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Effects of Phenolic Compounds Isolated from Tamarix Aphylla on E. Coli Carrying the Fim-H Gene Isolated from Patients with Urinary Tract Infections

Conflict of interest: nothing to declare.
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Abstract

Introduction. Antibiotic-resistant strains of numerous bacteria, including E. coli, the primary cause of urinary tract infections, have arisen as a result of the indiscriminate use of antibiotics, which affects individuals of all ages and cultures. Tamarix aphyllaa has antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory qualities and is common in the Iraqi environment.
Purpose. To determine the effects of Tamarix aphylla phenolic compounds on E. coli carrying the fim-H gene isolated from patients with urinary tract infections.
Materials and methods. An economical and low-cost approach was used to separate phenolic chemicals from the plant's leaves and stems; concentrations ranging from 100% to 5% (75%, 50%, 25%, 10%, and 5%) were created. Eosin-methylblue-Api-20E and 16srRNA were used to describe the E. coli bacteria that were recovered from patients with urinary tract infections. The criterion for the extract's antibacterial activity was the assessment of the zone of inhibition growth of bacteria cultured on Mueller-Hinton agar. The fim-H gene, one of the most well-known virulence factors in E. coli bacteria, was the focus of the genetic analysis of the bacteria. Genomic DNA was extracted using the Geneaid Kit.
Results. With the highest average inhibition zone of 17.8±3.4 at a 100% concentration and the lowest average concentration of 1.2±1.8 at a 5% concentration, tamarix aphylla displayed exceptional efficiency against E. coli bacteria. The study demonstrated that an increase in concentration is necessary for an increase in the inhibition zone. With the greatest effect (17.8) at 100% and the lowest at 5% (1.2), inhibition rose as concentration increased. The fim-H resistance gene was present in every isolate used in the investigation.
Conclusion. Tamarix aphylla possesses significant antibacterial activity against Escherichia coli isolated from urinary tract infections. The phenolic extracts showed a clear concentration-dependent inhibitory effect, with the highest antibacterial activity observed at the maximum concentration. The presence of the fim-H virulence gene in all isolates highlights the pathogenic potential of the studied strains and emphasizes the importance of identifying effective alternative antimicrobial agents.
Keywords: E. coli, tamarix aphylla, UTI, fim-H, phenol

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Влияние фенольных соединений растения Tamarix aphylla на выделенные у пациентов с инфекциями мочевыводящих путей штаммы E. coli, несущие ген fim-H

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Резюме

Введение. В результате бесконтрольного применения антибиотиков появились устойчивые к ним штаммы множества бактерий, в том числе E. coli – основной причины инфекций мочевыводящих путей, от которых страдают люди всех возрастов и рас. Растение Tamarix aphylla (тамарикс безлистный), повсеместно встречающееся в Ираке, обладает антибактериальными, противогрибковыми, противовирусными, антиоксидантными и противовоспалительными свойствами.
Цель. Определить эффект воздействия фенольных соединений Tamarix aphylla на бактерии E. coli, несущие ген fim-H, выделенные у пациентов с инфекциями мочевыводящих путей.
Материалы и методы. Исследовано 74 пробы мочи на предмет изучения влияния фенольного экстракта из листьев и стеблей растения тамарикс безлистный (Tamarix aphylla) на несущие ген fim-H бактерии E. coli, выделенные у пациентов с инфекциями мочевыводящих путей. Использован не требующий больших финансовых затрат простой в исполнении метод экстракции фенольных соединений с получением растворов с концентрацией от 100% до 5% (75%, 50%, 25%, 10% и 5%). Для описания бактерий E. coli, выделенных у пациентов с инфекциями мочевыводящих путей, было применено окрашивание эозин-метиленовым синим – Api-20E с последующим анализом нуклеотидных последовательностей генов 16srRNA. Критерием антибактериальной активности экстракта служила оценка зоны ингибирования роста бактерий, выделенных на агаре Mueller-Hinton. В центре внимания генетического анализа бактерий находился ген fim-H – один из наиболее известных факторов вирулентности бактерий E. coli. Экстракция геномной ДНК осуществлялась с использованием Geneaid kit.
Результаты. Судя по показателям средней площади зоны ингибирования, наибольший эффект воздействия фенольных соединений на бактерии наблюдался при использовании 100%-ного раствора экстракта из растения тамарикс безлистный (максимальная средняя площадь зоны ингибирования составила 17,8±3,4), наименьший – при концентрации 5% экстракта, при которой отмечена минимальная средняя

НА ПЕРВУЮ | НА СЛЕДУЮЩУЮ | НА ПРЕДЫДУЩУЮ | К СОДЕРЖАНИЮ

площадь зоны ингибирования – 1,2±1,8. Ингибирование усиливалось по мере увеличения концентрации раствора экстракта. Ген устойчивости fim-H был выявлен во всех изолятах, использованных в исследовании.

Заключение. Тамарикс безлистный обладает выраженной антибактериальной активностью в отношении Escherichia coli, выделенной из образцов мочи при инфекциях мочевыводящих путей. Фенольные экстракты проявили явный ингибирующий эффект, зависящий от концентрации раствора, причем наибольшая антибактериальная активность его наблюдалась при максимальной концентрации. Наличие гена вирулентности fim-H во всех изолятах свидетельствует о патогенном потенциале исследуемых штаммов и подчеркивает важность поиска эффективных альтернативных антимикробных препаратов.

Ключевые слова: E. coli, Tamarix aphylla (тамарикс безлистный), инфекции мочевыводящих путей (UTI), ген вирулентности fim-H, фенол

■ INTRODUCTION

Phenolic Substances "Polyphenols" and "plant phenolics" are secondary natural metabolites that originate biogenetically from either the shikimate/phenylpropanoid pathway, which directly creates phenylpropanoids, or the "polyketide" acetate/malonate system, which can produce simple phenols. These metabolites can generate polyphenols and monomeric and polymeric phenols, which have a wide range of physiological uses in plants [1].

Because they are present in the vacuole in mixed forms with sugars as heterosides, they are typically soluble in water. They both have an aromatic ring, but at least one hydroxyl group has taken the place of at least one aromatic compound ring [2].

Fruits, vegetables, cereals, bark, roots, stems, flowers, tea, and wine all contain phenolic structures. The positive effects of these natural items on health and endeavors are widely recognized [3]. Phenolic compound classification Simple and polyphenolic compounds are the two main categories of phenolic compounds simple phenol compounds with a basic skeleton consisting of at least one hydroxyl group joined to an aromatic ring [4].

Polyphenols: Phenolic compounds that contain more than one phenol unit are considered "polyphenol". Polyphenolic compounds have a C15 general skeleton representation. Plant foods are rich sources of phenolics, which are molecules that can act as antioxidants to prevent heart disease, reduce inflammation lower the incidence of cancers and diabetes as well as reduce rates of mutagenesis in human cells anti bacteria, antiviral and antifungal [2].

Tamarix aphylla Popular habitats include sand dunes, canals, riverbanks, wadi beds, salty deserts, salt marshes and coastal plains. The tree is drought, heat, salt and frost tolerant [3].

It is used as a carminative diuretic in tuberculosis, leprosy, and hepatitis. Various pharmacological properties have been shown by T. aphylla, such as antidiabetic, anti-inflammatory, antibacterial, antifungal, anticholinesterase, and wound-healing activity [5].

E. coli Straight, cylindrical rods that range in size from 1.1 to 1.5 to 2.0 to 6.0 microns can be found alone or in pairs as E. coli. Meet the general requirements of the Enterobacteriaceae family. Gram-negative, according to [6] either nonmotile or motile via

peritrichous flagella. Respiratory system that is both facultatively anaerobic and aerobic. Voges-Proskauer negative, methyl red positive, and glucose fermentation via the mixed acid route [7].

Strains of Escherichia coli (STEC) that produce the enterotoxin verotoxin, which is comparable to Shiga toxin [8].

E. coli strains, including urinary tract infection, septicemia, newborn meningitis, central nervous system and respiratory system infections. Several bacterial agents can cause diarrheal and urinary tract infections, among these E. coli strains are detected as an important cause of morbidity and mortality of diarrhea and UTI throughout the world [9]. The fim-H protein is produced as a precursor of 300 amino acids and is processed into a mature form of 279 amino acids. The fim-H gene is 903bp long and DNA sequence variations were common among commensal E. coli, intestinal pathogenic strains and extraintestinal pathogens. However, genetic variations were more common in intestinal pathogens and extraintestinal pathogens because stressful environment make these isolates adaptable by genotypic changes and consequently phenotypic variants were prevalent among these groups [10].

One of the serious risk factors for CKD is UTIs, which may also be the most common infectious disease associated with CKD patients [11].

UTI is an infection that occurs in any part of the urinary system, including the urethra, bladder, ureters, and kidneys. UTI was classified as either uncomplicated infections such as cystitis and pyelonephritis in people without any structural or neurological abnormalities of the urinary tract or complicated urinary tract infection include factors [12].

■ PURPOSE OF THE STUDY

To determine the effects of Tamarix aphylla phenolic compounds on E. coli carrying the fim-H gene isolated from patients with urinary tract infections.

■ MATERIALS AND METHODS

Pathogenic Bacteria Collection

At the Al-Hussein Teaching Hospital in the Thi-Qar Governorate, 74 urine samples from patients with UTIs were taken.

Midstream Clean-Catch: After the first portion of the urine is emptied, collect a midstream specimen into a sterile container. Bacteria culture on MacConkey [13].

Collection Plant Samples

The leaves and stems of the plant utilized in the study were obtained in the spring in the Nasiriyah region of southern Iraq during the beginning of March 2024. After drying, they were ground.

Separation of Polyphenols from Tamarix aphylla by Ultrasound-assisted Extraction

Phenolic chemicals were extracted from Tamarix aphylla using ultrasound and a 30 : 70 ml water – ethanol combination. The extraction's outcomes at 45 °C, 250 volts, 24 hours, and 50/Hz UAE is based on the principle of acoustic cavitation ultrasound that induced via a series of compression and rarefaction waves in the molecules of the medium

through which it is capable of damaging the cell walls of plant matrix as well as favoring the release of bioactive compounds [14, 15].

Each 50 g of Corymbia citriodora was crushed and added to 500 mL of 70% ethanol in 1000 ml brown bottles. The mixture was subjected to the previously mentioned ultrasonic therapy while in an ultrasonic bath. The extraction sample was separated using a centrifuge, and Whatmann No. 1 was employed for treatment.

The diluted liquid was gathered and put in a separatory funnel in a rotating evaporator under vacuum at 45 °C. To lower solvent quantities to one-third of their initial levels, the filtered liquid was separated five times using half volume chloroform to create two layers (aqueous and chloroform layers). After the aqueous layer was extracted four times with half volume using athyle acetate, the ethyl acetate layer was separated and evaporated under decreased pressure at a temperature of no more than 45 °C, resulting in a yellow/brown residue. After that, the extract is allowed to dry for a full day at room temperature [16].

Stock Solution Preparation

To make a 100% concentration solution, dissolve 1 g of extracted material in 10 ml of dimethyle sulfoxide (DMSO). Sterilize the concentrated solution for each extract using a Millipore microfiltration filter with a diameter of 0.22 mm, and then repeat the 5% concentration [17].

Determination of Minimum Bactericidal Concentration (MBC)

On Mueller-Hinton agar, the bacteria were punched into wells with a diameter of 6 mm. The wells were then filled with 100 µl of plant extracts, with DMSO serving as the negative control. After the plates were incubated at 37 °C for 18 to 24 hours, the diameter of the inhibition zones (mm) was used to estimate the antimicrobial activity. Every test was conducted three times, and the average of the outcomes was calculated. Negative controls were evaluated using the extraction solvents [18].

Genomic DNA Extraction

Genomic DNA was extracted from overnight bacterial cultures using the Geneaid kit. After centrifugation, cells were lysed with GST buffer and Proteinase K at 60 °C, followed by GSB buffer at 70 °C. Ethanol was added, and the lysate was applied to a GD column. The column was washed with W1 and Wash buffers, then dried. Pure DNA was eluted with pre-heated elution buffer. The primers of gene [18, 19] were listed in Table 1.

Table 1
Gene primer16Srna, fim-H estB

Gene name	Primer sequences	Annealing Temperature	Product Size (bp)
16srRNA E. coli	CGAGTGGCGGACGGGTGAGT	50 °C	727 bp
	TCGACATCGTTTACGGCGTGGA		
Fim-H	AACAGCGATGATTTCCAGTTTGTGTG	63 °C	465 bp
	TTGCGTACCAGCATTAGCAATGTCC		

■ RESULTS

41 (56%) of the 74 urine samples that were cultivated on blood and MacConkey agar media had no culture. Staph. aureus 3 (4%), E. coli 21 (28%), M. catarrhalis 0%, P. aeruginosa 5 (7%), Kleb. pneumoniae 1 (1%), Ent. cloacae 3 (4%). Eosin-methyl blue medium, API 20E, and 16Srna were used to diagnosis E. coli bacteria, as shown in Figure 1. All isolates contain fim-H gene see Figure 2.

Bioactive Phenolic Compounds

Minimum Bacterial Concentration (MBC). A very strong relationship emerged between increasing concentration and the inhibition zone, with the highest average

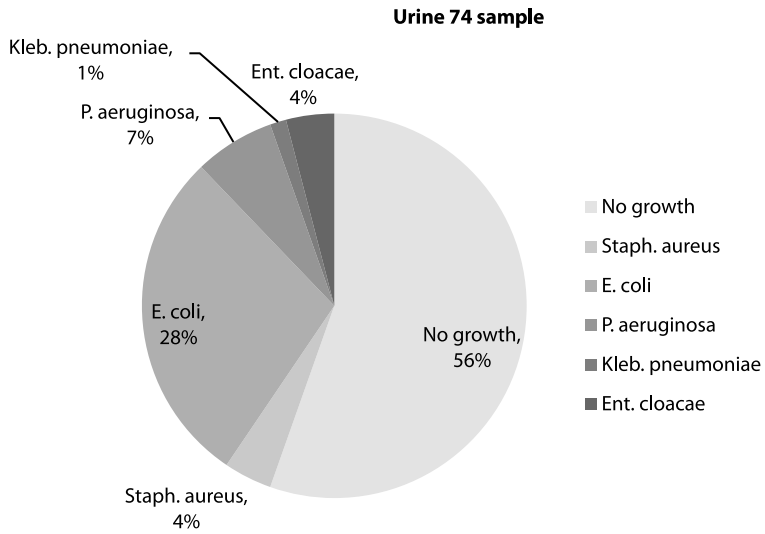

Fig. 1. Result of urine culture

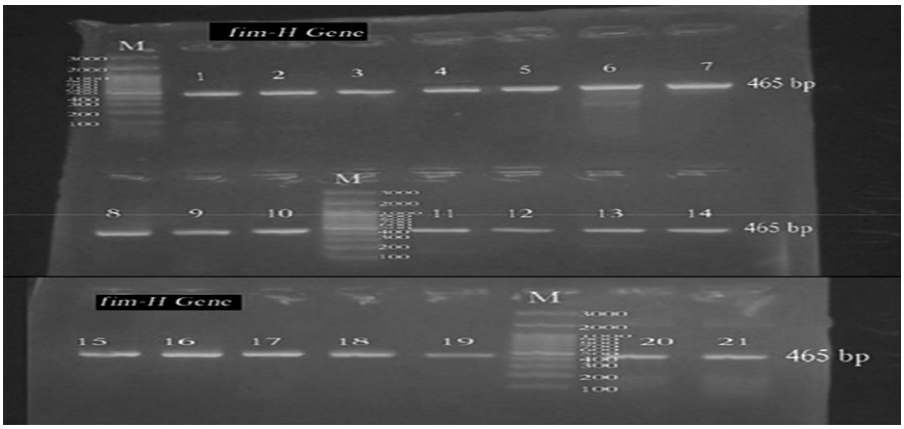

Fig. 2. Result of fim-H gene in E. coli

Table 2
Statistical analysis of the MBC areas of the extraction of Phenolic compounds of tamarix aphyla in bacteria, E. coli (At a probability level of <0.05)

	No.	Concentration	Mean±SD (mm)	p-value
E. coli	21	100%	17.8±3.4	<0.001
E. coli	21	75%	15.0±3.7	0.002
E. coli	21	50%	11.6±3.6	<0.001
E. coli	21	5%	7.9±3.0	<0.001
E. coli	21	10%	4.7±3.0	<0.001
E. coli	21	5%	1.2±1.8	<0.001

inhibition zones appearing at 100% concentration (17.8±3.4). These zones then gradually decreased with decreasing concentration, reaching their lowest value (1.2±1.8) at 5% concentration, result as shown in Table 2.

■ DISCUSSION

Seventy-four urine samples were cultured on blood agar and MacConkey agar, and bacteria were identified using various methods. E. coli was identified using eosin-methyl blue medium, with a detection rate of 21% (28%), which is consistent with what was reported with [20] E. coli formed of the total sample 21 (23.3%) in urine.

According to the information given, the Tamarix aphylla extract has strong, dose-dependent, and statistically significant ($p<0.001$) antibacterial activity against Escherichia coli. The mean zone of inhibition, which dropped from 17.8 mm at the highest concentration to 1.2 mm at the lowest, clearly showed an inverse connection with extract concentration This agrees with what was mentioned [21–23] earlier, where they showed that the excitability increases with increasing concentration, and as the concentration decreases, the effect decreases and the diameter of the inhibitory zones decreases.

The difference in response is due to the ability of the extract molecules to penetrate the cell wall and produce an effect that increases with the number of extract molecules. Increasing the concentration means increasing the number of molecules. Additionally, Tamarix aphylla possesses several pharmacological properties, such as antibacterial and anti-inflammatory effects. This is consistent with what he mentioned [24]. This activity is pharmacologically supported by the rich phytochemical makeup of Tamarix species and is consistent with their historic therapeutic usage.

■ CONCLUSION

Tamarix aphylla possesses significant antibacterial activity against Escherichia coli isolated from urinary tract infections. The phenolic extracts showed a clear concentration-dependent inhibitory effect, with the highest antibacterial activity observed at the maximum concentration. The presence of the fim-H virulence gene in all isolates highlights the pathogenic potential of the studied strains and emphasizes the importance of identifying effective alternative antimicrobial agents. Overall, Tamarix aphylla represents a promising natural source of antibacterial compounds that may contribute to the development of alternative treatments for antibiotic-resistant E. coli infections.

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