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Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF GENISTEINAND QUERCETIN AGAINST *ESCHERICHIA COLI* ISOLATED FROM CLINICAL SAMPLES

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ABSTRACT

Objective: The aim of this study was to investigate the antibacterial activity of Genistein and Quercetin against pathogens of the urinary tract infection (UTI).

Methods: An *in vitro* study was carried out using the following bacterial strains involved in UTI diseases using well diffusion (WD) testing: *Escherichia coli* (ATCC 25922) and 10 strains were compiled from Aleppo Hospital. It was from women and men have UTI. The antibacterial activity of Genistein and Quercetin was determined in the form of inhibition zone using agar WD testing.

Results: In all experiments, results obtained indicated that Genistein and Quercetin had inhibitory effects on *E. coli* (ATCC 25922) and some 10 strains. This study showed that Genistein and Quercetin were active against the tested bacterial strains.

Conclusion: The Genistein showed better antibacterial effect the tested bacterial strains. Therefore, the Genistein and Quercetin could be encouraged for further development in caries prevention and treatment.

Keywords: Urinary tract infection, *Escherichia Coli*, Genistein, Quercetin, Well diffusion testing.

INTRODUCTION

A urinary tract infection (UTI) is an infection of the urethra, bladder, and/or kidneys, the major structures composing the urinary tract. Infections of the urethra (called urethritis) and of the bladder (called cystitis) are more common than infections of the kidney (called pyelonephritis). A bladder infection, if left untreated, may lead to a kidney infection due to bacteria ascending from the bladder, up the ureters, to the kidneys. Since kidneys are vital organs, essential to life, it is important that medical care is sought if any symptoms of a UTI are present. UTIs develop when bacteria get into the urinary system, a part of the body which normally has no bacteria [1]. About 85% of these infections are caused by a normal intestinal bacterium named *Escherichia coli*, commonly called *E. coli.* While infection with *E. coli* is most common, many bacteria including *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Proteus*, and *Klebsiella* species may be responsible. *E. coli* is the most common bacterium isolated in urine UTIs. Figure 1 is shown the colony's *Escherichia coli* in Eosin Methylene Blue Agar (EMB) agar.

E. coli is considered a normal component of gastrointestinal and distal urogenital flora but it can ascend the urethra and gain entrance to the urinary tract. Specific virulence factors found in *E. coli* allow it to adhere to and invade host cells, produce toxins, utilize host nutrients, and evade the host's immune system.

Most UTIs are not serious, but some infections can lead to serious problems, such as kidney infections. Chronic kidney infections – infections that recur or last a long time – can cause permanent damage including kidney scars, poor kidney function, high blood pressure, and other problems. Some acute kidney infections – infections that develop suddenly – can be life threatening, especially if the bacteria enter the bloodstream, a condition called septicemia [2].

Most UTIs are caused by bacteria, which are treated with bacteria-fighting medications called antibiotics or antimicrobials. The choice of medication and length of treatment depend on the patient's history and the type of bacteria causing the infection. The synthetic drugs have been associated with severe side effects on human health. Due to these facts, it is urgent to explore an alternative antibacterial drug [3,4], which can affect the UTIs with less drug-resistance, for rebuilding/maintaining a "healthy" microbial community [5]. Recently, there has been a growing interest in the investigation and introduction of medicinal plants with various biological activities at the aspect of new drug development because of the advantages of ample materials source, ease of use, good efficacy, and small side effects [6].

Genistein and Quercetin are a natural extract of flavonoids Table1 is shown information on the Genistein and Quercetin, which widely exist in many parts of plants and attract much attention as natural molecules with a significant affinity toward targets of potential medicinal interest but also as a food supplement or prospective chemopreventive agent [7].

Many studies have demonstrated that Genistein and Quercetin have obvious pharmaceutical effects of anti-cancer [8,9], antioxidation [10,11], tyrosine kinase inhibiter [12], and anti-inflammatory [13,14]. Recently, Genistein showed significant antibacterial activity against *Pseudomonas syringae* (phytopathogenic bacteria) and *Vibrio fluvialis* (human pathogenic bacteria) [15], while the Quercetin have the ability to inhibit the acidogenicity of *Streptococcus mutans* [16].

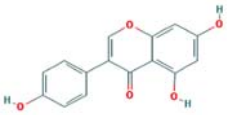
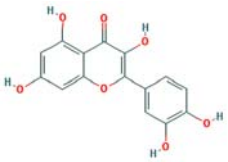
In this study, we aimed to evaluate the antibacterial ability of Genistein and Quercetin on the UTI diseases-associated pathogen including *E. coli*.

METHODS

Strains and culture media

The Genistein and Quercetin were tested on the following strains: *E. coli* (ATCC 25922) was kindly donated by Aleppo University. It used as references for the antibacterial assay of Genistein and Quercetin. Addition the 10 strains were compiled from Aleppo Hospital. It was

Table 1: : Structure of and information on the Genistein and Quercetin

Chemical name	IUPAC name	Information	Compound structure
Genistein	5,7-dihydroxy-3-(4 hydroxyphenyl) chromen-4-one	MW: 270.24 g/mol MF: $C_{15}H_{10}O_5$ H-bond donor: 3 H-bond acceptor: 5 Log p: 2.37	
Quercetin	2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	MW: 302.236 g/mol MF: $C_{15}H_{10}O_7$ H-bond donor: 5 H-bond acceptor: 7 Log p: 1.82	

E. coli: Escherichia coli

Table 2: Biochemical identifications of the isolated *E. coli*

E. coli	Biochemical test
Positive	Catalase
Negative	Oxidase
Not done	Coagulase
Positive	Indole
Positive	Methyl red test
Negative	Citrate
Negative	Urease
Acid in slant and butt with gas production	TSI
Positive	Nitrate

E. coli: Escherichia coli

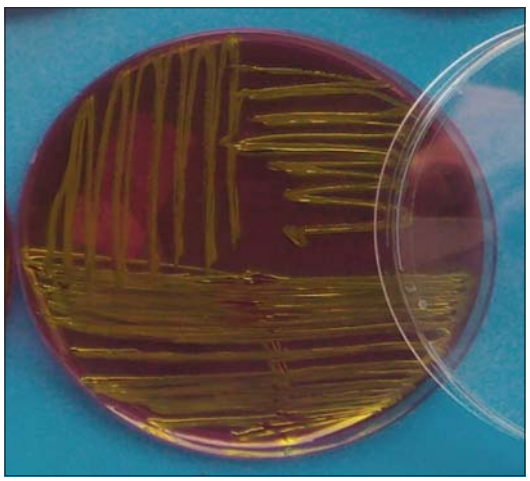


Fig. 1: *Escherichia coli* in eosin methylene blue agar

from women and men have UTI. Table 2 is shown biochemical test results.

Preparation of Genistein and Quercetin solution

To examine the antibacterial activity of Genistein and Quercetin on *E. coli*, the Genistein and Quercetin purchased from Sigma were dissolved in dimethyl sulfoxide (DMSO, Sigma) to obtain concentrations of 400 µg/100 µL.

Antibacterial test using the agar diffusion method (well)

The well diffusion (WD) test was carried out with Muller-Hinton agar (MHA). The inoculum was prepared using 24-hr plate cultures of *E. coli*. The colonies were suspended in 0.85% saline, and the turbidity was compared with the 0.5 McFarland standard (equal to 1.5×10^8 colony-forming units/ml). The suspension was loaded on a sterile cotton swab that was rotated several times and press firmly against the inside wall of the tube to remove excess inoculum from the swab. The dried surface of an MHA agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated two more times, rotating the plate approximately 60° each time to ensure a uniform distribution of inoculum. Next, where 7 mm wells were cut and filled with 100 µL of sample (400 µg/well). Ampicillin (AMP) (100 µL at a concentration of 1 mg/10 mL, equivalent to 10 µg/well) and ceftriaxone (CET) (100 µL at a concentration of 3 mg/10 mL, equivalent to 30 µg/well) were used as positive control and DMSO as a negative control. The Petri dishes were pre-incubated for 3 hrs at room temperature, allowing the complete diffusion of the samples [17]. Then, the plates were incubated at 37°C±1°C for 24 hrs. The antibacterial activity was determined by measuring of inhibition zone diameters (mm) and was evaluated according the parameters suggested by [18]: Inhibition zones <9 mm, inactive; 9-12 mm, less active; 13-18 mm, active; >18 mm, very active [3]. All assays were performed in triplicate and repeated at least three times.

RESULTS FOR ANTIBACTERIAL ACTIVITY

In this study, we focused on some Flavonoid compounds action against bacteria involved in UTI. The antibacterial activity of Genistein and Quercetin against *E. coli* (ATCC 25922) and 10 strains were tested and compared to that of antibiotics AMP and CET (Tables 3 and 4). Among the 10 strains of *E. coli* tested, we observed the inhibitory effect on the growth of 10 bacterial strains. The Genistein and Quercetin, when used in combination with antibiotics, were found to increase each other activity against test bacteria. The relationship between the Genistein, Quercetin, and antibiotics in most of the cases was synergism.

DISCUSSION

Many natural plants and their extracts have been used for the medical purpose for centuries, and their biological and pharmacological effects have attracted more and more investigations in recent years [19,20]. Flavonoids (polyphenols) are bioactive molecules. These biological activities are related to the molecules structures; by their hydroxyl groups or by phenolic ring, phenolic compounds have capacity to link with proteins and bacterial membrane to form complexes [21]. Thus, several studies have reported the antimicrobial activities of plants extracts from various parts such as leaves, seeds, and flowers [22,23]. These results often pointed out that crud extract possessed low antibacterial activities against enteric bacteria. However, our results are similar to other studies carried out with pure molecules of flavonoids where the table 3 and table 4 presented that Genistein and Quercetin have an antibacterial activity. Moreover, the Genistein has a high antibacterial activity due to its bigger inhibition zone than Quercetin has [24].

These molecules showed antibacterial activities higher than crud extract. This difference may be explained by the presence of some residue in crud extract, which prevents direct contact between phenolic compounds and bacteria.

Table 3: Antibacterial activity of Genistein against *E. coli*

Materials	Diameters of inhibition zone (mm)				
	Genistein concentration 400 µg/well	AMP	CET	Genistein+AMP	Genistein+CET
ATCC	17	20	25	19	20
Strain 1	R	R	R	R	R
Strain 2	15	R	R	R	R
Strain 3	15	20	25	19	20
Strain 4	15	20	24	18	20
Strain 5	20	21	25	22	25
Strain 6	15	R-	R	R	R
Strain 7	R	20	26	16	19
Strain 8	15	R	R	R	R
Strain 9	R	R	R	R	R
Strain 10	16	20	25	18	22

AMP: Ampicillin, CET: Ceftriaxone, *E. coli: Escherichia coli*

Table 4: Antibacterial activity of Quercetin against *E. coli*

Materials	Diameters of inhibition zone (mm)				
	Quercetin concentration 400 µg/well	AMP	CET	Quercetin+AMP	Quercetin+CET
ATCC	15	20	25	15	28
Strain 1	12	NA	NA	NA	NA
Strain 2	15	20	25	NA	25
Strain 3	14	20	25	22	30
Strain 4	15	20	24	23	30
Strain 5	15	21	25	22	28
Strain 6	15	21	25	NA	28
Strain 7	NA	NA	NA	NA	NA
Strain 8	12	NA	NA	10	28
Strain 9	NA	NA	NA	NA	NA
Strain 10	18	20	25	24	30

AMP: Ampicillin, CET: Ceftriaxone, *E. coli: Escherichia coli*

In fact, Cowan [6] supposed that phenolic compounds without free hydroxyl groups have more antibacterial activity than those, which are provided. That increases their chemical affinity to microbial lipid membrane. There are less hydroxyl groups in Genistein molecular structure than Quercetin that might justify the difference of antibacterial activity between these two compounds in our study.

The results of this work are clear indication for the possible use of Genistein and Quercetin for control of some UTI.

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