**Use of *Arctostaphylos uva-ursi* Extracts for the Treatment of Urinary Tract Infections**

**ABSTRACT**

Extracts of the low-lying shrub *Arctostaphylos uva-ursi*, which is commonly referred to as “bearberry” or simply as *Uva ursi*, have been widely promoted and frequently used as an herbal treatment for urinary tract infections. These extracts contain a wide range of organic chemicals such as arbutin (4-hydroxyphenyl-β-D-glucopyranoside), polyphenolic compounds, and flavonoids. *Arctostaphylos uva-ursi* extracts have been shown to inhibit the growth of uropathogenic Gram-negative bacteria. These include *Escherichia coli*, *Proteus mirabilis,* *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, Gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus saprophyticus*, and the pathogenic yeast *Candida albicans*. This review article summarizes the experimental work on the inhibition of microbial growth by *Uva ursi* extracts and their components. It describes the complementary studies on the effects of these extracts on two key virulence factors, the enzyme urease and the ability of the microbes to form biofilms. The article also reviews the antioxidant and anti-inflammatory properties of these extracts and their effects on other host functions. The preliminary animal and human studies with *Arctostaphylos uva-ursi* extracts are summarized, as are the human clinical trials that have been completed so far. These studies have shown that these extracts are relatively safe, although the key component arbutin can be metabolized to form the more toxic compound hydroquinone. Although the laboratory data indicate that *Uva ursi* preparations can effectively inhibit urinary tract pathogens and so may represent an alternative to traditional antibiotics, the clinical studies are far more limited and currently insufficient to justify their widespread medical use. Further areas of research might include the use of extracts from plants with low arbutin contents or the combination of *Arctostaphylos uva-ursi* extracts with those from other plants.

***Key Words:*** *antioxidant, anti-inflammatory, Arctostaphylos uva-ursi*, bearberry, biofilm, herbal medicine, urease, urinary tract infection

1. **INTRODUCTION**

One of the most active areas of research in herbal medicine is the identification of plant extracts with the potential for treating urinary tract infections (UTIs) [1-5]. This represents an extension of the traditional use of herbal preparations by Indigenous people in India, China, Africa, the South Pacific, and Europe [6-10]. This research has become particularly important because microbial resistance to traditional antibiotics has increased and there is consequently a growing need for alternative modes of therapy [11-14]. UTIs are among the most common human diseases and affect more than 150 million people worldwide each year [15]. UTIs can be classified according to the patient’s gender (male or female) or age (pediatric, adolescent, adult) [16-19]. Infections of the bladder are referred to as cystitis while infections of the kidneys are known as pyelonephritis [20]. Uncomplicated or community-acquired UTIs are due to microorganisms derived from the gastrointestinal tract, the genital system, or the external environment [21-22]. Complicated or catheter-associated UTIs are due primarily to the implantation of devices such as catheters in surgical or elderly patients [23-24]. Urinary tract infections may be acute or chronic [25-26], but many acute UTIs can recur even after treatment with antibiotics [27-28].

A number of different microorganisms can cause UTIs, including various Gram-negative bacteria, Gram-positive bacteria, and yeast [29-30]. Approximately 75% of uncomplicated UTIs and 65% of complicated UTIs are associated with Gram-negative uropathogenic *Escherichia coli* (UPEC). Other important Gram-negative bacteria associated with UTIs include *Proteus mirabilis*, *Klebsiella pneumoniae* (formerly *K. aerogenes* or *Enterobacter aerogenes*), and *Pseudomonas aeruginosa*. About 6% of uncomplicated UTIs are caused by the Gram-positive bacterium *Staphylococcus saprophyticus* while about 11% of complicated UTIs are associated with Gram-positive *Enterococcus* species and 3% with *Staphylococcus aureus.* The most common uropathogenic yeasts are *Candida* species, which are found primarily in complicated UTIs. There are important geographic differences in the epidemiology of these infections [31].

Much of the research on the use of herbal extracts for the treatment of UTIs has focused on the low-lying shrub *Arctostaphylos uva-ursi*, which is commonly referred to as “bearberry” or simply as *Uva ursi* [32-33]. This plant is a member of the family Ericaceae and is widely-distributed across North America, Europe, and Asia. The fruits form small grape-like clusters and have been found to be eaten by bears. Several medically-related internet sites describe the potential use of *Uva ursi* extracts for the treatment of urinary conditions [34-37]. Over-the-counter preparations derived from the leaves of the plants are widely available without a prescription as liquids or capsules in pharmacies or health food stores and from online-distributors [38]. This review summarizes the currently-available information about *Arctostaphylos uva-ursi* and the use of its extracts in the treatment of UTIs. Although there is a large amount of *in vitro* microbiological data indicating that *Uva ursi* preparations can inhibit urinary tract pathogens, the *in vivo* clinical studies are far more contradictory and are currently insufficient to justify its widespread medical use. It is thus important to understand both the potential uses and the limits of these extracts.

 **2. COMPOSITION OF *UVA URSI* EXTRACTS**

*Arctostaphylos uva-ursi* extracts contain a wide range of organic compounds [39]. High performance thin layer chromatography (HPTLC) of methanolic extracts of 16 samples of bearberry leaves from several countries in Europe and from sites in Mexico and Latin America revealed two general patterns [40]. The first contained multiple spots including a very intense one for arbutin (4-hydroxyphenyl-β-D-glucopyranoside). The second also contained multiple spots but the arbutin one was not as intense. These results were confirmed by 3H-nuclear magnetic resonance (NMR) analysis and by high performance liquid chromatography – diode array detector (HPLC-DAD) analysis. These results allowed the authors to differentiate between *Arctostaphylos uva-ursi* and

*Arctostaphylos pungens.* Ultra high-performance liquid chromatography-photodiode array (UHPLC-PDA) analysis of methanolic extracts of these leaf samples indicated more clearly the presence of many polyphenolic metabolites. These included gallic acid and gallotannins, several flavonoids such as quercetin, myricetin, kaempferol, and catechin [41]. The concentrations of these compounds differed between the two species. Gallic acid, quercetin, myricetin, kaempferol, catechin, and epigallocatechin gallate have all been extensively studied due to their antimicrobial, anti-inflammatory, and antioxidant activities as well as their potential medical applications [42-47]. Many of these same compounds have been found in other species of the Ericaceae including bilberry, lingonberry, and blueberry [48].

The earlier studies on the contents of *Uva ursi* extracts were recently extended using ion mobility-high resolution mass spectrometry (IMS-HRMS) to characterize nine leaf samples collected in Spain [49]. After extraction of ground leaf powder with 70% ethanol and separation with a high-performance liquid chromatography system, 88 different polyphenolic compounds were identified by mass spectrometry. The concentrations of 14 of these compounds were determined in the samples and found to vary significantly among these samples. The relative contents of arbutin and the flavonoids (+) catechin, myricetin, and quercetin glycoside were also analyzed in 42 wild populations of *Arctostaphylos uva-ursi* from the Iberian peninsula [50]. They were found to vary across both latitudinal and longitudinal gradients, reflecting differences in local microclimates. The populations of *Uva ursi* were also shown to differ genetically. An analysis of the total phenolic compound concentrations in three over-the-counter liquid preparations of *Uva ursi* using the colorimetric Folin-Ciocalteau reagent indicated they varied from 6.8 mg/ml (Honey Combs) to 93.6 mg/ml (Nature’s Answer) [51].

Of the various chemicals found in extracts of *Uva ursi*, arbutin is usually considered to be the most important in terms of the efficacy of these preparations as inhibitors of urinary tract pathogens [39,52-53]. This glycoside can occur as an α- or β-anomer depending on the configuration at the bond between D-glucose and the ring system (**Fig. 1**). The β-anomer occurs naturally in plants such as *Uva ursi* while the α-anomer can be synthesized in the laboratory and is used primarily as a skin whitening agent [53-54].



 α-arbutin β-arbutin

**Fig. 1.**  Structure of arbutin

β-arbutin is synthesized *in vivo* from hydroquinone and UDP-glucose (**Fig. 2**). The biochemical pathway leading to hydroquinone is based on the shikimate pathway that is normally used for the synthesis of aromatic amino acids [55-57]. Chorismate is first converted to *p*-hydroxybenzoate (PHB) by chorismate lyase. PHB is then transformed into hydroquinone by PHB-1-hydroxylase. The hydroquinone is attached to D-glucose using UDP-glucose as the glycosyl donor in a reaction catalyzed by a UDPG transglycosylase termed arbutin synthase. Recently, two groups achieved *in vitro* synthesis of β-arbutin using the same pathway by genetic engineering of microorganisms [58-59].



**Fig. 2.** Pathway of arbutin biosynthesis

Although arbutin is relatively stable, it may undergo spontaneous or acid-mediated hydrolysis to yield free D-glucose and hydroquinone (**Fig. 3**) [60-61]. The hydroquinone may then undergo spontaneous oxidation to form 1,4-benzoquinone, also called *p*-benzoquinone. Many derivatives of arbutin including acylated and benzoylated forms have also been reported to occur in various plant species including *Uva ursi* [62]. Plant extracts often contain varying concentrations of arbutin, hydroquinone, and 1,4-benzoquinone as determined by a colorimetric assay, analytical high performance liquid chromatography, or other methods [63-66].



**Fig. 3.** Degradation of arbutin

**3. INHIBITION OF UROPATHOGENIC MICROBES BY *UVA URSI* EXTRACTS**

Extracts of *Arctostaphylos uva-ursi* have the potential to inhibit the growth of those bacteria that can cause UTIs either alone or in combination with specific antibiotics. The results reported by various investigators have varied, however, depending on the type of extract employed, the bacterial species tested, and the specific inhibition assay. The solvents used to extract chemicals from *Uva ursi* leaves have included water, ethanol, methanol, and ethyl acetate. The species studied have been *Escherichia coli* or other Gram-negative bacteria and *Staphylococcus aureus* or other Gram-positive bacteria. The methods used to demonstrate growth inhibition have involved the measurement of zones of inhibition on agar plates around wells or paper disks to which *Uva ursi* extracts were added or the quantitation of growth as a minimum inhibitory concentration (MIC) in a series of tubes or wells in microtiter plates containing serial dilutions of extracts.

Holopainen et al. [67] prepared an extract of the aerial material from *Arctostaphylos uva-ursi* collected in Finland by macerating the tissue in 80% ethanol using a 5:1 ratio of solvent to plant material. A 10 ml portion of the extract was evaporated to dryness and resuspended in 80% ethanol at 100 mg/ml. Another 10 ml portion was evaporated, diluted 1:1 with water, and extracted again with ethyl acetate. The solvent was evaporated off and the residue suspended in 80% ethanol. The preparation was tested for inhibitory activity towards *Escherichia coli* (ATCC 11775), *Proteus vulgaris* ((DSM 30118), *Streptococcus faecalis* (ATCC 29212), and *Enterobacter aerogenes* (ATCC 13048). Aliquots (0.1 ml) of the bacteria in 0.9% saline containing about 107 bacteria/ml were spread on tryptone soya peptone agar plates. Wells were made in the agar to which 0.1 ml of a 10% or 5% test solution were added. The zones of inhibition were measured after overnight incubation at 32oC. The *E. coli*, *P. vulgaris*, and *S. faecalis* cultures showed good inhibition (21 to >24 mm ) with the ethanol extracts, but there was much less inhibition of *E. aerogenes.* There was a similar inhibition with the ethyl acetate-treated samples. HPLC analysis of the ethanol extract revealed the presence of arbutin, quercetin, kaempferol and several other compounds.

Vučić et al. [68] made extracts of the leaves of natural populations of *Uva ursi* from central Bosnia and Herzegovina by mixing 10 g of leaves with 200 ml of water, ethanol, or ethyl acetate as the solvent for 24 hr. The liquid was filtered through filter paper and the extraction process repeated with the same solvent two more times. The supernatants were combined and evaporated to dryness under vacuum. The total concentration of phenolic compounds in methanolic solutions of the extracts was determined using the colorimetric Folin-Ciocalteu reagent with gallic acid as the standard. The total concentration of flavonoids was determined using another spectrophotometric method with rutin as the standard. The aqueous and ethanol extracts had higher concentrations of both phenolic compounds and flavonoid compounds compared to the ethyl acetate extract. Ten clinical samples of *Escherichia coli* and ten samples of *Enterococcus faecalis* from the Institute of Public Health in Banja Luka, Bosnia and Herzegovina were tested for inhibition by each extract. The MIC was determined after 24 hr of incubation in Mueller Hinton broth. The MICs for the aqueous extracts ranged from 1.25 mg/ml to 5 mg/ml but varied widely for both of the bacteria. In general, the *Enterococcus faecalis* samples were more sensitive to the ethanol and ethyl acetate extracts (values from 1.25 mg/ml to 2.5 mg/ml) than the *E. coli* samples (values from 5 mg/ml to 10 mg/ml). The specific phenolic compounds or flavonoids associated with inhibition were not identified.

Hassan et al. [69] prepared an extract from the leaves of *Arctostaphylos uva-ursi* grown in the Thunder Bay region of Northern Ontario, Canada by combining 2 g of dried plant material with two 50 ml portions of 95% ethanol for 24 hr at room temperature. The extract was filtered through Whatman No 1 filter paper and evaporated to dryness under vacuum at 34oC. The dried extract was suspended in 99% methanol at a concentration of 10 mg/ml or 20 mg/ml. For the hole-plate diffusion assay, various Gram-positive or Gram-negative bacteria were diluted in phosphate buffered saline to give a concentration of 2 x 106 colony forming units (CFU)/ml and 100 µl aliquots were added to 25 ml of liquid LB agar. After the agar had gelled, 6 mm wells were bored into the agar and 40 µl of the extract (10 mg/ml in methanol) added. The plates were incubated at 32oC for 24 hr and the sizes of the zones of inhibition measured. For *E. coli*, this zone was 16.6 mm. For the determination of the MIC, 180 µl of serial dilutions of the extract in LB broth were added to the wells of a microtiter plate and 20 µl of a bacterial suspension at 2 x 106 CFU/ml added. The plates were incubated at 32oC for 24 hr. Resazurin (10 µl of 0.04% in phosphate buffered saline) was added to each well, and after 90 to 180 min in the dark, the color of the fluorescence detected with a short-wavelength UV lamp. The MIC was taken as the concentration at which the color changed from blue to pink. For *E. coli*, the MIC was >5 mg/ml.

Snowden et al. [70] prepared a different extract of *Uva ursi* by suspending 1 part of ground plant leaf and berry powder in 3 parts of a mixture of 95% ethanol/distilled water/glycerol (42/48/10) and incubating it for 14-21 days at room temperature. The liquid was expressed from the solid plant material and filtered through paper. A portion of the extract was dried and found to have a total concentration of nonvolatile solutes of 31 mg/ml. When a culture of *Staphylococcus aureus* ATCC 11632 growing at 37oC in tryptic soy broth was treated with increasing amounts of the extract up to 20 µg/ml, there was a progressive decrease in viable cell count over a period of 24 hrs. The concentration of the extract required to completely inhibit growth in 24 hr was determined to be 2.9 µl/ml for the liquid extract or 90 µg/ml for the nonvolatile solids. These values were in the more active range of all the botanical extracts tested (MIC <200 µg/ml) but higher than tetracycline or vancomycin. No specific components of the *Uva ursi* extract were identified as determining factors.

Samoilova et al.[71]studied the growth rate of the standard laboratory strain *E. coli* BW25113 in a liquid M9 minimal salts medium supplemented with 0.2% D-glucose, 0.2% casamino acids, and 10 µg/ml thiamine. This growth rate was reduced by about 30% when a boiled aqueous extract of *Uva ursi* was added. The growth rate was even slower in the presence of the extract and 4 mM H2O2. The *Uva ursi* extract also increased the susceptibility of the bacteria to growth inhibition and lysis by 30 µg/ml kanamycin and 10 µg/ml ampicillin.

Gerstel et al. [72] made a different extract of *Uva ursi* by combining one part of dried plant material (leaves and berries) with 3 parts of a mixture of 40% ethanol and 60% water. After incubation at room temperature and centrifugation at 3000 x *g* to remove cell debris, the liquid was filtered through a 0.2 µm filter. The solution was tested as an inhibitor of a standard strain of *S. aureus* (ATCC 14775), a penicillin-resistant strain (ATCC 11632), and a multidrug-resistant strain (ATCC BAA-44). For determination of the minimum inhibitory concentrations, 18 hr cultures containing 1 to 5 x 108 colony-forming units/ml were diluted 1:1000 into tryptic soy broth and incubated at 37oC for 24 hr. The MIC was defined as the concentration of extract needed to completely inhibit growth as measured by an increase in turbidity. The values were 0.12 µg/ml for ATCC 14775, 0.25 µg/ml for ATCC 11632, and 0.25 µg/ml for ATCC BAA-44. When growth was measured over a 12 hr period in tryptic soy broth liquid cultures containing the extract at a concentration of ½ the MIC or the full MIC, all three strains showed complete inhibition of growth at the full MIC as measured by colony-forming units. The standard strain ATCC 14775 showed no growth at ½ MIC and the penicillin-resistant strain and the multidrug-resistant strain exhibited delayed growth at ½ MIC.

Sumreen et al. [73] tested the effects of a commercial tincture of *Uva ursi* from the Schwabe Homeopathic Pharmaceutical Company in Germany on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The bacteria were grown in nutrient broth and then swabbed onto nutrient agar plates. Dried filter paper discs containing the tincture were placed on the agar surface and the zones of inhibition measured after 24 hr at 37oC. The sizes of the zones were compared to those with ampicillin. The zone for the Gram-positive bacterium *S. aureus* with the *Uva ursi* tincture (31 mm) was larger than those for the Gram-negative bacteria (13 to 18 mm).

Other studies have shown no bacterial inhibition by *Uva ursi* extracts. Dykes et al. [74] examined the effect of an ethanolic leaf extract of *Arctostaphylos uva-ursi* on 25 bacterial strains alone or in combination with nisin. Nisin is a polycyclic antibacterial peptide produced by the bacterium *Lactococcus lactis* which is used as a food preservative. The extract showed no inhibitory activity towards any of the bacteria as measured by spot tests on agar plates or by a microdilution method in which two-fold dilutions were added to the wells of a microtiter plate. On the other hand, nisin was inhibitory to many of the bacterial strains with MICs ranging from 31.3 to 500 µg/ml. When the *Uva ursi* extract at a concentration of 5 mg/ml was combined with the nisin, the MIC decreased for eight of the 14 Gram-positive samples. However it did not affect any of the Gram-negative samples.

Hafizović et al. [75] examined the effects of an herbal tea of *Arctostaphylos uva-ursi* prepared using samples from a pharmacy in Sarajevo, Bosnia and Herzegovina at full strength and double strength. Each was tested as an inhibitor of several strains of *Escherichia coli* (ATCC 14169, ATCC 12493), *Staphylococcus aureus* (ATCC 25923, ATCC 6538, ATCC 12493), *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (ATCC 10231), and *Pseudomonas aeruginosa* (ATCC 27853), The microbes were grown at 37oC in the wells of a microtiter plate containing 100 µl aliquots of sequential dilutions of the tea in tryptic soy broth for 24 hr. The amount of bacterial growth was then assessed by spreading the contents of the wells on Mueller Hinton agar plates. There was almost no inhibition of any of the samples.

Several laboratories have examined the effect of the compounds found in extracts of *Arctostaphylos uva-ursi* on the bacteria that can cause UTIs. Maslov et al. [76] compared the sensitivities of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6538, *Proteus vulgaris* NTCS 4636, *Pseudomonas aeruginosa* 27853, and the yeast *Candida albicans* ATCC 885/653 to β-arbutin, α-arbutin, and hydroquinone. In the hole-diffusion method, the microbes were spread on agar plates and a 0.003 mM solution of the test compound added to a well in the center of the plate. In the microdilution method, stock solutions at 0.2 M were serially diluted to determine the minimum inhibitory concentration. In the hole diffusion method, the two Gram-positive bacteria gave zones of inhibition of 20.0 to 21.0 mm for β-arbutin and α-arbutin and 23.0 for hydroquinone. The three Gram-negative bacteria and the yeast gave zones of inhibition of 14.0 to 18.0 mm with β-arbutin and 17.0 to 21.0 with α-arbutin. Hydroquinone gave more variable results. In the MIC assay, β-arbutin and α-arbutin were inhibitory to all of the microbes with most values falling between 0.00078 M and 0.00156 mM. The microorganisms were less sensitive to hydroquinone with MIC values two- or three-times higher and between 0.00313 M and 0.00125 mM.

Rauha et al. [77] tested the effects of a series of plant extracts and phenolic substances on 12 samples of bacteria including *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* DSM 2023. Although *Arctostaphylos uva-ursi* was not one of the plants tested, the compounds included gallic acid, (+) catechin, (+/-) catechin, flavone, quercetin, rutin, and kaempferol which have been found in some *Uva ursi* extracts. Inhibition was studied by a hole-diffusion assay in which the microorganisms were spread on agar plates and 500 µl of a 1 mg/ml solution in methanol added to wells punched in the agar with a cork borer. The zones of inhibition around the wells were measured after 3 days incubation at 25oC*. E. coli* ATCC 11775 was inhibited by the flavonoids quercetin, (+/-) catechin, and kaempferol. *Ps. aeruginosa* ATCC 9027 was inhibited by gallic acid, (+) catechin, (+/-) catechin, and kaempferol. *S. aureus* DSM 2023 was inhibited by flavone, quercetin, and kaempferol.

Shimizu et al. [78] isolated a phenolic compound called corilagen from an *Arctostaphylos uva-ursi* extract that had been found to inhibit methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria. The compound was purified to homogeneity and its structure determined by 3H-nuclear magnetic resonance (NMR) spectroscopy. When corilagen was tested alone against two MRSA strains, the minimum inhibitory concentration was found to be 128 µg/ml. However, when a lower concentration of 16 µg/ml was added along with a series of other antibiotics including the β-lactams oxacillin, cefmetazole, imipenem, and benzylpenicillin, the observed MIC was reduced. This was a very dramatic effect with some of the MRSA strain but less so for *S. aureus* strain 209P. In general, corilagen had no effect on the sensitivity of the bacteria to erythromycin, fosfomycin, ofloxacin, or streptomycin. When liquid cultures of *S. aureus* strain OM584 grown in Mueller Hinton broth were treated with 5 µg/ml oxacillin alone, there was a slight reduction in the growth rate over 24 hr. When the bacteria were treated with 16 µg/ml corilagen alone, there was also a slight reduction in growth rate. However, when the bacteria were treated with 5 µg/ml oxacillin along with 16 µg/ml corilagen, there was a dramatic decrease in the viable cell during the first five hours. This was indicative of a bactericidal effect for the combination of compounds.

More recently, Lewis et al. [79] examined the effects of several phenolic compounds found in extracts of *Uva ursi* including (+) catechin and epigallocatechin gallate on a uropathogenic isolate of *Escherichia coli* (UPEC). (+) Catechin had a very limited ability to inhibit the ability of the bacteria to adhere to and then invade bladder epithelial cells grown in culture. Epigallocatechin gallate, on the other hand, showed no effect on binding but caused a marked reduction in invasion of these cells as measured by the number of intracellular bacteria. The other compounds tested including caffeic acid, phenylethyl ester, and resveratrol were more effective as inhibitors of either binding or host invasion.

1. **INHIBITION OF UREASE ACTIVITY BY *UVA URSI* EXTRACTS**

Extracts of *Arctostaphylos uva-ursi* have been shown to inhibit the activity of several proteins that can contribute to the virulence of urinary tract pathogens. A key example is the enzyme urease (urea amidohydrolase, EC 3.5.1.5),which has become the focus of a large number of experimental studies[80-81]. Urease catalyzes the breakdown of urea to form two molecules of ammonia and one molecule of carbonic acid (**Fig 4**) [82]. The initial reaction in which one of the amino groups is removed is enzyme-catalyzed while the second reaction in which the other amino group is removed is spontaneous. Under physiological conditions, the carbonic acid picks up H+ to form bicarbonate (HCO3-) and the two ammonia molecules become protonated to form two ammonium ions (NH4+). The net decrease in the H+ concentration raises the pH. Alkalinization of urine as a result of urease activity can change the pH from less than 6 to more than 8.5.

 

urea carbonic acid

**Fig. 4**. Degradation of urea by urease

A major consequence of this change is the precipitation of salts and the formation of urinary infection stones in the kidneys and ureters (urolithiasis). It also can lead to the encrustation of catheters that may have been inserted into the urethra and bladder [83-84]. The precipitates are composed primarily of magnesium ammonium phosphate (MgNH4PO4.6H2O, struvite) and calcium phosphate carbonate (Ca10(PO4)6CO3, calcium apatite) [85]. Crystals of calcium oxalate or monoammonium urate can also be formed, particularly in the kidneys, although these depend more on diet and host metabolism than on urease activity [86]. Struvite infection stones are complex structures containing an organic matrix composed of glycosaminoglycans from the host mucosa and bacteria [87-88].

In addition to its effect on the pH of the urine, bacterial urease can directly damage host tissues, leading to inflammation and necrosis [89-90]. This is particularly a problem in the tubules of the kidney and associated with pyelonephritis caused by *Proteus mirabilis*. The ammonia formed by urease can react with the thioester linkage between cysteine and glutamate in the C3 protein of the complement system. This activates complement, leading to a respiratory burst and degranulation of polymorphonuclear leukocytes and monocytes. Although urine contains many other potential nitrogen sources, the ammonia derived from urea can be used for biosynthesis if these are limiting. The formation of ammonia by urease activity can thus also support the growth of other commensal bacteria in the urinary tract which are urease-negative.

All of the ureases from urinary tract pathogens are intracellular enzymes composed of multiple subunits which contain Ni2+ ions at the active site [81-82, 91-93]. They have a common catalytic mechanism and the three major subunits show significant sequence identity. The ureases differ, however, in the location of the genes that encode the subunits, the structure of the native enzyme, and the regulation of enzyme synthesis. Experiments have shown that the enzymes from *Staphylococcus saprophyticus* and *Proteus mirabilis* are essential virulence factors and that mutants lacking urease activity cannot cause a UTI [94-96]. Although *Escherichia coli* is the most common urinary tract pathogen, most clinical isolates are urease-negative.

In a survey of 14 over-the-counter plant extracts often used for the treatment of urinary tract infections, the urease from *Staphylococcus saprophyticus* was found to be susceptible to inhibition by the Nature’s Answer® *Uva ursi* extract [97]. Urease activity was measured with a cell-free extract of the bacteria using a standard colorimetric assay for ammonia. The activity was also inhibited by a green tea extract, but most of the others had no effect. The *Uva ursi* extract could also inhibit the urease activity in whole cells of *S. saprophyticus*. When bacterial cultures growing in an artificial urine medium were treated with 1/20 volume of this extract, the increase in pH which is normally observed did not occur and the viable cell count dropped to zero over 24 hr.

These studies were extended by examining the effects of four different commercial preparations of *Uva ursi* on both *S. saprophyticus* and *Proteus mirabilis* [98]. All of the preparations inhibited the urease activity in cell-free extracts *in vitro*. However, neither urease activity was inhibited by β-arbutin. On the other hand, both activities were inhibited by increasing amounts of hydroquinone. The enzyme from *S. saprophyticus* was inhibited about 20% by increasing amounts of 1,4-benzoquinone, but the one from *P. mirabilis* was completely inhibited by it. When the kinetics of hydroquinone inhibition were analyzed using Lineweaver-Burke plots, a mixed inhibition pattern was observed in both cases in which the *Vmax* gradually decreased and the *Km* gradually increased.

The Nature’s Answer® *Uva ursi* extract and pure hydroquinone also inhibited the urease activity in whole cells of both *S. saprophyticus* and *P. mirabilis*. Each bacterium was grown in an artificial urine medium to which 1% *Uva ursi* extract was added. For *S. saprophyticus*, there was a decrease in the growth rate, a delay in the increase in pH, and a reduction in the yield of cells over 9 hr. For *P. mirabilis*, there was an increase in the length of the lag phase but the pH rose rapidly and the final yield was the same. When *S. saprophyticus* was grown in an artificial urine medium to which 1 mg/ml hydroquinone was added, there was only a slight increase in the lag phase and the pH still increased. When *P. mirabilis* was grown in an artificial urine medium to which 50 µg/ml or 100 µg/ml hydroquinone was added, there was no effect. However, when similar experiments were done using cultures containing 20 µg/ml or 50 µg/ml 1,4-benzoquinone, there was a delay in the increase in pH at both concentrations; growth in 50 µg/ml 1,4-benzoquinone also showed a long delay. In related experiments, it was shown that (+) catechin and epigallocatechin gallate inhibited the urease activity in both cell extracts and whole cells of *S. saprophyticus* [99]. *Arctostaphylos uva-ursi* extracts have not been tested as inhibitors of the ureases from other urinary tract pathogens.

1. **INHIBITION OF BACTERIAL ADHERENCE AND BIOFILM FORMATION BY *UVA URSI* EXTRACTS**

Another key set of molecules that contribute to the virulence of urinary tract pathogens includes those which are involved in the adherence of the bacteria to host tissues and to the formation of biofilms. These include the proteins that form pili or fimbriae and curli attached to the cell surface, a wide range of adhesins found in the outer membrane or associated with the peptidoglycan layer of the cell wall, and polysaccharides that may be part of surface layers or capsules [100-103]. A number of standardized protocols have been developed for measuring bacterial adhesion to cultured mammalian cells[104-105]or for quantifying biofilm formation after growth of bacteria in the wells of plastic microtiter plates by staining with crystal violet [106-107]. In a survey of several over-the-counter herbal preparations, 0.5% Nature’s Answer® *Uva ursi* extract, 0.5% Sprouts green tea extract, and Wish Garden® UTI Herbal Supplement were the most effective in reducing biofilm formation by *Staphylococcus saprophyticus* ATCC 15305 during growth in either a rich medium or an artificial urine medium [108]. While (+) catechin, epigallocatechin gallate, gallic acid, hydroquinone, and arbutin caused some inhibition of growth, they did not individually affect biofilm formation as measured by the crystal violet staining method in these experiments.

Several derivatives of hydroquinone but not hydroquinone itself showed antibiofilm activity towards several methicillin-sensitive and methicillin-resistant strains of *S. aureus* [109]. On the other hand, aqueous extracts of *Arctostaphylos uva-ursi* and several other plants stimulated biofilm formation by *Escherichia -coli* BW25113 [110]. β-Arbutin has been combined with umbelliferon (150 mg or 300 mg) and N-acetyl-cysteine (150 mg or 400 mg) to form a mixture termed Itxasol© [111-112]. This formulation was found to reduce the attachment of a strain of *Enterococcus faecalis* isolated from a patient with a UTI to urinary catheters [113].

Several of the other compounds found in *Uva ursi* extracts have been found to affect biofilm formation. Subinhibitory concentrations of gallic acid isolated from the bacterium *Acinetobacter baumannii* were found to reduce biofilm formation by uropathogenic strains of *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans* by 30% to 70% [114]. Catechin isolated from the leaves of *Canarium patentinervium* Miq, a rare tropical plant used for wound healing in Malaysia, was tested as an inhibitor of three reference strains of *Escherichia coli* and 15 clinical *E. coli* isolates from patients with UTIs [115]. The material showed good antibacterial activity alone and in combination with various antibiotics. It also reduced biofilm formation by an average of 82% for the clinical isolates as measured in the wells of microtiter plates by the crystal violet assay after growth in Mueller-Hinton broth at 37oC for 24 hr. The clinical isolates were tested for expression of the gene *acrA* by real-time polymerase chain reactions (PCR). This gene together with *acrB* and *tolC* encode proteins that make up an efflux pump which has been implicated in the removal of antibiotics and biofilm formation [116]. The gene *acrA* was expressed in nine of the *E. coli* clinical isolates and the level of expression was reduced by catechin at a subinhibitory concentration of 0.5 mg/ml. In a separate study, catechin alone or a combination of catechin, protocatechuic acid, and vanillic acid was found to be inhibitory to a uropathogenic strain of *E. coli* (ATCC 700416) [117]. Catechin alone and the combination of catechin, protocatechuic acid, and vanillic acid also inhibited the formation of biofilms on silicone catheters as well as in the wells of plastic microtiter plates as measured by staining with crystal violet.

An extract of *Alnus japonica*, a Korean medicinal tree, was found to inhibit the formation of biofilms by the methicillin sensitive strains of *Staphylococcus aureus* ATCC 6538 and ATCC 25923, and by the methicillin-resistant *Staphylococcus aureus* (MRSA) isolate BAA-1707 [118]. Biofilm formation was again measured by crystal violet staining after growth of the bacteria in Luria-Bertani medium at 37oC in the wells of polystyrene plates. The key metabolites contributing to the anti-biofilm activity were identified by high performance liquid chromatography (HPLC) as quercetin and tannic acid, which inhibited biofilm formation in a dose-dependent fashion. Transcriptional analysis by a quantitative reverse transcription polymerase chain reaction (qRT-PCR) indicated that the *A. japonica* extract at a concentration of 20 µg/ml and purified quercetin and tannic acid reduced expression of a number of genes including *agrA, sigB, sarA, icaA,*and *icaD*. The genes *agrA, sigB,* and *sarA*  are key regulators in *S. aureus*, and *icaA* and *icaD* are involved in the formation of a key intercellular adhesin [119].

Quercetin also also found to inhibit biofilm formation by several antibiotic-resistant strains of *S. saprophyticus* [120]. By contrast, quercetin was found to decrease swarming behavior and increase biofilm formation by a wild-type strain of *Proteus mirabilis* HI4320 in a dose-dependent fashion [121]. These responses were associated with decreased expression of the genes *flaA, flhD, mrpJ, umoC, rcsB,* and *rcsD*, which are involved in flagella expression, and *glnA, hisG*, and *speB*, which are involved in polyamine synthesis. Interestingly, expression of the outer membrane protein gene *ompF* increased slightly. Quercetin in combination with the antibiotics tobramycin and amikacin inhibited biofilm formation by *Pseudomonas aeruginosa* strains obtained from patients with catheter-associated urinary tract infections [122].

Flavonoids and polyphenols isolated from *Morus* sp. leaves in Egypt were found to inhibit biofilm formation by clinical isolates of *Enterococcus faecalis* from patients with UTIs [123]. The effective compounds included kaempferol and quercetin as well their glycoside derivatives. Metabolites derived from *Vaccinium macrocarpon* (cranberry) capsules were recovered from the urine of human volunteers and tested as inhibitors of biofilm formation by *Candida albicans* [124]. A large number of compounds were identified including polyphenols, anthocyanins, and flavonoids. Some of the fractions reduced biofilm formation. In a more recent study, the flavonoid rutin was found to inhibit host cell adhesion and biofilm formation by *Pseudomonas aeruginosa* MMA83 and methicillin-resistant *Staphylococcus aureus* IBRS MRSA 011 [125]. Host cell adhesion was measured by binding of the bacteria to HeLa cells grown in tissue culture plates. Biofilm formation was assessed by binding of the bacteria to catheters and by binding of the dye Congo Red to the microbes. Rutin has been found in extracts of *Uva ursi* [39].

1. **ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *UVA URSI* EXTRACTS**

In addition to its ability to inhibit the enzyme urease and to reduce bacterial adhesion and biofilm formation, *Uva ursi* extracts have antioxidant and anti-inflammatory activities. The presence of pathogenic microbes in the bladder and other parts of the renal system leads to the activation of a complex set of innate defense mechanisms [126-129]. These defenses include the formation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, and superoxide anions which can kill the pathogen. They also involve the production and release of a large number of cytokines and chemokines that together lead to an inflammatory response. The activation of these defense mechanisms varies with the specific pathogen and site of infection [130]. The severity of an infection depends on the transcription of specific genes in both the bacteria and the host [131]. While host defense mechanisms can help eliminate the pathogen and are usually well-regulated and self-limiting, their activity and persistence can lead to host cell damage. Plant extracts like that from *Arctostaphylos uva-ursi* contain a variety of chemicals that can act as antioxidants. They may remove reactive oxygen species and may moderate host inflammation. The plant extracts can thus help minimize the clinical consequences of a UTI and give the host time to remove the pathogen.

An extract of *Arctostaphylos uva-ursi* leaves was prepared using a 50/50 (v/v) mixture of ethanol and water, and after filtration through Whatman filter paper, tested for its total phenolic content and antioxidant activity [132]. The total content was 102.11 mg of gallic acid equivalents per gram dry weight. Among the polyphenols detected were catechin, epigallocatechin gallate, and epigallocatechin. The antioxidation activity as measured in Trolox equivalents (TE) using the ABTS colorimetric method [133] was 90.42 mmol of TE per gram dry weight. Antioxidant activity towards free methoxy (CH3O\*) radicals was also demonstrated using electron paramagnetic resonance spectroscopy [134]. Another extract of bearberry containing 20% arbutin with a total phenolic compound concentration of 57.4 gallic acid equivalents/100 g was shown to reduce lipid oxidation in raw pork patties over a period of 12 days in a dose dependent fashion [135].

Samples of *Arctostaphylos uva-ursi* were collected across a range of heathland and forest habitats in Southern Europe and the leaves extracted with 70% ethanol/30% water [136]. Analysis of their chemical composition by high performance liquid chromatography indicated that they varied in arbutin content and in various phenolic compounds and flavonoids. The extracts were tested for antioxidant activity using the ABTS and DPPH [137] scavenging assays and for reducing power with Fe(III) chloride and for ferrous ion chelation ability. All of the extracts gave positive results which were positively correlated with their methyl arbutin and total phenolic concentrations. More recently a 70% ethanol/30% water extract of *Arctostaphylos uva-ursi* was shown to have antioxidant activity as measured by the DPPH anti-radical assay and scavenging assays for hydroxyl radical, superoxide radicals, and nitrosyl radicals [138]. Although not all compounds in an *Uva ursi* extract have been tested as antioxidants, many of those which have been isolated from other plants in the family Ericaceae such as cranberry have been shown to have this activity [139].

An ethanolic extract of *Arctostaphylos uva-ursi* leaves along with those of 98 other plants was tested for anti-inflammatory activity using TLR-4 based laboratory assays [140]. In one assay, a HeLa-TLR4 transfected reporter cell line was activated by lipopolysaccharide LPS-EB from *E. coli* O111:B4 in the presence of varying amounts of extract. The response was measured with luciferase compared to the 70% ethanol vehicle. In another assay, the myeloid THP-1 cell line TIB-202 was activated by LPS-EB and TLR4 stimulation measured by an enzyme-linked immunosorbent assay (ELISA) for IL-8. Cell viability in these experiments was measured with the Alamar Blue reagent. The extract of *Uva ursi* gave a concentration-dependent inhibitory response in both assays and only reduced viability at very high concentrations. In earlier experiments with a different test system, arbutin was found to inhibit nitric oxide (NO) production by lipopolysaccharide-stimulated mouse microglial macrophages [141]. It reduced expression of the genes for the pro-inflammatory cytokines interleukin-1β, TNF-α, COX-2, IL-6, and CXCL1 as measured by real-time polymerase chain reactions (PCR). Arbutin also reduced formation of the protein Ninj1 involved in cell adhesion. Although other compounds found in *Uva ursi* extracts were not tested for anti-inflammatory activity in these experiments, other studies have indicated that quercetin and epigallocatechin gallate can also interfere with TLR4 signaling pathways and reduce inflammation [142-143].

1. **INHIBITION OF OTHER HOST FUNCTIONS BY *UVA URSI* EXTRACTS**

Another enzyme that can be inhibited by extracts of *Arctostaphylos uva-ursi* is tyrosinase (TYR) or polyphenol oxidase (PPO). This copper-containing protein catalyzes the oxidation of L-tyrosine and L-dopamine and so plays a key role in the formation of melanin pigments [144]. It is able to oxidize a wide range of monophenolic and *o*-diphenolic compounds, leading to the formation of *o*-quinone. Many plant extracts contain phenolic compounds that can act as competitive, noncompetitive, or uncompetitive inhibitors of this enzyme [145]. Dried leaves and shoots of *Uva ursi* collected in Russia were treated with water or with various water/ethanol mixtures in a boiling water bath and the extract dried [138]. The residues were dissolved in dimethyl sulfoxide (DMSO) and serially diluted. They were tested for inhibitory activity in 96 well microtiter plates using purified enzyme and 2 mM L-tyrosine as the substrate. Absorbance was directly measured at 492 nm. All of the samples showed good anti-tyrosinase activity, comparable to that of Kojic acid, a standard inhibitor. In other experiments, polyphenol oxidase in combination with the enzyme asparaginase or a low-frequency laser was found to reduce the adhesion of *E. coli* strains obtained from patients with urinary tract diseases to buccal epithelial cells or erythrocytes [146-147]. This was believed to be due to interference with the binding of type I or type P pili to the mammalian cells. However, it is not yet clear how addition of a PPO or tyrosinase inhibitor might reduce the effects of a urinary tract infection.

Several isoforms of the enzyme UDP-glucuronosyl transferase have been found in human liver and intestinal cells [148]. These enzymes are believed to be involved in the metabolism of various endogenous metabolites including bilirubin, steroid hormones, thyroid hormones, and vitamins as well as a number of important oral drugs. An *Uva ursi* extract was found to inhibit the activity of several UGT isoforms as measured by the transfer of glucuronic acid or glucuronamide to β-estradiol *in vitro* [149]. The reaction was also inhibited by (+) catechin, gallotannin, myricetin, and several other compounds often found in *Uva ursi* preparations. Although this enzyme has not been identified in any of the urinary tract pathogens, the inhibition of host enzymes by *Uva ursi* extracts or their components may lead to unintended side effects.

An extract of dried leaves of *Arctostaphylos uva-ursi* and its components arbutin and hydroquinone were also tested for their effects on human bladder T24 cells [150]. Proteins were extracted and analyzed by high performance liquid chromatography and MALDI-TOF mass spectrometry. Compared to untreated cells, 17 proteins were upregulated following exposure to 320 µg/ml of hydroquinone for 2 hr. These included the stress-signaling proteins 14-3-3 protein epsilon and 14-3-3 protein zeta/delta, the heat shock proteins HSP 90-alpha and HSP 90-beta, the oxidative stress response protein peroxiredoxin-1, as well as several proteins involved in central carbon metabolism and several eukaryotic translation factors. These experiments were consistent with an observed dose-dependent cytotoxicity for hydroquinone. In a related study, arbutin was found to decrease cell proliferation by TCCSUP human bladder cancer cells [151]. This was shown to be due to inactivation of an extracellular signal-regulated kinase and up-regulation of the regulatory protein p21.

1. **PRELIMINARY ANIMAL AND HUMAN STUDIES WITH *UVA URSI* EXTRACTS**

There have been a number of studies examining the effects of extracts of *Arctostaphylos uva-ursi* and its components on animals as a prelude to clinical investigations with humans. Female Wistar rats were given several plant infusions including one made with *Uva ursi* as a replacement for tap water along with the standard lab diet [152]. After 12 days, 24 hr urine samples were collected and analyzed for total volume, pH, calcium, citrate, phosphate, or creatinine content. There were no significant differences compared to the control, which was taken to mean that none of the plant extracts can directly affect urolithiasis and kidney stone formation. In a related study, male Sprague Dawley rats were given a single dose (50 mg/kg body weight) of 16.6% extract of *Uva ursi* leaves in a hypotonic NaCl solution [153]. Urine flow was measured each hour for 8 hr and again after 24 hr. The concentrations of Na+ and K+ ions were determined for the 8 hr and 24 hr samples. With the *Uva ursi* extract, there was an increase in urine flow during the 8 hr test period, which remained above the control at 24 hr. There were no significant differences in the electrolyte concentrations over the 24-hr test period.

In a later study designed to evaluate the safety of *Arctostaphylos uva-ursi*, rabbits of both sexes were given oral injections of an extract of leaves (25 mg/kg body weight) at 24 hr intervals over a period of 90 days [154]. The animals were sacrificed and blood removed by cardiac puncture for analysis. Gender-based differences were observed in blood parameters, kidney function parameters, cardiac enzymes, liver enzymes, or lipids. Specifically, the urea concentrations were lower among females but higher among males compared to the controls. Lactate dehydrogenase (LDH) levels were higher in both males and females, but creatine phosphokinase (CPK) levels were lower in females but higher in males. Cholesterol, triglycerides, low density lipoproteins (LDL), and very low-density lipoproteins (VLDL) were higher in females compared to the controls. On the other hand, triglycerides and VLDL concentrations were lower in males and the high-density lipoproteins (HDL) concentration was higher compared to the control. Serum glutamate oxaloacetate transferase (SGOT) and serum glutamate pyruvate transferase (SGPT) levels were greatly elevated in males but not in females compared to the controls while alkaline phosphatase was decreased. However, histological analysis indicated there was no significant pathology in the heart, stomach, liver, or kidney tissues. The effects on serum parameters was attributed to the anti-inflammatory effects of compounds in the *Uva ursi* extract, but the gender differences were not explained and the authors concluded that such extracts were safe for use in human medicine.

Several groups have examined the metabolism of *Uva ursi* extracts in humans and animals. In one study, 16 healthy human volunteers were given a dry extract of bearberry leaves as film coated tablets containing 472 mg of extract with the equivalent of 105 mg of arbutin or as 100 ml of an aqueous solution containing 945 mg of extract with the equivalent of 210 mg of arbutin [155]. Urine was collected prior to drug administration and during the 12-24 hr and 24-36 hr periods afterwards. The urine was analyzed for hydroquinone, hydroquinone-glucuronamide, and hydroquinone sulfate using high performance liquid chromatography (HPLC). The mean amounts of total hydroquinone, hydroquinone-glucuronamide, and hydroquinone sulfate did not differ for the two treatments. Most of the excretion occurred in the first 4 hr and consisted primarily of hydroquinone-glucuronamide. No urinary excretion of hydroquinone could be detected after 24 hr. There was some intersubject variability but no unexplained pathological laboratory results.

In another study, three volunteers were given 150 mg of arbutin in the form of 150 ml of an aqueous bearberry extract [156]. Urine was collected before the treatment and at 2 hr intervals for 24 hr after the treatment. The urine was again analyzed by HPLC and revealed the excretion of hydroquinone glucuronamide and hydroquinone sulfate. About 50% of the applied arbutin was excreted after 4 hr and 79% to 99% after 24 hr. Only one of the test subjects excreted any hydroquinone. In a different study, 100 female rats were given 3 ml of 0.3 mg/ml or a 17 mg/ml solution of arbutin by gavage (direct feeding into the stomach through a tube) [157]. Blood samples (1 ml) were collected from the posterior orbital venous plexus at times up to 36 hr and centrifuged to separate the plasma. The plasma was analyzed for arbutin and hydroquinone by HPLC. The results indicated that the concentration of arbutin with either test solution reached a peak at 4 hr. The 0.3 mg/ml solution had a half-life of 43.0 hr and the 17 mg/ml solution had a half-life of 12.7 hr but no hydroquinone itself could be detected in the plasma.

1. **CONTROLLED HUMAN CLINICAL TRIALS WITH *UVA URSI* EXTRACTS**

Several studies have examined the use of *Arctostaphylos uva-ursi* extracts as an alternative or adjunct to standard antibiotic therapy for the treatment of human urinary tract infections. In one detailed investigation designated ATAFUTI, patients with a history of uncomplicated UTIs were matched and divided into four groups [158-159]. Those in Group 1 (n = 102) were given a daily dose of 1600 mg of *Uva ursi*extract containing 20% arbutin divided into three capsules along with advice to take 1200 mg of ibuprofen if necessary; those in Group 2 (n = 86) were given an *Uva ursi* placebo along with advice to take 1200 mg of ibuprofen as necessary; those in Group 3 (n = 97) were given the *Uva ursi* extract along with an ibuprofen placebo; and those in Group 4 (n = 97) were given both *Uva ursi* and ibuprofen placebos. The patients were asked to keep daily diaries in which they recorded urinary symptoms including burning, urgency, day time frequency and night time frequency on a 6-point scale with 1 = no problem and 6 = most severe after 2 to 4 days. An extensive statistical analysis of the patient records indicated there was no effect of either the *Uva ursi* extract or the ibuprofen on the severity of the symptoms.

In a separate detailed study (REGATTA) [160-161], patients with uncomplicated UTIs were treated either with an *Uva ursi* extract (n = 207) or fosfomycin (n = 191). The *Uva ursi* treatment involved 3 x 2 tablets containing 105 of arbutin) for five days while the fosfomycin treatment involved a single dose of 3 g or a placebo dissolved in orange juice. Participants completed a daily diary which included an 8-item symptom questionnaire for at least 7 days until the symptoms were resolved. A more detailed follow up occurred on day 28 with another follow-up phone call at three months. The number of all antibiotic courses from days 0 to 28 was reduced by 61.8% for the group treated with *Uva ursi* compared to the fosfomycin-treated group. On the other hand, the total burden of symptoms decreased more slowly from days 0 to 7 for the group treated with *Uva ursi* compared to the fosfomycin-treated group. Eight women in the *Uva ursi*-treated group developed pyelonephritis compared to only two in the fosfomycin-treated group. A new clinical trial (BRUMI) has been proposed but the results have not yet been reported [162]. Further large-scale clinical trials using *Uva ursi* extracts are clearly warranted.

Another study involved a retrospective review of the charts of 103 individuals who had undergone 82 different treatment regimens at naturopathic centers for UTIs [163]. Of these patients, 52 (50.5%) were treated with botanical products and 46 (44.7%) were treated with antibiotics. There were 43 patients who provided follow-up data, of which 65% reported non-pharmacological treatment success. Two of the treatment regimens involved *Uva ursi* leaf among several other constituents, one of which gave a statistically significant success rate.

1. **FUTURE PROSPECTS**

There is a growing interest in the use of natural plant-based products for the treatment of urinary tract infections as evidenced by the large number of recently published reviews and proposals [164-170]. Extracts from bearberry (*Arctostaphylos uva-ursi*) may be useful in this regard. However, as this review indicates, the laboratory-based evidence supporting its potential value is much stronger than the current clinical evidence. Extracts of *Uva ursi* and their components can inhibit the growth of both Gram-positive and Gram-negative uropathogens, particularly at high concentrations. They can also inhibit urease activity and decrease biofilm formation. However, they may also alter other host functions and so can have unintended side effects.

A major issue in the use of *Uva ursi* extracts is the potential toxicity of the hydroquinone that may be formed by the degradation of arbutin, a key component of these extracts. Hydroquinone has been found to be both mutagenic and carcinogenic *in vitro* and *in vivo*, although the risks to humans appear to be low [171}. A risk assessment of free hydroquinone derived from bearberry leaves indicated that human therapeutic doses were within acceptable safety limits [172]. One solution to this potential problem might be to use the tools of molecular biology to produce genetically-modified forms of *Arctostaphylos uva-ursi* which have little or no arbutin but still contain the polyphenols and flavonoids that an inhibit urease activity or limit biofilm formation. Because the use of a GMO may impact public acceptance of the modified *Uva ursi* extracts, an alternative solution would be to start with isolates of *Arctostaphylos uva-ursi* which naturally produce lower amounts of arbutin in the development of new products [50,65].

A different approach may be to combine extracts of *Uva ursi* with other chemicals that can also affect urinary tract pathogens. A mixture called Itxasol© composed of β-arbutin, umbelliferon, and n-acetyl cysteine has been found to be useful in treating UTIs [111]. It may prove effective to combine *Uva ursi* with D-mannose [173-174], cranberry (*Vaccinium macrocarpon*) extracts [175-176], or other medicinal herbal extracts that have been described [177-178]. A dry extract from *Uva ursi* was combined with D-mannose and extracts of several other plants and found to reduce the incidence of recurrent cystitis [179]. This has also been done with cranberry extract in the form of Crano-cure [180] and with various European [181-182], Chinese [183], and Indian [184] herbal preparations. These have been found to be useful in the treatment of urinary tract infections. Other combinations are possible and should be explored. It would be particularly important to ensure that these combinations have low toxicity risks.

1. **CONCLUSION**

Extracts from *Arctostaphylos uva-ursi* may be useful for the herbal treatment of certain urinary tract infections. They can reduce the growth of most uropathogenic microorganisms and can inhibit key virulence factors such as the enzyme urease and the formation of biofilms. They appear to be safe and can be produced and sold around the world at relatively low cost. However, the clinical data supporting their widespread medical use is still quite limited. Further randomized controlled trials comparing them to standard antibiotics and to other herbal preparations should be carried out using patients of varying gender and age with both uncomplicated community-acquired infections and complicated catheter-associated infections.

**DISCLAIMER**

Author hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc. and text-to-image generators have been used during the writing or editing of this manuscript.

**REFERENCES**

1. Yarnell, E. (2002). Botanical medicines for the urinary tract. *World J Urol* *20*(5), 285-293. https//:doi: 10.1007/s00345-002-0293-0.
2. Sihra, N., Goodman, A., Zakri, R., Sahai, A., & Malde, S. (2018). Nonantibiotic prevention and treatment of recurrent urinary tract infection. *Nat Rev Urol 15*(12), 750-776. https://doi: 10.1038/s41585-018-0106-x.
3. Das, S. (2020). Natural therapeutics for urinary tract infections – a review. *Futur J Pharm Sci 6*(1), 64. https://doi: 10.1186/s43094-020-00086-2.
4. Badiger A. S., Maruthi, K. R., Bajpe, S. N., Ramu, R. & Jaydev K. (2021). Urinary tract infection – a review on its prevalence and recent advances. *J Pharm Res Int 33*(46B), 582-592. https:// doe: 10.9734/JPRI/2021/v33i46B32980.
5. Tache, A. M., Dinu, L. D., & Vamanu, E. (2022). Novel insights on plant extracts to prevent and treat recurrent urinary tract infections. *Appl Sci* *12*, 2635. <https://doi>: 10.3390/app12052635.
6. Narayanan, A. S., Raja, S. S. S., Ponmurugan, K., Kandekar, S. C., Natarajaseenivasan, K., Maripandi, A. et al. (2011). Antibacterial activity of selected medicinal plants again multiple antibiotic resistant uropathogens: a study from Kolli Hills, Tamil Nadu, India. *Benef Microbes* *2*(3), 264-243. https://doi: 10.3920/BM2010.0033.
7. Flower, A., Wang, L. Q., Lewith, G., Liu, J. P., & Li, Q. (2015). Chinese herbal medicine for treating recurrent urinary tract infections in women. *Cochrane Database Syst Rev 2015*(6), CD010446. https://doi: 10.1002/14651858.CD010446.pub2.
8. Cock, I., Mavuso, N., & Van Vuuren, S. (2021). A review of plant-based therapies for the treatment of urinary tract infections in traditional Southern Africa medicine. *Evid Based Complement Alternat Med 2021*, 7341124. https://doi: 10.1155/2021/7341124.
9. Ferdosh, S. (2023). Ethnobotanical review of selected medicinal plants in Guam for the treatment of urinary tract ailments and their pharmacological properties. *Sci Pharm 91*, 45. https://doi: 10.3390/scipharm91030043.
10. Olas, B., Różański, W., Urbańska, K., Slawińska, N., & Bryś, M. (2024). New light on plants and their chemical compounds used in Polish folk medicine to treat urinary diseases. *Pharmaceuticals (Basel) 17*(4), 435. https://doi: 10.3390/ph17040435.
11. Mancuso, G., Midiri, A., Gerace, E., Marra, M., Zummo, S., & Biondo, C. (2023). Urinary tract infections: the current scenario and future prospects. *Pathogens 12*(4), 623. https://doi: 10.3390/pathogens12040623.
12. McCallin, S., Kessler, T. M., & Leitner, L. (2023). Management of uncomplicated urinary tract infection in the post-antibiotic era: select non-antibiotic approaches. *Clin Microbiol Infect 29*(10), 1267-1271. https://doi: 10.1016/j.cmi.2023.06.001.
13. Pothoven, R. (2023). Management of urinary tract infections in the era of antimicrobial resistance. *Drug Target Insights 17*, 126-137. https://doi: 10.33393/dti.2023.2660.
14. Simoni, A., Schwartz, L,, Junquera, G. Y., Ching, C. B, & Spencer, J. D. (2024). Current and emerging strategies to curb antibiotic-resistant urinary tract infections. *Nat Rev Urol* *21*(12), 707-722. https://doi: 10.1038/s41585-024-00877-9.
15. Öztürk, R., & Murt, A. (2020). Epidemiology of urological infections: a global world burden. *World J Urol 38*(11), 2669-2679. https://doi: 10.1007/s00345-019-03071-4.
16. Bhat, R. G., Katy, T. A., & Place, P. C. (2011). Pediatric urinary tract infections. *Emerg Med Clin North Am 29*(3):637-653. https://doi: 10.1016/j.emc.2011.04.004.

17. Tullus, K., & Shaikh, N. Urinary tract infections in children. (2020). *Lancet 395*(10237), 1659-1668. https://doi: 10.1016/S0140-6736(20)30676-0.

18. Korbel, L., Howell, M., & Spencer, J. D. (2017).The clinical diagnosis and management of urinary tract infections in children and adolescents. *Paediatr Int Child Health 37*(4), 273-279. https:// doi: 10.1080/20469047.2017.1382046.

19. Cortes-Penfield, N. W., Trautner, B. W., & Jump, R. L. P. (2017). Urinary tract infection and asymptomatic bacteriuria in older adults. *Infect Dis Clin North Am 31*(4), 673-688. https://doi: 10.1016/j.idc.2017.07.002.

20. Kolman, K. B. (2019). Cystitis and pyelonephritis: diagnosis, treatment, and prevention. *Prim Care 46*(2), 191-202. https:// doi: 10.1016/j.pop.2019.01.001.

21. Guay, D. R. P. (2008). Contemporary management of uncomplicated urinary tract infections. *Drugs 68*(9), 1169-1205. https://doi: 10.2165/00003495-200868090-00002.

22. Hooton, T. M. (2012). Clinical Practice. Uncomplicated urinary tract infection. *N Engl J Med 366*(11), 1028-1037. https://doi: 10.1056/NEJMcp1104429.

23. Flores-Mireles, A., Hreha, T. N., & Hunstad, D. A. (2019). Pathophysiology, treatment, and prevention of catheter-associated urinary tract infection. *Top Spinal Cord Inj Rehabil 25*(3), 228-240. https://doi: 10.1310/sci2503-228.

24. Clarke, K., Hall, C. L., Wiley, Z., Tejedor, S. C., Kim, J. S., Reif, L. et al. (2020). Catheter-associated urinary tract infections in adults: diagnosis, treatment, and prevention. *J Hosp Med* *15*(9)*,* 552-556. https:// doi: 10.12788/jhm.3292.

25. Kellum, J. A., Romagnani, P., Ashuntantang, G., Ronco, C., Zarbock, A., & Angers, H. J. (2021). Acute kidney injury. *Nature Rev Dis Primers 7*(1), 52. https://doi: 10.1038/s41572-021-00284-z.

26. Kalantar-Zadeh, K., Jafar, T. H., Nitsch, D., Neuen, B. L., & Perkovic, V. (2021). Chronic kidney disease. *Lancet 398*(10302), 786-802. https://doi: 10.1016/S0140-6736(21)00519-5.

27. Guglietta, A. (2017). Recurrent urinary tract infections in women: risk factors, etiology, pathogenesis and prophylaxis. *Future Microbiol 12*, 239-246. https://doi: 10.2217/fmb-2016-0145.

28. Peck, J., & Shepherd, J. P. (2021). Recurrent urinary tract infections: diagnosis, treatment, and prevention. *Obstet Gynecol Clin North Am. 48*(3), 501-513. https://doi: 10.1016/j.ogc.2021.05.005.

29. Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Rev Microbiol 13*(5), 269-284. https://doi: 10.1038/nrmicro3432.

30. Wagenlehner, F. M. E., Bjerkland, T. E., Cai, T., Koves, B., Kranz, J., Pilatz, A. et al. (2020). Epidemiology, definition and treatment of complicated urinary tract infections. *Nature Rev Urol 17*(10), 586-600. https://doi: 10.1038/s41585-020-0362-4.

31. Tandogdu, Z., & Wagenlehner, F. M. E.(2016). Global epidemiology of urinary tract infections. *Curr Opin Infect Dis 29*(1), 73-79. https:// doi: 10.1097/QCO.0000000000000228.

32. Anonymous (2002). USDA/NRCS Plant Fact Sheet for BEARBERRY *Arctostaphylos uva-ursi* (L.) Spreng. <https://plants.usda.gov/DocumentLibrary/factsheet/pdf/fs_aruv.pdf>.

33. Anonymous (2024).Wikipedia entry for *Arctostaphylos uva-ursi*.

<https://en.wikipedia.org/wiki/Arctostaphylos_uva-ursi>.

34. Anonymous (2025) WebMD entry for Uva Ursi – uses, side effects and more. <https://www.webmd.com/vitamins/ai/ingredientmono-350/uva-ursi>.

35. Anonymous (2025( Drugs.com entry for Uva Ursi. <https://www.drugs.com/npc/uva-ursi.html>.

36. Anonymous (2025) Mount Sinai entry for Uva ursi. <https://www.mountsinai.org/health-library/herb/uva-ursi>.

37. Anonymous (2025) Medicinenet entry for What is uva ursi used for and can you take it every day? <https://www.medicinenet.com/what_is_uva_ursi_used_for_can_take_it_every_day/article.htm>.

38. Anonymous (2025) Amazon.com entry for Uva Ursi. <https://www.amazon.com/s?k=uva+ursi&i=hpc&crid=H56ZEVVGJ7ZE&sprefix=uva+ursi%2Chpc%2C197&ref=nb_sb_noss_1>.

39. Shamilov, A. A., Bubenchikova, V. N., Chernikov, M. V., Pozdnyakov, D. I., Garsiya, E. R., & Larsky, M. V. (2021) Bearberry (*Arctostaphylos uva-ursi* (L.) Spreng.): chemcial content and pharmacological activity. *J Excipients and Food Chem* *12*(3), 49-66.

40. Gallo, F. R., Multari, G., Pagliuca, G., Panusa, A., Palazzino, G., Giambenedetti, M. et al.(2013). Bearberry identification by a multidisciplinary study on commercial raw materials*. Nat Prod Res* 27(8), 735-742. https://doi: 10.1080/14786419.2012.696253.

41. Panusa, A., Petrucci, R., Marrosu, G., Multari, G., & Gallo, F. R. (2015). UHPLC-PDA-ESI-TOF/MS metabolic profiling of *Arctostaphylos pungens* and *Arctostaphylos uva-ursi*. A comparative study of phenolic compounds from leaf methanolic extracts. *Phytochemistry 115*, 79-88. https://doi: 10.1016/j.phytochem.2015.01.002.

42, Bai, J., Zhang, Y., Tang, C., Hou, Y., Ai, X., Chen, X. et al. (2021). Gallic acid: pharmacological activities and molecular mechanisms involved in inflammation-related diseases. *Biomed and Pharmocother 133*, 110985. https:// doi: 10.1016/j.biopha.2020.110985.

43. Georgiou, N., Kakava, M. G., Routsi, E. A., Petsas, E., Stavridis, N., Freris, C. et al. (2023). Quercetin: a potential polydynamic drug. *Molecules 28*(24), 8141. https://doi: 10.3390/molecules28248141.

44. Agraharam, G., Girigoswami, A., & Girigoswami, K. (2022). Myricetin: a multifunctional flavonol in biomedicine. *Curr Pharmacol Rep 8*(1), 48-61. https://doi: 10.1007/s40495-021-00269-2.

45. Periferakis, A., Periferakis, K., Badarau, I. A., Petran, E. M., Popa, D. C., Caruntu, A. et al. (2022). Kaempferol: antimicrobial properties, sources, clinical, and traditional applications. *Int J Molec Sci 23*{23), 15054. https://doi: 10.3390/ijms232315054.

46. Baranwal, A., Aggarwal, P., Rai, A., & Kumar, N. (2022). Pharmacological actions and underlying mechanisms of catechin: a review. *Mini Rev Med Chem 22*(5), 821-833. https://doi: 10.2174/1389557521666210902162120.

47. Almatroodi, S. A., Almatroudi, A., Khan, A. A., Alhumaydhi, F. A,, Alsahli, A. M., & Rahmani, A. H. (2020). Potential therapeutic targets of epigallocatechin gallate (EGCG), the most abundant catechin in green tea and its role in the therapy of various types of cancer. *Molecules 25*, 3146. https:// doi: 10.3390/molecules25143146.

48. Ştefănescu, B. E., Szabo, K., Mocan, A., & Crişan, G. (2019). Phenolic compounds from five Ericaceae species leaves and their related bioavailability and health benefits. *Molecules 24*(11), 2046. https:// doi: 10.3390/molecules24112046.

49. Song, X. C., Canellas, E., Dreolin, N., Nerin, C., & Goshawk, J. (2021). Discovery and characterization of phenolic compounds in bearberry (*Arctostaphylos uva-ursi*) leaves using liquid chromatography-ion mobility-high resolution mass spectrometry. *J Agri Food Chem 69*(37), 10856-10868. https://doi: 10.1021/acs.jafc.1c02845.

50. Asensio, E., Vitales, D., Pérez, I., Peralba, L., Viruel, J., Montaner, C. et al. (2020). Phenolic compounds content and genetic diversity at population level across natural distribution range of bearberry (*Arctostaphylos uva-ursi*, Ericaceae) in the Iberian Peninsula. *Plants (Basel) 9*(9), 1250. https:// doi: 10.3390/plants9091250.

51. Deutch, C. E. (2022). Analysis of phenolic compound concentrations in over-the-counter herbal preparations. *Amer Biol Teacher 84*(3), 163-166. https://doi: 10.1525/abt.2022,84,3,163.

52. Upton, R. (2008) Uva-ursi Leaf. *Arctostaphylos uva-ursi* (L.) Spreng. Standard of Analysis, Quality Control and Therapeutics; American Herbal Pharmacopoeia: Scotts Valley, CA, USA.

53. Migas, P., & Krauze-Baranowska, M. (2015). The significance of arbutin and its derivatives in therapy and cosmetics. *Phytochem Lett 13*, 35–40. https://doi: 10.1016/j.phytol.2015.05.015.

54. Saeedi, M., Khezri, K., Zakaryaei, A. S., & Mohammadamini, H. A. (2015). A comprehensive review of the therapeutic potential of α-arbutin. *Phytother Res 35*(8), 4136-4154. https://doi: 10.1002/ptr.7076.

55. Yamaha, T., & Cardini, C. E. (1960). The biosynthesis of plant glycosides. I. Monoglucosides. *Arch Biochem Biophys 86*(1), 127-132. https://doi: 10.1016/0003-9861(60)90379-9.

56. Hefner, T., Arend, J., Warzecha, H., Siems, K., & Stöckigt, J. (2002). Arbutin synthase, a novel member of the NRD1β glycosyltransferase family, is a unique multifunctional enzyme converting various natural products and xenobiotics. *Bioorg Med Chem 10*(6), 1731-1741. https:// doi: 10.1016/s0968-0896(02)00029-9.

57. Zhou, H., Zhao, J., Li, A., & Reetz, M. T. (2019). Chemical and biocatalytic routes to arbutin. *Molecules 24*(18), 3303. https:// doi: 10.3390/molecules24183303.

58. Shang, Y., Wei, W., Zhang, P., & Ye, B. C. (2020). Engineering *Yarrowia lipolytica* for enhanced production of arbutin. *J Agri Food Chem 68*(5), 1364-1372. https://doi: 10.1021/acs.jafc.9b07151.

59. Yang, J., Yang, L., Zhao, F., Ye, C., & Han, S. (2024). De novo biosynthesis of β-arbutin in *Komagataella phaffii* based on metabolic engineering strategies. M*icrob Cell Fact 23*(1), 261. https://doi: 10.1186/s12934-024-02525-8.

60. Avonto, C., Wang, Y. H., Avula, B., Wang, M., Rua, D., & Khan, I. A. (2016) Comparative studies on the chemical and enzymatic stability of alpha- and beta-arbutin. *Int J Cosmet Sci 38*(2), 187-193. https://doi: 10.1111/ics.12275.

61. Braga, V. C. C., Pianetti, G. A., & César, I. C. (2020). Comparative stability of arbutin in *Arctostaphylos uva-ursi* by a new comprehensive stability-indicating HPLC method. *Phytochem Anal 31*(6), 884-891. https://doi: 10.1002/pca.2953.

62. Xu, W. H., Liang, Q., Zhang, Y. J., & Zhao, P. (2015). Naturally occurring arbutin derivatives and their bioactivities. *Chem Biodivers 12*(1), 54-81. https:// doi: 10.1002/cbdv.201300269.

63. Parejo, I., Viladomat, F., Bastida, J., & Codina, C. (2001). A single extraction step in the quantitative analysis of arbutin in bearberry (*Arctostaphylos uva-ursi*) leaves by high-performance liquid chromatography. *Phytochem Anal* *12*(5), 336-339. https://doi: 10.1002/pca.602.

64. Thongchai, W., Liawruangrath, B., & Liawruangrath, S. (2009). Arbutin determination in medicinal plants and creams. *Int J Cosmet Sci 31*(2), 87-96. https://doi: 10.1111/j.1468-2494.2008.00481.x.

65. Sugier, P., Sęczyk, L., & Sugier, D. (2022). Variation in population and solvents as factors determining the chemical composition and antioxidant potential of *Arctostaphylos uva-ursi* (L.) Spreng. leaf extracts. *Molecules 27*(7), 2247. https://doi: 10.3390/molecules27072247.

66. Repert, S., Matthes, S., & Rozhon, W. (2022) Quantification of arbutin in cosmetics, drugs and food supplements by hydrophilic interaction chromatography. *Molecules 27*(17), 5673. https://doi: 10.3390/molecules27175673.

67. Holopainen, M., Jabodar, L., Seppänen-Laakso, T., Laakso, I., & Kauppinen, V. (1988). Antimicrobial activity of some Finnish Ericaceous plants*. Acta Pharmaceu Fennica 97*, 197-202.

68. Vučić, D. M., Petković, M. R., Rodić-Grabovac, B. R., Vasić, S. M., & Čomić, L. R. (2013). *In vitro* efficacy of extracts of *Arctostaphylos uva-ursi* L. on clinical isolated *Escherichia coli* and *Enterococcus faecalis* strains. *Kragujevac J Sci* *35*, 107-113.

69. Hassan, H. M., Jiang, Z. H., Asmussen, C., McDonald, E., & Qin, W. (2014). Antibacterial activity of northern Ontario medicinal plant extracts. *Can J Plant Sci 94*, 417-424. <https://doi>: 10.4141/cjps2013-258.

70. Snowden, R., Harrington, H., Morrill, K., Jeane, L., Garrity, J., Orian, M. et al. (2014). A comparison of the anti-*Staphylococcus aureus* activity of commonly used medicinal plants. *J Altern Complement Med 20*(5), 375-382. https://doi: 10.1089/acm.2013.0036.

71. Samoilova, Z., Smirnova, G., Muzyka, N., & Oktyabrsky, O. (2014). Medicinal plant extracts variously modulate susceptibility of *Escherichia coli* to different antibiotics. *Microbiol Res 169*(4), 307-313. https://doi: 10.1016/j.micres.2013.06.013.

72. Gerstel, J., Turner, T., Ruiz, G., Wise, J., Stein, A., Jones, G. et al. (2018). Identification of botanicals with potential therapeutic use against methicillin-resistant *Staphylococcus aureus* (MRSA) infections. *Phytother Res 32*(12), 2577-2585. https://doi: 10.1002/ptr.6198.

73. Sumreen, L., Shaheen, G., Shamim, T., Rehman, T., Tanveer, R., Shirazi, J. H, et al. (2022). The antibacterial effect of *Uva ursi* plant tincture against some uropathogens*. Plant Biotech Mol Biol 23*(7&8), 94-103.

74. Dykes, G. A., Amarowicz, R., & Pegg, R, B. (2003). Enhancement of nisin antibacterial activity by a bearberry (*Arctostaphylos uva-ursi*) leaf extract. *Food Microbiol 20*, 211-216. https:// doi: 10.1016/S0740-0020(02)00107-7

75. Hafizović, L., Karup, S., & Hadžialić A. (2021). Effect of *Vaccinium vitis-idaea* tea and *Arctostaphylos uva-ursi* tea on growth of causative agents of urinary tract infections. *J Nat Sci Engineer 3*(1):1-7.

76. Maslov, O., Komisarenko, M., Ponomarenko, S., Osolodchencko, T., Marchenko, A., Pils, D. et al. (2024). Antimicrobial action of α-arbutin, β-arbutin and hydroquinone: truth and fiction. *Annals Mechnikov's Institute 3*, 10-19.

77. Rauha, J. P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T. et al. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol 56*(1), 3-12. https://doi: 10.1016/s0168-1605(00)00218-x.

78. Shimizu, M., Shiota, S., Mizushima, T., Ito, H., Hatano, T., Yoshida, T. et al. (2001). Marked potentiation of activity of β-lactams against methicillin-resistant *Staphylococcus aureus* by Corilagin. *Antimicrob Agents Chemother 45*(11)**,** 3198-3201. https://doi: 10.1128/AAC.45.11.3198-3201.2001.

79. Lewis, A. J., Richards, A. C,, Mendez, A. A., Dhakal, B. K., Jones, T. A., Sundsbak, J. L. et al. (2024). Plant phenolics inhibit focal adhesion kinase and suppress host cell invasion by uropathogenic *Escherichia coli*. *Infect Immun 92*(5), e0008024.https://doi: 10.1128/iai.00080-24.

80. McLean, R. J. C., Nickel, J. C., Cheng, K. J., & Costerton, J. W. (1988). The ecology and pathogenicity of urease-producing bacteria in the urinary tract. *Crit Rev Microbiol 16*(1), 39-79. https://doi: 10.3109/10408418809104467.

81. Deutch, C. E. (2024). Ureases as drug targets in urinary tract infections. In R. Ligabue-Braun & C. R. Carlini (Eds.). Ureases: functions, classes, applications. pp 297-358. Academic Press/Elsevier

82. Mobley, H. L.T., & Hausinger, R. P. (1989). Microbial ureases: significance, regulation, and molecular characterization. *Microbiol Rev 53*, 85-108.

83. Griffith, D. P., Musher, D. M. & Itin, C. (1976). Urease: the primary cause of infection-induced urinary stones. *Invest Urol 13*(5), 346-350.

84. Hedelin, H. (2002). Uropathogens and urinary tract concretion formation and catheter encrustations. *Int J Antimicrob Agents 19*(6), 484-487. https://doi: 10.1016/s0924-8579(02)00095-x.

85. Miano, R., Germani, S., & Vespasian, G. (2007) Stones and urinary tract infections. *Urol Int 79*(Suppl 1), 32-36. https:// doi: 10.1159/000104439.

86. Marien, T., & Miller, N. L. (2015). Treatment of the infected stone. *Urol Clin North Am 42*(4), 459-472. https://doi: 10.1016/j.ucl.2015.05.009.

87. McLean, R. J. C., & Nickel, J. C. (1994) Glycosaminoglycans and struvite calculi. *World J Urol 12*:49-51. https://doi: 10.1007/BF00182051

88. Torzewska, A., & Różalski, A. (2013). In vitro studies on the role of glycosaminoglycans in crystallization intensity during infections urinary stones formation. *APMIS 122*(6), 505-511. https://doi: 10.1111/apm.12191.

89. Broomfield, R. J., Morgan, S. D., Khan, A., & Stickler, D. J. (2009) Crystalline bacterial biofilm formation on urinary catheters by urease-producing urinary tract pathogens: a simple method of control. *J Med Microbiol 58*(Pt 10), 367-1375. https://doi: 10.1099/jmm.0.012419-0.

90. Clark. E. C., Nath, K. A., Hostetter, M. K., & Hostetter, T. H. (1990). Role of ammonia in tubulointerstitial injury. *Miner Electrolyte Metab 16*, 515-521.

91. Mobley, H. L. T., Island, M. D., & Hausinger, R. P. (1995). Molecular biology of microbial ureases*. Microbiol Rev 59*, 451-480. https:// doi: 10.1128/mr.59.3.451-480.1995.

92. Kappaun, K., Piovesan, A. R. Carlini, C. R., & Ligabue-Braun, R. Ureases: historical aspects, catalytic and non-catalytic properties – a review. *J Adv Res* 13:3-17. https:// doi: 10.1016/j.jare.2018.05.010.

93. Deutch, C. E. (2025)Ureases from pathogenic Gram-positive bacteria in the genus *Staphylococcus*. *Curr Topics Biochem Res* *25*:1-34.

94. Gatermann, S., John, J., & Marre, R. (1989) *Staphylococcus saprophyticus* urease: characterization and contribution to uropathogenicity in unobstructed urinary tract infection in rats. *Infect Immun 57*(1), 110-116. https://doi: 10.1128/iai.57.1.110-116.1989.

95. Jones, B. D., Lockatell, C. V., Johnson, D. E., Warren, J. W., & Mobley, H. L..T. (1990). Construction of a urease-negative mutant of *Proteus mirabilis*: analysis of virulence in a mouse model of ascending urinary tract infection. *Infect Immun 58*(4), 1120-1123. https://doi: 10.1128/iai.58.4.1120-1123.1990.

96. Johnson, D. E, Russell, R. G., Lockatell, C. V., Zulty, J. C., Warren, J. W., & Mobley, H. L. T. (1993). Contribution of *Proteus mirabilis* urease to persistence, urolithiasis, and acute pyelonephritis in a mouse model of ascending urinary tract infection. *Infect Immun 61*(7), 2748-2754. https://doi: 10.1128/iai.61.7.2748-2754.1993.

97. Deutch, C, E. (2017). Limited effectiveness of over-the-counter plant preparations used for the treatment of urinary tract infections as inhibitors of the urease activity from *Staphylococcus saprophyticus*. *J Appl Microbiol 122*(5), 1380-1386. https://doi: 10.1111/jam.13430.

98. Deutch, C. E. (2021). Inhibition of urease activity in the urinary tract pathogens *Staphylococcus saprophyticus* and *Proteus mirabilis* by Uva Ursi*. Int J Herb Med 9*(5), 17-24.

99. Loes, A. N., Ruyle, L., Arvizu, M., Gresko, K, E., Wilson, A. L,, & Deutch, C. E. (2014). Inhibition of urease activity in the urinary tract pathogen *Staphylococcus saprophyticus*. *J Appl Microbiol 58*(1), 31-41. https://doi: 10.1111/lam.12153.

100. Chahales, P., & Thanassi, D. G. (2015). Structure, function, and assembly of adhesive organelles by uropathogenic bacteria. *Microbiol Spectrum 3*(5), UTI-0018-2013. https://doi: 10.1128/microbiolspec.UTI-0018-2013.

101. Paharik, A. E., & Horswill, A. R. (2016). The staphylococcal biofilm: adhesins, regulation, and host response. In I. T. Kidva, N. A. Comick, P. J. Plummer, Q. Zhang, J. P. Nicholson, J. P. Bannatine, & B. H. Bellaire (Eds). Virulence Mechanisms in Bacterial Pathogens, Edn 5, pp 529-566. American Society for Microbiology, Washington DC..

102. Yu, Y., Singh, H., Tsitrin, T., Bekele, S., Lin, Y. H., Sikorski, P. et al. (2021). Urethral catheter biofilms reveal plasticity in bacterial composition and metabolism and withstand host immune defenses in hypoxic environment*. Front Med (Lausanne) 8,* 667462. https://*doi: 10.3389/fmed.2021.6674628*:667492.

103. Abu Lila A. S., Rajab, A. A. H., Abdallah, M. H., Rizvi, S. M. D., Moin, A., Khafagy, E. S. et al. (2023). Biofilm lifestyle in recurrent urinary tract infections. *Life 13,*148. <https://doi>: 10.3390/life13010148,

104. Letourneau, J., Levesque, C., Berthiaume, F., Jacques, M., & Mourez, M. (2011). *In vitro* assay of bacterial adhesion onto mammalian epithelial cells. *J Vis Exp 16*(51),2783. https://doi: 10.3791/2783.

105. Pedersen, R. M., Grønnemose, R. B., Staerk, K., Asferg, C. A., Andersen, T. B., Kolmos, H. J. et al. (2018). A method for quantification of epithelium colonization capacity by pathogenic bacteria. *Front Cell Infect Microbiol* *8*, 6. https://doi: 10.3389/fcimb.2018.00016.

106. Merritt, J. H., Kadouri, D. E., & O’Toole, G. A. (2005). Growing and analyzing static biofilms. *Curr Protoc Microbiol* *Chapter 1*(Unit 1B.1.) https://doi: 10.1002/9780471729259.mc01b01s00.

107. Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M, et al. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol 22*(6), 996-1006. https://doi: 10.1128/jcm.22.6.996-1006.1985.

108. Deutch, C. E. (2020). Effects of over-the-counter herbal preparations on biofilm formation by the urinary tract pathogen *Staphylococcus saprophyticus*. *Int J Herb Med 8*(5), 116-124.

109. Samoilova, Z., Muzyka, N., Lepekhina, E., Oktyrabrsky, O., & Smirnova, G. (2014). Medicinal plant extracts can variously modify biofilm formation in *Escherichia coli*. *Antonie van Leeuwenhoek 105*(4), 709-722. https://doi: 10.1007/s10482-014-0126-3.

110. Kim, S., Lee, jJ. H., Kim, Y. G., Tan, Y., & Lee, J. (2022). Hydroquinones inhibit biofilm formation and virulence factor production in *Staphylococcus aureus*. *Int J Molec Sci 23*(18), 10683. https://doi: 10.3390/ijms231810683.

111. Cela-López, J. M., Camacho Roldán, C. J., Gómez-Lisarraga, G., & Martinez, V. (2021). A natural alternative treatment for urinary tract infections: Itxasol©, the importance of the formulation. *Molecules 26*(15), 4564. https://doi: 10.3390/molecules26154564.

112. Cela-López, J. M., Camacho Roldán, C. J., Gómez-Lisarraga, G., & Martinez V. (2021). Effects of Itxasol© components on gene expression in bacteria related to infections of the urinary tract and to the inflammation process. *Int J Molec Sci 22*(23), 12655. https://doi: 10.3390/ijms222312655.

113. Cai, T., Gallelli, L., Meacci, F., Brugnolli, A., Prosperi, L., Roberta, S. et al. (2016). The efficacy of umbelliferon, arbutin, and N-acetylcysteine to prevent microbial colonization and biofilm development on urinary catheter surface: results from a preliminary study. *J Pathogens 2016*,1590952. https://doi: 10.1155/2016/1590952.

114. Abdulshaheed, A. A., Hanafiah, M. M., Nawaz, R., & Muslim, S. N. (2024). Evaluation of antibacterial, antifungal and antibiofilm activities of *A. baumannii*-derived tannase and gallic acid against uropathogenic microorganisms. *Microb Pathog 187*, 106534. https://doi: 10.1016/j.micpath.2024.106534.

115. Jubair, N., R., M., Fatima, A., Mahdi, Y. K., & Abdullah, N. H. (2022). Evaluation of catechin synergistic and antibacterial efficacy on biofilm formation and *acrA* gene expression of uropathogenic *E. coli* clinical isolates. *Antibiotics (Basel) 11*(9), 1223. https://doi: 10.3390/antibiotics11091223.

116. Alav, I., Sutton, J. M., & Rahman, K. M. (2018). Role of bacterial efflux pumps in biofilm formation. *J Antimicrob Chemother 73*(8), 2003-2020. https://doi.org/10.1093/jac/dky042.

117. Bernal-Mercado, A. T., Vazquez-Armenta, F. J., Tapia-Rodriguez, M. R., Islas-Osuna, M. A., Mata-Haro, V., Gonzalez-Aguilar, G. A. et al. (2018). Comparison of single and combined use of catechin, protocatechuic, and vanillic acids as antioxidant and antibacterial agents against uropathogenic *Escherichia coli* at planktonic and biofilm levels. *Molecules 23*(11), 2813. https://doi: 10.3390/molecules23112813.

118. Lee, J. H., Park, J. H., Cho, H. S., Joo, S. W., Cho, M. H., & Lee, J. (2013). Antibiofilm activities of quercetin and tannic acid against *Staphylococcus aureus*. *Biofouling 29*(5), 491-499. https://doi: 10.1080/08927014.2013.788692.

119. Arciola, C. R,, Campoccia, D., Ravaioli, S., & Montanaro, L. (2015). Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. *Front Cell Infect Microbiol 5*, 7. https://doi: 10.3389/fcimb.2015.00007.

120. da Costa Junior, S. D,, de Oliveria Santos, J. V., de Almeida Campos, L. A., Pereira, M. A., Santos Magalhães, N. S., & Ferro Cavalcanti, I. M. (2018). Antibacterial and antibiofilm activities of quercetin against clinical isolates of *Staphylococcus aureus* and *Staphylococcus saprophyticus* with resistance profile. *Int J Env Agri Biotechnol 3*(5), 9481958. http://dx.doi.org/10.22161/ijeab/3.5.50

121. Aygül, A., Öztürk, I., Çilli, F. F., Ermertcan, Ş. (2019). Quercetin inhibits swarming motility and activates biofilm production of *Proteus mirabilis* possibly by interacting with central regulators, metabolic status or active pump proteins. *Phytomedicine 57*, 65-71. <https://doi>: 10.1016/phytomed.2018.12.014

122. Vipin, C., Saptami, K., Fida, F., Mujeeburahiman, M., Rao, S. S., Athmika, et al. (2020). Potential synergistic activity of quercetin with antibiotics against multidrug-resistant clinical strains of *Pseudomonas aeruginosa*. *PloS ONE 15*(11), e0241304. https://doi: 10.1371/journal.pone.0241304.

123. Salem, M. A., Salama, M. M., Ezzat, S. M., & Hashem, Y. A. (2022). Comparative metabolite profiling of four polyphenol-rich *Morus* leaves extracts in relation to their antibiofilm activity against *Enterococcus faecalis*. *Sci Rep 12*(1), 20168. https:/ doi: 10.1038/s41598-022-24382-4.

124. Baron, G., Altomare, A., Regazzoni, L., Fumgalli, L., Artasensi, A., Borghi, E. et al. (2020). Profiling *Vaccinium macrocarpon* components and metabolites in human urine and the urine *ex-vivo* effect on *Candida albicans* adhesion and biofilm formation. *Biochem Pharmacol 173*, 113726. https://doi: 10.1016/j.bcp.2019.113726.

125. Ivanov, M., Novović, K., Malešević, M., Dinić, M,, Stojković, D., Jovičić, B. et al. (2022). Polyphenols as inhibitors of antibiotic-resistant bacteria – mechanisms underlying rutin interference with bacterial virulence. *Phamaceuticals (Basel) 15*(3), 385. https:// doi: 10.3390/ph15030385.

126. Abraham, S. N., & Miao, Y. (2015). The nature of immune responses to urinary tract infections. *Nat Rev Immunol 15*(10), 655-663. https://doi: 10.1038/nri3887.

127. Mariano, L. L., & Ingersoll, M. A. (2020). The immune response to infection in the bladder. *Nat Rev Urology 17*, 439-458. <https://doi>: 10.1058/s41585-020-0550-8.

128. Munoz, J. A., Uhlemann, A. C., & Barash, J. (2022). Innate bacteriostatic mechanisms defend the urinary tract. *Annu Rev Physiol 84*, 533-558. https://doi: 10.1146/annurev-physiol-052521-121810.

129. Kuhn, H. W., Hreha, T. N., & Hunstad, D. A. (2023). Immune defenses in the urinary tract. *Trends Immunol 44*(9), 701-711. https://doi: 10.1016/j.it.2023.07.001.

130. Li, L., Li, Y., Yang, J., Xie, X., & Chen, H. (2022). The immune responses to different uropathogens call individual interventions for bladder infection. *Front Immunol 13*, 953354. https://doi: 10.3389/fimmu.2022.953354.

131. Ambite, I., Butler, D., Wan, M. L.Y., Rosenblad, T., Tran, T. H., Chao, S. M. et al. (2021). Molecular determinants of disease severity in urinary tract infection*. Nat Rev Urology* 18, 468-486. https://doi: 10.1058/s41585-021-00477-x.

132. Azman, N. A. M., Gallego, M. G., Segovia, F., Abdullah, S., Shaarani, S. M., & Pablos, M. P. A. (2016). Study of the properties of bearberry leaf extract as a natural oxidant in model foods. *Antioxidants 5,* 71. <https://doi>: 10.3390/antiox5020011,

133. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med 26*(9-10), 1231-1237. https:/ doi: 10.1016/s0891-5849(98)00315-3.

134. Azman, N. A, M., Peiró, S., Fajari, L., Julià, L., & Almajano, M. P.(2014). Radical scavenging of white tea and its flavonoid constituents by electron paramagnetic resonance (EPR) spectroscopy. *J Agri Food Chem 62(*25), 5743-5748. https://doi: 10.1021/jf501707p.

135. Carpenter, R., O’Grady, M. N,, O’Callaghan, Y. C., O’Brien, N. M., & Kerry JP. (2007). Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Sci 76*(4), 604-610. https://doi: 10.1016/j.meatsci.2007.01.021.

136. Sugier, P., Sęczyk, L., Sugier, D., Krawczyk, R., Wójcik, M., Czarnecka, J. et al. (2021). Chemical characteristics and antioxidant activity of *Arctostaphylos uva-ursi* L. Spreng. at the southern border of the geographic range of the species in Europe. *Molecules 26*(24), 7692. https://doi: 10.3390/molecules26247692.

137. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss U Technol 28,* 25-30.

138. Shamilov, A. A., Olennikov, D. N., Pozdnyakov, D. I., Bubenchikova, V. N., & Garsiya, E. R. (2022). Investigation of phenolic compounds at the leaves and shoots *Arctostaphylos* spp. and their antioxidant and antityrosinase activities. *Nat Prod Res 36*(24), 6312-6317. <https://doi>: 10.1080/14786419.2021.2025370.

139. Martău, G. A., Bernadette-Emöke, T., Odocheanu, R., Soporan, D. A., Bochiş, M., Simon, E. et al. (2023). *Vaccinium* species (Ericaceae): phytochemistry and biological properties of medicinal plants. *Molecules 28*(4), 1533. https://doi: 10.3390/molecules28041533.

140. Schink, A., Neumann, J., Leifke, A. L., Ziegler, K., Fröhlich-Nowolsky, J., Cremer, C. et al. (2018). Screening of herbal extracts for TLR2- and TLR-4-dependent anti-inflammatory effects. *PloS ONE 13*(10):e0203907. https://doi: 10.1371/journal.pone.0203907.

141. Lee, H. J., & Kim, K. W. (2012). Anti-inflammatory effects of arbutin in lipopolysaccharide-stimulated BV2 microglial cells. *Inflamm Res 61*, 817-825. https://doi: 10.1007/s00011-012-0474-2.

142. Avila-Carrasco, L., Garcia-Mayorga, E. A., Diaz-Avila, D. L., Garza-Veloz, I., Martinez-Fierro, M. L., & González-Mateo, G. T. (2021). Potential therapeutic effects of natural plant compounds in kidney disease. *Molecules 26*(20), 6096. https://doi: 10.3390/molecules26206096.

143. Saleh, H. A., Yousef, M. H., & Abdelnaser, A. (2021). The anti-inflammatory properties of phytochemicals and their effects on epigenetics mechanisms involved in TLR4/NF-kB-mediated inflammation. *Front Immunol 12*, 606069. https://doi: 10.3389/fimmu.2021.606069.

144. McLarin, M. A , & Leung, I. K. H. (2020) Substrate specificity of polyphenol oxidase. *Crit Rev Biochem Mol Biol 55*(3), 274-308.https://doi: 10.1080/10409238.2020.1768209

145. Zolghadri, S., Bahrami, A., Khan, M. T. H., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F. et al. (2010). A comprehensive review on tyrosinase inhibitors. *J Enz Inhib Med Chem 34*(1), 279-309. https://doi: 10.1080/14756366.2018.1545767.

146. Kolganova, T. V,, Ermolaev, A. V., & Doyle, R. J. (2002). Effects of asparaginase and polyphenol oxidase on adhesive characteristics of microorganisms*. Bull Exp Biol Med 133*(1), 71-74. https://doi: 10.1023/a:1015108628195.

147. Kolganova, T. V., Dzharadat, T., Ermolaev, A. V., Osipova, I. G., Vasil’eva, E. A., Dalin, M. V. et al. (2002). Combined effects of low-frequency laser and polyphenol oxidase on adhesive activity of *Escherichia coli* strains isolated from patients with urinary diseases. *Bull Exp Biol Med 134*(7), 63-65. https://doi: 10.1023/a:1020660706121.

148. Kiang, T. K. L., Ensom, M. H. H., & Chang, T. K. H. (2005). UDP-glucuronosyltransferases and clinical drug interactions. *Pharmacol Ther 106*(1), 97-132. <https://doi>: 10.1016/j.pharmthera.2004.10.013.

149. Park, J. B., Kim, D., Min, J. S., Jeong. S., Cho, D. Y., Zheng, Y. F. et al. (2018). Identification and characterization of *in vitro* inhibitors against UDP-glucuronosyltransferase 1A1 in uva-ursi extracts and *in vivo* uva-ursi-drug interactions. *Food Chem Toxicol* *120*, 651-661. <https://doi>: 10.1016/j.fct.2018.07.058.

150. Turkovic, A. H., Gunjača, M., Marjanovic, M., Lovrić, M., Butorac, A., Rašić, D. et al. (2022). Proteome changes in human bladder T24 cells induced by hydroquinone derived from *Arctostaphylos uva-ursi* herbal preparation. *J Ethnopharm 289*, 115092. <https://doi>: 10.1016/j.jep.2022,115092.

151. Li, H., Jeong, Y. M., Kim, S. Y., Kim, M. K., & Kim, D, S. (2011). Arbutin inhibits TCCSUP human bladder cancer cell proliferation via up-regulation of p21. *Pharmazie 66*(4), 306-309.

152. Grases, F., Melero, G., Costa-Bauza, A., Prieto, R., & March J, G. (1994). Urolithiasis and phytotherapy. *Int Urol Nephrol 26*(5), 507-511. https://doi: 10.1007/BF02767650.

153. Beaux, D., Fleurentin, J., & Mortier, F. (1999). Effect of extracts of *Orthrosiphon stamineus* Benth, *Heiracium pilosella* L, *Sambucus nigra* L., and *Arctostaphylos uva-ursi* (L.) Spreng. in rats. *Phytother Res 13*(3), 222-225. https://doi: 10.1002/(SICI)1099-1573(199905)13:3<222::AID-PTR447>3.0.CO;2-P.

154. Saeed, F., Mehjabeen, Jahan, N., & Ahmad, M. (2014). *In vivo* evaluation & safety profile evaluation of *Arctostaphylos uva-ursi* (L.) Spreng. extract in rabbits. *Pak J Pharm Sci 27*(6), 2197-2205.

155. Schindler, G., Patzak, U., Brinkhaus, B., van Niecieki, A., Wittig, J., Krähmer, N. et al. (2002). Urinary excretion and metabolism of arbutin after oral administration of *Arctostaphylos uva-ursi* extract as film-coated tablets and aqueous solution in healthy humans. *J Clin Pharmacol 42*(8), 920-927. https://doi: 10.1177/009127002401102740.

156. Quintus, J., Kovar, K. A., Link, P., & Hamacher, H. (2005). Urinary excretion of arbutin metabolites after oral administration of bearberry leaf extracts. *Planta Med 71*, 147-152. <https://doi>: 10.1055/s-2005-837752.

157. Gallo, L. R., Pagliuca, G., Multari, G., Panzini, G., D’Amore, E., & Altieri I. (2015). New high performance liquid chromatography-DAD method for analytical determination of arbutin and hydroquinone in rat plasma. *Indian J Pharm Sci 77*(5), 530-535. <https://doi>: 10.4103/0250-474x.169031.

158. Moore, M., Trill, J., Simpson, C., Webley, F., Stanton, L., Maishman, T. et al.(2019). Uva-ursi extract and ibuprofen as alternative treatments for uncomplicated urinary tract infection in women (ATAFUTI): a factorial randomized trial. *Clin Microbiol Infect 25*(8), 973-980. https://doi: 10.1016/j.cmi.2019.01.011.

159. Datta, R., & Juthani-Mehta, M. (2019). Antibiotic-sparing agents for uncomplicated cystitis: uva-ursi and ibuprofen not ready for primetime. *Clin Microbiol Infect* 25(8), 922-924. https://doi: 10.1016/j.cmi.2019.04.022.

160. Afshar K, Fleischmann N., Schmiemann, G., Bleidorn J, Hummers-Pradier E, Friede T, et al. (2018). Reducing antibiotic use for uncomplicated urinary tract infection in general practice by treatment with uva-ursi (REGATTA) – a double-blind, randomized, controlled comparative effectiveness trial. *BMC Complement Altern Med 18(*1), 203. https://doi: 10.1186/s12906-018-2266-x.

161. Gágyor, I., Hummers, E.,., Friede, T., Pfeiffer, S., Afshar, K. et al. (2021). Herbal treatment with uva ursi extract versus fosfomycin in women with uncomplicated urinary tract infection in primary care: a randomized controlled trial. *Clin Microbiol Infect 27*(10), 1441-1447. https:// doi: 10.1016/j.cmi.2021.05.032.

162. Tóth, B., Javorhaázy, A., Nyirády, P., Csupor-Löffler, B. Birinyi, P., Zhanel, G. et al. (2022). Bearberry in the treatment of acute uncomplicated cystitis (BRUMI): protocol of a multicenter, randomized double-blind clinical trial. *BMJ Open 12*(6), e057982. https://doi: 10.1136/bmjopen-2021-057982.

163. Garofalo, L., Zwickey, H., Bradley, R., & Hanes, D. (2021). Naturopathic management of urinary tract infections: a retrospective chart review. *J Altern Comple Med 27*(12), 1116-1126. https://doi: 10.1089/acm.2021.0163.

164. Barea, B. M, Veratterapillay, R., & Harding, C. (2020). Nonantibiotic treatments for urinary cystitis: an update. *Curr Opin Urol 30*(6), 845-852. https:// doi: 10.1097/MOU.0000000000000821.

165. Heinz, J., Röver, C., Furajat, G., Kaußner, Y., Hummers, E., Debray, T. et al (2020). Strategies to reduce antibiotic use in women with uncomplicated urinary tract infection in primary care: protocol of a systematic review and meta-analysis including individual patient data. *BMJ Open10*(10), e035883. https://doi: 10.1136/bmjopen-2019-035883.

166. No Author. (2022). Bladder infection: Do herbal remedies help with recurrent bladder infection? Cologne (Germany): Institute for Quality and Efficiency in Health Care (IQWiG). PMID: 37023241.

167. van Wietmarschen, H., van Seenberger, N., van der Weft, E., & Baars, E. (2022). Effectiveness of herbal medicines to prevent and control symptoms of urinary tract infections and to reduce antibiotic use: a literature review. *Integr Med Res 11*(4), 100892. https://doi: 10.1016/j.imr.2022.100892.

168. Garofalo, L., Nakama, C., Hanes, D., & Zwickey, H. (2022). Whole-person, urobiome-centric therapy for urinary tract infection. *Antibiotics 11*(2), 218. https://doi: 10.3390/antibiotics11020218.

169. Hou, P. J., Lin, P. Y., Lin, W. L., & Hsueh, T. P. (2024). Integrated traditional herbal medicine for recurrent urinary tract infection treatment and follow-up: a meta-analysis of randomized controlled trials. *J Ethnopharmacol* *321*, 117491. https://doi: 10.1016/j.jep.2023.117491.

170. Burrows, L. L. (2024). It’s uncomplicated: prevention of urinary tract infections in an era of increasing antibiotic resistance. *PLoS Pathog 20*(2), e1011930. https://doi: 10.1371/journal.ppat.1011930.

171. McGregor D. (2007). Hydroquinone: an evaluation of human risks from its carcinogenic and mutagenic properties. *Crit Rev Toxicol 37*(10), 887-914.

doi: 10.1080/10408440701638970.

172.

172. Garcia de Arriba, S., Nasser B., & Nolte, KU. (2013). Risk assessment of free hydroquinone derived from *Arctostaphylos Uva-ursi folium* herbal preparations.

*Int J Toxicol 32*(6), 442-53. doi: 10.1177/1091581813507721.

173. Lenger, A. M., Bradley, M. S., Thomas, D. A., Bertotet, M. H., Lowder, J. L., & Subcliffe, S. (2020). D-mannose vs other agents for recurrent urinary tract infection prevention in adult women: a systematic review and meta-analysis. *Am J Obstet Gyncol 223*(2), 265.e1-265.e13. https//doi: 10.1016/j.ajog.2020.05.048.

174. Cooper, T. E., Teng, C., Howell, M., Teixeira-Pinto, A., Jaure, A. & Wong, G. (2022). D-mannose for preventing and treating urinary tract infections. *Cochrane Database Syst Rev 8*(8), CD013608. https://doi: 10.1002/14651858.CD013608.pub2.

175. Gonzáles de Llano, D., Moreno-Arribas, M. V., & Bartolomé, B. (2020). Cranberry polyphenols and prevention against urinary tract infections: relevant considerations. *Molecules 25*(15), 3523. https// doi: 10.3390/molecules25153523.

176. Gbinigie, O. A., Spencer, E. A., Heneghan, C. J., Lee, J. J., & Butler, C. C. (2021). Cranberry extract for symptoms of acute, uncomplicated urinary tract infection: a systematic review. *Antibiotics (Basel) 10*(1), 12. https://doi: 10.3390/antibiotics10010012.

177. Fazly Bazzaz, B. S., Fork, S. D., Ahmadi, R., & Khameneh, B. (2021) Deep insights into urinary tract infections and effective natural remedies. *Afr J Urol 27*, 6. <https://doi>: 10.1186/s12301-020-00111-z.

178. Panda, S. R., Meher, A., Prusty, G., Behera, S,, & Prasad, B. R. (2025). Antibacterial properties and therapeutic potential of few medicinal plants: current insights and challenges. *Discover Plants 2*, 21. <https://doi>: 10.1007/s44372-025-00097-4.

179. Genovese, C., Davinelli, S., Mangano, K., Tempera, G., Nicolosi, D., Corsello, S. et al. (2018). Effects of a new combination of plant extracts plus D-mannose for the management of uncomplicated recurrent urinary tract infections. *J Chemother 30*(2), 107-114. https:// doi: 10.1080/1120009X.2017.1393587.

180. Ur Rehman, J., Iqbal, A., Asif, H. M., Hadi, F., Ahmad, K., Ahmad, M. et al. (2024). Alternative approaches to the management of urinary tract infection with Crano-cure: a randomized clinical trial. *Altern Ther Health Med 30*(7), 12-19.

181. Heller, M., Steindl, H., Abramov-Samaria, D., Wagenen, F., Naber, K. G., & Kostek, K. (2021). Treatment of urinary tract infections with Canephor® in Germany: a retrospective database analysis. *Antibiotics (Basel) 10*(6), 685. https://doi: 10.3390/antibiotics10060685.

182. Kassner, N., Wonnemann, M., Ziegler, Y., Vahlensieck, W., Kranz, J., & Kostev, K. (2024). Effectiveness of a combination of Nasturtium herb and Horseradish root (Angocin® *Anti-Infekt N*) compared to antibiotics in managing acute and recurrent urinary tract infections: a retrospective real-world cohort study. *Antibiotics (Basel) 13*(11), 1036. https:// doi: 10.3390/antibiotics13111036.

183. Zheng, H., Yu, X., Wang, C., Guo, X., Gao, C., Chen, K. et al. (2025). Elucidation of the mechanism of the Yinhua Miyanling tablet against urinary tract infection based on a combined strategy of network pharmacology, multi-nomics, and molecular biology. *J Ethnopharmacol* 337(Pt 1), 118835. https://doi: 10.1016/j.jep.2024.118835

184. Qureshi, A., ur Rahman, R., & Shamsi, Y. (2024). Efficacy and safety of a polyherbal formulation in the management of *Escherichia coli* urinary tract infection. *Drug Metal Pers Ther 39*(4), 221-230. https://doi: 10.1515/dmpt-2024-0070