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Muhammad Ajmal Shah
Department of Pharmacognosy, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.

Sikandar Khan Sherwani
Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.

Mohammad Sualeh
Department of Pharmacognosy, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.

Sandaleen Kanwal
Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.

Haq Nawaz Khan
Department of Pharmaceutics, University of Karachi, Pakistan.

Shahana Urooj Kazmi
Department of Microbiology, University of Karachi, Pakistan.

Correspondence:
Muhammad Ajmal Shah
Department of Pharmacognosy, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.
Email: ajmal.shangla@gmail.com

In vitro anthelmintic and antiurolithic assessment of *Berberis lycium* root bark

Muhammad Ajmal Shah, Sikandar Khan Sherwani, Mohammad Sualeh, Sandaleen Kanwal, Haq Nawaz Khan, Shahana Urooj Kazmi

Abstract
Berberis lycium is one of the species belong to family Berberidaceae, an evergreen shrub growing in the Himalayan region. The various parts of the plant i.e. Root; bark, stem, leaves and fruits are used by local inhabitants as a medicine or food. Root bark of *Berberis lycium* has tremendous phytotherapeutic effects. The present study was conducted to evaluate *Berberis lycium* root bark for its anthelmintic and antiurolithic potential. During this study, *in vitro* anthelmintic as well as antiurolithic effects were assessed in both aqueous *Berberis lycium* root bark extracts i.e. decoction and infusion. Three concentrations, i.e. 25, 50 and 100 mg/ml of both infusion and decoction were evaluated in anthelmintic bioassay, which include the measurement of paralysis and death time of earthworm i.e. *Pheretima posthuma*. Both aqueous extracts showed considerable anthelmintic activity at a maximum concentration of extracts i.e. 100 mg/ml; both the paralysis and death times taken by decoction are lesser than infusion indicating its better efficacy. The reference standard Piperazine Citrate and control normal saline were used in the same concentration as that of extracts. The antiurolithic effect of *Berberis lycium* root bark was studied on different phases of calcium oxalate crystallization in artificial urine. Results showed that both decoction and infusion gave 92.4% and 80.2% inhibition of calcium oxalate crystallization at the concentration of 100% respectively. Furthermore, it has been observed that both aqueous extracts have a satisfactory inhibitory effect on the nucleation and growth phases of calcium oxalate crystallization.

Keywords: anthelmintic, antiurolithic, *Berberis lycium*, root bark

1. Introduction
Medicinal plants play a vital role in the healthcare of human being, according to World Health Organization, 80% of the world population still rely on medicinal plants for their primary health care needs, whereas more than 30% of the pharmaceutical preparations are plant-based [1]. The genus Berberis belongs to family Berberidaceae, where a majority of its species has tremendous phytotherapeutic potential. *Berberis lycium* is an evergreen plant known as Barberry in English or *Kashmal* in Hindi and *Ishkeen* in Urdu languages [2]. In appearance, it is a 2 to 3-meter sub-erect, rigid, spiny shrub [3]. *Berberis* is widely distributed in major continents of the world such as America, Europe and Asia. Like other species of *Berberis*, all parts of *Berberis lycium* possess significant phytotherapeutic benefits such as its root, bark, stem and fruits have antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, hepatoprotective, anticoccidial, pesticidal, antimutagenic and wound-healing properties [2-10]. Reported phytochemicals in *Berberis lycium* include Berberine, Palmatine, Palmatine chloroform, Berbamine, Aromoline, Oxyacanithine, Umbellatine, ß-sitosterole, Punjabine, Balochistanamine, Oxyberberine, Berberine chloroform [11]. Due to prevailing resistance to available anthelmintic agents and diverse therapeutic potential, physiotherapy is considered to be a treatment of choice in helminthes infections and urolithiasis [12-14]. Helminthes infections are among the most prevalent human infections globally. Such infections occur in human intestine due to parasitic worm infestation through contaminated water and food, resulting in various disorders such as weakness, loss of appetite, reduced feed efficiency, reduced weight gain and decreased efficiency. Worms take nutrients from the human body, causing malnutrition, which ultimately results in growth retardation [15-16]. Certain mixed and chronic types of helminthes infections are associated with life threatening disorders such as parasitic gastroenteritis, retarded cognitive development; iron-deficiency anemia [17]. Urolithiasis is one of the oldest globally occurring human disorders in which stone formation occurs in any part of the urinary system mainly associated with renal calculi and hyperoxaluria. Some parts of the world which are exceptional where urolithiasis rarely occur, such as Greenland and some coastal areas of Japan [14].

Among all kinds of renal calculi calcium containing stones are most common which are in the form of pure calcium oxalate i.e. 50 % or calcium phosphate i.e. 5 % or a combination of both i.e. 45 % [18]. Mechanism of kidney stone formation is not clearly understood, but it is generally believed to be a multistep process, including nucleation, aggregation and growth of insoluble crystals [19]. In normal individual's urine has the capability to restrain the crystallization process by its supersaturated nature with macromolecules, citrate and magnesium so the individuals with urolithiasis have insufficiency of such natural inhibition [20]. Urinary calculi are the third most prevalent disorder of the urinary system affecting 10% male and 3% female in their adult lives [20]. Urolithiasis is mostly a persistent ailment with a reversion rate of approximately 50% in 5–10 years and 75% in 20 years [21]. Few clinical procedures, are presently available for eradicating renal calculi such as extracorporeal shock wave lithotripsy, ureteroscopy and percutaneous nephrolithotomy but these are ineffective due to their high cost and side effects [13, 14, 22]. In urolithiasis many metabolic disorders are involved so a single chemical entity is clinically less effective. Phytotherapeutics having multiple constituents are considered to be more effective in comparison to chemical agents as they are multi targets with fewer side effects [23-26]. It has been clinically observed that urolithic patients feel severe spasmodic pain; this complication can also overcome as *Berberis lycium* root has the spasmolytic effect [27]. The present experiments were performed to investigate anthelmintic and antiurolithic potential of *Berberis lycium* root bark. Aqueous extracts, i.e. Infusion and decoction of *Berberis lycium*, were assessed *in vitro* for combating helminthes infections and urolithiasis.

2. Materials and Methods

2.1 Collection and authentication of plant

Berberis lycium was collected in Lilownai valley, District Shangla, Khyber Pakhtunkhwa in April, 2012. Complete plant with all parts was brought to Pakistan Natural History Museum, Islamabad, Pakistan where Dr. Seyed Anil Gilani, Senior Curator identified and authenticated the plant as *Berberis lycium.*

2.2 Aqueous extract preparation

The roots of the plant were brought to Research Laboratory, Department of Microbiology, Federal Urdu University of Arts, Science &Technology (FUUAST), Karachi, Pakistan. Roots were thoroughly washed with deionized distilled water; the bark was peeled off carefully and dried under shade. Dried bark was grinded for further study.

2.2.1 Preparation of infusion:

5 % Infusion of *Berberis lycium* root bark has been prepared by taking 5 grams of grinded bark in 100 ml deionized distilled water and left for 48 hours with casual shaking at room temperature [32] and later filtered with Whatman No. 1 filter paper to obtain a clear infusion. The filtrate was then passed through 0.22 micron filter and stored the infusion in small Eppendorf tubes in freezer for further work [28].

2.2.2 Preparation of decoction:

5 % Decoction of *Berberis lycium* root bark has been prepared by boiling 5 grams of grinded bark in 100 ml deionized distilled water in conical flask and later filtered with Whatman No. 1 filter paper to obtain clear decoction. The filtrate was then passed through 0.22 micron filter and stored the decoction in small Eppendorf tubes in freezer for further work [29].

2.3 Anthelmintic activity

The anthelmintic activity was conducted by following method adapted by Sherwani *et al*., 2013. Earth worm, i.e. The *Pheretima posthuma* model was used as it is much resembled with human intestinal parasite, round worms in terms of its anatomy and physiology as well as it is easily available [30-32].

2.3.1 Earth worm collection and authentication

Earthworms having the size range of 8 cm were collected from moist garden soil of FUUAST, Karachi, Pakistan and authenticated as *Pheretima posthuma* by Dr. Uzair Khan, MRCC, University of Karachi, Pakistan. All the collected earth worms were washed with saline and preserved in buffer saline [28].

2.3.2 Bioassay

Total eighteen earthworms were segregated into three groups i.e. each group comprises of six worms. The control group was treated with normal saline, standard with Piperazine Citrate and third group was treated with plant extracts. The concentrations used in all the three groups used in the experiment were 25 mg/ml, 50mg/ml and 100mg/ml [28, 31]. The mean paralysis time and death time of each earth worm was observed and recorded in minutes. Earthworm was considered to be paralyzed when it was not able to move even in normal saline whereas the earthworm was considered to be died when the worm completely lost its motility and its body color vanished [28, 32].

2.4 Antiurolithic activity

The antiurolithic effect of *Berberis lycium* root bark extract on Calcium Oxalate crystallization was determined by the time course measurement of turbidity changes owing to the crystallization in artificial urine on adding of 0.01M sodium oxalate solution. The Precipitation of calcium oxalate was measured in term of turbidity via UV/Visible spectrophotometer (620 nm) at temperature of 37°C and pH 6.8.

2.4.1 Synthesis of Calcium Oxalate crystals

The inhibitory effect of aqueous extracts on calcium oxalate crystallization was observed in the form of turbidity due to the crystal nucleation and aggregation while adding 0.01M sodium oxalate to synthetic urine. It was observed that calcium oxalate was precipitated at pH 6.8, temperature 37 °C and wavelength 620 nm via spectrophotometer in the form of turbidity.

2.4.2 Preparation of artificial urine

The artificial urine was formulated by following the method of Finlayson *et al.*, 1978, at a constant temperature of 37 ^{o}C in capped bottle. This standard model was selected because it is highly simple and easy to do. Following formula was followed for making artificial urine. All the chemical reagents were dissolved in deionized water and the pH was adjusted to 6.0 as shown in Table-1.

2.4.3 Observation without the addition of plant extract

1.0 ml of artificial urine and 0.5 ml distilled water was transferred into the cell and blank reading was taken on a spectrophotometer. Then 0.5 ml of 0.01 sodium oxalate was added and readings were taken with a time period of 10 minutes.

2.4.4 Observation in the presence of Berberis lycium root bark infusion and decoction

Different concentrations of *Berberis lycium* root bark decoction and infusion i.e. 25 %, 50%, 75% and 100% were tested for

calcium oxalate crystallization inhibition. 0.5 ml of each concentration was added to 1 ml of artificial urine and blank reading was taken through a spectrophotometer at 620 nm, then 0.5 ml of 0.01 M sodium oxalate was further added and the measurement was started for a period of 10 minutes. Three replicates were run for each experiment.

The percentage inhibition was determined by the following formula:

$$\%\text{inhibition} = \{1-[Si / Sc]\} X\ 100$$

Where; Si: slope of graph in the presence of extract (Test),
Sc: slope of without extract (Control).

2.4.5 Microscopic study
Artificial urine samples were centrifuged and the sediments were studied under a microscope for the appearance of calcium oxalate crystals, pictures were taken by digital camera. (Figure-1 and Figure-2)

3. Results and Discussion

Neglected tropical diseases classified by WHO are mainly associated with helminthes infections i.e. schistosomiasis, ascariasis and hookworm infection. These debilitating infections have persisted to impose stern disability and often fatalities. It is more prominent among the indigent inhabitants existing in marginalized regions of the [33, 34]. In developing world due to poor hygienic conditions helminthes infections are one of the major health related [16, 35]. Synthetic anthelmintic agents are used with 99% effectiveness rate against different susceptible worms, but a very small number of helminthes which left resistant lead to a resistant generation of worms resulting in anthelmintic resistance due to genetics, biological and operational factors [12].

In the present study, we explore anthelmintic and antiurolithic potential of *Berberis lycium* root bark. In anthelmintic assay control, i.e. Normal saline showed a paralysis time of earthworm at the concentration of 25 mg/ml, 50 mg/ml and 100 mg/ml i.e.96.46±0.46 minutes, 78.23±0.25 minutes and 62.43±0.11 minutes, whereas the death time was 160.06±0.11minutes, 142.2±0.34 minutes and 111.7±0.26 respectively. Comparatively Piperazine citrate, which was used as standard showed paralysis time at the concentration of 25 mg/ml, 50 mg/ml and 100 mg/ml i.e. 18.36±0.15minutes, 12.00±0.00 minutes, 08.84±0.03 minutes while the death time on the same concentrations were 54.36±0.40 minutes, 46.46±0.40minutes and 13.60±0.17 minutes respectively which is given in Table-2. Decoction of *Berberis lycium* root bark showed paralysis time at the concentration of 25 mg/ml, 50 mg/ml and 100mg/ml i.e. 39.20±0.11minutes, 19.13±0.25minutes and 13.23±0.22 minutes, whereas the death time on the same concentrations was 73.13±0.32minutes, 64.22±0.11minutes and 23.14±0.31minutes respectively as shown in Table-3. Similarly infusion of *Berberis lycium* root bark paralyzed the earthworm on 25 mg/ml, 50 mg/ml and 100 mg/ml concentrations i.e. 40.21±0.27 minutes, 21.15±0.13 minutes and 18.7±0.33minutes, while earthworm death time on the same concentrations i.e. 80.61±0.13 minutes, 73.2±12 minutes, 40.9±0.22 minutes given in Table-4. In urinary system, lipid peroxidation occur which produce oxygen free radicals resulting in cellular injury which facilitated aggregation of calcium oxalate crystals and their growth. The antioxidant potential of *Berberis lycium* root is already reported which might be responsible for its antiurolithic effect [11, 23]. In the present study, we observed the crystal synthesis in artificial urine, after adding the oxalate salt just in 5 minute crystal started to develop as shown in Figure-1. At the interval of 5 minutes 578 calcium oxalate crystals/ mm^3 were developed and aggregated initially, with the passage of time, number of crystals increases and it was noted that at the interval of 40 minutes 853 crystals/mm^3 were developed and aggregated as given in Table-5. In appearance calcium oxalate crystals synthesized in artificial urine were found to be similar as present in urine of urolithic patient. Both decoction and infusion of *Berberis lycium* root bark have an inhibitory action on the nucleation and growth stages of calcium oxalate crystallization as shown in Table-6 but have no promising effect on the aggregation stage of the crystallization. It was observed microscopically that both the plant extracts have a significant inhibitory effect on the size and growth of the crystals as shown in Figure-2, decoction showed 92.4% inhibitory effect on its highest concentration, i.e. 100% whereas infusion showed 80.2% inhibition on its highest concentration as shown in Table-7.Microbial infections are associated with urolithiasis so *Berberis lycium* is very useful antiurolithic agent as it has been reported to have anti-antimicrobial potential [3, 6-7] so Phytotherapeutics having numerous activities are much useful in urolithiasis where different therapeutic targets are needed [11].

Tables and figures:

Table 1: Chemical composition of artificial urine

S. No	Reagent	Quantity (mmol/liter)
01	sodium chloride	105.5
02	sodium phosphate	32.3
03	sodium citrate	3.21
04	magnesium sulfate	3.85
05	sodium sulfate	16.95
06	potassium chloride	63.7
07	calcium chloride	4.5
08	sodium oxalate	0.32
09	ammonium hydroxide	17.9
10	ammonium chloride	0.0028

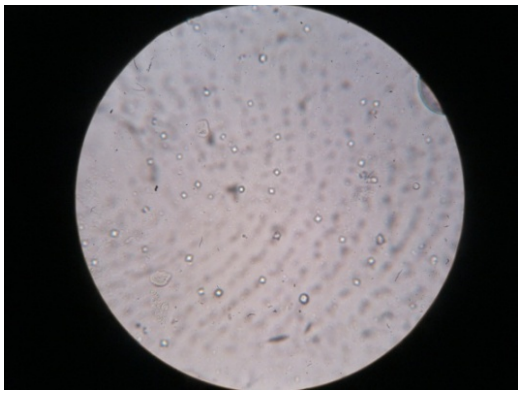

Fig 1: The calcium oxalate crystals in the synthetic urine sample in the absence of *Berberis lycium root bark* extracts.

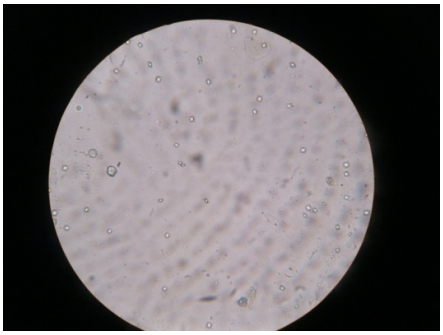

Fig 2: Inhibitory effect of 100% concentration of *Berberis lycium root bark* decoction on calcium oxalate crystallization.

Table 2: Anthelmintic activity of control and standard; Values are the mean ± S.E.M. of control and standard drug on three earthworms. Control is normal saline while standard drug is Piperazine citrate.

Concentration mg/ml	Control		Standard	
	Paralysis time (minutes)	Death time (minutes)	Paralysis time (minutes)	Death time (minutes)
25 mg/ml	96.46 ± 0.46	160.06± 0.11	18.36 ± 0.15	54.36 ± 0.40
50 mg/ml	78.23 ± 0.25	142.2± 0.34	12.00± 0.00	46.46 ±0.40
100 mg/ml	62.43± 0.11	111.7± 0.26	08.84 ± 0.03	13.60± 0.17

Table 3: Anthelminthic activity of decoction; Values are the mean ± S.E.M. of aqueous extract on three earthworms

Concentration mg/ml	Crude extract of *Berberis lycium* root bark decoction	
100 mg/ml	Paralysis time(minutes)	Death time(minutes)
	13.23± 0.22	23.14± 0.31
25 mg/ml	39.20± 0.11	73.13 ±0.32
50 mg/ml	19.13± 0.25	64.22±0.11

Table 4: Anthelminthic activity of infusion; Values are the mean ± S.E.M. of aqueous extract on three earthworms

Concentration mg/ml	Crude extract of *Berberis lycium* root bark decoction	
	Paralysis time(minutes)	Death time(minutes)
25 mg/ml	40.21± 0.27	80.61 ±0.13
50 mg/ml	21.15± 0.13	73.2±0.12
100 mg/ml	18.7 ± 0.33	40.9± 0.22

Table 5: Synthesis of Calcium oxalate crystals with the passage of time

Time (minutes)	Number of Calcium oxalate crystals /mm3	Calcium oxalate aggregation/mm3	Total
5	498	80	578
10	545	99	644
15	600	121	721
20	612	127	739
25	674	164	838
30	700	142	842
35	710	149	859
40	688	165	853

Table 6: Effect of *Berberis lycium* root bark extracts on different phases of crystallization

Berberis lycium root bark extracts	Nucleation	Growth	Aggregation
Decoction	+	+	-
Infusion	+	+	-

Table 7: Effect of *Berberis lycium* root bark extracts on calcium oxalate crystallization

Plant extracts	25%	50%	75%	100%
Decoction	70.5	75.3	82.2	92.4
Infusion	63.3	70.7	74.3	80.2

4. Conclusion

The experimental work indicated that both aqueous extracts of *Berberis lycium* root bark i.e. infusion and decoction contain active phytopharmaceuticals responsible for helminthes infections and urolithiasis. Therefore, more emphasis towards identification and isolation of active constituents in future studies is recommended for developing novel, potent, safe and effective Phytotherapeutics in curing helminthes infections and urolithiasis.

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