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Evaluation of the synergistic effect of cinnamon and probiotic filtrate against UTI

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***Abstract*---**This study was designed to evaluate the effects of cinnamon and probiotics both alone and in combination against pathogenic bacteria isolated from UTI patients. One hundred and ten male and female patients with a urinary tract infection (UTI) who visited private health care facilities were asked to provide urine samples. The urine samples were inoculated on Blood and MacConkey agars and incubated for 24 hrs. at 37 degrees Celsius. After 24 hours of incubation, no development was seen in the cultures, thus they were kept in the incubator for a further 48 hours. Gram staining was used to classify bacteria, and then conventional microbiological culture and biochemical properties were used to identify the isolates. Cinnamomum bark oil was extracted manually. Mueller Hinton (MH) agar plates were infected with 10^8 CFU/ml suspensions of microorganisms; this method was used to determine the agar disc diffusion test. Discs of sterile Whatman paper No. 3 impregnated with 10 g of the compounds were placed on top of MH agar that had been infected with bacteria. For 18 hours, the plates were kept at 37°C. Inhibition zones against the various bacterial strains were used to evaluate the antibacterial impact. Inhibition diameter measurements are shown as the mean standard deviation for each sample as follows: For a diameter lower than 8 mm, not sensitive, moderately sensitive (+) between 8 and 14 mm, and sensitive (++) between 15 and 20 mm, more than 20 mm it highly sensitive. A total of 110 male and female individuals matched our inclusion criteria and were enrolled in the research. Out of them, 84 (76.4 percent) isolates were Gram-negative bacteria, and 26 (23.6 percent) were Gram-positive. The most often isolated species were *E. coli* (51.2 percent), *P. aeruginosa* (21.4 percent), *Klebsiella pneumonia* (17.9 percent), *Staphylococcus spp.* (15.4 percent), and *Staphylococcus epidermidis* (26.9 percent). Cinnamon's antibacterial activity varied depending on the isolates studied. From whole, all the studied isolates were showed different sensitivity for cinnamon extract. The most sensitive isolate were *Proteus mirabilis, Staphylococcus epidermidis, P. aeruginosa, E. coli*. The combination of cinnamon oil extract Plus probiotic filtrate showed

a high effectivity against all bacterial isolates. In conclusion, *E. coli* was the most prevalent bacteria isolated from cases of UTI. Cinnamon showed good antibacterial effects against most bacterial isolates of UTI. The combination of cinnamon oil extract Plus probiotic filtrate showed a high effectivity against all bacterial isolates more than both alone.

***Keywords*---**cinnamon, UTI, bacteria.

Introduction

One of the most prevalent bacterial illnesses is an infection of the urinary tract (UTI) (1). An estimated 150 million individuals are diagnosed with UTI each year (2), resulting in over $6 billion in medical expenses. Human urinary tract infections (UTIs) represent a wide range of disorders, from mild cystitis to more severe infections such as necrotizing pyelonephritis (3). As the female urethra is physically proven to be less efficient at preventing bacterial entrance, UTI is more frequent in women than in men (4). In addition, variables such as age, prior use of antibiotics, hospitalization, and catheterization all have a role in the likelihood of UTIs. Almost all urinary tract infections are caused by a single type of bacteria, according to research. In acute infections, E. coli is the most common bacteria to cause illness (5). According to recent studies in Iraq, the three most frequent pathogens responsible for UTIs, E. coli, Staphylococcus spp. and K. pneumonia, are all antibiotic-resistant (6, 7).

Urinary tract infections (UTI) that are diagnosed and treated early are less likely to result in morbidity (8). An adequate empirical treatment requires an understanding of the most common bacteria that cause urinary tract infections and their distinct antibiotic susceptibility patterns (9). Antibiotic sensitivity patterns of bacteria may be determined, which allows for better treatment results, limits the growth of antimicrobial prescriptions, and helps in the fight against antimicrobial resistance, which is a global public health issue. While male germs were shown to be more antibiotic-resistant, female pathogens were found to be less resistant. Gender should thus be taken into consideration when selecting an experimental antimicrobial medication (10).

Several trees from the Cinnamomum genus are endemic to Sri Lanka and are used to produce cinnamon, a tropical Asian spice (11–13). In addition to being used in cooking, cinnamon is also a prominent ingredient in both traditional and contemporary medicine (13). Antibacterial (14), antifungal (15), antioxidant (16), antidiabetic (17), anti-inflammatory (18,19), nematicidal (20), insecticidal (21), and anticancer (22) properties have been reported for the barks and leaves.

There are non-pathogenic bacteria known as probiotics, which have a positive impact on a person's health by altering their gut microbiota balance. They may be termed functional foods if ingested in adequate quantities and in a usable form. Nevertheless, their impact on diabetes is still debatable (23). In the digestive tract, Lactobacillus and Bifidobacterium, which produce lactic acid, are considered probiotics. By altering the natural flora of the gut, oral treatment of these

probiotics may ameliorate metabolic diseases such as diabetes (24, 25). This study was designed to evaluate the effects of cinnamon and probiotics both alone and in combination against pathogenic bacteria isolated from UTI patients.

Materials and Methods

One hundred and ten male and female patients with a urinary tract infection (UTI) who visited private health care facilities were asked to provide urine samples. To prevent contamination, we used a sterile, single-use container to collect midstream-clean catch urine from patients. Afterwards, the urine samples were inoculated on Blood and MacConkey agars and incubated for 24 hrs. at 37 degrees Celsius. After 24 hours of incubation, no development was seen in the cultures, thus they were kept in the incubator for a further 48 hours. Gram staining was used to classify bacteria, and then conventional microbiological culture and biochemical properties were used to identify the isolates. Methods used to identify microorganisms included (26).

There were *Cinnamomum* (cinnamon bark) purchases made at a local market (Iraq). Using a Clevenger-type modified equipment, 600 ml of distilled water was used to hydrodistill 100 g of cinnamon barks for three hours. As the hydrosol was collected, the heavy oil was decanted from the flask, while the hydrosol water was recycled back into a flask that contained boiling plant material. Before analysis, the EO was collected and kept at 4°C. Colonies from 24-hour cultures were used to make the inoculum solution. NaCl solution (0.9%) was used to suspend the colonies. The turbidity of a 0.5 McFarland Standard (10^8 CFU/mL) was used to alter the density.

Mueller Hinton (MH) agar plates were infected with 10^8 CFU/ml suspensions of microorganisms; this method was used to determine the agar disc diffusion test. Discs of sterile Whatman paper No. 3 impregnated with 10 g of the compounds were placed on top of MH agar that had been infected with bacteria. For 18 hours, the plates were kept at 37°C. Inhibition zones against the various bacterial strains were used to evaluate the antibacterial impact. Inhibition diameter measurements are shown as the mean standard deviation for each sample as follows: For a diameter lower than 8 mm, not sensitive, moderately sensitive (+) between 8 and 14 mm, and sensitive (++) between 15 and 20 mm, more than 20 mm it highly sensitive (27). Probiotic was purchased from local market. Antimicrobial activity of probiotic against test pathogens was determined by agar-well diffusion method as per (28).

Results and Discussions

A total of 110 male and female individuals matched our inclusion criteria and were enrolled in the research. Out of them, 84 (76.4 percent) isolates were Gram-negative bacteria, and 26 (23.6 percent) were Gram-positive. The most often isolated species were *E. coli* (51.2 percent), *P. aeruginosa* (21.4 percent), *Klebsiella pneumonia* (17.9 percent), *Staphylococcus spp.* (15.4 percent), and *Staphylococcus epidermidis* (26.9 percent) (Table 1).

Table 1. Bacterial isolates from UTI cases

Gram Positive bacteria	No. (%)	Gram negative bacteria	No. (%)
Staphylococcus spp.	4 (15.4)	*Escherichia coli*	43 (51.2)
Staphylococcus epidermidis	7 (26.9)	*Pseudomonas aeruginosa*	18 (21.4)
Streptococcus spp.	9 (34.6)	*Klebsiella pneumonia*	15 (17.9)
Staphylococcus aureus	6 (23.1)	*Proteus mirabilis*	8 (9.5)
Total	26	*Total*	84

It is one of the most common illnesses in the world, according to the CDC (1). More than half of all community-acquired urinary tract infections (UTIs) are caused by *E. coli*, which is the most common uropathogen (29). Gender, age, geographic location, past antibiotic usage, hospitalization, and catheterization all have a role in UTI prevalence (30).

Because it is a member of the normal flora of the human intestine and thus easily colonizes the urinary tract, *E. coli* is the most commonly isolated etiological agent responsible for 80 percent to 90 percent of uncomplicated UTIs and can exhibit multidrug resistance (31). E. coli has also been found to be the most common cause of UTIs in males, according to other studies (32, 33). *E. coli* was shown to be the most frequent bacteria responsible for urinary tract infections (UTIs) in earlier research done in Iraq (7, 34).

This study's findings are in line with those of an earlier Iranian investigation that identified *P. aeruginosa* as the second most prevalent gram-negative cause of UTI in human (35). *P. aeruginosa* was shown to be the second most prevalent cause of urinary tract infection (UTI) in men in another study from Ethiopia (36). UTIs are more often caused by the bacteria *P. aeruginosa*, which has been shown to be an opportunistic infection in the general population (8). Due to changes in sampling, research design, and inclusion criteria, the results reported in various studies may vary. Table 2 showed that Cinnamon had different antibacterial properties against tested isolates. In total, all studied isolates were showed different sensitivity for cinnamon extract. The most sensitive isolate were *Proteus mirabilis*, *Staphylococcus epidermidis, P. aeruginosa, E. coli*.

Table 2. zone of inhibition for cinnamon oil extract

Bacteria	inhibition zone of isolates (mm)			
	n (%) (DIZ < 8 mm) Not sensitive	n (%) (DIZ = 8-14 mm) Moderately sensitive	n (%)(DIZ = 15-20 mm) Sensitive	n (%) (DIZ > 20 mm) Very Sensitive
Staphylococcus spp.	0 (0%)	2 (50%)	1 (25%)	1 (25%)
Staphylococcus epidermidis	1 (14.3%)	3 (42.9%)	3 (42.9%)	0 (0%)
Streptococcus spp.	1 (11.1%)	5 (55.6%)	2 (22.2%)	1 (11.1%)
Staphylococcus aureus	2 (33.3%)	1(16.7%)	2 (33.3%)	1 (16.7%)
Escherichia coli	2 (4.6%)	17 (39.5%)	14 (32.6%)	10 (23.3%)
Pseudomonas aeruginosa	0 (0%)	7 (38.9%)	5 (27.8%)	6 (33.3%)

Klebsiella pneumonia	1 (6.7%)	8 (53.3%)	4 (26.7%)	2 (13.3%)
Proteus mirabilis	2 (0%)	4 (37.5%)	1 (50%)	1 (12.5%)
Total (110)	9 (8.2%)	47 (42.7%)	32 (29.1%)	22 (20%)

Cinnamon oil was shown to be effective against several bacterial UTI infections in a recent study. The presence of chemicals like cinnamaldehyde as well as eugenol, that have been shown in various researches to have antibacterial characteristics [37,38,39], is responsible for these results.

Cinnamon's primary mode of action against germs is the breakdown of the cell membrane. Even yet, a number of studies have shown that cinnamon has antibacterial properties against a wide range of pathogenic bacteria from the clinical and dietary origins as well as conventional bacteria [40- 42]. Our findings are consistent with those of Intorasoot *et al.* [43], who found that the volatile oil from cinnamon exhibited excellent antibacterial action against *E. coli*, *S. aureus, P. aeruginosa* as well as MDR clinical isolates. Also, many authors reported that the antimicrobial effects of cinnamon on the food-borne pathogens, typical strains, and food deteriorating bacteria have been examined in the majority of prior investigations [40–42]. The probiotic filtrates showed some antimicrobials effects against all bacterial strains especially against *Proteus mirabilis, Streptococcus spp., E. coli* (Table 3).

Table 3. zone of inhibition for probiotic filtrate

Bacteria	inhibition zone of the isolates (mm)			
	n (%) (DIZ < 8 mm) Not sensitive	n (%) (DIZ = 8-14 mm) Moderately sensitive	n (%)(DIZ = 15-20 mm) Sensitive	n (%) (DIZ > 20 mm) Very Sensitive
Staphylococcus spp.	0 (0%)	3 (75%)	1 (25%)	0 (0%)
Staphylococcus epidermidis	1 (14.3%)	4 (57.2%)	2 (28.6%)	0 (0%)
Streptococcus spp.	2 (22.2%)	7 (77.8%)	0 (0%)	0 (0%)
Staphylococcus aureus	3 (50%)	2(33.3%)	1 (16.7%)	0 (0%)
Escherichia coli	5 (11.6%)	23 (53.5%)	14 (32.6%)	1 (2.3%)
Pseudomonas aeruginosa	3 (16.7%)	13 (72.2%)	1 (5.6%)	1 (5.6%)
Klebsiella pneumonia	4 (26.7%)	4 (26.7%)	4 (26.7%)	3 (20%)
Proteus mirabilis	0 (0%)	3 (37.5%)	4 (50%)	1 (12.5%)
Total (110)	18 (16.4%)	59 (53.6%)	27 (24.5%)	6 (5.5%)

In order to find new and effective probiotics, antimicrobial activity is one of the most crucial selection patterns. Bacteriocins, organic acids, hydrogen peroxide, low-molecular-weight antimicrobial compounds, and bacteriocins are some of the antimicrobial chemicals produced by the isolates (44). A broad variety of intestinal pathogens may be inhibited by the use of probiotics, including *Lactobacillus, Bifidobacterium*, and *Streptococcus sp.* The formation of colon tumors seems to be protected against by the beneficial effects of probiotic bacteria, in addition to those against illness induced by an imbalance in the gut microflora.(45)

Several clinically significant pathogens, such as *Enterotoxigenic E. coli* (4.2 mm), *Salmonella typhimurium* (4.3 mm), and *Listeria monocytogenes*, were successfully combated by *Lactobacillus spp.* (5.0 mm) isolated from fermented dairy products in the research by (46). The *Lactobacillus spp.* isolates pale in comparison to the antibacterial abilities of the isolates from this investigation. In contrast to the *Lactobacillus plantarum* and *Lactobacillus salivarius* isolates by (47) from a plant-related probiotic, our isolates exhibited essentially identical antagonistic activity against *E. coli* and *S. typhimurium*. (47). Tea-leaf isolated *Lactobacillus paraplantarum* strains showed high inhibitory activity against *S. typhii* (65 mm), *E. coli* (30 mm), *S. aureus* (56 mm), *E. faecalis* (55 mm), and *Citrobacter species* (60 mm) (48). The combination of cinnamon oil extract Plus probiotic filtrate showed a high effectivity against all bacterial isolates (Table 4).

Table 4. zone of inhibition for cinnamon oil extract Plus probiotic filtrate

Bacteria	inhibition zone of the isolates (mm)			
	n (%) (DIZ < 8 mm) Not sensitive	n (%) (DIZ = 8-14 mm) Moderately sensitive	n (%)(DIZ = 15-20 mm) Sensitive	n (%)(DIZ > 20 mm) Very Sensitive
Staphylococcus spp.	0 (0%)	1 (25%)	2 (50%)	1 (25%)
Staphylococcus epidermidis	0 (0%)	3 (42.9%)	3 (42.9%)	1 (14.3%)
Streptococcus spp.	0 (0%)	2 (22.2%)	5 (55.6%)	2 (22.2%)
Staphylococcus aureus	0 (0%)	2(33.3%)	3 (50%)	1 (17.7%)
Escherichia coli	0 (0%)	12 (27.9%)	26 (60.5%)	5 (11.6%)
Pseudomonas aeruginosa	0 (0%)	7 (38.9%)	9 (50%)	2 (11.1%)
Klebsiella pneumonia	0 (0%)	2 (13.3%)	8 (53.3%)	5 (33.3%)
Proteus mirabilis	0 (0%)	1 (12.5%)	5 (62.5%)	2 (25%)
Total (110)	0 (0%)	30 (27.3%)	61 (55.5%)	19 (17.2%)

There was a lack of studies about using of cinnamon and probiotic against bacteria, for prebiotic qualities and medicinal uses, cinnamon is one of the most well-known spices. Cinnamon's prebiotic qualities, polyphenols, antioxidants, and essential oils may be responsible for its health benefits (49). The combination of probiotic bacteria and a prebiotic compounds form synbiotics (50). According to the previous in vitro studies, merely an application of a synbiotic is more advantageous than prebiotic and probiotic (51).

Conclusion

E. coli was the most prevalent bacteria isolated from cases of UTI. Cinnamon showed good antibacterial effects against most bacterial isolates of UTI. The combination of cinnamon oil extract Plus probiotic filtrate showed a high effectivity against all bacterial isolates more than both alone.

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