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In-vitro Anti-Urolithiatic Activity of some Yemeni Medicinal Plants Extracts

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Abstract:- Kidney stone is one of the most prevalent diseases worldwide. Phytochemicals are responsible for the medicinal activity of plant species. They have the ability for curing various ailment and possesses potential anti-inflammatory, anti-bacterial, anti-oxidant, and anti-fungal properties. Natural products from medicinal plants, either as pure compounds or as extracted out, provide opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to the rising demand for chemical diversity nowadays in screening programs, seeking therapeutic drugs from herbal products, are quite interesting throughout the world. The present study aims at studying the anti-urolithiatic activity of aqueous, methanolic, and hot aqueous extracts of the leaves of *Indigofera oblangifolia (Fabaceae)*, leaves and barks of *Capparis Catrilaginea* (*Capparisaceae*) and vegetable collection of *Fagonia indica Burm.f.* (*Zygophyllaceae*). First, in-vitro study was conducted to assess anti-urolithiatic effect of plants for all extracts with a standard drug namely Cystone as a control. Turbidity method and calcium oxalate dissolution method were practiced to access the inhibition of stone formation and dissolution of stone crystals respectively. Secondly, extracts were prepared and arranged in different concentrations. The homogenous precipitation method was used to prepare an artificial stone such as calcium oxalate and a semi-permeable membrane of eggs was used as dissolution bags. Dissolution models were incubated for 72hrs and after that, the entire content in dissolution bags was estimated spectrophotometrically. The inhibitory activity of extracts on the nucleation of calcium oxalate crystals and the rate of aggregation in calcium oxalate crystals were determined by spectrophotometric assay besides a titration method. Aqueous & alcoholic extracts of *Indigofera oblangifolia* leaves showed the highest solubility, inhibition, and non-formation of calcium oxalate followed by *Capparis Catrilaginea* bark, the results were very close to the Cystone control. On the other hand, the lowest effects were for *Fagonia indica Burm.f.* leaves and *Capparis Catrilaginea* leaves. *Indigofera oblangifolia* leaves and *Capparis Catrilaginea* bark in some extracts exhibited significant in-vitro anti-urolithiatic activity. Aqueous and alcoholic extracts of *Indigofera oblangifolia* leaves and *Capparis Catrilaginea* bark had similar effects as commonly used Cystone medicine for preventing as well as treating renal stones, even in its crude form. Results guide us for the further detailed investigation and development of new drugs from these medicinal plants.

Keywords:- *Kidney and Renal Stones, Extracted Plants, Cystone, Anti-Urolithiatic Activity, in-Vitro Study.*

I. INTRODUCTION

All over the world especially in developing countries, approximately 80% of the population continues to use traditional medicine for primary medical problems in the past decade, therefore, research has been focused on the scientific evaluation of traditional drugs of plant origin. There is an urgent need to systematically evaluate the plants used in traditional medicine. Such research could lead to new drug discoveries or advanced use of indigenous herbal medicines for treatment. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than the costly synthetic drugs many of which have adverse side effects [1].

Stone formation is the oldest and most serious painful urologic disease with significant prevalence in the population due to changes in lifestyle and dietary factors. Stone formation or lithiasis is characterized by calculi formation. It has two main types such as nephrolithiasis and urolithiasis. Calculi formation in the urinary bladder, ureter, or any part of urinary tract rather than kidney is known as urolithiasis while nephrolithiasis is characterized by calculi formation in kidney [2]. Generally, calcification for the formation of bone and teeth takes place in controlled biological situations. Uncontrolled pathological crystallization occurs when fluid becomes supersaturated leading to the formation of precipitates in the body called kidney stones [3, 4].

Oxalic acid is biosynthesized from ascorbic acid, glycolate, and glyoxylate in the metabolism of higher plants. A significant loss of minerals is more prevalent in the body when it is consumed in large content of oxalate-rich foods

[5]. When calcium ions present in the body bind with free oxalic acid/oxalate they precipitate as insoluble calcium oxalate crystals and may lead to hypocalcemia and urolithiasis [3] [6]. Generally, kidney stones are comprised of a high concentration of calcium oxalate [7] with a subsequent minute amount of calcium carbonate, and calcium phosphate [5]. The pathogenesis of calcium oxalate stone formation is involved nucleation, crystal growth, crystal aggregation, and crystal retention in a multistep process [8, 9].

Procedures of dietary supplementation for forestalling calcium oxalate stones and arrangement admission of oxalate-rich food varieties ought to be restricted. Oxalate-rich food such as spinach, rhubarb, beets, nuts, chocolate, tea, wheat bran, and strawberries have been shown to influence raising oxalate levels and a significant increase in urinary oxalate excretion [10]. It is well-known that vitamin C can convert to oxalate therefore; supplementation of higher-dose vitamin C may induce to increase oxaluria and an increased risk of stone formation. Excess of fluid intake, restricted sodium, and protein intake are advisable [11]. Calcium intake during mealtime is advised in order to avoid calcium oxalate formation [12].

Urolithiasis is characterized by the formation of a stone in the kidneys or urinary tracts. A large number of people, nearly 4–15% of the human are suffering from urinary stone problems all over the globe [13]. The crystals of calcium oxalate (CaC_2O_4) are the primary constituent of more than 60% of the majority of human kidney stones; they exist in the form of calcium oxalate monohydrate ($CaC_2O_4.H_2O$) and calcium oxalate dihydrate ($CaC_2O_4.2H_2O$) [14]. The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes nucleation, crystal growth, crystal aggregation, and crystal retention. The stone formation requires supersaturated urine. Supersaturation also depends on urinary pH, ionic strength, solute concentration, and complexations [15]. In spite of substantial progress in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Endoscopic stone removal and extracorporeal shock wave lithotripsy are prohibitively costly and recurrence is quite common with these procedures [16, 17]. Thus a drug for the prevention of this disease or its recurrence would be of great interest.

Several medicinal plants extracts have been reported [18-21]. A current study inveistigated *Indigofera oblangifolia* (*Fabaceae*), *Capparis Catrilaginea* (*Capparisaceae*), and *Fagonia indica Burm. f.* (*Zygophyllaceae*) plants (see **Supplementary** 1) for in-vitro anti-crystallization activities to find out the stone formation inhibitor effect and stone dissolving effect of extracts.

II. MATERIALS AND METHODS

For the control treatments, herbal formulation, Cystone was used and its stock solutions were prepared by suspending them in DMSO solution (50mg/ml) and filtered through a 0.22 mm pore size filter paper [22].

The data were statistically analyzed using a completely Randomized Design (CRD), with three replications. Means of treatments were compared using least Significant Differences (LSD) at 5% level, with the help of Genstat5 software, version2018 (National Statistical Services Center).

- *In-Vitro Anti-lithiatic Activity of Plant Study Extracts & Inhibition of Kidney Stones Formation by Turbidity Method*

In-vitro anti-lithiatic activity of the medicinal plants was tested in terms of inhibition of calcium oxalate formation by the extracts in the presence and absence of inhibitors (standard drugs and extracts).

The precipitation of calcium oxalate at 37°C and pH 6.8 has been studied by the measurement of turbidity at 620nm. A UV/vis spectrophotometer was employed to measure the turbidity caused due to formation of calcium oxalate in treatments [23]. The method used was similar to that described by Burns and Finlayson [24] with minor modifications.

First of all, in-vitro growth of stone nucleus in the absence of any inhibitor was done. For this, a volume of 1.0 ml of 0.025M $CaCl_2$ and 2ml of Tris-buffer (pH 7.4) was added to a test tube. Formation of the turbidity results immediately after mixing of the above chemicals (up to 10 min) and then the measurement of turbidity formed (in terms of absorption at 620 nm in UV/Vis spectrophotometer).

Absorptions were noted down and obtained data were used as the uncontrolled growth of the stone nucleus for the comparison of growth in the presence of the standard drugs and plant extracts.

For the study of the effect of plants extracts against stone nucleus formation, four sets of test tubes with 1ml of 0.025M calcium chloride, 2ml Tris-buffer and 1ml (50 mg/ml solution) of three plant extracts were taken. Two more sets of test tubes were prepared same as the above in which synthetic drug of the polyherbal formulation, Cystone was administered. Then 1 ml volume of 0.025M sodium oxalate was added to each test tube. Each set was replicated six times.

Immediately after the mixing of sodium oxalate, measurement of change in turbidity of the solution was done up to the period of 10 min post mixing. Inhibition in stone nucleus formation was calculated by the graphical method using the following mathematical formula:

$$Inhibition\ \% = 1 - \frac{Si}{Sc}\ x100 \quad \rightarrow (1)$$

Where Si: slope of graph in the presence of inhibitor (drugs/extracts); Sc: slope of without inhibitor (Control).

- *Dissolution Study of Kidney Stones by Spectrophotometric Method of Elements*

To know the role of plant extract in dissolving the already formed stones nucleus in the renal system, artificial

calcium oxalate crystals, potassium permanganate, and semi-permeable membrane from egg were prepared in the laboratory by standard method [25-29].

Three groups (Group I : 1ml of calcium oxalate (1mg/ml) + 1ml of distilled water ;Group II : 1ml of calcium oxalate (1mg/ml) + 1ml of Cystone solution (400mg/ml); Group III : 1ml of calcium oxalate (1mg/ml) + 1ml of hot aqueous extract (20mg/ml)) were prepared and packed together in egg semi-permeable membrane tied with thread at one end and were suspended in a conical flask containing 150 ml 0.1 M Tris buffer each. At another end of the thread, a membrane tied by a stick was placed on the mouth of conical flask and covered with aluminum foil. The groups were kept in an incubator, preheated to 37 °C for 4hrs, for three days (Fig 1). The entire content of each group was removed from sutured semi-permeable membrane and was transferred into a test tube individually. 4ml of 1M H_2SO_4 and about 70µl of 0.02M $KMnO_4$ were added and kept aside for 2 hrs. Color change from dark pink to colorless was observed after hrs. Change of color intensity was measured against λ_{max} (620nm) spectrophotometrically. Concentration of undissolved calcium was determined from standard calibration curve of calcium oxalate solutions by measuring absorbance and applying Beer-Lambert law.

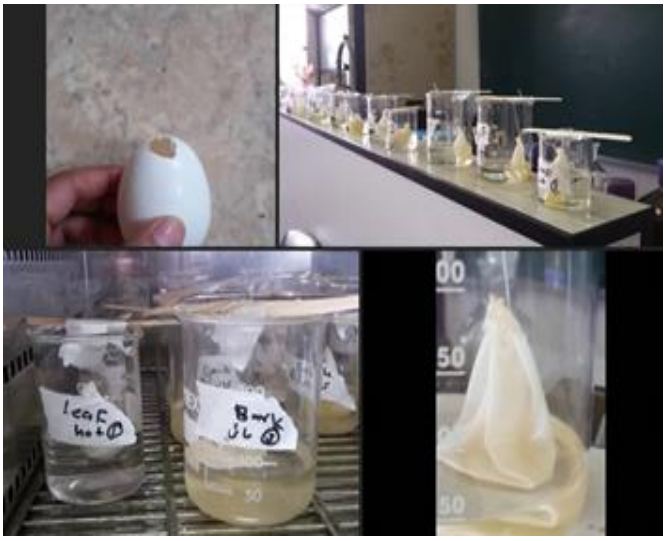

Fig 1 In-Vitro Anti - Urolithiatic Model Using Egg Membrane for Urolithiasis Activity

Inhibition % was calculated using the equation 2 below:

$$Inhibition\ \% = \frac{Abs(Control) - Abs(Sample)}{Abs(Control)} X100 \rightarrow (2)$$

Where Abs (Control): absorbance of control at λ_{max}, Abs (Sample): absorbance of sample at the same λ_{max}.

➢ *Dissolution Study of Kidney Stones by Titration Method*

The dissolution percentage of calcium oxalate was evaluated as represented in Figures 2 and 3 below:

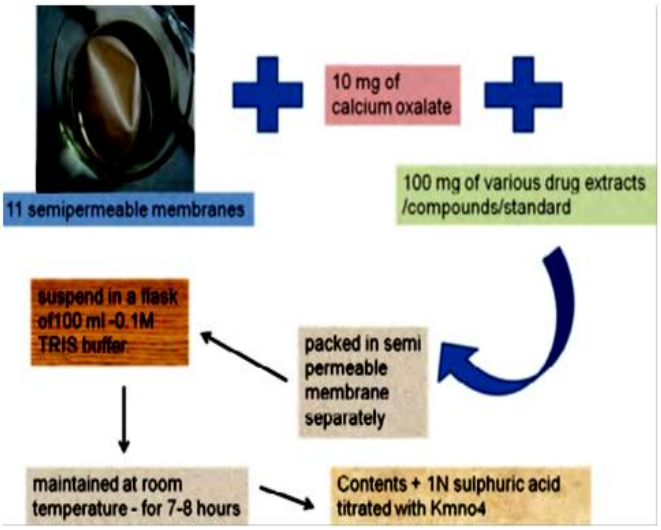

Fig 2 Experimental Protocol

Fig 3 Titration Result Shows Pink Color as the Endpoint

III. RESULTS AND DISCUSSION

A. Inhibition of Kidney Stones Formation by Turbidity Method

For seeing in the medicines fixation reliant, several different concentrations have been worked out (i.e. 25-125 mg/ ml) and initial steep rising was noted in turbidity (nucleation) followed by a decrease in turbidity (aggregation). The concentration for all studied plants was summarized in (Table1) to show its effectiveness in extracting kidney stones.

At concentration (50 mg/ml), the results represented that the highest inhibition was for the aqueous and methanol extracts of *Indigofera oblangifolia* leaves (Table2). The inhibition was parallel to Cystone control, and the least was the hot aqueous extract of *Capparis catrilaginea* leaves and *Fagonia indica Burm.f* vegetable collection. Significant differences were found between all studied plants.

The previous researches did not examine our mentioned plants in terms of their effectiveness on kidney stones. On the other hand, the way of examining their effectiveness was found in other plants. There was a great convergence between the inhibition of Cystone in the study of [22] and the recent study.

It was reported that most of the plant studies contain alkaloids, saponins, tannins, steroids, amino acids, flavonoids, etc. which can be confirmed from qualitative phytochemical tests. Flavonoids show anti-oxidant property which places an important role as it is a part of mechanism of dissolving kidney stone, Saponins are known to have anti-crystallization properties by disaggregating the suspension of mucoproteins, the promoters of crystallization. Also, calcium oxalate monohydrate is an insoluble form of calcium responsible [30, 31].

For aggregation and crystal growth leads to kidney stone formation. The factors which convert insoluble form to soluble form lead to the dissolution of kidney stones. At acidic pH calcium oxalate is converted to oxalic acid and calcium chloride, both are the soluble form, and also potassium citrate is given for the treatment of calcium oxalate renal calculi to raise the pH but, as per the studies it is seen that alkaline pH is not the treatment of this type of calculi. Rather, potassium salts increase the solubility by the principle of ionic strength and activity coefficient, while citrate gives a chelating effect. By that, they increase the solubility of calcium oxalate [32].

Table 1 Inhibition Model of Dissolution of Calcium Oxalate.

Plant (P)	**Extraction (E)**	**Concentration (C) mg/ml**					**Interaction (P) * (E)**
		25mg/ml	**50mg/ml**	**75mg/ml**	**100mg/ml**	**125mg/ml**	
Indigofera oblangifolia (Leaves)	**E_1**	0.5760	0.5100	0.4970	0.4010	0.326	**0.4540**
	E_2	0.5720	0.5400	0.5100	0.4957	0.3870	**0.5009**
	E_3	0.6020	0.5550	0.5370	0.5193	0.4990	**0.5425**
Capparis catrilaginea (Barks)	**E_1**	0.6150	0.5610	0.5430	0.5250	0.5090	**0.5506**
	E_2	0.5960	0.5487	0.5270	0.5100	0.4753	**0.5314**
	E_3	0.6560	0.6010	0.5720	0.5590	0.5353	**0.5847**
Capparis catrilaginea (Leaves)	**E_1**	0.7670	0.7400	0.7380	0.7250	0.7110	**0.7362**
	E_2	0.8090	0.7790	0.7530	0.7500	0.7460	**0.7674**
	E_3	0.8313	0.8127	0.7940	0.7870	0.7640	**0.7978**
Fagonia indica burm.f (Vegetable collection)	**E_1**	0.6920	0.6120	0.5770	0.5703	0.5410	**0.5985**
	E_2	0.6313	0.5740	0.5600	0.5310	0.5170	**0.5627**
	E_3	0.8210	0.7820	0.7760	0.7610	0.780	**0.7796**
							Mean of (P)
(P) * (C) Interaction	**P_1**	0.5167	0.5350	0.5147	0.4720	0.4040	**0.4885**
	P_2	0.6233	0.5702	0.5473	0.5313	0.5066	**0.5556**
	P_3	0.8024	0.7772	0.7617	0.7540	0.7403	**0.7671**
	P_4	0.7148	0.6560	0.6377	0.6208	0.6053	**0.6469**
							Mean of (E)
(E) * (C) Interaction	**E_1**	0.6125	0.6058	0.5888	0.5553	0.5218	**0.5768**
	E_2	0.6521	0.6104	0.5875	0.5717	0.5313	**0.5906**
	E_3	0.7276	0.6877	0.6698	0.6566	0.6391	**0.6761**
Mean of (C)		**0.6641**	**0.6346**	**0.6153**	**0.5945**	**0.5641**	
L D S 5%	**P=0.01760, E=0.01524, C=0.01968, P*E=0.03048, P*C=0.03935, E*C=NS, P*E*C=NS**						
CV%	**6.9%**						
NS= Not Significant, **E1**= Aqueous, **E2**= Methanol, **E3** = Hot aqueous							

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Table 2 Reduction of CaC_2O_4 Nucleus formation by plant extracts compared with a control drug.

Treatment Groups	Absorbance at 620nm (after 180 min)	Reduction in turbidity (In%)
IndigoFera oblangifolia Leaves (50mg/ml)		
Buffer	1.599	**0**
Cystone (Control)	0.291	**81..8**
Aqueous extract	0.510	**68.11**
Methanol extract	0.540	**66.23**
Aqueous hot extract	0.555	**65.29**
Capparis Catrilaginea Barks (50mg/ml)		
Buffer	1.599	**0**
Cystone (Control)	0.291	**80.6**
Aqueous extract	0.561	**64.92**
Methanol extract	0.549	**65.66**
Aqueous hot extract	0.601	**62.41**
Capparis Catrilaginea Leaves (50mg/ml)		
Buffer	1.599	**0**
Cystone (Control)	0.291	**80.6**
Aqueous extract	0.740	**53.72**
Methanol extract	0.779	**51.28**
Aqueous hot extract	0.813	**49.16**
Fagonia indica Burm.f. Vegetable Collection (50mg/ml)		
Buffer	1.599	**0**
Cystone (Control)	0.291	**80.6**
Aqueous extract	0.612	**61.73**
Methanol extract	0.574	**64.10**
Aqueous hot extract	0.782	**51.09**

B. Dissolution Study of Kidney Stones by Spectrophotometric Method

To explore anti-urolithiatic activities of the studied plants extract by utilizing different in-vitro models, and to investigate the inhibitory effect of extract on in-vitro crystallization through analyzing nucleation and aggregation assays. The aqueous, methanol, and hot aqueous extracts for the studied plants were prepared in different concentrations (25-125 mg/ml). Homogenous precipitation method was used to prepare artificial stones calcium oxalate, and semi-permeable membrane of eggs was used as dissolution bags. The entire content in dissolution bags was estimated spectrophotometrically. The inhibitory activity of the studied plants extract on the nucleation of calcium oxalate crystals and the rate of aggregation in calcium oxalate crystals was determined by spectrophotometric assay [33].

Table 3 shows that the highest values for the solubility and non-formation of calcium oxalate were for all extracts of the *Indigofera oblangifolia* plant, and the lowest value for the solubility of the gravel and the weak effect was for the hot water extract of *Capparis catrilaginea* (leaves) and *Figonia indica Burm.f.* Significant differences were found between all studied plants. Each plant was shown separately in terms of its effectiveness in Table 4 & Figures (4, 5).

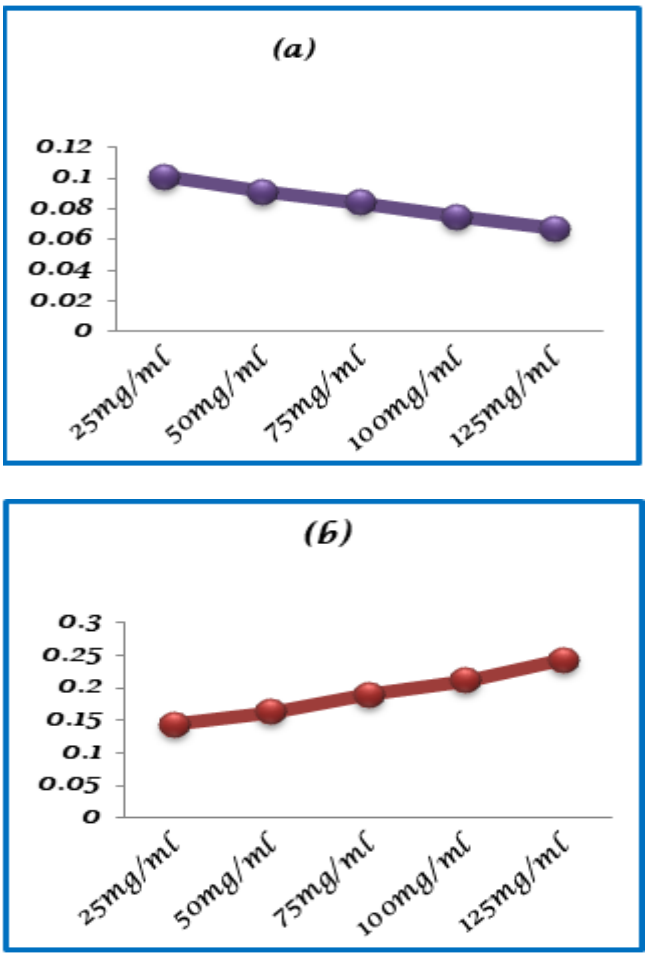

Fig 4 Calibration Curve of (a) Cystone (b) Calcium Oxalate CaC_2O_4

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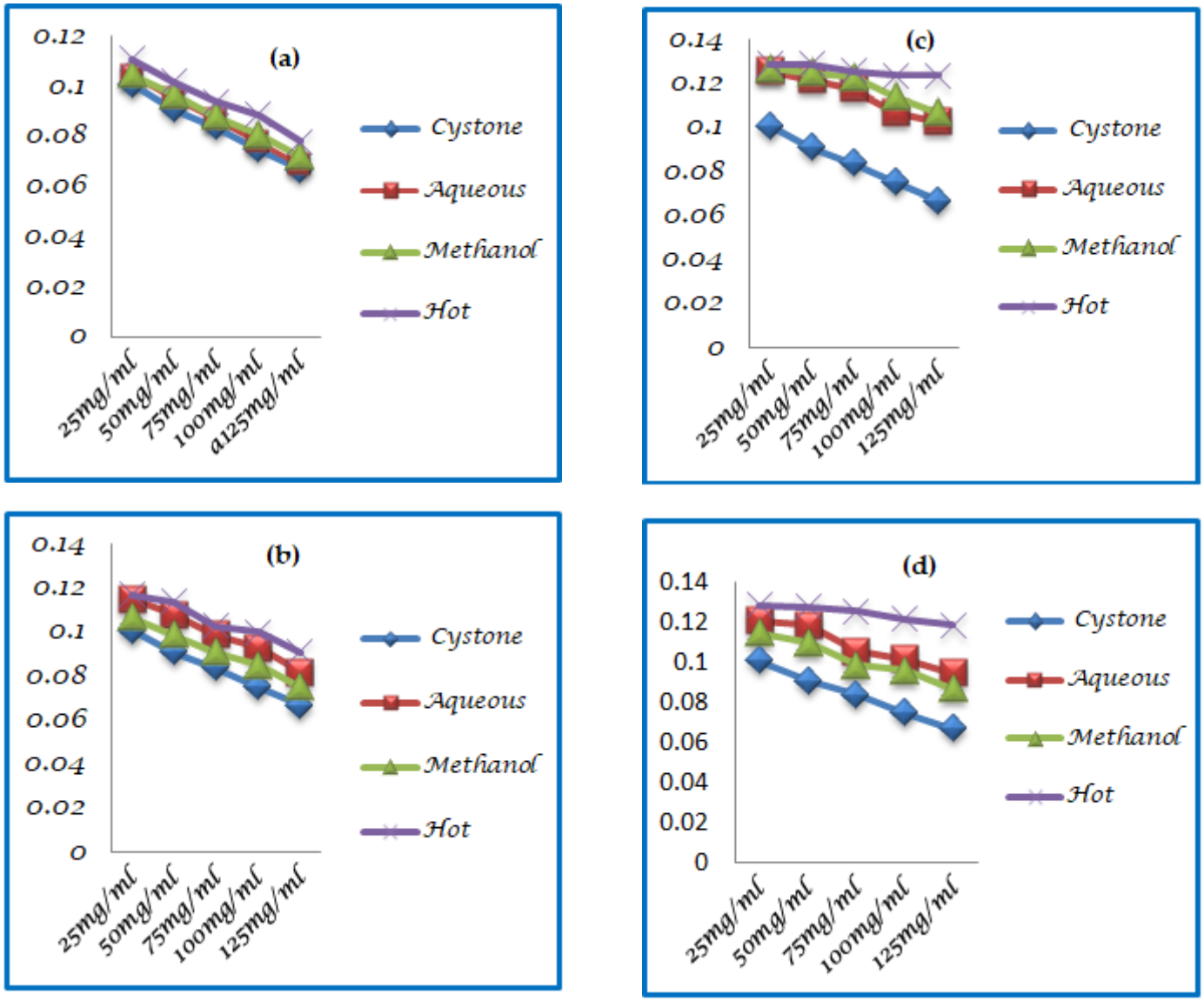

Fig 5 Dissolution of Calcium oxalate in different extracts of studied plants and Cyston. (a) *IndigoFera oblangifolia* leaves (b) *Capparis Catrilaginea* bark (c) *Capparis Catrilaginea* leaves (d) *Fagonia indica Burm.f.* vegetable collection.

According to our knowledge, there were no previous investigation in terms of recent plants and their effectiveness on the kidney stones. On the other hand, we followed the protocol and standard methods same as the previous studies. There was a great convergence between the values of control and the standard solutions in the study of Phatak and Gender [34], and between the values of the Cystone and the standard solution obtained in our work.

Table 3 Spectroscopy Method Results in the Dissolution of Calcium Oxalate by Plants Extracts at Different Concentrations

Plant (P)	Extraction (E)	Concentration (C) mg/ml					Interaction (P) * (E)
		25mg/ml	50mg/ml	75mg/ml	100mg/ml	125mg/ml	
1- Indigofera oblangifolia (Leaves)	E_1	0.10433	0.09500	0.08733	0.07800	0.06900	0.08673
	E_2	0.10500	0.09700	0.08800	0.08100	0.07200	0.08860
	E_3	0.11100	0.10233	0.09433	0.08900	0.07800	0.09493
2- Capparis catrilaginea (Bark)	E_1	0.07733	0.10800	0.09900	0.09300	0.08233	0.09193
	E_2	0.10733	0.09933	0.09100	0.08500	0.07500	0.09153
	E_3	0.11700	0.11367	0.10400	0.10000	0.09033	0.10500
3- Capparis catrilaginea (Leaves)	E_1	0.12533	0.12200	0.11800	0.10700	0.10300	0.11507
	E_2	0.12667	0.12500	0.12333	0.11400	0.10733	0.11927
	E_3	0.12900	0.12900	0.12633	0.12400	0.12400	0.12647
4- Fagonia indica burm	E_1	0.12100	0.11867	0.10600	0.10200	0.09500	0.10853

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.f (Vegetable collection)	**E_2**	0.11500	0.11000	0.09867	0.09633	0.08700	0.10140
	E_3	0.12900	0.12800	0.12600	0.12233	0.11867	0.12480
							Mean of (P)
(P) * (C) Interaction	**P_1**	0.10678	0.09811	0.08989	0.08267	0.07300	0.09009
	P_2	0.10056	0.10700	0.09800	0.09267	0.08256	0.09616
	P_3	0.12700	0.12533	0.12256	0.11500	0.11144	0.12027
	P_4	0.12167	0.11889	0.11022	0.10689	0.10022	0.11158
							Mean of (E)
(E) * (C) Interaction	**E_1**	0.10700	0.11092	0.10258	0.09500	0.08733	0.10057
	E_2	0.11350	0.10783	0.10025	0.09408	0.09408	0.10020
	E_3	0.12150	0.11825	0.11267	0.10883	0.10275	0.11280
Mean of (C)		**0.11400**	**0.11233**	**0.10517**	**0.09931**	**0.09181**	
L D S 5%	P= 0.003726 E=0.003227 C=0.004166 P*E=0.006454 P*C= 0.008332 E*C=0.007216 P*E*C=0.014432						
CV%	8.5%						
E1= Aqueous , E2= Methanol , E3 = Hot aqueous							

Table 4 Dissolution of Calcium Oxalate by Investigated Plants

Group	**Concentration (mg/ml)**	(%)**Dissolution of Calcium oxalate**			
Plant		**Indigofera oblangifolia (Leaves)**	**Capparis catrilaginea (Bark)**	**Capparis catrilaginea (Leaves)**	**Fagonia indica burm .f (Vegetable collection)**
Control Calcium oxalate (Abs= 0.130)	1	--	--	--	--
Aqueous	25	20	11.5	3	6.9
	50	26.9	16.9	6.15	8.5
	75	33.1	23.8	9.2	18.46
	100	40	28.46	17.7	21.53
	125	46.92	36.9	20.77	26.92
Methanol	25	19	17.69	2.3	11.5
	50	25.4	23.85	3.8	15.38
	75	32.31	30	5.4	23.85
	100	37.7	34.6	12.3	26.15
	125	44.6	42.30	17.7	33.07
Hot Aqueous	25	14.62	10	0.7	0.7
	50	21.5	12.31	0.7	1.54
	75	26.7	20.77	3	3
	100	31.5	23.07	3	6.15
	125	40	30	3	8.5

C. Dissolution Study of Kidney Stones by Titration Method

This study evaluated the anti-urolithiatic activity of extracts of the studied plant with different concentrations as it is evident in (Table5) for calculating the percentage dissolution of kidney stones through calibration. This study has given primary evidence for the studied plants as they are possessing anti-urolithiatic activity.

No previous studies have been found regarding the activities of the studied plants in terms of their effectiveness on kidney stones.

The results in Table 5 show that the highest values for the solubility and disappearance of calcium oxalate stones were for the aqueous and alcoholic extracts of the *Indigofera oblangifolia* plant, and the lowest solubility and disappearance value was for the hot aqueous extract of *Capparis catrilaginea* leaves and *Figonia indica Burm. f.* vegetable collection. Significant differences were found between all studied plants. Each plant was shown separately in terms of efficacy, as well as the standard solutions and control (Tables 6,7).

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Table 5 Dissolution of Calcium Oxalate by Plants Extracts- A Titration Method Results

Plant (P)	Extraction (E)	Concentration (C) mg/ml					Interaction (P) * (E)
		25mg/ml	50mg/ml	75mg/ml	100mg/ml	125mg/ml	
Indigofera oblangifolia (Leaves)	E_1	4.000	3.700	3.400	3.100	2.800	**3.400**
	E_2	4.100	3.700	3.500	3.100	2.967	**3.473**
	E_3	4.200	3.800	3.500	3.300	3.033	**3.567**
Capparis catrilaginea (Bark)	E_1	4.167	3.833	3.600	3.400	3.000	**3.600**
	E_2	4.100	3.700	3.500	3.200	2.933	**3.487**
	E_3	4.300	3.933	3.600	3.500	3.233	**3.713**
Capparis catrilaginea (Leaves)	E_1	4.367	4.100	3.700	3.733	3.400	**3.860**
	E_2	4.400	4.100	3.700	3.733	3.500	**3.887**
	E_3	4.533	4.467	4.400	4.200	4.000	**4.320**
Fagonia indica burm .f (Vegetable collection)	E_1	4.300	4.033	3.733	3.600	3.300	**3.793**
	E_2	4.300	3.933	3.600	3.500	3.067	**3.680**
	E_3	4.500	4.333	4.300	3.900	3.500	**4.107**
							Mean of (P)
(P) * (C) Interaction	P_1	4.100	3.733	3.467	3.167	2.933	**3.480**
	P_2	4.189	3.822	3.567	3.367	3.056	**3.600**
	P_3	4.433	4.222	3.933	3.889	3.633	**4.022**
	P_4	4.367	4.100	3.878	3.667	3.289	**3.860**
							Mean of (E)
(E) * (C) Interaction	E_1	4.208	3.917	3.608	3.458	3.125	**3.663**
	E_2	4.225	3.858	3.575	3.383	3.117	**3.632**
	E_3	4.383	4.133	3.950	3.725	3.442	**3.927**
Mean of (C)		**4.272**	**3.969**	**3.711**	**3.522**	**3.228**	
L D S 5%	**P=0.0648 E=0.0561 C=0.0725 P*E= 0.1123 P*C=0.1449 E*C=0.1255 P*E*C=0.2510**						
CV%	4.2%						

Table 6 % Dissolution of Calcium Oxalate by Cystone Drug

Control				
	Vol. of 0.02M $KMnO_4$ (ml)	**Wt .of estimated Calcium oxalate (mg)**	**Wt .of reduced calcium oxalate (mg)**	(%)**Dissolution of Calcium oxalate**
---	4.5	0.854	---	---
Cystone				
Concentration (mg/ml)	**Vol. of 0.02M $KMnO_4$ (ml)**	**Wt .of estimated Calcium oxalate (mg)**	**Wt .of reduced calcium oxalate (mg)**	(%)**Dissolution of Calcium oxalate**
25	3.9	0.74	0.11	12.9
50	3.3	0.63	0.22	25.88
75	3	0.569	0.281	33.05
100	2.8	0.53	0.32	37.65
125	2.5	0.475	0.375	44.12

Table 7 % Dissolution of Calcium Oxalate in the Studied Plants

Group	(%)Dissolution of Calcium oxalate	Vol. of 0.02M $KMnO_4$ (ml)
IndigoFera oblangifolia leaves		
Reference	4.5	--
Standard Cystone	2.5	44.12
Aqueous	2.8	37.94
Methanol	2.9	35.59
Hot Aqueous		33.3
Capparis Catrilaginea bark		
Reference	4.5	--
Standard Cystone	2.5	44.12
Aqueous	3	33.26
Methanol	2.9	35.6
Hot Aqueous	3.2	28.57

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Capparis Catrilaginea leaves		
Reference	4.5	--
Standard Cystone	2.5	44.12
Aqueous	3.4	23.89
Methanol	3.5	22.72
Hot Aqueous	4	11.01
Fagonia indica Burm .f. vegetable collection		
Reference	4.5	--
Standard Cystone	2.5	44.12
Aqueous	3.3	26.7
Methanol	3.1	31.15
Hot Aqueous	3.5	22.25

IV. CONCLUSIONS

In-vitro studies have shown that extracts of the plants studied have a dissolving effect on kidney stones, and the most effective plants as anti-calculus agents are the aqueous and alcoholic extracts of the *Indigofera oblangifolia* plant. In comparison, alcoholic extract of the bark of *Capparis Catriaginea* - *Fagonia indica Burm. f.* vegetable harvesting of alcohol extracts, *Capparis Catriaginea* leaves have the lowest potency.

This in-vitro study provides very valuable data and suggests that these extracts have potential anti-urolithic activity. Different phytochemicals have different protective and therapeutic effects, which are essential for disease prevention.

The folk plants have many bioactive compounds that serve as a potential source of herbal remedies.

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SUPPLEMENTARY (1)

Indigofera oblongifolia (Sup1) is an erect perennial plant with many branched stems that become more or less woody, especially near the base, and persist.

The plant is harvested from the wild for local use as a medicine and a dye. It is occasionally cultivated, as a living fence in Sudan; for green manure in southern India; and as dye plant perhaps in Mali and Zimbabwe.

Ref: Tropical Plants Database, Ken Fern. tropical.theferns.info. 2022-10-09. < tropical.theferns.info/viewtropical.php?id =Indigofera+oblongifolia>

Supp 1 Indigofera Oblangifolia *(Fabaceae)* Plant

Capparis Catrilaginea is harvested from the wild for local use as a food and a medicine. It found in Northeastern and eastern Africa, through the Arabian Peninsula and the Levant, eastwards to Pakistan and India.

Ref.: Tropical Plants Database, Ken Fern. tropical.theferns.info. 2022-10-09. <tropical.theferns.info/viewtropical.php?id= Capparis+spinosa+cartilaginea>

Supp 2 Capparis Catrilaginea (*Capparisaceae*) Plant

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Fagonia indica Burm.f. is widely distributed in Asian and African deserts.

Ref.: Mohamed S. Elshikh, Mohammad Ajmal Ali, Fahad Al-Hemaid, Soo Yong Kim, Meena Elangbam, Arun Bahadur Gurung, Prasanjit Mukherjee, Mohamed El-Zaidy, Joongku Lee,"Insights into plastome of Fagonia indica Burm.f. (Zygophyllaceae): organization, annotation and phylogeny,"Saudi Journal of Biological Sciences, Volume 29, Issue 3,2022,Pages 1313-132. https://doi.org/10.1016/j.sjbs.2021.11.011

Supp 3 Fagonia Indica Burm. f. (Zygophyllaceae) Plant