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The antimicrobial activity of cranberry juice (*Vaccinium macrocarpon L.*) Ethanol extract against uropathogenic bacteria.

[1] Lecturer Hayder Kamil Jabbar Al-Kaabi
[2]Professor dr.Baheeja A.Hmood Al-Khalidi

[1,2]Department of medical and Basic sciences, College of nursing, University of AL-Qadisiyah,Diwaniyah, Iraq.

Abstract

Cranberry fruit is distinguished by its complex phytochemical profile, which includes flavonoids for example: flavonols, anthocyanins, proanthocyanidins; catechins, phenolic acids and triterpenoids . Cranberry fruit extracts exhibit antiviral activity in addition to their antibacterial and antifungal effects. In the current research, an ethanol extract of cranberry juice (*Vaccinium macrocarpon L*.) was tested for its antibacterial activity against bacteria that cause urinary tract infections in pregnant women. These bacteria include e. coli and salmonella.

It is evident that the ethanol extract of cranberry juice (*Vaccinium macrocarpon L*.) had excellent inhibitory effects on all of the selected bacteria, particularly with standard concentrations (100%) it clearly shows that it had noticeable effectiveness in comparison to antibiotics all bacteria as a result of having the largest zone of inhibition The impact of cranberry juice, which is made from the *Vaccinium macrocarpon L*. plant, differed depending on the strain of bacteria that was used and the concentration of the extract. The CNS was more sensitive to the cranberry juice (*Vaccinium macrocarpon L*.), with a rate of inhibition of 100%, whilst the E. coli strain seemed to be less responsive to the extract, with a rate of inhibition of 60%. The percentage of inhibition of Staph. aureus was (90%), whereas the rate of inhibition for P. valgarus was 75%. In this study, *Vaccinium macrocarpon L*, more familiary known as cranberry juice, exhibited dose-dependent inhibitory effects on the growth of all of the organisms being studied.

KEY WORDS: cranberry juice ,(*Vaccinium macrocarpon L.*) Ethanol extract,*E.col*

INTRODUCTION

When bacteria enter the urinary system, they may cause an illness called as a urinary tract infection (UTI). In most cases, *E. coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, Staphylococcus saprophyticus, Streptococcus agalactiae, Pseudomonas aeruginosa, Candida albicans,* and other kinds of fungus are to blame for these infections. Pain, burning, urgency, frequency, blood in the urine, and even fever might be symptoms of a urinary tract infection (UTI). The length of time that symptoms are present might range anywhere from a few days to many weeks. If not treated, a urinary tract infection (UTI) may result in damage to the kidneys or even death. .(Schnarr and Smaill 2008).

During pregnancy, the kidneys grow larger. This growth happens because the body needs extra water and nutrients to help the baby grow. The kidneys also need to get rid of waste products like salt and potassium. As the kidneys become bigger, they also stretch out the ureters, bladder, and pelvic area. These changes happen throughout your pregnancy..(Parveen *etal.*,2011)

Urinary tract infection UTIs are caused by many different types of microorganisms, including viruses, fungi, and even parasites. However, the main cause of UTIs is usually bacterial infections with percent 95%.(Farajnia *etal*.,2009 ;Moroh *etal*.,2014,4]

Infections caused by antibiotic resistant bacteria are a growing problem around the world. Antibiotics are often prescribed when there is not an immediate need for them, leading to the development of antibiotic resistance. Bacteria become resistant to antibiotics because they produce enzymes that break down the drugs. Beta lactamases are the name given to these enzymes. Antibiotics known as beta-lactams are often prescribed for patients suffering from infections caused by gram-negative bacteria. On the other hand, certain forms of beta-lactam resistance have surfaced. Extended spectrum beta-lactamse (ESBL) ;metallobeta lactamases (MBLs), and AmpC beta-lactamase. So continual production of these enzymes by bacteria has led

to a restricted range of antibiotics. Because of this, it is of the highest importance to research and develop novel antibacterial agents.(Baral ,*etal.*,2012; Sultan,*etal*.,2014)

Antibiotic therapy is often prescribed for UTIs, but the development of drug-resistant strains of bacteria has become an increasing problem. As a result, alternative therapies are being explored (Arrigo *etal.*,2010).., Medicinal plants have become an important source of natural products with medicinal properties. They are rich in bioactive compounds and have been used since ancient times. These plants are also used as food supplements, cosmetics, and nutraceuticals. Some of them are used in traditional medicine, while others are used in modern medicine..(Ahmad and Beg (2001). Cranberry (*Vaccinium macrocarpon L*.), one of several plant-based spices that have been utilised for various therapeutic reasons and as food preservatives, is one example. Cranberries are an excellent food choice for their high levels of vitamin C, fibre, and antioxidants, as well as their high mineral content. In addition to that, they contain anthocyanins, tannins, flavonoids, and proanthocyanidins. These compounds help prevent urinary tract infections, reduce inflammation, and protect against cancer..(Okmen *etal*.2018).

Cranberries are rich sources of polyphenolic antioxidants, including flavonoids, phenolic acid derivatives, anthocyanins, ellagitannin, proanthocyanidins, and hydroxycinnamic acids. Cranberries contain a large number of lipids, mainly triacylglycerol, phosphatidylcholine, free fatty acids, sterols, stanols, and waxes. Cranberries are considered a functional food because of their antioxidant properties, ability to inhibit pathogenic microorganisms, and other health benefits. The results of GC-MS tests have shown that fresh berries contain a significant quantity of C18 unsaturated fatty acids as well as phytosterols. These results were shown to be true. (Klavins ,*etal*.,2016).

Benzyl alcohol terpineol and 2methylbutyric acid were found to be the three primary constituents in the wild cranberry (V. oxycoccos) extracts that were derived from fresh fruit that originated in Russian Siberia and was grown in its natural environment. The results of the GC-MS analysis revealed these three chemicals to be

the predominant ones. Other prominent components were ascorbic acid, malic acid, citric acid, benzoic acid, and cinnamic acid, ALSO to malic acid and citric acid (vitamin C) . (Lyutikova and Turov. 2011).

According to research conducted by Puuponen-Pimea et al. (2011), berry fruits are a rich source of phenolic and organic acids. Both of these types of acids have the potential to inhibit the growth of bacteria . Cranberry is a kind of berry fruit that is native to North America . It is known by its scientific name, *Vaccinium macrocarpon L.*, and it is packed with of polyphenolic compounds. On the other hand, it is also manufactured and put to extensive use in the traditional folk medicine of EU as a treatment for a variety of microbiological ailments. The consumption of cranberry fruits, which provide considerable benefits to one's health, is not only an important component of one's diet but also a component that is enjoyed by consumers. (Howell ,2007;Nowack , 2007;Avorn,*etal*.,1994).

The purpose of the present research was to explore the antimicrobial activity of cranberry against Gram-negative bacteria that were isolated from instances of urinary tract infections. This was done because of the importance of cranberry (Vaccinium macrocarpon L.) as an antibacterial agent.

Materials and Methods

1- *Vaccinium macrocarpon* L.Fruits

The fruit components were gathered from the various local marketplaces. The Omega 8006 juicer was used in the preparation of the fruit juices (Omega, USA). Following that, the liquid went through a filter made of Whatman paper No. 1. (Whatman Ltd., in the United Kingdom) The filtrate was oven-dried at a temperature of 30 degrees Celsius. The powders were kept at a temperature of 4 degrees Celsius until they were used again.

2-Preparation of Alcohol plant extract

The powder of cranberry juice (Vaccinium macrocarpon L.) that was prepared in the previous step was soaked in 2.5 litres of ethanol that was 70% while being

stirred every 10 hours with a sterile glass rod. This process took between 8 and 10 days. This procedure was carried out several times. After the extraction procedure had been finished, the product was filtered using Whatman filter paper No. 1 to remove any remaining impurities (Whatman Ltd., England). The ethanol filtrate was heated in a water bath at a temperature of 40 degrees Celsius until it reached the desired consistency of a sticky semisolid mass. After this, the filtrate was placed in a refrigerator at a temperature of 4 degrees Celsius until it was needed. We were able to make a stock solution with a concentration of 100 mg/ml by first dissolving the dried extract in dimethyl sulfoxide and then filtering the solution (DMSO). (Ahmad and Aqil 2007).

2-Bacterial isolates and *Candida albicans*

E.coli ,*Proteus vilgarus* , *Staphylococcus auerus* ,*Enterococcus* sp & CoagulaseNegative Staphylococci (CNS) obtained from laboratory of Maternity and Children's Hospital in Al-Diwaniyah Governorate. Brian –Heart Infusion (BHI/Oxoid) media werev used tin growing of bacterial isolates and incubated in 37C ̊ for 18 h.

3-Antibacterial activities of Fruit extracts

The disc-diffusion technique was used in order to determine whether or not an ethanolic extract of cranberry juice (*Vaccinium macrocarpon L.*) has antibacterial properties. The turbidity of the culture was maintained at 0.5 McFarlands after certain adjustments were made. After that, the suspension was seeded into Mueller Hinton Agar (MHA) plates. After being saturated with 20 l of fruit extracts at various concentrations (100, 50, 25, & 12.5) mg/ml, sterile paper discs measuring 6 millimetres in diameter and made by HiMedia's oxoid division were then put on agar that had been infected. For the purpose of the negative control, discs that had been saturated with 20L of 70% ethanol and put in the middle of inoculated MHA were used. For the purpose of the positive control, amoxicillin (30 g) was employed. The culture plates were kept in an incubator at 37 degrees Celsius for a whole day. After

the duration of incubation, the zone of inhibition was evaluated and analysed. (Efstratiou ,*etal*.,2012).

The antibacterial activity was examined by the use of the disc diffusion method, and the experiment was carried out three times. If there was a zone of inhibition around the discs, which could be identified by using a ruler to measure the diameter of the area around the discs, then there was an observation of growth. This was the case if there was an inhibition region (mm).

4- Data analysis

The data are given using the mean as well as the standard deviation (SD). The ANOVA test using the SPSS programme was used to assess significance. When p was less than $P<0.05$, we judged the differences to be significant.

Results and Discussion

Results sunnerized in table (1) and Figure (1) appeared that the ethanolic cranberry juice *(Vaccinium macrocarpon L.*) extract in standard concentrations (100%) had accurate inhibitory effects on all tested bacteria ,where these extract appear the largest zone of inhibition compared with antibiotics against all bacteria .The impact of cranberry juice (*Vaccinium macrocarpon L.*) were different according to type of bacteria and of extracts' concentration that used in test. where *CNS* was more responsible (100%) for the cranberry juice *(Vaccinium macrocarpon L.*) *Staph.aureus* with rate (90 %), *P. valgarus* with inhibition rate (75%). while *E.coli* appear less sensitive to extract with rate of inhibition (60%) , Cranberry juice *Vaccinium macrocarpon L* presented dose dependent inhibitory effects against growth of all organisms for this research .

Table (1) Antimicrobial activity of cranberry juice (*Vaccinium macrocarpon L.*) and Amoxicillin against Uropathogenic bacteria

Bacterial isolates	Amoxicillin	Concentration(mg/ml) / mean of Zone inhibition				Rate of Inhibition
	30µg	12.5	25	50	100	
E.coli	9±1.2	12± 1.2	17±1.3	22±1.0	25±1.6	60%
P.vilgarus	11±1.0	13±1.0	18±1.4	24±1.8	26±1.1	75%
Staph.aureus	12±1.5	15±1.2	20±1.2	23±1.4	30±1.4	90%
Enterococcus sp	11±1.7	14±1.4	18±1.3	21±1.7	28±1.3	85%
CNS	12±1.5	15±1.3	19±1.7	22±1.2	26±1.7	100%

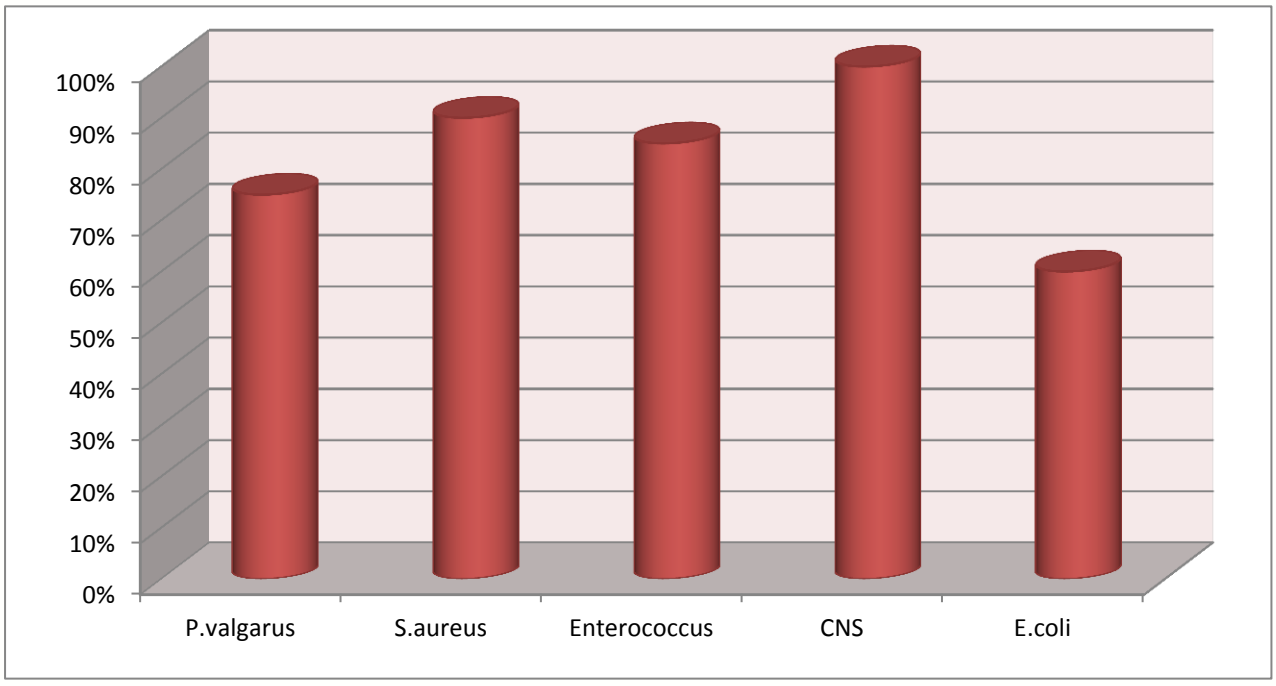

Figure (1) Inhibition rate of cranberry juice *(Vaccinium macrocarpon L.)* against Uropathogenic bacteria

Since cranberries are known to possess both of these components, the antibacterial effect of cranberry juice (*Vaccinium macrocarpon L.*) may be related to

the breakdown of bacterial cell wall or adherence to cell membranes. This is because cranberries contain both of these components. By changing the membrane's permeability by adsorption to the negatively charged bacterial cell wall, the cytoplasmatic membrane of bacteria is able to generate a permeability barrier that prevents the passage of tiny cations such as hydrogen ions, potassium ions, and sodium ions. When there is a negative charge on the membrane, this activity might take place. The structure and chemical makeup of the cell wall of the bacterial organism are what maintain this impermeability and even regulate it to some degree. If there is an increase in the quantity of potassium that is lost through the membrane, this is an indicator that the permeability barrier has been broken, and as a consequence, the ion homeostasis has been thrown off. It is crucial to the preservation of the cell's energy status as membrane bound energy dependent functions such as the management of turgor pressures and motility that the ion balance be maintained. Additional examples include the intake of solutes, the control of metabolic processes, and the management of turgor pressures. Therefore, even relatively minor disruptions in the structural integrity of the cell membrane can have a negative impact on the function of the cell, which can ultimately result in the death of the cell and the production of reactive oxygen species. This can happen even if the cell membrane is only slightly damaged (ROS).

The study's results are comparable to the results of other studies, such as the study by Agata Stobnicka et al. (2013), in which the researchers discovered that *Vaccinium macrocarpon L*. was cytotoxic to bacteria; the studies by Lian et al. (2012) and Lacombe et al. (2013), in which the researchers discovered; and so on. The results of this research are similar to those of other studies. It has been shown that the antibacterial action of American cranberry concentrates is particularly efficient against many bacterial infections, including Staphylococcus aureus and E. coli O157:H7. They found that this extract was effective against a variety of bacterial strains, which is a significant finding. Therefore, Rauha et al. (2000) used an alcoholic extract of Finnish berries to prove this fact also for *Vaccinium macrocarpon L* fruits. It has been shown to have very little antibacterial activity against Staphylococcus aureus; on the other hand, it has been discovered to have good antimicrobial activity

against Escherichia coli. The yeast Candida albicans, Staphylococcus epidermidis, Bacillus subtilis, and Micrococcus luteus all developed normally despite the fact that the berry extract did not have any influence on their growth. Additionally, the development of the mould known as Aspergillus niger was not inhibited by it. In addition, Esonien et al. (2009) investigated the antibacterial activities of numerous wild clones of European cranberry by using the agar well diffusion method. This method was used in order to determine whether or not the clones were efficient in combating the bacterium in issue. It has been shown that extracts of European cranberries may inhibit the growth of a wide range of Gram-negative (such as Escherichia coli and Salmonella typhimurium) and Gram-positive (such as Candida albicans) human pathogenic bacteria (Enterococcus faecalis , Listeria monocytogenes, Staphylococcus aureus and Bacillus subtilis).

According to Hellstrom and colleagues' research, the antibacterial inhibitory effect of European cranberry may be explained by its high amount of polyphenols (2009). analysed, is provided by the polyphenolic subfraction at a concentration of 5 mg/100 ml and proanthocyanidins at about 400 mg/100 g. It is well established that proanthocyanidins inhibit the ability of certain bacteria to adhere. It has been discovered that European cranberries include A-type dimers and trimers, as well as lingo berries and American cranberries, which have a significant quantity of these compounds (Netto . 2007). Kylli et al. (2011) came to the same conclusion, demonstrating that the antibacterial properties of V. microcarpon are due to the presence of proanthocyanidins. Cranberry polymeric proanthocyanidin proved to have a good antibacterial activity against Staphylococcus aureus; however, these compounds did not demonstrate any impact against other kinds of bacteria (S. enterica sv. Typhimurium, Lactobacillus rhamnosus , and Escherichia coli).

Extract from European cranberry was shown to be an attractive option for use as anatural preservative of minced pig meat, according to research conducted by Stobnicka and Gniewosz (2018). S. aureus, L. monocytogenes, S. enteritidis and E. coli were inoculated into fresh minced pork meat that contained 2.5% extract. Water, ethanol fruit, and pomace extracts were tested due to their antimicrobial activity as

the growth inhibitors of these bacteria. Extracts were more effective in inhibiting Gram-positive bacteria strains than Gram-negative bacteria strains, however yeasts were resistant to the extracts' antifungal properties. The bactericidal activity of water–ethanol fruit and pomace extracts was found to be much higher than that of ethanolic fruit and pomace extracts and aqueous fruit and pomace extracts. Cranberry pomace extracts contained stilbenes (resveratrol), as well as more organic acids and flavonols than fruit extracts; however, ethanol extracts of both types contained terpenes (ursolic acid).

Conclusions

The current research work demonstrates that cranberry juice, also known as *vaccinium macrocarpum L.*, possesses antimicrobial properties against Escherichia coli, Pseudomonas valgaris, Staphylococcus aureus, Enterococci sp, and CNS. This knowledge contributes to the body of knowledge regarding natural antibacterial substances. The results provide encouraging preliminary information about the possible use of cranberries as a therapy for urinary tract infections. Cranberries may also be investigated further as a natural remedy for bacterial infections.

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