Evaluation of Efficiency of Some Plant Extracts Against Two Bacterial Pathogens

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الخلاصة

انجزت هذه الدراسة لبحث تقييم فعالية التثبيط للمستخلص المائي والكحولي لبذور الجرجير Nasturtium officinale والقشور الرقيقة للرمان Punicagranatum ضد بكتريا Escherichia coli وبكتريا الـ Staphylococcus aureus والمعزولة من الانسان. تمدراسةتأثيرتراكيزمختلفة منالمستخلصاتالمائيةوالكحولية على نموالبكتريا قيدالدراسة باستخدام طريقة الانتشار. اشارتالنتائجالى عدم تاثيرالمستخلص الكحولي لبذور الج رجيرعلينموالبكتريا الخاضعةللدراسة.أمابقية المستخلصاتفقدأعطتتأثيراتمتباينة. اشارتنتائج التحليل الاحصائي للنتائج ان المستخلص المائي لقشور الرمان بتركيز (10%) و(25 %) والتي حضرت من المحلول القياسي (100 ملغم / مل) كان اكثرها معنوية في تثبيط بكتريا المكورات العنقودية (P = 0.027) و(P = 0.036) على التوالي بينما كان تركيز (75%) من المستخلص المائي لقشور الرمان اكثرها تثبيطا على بكتريا القولون.

الكلمات المفتاحية

رمان، المستخلصات المائية، بكتريا القولون، المكورات العنقودية، تثبيط البكتريا.

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Abstract

This study was carried out to investigate the effectiveness of inhibition by aqueous and alcoholic extracts of watercress seeds Nasturtium officinale and thin peels of pomegranate Punicagranatum against two of human pathogens Escherichia coli and Staphylococcus aureus. The diffusion method was used for investigating the effect of different concentrations of aqueous and alcoholic extracts on the growth of bacteria, and the results showed that the alcoholic extract of the seeds of watercress did not affect the growth of bacteria under the study. The rest of the extracts have given mixed results; statistical analysis showed that the aqueous extract of the peel pomegranateconcentrations of 10% and 25%, prepared from the stock solution (100 mg. ml^{-1}), were more significantly affected the growth of St. aureus ($P = 0.027$ and $P = 0.036$), respectively. On the other hand, 75% of the aqueous extract of pomegranate peel was more significantly affected the growth of E. coli.

Keywords

pomegranate; watercress,Escherichia coli,Staphylococcus aureus, antibacterial activity.

1. Introduction

Escherichia coli, which is considered a coliform bacteria belonging to the Enterobacteriaceae family,inhabits the digestive tract of animals and humans[1].

E. coli can cause serious infectionsincludinginflammatory bowel disease, diarrhea and colitis if they escape from the digestive tract as a result of surgical operations and enter the bloodstream andtissues [2].Staphylococcus aureus is one of the bacteria that cause diseases in humans (gastroenteritis) by producing a highly heat-stable protein toxin. St. aureusis a facultative anaerobic bacteria, catalase and coagulase positiveand Gram-positive cocci[3].

Diseases caused by these pathogens are normally treated by using antibiotics. The misuse of antibiotics led to serious risks such as the increase of antibiotic resistance [4], the increasing occurrence bacterial strains with multi-drug resistance and new occurrence of strains with decreased sensitivity to antibiotics [5]. In addition, antibiotics are commonly associated with adverse effects on the host including immune suppression, allergic reactions and hypersensitivity [4]. Furthermore, commensal bacteria are also affected by antibiotics.

Serious attention has been paid towards searching for new antimicrobial substances. Therefore, significant attention has recently been paid to explore alternatives to antimicrobial drugs for the treatment of diseases from medicinal plants. A growing body of literature is available on the application of various plant extracts in different parts of the world.

Many diseases including dysentery, haemorrhage, microbial infections and respiratory pathologies have been effectively treated by the fruits of pomegranate, Punicagranatum L. [6]. Furthermore, pomegranate extracts have shown an antiviral activities against the herpes virus [7]. Although whole fruits of pomegranate have been widely investigated, thin peels of pomegranate were rarely studied.

Watercress (Nasturtium officinale) is a perennial plant belonging to the Brassicaceae family which found in clear, cold water in Europe, America and Asia [8]. Watercress is used as a herbal medicine in treatment of some diseases including oxidative stress, asthma and diabetes [9, 10]. Watercress is a high source of vitamins and pro-vitamin A, glucosinolates, folic acid, protein, iron, sulphur and calcium compounds [11].

The present study was carried out to investigate the antibacterial activity of thin peels of pomegranate Punicagranatum and watercress seeds Nasturtium officinale against two human pathogens E. coli and St. aureus.

2. Materials and methods

2.1. Plant specimens

Watercress seeds and thin peels of pomegranate were obtained from a local market and transferred to the laboratory, and ground into fine powder using an electric blender. The powder was dried in an oven at 40°C for 24 h. Then plant pellets were collected in polyethylene and stored at 4°C until extraction.

2.2. Preparation of the extract

2.2.1. Preparation of aqueous extracts

Separately, ten grams (10 g) of watercress seeds and thin peels of pomegranatewere extracted with (100) ml of hot distilled water in a conical flask (250) ml with a rubber stopper. Suspension was left in a shaking incubator (at room temperature) for (24) hours. After that, plant extracts were filtered off using several layers of sterile gauze medical pads into another clean conical flask. The plant extracts obtained were secondly filtered using sterile filter paper (Whatman no. 1) into another clean conical flask. Finally, plant extracts were then centrifuged at (3000) rpm for (10) min. Supernatants were placed in sterile Petri dishes and the extracted liquid was subjected to rotary evaporation in order to remove the liquid. After drying, the dried extracts were scraped off using a clean and sterile knife. The obtained extracts were then stored at (4) °C for antibacterial activity test. Different concentrations were prepared (7.5, 10, 25, 75 and 100%) by adding the required amount of the extract to a suitable volume of distilled water (D.W.) immediately before use.

2.3. Ethanol extraction

Ten grams (10 g) of watercress seeds and thin peels of pomegranatewere separately suspended in (100) ml of ethanol (95%) in a conical flask (250) ml and covered with a rubber stopper. After that, the same steps were used as in hot water extraction.

2.4. Bacterial isolates

Two bacterial strains were used in the study: one Gram negative, namely E. coli and one Gram positive, namely St. aureus. The tested strains were obtained from Kerbala Public Health Laboratory, Iraq.

2.5. Inoculum preparation

The inocula were prepared from stock cultures, which were maintained on nutrient agar slant at (4) °C and subcultured on to nutrient broth.

To calculate the bacterial cellsin the suspension, (0.1) ml of suspension was diluted with (0.9) ml sterile of phosphate buffered saline (PBS; pH (7.3); Oxoid, UK). This solution was then serially diluted tenfold to 10-7 with PBS and (100)µl of each dilution was spread on duplicate Nutrient Agar (Oxoid, UK) plates and incubated aerobically at (37) °C for (24) h. The colony forming units (CFU) were counted on all plates containing (30–300) CFU to determine the inoculum size to reach an inoculum of approximately log (107) CFU ml-1 to be used in assays.

3. Antibacterial activity

Antibacterial activity of the four different samples: hot water extract of watercress seeds, hot water extract of thin peels of pomegranate, ethanol extract of watercress seeds and ethanol extract of thin peels of pomegranate,were separately investigated against the studied organisms.

In vitro, antibacterial activity was then conducted by an agar-well diffusion method us-

ing (100)µl of final suspension of investigated bacteria (107 CFU ml-1) spread on Mueller-Hilton agar plates. Plates were left at room temperature for 15 minutes for adsorption and wells were cut from the agar by using a sterile tip (5 wells for different concentrations for each plant extract and one for control with sterile distilled water in each plate). Fifty µl (50) µl from each concentration were transferred to the well for each plant extract and at the same time 50 µl D.W. was transferred to a negative control well. The plates were incubated at (37) °C for (24) h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone in centimeters (cm) against the invesitgated bacteria.

4. Statistical analyses

The means and standard deviations were calculated for all data and a one-way analysis of variance (ANOVA), followed by post-hoc Tukey's HSD test, were applied to test for significant differences between different concentrations in each type of plant extract and between each concentration for plant extracts. Data analysis was conducted using MiniTab statistical software version 16 (IBM, Pennsylvania, USA). The accepted level of significance was $P< 0.05$.

5. Results

5.1. Escherichia coli

All types of plant extracts except the ethanol extraction of watercress seeds showed different levels of antibacterial action against St. aureusand E. coli. Fig (1) showed that all concentrations of aqueous extract of watercress seeds displayed different levels of antibacterial action against E. coli bacteria. Although the greatest effect among all concentrations was at (25%) for which diameter of zones of inhibition was (2.2) cm, no significant differences were found between the concentrations ($P = 0.127$).

Ethanol extract of thin peels of pomegranate exhibited various antibacterial effects against E. coli; the pathogen was highly sensitive to (75%) and (100%), with diameter of inhibition zones being (2.6) and (2.9) cm, respectively. Significant differences were shown between these concentrations and the others ($P = 0.011$) Fig (2).

Fig (3) shows that all concentrations of aqueous extract ofthin peels of pomegranate exhibited different levels of antibacterial activity against E. coli. The highest effect was observed at (100) % with significant differences among other concentrations ($P = 0.002$).

5.2. Staphylococcus aureus

The ethanol extract of watercress seeds had no activity against St. aureus, unlike that shown with E. coli.

Although the highest effect of aqueous extract of watercress was for (75%), for which the diameter of zones of inhibition was (2.9) cm, other concentrations showed antibacterial activity as well. Significant differences were found

between the concentrations (P = 0.127) Fig (4)

Fig.(5)reflects the results of antibacterial activity ofethanol extract of thin peels of pomegranate against St. aureus. The highest action was for (100)mg ml-1 with significant differences among the concentrations (P = 0.002).

Fig.(6)shows that all concentrations of aqueous extract ofthin peels of pomegranate exhibited different levels of antibacterial activity against St. aureus. No significant differences were found between the concentrations (P = 0.407).

6. Discussions

Results of the current work revealed that aqueous and ethanolic extracts of two plants exhibited antibacterial activity against two bacterial pathogens. Experimental findings showed that all the extracts displayed different degrees of antimicrobial activity on the invesitgated bacteria, but the aqueous extract was more effective than the ethanolic extract for controlling the bacteria.

The ethanolic and aqueous extracts of thin peels of pomegranate displayed strong antibacterial activity on the growth of the tested pathogenic bacteria.

This work is in agreement with Dahhamet al. [12], who found that the peel extract gave highest antimicrobial activity compared to seed, juice and whole fruit of pomegranate extracts on St. aureus. In earlier research conducted by other researchers, the growth ofListeria monocytogenes,St. aureus,E. coliandYersinia enterocoliticawas inhibited by using alcohol extracts of pomegranate peels [13]. Recently, Gullonet al. [14]reported that pomegranate peel flour had antibacterial activity on Listeria monocytogenes,Listeria innocua,St. aureus, Pseudomonas aeruginosa,E. coliand-Salmonellasp. Furthermore, the current results confirmed the study of Pagliaruloet al.[15] who found thatSt. aureusandE. coli, were affected by both pomegranate aril and peel extracts. In all work mentioned, pomegranate revealed strong antibacterial activity against tested microorganisms due to their phenolic and anthocyanin content of fruits including alkaloids, tannins, phenolic compounds, flavonoids, polyphenols, sugars, fatty acids, aromatic compounds, and amino acids[16, 17].

In the current study, aqueous extract of watercress exhibited higher antibacterial activity on the growth of pathogenic bacteria tested. In agreement with the current results, acetone/dichloromethane, ethanol and aqueous extracts of twelve common medicinal plants, among them watercress, were tested against St.aureus, Bacillus subtilis, E. coli, and Ps. aeruginosa[18]. Results obtained from this study showed that watercress revealed antibacterial activity against pathogenic bacteria. In a study Bocanegra-Garcíaet al. [19] found that the watery extract of watercress displayed antibacterial activity against the microorganisms tested in that study including E. coli and St.aureus. Furthermore, in agreement with the current resultsaqueousextract of watercress has previously been reported

to exhibit antibacterial activity against Mycobacterium tuberculosis [20].

Results of the current study showed that the ethanol extract of watercress exhibited no antibacterial activity in the growth of bacteria tested. These results contrast with the above-mentioned studies which indicated that all extracts of watercress including ethanolic extract revealed antibacterial activity against tested bacteria. The reason is not clear but it could be attributed to the method used to obtain the extract which affected the potency of the extract. In addition, the differences in antimicrobial activities of the plant extracts are influenced by such factors including freshness of the plant material, age of the plant used, microbial contamination, physical factors (light, water, or temperature), and incorrect preparation of the plant [21].

7. Conclusions

The results of the present study have provided evidence and confirmed previous results that watercress and pomegranates revealed an antimicrobial activity against E.coli and St. aureus. The inhibition of these bacteria by the extracts of pomegranate and watercress could become the promising plant antimicrobial agents therapeutic

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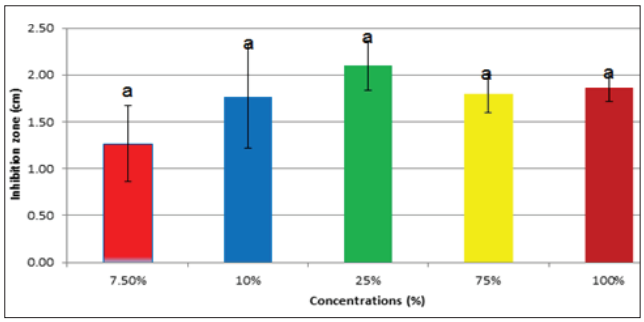

Fig (1): Antibacterial activity (diameter of zone of inhibition; cm) of various concentrations of aqueous extract of watercress seeds against E. coli. Results are presented as mean values ± SD (n = 3). No significant differences were found between the concentrations used in the present study (P = 0.127).

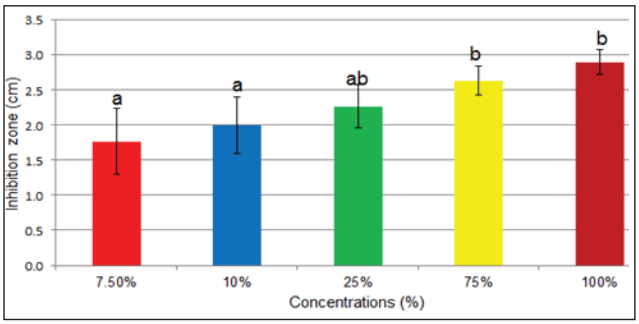

Fig (2): Antibacterial activity (diameter of zone of inhibition; cm) of various concentrations of ethanol extract of thin peels of pomegranate against E. coli. Results are presented as mean values ± SD (n = 3). Columns with different letters are significantly different (P = 0.011).

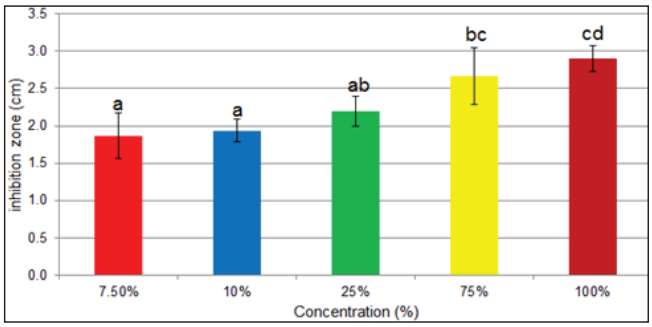

Fig (3): Antibacterial activity (diameter of zone of inhibition; cm) of various concentrations of aqueous extract of thin peels of pomegranate against E. coli. Results are presented as mean values ± SD (n = 3). Columns with different letters are significantly different (P = 0.002).

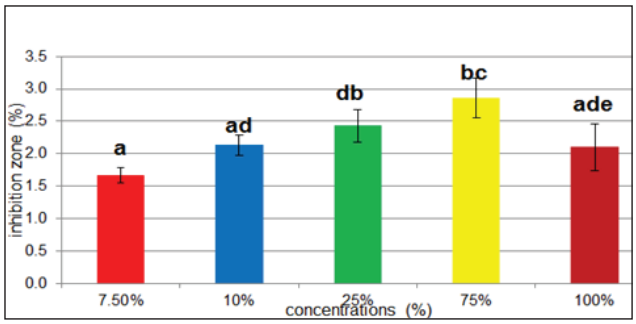

Fig (4): Antibacterial activity (diameter of zone of inhibition; cm) of various concentrations of aqueous extract of watercress seeds against St. aureus. Results are presented as mean values ± SD (n = 3). Columns with different letters are significantly different (P = 0.002).

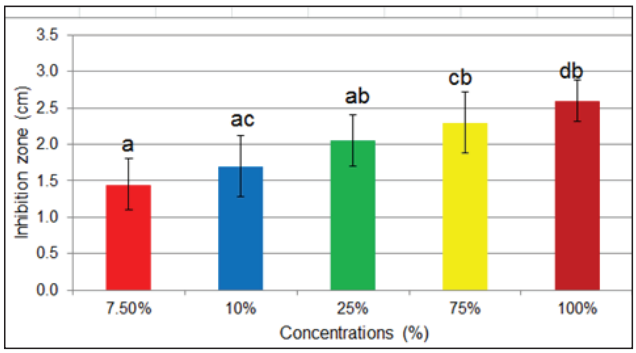

Fig (5): Antibacterial activity (diameter of zone of inhibition; cm) of various concentrations of ethanol extract of thin peels of pomegranate against St. aureus. Results are presented as mean values ± SD (n = 3). Columns with different letters are significantly different (P = 0.002).

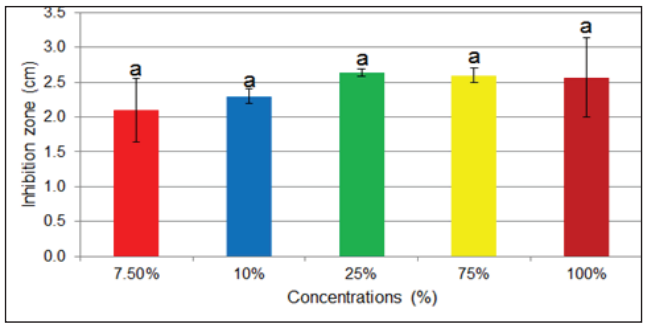

Fig (6): Antibacterial activity (diameter of zone of inhibition; cm) of various concentrations of aqueous extract of thin peels of pomegranate against E. coli. Results are presented as mean values ± SD (n = 3). No significant differences were found between the concentrations used in the present study (P = 0.407).