to the genus Escherichia. It is facultative anaerobic and typically found in the lower intestine of warm-blooded animals. Within this genus, there exists a diverse array of strains, with the majority serving as commensal organisms residing in the lower gastrointestinal tract (GIT) of mammals. However, certain strains exhibit pathogenic behavior, which can be classified into two main groups based on the site of infection: intestinal pathogenic E. coli (IPEC) and extra-intestinal E. coli (EXPEC) (O'Sullivan et al., 2007).

The commensal strains of E. coli play a role in the gut microbiota and serve as indicators of fecal contamination. Pathogenic strains, on the other hand, possess virulence factors that enable them to cause various disease syndromes. Among the intestinal pathogenic E. coli are enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC), enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), diffusely adherent E. coli (DAEC), and verocytotoxigenic E. coli (VTEC) (O'Sullivan et al., 2007).

In addition to gastrointestinal infections, E. coli can also cause diseases affecting other systems in the body. Extra-intestinal pathogenic E. coli include uropathogenic E. coli (UPEC), meningitis-associated E. coli (NMEC), and sepsis-causing E. coli (SEPEC) (Kirk et al., 2015). These pathogenic strains are often transmitted through the fecal-oral route via contaminated food, water, animals, or environmental sources. Symptoms of E. coli infection vary depending on the pathotype and system involved, ranging from watery or bloody diarrhea to abdominal cramps, urinary tract infections, and meningitis. In severe cases, complications such as hemorrhagic uremic syndrome (HUS) can arise. Outbreaks of E. coli-related illnesses have been documented as instances of food poisoning, travel-related illnesses, or diseases contracted through contact with contaminated animals or environments. Cattle can harbor E. coli strains without exhibiting symptoms, serving as potential sources of contamination for water and food products (Naylor et al., 2005). Consequently, the shedding of these enteric pathogens in cattle feces poses risks for contamination, highlighting the importance of hygiene and sanitation measures in food and water safety. Escherichia coli, commonly abbreviated as E.

coli, is a Gram-negative, facultative anaerobic bacterium with a characteristic rod-shaped morphology, belonging to the genus Escherichia. Typically, it thrives in the lower intestine of warm-blooded organisms. Within this genus, a vast array of bacteria is found, predominantly serving as commensals in the lower gastrointestinal tract (GIT) of mammals. However, some strains exhibit pathogenic behavior and are classified into two main groups based on the site of infection.

Those strains of E. coli that instigate infections and induce disease syndromes within the gastrointestinal tract are identified as intestinal pathogenic E. coli (IPEC). Conversely, strains causing disease syndromes outside the gastrointestinal tract are classified as extra-intestinal E. coli (EXPEC). The commensal E. coli group is integral to the gut microbiota and is widely employed as indicator bacteria for fecal contamination.

The pathogenic group of E. coli encompasses numerous strains, which can be categorized based on their virulence factors or the pathological effects they induce. Examples of intestinal pathogenic E. coli include enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC), enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), diffusely adherent E. coli (DAEC), and verocytotoxigenic E. coli (VTEC) (O'Sullivan et al., 2007; Kirk et al., 2015; Naylor et al., 2005). These references provide a comprehensive understanding of the diversity and pathogenicity of E. coli strains.

MATERIALS AND METHODS

Nanoparticles synthesis

Nanoparticles are a small object that have size ranging from 1-100 nm (nm=10-9 m) (Anselmo and Mitragotri ,2015). Nanoparticles are applied vastly today in medicine, health care, and environmental field. In medicine, the antimicrobial activity of nanoparticles is the ability to destroy wide spectrum of pathogens and multidrug-resistant bacteria, especially biofilm forming pathogens (Abinaya *et al* .,2017). Many methods are used in the synthesis of nanoparticles such as physical, chemical and biological or green synthesis methods. The safer one is the green synthesis, especially plant synthesis method because it's applied on non-toxic materials in addition to being, eco-environmental friendly, less costly and they utilize renewable materials. However, the use of antibiotics has various side effects, such as the increase in bacterial resistance). The exploitations of novel substitutes to antibiotics, especially on inorganic nanoparticles, have recently attracted more attention.

Phenylethanoid nanoparticles

Several studies have highliged phenylethanoid that phenylethanoid and its derivatives possess antioxidant properties (Rietjens *et al.,* 2007), antimicrobial actions (Omar, 2010), Years ago, olive oil and extracts from olive leaves were identified as antimicrobial agents with activity against *Escherichia coli, Candida albicans, Kluyveromyces marxianus, Clostridium perfringens, Streptococcus mutans, Shigella sonnei, Salmonella enterica*, and others (Medina E, *et al*., 2007). It appears that the main components of olives and olive leaves responsible for the antimicrobial effect are the dialdehyde and decarboxymethyl forms of elenolic acid together with phenylethanoid (Medina E, *et al*., 2007).

Synthesizing phenylethanoid nanoparticles and studying their antibacterial effects would involve several steps. Here's a general outline of the process:

1. Synthesis of Phenylethanoid Compounds: Phenylethanoids are a class of natural compounds found in various plant sources like olive, verbena, and honeysuckle. Common examples include verbascoside, echinacoside, and acteoside. These compounds can be extracted from plant sources or synthesized chemically.
2. Nanoparticle Formation: Once the phenylethanoid compound is obtained, nanoparticles can be synthesized using various methods
3. Characterization of Nanoparticles: Characterization techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM).
4. Study of Antibacterial Effects.

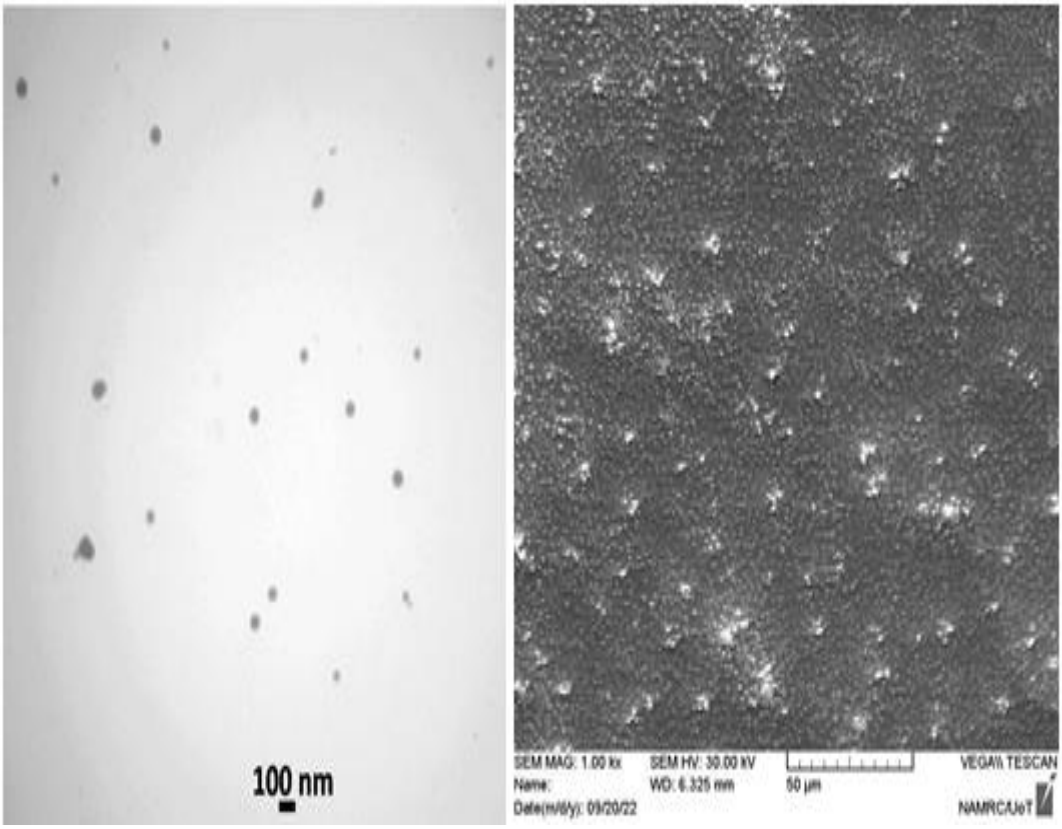

Figure 1: SEM and TEM analysis of Phenylethanoid nanoparticles

Samples collection

A total of 16 bovine fecal samples were collected by direct rectal retrieval using disposable gloves. Each glove was inverted and sealed immediately after collection.

Isolation and Identification of *E. coli*

One gram of each sample was enriched by inoculation in 10 ml of peptone water. They were then homogenized and incubated at 37°C. After 24 h, the samples were plated onto eosin methylene blue and were incubated at 37°C for 18–24 h. Colonies of presumptive *E. coli* strains were randomly picked from each sample were confirmed by biochemical identification, including the sugar fermentation test, the indole production test, and the citrate test (Brenner *et al* ., 2008).

Preparation of phenylethanoid

The phenylethanoid powder was boug phenylethanoid from Transhuman Technologies Company, UK. The doses of phenylethanoid was daily prepared by dissolving the phenylethanoid in distilled water at room temperature and keeping the solution dark bottle, for drenching the animals (Yuan *et al.*, 2017, Liu *et al.*, 2019).

Preparation of phenylethanoid- nanoparticles

Nanoparticles of phenylethanoid were prepared by the nanoprecipitation method according to (Gaonkar*et al.*, 2017 ; jasim *et al*.,2019) with mild modification.

Measurement of Antimicrobial Activity of phenylethanoid

Evaluation was done by agar well diffusion method, this method was used to assess the antimicrobial activity of zinc oxide using Muller Hinton plate inoculated with tested bacteria at inoculum 1.5 x 108 CFU/ml . A cork borer was used to make wells in the center of plate, these wells were filled with 100 µl of filtered zinc oxide and incubated at 37°C for 24 hrs. at dark conditions. After that, the diameter of the inhibition zone was measured using the ruler .

RESULTS AND DISCUSSION

Isolation and Identification of *E. coli*

A total of 16 bovine fecal samples were collected by direct rectal retrieval , six isolates of these samples give positive result for *E. coli*

Table (1):- Biochemical tests of *E. coli* isolated from bovine feces

Characteristics	*E. coli*
Gram Staining	Negative
MR	Positive (+ve)
VP	Negative (-ve)
Indole	Positive (+ve)

Citrate	Negative (-ve)
Catalase	Positive (+ve)
Oxidase	Negative (-ve)

E.coli is an important pathogen of intestinal and extraintestinal infections. Bovine E. coli strains can produce Shiga-like toxins (Stx), heat-labile (LT) or heat-stable (ST) enterotoxins, cytotoxic necrotizing factors (CNF1 and CNF2) and hemolysins (a-Hly and E-Hly) (Gay and Besser , 1994).

Antimicrobial effects of phenylethanoid NPs

The test was done by agar well diffusion method , the result showed a pronounced antimicrobial activity of phenylethanoid NPs in comparison with plant extract against the tested bacteria with different inhibition zones as mentioned in table (2).

Table (2):- Antimicrobial activity of phenylethanoid NPs against *E. coli* isolates

Samples	Inhibition zone (mm) of phenylethanoid NPs (0.05)m	Inhibition zone (mm) of plant extract (0.05)m
1	20	14
2	16	13
3	16	14
4	19	15

The antibacterial properties of phenylethanoid have been extensively studied, with research indicating that factors such as concentration, size, and temperature can influence its effectiveness. Compared to other organic reagents, phenylethanoid demonstrates greater stability as an organic antibacterial agent (Sawai, 2003). Various mechanisms have been proposed to explain the antimicrobial action of phenylethanoid. Firstly, it is suggested that hydrogen peroxide, produced from the surface of hydroxytyrosol, can permeate the cell membrane, causing cellular damage and inhibiting cell growth (Yamamoto, 2001). Secondly, the interaction between phenylethanoid and bacterial cells plays a crucial role in its antibacterial activity (Stoimenov et al., 2002). These studies shed light on the diverse mechanisms through which phenylethanoid exhibits its antibacterial effects.

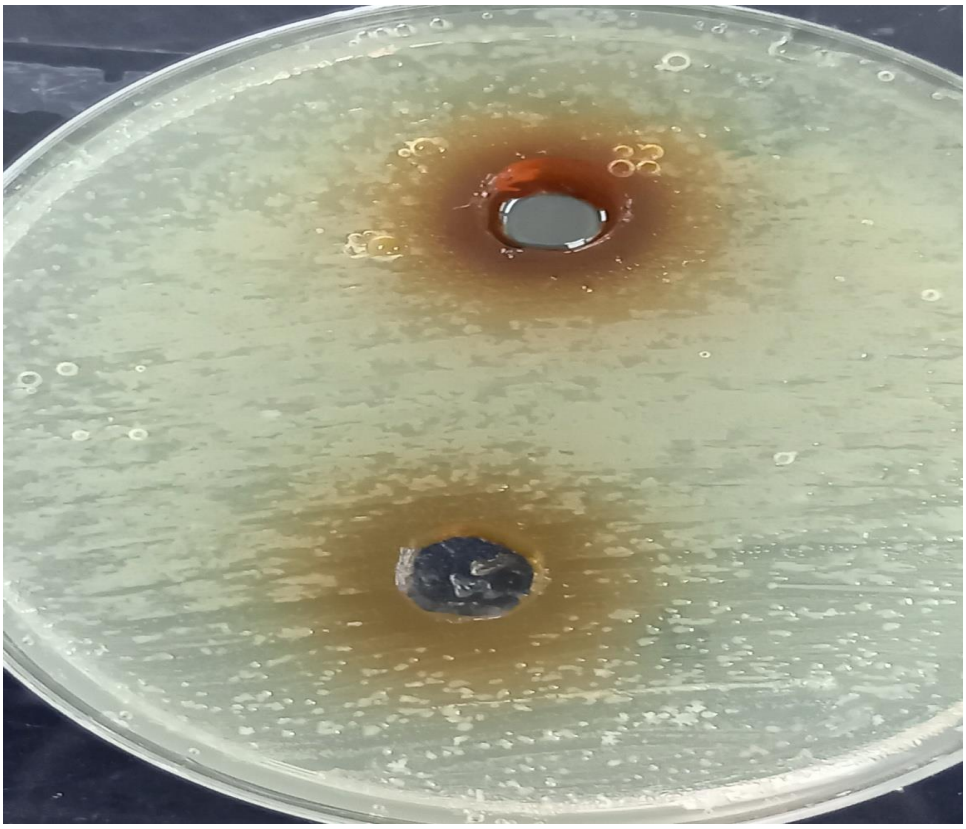

Figure 2: Antibacterial effect of phenylethanoid NPs against *E.coli* by agar well diffusion.

Phenylethanoid nanoparticles (NPs) have demonstrated potent antimicrobial activity against various pathogenic microorganisms, including Pseudomonas aeruginosa, Campylobacter jejuni, and others. These NPs are believed to impede bacterial cell growth by disrupting the structure of bacterial membranes and accumulating within the cytoplasm.

Moreover, the size of these nanoparticles plays a critical role in the mechanisms of particle internalization by cells and their distribution in tissue, leading to significant differences in delivery effectiveness (Mundargi et al., 2008). Small phenylethanoid NPs can passively target vessels through the enhancement of permeation and retention effects, thereby reducing the frequency of drug administration (Zhang et al., 2018). In the current study, the Nano precipitation technique was utilized to encapsulate the drug, resulting in widely varying drug encapsulation efficiency depending on specific drug properties, particle size, and emulsifier characteristics. Encapsulation of hydrophobic drugs via a single emulsion may facilitate the production of ultra-small NPs compared to the double-emulsion method described by Zhang and Feng (2006) and Madani et al. (2018).

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