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Comparative Analysis of Antibacterial Effect of Cranberry Tea vs Cranberry Fruit on Gram-Negative Bacteria Using Agar Well Method

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Abstract

The word "cranberry" originated from the Pilgrims, who called it "craneberry" because the small, pink blossoms seen in spring resemble the head and bill of a Sandhill crane. Cranberries come from North America, where they are native to the cooler regions of the continent. They grow naturally in acidic bogs, marshes, and wetlands in areas such as the northeastern United States and eastern Canada. Cranberries are also cultivated in other parts of the world with suitable climates, including parts of Europe and Asia. There are two known species of cranberry those are: the American cranberry (Vaccinium macrocarpon) and the European cranberry (V. oxycoccos). For centuries, Cranberry (Vaccinium macrocarpon) has served dual purposes both as a food and as medicine. Cranberry's efficacy in treating urinary infections is widely acknowledged and supported by empirical evidence. This natural remedy has been consistently utilized to address urinary tract infections, showcasing its therapeutic potential and longstanding reputation as a reliable treatment option. Cranberries contain proanthocyanidins that interfere with the ability of bacteria to grow, specifically Gram-negative bacteria. These compounds may prevent the bacteria from sticking to the urinary tract walls, making it more difficult for them to cause infections such as urinary tract infections (UTIs). Additionally, cranberries contain other bioactive compounds that may have antibacterial properties, further contributing to their potential effects against Gram-negative bacteria. In this experimental study, we employed the agar well technique to investigate the impact of various cranberry formulations on Gram-negative bacteria. Our approach involved examining multiple types of cranberries to distinguish their comparative efficacy in inhibiting the growth of Gram-negative bacterial strains. By analyzing the size and extent of inhibition zones produced by different cranberry forms, our aim was to identify the most effective form for combating Gram-negative bacterial infections.

Keywords: Cranberry, Agar well, Gram-Negative Bacteria

Introduction

The History of Cranberry

The term "cranberry" originates from the Pilgrims, who called the fruit "craneberry" due to its resemblance to the head and bill of a Sandhill crane. Cranberries are indigenous to North America, thriving in cooler regions of the continent. They typically grow in acidic bogs, marshes, and wetlands, primarily in the northeastern United States and eastern Canada. Although native to North America, cranberries are also cultivated in other suitable climates worldwide, including parts of Europe and Asia. There are two recognized species of cranberry: the American cranberry (Vaccinium macrocarpon) and the European cranberry [1]. Folklore recordings by the Native Americans from the 1600s have information about the treatment of urinary infections with cranberry, also cranberries were used to cure blood poisoning. Aside from the cranberry fruit, cranberry leaves were

used as well for urinary infections, diarrhea, and diabetes. In the 1980s research on the health benefits of cranberry started, the research took 25 years altogether to find the active substances of cranberry. The research took so long because, in the first trials, there were inconsistent conclusions given, this is due to the experiments not being in a controlled environment. The first conclusive research came in the 1990s by Avron et al. In this research experiment fifty-three female patients from a nursing home, with an average age of 78.5 years old. The study included a large number of patients who had a long period of treatment. This study showed that the occurrence of bacterial urinary infections was lowered by 50 % following daily consumption of cranberry juice. The experiment showed the positive effect of cranberry use on urinary infections. Another study concluded at the same time was the Walker et al. study, which has also had similar positive conclusion as well as the previously mentioned study. The difference in this study is that the test subjects were given two 400 mg capsules of cranberry powder to ingest once a day for three months. In the 2000s research on cranberry continued, Kontiokari et al researched the effect of cranberry juice as well as tablets in the prevention of urinary infections. The tested patients were healthy females ages 21-72 years of age, with a long history of resistant UTIs. Pediatric trials have been conducted throughout the 2010s. The research continued in the 21st century but more focused on other beneficial factors [2].

Antibacterial Properties of Cranberry

In this era of antibiotic resistance, there is a turn to traditionally used medicine. Traditionally used medician such as cranberry. Cranberries in different forms have generally been used to treat UTIs for centuries [3]. Cranberries contain bioactive compounds such as proanthocyanidins, anthocyanins, phenolic acids, flavonols, and triterpenes. These compounds have antibacterial, antioxidant, anti-inflammatory, and other biological effects. For instance, anthocyanins inhibit bowel inflammation and modulate gut bacteria, while type A proanthocyanidins reduce E. coli adhesion in the urinary tract. Triterpenes have anti-inflammatory and anti-cancer properties [4]. UTIs are primarily caused by uropathogenic Escherichia coli (E.coli) that adhere to uroepithelial cells through Type-1 and P-fimbriae. UTIs have been a major focus point of prevention studies because of increasing bacterial resistance to antibiotics, which makes treatment of infections more difficult and costly. Recently, there has been increased interest in alternative measures and remedies, including the use of naturally available herbal solutions, which are affordable, cost-effective, and very potent. Cranberry juice, Vaccinium macrocarpon, has been used for over 200 years, especially by native Americans to help prevent UTI. Studies show that not the acidic pH of urine but an agent in cranberries prevents E-coli from adhering to uroepithelial cells. Cranberries contain fructose, an inhibitor of Type-1 fimbriae, and proanthocyanidins, an inhibitor of P-fimbriae. Proanthocyanidins inhibit the adhesion of both sensitive and trimethoprim-sulfamethoxazole-resistant E. coli and asymptomatic bacteriuria in patients with ileal enterocystoplasty. However, the effect of proanthocyanidins against multi-drug-resistant uropathogens has not been thoroughly investigated [5]. Condensed tannins (cPAC) from cranberries can inhibit the attachment of bacteria to surfaces, compromise bacterial motility, cause iron limitation, and disrupt quorum sensing. Data also indicate that cranberry consumption prevents bacterial infections, and a trial showed that cranberry derivatives that lack proanthocyanidins combined with β-lactam antibiotics are effective against Gram-positive bacteria. cPAC can also inhibit the formation of biofilm and enhance the efficacy of gentamicin against P.aeruginosa. However, the potential of cPAC in preventing antibiotic resistance development or in restoring antibiotic efficacy has yet to be tested in depth. The present study shows that cPAC can prevent the development of resistance to tetracycline in E. coli and P.aeruginosa. It shows that cPAC has broad-spectrum antibiotic-potentiating activity against a range of Gram-negative bacteria in vitro and in vivo. It also represses two key mechanisms of antibiotic resistance: selective membrane permeability and the activity of multiple drug efflux pumps [6].

Material and Methods

Material

Cranberry

For this research project a few forms of cranberries were used, those were cranberry tea, dried cranberry, and fresh cranberries.

Bacterial Strains

- Escherichia coli ATCC 14169
- Escherichia coli ATCC 25922
- ESBL Escherichia coli 35218
- Pseudomonas aureginosa ATCC 10145
- Pseudomonas aureginosa ATCC 27855

Chemicals

In this research project, we used Tryptic Soy Broth (TSB). That was made by dissolving the TSB in 1 L of distilled water. The broth was stirred on the magnetic stirrer. Miller-Hinton plates were made by dissolving MH in 1L of distilled water.

Methods

Agar well Method

Miller-Hinton agar plates were used, which are made by weighing 38g of MH and dissolving it in 1L of distilled water. In the MH plates wells were made by pressing pipetting tips, that beforehand were autoclaved and scorched with a lighter, and then the excess of the MH plate was removed by using a loop. Into the well 80 µL of cranberry tea was added, in two concentrations, one tea and two tea bags. The tea was prepared in 300 mL of sterile water that was cooked until boiling. The dry cranberry was weighed in Eppendorf tubes 0,1 g and those 0,1 grams were inserted by tweezers into the wells. Fresh cranberry was processed using a mortar and pestle after 0,1 g was weighed into Eppendorf tubes, and that content was inserted into the wells. Zone of inhibition we read in millimeters after 24 h in the incubator at 37 °C.

Results

Table 1: Agar well method results for fresh and dried cranberry.

Bacterial strains	Fresh Cranberry 0.1 g	Dried Cranberry 0.1 g
· Escherichia coli ATCC 14169	17 mm	25,3 mm
· Escherichia coli ATCC 25922	18,6 mm	27 mm
· ESBL Escherichia coli 35218	14,3 mm	29 mm
· Pseudomonas aureginosa ATCC 10145	15,3 mm	R
· Pseudomonas aureginosa ATCC 27855	16,6 mm	R

Table 2: Agar well method results for cranberry tea, one and two tea bags in 300 mL of water.

Bacterial strains	Cranberry tea one bag	Cranberry tea two bags
· Escherichia coli ATCC 14169	R	R
· Escherichia coli ATCC 25922	R	R
· ESBL Escherichia coli 35218	R	R
· Pseudomonas aureginosa ATCC 10145	R	R
· Pseudomonas aureginosa ATCC 27855	R	R

Discussion

Cranberry has long been used in folk medicine due to its potent health properties. Research has established that cranberries contain active components that have remarkable antibacterial effects on gram-negative bacteria. In our research experiment, we investigate and quantify the antibacterial properties of cranberry against five specific gram-negative pathogenic bacteria, including Escherichia coli ATCC 14169, Escherichia coli ATCC 25922, ESBL Escherichia coli 35218, Pseudomonas aeruginosa ATCC 10145, and Pseudomonas aeruginosa ATCC 27855. The agar well method was the technique of choice for conducting this study, considering it a very established method to detect the antibacterial activity of various substances. This technique involves the formation of wells in an agar plate where bacteria are grown and the substance being tested—in this case, different forms of cranberry extract—is introduced into the wells. Different forms of cranberry were tested to determine which type had the best antibacterial effect. These included fresh cranberries, dried cranberries, and cranberry tea. Each form was prepared under standardized protocols for consistency and reliability in the results.

Conclusion

Our findings revealed that all forms of cranberry showed some degree of antibacterial activity on gram-negative bacteria, although effectiveness varied immensely according to the form of cranberry utilized. More specifically, fresh and dried cranberries provided inhibition zones to the gram-negative bacterium, although their size and presence vary among the various strains tested. Fresh cranberries generated zones of inhibition on all the bacteria tested for, which evidently shows broad-spectrum antibacterial activity. Although the inhibition zones were fairly smaller as compared to the other forms of cranberries, inhibition on all the bacterial strains, including Escherichia coli ATCC 14169, Escherichia coli ATCC 25922, ESBL Escherichia coli 35218, Pseudomonas aeruginosa ATCC 10145, and Pseudomonas aeruginosa ATCC 27855, shows their potential as a versatile antibacterial agent. Dried cranberries showed more selective antibacterial activity. Their inhibition zones against the Escherichia coli strains were bigger. The drying action may concentrate the active compounds in cranberries, increasing their activities against some bacteria. However, it is important to note that dried cranberries did not have any significant effect on Pseudomonas aeruginosa strains. This lack of effect, therefore, expresses the fact that while being effective against some bacteria, dried cranberries are not universally effective against all gram-negative pathogens. All tested bacteria strains were resistant to the effect of cranberry tea in both concentrations, implying that either the active antibacterial compounds found in fresh or dried cranberries are lost or highly reduced in making cranberry tea. This may be because boiling water can partly denature some of the heat-labile bioactive compounds, or it may be that the concentration of these compounds is too low to have a measurable effect. These results demonstrate that cranberries, especially the fresh and dried forms, have significant potential as natural antibacterial agents. Conclusively, our study showed the variant degrees of the antibacterial activity of different forms of cranberries against gram-negative bacteria. Fresh cranberries exert broad-spectrum activities of antibacterial effects, while the dried form demonstrates higher activity against specific strains. No high antibacterial activities are noted with cranberry tea. These insights put focus on the potential role of cranberries in the development of novel, natural antibacterial treatments and stress the further need for optimization of preparation methods to gain full health benefits.

References

1. Neto C, Vinson C, Cranberry JA (2011) In Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis. https://www.ncbi.nlm.nih.gov/books/NBK92762/
2. Zhao S, Liu H, Gu L (2020) American cranberries and health benefits - an evolving story of 25 years. J Sci Food Agric 100: 5111-5116.
3. Caceres C, Kim M, Bae BM (2021) The effect of cranberry consumption on lipid metabolism and inflammation in human apo A-I transgenic mice fed a high-fat and high-cholesterol diet. British Journal of Nutrition 126: 183-190.

4. Šedbarė R, Pašakinskienė I, Janulis V (2023) Changes in the Composition of Biologically Active Compounds during the Ripening Period in Fruit of Different Large Cranberry (Vaccinium macrocarpon Aiton) Cultivars Grown in the Lithuanian Collection. Plants 12: 202.
5. Gupta A, Dwivedi M, Mahdi A, Nagana A, Gowda GA, et al. (2011) Inhibition of adherence of multi-drug resistant E. coli by proanthocyanidin. Urological Research 40: 143-150.
6. Maisuria V, Okshevsky B, Déziel ME, Tufenkji N (2019) Proanthocyanidin Interferes with Intrinsic Antibiotic Resistance Mechanisms of Gram-Negative Bacteria. Advanced Science 6: 1802333.