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Inhibition of urease activity in the urinary tract pathogens *Staphylococcus saprophyticus* and *Proteus mirabilis* by Uva Ursi

Charles E Deutch

Abstract
This study was designed to compare the sensitivities of ureases from *Staphylococcus saprophyticus* and *Proteus mirabilis* to different over-the-counter preparations of Uva Ursi (*Arctostaphylos uva-ursi*) and to determine if they can inhibit the increase in pH that occurs in cultures as a result of urease activity. The Nature's Answer® Uva Ursi preparation was the more effective inhibitor in cell-free extracts. Neither enzyme was inhibited by arbutin, but the enzymes were partially inhibited by hydroquinone and 1,4-benzoquinone. The Nature's Answer® Uva Ursi preparation and 1,4-benzoquinone could partially inhibit urease in whole cells of *S. saprophyticus* and *P. mirabilis*. Exposure of *S. saprophyticus* cultures in artificial urine medium to Nature's Answer® Uva Ursi slowed the increase in pH. Uva Ursi preparations should be used with caution for the treatment of urinary tract infections since they vary in concentration and bacterial pathogens with urease activity differ in their sensitivity to them.

Keywords: 1,4-benzoquinone, hydroquinone, *Proteus mirabilis*, *Staphylococcus saprophyticus*, urease, Uva Ursi

1. Introduction
Urinary tract infections (UTIs) commonly occur in infants and small children, in adolescent and adult women, and in patients or older adults fitted with catheters [1-4]. The primary etiological agents are the Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumonia*, and *Proteus mirabilis* and the Gram-positive bacteria *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Staphylococcus aureus* [5-7]. While *S. saprophyticus* is most commonly associated with community-acquired UTIs in young women [8, 9], *P. mirabilis* is more often involved in catheter-associated UTIs in older adults or post-surgical patients [10]. Although most UTIs can be treated with antibiotics [11], resistant microorganisms are frequently recovered from infected individuals [12] and recurrent infections are common [13].
A key virulence factor for both *S. saprophyticus* and *P. mirabilis* is the enzyme urease (urea amidohydrolase, EC 3.5.1.5), which catalyzes the hydrolysis of urea to form ammonium ions and carbonic acid [14, 15]. The ammonium ions raise the urinary pH and lead to the formation of urinary stones and catheter encrustations [16, 17]. Urease is synthesized by most strains of *S. saprophyticus* and *P. mirabilis* and by some plasmid-containing strains of *Escherichia coli* [18]. The protein typically contains three types of subunits and two Ni^{2+} ions at the active site. Urease activity is constitutive in *S. saprophyticus* but inducible by urea in *P. mirabilis*. Because antibiotics can be relatively expensive and may require prescriptions from physicians, there is a great deal of interest in alternative approaches to preventing or treating UTIs [19]. These include the use of over-the-counter plant preparations such as those derived from fruits like cranberries or various herbs [20-23]. Many plant extracts have been tested as urease inhibitors using the enzymes from Jack Beans (*Canavalia ensiformis*), *Proteus mirabilis*, or *Helicobacter pylori* as model systems [24-30]. These extracts may contain inhibitors with diverse chemical structures [31].
In a survey of 14 over-the-counter plant preparations that might be used to treat UTIs, the urease from *S. saprophyticus* was found to be susceptible to inhibition by Nature's Answer® extract from Uva Ursi (*Arctostaphylos uva-ursi* or bearberry) [32]. Many other commercial preparations of Uva Ursi are available in natural food stores and pharmacies or from online distributors. They are promoted on various web sites for the treatment of both urinary tract infections and prostate disorders [33-37]. Uva Ursi extracts have been shown to have antimicrobial activity [38-40], antioxidant activity [41, 42], and anti-inflammatory activity [43, 44]. Although these extracts contain a wide range of organic compounds [45, 46], the clinical effects of Uva Ursi extracts have been attributed to arbutin (4-hydroxyphenyl-β-D-glucopyranoside) [47-49]. Arbutin undergoes hydrolysis to yield free D-glucose and hydroquinone, which may

then undergo oxidation to form 1,4-benzoquinone (*p*-benzoquinone). I now describe additional experiments on the inhibition of bacterial ureases by different preparations of Uva Ursi and by arbutin, hydroquinone, and 1,4-benzoquinone.

2. Materials and Methods

2.1 Bacterial strains and growth conditions

S. saprophyticus strain ATCC 15305 was obtained from the American Type Culture Collection; *P. mirabilis* strain BB2000 was part of a microbiology teaching collection. Bacteria were maintained on Difco™ tryptic soy agar (Becton, Dickinson and Company) containing 0.5% yeast extract and cultured at 37 ºC. Liquid P medium was prepared as described by Gatermann *et al.* [14] and contained per liter: 10 g peptone, 5 g yeast extract, 1 g Na_2HPO_4, and 1 g D-glucose. Liquid LB medium contained per liter: 10 g tryptone, 5 g yeast extract, and 10 g NaCl. To induce urease activity in *P. mirabilis*, LB medium was supplemented with 100 mM urea. The artificial urine medium (AUM) used in these studies was based on one described by Minuth *et al.* [50] and contained per liter: 0.65 g $CaCl_2$.2 H_2O, 0.65 g $MgCl_2.6H_2O$, 4.6 g NaCl, 2.3 g Na_2SO_4, 2.8 g KH_2PO_4, 1.6 g KCl, 1.0 g NH_4Cl, 12 g urea, 1.1 g creatinine, and 10 g tryptic soy broth. The pH was adjusted to 6.5and the solution sterilized by filtration. All liquid cultures were grown at 37 ºC and shaken at 250 rpm in flasks containing less than 10% of the total volume as medium.

2.2 Over-the-counter Uva Ursi preparations and other chemicals

Liquid Uva Ursi preparations (*Arctostaphylos uva-ursi*) from Nature's Answer®, Hawaii Pharm LLC, and Honey Combs Industries LLC were obtained from local natural food stores in the United States or from internet suppliers. Solaray® Uva Ursi whole leaf capsules were obtained from a local health food store. To prepare an aqueous extract from the leaves, three capsules were opened and the material suspended in 3 ml of urease resuspension buffer (50 mM HEPES, 1 mM EDTA, pH 7.5). After incubation overnight at room temperature, another 3 ml of urease resuspension buffer was added and the sample centrifuged at 10,000 x *g* for 5 min. The brown-colored solution was saved as the Uva Ursi extract. Arbutin (95%) was obtained from Acros Organics. Hydroquinone (>99%) and 1,4-benzoquinone (*p*-benzoquinone, >99%) were purchased Chemsavers and4-Aminoantipyrine was from Sigma-Aldrich.

2.3 Analysis of Uva Ursi preparations

The concentration of arbutin in the Uva Ursi preparations was determined by the method of Thongchai *et al.* [51]. Varying amounts of a sample containing arbutin were combined with water to give a sample volume of 100 µl. 700 µl of 0.2 M Na borate buffer, pH 9.4, 100 µl of 0.1 M 4-aminoantipyrine, and 100 µl of 0.1 M potassium ferricyanide in borate buffer were added. After mixing, the absorbance of the solution was measured at 514 nm in a Shimadzu 160U UV-visible spectrophotometer. The amount of arbutin in the extracts was determined from an arbutin standard curve. The presence of arbutin and other glycosides in the Uva Ursi extracts was visualized by thin-layer chromatography on Kieselgel 60 silica gel sheets using a solvent of ethyl acetate-methanol-water (85:17:13) and Berlin Blue (10 g ferric chloride, 0.5 g potassium ferricyanide in 100 ml water) as the spray reagent.

2.4 Preparation of whole cell suspensions

S. saprophyticus or *P. mirabilis* was grown at 37 ºC with aeration in 25 ml of P medium or LB medium containing 100 mM urea to late exponential phase (75 to 100 Klett Unitsin a Klett Summerson colorimeter with a red 660_{nm} filter). The bacteria were harvested by centrifugation for 10 min at 10,000 x *g* in a Bio-Lion XC-H165 centrifuge at room temperature, washed once with 0.85% (w/v) NaCl, and resuspended in 0.85% (w/v) NaCl to give a suspension equal to 100 Klett Units. The suspension was kept on ice and used within 24 hr.

2.5 Preparation of cell extracts

S. saprophyticus or *P. mirabilis* was grown at 37 ºC with aeration in 900 ml of P medium or in 600 ml of LB medium containing 100 mM urea to late exponential phase (75 to 100 Klett Units in a Klett Summerson colorimeter with a red 660_{nm} filter. The bacteria were harvested by centrifugation for 10 min at 10,000 x *g* in a Bio-Lion XC-H165 centrifuge at room temperature, washed once with 0.85% (w/v), and stored as a pellet at -20 ºC. The cells were thawed and resuspended in 30 ml of urease resuspension buffer (50 mM HEPES, 1 mM Na_2EDTA, pH 7.5). The bacterial suspension was combined with 1 mm glass beads and disrupted by 5 one-minute cycles in a Bead-Beater® (Biospec Products, Inc.). After allowing most of the beads to settle, the remaining beads, unbroken cells, and debris were removed by centrifugation in a Bio-Lion XC-H165 centrifuge at room temperature for 5 min at 2,000 x *g* and then for 10 min at 10,000 x *g*. The supernatant fraction was saved as the cell extract. Because the extract of *S. saprophyticus* was particularly active, it was diluted 1/3 with urease resuspension buffer before further use.

2.6 Urease assays

Urease activity was determined using a colorimetric assay in which ammonium formation was measured by the phenol-hypochlorite method [52]. A sample of a cell extract or a whole cell suspension was added to a 50 mM HEPES, 1 mMNa_2EDTA, 25 mM urea buffer, pH 7.5, without or with a particular inhibitor to give a total volume of 2000 µl (2.0 ml).In most cases, 10 to 15µl of a cell extract or 200 µl of exponential-phase cells were used. After 30 to 120min at 37 ºC depending on the amount of activity, three replicate 100 µl samples were removed and added to 1.5 ml of Solution A (1.0 g phenol, 5 mg sodium nitroprusside [sodium nitroferricyanide (III)] in 100 ml water). 1.5 ml of Solution B (0.5 g NaOH, 870 µl sodium hypochlorite in 100 ml water) were immediately added and the solution rapidly mixed. When all of the samples were collected, they were incubated at 37 ºC for 30 min and the absorbance determined at 625nm in a Shimadzu U160 UV-visible spectrophotometer. Ammonium concentrations were determined using an NH_4Cl standard curve. Results were normally expressed as a percent of the control reaction ±one standard deviation of the replicate assays. All experiments were done at least twice.

2.7 Inhibition studies in artificial urine medium

To assess whether some of the urease inhibitors could affect the increase in pH that normally occurs when the bacteria are grown in urine or an artificial urine medium, an overnight culture of *S. saprophyticus* or *P. mirabilis* was diluted 1/50 or 1/100 into 25 ml of artificial urine medium without or with a potential inhibitor. The cultures were incubated with aeration at 37 ºC. Each hour for 6 to 8 hr, the turbidity of the culture was determined in a Klett Summerson colorimeter with a red 660_{nm} filter and a 2.0 ml sample was removed for the determination of pH. The bacterial suspension was

centrifuged in a microcentrifuge for 5 minto remove the bacteria and any insoluble material and the pH of the medium determined with a micro-combination electrode and a Thermo Scientific 720A+ pH meter.

3 Results

3.1 Inhibition of urease activity in extracts of *S. saprophyticus* and *P. mirabilis* by Uva Ursi

To compare the sensitivities of the ureases from *S. saprophyticus* and *P. mirabilis* to inhibition by different Uva Ursi over-the-counter preparations, increasing volumes of four different samples were added to standard urease assay reactions. The enzyme from *S. saprophyticus* was most sensitive to inhibition by the Nature's Answer® sample (Fig 1 panel A). Activity was reduced about 70% by 100 µl of the extract. Larger volumes of the extract appeared to have no effect because of the dark color of the Uva Ursi preparation. The preparations from Hawaii Pharm LLC and Honey Combs Industries LLC were less inhibitory while the extract from Solaray® Uva Ursi leaves had an intermediate effect. Similar results were obtained with the enzyme from *P. mirabilis*, but the urease was more sensitive to all of the preparations (Fig 1 panel B).

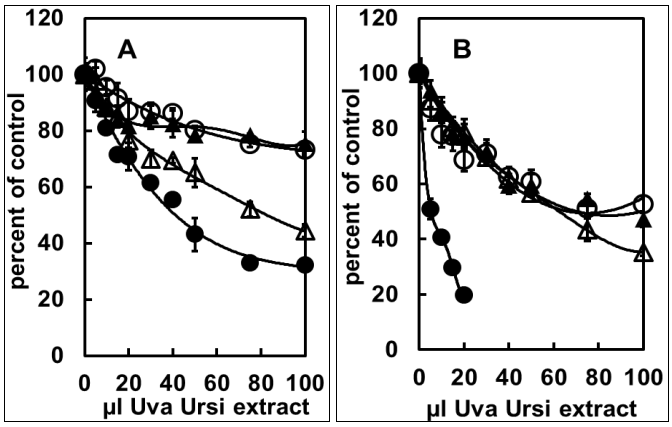


Fig 1: Inhibition of urease activity in an extract from *Staphylococcus saprophyticus* (panel A) and *Proteus mirabilis* (panel B) by Uva Ursi preparations. The specific activities of the urease extracts were 420 nmol/min (mg protein) and 98.4 nmol/min (mg protein), respectively. Each panel shows the effects of the Nature's Answer Uva Ursi preparation (●), the Hawaii Pharm LLC preparation (○), the Honey Combs Industries LLC preparation (▲), and the Solaray Uva Ursi leaf extract (△). Increasing volumes of inhibitor were added to standard 2.0 ml reaction mixtures and incubated at 37 ºC for 30 min. Three replicate 100 µl aliquots were removed and the ammonium ions determined using the colorimetric assay. The results were expressed as a percent of the control activity ± one standard deviation.

Commercial Uva Ursi products have no standardized information about their content, and although the active component of Uva Ursi extracts is thought to be arbutin [47-4] the concentration of this compound is not indicated on the labels. To quantitate the amount of arbutin in these samples, a colorimetric assay based on the reaction of this glycoside with 4-aminoantipyrine was used [51]. Based on an arbutin standard curve, the concentrations of arbutin equivalents were 65.8 mg/ml for the Nature's Answer® extract, 9.91 mg/ml for the Solaray® extract, 3.44 mg/ml for the Hawaii Pharm LLC extract, and 1.25 mg/ml for the Honey Combs LLC extract. This was generally consistent with their effectiveness as inhibitors of both the *S. saprophyticus* and *P. mirabilis* ureases. However, the colorimetric reaction is not entirely specific for arbutin [53]. In similar reactions with catechin or epigallocatechin gallate, these other glycosides showed about 15% of the reactivity as arbutin. Thin layer chromatography of arbutin and hydroquinone along with the Uva Ursi extracts on Silica Gel sheets revealed characteristic blue spots for the standards but complex streaks for the extracts after spraying with Berlin Blue reagent.

3.2 Inhibition of urease activity in extracts of *S. saprophyticus* and *P. mirabilis* by arbutin, hydroquinone, and 1,4-benzoquinone

To refine these results, the sensitivities of the ureases from *S. saprophyticus* and *P. mirabilis* to pure samples of arbutin, hydroquinone, and 1,4-benzoquinone were determined. The enzyme from *S. saprophyticus* was not inhibited by increasing volumes of50 mg/ml arbutin; it was inhibited about 80% by 100 µl of 50 mg/ml hydroquinone and only slightly sensitive to 10 mg/ml 1,4-benzoquinone (Fig 2 panel A). The enzyme from *P. mirabilis* also was not inhibited by increasing volumes of 50 mg/ml arbutin, but was much more sensitive to both hydroquinone and 1,4-benzoquinone (Fig 2 panel B). It was inhibited more than 95% by 100 µl of 1 mg/ml hydroquinone or by 100 µl of 0.1 mg/ml 1,4-benzoquinone.

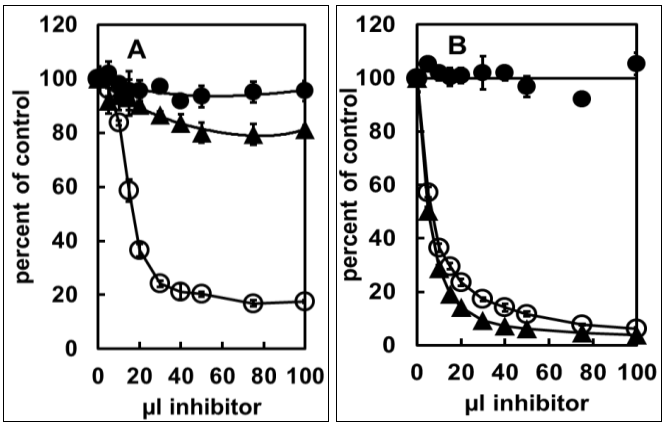


Fig 2: Inhibition of urease activity in soluble extracts of *Staphylococcus saprophyticus* (panel A) and *Proteus mirabilis* (panel B) by chemicals in Uva Ursi preparations. The specific activities of the urease extracts were the same as in Fig. 1. For panel A, the concentration of arbutin (●) was 50 mg/ml, the concentration of hydroquinone (○) was 50 mg/ml, and the concentration of 1,4-benzoquinone (▲) was 10 mg/ml. For panel B, the concentration of arbutin (●) was 50 mg/ml, the concentration of hydroquinone (○) was 1 mg/ml, and the concentration of 1,4-benzoquinone (▲) was 0.1 mg/ml. Increasing volumes of inhibitor were added to standard 2.0 ml reaction mixtures and incubated at 37 ºC for 30 min. Three replicate 100 µl aliquots were removed and the ammonium ions determined using the colorimetric assay. The results were expressed as a percent of the control activity ± one standard deviation.

3.3 Kinetics of urease inhibition

The kinetics of inhibition of the *S. saprophyticus* urease by hydroquinone was determined using varying concentrations of urea and several volumes of 50 mg/ml hydroquinone. A Line weaver-Burke plot of the data was consistent mixed inhibition in which the V_{max} gradually decreased and the K_m gradually increased (Fig 3 panel A). The lines intersected at the left of the Y axis. Similar mixed inhibition kinetics were observed for the *P. mirabilis* urease using varying concentrations of urea and several volumes of 1 mg/ml hydroquinone (Fig 3 panel B) or several volumes of 0.1 mg/ml 1,4-benzoquinone (Fig 3 panel C). Lower concentrations of these inhibitors were used with the *P. mirabilis* urease because of its greater

sensitivity to them. The inhibition constant (K_i) for each case was determined by plotting the slopes of the lines (K_m/V_{max}) as a function of the inhibitor concentration [55, 56]. The values were found to be 2.4 mM for hydroquinone and the *S. saprophyticus* urease, 1.0 µM for hydroquinone and the *P. mirabilis* urease, and 0.46µM for 1,4 -benzoquinone and the *P. mirabilis* urease, respectively.

3.4 Inhibition of urease activity in whole cells of *S. saprophyticus* and *P. mirabilis*

The Nature's Answer® Uva Ursi extract and its active components also inhibited the urease activity in whole exponential-phase cells of *S. saprophyticus* and *P. mirabilis* after growth in P medium or in LB medium containing 100 mM urea, respectively. The urease in *S. saprophyticus* was inhibited by increasing volumes of Nature's Answer® Uva Ursi or 50 mg/ml hydroquinone (Fig 4 panel A). The enzyme was more sensitive to the pure hydroquinone. The urease in *P. mirabilis* was inhibited by increasing volumes of Nature's Answer® Uva Ursi, 1 mg/ml hydroquinone, or 0.1 mg/ml 1,4-benzoquinone (Fig 4 panel B). Again, lower concentrations of inhibitor were needed with the *P. mirabilis* enzyme.

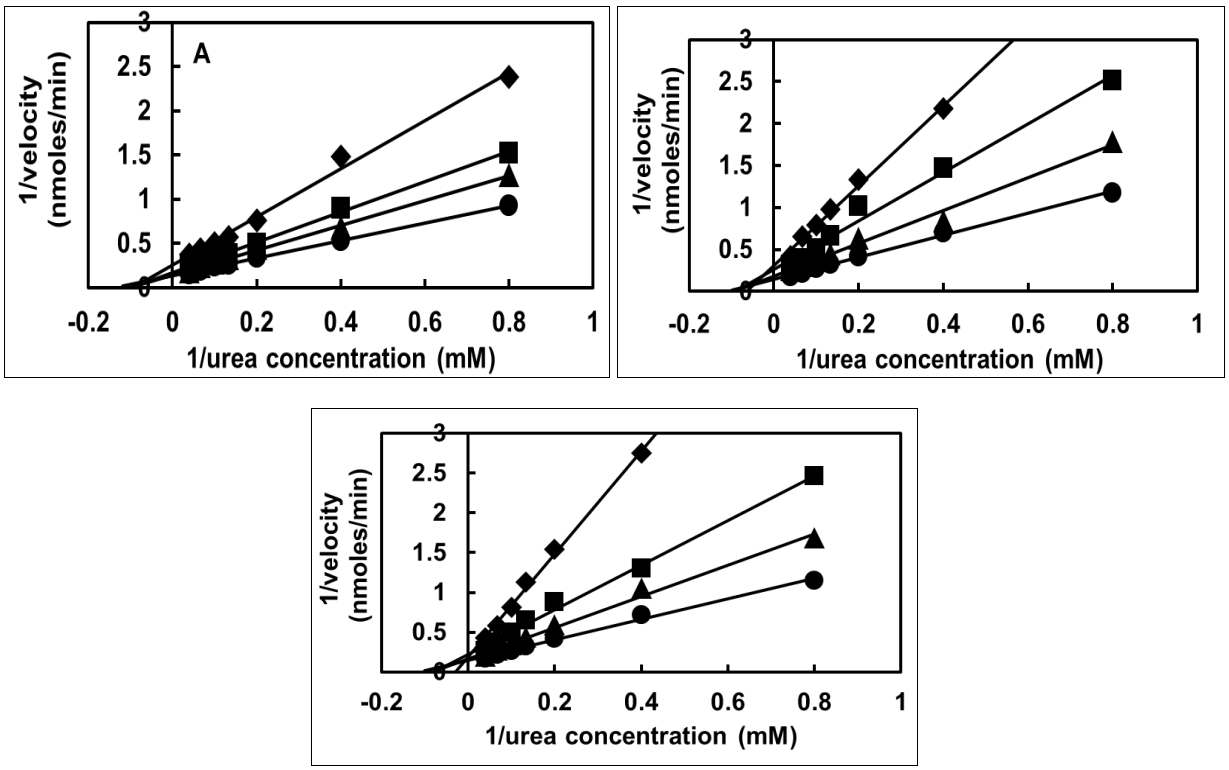


Fig 3: Kinetics of inhibition of urease activity by components of Uva Ursi extracts. Panel A shows a Line weaver-Burke plot of the data for the *S. saprophyticus* extract with 0 (●), 10 µl (▲), 15 µl (■), or 20 µl (◆) of 50 mg/ml hydroquinone. Panel B shows A Line weaver-Burke plot of the data for the *P. mirabilis* extract with 0 (●), 3 µl (▲), 5 µl (■), or 8 µl (◆) of 1 mg/ml hydroquinone. Panel C shows a Lineweaver-Burke plot of the data for the *P. mirabilis* extract with 0 (●), 3 µl (▲), 5 µl (■), or 8 µl (◆) of 0.1 mg/ml 1,4-benzoquinone. The reactions were run for 30 min at 37 ºC. The specific activities of the enzyme solutions were the same as in Fig. 1. All of the data points reflect triplicate assays that varied by <10%.

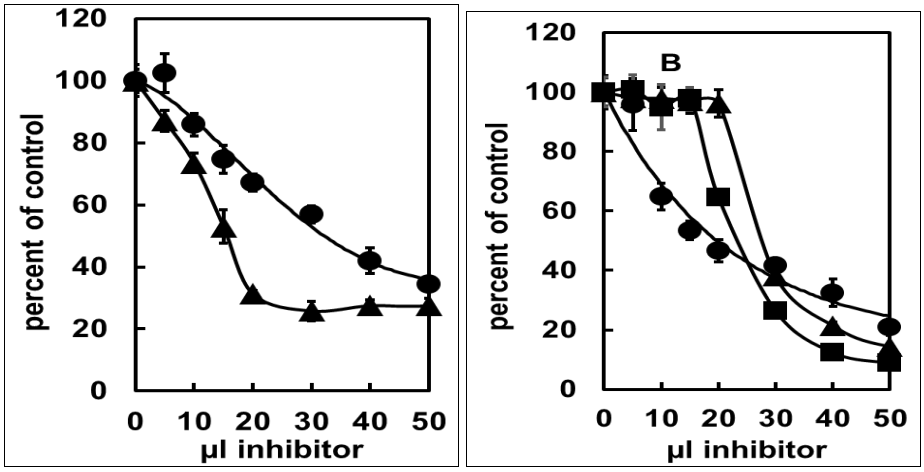
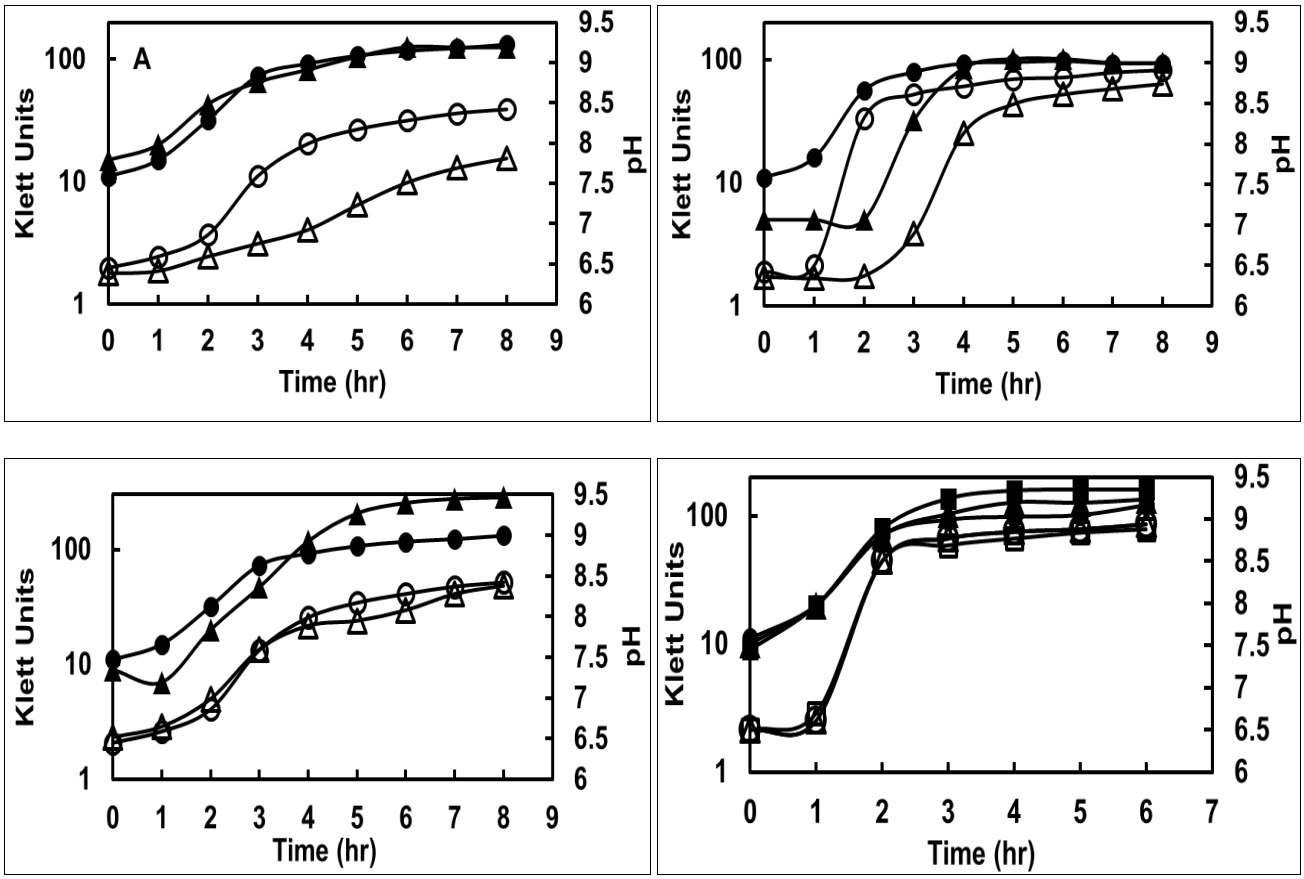


Fig 4: Inhibition of urease activity in exponential-phase whole cells. Panel A shows the effects of increasing volumes of Nature's Answer® Uva Ursi (●) or 50 mg/ml hydroquinone (▲) on urease activity in *Staphylococcus saprophyticus* grown in P. medium. Panel B shows the effects of increasing volumes of Nature's Answer® Uva Ursi (●), 1 mg/ml hydroquinone (▲), or 0.1 mg/ml 1,4-benzoquinone (■) on urease activity in *Proteus* mirabilis grown in LB medium containing 100 mM urea. All reactions contained a total of 2.0 ml and were incubated at 37 ºC for 120 min for *S. saprophyticus* for 30 min for *P. mirabilis*. Three replicate 100 µl aliquots were removed and the ammonium ions determined using the colorimetric assay. The results were expressed as a percent of the control activity ± one standard deviation.

3.5 Effect of Uva Ursi and its components on the pH of artificial urine medium cultures

In previous studies, we grew *S. saprophyticus* in an artificial urine medium and tested the effects of various compounds on the increase in pH that can occur as a result of urease activity [32, 50]. We found that some chemical inhibitors such as fluorofamide and acetohydroxamic acid and some over-the-counter products such as green tea extract could delay the increase in pH; however, others such as catechin had no effect. To extend these studies to Uva Ursi, *S. saprophyticus* and *P. mirabilis* were grown in an artificial urine medium without or with 1% (v/v) Nature's Answer® Uva Ursi. For *S. saprophyticus* grown in this medium in the absence of Uva Ursi extract, there was a gradual increase in pH as the bacteria grew (Fig. 5 panel A). Addition of 1% (v/v) Uva Ursi extract slowed the growth and delayed the increase in pH. For *P. mirabilis* grown in artificial urine medium in the absence of Uva Ursi extract, growth was more rapid but the final turbidity was lower (Fig. 5 panel B). The pH rose more rapidly and reached a more basic level than seen with *S. saprophyticus*. Addition of 1% (v/v) Uva Ursi extract increased the length of the lag phase with *P. mirabilis* but the final turbidity was about the same. There was some delay in the increase in pH, but this was less dramatic then seen with *S. saprophyticus* and the final pH was still about 8.9.

To determine if hydroquinone affected bacterial growth and pH in artificial urine medium, *S. saprophyticus* was grown in the absence or presence of 1 mg/ml hydroquinone (Fig. 5 panel C). There was no inhibition of bacterial growth although there was a slightly longer lag phase. The cultures became darker and darker in red color as growth occurred and this color contributed to the higher apparent turbidity of the culture. A comparison of the absorption spectrum of the medium with that of pure 1,4-benzoquinone indicated that they were the same. The red color thus appeared due to the spontaneous conversion of hydroquinone to 1,4-benzoquinone. The increase in pH in the culture containing hydroquinone was the same as the control culture. In parallel experiments, *P. mirabilis* was grown in artificial urine medium in the absence or presence of 50 µg/ml or 100 µg/ml hydroquinone (Fig. 5 panel D). Again, there was no inhibition of growth and the pH increased in the same way as the control culture. Again, the cultures turned red in color as growth occurred. In similar experiments, *P. mirabilis* was grown in artificial urine medium in the absence or presence of 20µg/ml or 50 µg/ml 1,4-benzoquinone (Fig. 5 panel E). There was a noticeable increase in the length of the lag phase with both 20 µg/ml or 50 µg/ml 1,4-benzoquinone. However, the growth rates and final yields were similar to the control cultures. The increase in pH in the cultures containing 1,4-benzoquinone also showed a similar lag phase but then rapidly rose to the same final value. Lower concentrations of 1,4-benzoquinone had no effect and higher concentrations were completely inhibitory.



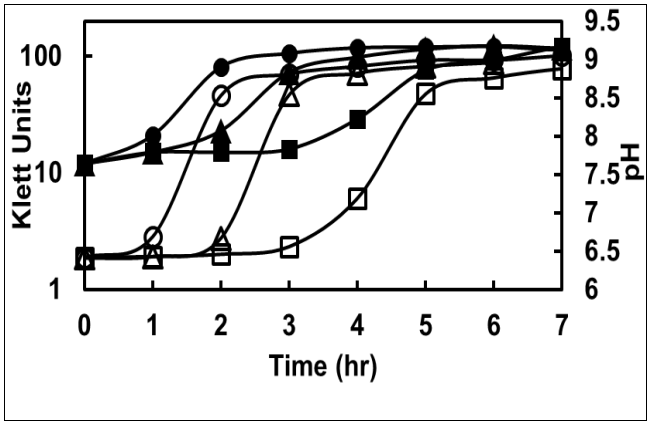


Fig 5. Effects of Uva Ursi extract, hydroquinone, and 1,4-benzoquinone on the growth and pH of cultures of *Staphylococcus saprophyticus* and *Proteus mirabilis* in an artificial urine medium. Panel A shows the turbidities of a control culture (●) of *S. saprophyticus* and one containing 1% (v/v) Nature's Answer Uva Ursi (▲) in artificial urine medium. It also shows the pH of the control culture (○) and the one containing Uva Ursi (△) at each time point. Panel B shows the turbidities of a control culture (●) of *P. mirabilis* and one containing 1% (v/v) Nature's Answer Uva Ursi (▲) in artificial urine medium. It also shows the pH of the control culture (○) and the one containing Uva Ursi (△) at each time point. Panel C shows the turbidities of a control culture (●) of *S. saprophyticus* and one containing 1 mg ml^{-1} hydroquinone (▲) in artificial urine medium. It also shows the pH of the control culture (○) and the one containing hydroquinone (△) at each time point. Panel D shows the turbidities of a control culture (●) of *P. mirabilis*, one containing 50 µg/ml hydroquinone (▲), and one containing 100 µg/ml hydroquinone (■) in artificial urine medium. It also shows the pH of the control culture (○), the one containing 50 µg/ml hydroquinone (△), and the one containing 100 µg/ml hydroquinone (□) at each time point. Panel E shows the turbidities of a control culture (●) of *P. mirabilis*, one containing 20 µg/ml 1,4-benzoquinone (▲), and one containing 50 µg/ml 1,4-benzoquinone (■) in artificial urine medium. It also shows the pH of the control culture (○), the one containing 50 µg/ml 1,4-benzoquinone (△), and the one containing 100 µg/ml 1,4-benzoquinone (□) at each time point.

4. Discussion

These studies indicated that extracts of Uva Ursi (*Arctostaphylos uva-ursi*) were effective inhibitors of the urease activity in *S. saprophyticus* and *P. mirabilis*. Different commercial preparations varied in their activity which was generally correlated with their concentration of arbutin equivalents. The enzyme from *P. mirabilis* was consistently more sensitive to Uva Ursi extracts than the enzyme from *S. saprophyticus*. Neither enzyme was inhibited by arbutin. The enzyme from *S. saprophyticus* was partially inhibited by high concentrations of hydroquinone but not by 1,4-benzoquinone. The enzyme from *P. mirabilis* was more sensitive to hydroquinone and strongly inhibited at even lower concentrations by 1,4-benzoquinone. Kinetic analysis revealed hydroquinone and 1,4-benzoquinone were mixed inhibitors of urease from both *S. saprophyticus* and *P. mirabilis*, indicative of their binding to both free enzyme and enzyme-substrate complexes. Previous studies have indicated that hydroquinone and 1,4-benzoquinone can inhibit urease activity through interactions with cysteine residues at the active site [57, 58]. While such residues occur in the enzyme from *P. mirabilis*, they are absent from all of the subunits of urease from *S. saprophyticus* [59-61]. We previously showed that the enzyme from *S. saprophyticus* was resistant to inhibition by omeprazole and other compounds that react with cysteine [32, 56].

The Nature's Answer® Uva Ursi extract also inhibited urease in whole cells of *S. saprophyticus* and *P. mirabilis*. No membrane transporter appeared to be required. Again, the enzyme from *P. mirabilis* was more sensitive to inhibition that the one from *S. saprophyticus*. As with the extracts, hydroquinone had a modest effect on the urease from *S. saprophyticus* and 1,4 benzoquinone had no effect. Both hydroquinone and 1,4-benzoquinone were good inhibitors of the urease in whole cells of *P. mirabilis*. Addition of 1% (v/v) Nature's Answer® Uva Ursi extract to bacterial cultures in an artificial urine medium slowed the growth and delayed the increase in pH. The pH in cultures of *S. saprophyticus* did not rise as fast and did not reach as high a final level as in the control cultures. The pH in cultures of *P. mirabilis* rose more rapidly and reached a level similar to the control cultures; the final pH was more basic level than that seen with *S. saprophyticus*.

Addition of 1 mg/ml hydroquinone to cultures of *S. saprophyticus* in artificial urine medium had little effect. Surprisingly, addition of 50 µg/ml or 100 µg/ml hydroquinone to cultures of *P. mirabilis* in artificial urine medium also had little effect. These experiments were complicated by the oxidation of hydroquinone to 1,4-benzoquinone, which had a noticeable brown color and tended to obscure its effect on growth. Addition of 20 µg/ml or 50 µg/ml 1,4-benzoquinone to cultures of *P. mirabilis* in artificial urine medium led to a noticeable increase in the length of the lag phase but the final growth rates, yields, and pHs were similar to the controls. The effects thus seemed to be nonspecific rather than a direct consequence of the inhibition of urease activity.

The observations that arbutin did not inhibit urease activity and that hydroquinone and 1,4-benzoquinone were not able to prevent the increase in pH associated with urease activity suggest that these compounds are unlikely to be effective as pharmacological treatments for urinary tract infections. On the other hand, the Nature's Answer® Uva Ursi extract did work well, particularly for *S. saprophyticus*. The extracts are complex mixtures [45, 46], and so these results suggest that other components of the Uva Ursi extract may be acting as urease inhibitors. As the next phase of this project, we propose to fractionate the Uva Ursi extract more completely and to test other components as urease inhibitors both *in vitro* and in bacterial cultures.

5. Conclusions

Over-the-counter herbal preparations of Uva Ursi are complex mixtures which vary in their concentrations of arbutin and other compounds that may prevent or control urinary tract infections. The Nature's Answer® Uva Ursi extract inhibited

the urease activity in extracts and whole cells of *S. saprophyticus* and *P. mirabilis*. Although neither enzyme was inhibited by arbutin, hydroquinone was a good inhibitor of the *S. saprophyticus* urease and hydroquinone and 1,4-benzoquinone were good inhibitors of the *P. mirabilis* urease. Hydroquinone could partially inhibit urease in whole cells of *S. saprophyticus* and both hydroquinone and 1,4-benzoquinone could inhibit urease in whole cells of *P. mirabilis*. Addition of Nature's Answer® Uva Ursi to cultures of *S. saprophyticus* in artificial urine medium slowed the increase in pH that results from urease activity. The preparation was less effective against *P. mirabilis* cultures in artificial urine, although the length of the lag phase increased in cultures treated with 1,4-benzoquinone and there was a similar lag in the change in pH. These results indicate that Uva Ursi preparations should be used with caution for the treatment of urinary tract infections since they vary in concentration and the bacterial pathogens with urease activity differ in their sensitivity to them.

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7. Conflicts of Interest
I declare that there are no conflicts of interest.

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