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The Antimicrobial of Selenium Nanoparticles and Combination with Cinnamon Oil Against *Salmonella Typhi* Which Cause Diarrhea in Human

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ABSTRACT

Many bacterial species can cause diseases in humans, such as the bacteria that cause diarrhea as a result of having genetic characteristics that can form pathogenic factors, which help the bacterium to produce harmful effects in the host's body. From (60) fecal samples of diarrheal cases of human, *Salmonella spp.*42(70%) where *Salmonella typhi* consist29(48.33%) isolates and *Salmonella paratyphi* consist13(21.67%) isolates, *E. coli* 10(16,66%), *Enterobacter* 4(6,66%) and *Shigella* 4(6,66%). On Congo red agar (CRA) plates, among (29) *S. typhi bacterial* isolates from human, all the isolates (100%) were showed biofilm formation through formation of brown colonies with a dry crystalline quality. Antibiotic susceptibility profile of *Salmonella typhi.* isolated from fecal sample of human were determined by using Vitek2 system, the results revealed that *Salmonella typhi* were100% resistant to Ticarcillin and 89.7% to Aztreonam while the rest of the antibiotics were less resistant. Selenium nanoparticles (Se-NPs) are green synthesized using cinnamon extract. The antibacterial activity of the cinnamic ethanolic extract and synthesized SeNPs was investigated against isolates of human source *Salmonella typhi* by using the agar well diffusion method and tested by using different concentrations (25,50,100 µg/mL).According to the data, cinnamon extract only produced an inhibition of 10.66 mm at the same dose of 100 mg/ml, but Se-NPs produced a good inhibition at that concentration of 14.33 mm.

KEYWORDS: SeNPs; Cinnamon extract; Biofilm; Antibacterial.

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INTRODUCTION

Diarrhea is a serious public health risk for people and is brought on by bacteria, viruses, fungi, protozoa, nutritional deficiencies, chemical agents, and environmental factors. These elements contribute to the development of diarrhea either separately or in concert. [1]. The most common diarrhea is caused by the ingestion of bacteria capable of producing enterotoxin inside the intestine or the ingestion of bacterial exotoxin that forming contaminated food. Diarrhea resulted from the action of these toxins on epithelial cell receptors. Children's diarrhea requires antibiotic treatment. The whole course of treatment and ongoing, indiscriminate use of antibiotics to treat diarrheal infections in humans may have resulted in the development of a new group of persistent, deadly bacteria. As a result, the microbial pathogens preserve genes that are multidrug resistant and contagious. To combat this resistance to conventional antibiotics, new antimicrobial drugs are required [2]. In general, the interaction of the bacterial cells, the substrates, and the surrounding medium is what leads to the creation of bacterial biofilms [3],[4]. Numerous scientific studies on the medicinal properties of cinnamon, including as its antibacterial, antioxidant, gastro protective, and immunomodulatory actions, have shown encouraging results [5]. Plants continue to be crucial to human health care despite advancements in biomedicine. Growing in popularity and use in the treatment of many chronic conditions are medicinal plants [6] Selenium was discovered and identified as an element in1817 by Berzelius, who named the new element selenium, after the Greek work Selene, meaning the moon or moon goddess. SeNPs has recently gained more attention in the medical sector as a promising candidate for therapeutics [7]. The present work was aimed to isolates pathogenic bacteria from diarrheal patients and screening the activity of SeNPs in culture media.

MATERIAL AND METHODS

Bacterial Isolates:

Sixty samples from diverse clinical samples were collected in the Babil Governorate between August 2022 and January 2023. Swab samples from the diarrhea of 60 people who were affected with various diseases were used to get these samples.

Prepare cinnamon extract:

An alcoholic extract of cinnamon was prepared according to the method described by [8].

SeNPs synthesis green:

The green synthesis of selenium nanoparticles was prepared according to the method described by [9].

Biofilm formation by Congo red agar

This material was tested for its capacity to create biofilms. Agar treated in Brain Heart Infusion Broth with 50 g/l sucrose and 8 g/l Congo red

Statistical analysis

A one-way analysis of variance (ANOVA) was done statistically on the results using the software SPSS, version 32, $P<0.05$ was consider significant [10].

RESULTS AND DISCUSSION

Isolation of *S. typhi* by culture and biochemical analysis

From (60) fecal samples of diarrheal cases of human, *Salmonella typhi* consist 29(48.33%) isolates and *Salmonella paratyphi constitute* 13(21.67%) isolates, while *E. coli* 10(16,66%), *Enterobacter* 4(6,66%) and *Shigella* 4(6,66%) Figure (1).

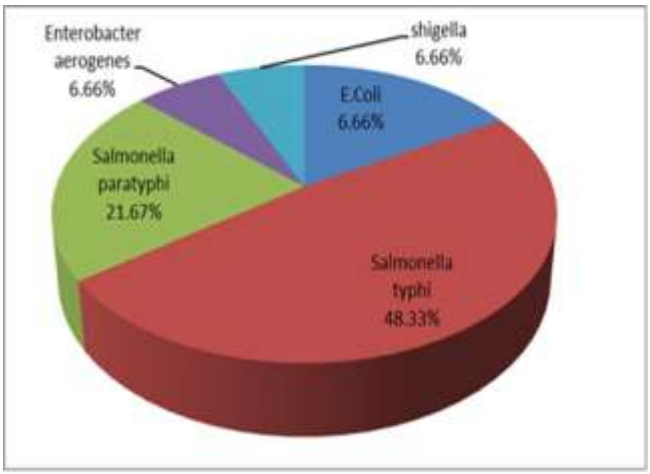

Fig (1): Bacterial isolates in human samples

With 20.4 confirmed cases per 100,000 people in 2011 [11,12], salmonellosis is the second most often reported gastrointestinal illness. The findings of this investigation were consistent with those of [13], who discovered that Salmonella Typhi, which makes up nearly 50% of all isolated bacteria, is the most often isolated bacterium from human stool samples.

Salmonella Typhoid biofilm formation:

On Congo red agar (CRA) plates, among [14] *S. typhi* bacterial isolates from human, all the isolates (100%) were showed biofilm formation through formation of brown colonies with a dry crystalline quality. All isolates that generated brown crystalline colonies on CRA were deemed to have formed biofilms, but those that generated red or pink colonies were deemed to have not done so. as seen in Figure 2.

Figure (2): *Salmonella typhi* biofilm formation

Biofilms are surface-attached collections of cells that are encased in extracellular polymeric substances (EPSs) that they self-produce (15). Antibiotics, UV exposure, host defense systems, and other threats are all mitigated while the bacteria are contained within a biofilm. As a result, biofilms are crucial to the environment, business, and health care [16]. Antibiotics and biofilm both shield bacteria from host defense systems [17]. Research by [18] using the Congo red technique revealed that 59.4% of the strains were biofilm producers. The findings of this investigation were consistent with those of [19], [20], who showed that every isolate of *Salmonella typhi* included in the study was capable of creating biofilm. Biofilm-forming bacteria are a major cause of complex infections, which are typically brought on by recurring infections. In their investigation [21],[22] showed that every human-isolated *salmonella typhi* was generating biofilm. About 80% of chronic infections are thought to be caused by biofilms, which raise hospital admission rates, medical expenses, morbidity, and mortality [23]. Antimicrobial treatment is less effective against bacteria grouped in biofilms.

Antibiotic susceptibility of Salmonella spp.:

Using the Vitek2 analysis method, the antibiotic susceptibility profile of *Salmonella typhi* isolated from human fecal sample was examined. Table (1) displays the MIC and antibiotic susceptibility profile of *Salmonella typhi*. The results revealed that 29 (100%) of the isolates were Ticarcillin resistant; additional investigations [24], [25] found comparable results (99% and 100%, respectively).

Table (1) Antibiotic Susceptibility Profile of human isolates *Salmonella typhi.*

Antibiotic class	Type	Sensitivity rate%	MIC	Resistance rate%	MIC
Penicillin's	Ticarcillin	0(0%)	≤ 8	29(100%)	32
Beta Lactam Inhibitors	Piperacillin/Tazobactam Ticarcillin-clavulanate	21(72.4%) 23 (79.3%)	≤ 4 16	8(27.6%) 6(20.7%)	≥32/4 ≤ 8
Cephalosporines	Cefepime	21(72.4%)	4	8(27.6%)	≥ 16
Monopactam	Aztreonam	3(10.3%)	≤ 4	26(89.7%)	≥ 64
Carbapenem	Imipenem Meropenem	27(93.1%) 24(82.8%)	0.5 ≤0.25	2(6.9%) 5(17.2%)	≥ 4 ≥ 4
Aminoglycosides	Amikacin Gentamicin Tobramycin	25(86.2%) 23 (79.3%) 21(72.4%)	8 2 2	4(13.8%) 6(20.7%) 8(27.6%)	≥ 64 ≥ 16 ≥ 16
Quinolones and fluroquinolones	Ciprofloxacin	23 (79.3%)	≤0.25	6(20.7%)	2
Folate pathway antagonist	Trimethoprim-sulfamethoxazole	23 (79.3%)	≤20	6(20.7%)	≥320
Retracycline	Minocycline	18(62.1%)	2	11(37.9%)	≥ 16

The result of the current study for resistance rate of Piperacillin-Tazobactam and Ticarcillin-clavulanate sensitivity rate in this study was (72.4% and 79.3%) respectively, this observation has been corroborated by [26] who demonstrated that (76%) and (81.9%) of isolates were sensitive to Piperacillin-Tazobactam and Ticarcillin-clavulanate respectively. The beta lactam family suppresses the development of the peptidoglycan layer, a component of the cell wall, by connecting with proteins termed Penicillin-binding proteins at a particular place on bacterial cells [27] The current results showed high resistance of *Salmonella typhi* to this class, and that belonged to the frequent and sporadic uses of this antibiotics as a result, leading to the appearance of MDR new bacterial strains. In comparison to other classes of antibiotics, this class is significant and frequently utilized [28]. Bacteria can resist this family of antibiotics by a variety of ways, such as the production of extended spectrum ESBLs and beta lactamase enzymes or reduced membrane permeability. Additionally, the presence of several microorganisms and efflux pumps contributes to the manifestation of the multi-drug phenomenon [29]. The resistance rate for cefepime in the current investigation was close to the finding made by [30], who discovered that cefepime resistance was (30%). The ability of the bacteria to produce the beta-lactamase, which includes the enzymes penicillinase and cephalosporinase, which have the ability to destroy the beta-lactam ring and inhibit the antibiotics of this class, as well as the change in the permeability of the bacterium's outer membrane and the presence of efflux pumps that are members of the RND family, all contribute to the bacterium's antibiotic resistance [31].

The low resistance rates against imipenem and meropenem seen in this investigation were consistent with the findings of [32]. Aminoglycosides (amikacin, gentamicin, and tobramycin) had resistance rates of 4, 6, and 8, respectively, of 13.8%, 20.7%, and 27.6%; these results were comparable to those of [33]. According to [34], who discovered that the resistance rate for ciprofloxacin was (23%), the results of the current investigation for quinolones (ciprofloxacin) were consistent. This study's findings on the resistance to trimethoprim-sulfamethoxazole in Salmonella typhi isolates were in agreement with those of [35], who discovered that the resistance rate was 22.5%. Dihydrofolate Reductase (DHFR) and Dihydropteroate Synthase (DHPS) genes are frequently associated with trimethoprim-sulfamethoxazole resistance in integrons. Antibiotic resistance was brought on by the development of bacterial biofilms, which have 10-1,000 times higher survival rates than planktonic cells. Additionally, the tight cooperation of bacteria in a biofilm might encourage the transmission of antibiotic genes [36]. Antibiotic clearance is hampered by biofilm resistance, which is caused by complicated, poorly understood processes that frequently include numerous species.

Antibacterial Activity of green SeNPs:

Agar Well Diffusion Method:

Investigators tested the cinnamic ethanolic extract's and manufactured SeNPs' antibacterial efficacy against isolated salmonella typhi by using the agar well diffusion method, and tested with various concentrations (25,50,100 µg/mL). Table (2) and figure (3), showed the zone of inhibition of tested substance on *salmonella typhi*.

Table (2): Zone of inhibition(mm) of tested substance in human *S.typhi* isolates

Concentration of materials (mg/mL)	**Human isolates**	
	Cinnamon extract	Selenium nanoparticles
25	**10.33±1.2**	**13.33±0.88**
50	**11±0.57**	**14±0.57**
100	**12.33±0.33**	**14.33±0.31**

C	**0±0**	**0±0**
LSD(P<0.05)	**0.272**	

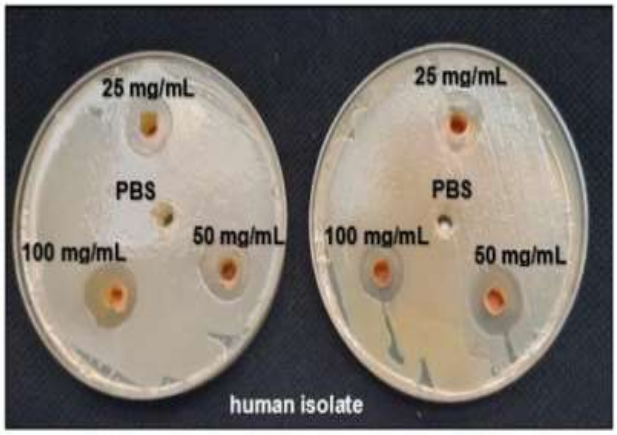

Figure (3): Antibacterial activity of cinnamon extract selenium nanoparticles on human isolates

The findings in Table 2 show that Se-NPs had a positive inhibitory effect at a concentration of 100 mg/ml, which was 14.33 mm, while the cinnamon extract at the same concentration gave an inhibition of 10.66 mm. [37] reported that the inhibition diameter of green synthesized SeNPs at(100mg\ml) concentration on human isolates *salmonella typhi* was more than the diameter in concentration (40and15mg\ml). Results obtained by [38] reported that the salmonella typhi zone of inhibition was increased for SeNPs at the maximum dose (100 mg/ml).

CONCLUSION

In the present research, All *Salmonella typhi* isolates showed resistant to most tested antibiotics, and have the ability to form biofilm. Green SeNPs synthesized by cinnamon extract showed excellent inhibitory effect against *Salmonella typhi*. Eventually, the biosynthesized Se-NPs will include potential antibacterial microbes and be helpful to the medical sector.

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