# Inhibitory Action of Plant Extracts (Maidenhair, Cranberry, and Vitis vinifera) Against Gram-Negative Bacteria Isolated from Urinary Tract Infection

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#### Abstract

Background: According to the Center for Disease Control and Prevention, urinary tract infections (UTIs) require healthcare. Likewise, UTIs are the most common infections that result in prescriptions for antibiotics following a doctor's visit. Because UTI symptoms are generally mild and overuse of antibiotics may contribute to antibiotic resistance, it is crucial to establish appropriate criteria for antibiotic therapy. Finding alternatives to antibiotics, such as extracts or nanomaterials. Objective: The current study was carried out to investigate the inhibitory action of three plant extracts (Maidenhair, Cranberry, and Vitis vinifera) against gram-negative bacteria isolated from UTI. Materials and Methods: Aquatic extracts were prepared from three different plant species (Maidenhair, Cranberry, and V. vinifera). The antimicrobial activity of the aquatic extracts from Maidenhair, Cranberry, and V. vinifera was tested in vitro using agar well diffusion assays, biofilm formation assays, and adherence tests. Results: The findings demonstrated that herbal plants had a potent inhibitory effect on gram-negative bacteria (Proteus vulgaris, Proteus merabilis, Pseudomonas aeruginosa, Pseudomonas flourscences, Escherichia coli, Enterobacter aerugenes, Klebsiella pneumoniae, and Serratia spp.) isolated from UTI. Additionally, we discovered that, against gram-negative bacteria, plant extracts outperformed the broad-spectrum antibiotic ciprofloxacin in terms of bacterial eradication. The virulence factors, including biofilm and anti-adhesion that are present in pathogenic bacteria, can be inhibited by Maidenhair and Cranberry plant extracts, with levels of inhibition ranging from high against six pathogenic species to moderate against two pathogenic isolates. However, V. vinifera extract was less successful in thwarting virulence factors for the isolates examined. Conclusion: The effectiveness of the aquatic extract for Maidenhair, Cranberry, and V. vinifera to inhibit growth, virulence factors of gram negative isolates and reduce UTIs.

Keywords: Cranberry, Maidenhair, plant extracts, Vitis vinifera

#### INTRODUCTION

Urinary tract infections (UTIs) are most frequently caused by gram-negative bacteria, but gram-positive pathogens can also be at risk. A single type of bacterium (monobacterial) accounts for approximately 95% of simple UTIs. In 75%–95% of cases, *Escherichia coli* (*E. coli*) is the most frequent pathogen for uncomplicated UTIs. The gram-negative pathogens *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B streptococci, and *Proteus mirabilis* are also frequently responsible for UTIs.<sup>[1]</sup> Depending on patient demographics and the severity of the infection,

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several bacteria can cause UTIs. Both straightforward and complex UTIs can be produced by *E. coli*. However, difficult UTIs are more frequently caused by *P. mirabilis*, *Pseudomonas aeruginosa*, and *Enterococcus* species, which are also frequently isolated in hospitals and long-term



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care facilities.<sup>[2]</sup> UTIs and elevated antibiotic resistance are associated with multidrug resistance (MDR), which poses a significant financial and medical burden to healthcare systems.<sup>[3-5]</sup> Microorganisms use a variety of resistance mechanisms, including mutations, genetic recombination, and horizontal gene transmission (such as mobile genetic elements and phages) to resist the effects of antibiotics. Antimicrobial resistance is a serious medical concern.<sup>[6]</sup>

Human infectious diseases can be cured using herbal and medicinal plants. Plants have been a valuable source of traditional medicine for thousands of years.<sup>[7]</sup> Given the chemical diversity inherent to many species, medicinal plants are a substantial source of chemotherapeutic compounds.<sup>[8,9]</sup> In recent years, some plants have been shown to exhibit antibacterial and antibiotic-modulating effects.<sup>[10-12]</sup> This prompted researchers to speculate that herbal remedies, which have been used for centuries to treat infectious disorders, and may contain compounds that act as antibacterial and/or antibiotic resistance modulators. The purpose of this study was to investigate the antibacterial effects of aqueous extracts of Maidenhair, Cranberry, and *Vitis vinifera* on gram-negative bacteria *in vitro*.

# MATERIALS AND METHODS

#### **Plant extract**

Aquatic plant extract of Maidenhair, Cranberry, *and V. vinifera* with thirty present of concentration and an antibiotic, called Ciprofloxacin.<sup>[13]</sup>

### **Clinical specimens**

Eight bacterial cell isolates (clinical samples from UTI) were used. Table 1 lists the isolates of the bacterial species. Isolates of bacterial cells were stimulated on nutrient agar multiple times in a row, before being preserved at 4°C as an agar slant. Various biochemical and vitic tests were used to establish the isolated documentation.<sup>[14]</sup>

### Antimicrobial activity test

There are two ways to evaluate antibacterial activity: the agar well diffusion assay is performed *in vitro*.<sup>[15]</sup>

Agar-disc diffusion was used to determine antibacterial activity according to Forbes (2007)<sup>[14]</sup> (the test was performed in triplicate on Muller Hinton agar).

Table 1: Gram-negative bacteria
Gram negative bacteria
Proteus vulgaris
Proteus merabilis
Pseudomonas aeroginosa
Pseudomonas flourscences
Escherichia coli
Enterobacter aerugenes
Klebsiella pneumonia
Serratia spp.

Assay for biofilm formation: The antibiofilm activities of plant extracts against bacteria playd an essential role in medical device biofilm-associated infections.<sup>[15,16]</sup> The microtiter plate assay was defined by Christensen *et al.*<sup>[17]</sup> as a conventional method for identifying biofilm production. In this experiment, bacteria were cultured in microtiter plate wells and the optical density of the biofilm was determined using spectrophotometry. The mean value of all negative controls included in the tests is less than 0.120, which is regarded as moderate; and values beyond 0.150 are regarded as high.

#### Adherence examination

To establish an infection, a bacterium must adhere to the epithelium and multiply before the mucus and extruded epithelial cells are swept away. To accomplish this, bacteria have evolved attachment mechanisms, such as pili that attach the bacteria to cells.<sup>[18]</sup>

To detect the bacterial adherence to the epithelial layer of the patient, the method described by Mateveki *et al.* and Avila-Campos *et al.* was used.<sup>[19,20]</sup>

#### **Ethical approval**

Not Applicable.

## RESULTS

The antibacterial activities of three medicinal plants were assessed in this study: *V. vinifera*, Cranberry, and Maidenhairs. Gram-negative bacteria (*Proteus vulgaris*, *P mirabilis*, *P. aeruginosa*, *P. fluorescens*, *E. coli*, *Enterobacter aerogenes*, *K. pneumoniae*, and *Serratia* spp.) are frequently isolated from UTIs. As shown in Figure 1, the extract from the Maidenhair plant exhibited the highest rate of inhibition against gram-negative bacteria, ranging from 33 mm to 38 mm.

Cranberries can destroy bacteria that cause UTIs. A previous study revealed that Cranberry extract exhibited







Figure 2: The antibacterial effect of Cranberry extract against gramnegative bacteria was determined using the agar-well method



Figure 3: The antibacterial effect of the *Vitis vinifera* extract against gram-negative bacteria was evaluated using the agar well method

inhibitory effects on several bacteria that cause UTIs, such as *E. aerogenes*, *P. vulgaris*, *P. mirabilis*, *E. coli*, *P. aeruginosa*, *P. fluorescens*, and *Serratia* spp. The 30–34mm range was covered by inhibitory actions [Figure 2].

Numerous compounds found in *V. vinifera* plants have the ability to prevent bacteria from proliferating in the urinary tract. This is shown in Figure 3, where the inhibitory effects of black sultana plant extract were comparable to those of other extracts that have been demonstrated to be successful in combating bacteria that cause urinary tract infections.

When plant extracts were compared to the broadspectrum antibiotic ciprofloxacin against gram-negative bacteria, it was found that the extracts were more efficient in suppressing the germs isolated from the urethral tract of patients suffering from urinary tract infections. Figure 4 illustrates how the antibiotic inhibition rate changed from 10 to 15 mm.



**Figure 4:** Antibacterial effects of ciprofloxacin against gram-negative bacteria using the agar-disc diffusion method

Table 2	: Effectiveness	of the	aquatic	extract	for	Maidenhair
to inhib	it virulence fac	tors in	gram-n	egative i	sola	ates

Gram negative bacteria	Anti-adherence activity	Anti biofilm activity
Proteus vulgaris	High	High
Proteus mirabilis	High	High
Pseudomonas aeroginosa	High	High
Pseudomonas flourscences	High	High
Escherichia coli	High	High
Enterobacter aerugenes	Moderate	Moderate
Klebsiella pneumonia	Moderate	Moderate
Serratia spp.	High	High

Table 2 illustrates how the virulence factors of various pathogenic bacteria are inhibited by Maidenhair plant extracts. Regarding the six different types of bacteria, the percentage of inhibition was high and medium for the two types. The preceding table illustrates that the extract also exhibited the capacity to decrease biofilm formation by the pathogenic bacteria. For the two pathogenic isolates, the inhibition varied from high against six pathogenic species to moderate.

Table 3 illustrates how well the plant extract suppressed virulence factors implicated in anti-adherence and biofilm formation.

Table 4 demonstrates that the virulence factors of the investigated isolates were less effectively inhibited by *V. vinifera* extract. The extract had a moderate level of efficacy against five isolates; however, it was only effective against three isolates.

#### DISCUSSION

Moreover, the antibacterial effect of Maidenhair against gram-negative bacteria, this agrees with earlier

Table 3: The effectiveness of aquatic extract for Cranberry to
inhibit virulence factors of gram-negative isolates

Gram negative bacteria	Anti-adherence activity	Anti biofilm activity	
	High	High	
Proteus merabilis	High	High	
Pseudomonas aeroginosa	High	High	
Pseudomonas flourscences	High	High	
Escherichia coli	High	High	
Enterobacter aerugenes	Moderate	Moderate	
Klebsiella pneumoniae	Moderate	Moderate	
Serratia spp.	High	High	

# Table 4: Effectiveness of the aquatic extract for Vitis vinifera to inhibit virulence factors in gram-negative isolates

Gram negative bacteria	Anti-adherence activity	Anti biofilm activity
Proteus vulgaris	High	High
Proteus merabilis	High	High
Pseudomonas aeroginosa	Moderate	Moderate
Pseudomonas flourscences	Moderate	Moderate
Escherichia coli	High	High
Enterobacter aerugenes	Moderate	Moderate
Klebsiella pneumoniae	Moderate	Moderate
Serratia spp.	Moderate	Moderate

research that showed potent antibacterial activity of Maidenhair against a range of pathogens,<sup>[20]</sup> such as *Salmonella typhi, P. aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus.* According to a study by Medrar *et al.*, plant extracts are more effective than amoxicillin in combating *P. aeruginosa*(2014).<sup>[21]</sup> Numerous pathogenic bacteria, such as Providencia, *K. pneumoniae, Shigella, V. cholera, S. aureus, S. typhi,* and *P. vulgaris,* have been demonstrated to be inhibited in their growth by plants. These effects have been observed in both aqueous and alcoholic plant extracts, including those from the roots, stems, and leaves.<sup>[22,23]</sup>

Furthermore, the UTIs have traditionally been treated with cranberries and blueberries.<sup>[24,25]</sup> Proanthocyanidins (PACs) and fructose are believed to be responsible for the anti-UTI characteristics of cranberries' and blueberries.'To prevent bacteria from colonizing and infecting the urinary tract wall, PACs hinder their adhesion to the wall.<sup>[26]</sup>

According to a previous study, the acetonic extract of *V. vinifera* is especially useful in combating *Salmonella typhimurium*, *P. aeruginosa*, *E. coli*, and *S. aureus*. *Salmonella typhimurium* and *E. coli*, however, were less vulnerable to the acetonic extract. Additional research has demonstrated that vinegar and crude extracts from *V. vinifera* plants, which both contain compounds from the plant, may be useful against a broader variety of

bacteria that cause UTIs, such as Salmonella typhi, E. coli, S. aureus, S. pneumoniae, Streptococcus faecalis, Streptococcus mutans, and S. faecalis.<sup>[27-29]</sup> Overall, the data indicate that V. vinifera is a potential natural treatment for tract infections. Further research is necessary to validate these results and establish the ideal dosage and form of V. vinifera for the treatment of UTIs.

On the other hand, in a study to evaluate the range and antibiotic resistance of uropathogens between 2004 and 2015, it was discovered that the five most common bacteria were E. coli, E. faecalis, K. pneumoniae, P. aeruginosa, and P. mirabilis. E. coli resistance to Ciprofloxin rose sharply from 19% to 25% over this period, and K. pneumoniae resistance to cephalosporins was extremely high.<sup>[30]</sup> The chemical components discovered in these herbs are responsible for the substantial antibacterial activity of the plant extracts.<sup>[31,32]</sup> Plant extracts have a high rate of inhibition, making them a promising treatment option for tract infections instead of antibiotics. The extensive application of antimicrobials has resulted in the emergence of bacterial resistance, thereby significantly diminishing their efficacy. Urinary tract infections can be effectively treated with plant extracts to overcome bacterial resistance.

Regarding effectiveness of the aquatic extract for Maidenhair to inhibit virulence factors, the results of the experiment demonstrated that the Maidenhair plant extract significantly reduced the virulence factors of pathogenic bacteria. Six different varieties of bacteria were shown to inhibit their development, and the capacity of the two types of bacteria to create biofilms was decreased by the extract. These results imply that the extract from the Maidenhair plant could be a useful natural treatment for diseases caused by harmful bacteria. Further investigation is necessary to validate these results and establish the best form and dosage of Maidenhair plant extract for the treatment of infection.

These findings imply that the virulence factors of pathogenic bacteria may not be as effectively inhibited by *V. vinifera* extract as they are by the other plant extracts. To validate these results and establish the ideal amount and form of *V. vinifera* extract for the treatment of infections, further investigation is necessary.

The way antibacterial compounds permeate bacterial cells, their capacity to interact with the cells, the pH of the surrounding environment, and temperature are some of the variables that affect their effectiveness.<sup>[33]</sup> Aqueous extracts of plant materials containing a variety of active chemicals have been found to be effective against bacteria. This is because they can directly destroy bacteria, inhibit their adherence to epithelial cells, stop the development of biofilms, stop their growth, balance the composition of gut bacteria, strengthen the immune system, and function as antioxidants.<sup>[34,35]</sup> Phenolic chemicals are a class of active materials present in plants

that have antibacterial properties. They function by changing the permeability of bacterial cells, which has the potential to modify macromolecules present within the cells. This has the potential to deactivate enzymes necessary for bacterial growth and reproduction as well as pathogenicity. Flavonoids, palmitic acid, unsaturated fatty acids, linolenic acid, terpenoids, saponins, tannins, alkaloids, and glycosides possess antibacterial properties. It has been demonstrated that two plants, cranberries and blueberries, are useful in both preventing and treating UTIs.<sup>[36]</sup> They contain proanthocyanidins, which can prevent bacteria from sticking to the wall of the urinary tract. Thus, UTIs may be avoided, and the study evaluated the beneficial effects of Maidenhair water extract on the urinary tract.

The number of crystals was significantly reduced, and the serum levels of blood urea, calcium, and phosphorus were also significantly lower. This implied that the extract may prevent bacterial growth. Additionally, the extract proved to be efficient against every type of bacterium examined. Anti-UTI qualities are attributed to PACs and fructose in cranberries and blueberries. Pathogens prevent colonization of the urinary system by PACs, which suppress virulence factors (VFs), such as *P. fimbria*. *V. vinifera* and *V. vinifera* vinegar have strong antibacterial and fungicidal properties. Oral epithelial cells can prevent microbial cell adhesion, motility, and biofilm formation.<sup>[18]</sup>

### CONCLUSION

The effectiveness of the aquatic extract for Maidenhair, Cranberry, and *V. vinifera* to inhibit growth, virulence factors of gram negative isolates and reduce urinary tract infections.

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#### **Conflict of interest**

There are no conflicts of interest.

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