Research Article

Assessment of in vitro anti-urolithiasis potential of Berberis aristata DC.

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Abstract

Urolithiasis is a highly prevalent and excruciating urological condition characterized by the accumulation of stones within the urinary system's walls. Kidney stones also referred to as nephrolithiasis, urolithiasis, or ureterolithiasis, are classified based on their specific location within the urinary system. Traditional approaches to manage urolithiasis include allopathic medications, surgical interventions, and shockwave lithotripsy, all of which are associated with various side effects. Due to these associated drawbacks, increased focus on exploring natural medicine for both prevention and management of urolithiasis has taken momentum. Anti-urolithiatic activity encompasses the capacity of specific plants and their extracts to prevent or alleviate urolithiasis, commonly known as kidney stones. Throughout various cultures, numerous plants have traditionally been employed as natural interventions to ward off or address kidney stones. One such potential herbal plant for the management of urolithiasis is Berberis aristata (Indian barberry or Tree Turmeric) which is known for its use in the management of urolithiasis in folk medicine. This plant is notably rich in alkaloids, with berberine being the primary alkaloid that contributes to its antioxidant as well as anti-inflammatory effects. In the present in vitro study, aqueous root extract of Berberis aristata (BAE) at varying concentrations, was examined for its effectiveness in preventing the formation of calcium oxalate crystals when introduced alongside the extract. The results revealed the effectiveness of the extract in preventing crystallization, suggesting its potential utility in managing urinary stone formation. Nevertheless, further investigation is required, particularly in suitable animal models, to comprehensively assess the anti-urolithiatic activity of Berberis aristata.

Keywords aggregation assay, calcium oxalate, simultaneous flow static model, urolithiasis

Introduction

Urolithiasis is a disorder within the urinary system that is characterized by the development of calculi/ stones that can be formed in different parts of the urinary system, such as the kidneys, bladder and ureter, giving rise to associated conditions known as nephrolithiasis, cystolithiasis and ureterolithiasis, respectively [1]. The presence of kidney stones can have an adverse impact on one’s health and pose a financial load. Kidney stone disease is not isolated but rather connected to a range of other medical conditions, including fractures, hypertension, metabolic disorders, chronic kidney disease, a heightened risk of coronary artery disease (CAD), and diabetes (particularly insulin-dependent diabetes).
This interconnection has led to its classification as a systemic disorder [2]. Urolithiasis is one of the oldest known medical conditions with a continuous rise in its incidence and prevalence [3]. There has been a 48.57% rise in cases of urolithiasis since the year 1990 to the year 2019 [4]. The north-western region of India is termed a stone belt as the prevalence of urolithiasis is very high in this region and the formation of calculi in the urinary system affects about 12% of the total world population [5]. The incidence rate of urolithiasis is about 11% in males and 7% in females [6]. Urolithiasis manifests with symptoms such as hematuria (the presence of blood in urine), dysuria (painful urination), pyuria (the presence of pus in urine), renal colic, and oliguria (reduced urine excretion). These symptoms occur due to the obstruction of the urinary tract [7]. The blockage prompts the precipitation of less soluble and insoluble salts, primarily phosphate and oxalate salts. Within the Indian context, kidney stone formation is a prevalent issue marked by a tendency for recurrence, and its impact is not limited to this country but extends across the globe. Urolithiasis is a condition in which the renal stones formed within the kidney (a condition termed nephrolithiasis) exit the renal pelvis and move into the remaining urinary collecting system which includes ureters, bladder, and urethra [8]. The formation of stones begins with minuscule crystals which then later grow into stones and build up in the walls of the urinary system. The nomenclature of kidney stones is as per their location in the urinary system like nephrolithiasis, urolithiasis, and ureterolithiasis [9]. The relapse rate of urolith development in the renal system is about 50% in 5-10 years [3]. Most commonly the uroliths consist of calcium oxalate stones (75-90%), followed by struvites i.e., magnesium ammonium phosphate (10-15%), uric acid (3-10%), and cystine (0.5–1%) stones [10]. The process of supersaturation and nucleation are major etiology involved behind urolithiasis. Supersaturation of salts for example, calcium oxalate followed by nucleation and union of ions in the solution to create a solid phase leads to stone/ urolith formation [11]. According to epidemiological studies, dietary preferences are one of the significant risk factors for urinary stones formation. Other factors responsible for de novo synthesis of uroliths include age, gender, and several metabolic abnormalities [12]. There are certain elements that play a crucial role in the development of urolithiasis including non-urinary factors like abnormal calcium metabolism and urinary factors such as excessive urinary calcium excretion, hypocitraturia, urine oxalate, and uric acid excretion [13]. Certain dietary elements such as sodium, protein, oxalate, calcium, and amount of fluid consumption can affect the excretion of urinary lithogenic substances and contribute to the risk of urolithiasis [14]. India has a longstanding tradition of embracing naturopathy as a potential therapeutic approach, with its roots extending back to Vedic times (1500-1000 B.C.). Although different modern treatment protocols have been adopted for the management of urolithiasis the traditional ethnobotanical preparations have been found very effective in the management of urolithiasis and, as they are effective in action and cheaper as compared to their chemical counterparts with fewer side effects.

There are several evidences that suggest that the addition of plant-based food, medicinal and herbal supplements to the staple diet of people can prevent urolithiasis and the anti-urolithic activity ascribed because of their antioxidant, anti-spasmodic, diuretic effects and inhibition of crystallization, nucleation, and crystal aggregation [15]. The medicinal plants from the genus Berberis of the family Berberidaceae are very important in the traditional medicine system [16]. One such important plant is Berberis aristata DC. of the family Berberidaceae which is commonly known in Sanskrit as Daruharidra and in English as Tree Turmeric which is known for its use in the management of urolithiasis in folk medicine. The major alkaloid present in Berberis aristata is berberine which possesses very important pharmacological activities and may be present either in roots, stem bark, or leaves followed by palmatine [17]. The HPLC chromatogram indicated the presence of berberine chloride as the main component and the other components were karachine, aromoline, oxyberberine, oxyacanthine, and berbamine in the crude ethanolic extract of the Berberis aristata roots [18]. The plant is widely used in the treatment of urinary issues, skin disorders, pores, syphilis, rheumatism, and diarrhea and it is widely used as a tonic, demulcent, diaphoretic, and diuretic [19]. Thus, from the wide range of pharmacological research, it is clear that the Indian barberry possesses various properties such as antioxidant, anti-inflammatory, anti-cancer, anti-bacterial, anti-microbial, anti-ulcer, anti-coagulant, and anti-diabetic [20]. Berberis aristata is a good source of antioxidants and can normalize the levels of endogenous antioxidants viz. glutathione, catalase, and superoxide dismutase, and decrease the level of pro-oxidants viz. TBARS and Nitric oxide [21].
antioxidant properties may be responsible for the anti-urolithiatic properties of *Berberis aristata* [22]. *Berberis aristata* root bark decoction is found efficient against cisplatin-induced nephrotoxicity in rats [23]. The present study is conducted to evaluate the anti-urolithiatic potential of aqueous root extract of *Berberis aristata* by using various *in vitro* assays.

**Methodology**

**Chemicals**
AR-grade chemicals were used in the study viz., Calcium chloride, Calcium acetate, Sodium carbonate, sodium oxalate, sodium chloride, Trisodium phosphate, and Tris. These chemicals were procured from Himedia (India) and SRL (India). Various buffers and solutions were prepared using autoclaved distilled water.

**Collection of Plant Material**
The roots of the plant bioresource used in the present study were collected from the Kumaon region (Berinag, Distt. Pithoragarh) of Uttarakhand and authenticated by Dr. D.S. Rawat, Assistant Professor, Department of Biological Sciences, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India (Voucher specimen No.- 1207).

**Preparation of the plant extract**
The process of extract preparation included various steps such as proper cleansing of plant material using tap water followed by distilled water, shade drying till the moisture gets evaporated, and after complete drying the plant material was pulverized using an electric grinder to make a fine powder. 50 grams of the dried powder was immersed in 250 mL of double distilled water followed by homogenization at 37°C for a period of 72 hours in an incubator cum shaker. Afterward, this mixture was filtered through the muslin cloth followed by Whatmann filters paper No. 1. The aqueous extract of *Berberis aristata* (BAE) was then kept in a water bath at 37°C-40°C to evaporate the water followed by lyophilisation and then it was stored at -20°C till further use.

**In vitro assays to evaluate the anti-urolithiatic activity of the plant extract**
Nucleation, aggregation, and calcium oxalate mineralization inhibition assays were used to estimate anti-urolithiatic activity of the plant extract. Crystallization/ inhibition assays were conducted with BAE and without BAE to estimate its inhibition capability.

**Nucleation assays**
The method used for nucleation assay was illustrated by Patel et al. [24] with slight alterations. A solution composed of calcium chloride (CaCl₂) and sodium oxalate (Na₂C₂O₄) at a concentration of 5 mmol/L and 7.5 mmol/L respectively, was prepared in a buffer comprising of Tris-HCl (0.05mol/L) and sodium chloride, NaCl (0.15mol/L) buffer at a pH of 6.5. In distilled water, several dilutions of BAE ranging from 100-1000 µg/mL were formulated. 100 µl of BAE of different dilutions was mixed with 950 µl of CaCl₂ solution followed by the addition of 950 µl of Na₂C₂O₄ solution which started the crystallisation. The final mixture was incubated for a period of 1 hour at 37°C. 100 µL of buffer was added to CaCl₂ solution which was then used as a control in the present experiment and it was also incubated for a period of 1 hour at 37°C. The crystal nucleation was then analyzed under the microscope at 40X magnification.

**Aggregation assay**
The methodology used to perform the aggregation assay was as illustrated by Hess et al., [25] with few minor adjustments. CaCl₂ and Na₂C₂O₄ were then mixed to prepare a solution to make calcium oxalate (CaOx) crystals also known as “seed” in the experiment. Then, a solution consisting of CaCl₂ and Na₂C₂O₄ at a concentration of 6.0 mmol/L and 6.5 mmol/L respectively, was constituted in a buffer comprising of Tris-HCl (0.05mol/l) and NaCl (0.15mol/L) buffer at a pH of 6.5. Then, the degree of inhibition of
aggregation was assessed by the turbidity of the samples in the presence of BAE at different concentrations ranging from 100-1500 µg/mL, for which 950 µL of CaCl₂ solution was mixed with 100 µL of different dilutions of BAE. After this 950 µL of Na₂C₂O₄ was added which started crystallization. A mixture of 100 µL of buffer and CaCl₂ was used as a control in the present experiment. The final mixture was incubated for a period of 1 hour at 37°C. Then, the optical density (OD) of the crystallized suspension was observed at 620 nm and the turbidity obtained in the presence of BAE was compared to that of the control to monitor the inhibition of percentage aggregation.

The following formula was used to calculate the inhibition of percentage aggregation:

% Inhibition = [1 - (Turbidity of the sample / Turbidity of the control)] × 100

Inhibition of Calcium Oxalate mineralization

The four different experimental models viz., ‘simultaneous flow static model’ (S.S.M.), ‘simultaneous flow dynamic model’ (S.D.M.), ‘reservoir static model’ (R.S.M.) and ‘reservoir dynamic model’ (R.D.M.) were used to access the in vitro inhibition of mineralization of calcium oxalate due to BAE.

Simultaneous flow static model (S.S.M.)
The S.S.M approach was illustrated by Farook et al., [26] with a few minor adjustments. For this method, three separate burettes were taken, which were filled with 50 mL of Na₂C₂O₄ (0.01M), 50 mL of calcium acetate, Ca(C₂H₃O₂)₂ (0.01M), and 50 mL of BAE (1500 µg/mL). After that, all three solutions present in the three different burettes were allowed to drip gradually in a synchronous manner and same pace into a 250 mL beaker. After this step, the obtained mixture was allowed to digest in a hot water bath for a duration of 10 minutes which was then cooled down to ambient room temperature. A small volume of the obtained mixture was taken in a pre-weighed centrifuge tube which was then centrifuged and the precipitate was gathered and the supernatant liquid was discarded. The precipitate-filled tubes were then dried at 120°C using a hot air oven, followed by cooling down to ambient room temperature, and then weighed.

Simultaneous flow dynamic model (S.D.M.)
The procedure was the same as S.S.M. except that there was a continuous stirring of the reaction mixture in the beaker using a magnetic stirrer during the gradual dripping of the inhibitors and salt-forming solutions [26].

Reservoir static model (R.S.M.)
In R.S.M. both the salt-forming solutions were allowed to fall gradually in a dripping manner via burettes into a beaker containing BAE (50 mL), thus forming a reservoir of inhibitor into which the salt-forming solutions ran down. The remaining protocol was alike the S.S.M. [26].

Reservoir dynamic model (R.D.M.)
The methodology used in the R.D.M. was the same as that of R.S.M. except that there was a continuous stirring of the reaction mixture in the beaker using a magnetic stirrer during the experiment [26].

Results and Discussion

Effect of BAE on Nucleation Assay
In the present study, it was found that with the increase in the concentration of BAE there was a decrease in the aggregation of CaOx crystals and an increase in the percentage reduction of CaOx crystallization as presented in Figure 1.
Figure 1. Nucleation assay at different concentration of BAE observed under Microscope (40X)
(A) Control (B) 25 µg/mL (C) 50 µg/mL (D) 100 µg/mL (E) 250 µg/mL (F) 500 µg/mL (G) 750 µg/mL (H) 1500 µg/mL

Effect of BAE on calcium oxalate crystallization through aggregation assay
The in vitro aggregation assay showed that the BAE exhibits a dose-dependent inhibition of CaOx crystallization. An increase in the concentration of BAE led to an increase in the percent inhibition of aggregation. At 1500 µg/mL of BAE the maximal percentage aggregation inhibition of 84.16% was achieved. The results are presented in Table 1 and Figure 2.

Table 1. Percentage inhibition of calcium oxalate crystallization by BAE in aggregation assay

<table>
<thead>
<tr>
<th>SN.</th>
<th>Different concentration of BAE</th>
<th>Percent inhibition of Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>25 µg/ml</td>
<td>9.53</td>
</tr>
<tr>
<td>2.</td>
<td>50 µg/ml</td>
<td>30.50</td>
</tr>
<tr>
<td>3.</td>
<td>100 µg/ml</td>
<td>41.52</td>
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<td>4.</td>
<td>250 µg/ml</td>
<td>51.85</td>
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<tr>
<td>5.</td>
<td>500 µg/ml</td>
<td>61.26</td>
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<tr>
<td>6.</td>
<td>750 µg/ml</td>
<td>68.12</td>
</tr>
<tr>
<td>7.</td>
<td>1000 µg/ml</td>
<td>70.24</td>
</tr>
<tr>
<td>8.</td>
<td>1500 µg/ml</td>
<td>78.61</td>
</tr>
<tr>
<td>9.</td>
<td>1250 µg/ml</td>
<td>84.16</td>
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</table>

Effect of BAE on Inhibition of Calcium Oxalate mineralization
The percent reduction of BAE against CaOx crystal formation has been studied in different dynamic models such as S.S.M., S.D.M., R.S.M., and R.D.M. For this BAE of 1500 µg/mL concentration was taken and the precipitate formed was calculated at last and then percent inhibition of crystallization was calculated in different models. It was found to be highest in the reservoir static model (58.18%) and least in the simultaneous flow static model (32.78%). The result of percent inhibition due to BAE in different dynamic models is presented in Table 2 and Figure 3. Rhizomes and seeds of *Berberis aristata* are found to be useful in the management of stone diseases. Rhizome of *Berberis aristata* is one of the important constituents of commercial herbal formulation named Neeri that is used in the management of uroliths [27]. *Berberis aristata* leaves when formulated as a boiled potion are suggested to have curative efficacy against different kidney troubles as well as urinary tract infection (UTI) [28]. Berberine is an important isoquinoline alkaloid present in *Berberis aristata*. Berberine was also found to show potent protective efficacy against iron-induced nephrotoxicity in rats which is due to its antioxidant potential and
iron chelation [22]. Leaf extract of *Berberis aristata* was used to prepare the ZnO nanoparticles which were found to have potent anti-bacterial activity against important UTI-causing organisms viz. *Serratia marcescens, Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus,* and *Escherichia coli* and it was also having a moderate anti-oxidant potential [29]. Several species of the genus *Berberis* are known for their protective efficacy against renal stone formation. *Berberis trifoliata* is one such important plant of the family Berberidaceae whose methanolic extract was found to have anti-urolithiatic activity in rats [30]. Another plant of the same family is *Berberis vulgaris* and its crude aqueous-methanol extract was found to have anti-urolithiatic effect in rats with ethylene glycol-induced urolithiasis and as a result it can be used in managing condition of urolithiasis [31]. A homeopathic preparation of *Berberis vulgaris* root bark in an ultra-diluted dose was found to have anti-urolithiatic activity in rats [32]. In vitro crystallization, studies have shown that *Berberis vulgaris* inhibits calcium oxalate crystallization at nucleation and aggregation level which can be the mechanism involved behind the protective action of homeopathic preparation of the *Berberis vulgaris* against stone formation [33]. A homeopathic preparation of *Berberis vulgaris* root bark has a nephroprotective property that helps in reducing kidney stone-associated oxidative damage by enhancing the redox status of the kidney [34]. Overall, reduction in oxidative stress by neutralizing free radicals, and berberine may help protect against such damage. *Berberis aristata* exhibits anti-inflammatory properties and chronic inflammation can create an environment conducive to stone formation. The risk associated with calculi formation in the urinary tract can be mitigated by reducing inflammatory conditions in the tract. Thus, the in vitro assays performed in the current study suggest that aqueous extract of *Berberis aristata* (BAE) has critical anti-urolithiatic activity based on results of nucleation assay.

<table>
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<th>SN</th>
<th>Models</th>
<th>Percent Inhibition</th>
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</thead>
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<tr>
<td>1</td>
<td>Simultaneous flow static model</td>
<td>32.78</td>
</tr>
<tr>
<td>2</td>
<td>Simultaneous flow dynamic model</td>
<td>40.10</td>
</tr>
<tr>
<td>3</td>
<td>Reservoir static model</td>
<td>58.18</td>
</tr>
<tr>
<td>4</td>
<td>Reservoir dynamic model</td>
<td>33.30</td>
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</table>

Figure 2. Percentage inhibition of calcium oxalate crystallization by BAE in aggregation assay
aggregation assays and different dynamic models as there was suppression of nucleation along with inhibition of aggregation of calcium oxalate (CaOx) crystals which is a common component of kidney stones. Significant efficacy was found in the highest concentration of 1500 µg/mL. To further investigate the mechanism of action of the aqueous extract of *Berberis aristata*, more *in vivo* research is required. The active phytoconstituents present in the plant extract may be involved in the anti-urolithiatic activity of the plant which will require further characterization and isolation of the active compounds.

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References


