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Evaluation of the antimicrobial activity of plant extracts on *Escherichia coli* and *Candida albicans*

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ABSTRACT

This study aimed to investigate the antimicrobial activity of watermelon extracts of *Teucrium polium* and *Citrullus colocynthis* on *Escherichia coli* and *Candida albicans* isolated from women's Urinary tract infections. *T. polium* and *C. colocynthis* were collected from the Sistan region of Iran. Extraction was performed with ethyl acetate, methanol, acetone, and ethanol solvents using a rotary apparatus. *E. coli* strains were isolated from urinary infections. The minimum inhibitory concentration and the Minimum Bactericidal Concentration were prepared by dilution method in the well. The lowest inhibitory concentration of *C. colocynthis* ethyl acetate extract was equal to 3.1 ppm, in which 2 strains were inhibited. In comparison, the highest inhibitory concentration was 100 ppm, in which one strain was inhibited in this concentration. The lowest inhibitory concentration of *T. polium* ethyl acetate extract of *E. coli* was 3.1 ppm, with 2 strains inhibited. In comparison, the highest inhibitory concentration was 50 ppm, with one strain at this concentration. This study showed that the lower inhibitory concentration of *C. colocynthis* ethyl acetate extract against *C. albicans* was 25 ppm. The lowest inhibitory concentration of T.polium methanol extract against *C. albicans* was 12.5 ppm, which was unilaterally inhibited in this concentration. According to the obtained results and the increasing resistance of bacteria to chemical antibiotics, it is suggested that with further studies on this plant, the antibacterial compounds of this plant can be used in the treatment of bacteria.

1. Introduction

E. coli was a gram-negative, gram-negative, voluntary anaerobic bacterium that belongs to the Enterobacteriaceae family. This bacterium grows and multiplies mainly in the intestinal tract of humans, mammals and birds. *E. coli* strains are usually limited to the intestinal lumen. Still, due to a weak host immune system or a defect in the gastrointestinal tract, this bacterium can cause infectious diseases[1, 2]. It also causes respiratory disease in birds with *E. coli* pathogenic strains[3].

Pathogenic strains of *E. coli* in birds lead to various diseases mainly outside the intestines and cause extensive damage in the poultry industry[4]. It also includes inflammation of the ovarian duct, which leads to reduced egg production and low mortality in laying hens and mother flocks, and salpingitis with peritonitis. One of the most important therapeutic challenges is to deal with the

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causes of infectious and toxic diseases due to their high prevalence and spread. Improper use of antibiotics has increased drug resistance in most bacteria, so finding new antimicrobial compounds with minimal side effects seems necessary[5]. Due to the diversity of climate and large area, Iran has a wide range of medicinal plants that form the basis of traditional medicine [2, 6, 7].

T. polium to the genus Mint, a perennial herbaceous plant with almost 30 cm tall shrubs and has a white and cotton appearance. The flowers in this plant are white, whitish-yellow or yellow. This variability can be seen not only in the colour of the flower but also in the position of the plant stem, which becomes branched or dormant. This plant grows in barren, rocky and sandy areas of Europe, the Mediterranean, North Africa and southwest Asia, including Iran [8].

Traditional healers have widely used this plant to treat rheumatism, wound healing, inflammation treatment, and blood sugar control [9].

This plant contains tannins, terpenoids, saponins, flavonoids, glycosides-alpha, sterols, leucoanthocyanins, beta-caryophyllene, hemolen, cariophylene oxide, diterpenoids, asparagine and ditrin[10]. *C. colocynthis* from the *Cucurbitaceae* family of the order Cucurbitales is one of the valuable medicinal plants used in traditional medicine to treat many diseases.

It has been used, and numerous laboratory studies have proven its healing properties[11].

The fruit extract of this plant contains alkaloids, flavonoids, saponins, triterpenoids and glycosides[12]. *C. colocynthis* fruit as anti-glycemic, anti-hypertensive, anti-tumour, anti-fever, antimicrobial, and the treatment of diabetes, haemorrhoids, hyperlipidemia, gastric ulcer, and urinary diseases rheumatism, intestinal weakness, liver disease, edema and also as a laxative Strongly used [13].

T. polium, something belonging to the genus Mint, is a perennial, herbaceous plant with almost woody shrubs up to 30 cm in height and has a white and cotton appearance. The flowers in this plant are white, yellowish-white or yellow. This variable condition can be seen not only in the colour of the flower but also in the position of the plant stem, which becomes branched or dormant. This plant grows in barren and sandy areas and sandy soils of different Europe, Mediterranean, North Africa and southwest Asia, including Iran [8]. Traditional healers have widely used this plant to treat rheumatism, wound healing, inflammation treatment, and blood sugar control[9].

The aim of this article was Evaluation of antimicrobial activity of *C. colocynthis* and *T. polium* on *Escherichia coli* and *C. albicans* isolation of the woman

2. Material and Methods

2.1 Isolation of *E. coli*

Different strains of *E. coli* used in this study were isolated from urinary infection in zabol and cultured on a Nutrient *agar* medium. The purified strains were identified on artificial media using genes-specific tests. Samples with Gram-negative results were inoculated on plates of nutrient agar, Clede agar, MacConkey's, and blood agar (Merck, Germany) and then incubated at 37°C for 24 hours. The colony that showed fermenting of lactose on MacConkey agar and Cled agar media were purified and identified according to their morphology as circular, rose-pink to red colonies on MacConkey agar medium and yellow colonies on Cled agar. In addition, the isolates were identified by some biochemical reactions (e.g. catalase enzyme, potassium hydroxide test, Indole and methyl red test, Voges Proskaur reaction, urease and citrate, H_2S and oxidase test).

Fig. 1. The properties of *C. colocynthis* (A) and *T. polium* (B)(Take in from: **http://www.stridvall.se;** Copyright under the terms and conditions of the Creative Commons Attribution (CC BY) license (**http://creativecommons.org/licenses/by/4.0/**)

2.2 Isolates of *C. albicans*

After sampling the vaginal using the sterile swap and Falcon tube by the gynaecological specialists, four samples were isolated and transferred to the laboratory and cultivated on agar dextrose Saburo and broth dextrose Saburo according to the manufacturer's instructions. After the growth of each sample, lam was prepared, and the candidate samples were identified. Colonies of *C. albicans* were ready in the media of agar dextrose Saburo at 37°C in homogenous suspension sterile physiology serum. The suspension's light passing rate was measured using the spectrophotometry device with 530 nm. The speed of the passing light of 90% is necessary for preparing a suspension with nearly 10^6fungi cells per mL. For determining the inhibitory concentration of the extracts, incubation in media was used (the concentration of 25, 50 and 100 ppm were used). Finally, they were placed in the incubator, and the samples were analyzed after 24 - 48 hours.

2.3 Extract preparation and investigation of the antimicrobial effects of the plant extract

The *C. colocynthis, T. polium,* used in this study was gathered from Sistan and Baluchistan (Southeastern Iran) provinces. They were detected to be *C. colocynthis, T. polium,* by a researcher from the University of Zabol. Each of 20 g grinded powders was soaked in 60 mL of methanol, ethanol, acetone, ethyl acetate separately for one day (shaken occasionally with a shaker). After one day of the dissolving process, filtered materials (Whatman no. 1 filter paper). Then the filtrates were evaporated using a rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4°C in an airtight screw-cap tube.

2.4 MIC and Minimum Bactericidal Concentration (MBC) of Plant Extracts

The sensitivity of the bacteria samples with multiple resistances to the *C. colocynthis, T. polium,* and extract against *E. coli* was analyzed by dilution method in broths.

All tests were performed in Mueller Hinton broth (MHB) supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranging from 0.3 mg/mL to 10.00 mg/mL. To each well, 10 µL of indicator solution (prepared by dissolving a 10-mg extract in 2 mL of DMSO) and 10 µL of Mueller Hinton Broth. Finally, 10 µL of bacterial suspension (10^6 CFU/mL) was added to each well to achieve a 104 CFU/mL concentration. The plates were wrapped loosely with cling film to ensure that the bacteria were not dehydrated. The leaves were prepared in triplicates, and then they were placed in an incubator at 37°C for 18 - 24 hours. The first broth inhibiting the growth of bacteria after being positioned in the incubator was considered as MIC. Then, 10 ml of the light broths was transferred to the Moller environment for more precision. After 24 hours, the first concentration removing 99.9% of the bacteria was MBC.

2.5 Isolation of Fungal

A cross-sectional study was performed. Fungal clinical were isolated from patients' vaginas that were referred to the medicinal centre using a sterile swab at the Amir Al Momenin hospital, Zabol, south-eastern Iran during the years 2015 - 2016. They were examined at the Medical Science University by a gynaecologist and then quickly transferred to a sterile falcon tube comprising sterile water for further culture and including solid culture medium: Sabouraud Dextrose Agar (Merck, Germany) was used for fungal culture and purification

3. Result

The lowest inhibitory concentration of *C.colocynthis* ethyl acetate extract was equal to 3.1 ppm, in which 2 strains were inhibited. In comparison, the highest inhibitory concentration was 100 ppm, in which one

strain was inhibited in this concentration (Table 1, Figure 2).

The lowest inhibitory concentration of *C. colocynthis* methanolic extract was equal to 12.5 ppm, in which 4 strains were inhibited. In comparison, the highest inhibitory concentration was 100 ppm, in which one strain was inhibited (Table 1, Figure 2).

The lowest inhibitory concentration of Estonian extract of *C. colocynthis* was equal to 3.1 ppm, in which 3 strains were inhibited, and the highest inhibitory concentration was 25 ppm, of which 2 strains were inhibited in this concentration (Table 1, Figure 2).

The lowest inhibitory concentration of the ethanolic extract was 6.25 ppm in which 2 strains were inhibited, while the highest inhibitory concentration was 50 ppm in which 100 strains were inhibited at this concentration (Table1)

Among the plant essential oils of thyme essential oil, the combination of thyme essential oil with fennel and the variety of thyme essential oil with marjoram had a more significant growth inhibition diameter than other essential oils (Table 1).

The lowest inhibitory concentration of *T. polium* ethyl acetate extract of *E. coli* was 3.1 ppm, with 2 strains inhibited at this concentration. The highest inhibitory concentration was 50 ppm, with one strain at this concentration. The lowest inhibitory concentration of *T. polium* metallic extract was 6.25 ppm, one strain was inhibited at this concentration, and the highest inhibitory concentration was 100 ppm, in which two strains was inhibited (Table 2)

The lowest inhibitory concentration of *T. polium* acetone extract was 12.5 ppm, in which 3 strains were inhibited, and the highest inhibitory concentration was 100 ppm, in which 2 strains were inhibited. The lowest inhibitory concentration of the ethanolic extract was 12.5 ppm, in which 2 strains were inhibited, and the highest inhibitory concentration was 50 ppm, in which 4 strains were inhibited in this concentration (Table2).

This study showed that the lowest inhibitory concentration of *Teucrium* extract was 25 ppm with 3 strains inhibited at this concentration and the highest inhibitory concentration was 100 ppm with 3 strains inhibited at this concentration (Table 2).

This study showed that the lowest inhibitory concentration against *C. albicans* was related to ethyl acetate extract of *C. colocynthis* at a concentration of 6.25 ppm, which inhibited *C. albicans*. In comparison, the highest inhibitory concentration, the equivalent of 200 ppm, was related to the aqueous extract of *C. colocynthis* (Table 3).

The results of this study showed that the lowest inhibitory concentration against *C. albicans* was related to methanolic extract of *Teucrium*, in which 3 strains were inhibited at a concentration of 12.5 ppm, while the highest inhibitory concentration was related to aqueous extract of *Teucrium* (Table 4)

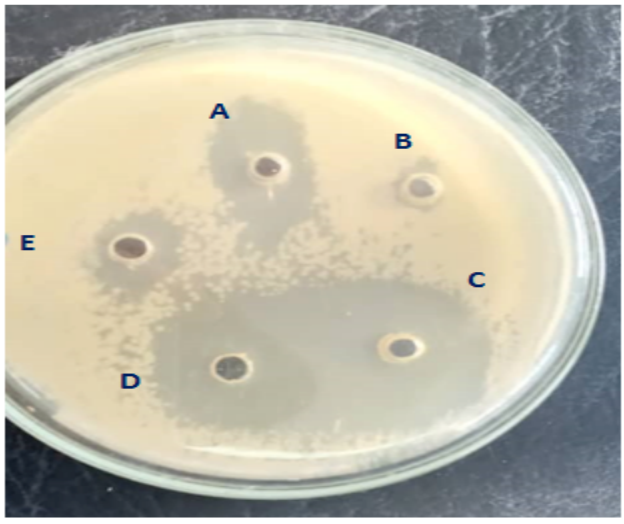

Fig. 2. The effects of Ethanol(A), Ethyl acetate (B), methanol(C), Acetone(D), and water(E) *C.colocynthis* extract on *E. coli*

Table 1. MIC and MBC of *C. colocynthis* extract with different solvents on antibiotic-resistant *E. coli*

Bacterial code	ethanol	Acetone	methanol	water	Ethyl acetate
1	12.5-25	6.25-12.5	25-50	100-200	12.5-25
2	25-50	25-50	12.5-25	50-100	25-50
3	6.25-12.5	6.25-12.5	50-100	25-50	50-100
4	25-50	25-50	50-100	100-200	12.5-25
5	50-100	6.25-12.5	12.5-25	50-100	6.25-12.5
6	25-50	3.1-6.25	25-50	100-200	25-50
7	6.25-12.5	6.25-12.5	100-100	50-100	3.1-6.25
8	25-50	3.1-6.25	12.5-25	50-100	100-200
9	12.5-25	12.5-25	25-50	25-100	3.1-6.25
10	25-50	3.1-6.25	12.5-25	50-100	12.5-25

Table 2. MIC and MBC of Teucrium extract with different solvents on antibiotic-resistant *E. coli*

Bacterial code	Ethanol	Acetone	Methanol	Ethyl acetate	Water
1	50-100	100-200	6.25-12.5	12.5-25	50-100
2	25-50	12.5-25	50-100	6.25-12.5	100-100
3	50-100	50-100	100-100	3.1-6.25	25-50
4	12.5-25	25-50	50-100	12.5-25	50-100
5	50-100	50-100	6.25-12.5	12.5-25	50-100
6	25-50	100-200	50-100	3.1-6.25	100-200
7	100-200	25-50	100-200	12.5-25	100-200
8	50-100	50-100	12.5-25	6.25-12.5	50-100
9	12.5-25	12.5-25	12.5-25	50-100	25-50
10	50	12.5-25	50-100	12.5-25	25-50

Table 3. MIC and MFC of *C. colocynthis* extract with different solvents on *C. albicans*

C. albicans	ethanol	Acetone	methanol	water	Ethyl acetate
1	12.5-25	12.5-25	25-50	200-200	12.5-25
2	50-50	50-100	12.5-25	50-100	6.25-12.5
3	25-50	12.5-25	50-100	50-50	25-50
4	25-50	25-50	50-100	100-200	12.5-25

Table 4. MIC and MFC of *Teucrium* extract with different solvents on *C. albicans*

C. albicans	ethanol	Acetone	methanol	water	Ethyl acetate
1	12.5-25	12.5-25	25-50	200-200	12.5-25
2	25-50	50-100	12.5-25	50-100	25-50
3	25-50	12.5-25	12.5-25	100-50	25-50
4	25-50	25-50	12.5-25	100-200	12.5-25

5. Discussion

It is reported that the use of tulip extract is helpful in infections of the gastrointestinal tract and urinary tract that are of infectious origin with gram-negative microorganisms[14]. The study investigates the effect of teat mouthwash on *Streptococcus* mutans, the main microorganism that causes tooth decay. The results showed that the two groups did not have a statistically significant difference in the number of *Streptococcus* mutans colonies before mouthwash[15].

In a study conducted in Kerman, the effect of the methanolic extract on *Bacillus cereus* and *Staphylococcus aureus* was reported[16]. In another report on several plants, the antibacterial effect of rasia extract was observed not only on *Bacillus cereus*[17].

It is examined the antimicrobial activity of *C.colocynthis*; the results showed that the ethanolic extract of fruit, leaves, roots and stems was an inhibitor of *Bacillus pumilus*, *Staphylococcus aureus*, while the fruit and root

extract was a promising inhibitor of Bacillus subtilis[18].

It is studied the antimicrobial activity of *C.colocynthis*; the results showed that *C.colocynthis* watermelon extract with a concentration of 50 mg/ml inhibitory auras was 8.5 ، 0.5, 19.5 1 1.5, 5 0.5 / 0.12, 0.13 0 0.1, 8.25 25 0.25, 17, 10.75 0 0.75 mm against Proteus viola, *E. coli*, *Bacillus cereus*, *Salmonella typhimurium*, *Klebsiella Pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *C. albicans* [19] are consistent with the results of our study.

In another study, the MIC against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Micrococcus luteus*, and *Listeria monocytogenes* was 0.312, 0.625, 0.625, 0.625, 0.625 and 0.625 µg / ml, respectively [20, 21].

Conclusion

This study showed that the plant extract was an inhibitor of E. coli bacteria used to treat the infection.

Conflict of interest

The authors declare no conflict of interest

Ethical approval

This article does not contain any studies with human participants or animals performed by any authors.

Consent for publications

The author read and proved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

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Reference

1. Dziva F, Hauser H, Connor T R, van Diemen P M, Prescott G, Langridge G C, Eckert S, Chaudhuri R R, Ewers C, Mellata M. (2013). Sequencing and functional annotation of avian pathogenic *Escherichia coli* serogroup O78 strains reveal the pathogenic evolution of *E. coli* lineages for poultry via distinct mechanisms. Infection and immunity, 81(3): 838-849. https://doi.org/10.1128/IAI.00585-12
2. Valizadeh M, Beigomi M, Fazeli-Nasab B. (2020). Antibacterial and Antibiofilm effects of ethanol and acetone leaf extract of *Momordica charantia* and *Tecomella undulat*e against *Acinetobacter baumannii*. Int. J. Adv. Biol. Biomed. Res., 8(4): 403-418. https://doi.org/10.33945/sami/ijabbr.2020.4.6
3. M Ammara A, K Abd El-Aziz N, A Nasef S, R Bakry N, M El Atfehy N, M Erfan A, G Shalaby A. (2014). Use of Multiplex PCR for Detection of Bacterial Respiratory Infections in Poultry. Zagazig Veterinary Journal, 42(3): 133-144. https://dx.doi.org/10.21608/zvjz.2014.60059
4. Abbas N, Suleman M, Khan N A, Ijaz A, Rauf M, ur Rahman S. (2018). Prevalence of Mycoplasma gallisepticum in poultry and wild life birds suspected of chronic respiratory disease in northern Pakistan. Pakistan Journal of Zoology, 50(3): 1071-1077. http://dx.doi.org/10.17582/journal.pjz/2018.50.3.1071.1077
5. Trick W E, Weinstein R A, DeMarais P L, Kuehnert M J, Tomaska W, Nathan C, Rice T W, McAllister S K, Carson L A, Jarvis W R. (2001). Colonization of skilled-care facility residents with antimicrobial-resistant pathogens. Journal of the American Geriatrics Society, 49(3): 270-276. https://doi.org/10.1046/j.1532-5415.2001.4930270.x
6. Rahim Z, Sanyal S, Aziz K, Huq M, Chowdhury A. (1984). Isolation of enterotoxigenic, hemolytic, and antibiotic-resistant Aeromonas hydrophila strains from infected fish in Bangladesh. Applied and environmental Microbiology, 48(4): 865-867. https://doi.org/10.1128/aem.48.4.865-867.1984
7. Fazeli-Nasab B, Sirousmehr A, Mirzaei N, Solimani M. (2017). Evaluation of total

phenolic, flavenoeid content and antioxidant activity of Leaf and Fruit in 14 different genotypes of *Ziziphus mauritiana* L. in south of Iran. Eco-Phytochemical Journal of Medicinal Plants, 4(4): 1-14.
8. Esmaeili A, Amiri H. (2008). Effects of antimicrobial and identification of composition of essential oil of *Teucrium polium* L. Persian. J Esfahan Univ, 31(2): 15-22.
9. Niazmand S, Ahmadpour E, Mousavian M, Saberi Z. (2008). The inotropic and chronotropic effects of aqueous ethanolic extract from *Teucrium polium* L. On guinea pig isolated heart. Journal of Babol University Of Medical Sciences, 10(1): 7-13.
10. Oganesyan G, Galstyan A, Mnatsakanyan V, Shashkov A, Agababyan P. (1991). Phenylpropanoid glycosides of *Teucrium polium*. Chemistry of natural compounds, 27(5): 556-559. https://doi.org/10.1007/BF00630353
11. Kouadri I, Satha H. (2018). Extraction and characterization of cellulose and cellulose nanofibers from *Citrullus colocynthis* seeds. Industrial Crops and products, 124: 787-796. https://doi.org/10.1016/j.indcrop.2018.08.051
12. Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A, Aouni M. (2010). Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. Journal of Ethnopharmacology, 128(1): 15-19. https://doi.org/10.1016/j.jep.2009.11.027
13. Hameed B, Ali Q, Hafeez M, Malik A. (2020). Antibacterial and antifungal activity of fruit, seed and root extracts of *Citrullus colocynthis* plant. Biological and Clinical Sciences Research Journal, 2020(1): 1-12. https://doi.org/10.54112/bcsrj.v2020i1.33
14. Shahraki M, Mirshekari H, Palen M. (2006). Comparison of analgesic effect of aqueous extract of Kalporeh and morphine in the rat. Persian. Ofogh-e-Danesh, 12(1): 10-14.
15. Khoramian Tusi S, Manzari Tavakoli Z, Bahram Abadi Nejhad R, Zeynali B. (2014). Evaluation of teucrium polium mouthwash effect on salivary *streptococcus mutans* count. Journal of Mashhad Dental School, 38(4): 321-330.
16. Bonjar G S. (2004). Antibacterial screening of plants used in Iranian folkloric medicine. Fitoterapia, 75(2): 231-235. https://doi.org/10.1016/j.fitote.2003.12.013
17. Memon U, Brohi A H, Ahmed S W, Azhar I, Bano H. (2003). Antibacterial screening of *Citrullus colocynthis*. Pakistan journal of pharmaceutical sciences, 16(1): 1-6. PMID: 16414561
18. Aldamegh M A, Abdallah E M, Hsouna A B. (2013). Evaluation of antimicrobial and antioxidant properties of leaves of *Emex spinosa* and fruits of *Citrillus colocynthis* from Saudi Arabia. African Journal of Biotechnology, 12(34). https://doi.org/10.5897/AJB2013.12987
19. Hsouna A B, Alayed A S. (2012). Gas chromatography-mass spectrometry (GC-MS) analysis and in vitro evaluation of antioxidant and antimicrobial activities of various solvent extracts from *Citrullus colocynthis* (L.) roots to control pathogen and spoilage bacteria. African Journal of Biotechnology, 11(47): 10753-10760. https://doi.org/10.5897/AJB12.417
20. Chawech R, Mhalla D, Trigui M, Mihoubi M, Fabre N, Jarraya R. (2015). Chemical composition and antibacterial activity of extracts and compounds isolated from *Citrullus colocynthis* (L.) Schrad. Journal of Pharmacognosy and Phytochemistry, 4(4): 197-203.
21. Alzoreky N, Nakahara K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology, 80(3): 223-230. https://doi.org/10.1016/S0168-1605(02)00169-1