The Efficacy of Citric Acid for Dissolving Calcium Oxalate
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Abstract
Calcium oxalate renal calculi are one of the most common types of kidney stones. Research has shown that citric acid can prevent calcium oxalate crystallization and that extracts of Bryophyllum pinnatum may be able to reduce the size of existing calcium oxalate crystals in vivo. Bryophyllum pinnatum extracts contain a high concentration of citric acid, which may contribute to its ability to dissolve renal calculi. The purpose of this study was to determine the efficacy of citric acid for dissolving calcium oxalate. Calcium oxalate powder, contained in dialysis tubing, was incubated in solutions of water, citric acid or EDTA for ten days and the change in mass was measured. Citric acid was shown to have no significant effect, compared with water alone and is unlikely to be the component in B. pinnatum extracts that are responsible for reducing the size of calcium oxalate calculi.

Introduction
Renal calculi are caused by excessive salt concentrations in urine (Coe et al. 2005). Common renal calculi may consist of calcium oxalate, calcium phosphate, struvite (magnesium ammonium phosphate), uric acid, cystine, or ammonium acid urate; imbalances in these salts can precipitate into insoluble solids that may cause extreme discomfort and require invasive surgery to remove (Coe et al. 2005). While the cause of kidney stone formation is largely unknown, the analysis of microRNA expression profiles, which have been demonstrated to accurately predict susceptibility for kidney stones, suggests that there may be a genetic component to kidney stone formation (Wang et al. 2014). A recent analysis of renal calculus demonstrated the presence of specific bacteria, including Escherichia coli, Enterococcus spp., Klebsiella spp., Enterobacter spp., and Proteus mirabilis, that are commonly embedded throughout the stone matrix, including the nidus (Tavichakorntrakoo et al. 2012). Subsequent research has demonstrated that P. mirabilis and E. coli actively promote calcium oxalate crystallization in vitro suggesting that bacterial infections may be the cause of at least some kidney stones (Tavichakorntrakoo et al. 2012, Ong 2014). Citric acid has been shown to inhibit the formation of calcium oxalate kidney stones (Meyer and Smith, 1975, Ebrahimipour et al. 1991), and recent research has demonstrated that extracts of Bryophyllum pinnatum, a plant traditionally used in many parts of the world to treat kidney stones, are effective in reducing the size of existing kidney stones (Gahlaut et al. 2012). Extracts of this plant reportedly contain high levels of citric acid, potassium malate, ascorbic acid, and malic acid (Okwu and Josiah 2006), raising the possibility that citric acid may also play a role in dissolving calcium oxalate stones. The purpose of this study was to investigate the efficacy of citric acid in dissolving calcium oxalate crystals.

Methods
The ability of citric acid to dissolve calcium oxalate was determined by placing 0.2 g calcium oxalate powder inside sealed packets of dialysis tubing (Spectra/Por Regenerated Cellulose 6-8KD MWCO 32 mm) and soaking in solutions of deionized water, citric acid (1, 10 and 100 mM), or EDTA (1, 10 and 100 mM) for ten days at room temperature with vigorous stirring. Calcium oxalate packets were dried and weighed before and after treatment and the difference in mass was averaged for each treatment. The means of the differences were analyzed by ANOVA.

Figure1. The dialysis tube contained 0.2 g calcium oxalate powder

Results
Following the 10 day incubation period the differences in mass of calcium oxalate was determined for each treatment (Table 1). While 100 mM citric acid was observed to reduce the mass of calcium oxalate by an average of 38.8% grams, the values obtained were not significant relative to water alone (6.1%, p = 0.131). The ability of 100 mM EDTA, which, as a chelator, is known to dissolve calcium oxalate crystals, was demonstrated to significantly reduce the mass of calcium oxalate (56.6%, p = 0.006) (Table 1).

Table 1. The average difference and p-value of each treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average Change (grams)</th>
<th>Percent Change (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.015 ± 0.001</td>
<td>6.12%</td>
<td>n/a</td>
</tr>
<tr>
<td>1 mM Citric acid</td>
<td>0.014 ± 0.001</td>
<td>7.22%</td>
<td>1.000</td>
</tr>
<tr>
<td>10 mM Citric acid</td>
<td>0.015 ± 0.015</td>
<td>7.76%</td>
<td>1.000</td>
</tr>
<tr>
<td>100 mM Citric acid</td>
<td>0.126 ± 0.111</td>
<td>38.84%</td>
<td>0.131</td>
</tr>
<tr>
<td>1 mM EDTA</td>
<td>0.040 ± 0.044</td>
<td>19.22%</td>
<td>0.990</td>
</tr>
<tr>
<td>10 mM EDTA</td>
<td>0.087 ± 0.034</td>
<td>34.43%</td>
<td>0.237</td>
</tr>
<tr>
<td>100 mM EDTA</td>
<td>0.187 ± 0.017</td>
<td>56.63%</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Discussion
Recent research has demonstrated that potassium citrate can prevent the formation of calcium oxalate kidney stones both in vitro and in vivo (Ebrahimipour et al. 1991, Ho et al. 2013). Reports that B. pinnatum extracts, which are rich in citric acid, actively reduce the size of both calcium oxalate and struvite kidney stones suggest that citric acid may also help dissolve pre-existing calcium oxalate stones (Gahlaut et al. 2012). However, this study failed to demonstrate the ability of citric acid to dissolve calcium oxalate, at concentrations up to 100 mM. While EDTA, a chelator of divalent metals, is able to dissolve calcium oxalate crystals, this compound is not absorbed through the epithelial cells of the intestinal tract; therefore, oral administration of EDTA is unlikely to accumulate to appreciable levels in the kidney (Downes and McDonald 1964). Further studies should be performed to determine if other components of the B. pinnatum extract directly confer an ability to reduce the size of existing calcium oxalate kidney stones or if components of the extract mediate changes in host gene expression that affect stone integrity.

Conclusions
Citic acid, a highly component of Bryophyllum pinnatum, has investigated that it has no direct function of dissolving calcium oxalate, the kidney stone major component. Other substances such as potassium malate, ascorbic acid, and malic acid are also found in B. pinnatum may have the ability of dissolving calcium oxalate, and be used for the treatment of common kidney stone.

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References