

## HIGH IN VITRO ANTIUROLITHIATIC EFFECT OF *PITURANTHOS SCOPARIUS* ROOTS EXTRACTS

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### Abstract

The richness of the steppe zone by the medicinal plants and their diversity traditional uses in the region of Laghouat, allowed us to study the inhibitory effect of *Herniaria fontanesii* J.Gay, *Pituranthos scoparius* and *Cynodon dactylon* (L) extracts on the formation of calcium oxalate kidney stones. Firstly, we have carried out phytochemical screening and quantitative analysis of phenolic compounds of various extracts from the three plants. The obtained results proved that our extracts are rich in catechic tannins and C-, O-heterosides, with a total phenolic content ranged from 0.867 to 38.835 mg of gallic acid equivalent per 1 g of dry matter.

The *in vitro* antiurolithiatic activity of different plants extracts has been carried out by two different models. In the turbidimetric assay, we have determined spectrophotometrically the effect of the extracts (1 g/L) on the oxalocalcic crystallization, induced by addition of oxalate in urines from healthy subject. Whereas, the gravimetric assay is based to measure the variation of calcium urate and uric acid renal calculi weight, after putting them in contact with 5 ml of the extracts (5g/L) during 15 days. In the two assays, the antiurolithiatic activity was compared with that of two antiurolithogenesis inhibitory standards: sodium citrate and Succinimide parbiol. The achieved results measured by the two tests, show clearly that the hydromethanolic extracts of *Pituranthos scoparius* roots have provided very important antiurolithiasic power (>40% of inhibition) compared to the standard inhibitors. However, this result is a great step forward towards the search for an effective treatment for the urinary calculi formation. But, this work will have to be confirmed by *in vivo* experiments in order to validate these *in vitro* observations on the contributions of these three plants in the treatment of the oxalocalcic renal calculi.

**Key Words** Medicinal plants, *Pituranthos scoparius* roots, phytochemical screening, kidney stones, Calcium oxalate, antiurolithiasic.

## Introduction

During the past decades, the use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health-care settings [1]. However, the safety of herbal medicines has become a major concern to both national health authorities and the general public. The world health organization (WHO) requested to compile a list of medicinal plants and to establish international specifications for the most widely used medicinal plants and simple preparations. The WHO estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. Therefore, the recent studies were focused on the scientific evaluation of traditional drugs of the vegetable which are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids [2].

In Algeria, the alternative medicine is still largely requested by the population for several reasons which are being the same ones in other countries. Essentially, patients are not satisfied with the treatments which they receive and preferred herbal remedies which appear to be more natural without harmful side-effects are also inexpensive. For example, the conventional urinary calculi medication like diuretics and anti-inflammatory drugs are the most common prescription used to treat calcium renal calculi oxalocalcic, but they can cause serious side effects [3]. It affects about 2-15% of the world population mostly in the industrialized countries. It is the third most prevalent disorder in the urinary system with recurrence rate of 50% [4]. The highest average frequency is between 30 and 50 years of age, with an obvious male prevalence [5]. There are mainly four types of calculi. About 75% of stones are calcium containing, composed of calcium oxalate or calcium oxalate mixed with calcium phosphate and 15% of stones are the so-called triple phosphate or struvite stones, composed of magnesium ammonium phosphate. It is almost associated with infection. Uric acid stones from 6% and cystine stones 1-2% [6]. A large number of plant drugs have been used in Algeria since ancient times which claim efficient cure of urinary stones. Amongst the medicinal plants used in urolithiatic are *Cynodon dactylon* (L) and *Herniaria fontanesii* J.Gay. *Pituranthos scoparius* (Guezzah) family Asteraceae is an aphyllous perennial plant where its leaves are used by the local population as jaundice and antiurolithiatic natural remedies.

However, no scientific study has been reported regarding the anti-urolithiatic property of *Pituranthos scoparius* root extract. Therefore, the objective of the present study was to investigate and to validate the antiurolithiatic property of *Pituranthos scoparius* extracts in the inhibition/dissolution of calcium oxalate and uric acid stones.

## Material and methods

### Plants and reagents

Plants (*Cynodon dactylon* (L), *Pituranthos scoparius* and *Herniaria fontanesii* J.Gay.) were collected from a local herbalist in December 2014 flowering times between March and May in the area of the Algerian Saharian Atlas at 40 km (at 40 km at the north of Laghouat). The samples were identified at the Agronomic National Institute of Alger, and the voucher specimens were deposited at the laboratory of Fundamental Sciences, University of Laghouat. The investigated plants were dried and stored in dark. All chemicals were of the highest quality available and were purchased from Sigma-Aldrich (France).

### Preparation of the extracts

The extracts have been prepared by maceration and decoction. The decoction of dried plant material was prepared by boiling 2 g of powder in 100 ml of distilled water for 5 min. While, the maceration of 2 g of powder was soaked in 100 ml of 4 different mixture solvents: acetone (100%), methanol (100%), acetone-water (50%-50%) and methanol-water (50%- 50%) at room temperature for 24 hours with occasional shaking. Then, each extract was filtered through a single layer of muslin cloth, and then final filtrate was collected by passing it through a Whatman grade 1 filter paper in a Büchner funnel under vacuum. The filtrates were evaporated to dryness on a Rotary Evaporator under reduced pressure. Dried extracts were dissolved, placed in a bottle, stopped and then stored at -4 °C until used. We obtained then, 5 acetic extracts, 5 methanolic extracts, 5 aqueous extracts, 5 hydromethanolic extracts and 5 hydroacetic extracts.

### Phytochemical analysis of the extracts

The phytochemical screening on 25 prepared extracts was carried out to determine the presence of alkaloids, flavonoids, tannins, saponins, starch, glycosides, anthocyanes, coumarins, sterols/triterpenes, anthraquinones and reducing compounds by using standard qualitative procedures as described by Trease and Evans [7], Sofowora [8] and Harborne [9].

### **Determination of phenolic content**

The concentration of total phenolics in different plant extracts was estimated by the Folin-Ciocalteu procedure [10], which is considered as the best method for total phenolics (included tannins) determination. The total phenolic content of the samples was expressed as gallic acid equivalents (GAE), which reflected the phenolic content as the amount of gallic acid (mg) in 1 gram of dry material.

### **Thin layer chromatography (TLC) Analysis**

The presences of different active constituents in hydromethanolic extract of *Pituranthos scoparius* roots were analyzed through two-dimensional TLC analysis. The one-dimensional TLC analysis chromatogram was developed by using commercially available aluminum sheets of Silica gel 60 F254 (Merck) with ethyl acetate /dichloromethane/cyclohexane in the volume ratio 4.7/3.5/1.76 as eluent. The mobile phase for two-dimensional TLC analysis was: dichloromethane / methanol / water in volume ratio 5/4.16/0.84. Spots were observed under UV light at 366 nm and 254 nm before and after spraying with Dragendorff reagent, 10% ethanolic aluminum chloride, Liebermann-Burchard reagent and vanillin sulphuric acid reagent.

### **In vitro antilithiatic activity**

#### **Turbidimetric method**

The *in vitro* antilithiatic activity of the extract was tested in terms of inhibition of calcium oxalate formation by the extracts in the presence and absence of inhibitors (standard drugs and extract) [11]. The Precipitation of calcium oxalate at 37°C and pH 6.8 has been studied by the measurement of turbidity at 620nm. A spectrophotometer UV/Visible (Shimadzu 1800) was employed to measure the turbidity caused due to formation of calcium oxalate in treatments.

Twenty four hours urine sample was collected from a healthy subject in a propylene bottle containing sodium azide as an antibacterial agent. 100 µl of inhibitor solution at 1 mg/ml were added to an aliquot of 1 ml of urine and allowed to warm up to 37°C. Finally, 1 ml of 25 mM sodium oxalate solution was added and tubes were incubated at 37°C for 300 sec. Each set was replicated five times. At the end of the experiment, tubes were read at 620 nm. Tubes with no extract added were used as control. The antilithiatic activity obtained for each plant extract was compared with that of Succinimide Pharbiol and sodium citrate as positive controls. The rate of nucleation was estimated by

comparing the induction time in the presence of the extract with that of control. Data was represented in percentage inhibition and calculated using the following mathematical formula:

$$\text{Inhibition \%} = \{1 - [A_i / A_c]\} \times 100$$

Where;  $A_i$ : Absorbance in the presence of inhibitor (drugs/extracts),  $A_c$ : Absorbance without Inhibitor (Control).

#### **Gravimetric method**

The dissolution percentage of renal calculus (C1: uric acid; C2: sodium urate) was evaluated by the ratio of these weight losses using analytical balance. Pure renal stones were incubated with 5 ml of plant extracts or standard drugs at 5 g/l during 2 weeks under magnetic stirring. Only, the extracts that have showed inhibition percent higher than 40% in the turbidimetric model, have been tested in this assay. Each experiment was performed in triplicate. The mass loss of the stones was measured after the two weeks period. The amount of remaining undissolved stones is subtracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved. The antiurolithiasic activity was expressed by the percentage of dissolution of the renal calculus (D %).

$$D \% = ((m_0 - m_f) / m_0) \times 100$$

Where; **D%**: Rate of dissolution,  $m_0$ : Initial stone weight,  $m_f$ : Undissolved stone weight.

#### **Statistical analysis**

All the treatments were performed in triplicates and each data point in the results is the mean of three replicates. All experiments were repeated at least once. The statistical significance of the treatment effect was expressed as mean ± SEM.

### **Result and discussion**

#### **Determination of total phenol content**

The obtained crude extracts present thick viscous appearance with different colors. The percentage yields of the extracts were ranged between 2.40% and 38.83% (Table 1). *Herniaria fontanesii* J. Gay aerial parts exhibited the highest crude extract content, while the lowest percent yield is revealed in *Pituranthos scoparius* roots extract. Moreover, the average of percent yields of acetonetic extracts is at least lower than that of aqueous or methanolic extracts. Recent studies have shown that phenolic plant extracts contribute significantly to the

antilithiatic activity [12, 13]. Therefore, one of the purposes of this study were to investigate the phenolic content of different parts of plants and to identify the best extraction solvent to further characterize the antilithiatic properties of these extracts and their effect on health.

The average of total phenol contents of twenty five plant extracts measured by Folin-Ciocalteu assay is presented in Table 1. The average total phenols content of ethanolic extracts was significantly different ( $p < 0.05$ ) from aqueous, acetonic or methanolic extracts. The amount of total phenolics changes in different plants varies from 0.867et 5 .160 mg GAE/g of dry material. The highest total phenolic levels have been detected in the methanolic and hydromethanolic fractions, and the lowest in acetonic extracts. Also, results show that among tested samples, aerial plant extracts have significantly higher ( $p < 0.005$ ) total phenols content than those found in roots for the same plant.

According to recent studies [14-17], the difference in polarities of extracting solvents might influence the solubility of chemical constituents in a sample and its extraction yield. Therefore, the selection of an appropriate solvent system is one of the most relevant steps in optimizing the recovery of total phenolics and other bioactive compounds from extracts.

### **Phytochemical analysis of extracts**

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates terpenoids, steroids and flavonoids. Thus, qualitative phytochemical studies were performed on extracts using suitable chemicals and reagents to confirm the presence of alkaloids, flavonoids, tannins, saponins, starch, glycosides, anthocyanins, coumarins, sterols/triterpenes, anthraquinones and reducing compounds. The phytochemical characteristics of the twenty five extracts of three medicinal plants tested were summarized in the Table 2. Phytochemical analysis showed the presence and absence of certain chemical constituents. Moreover, the results revealed the presence of medically active compounds in the different studied extracts. The results of qualitative phytochemical studies indicate that the maximum numbers of chemical constituents were present in the aqueous extract of *Pituranthos scoparius* aerial part when compared to the other extracts. As well, it could be seen that tannins and heterosides were present in all the plant extracts.

Saponins were present only in the aqueous extracts of all plant parts. Moreover, alkaloids are present in all *Herniaria fontanesii* J. Gay extracts with Wagner reaction but they are absent with Mayer reagent. The appearance of the reducing compounds is also verifiable in almost extracts of *Pituranthos scoparius* and *Cynodon dactylon* (L.) in particular those which contain water. The flavonoids are also present in almost all plants extracts except the *Cynodon dactylon* (L.) roots extracts. They are presented in variable quantities but with higher contents in the *Herniaria fontanesii* J. Gay aerial part extracts. Therefore, starch, glycosides, anthocyanins, coumarins, sterols/triterpenes and anthraquinones were absent on all plants extracts.

However, the absence of some phytochemical constituents in all extracts like coumarins, anthocyanins, sterols and terpenes does not justify their total absence in these plants but, the extraction methods used in our work are not satisfactory to extract desired metabolites. For example, phytochemical analyses which were carried out by some researchers [18-20] on the two parts of *Pituranthos scoparius* extracts revealed the presence of coumarins in certain extracts. Moreover, terpenoids are present also in almost all the studied extracts. Furthermore, some researchers [21, 22] reported that *Cynodon dactylon* (L.) contains sterols and terpenoids, which does not corroborate with our results.

### **Evaluation of antiurolithiatic activity**

Urinary calculi are the third prevalent disorder in the urinary system. Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system [23]. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However; these treatments are relatively costly, painful and require expert hands with availability of appropriate equipments [24]. Currently, phytotherapy or traditional medicine was employed for the the evaluation of antiurolithiatic activity, and certain medicinal herbs has been used in traditional medicine to treat urinary stones. Hence, caryophyllaceae, fabaceae and apiaceae are the most widely plant families used for the treatment of kidney stones [25].

The objective of the present study was to investigate and to validate the antiurolithiatic property of *Herniaria fontanesii* J. Gay, *Pituranthos scoparius* and *Cynodon dactylon* (L.) extracts in experimentally-induced urolithiatic *in vitro*. The choice of our investigated plants is based on two criteria: first,

in this domain there are few studies in Algeria that deals with these plants, and the second is that these plants have ethnopharmacological data indicating their traditional utilization against urinary stones. The formation of such urinary calculus involves several physicochemical events, e.g. nucleation, growth and aggregation, but the mechanism of these processes remains incompletely understood. The inhibiting potential of normal urine is attributed to the organic substances present in all urines. Most of the natural inhibitors are not present in sufficient concentrations [26]. Therefore, to prevent this physiological malfunction, the precipitation of calcium oxalate was carried out in the absence and the presence of twenty five extracts which compared to two standard inhibitors (sodium citrate and Succinimide pharbiol).

In our study, the incubation of healthy subject man urine with sodium oxalate at 37°C and pH 6.8 results the calcium oxalate precipitation. Measurements of the turbidity with the presence and without of inhibitor at 620 nm during 300 seconds provide the following curves (Fig. 1-7). Then the percentage of inhibition was calculated at the same concentration of all inhibitors (1 g/l). All the extracts showed inhibition of calcium oxalate crystallization at this concentration (Table 3) when compared with sodium citrate. But, the hydromethanolic extract of *Pituranthos scoparius* aerial part and the aqueous extract of *Cynodon dactylon* (L.) roots showed significant inhibition on calcium oxalate crystallization (51,14 and 50,59% respectively) when compared with Sussinimide pharbiol. Moreover, best inhibition in stone nucleus formation (turbidity) was seen by the hydromethanolic and hydroacetic extracts as compared to the control which is significantly different from other extracts. Furthermore, there is no significant linear correlation observed between the antiurolithiatic activity and total phenolic content According to the phytochemical analysis, extracts which found to contain flavonoids showed good anticristallo-oxalocalcic activities. This observation is agreeing with that of literature investigation which indicates that flavonoids can reduce oxalocalcic crystallization [27]. Thus, the hydroacetic extract of the aerial part of *Herniaria fontanesii* J.Gay which is traditionally used for the treatment of kidney stones 4 have provided high percent inhibition (47.05%). This is probably due to the presence of polyphenols which can form a complex with the calcium cations [26]. Also, the hydromethanolic extracts of the aerial part and roots of *Pituranthos scoparius* prevent 51.14% and

40, 35% of calcium oxalate formation respectively. This result can be interpreted that extracts have oxalocalcic inhibitors such as tannins. These compounds which contain acid functional group may directly imply complexation of calcium ions, and consequently the reduction of oxalocalcic crystallization [28, 29]. As well, hydromethanolic and hydroacetic extracts of *Cynodon dactylon* (L.) aerial part inhibit oxalocalcic crystallization with percentages exceeding 35%. That can be related to the presence of alkaloids. Moreover, the acetic and aqueous roots extracts of the same plant exhibit good inhibitions properties (higher than 40.50%), which can be due to their high content in saponins [30]. Thus, we noted that extracts exhibiting oxalocalcic inhibition percentage upper than 47%, contain catechic tannins, C-Heterosides and O-Heterosides. Subsequently, it is thought that the anticristallo-oxalocalcic activity of these extracts could be due to the existence of these three phytochemical families.

After the screening of anticristallo-oxalocalcic effect of our extracts by using the turbidimetric model, another *in vitro* assay (gravimetric model) was carried out to select the most effective extracts providing kidney stones dissolution. For this reason, extracts which having anticristallo-oxalocalcic inhibition percentages higher than 40% have been testing for their dissolution power against two types of kidney stones (uric acid: CAS number is 54495-64-6; formula  $C_5H_4N_4O$  and calcium urate: CAS number is 36-1619; formula  $C_{10}H_6CaN_8O_6$ ). Kidney stones were exposed for two week, in contact with extracts and the percentage of dissolution was calculated by comparing the residual weight of calculus to their initial weight [31]. The obtained results of this assay are shown in Table 4. According to the results exposed in Table 4, we can see that plants extracts were appeared to be more effective than the sodium citrate solution and less effective than Succinimide pharbiol used as standards. Therefore, the dissolution rates of sodium citrate solution for acid uric and sodium urate stones were 11.72% and 6.88% respectively. While, Succinimide pharbiol drug exhibited higher dissolving power than plant extracts, with dissolution rates of 42.47% and 75.95% for acid uric and sodium urate calculus respectively. However, the hydromethanolic extract of *Pituranthos scoparius* roots had the most efficient dissolving power for acid uric calculi with 46.39% dissolution rate. Whereas, the hydroacetic extract of *Pituranthos scoparius* aerial part and the methanolic extract of *Cynodon dactylon* (L.) roots revealed a dissolution rates of 30.33% and 28.6% respectively,



for sodium urate calculi. Thus, methanolic and acetic extracts of the two parts of *Cynodon dactylon* (L.) exhibited a weak dissolving activity with percentage of dissolution ranging between 1.37 and 9.11%. Moreover, uric acid calculi were better dissolved by some extracts than sodium urate and vice versa. Hence, the good dissolving power of hydromethanolic extract of *Pituranthos scoparius* roots can be resulted from the interaction between uric acid and molecules present in this extract during the dissolution process. According to the phytochemical analysis of this extract, we suppose that its higher activity could be due to the presence of tannins, flavonoids or anthracene derivative. This observation is supported by certain research studies [32, 33].

Moreover, phytochemical screening by one and two-dimensional thin-layer chromatography of this extract allowed us to highlight the presence of flavonoids (AlCl<sub>3</sub> Reagent, yellow fluorescence spot under UV light) (Fig. 7-10). It is also possible to deduce that the polar compounds, which appear as brown spot using vanillin-sulphuric reagent, were glycosides (Fig. 11). Furthermore, TLC plates which showed blue-violet coloured spots with vanillin-sulphuric acid reagent (Fig. 11) and blue fluorescent spots with Liebermann-Burchard reagent (Fig. 12) indicates the presence of terpenoids. These observations were also made by some researchers [34]. In conclusion, these *in vitro* results should be confirmed *in vivo* in order to develop a potent antilithiatic agent from this plant, as this property of the extract is advantageous in preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of their retention in the urinary tract. The mechanism by which the plant exerts its effects remains unknown and could be the objective of study in future. The plant extract may contain phytochemicals that inhibit the growth of calcium oxalate monohydrate crystals, thus phytochemicals responsible for this activity could be analyzed in future studies.

### Conclusion

Upto date, the scientific documentation regarding *in vitro* antiurolithiatic activity of *Cynodon dactylon* (L.) extracts has been reported, although it has not reported for *Herniaria fontanesii* J. Gay and *Pituranthos scoparius* extracts up till now. Subsequently, we have explored in our study the antiurolithiatic activities of twenty five extracts of three local medicinal plants by utilizing two different *in vitro* models. The present investigation

provides useful information on antiurolithiatic activity of these plant extracts, and the hydromethanolic extract of *Pituranthos scoparius* roots was showed maximum antiurolithiatic power of both *in vitro* assays in comparison to the other all extracts and standards.

From the present study it is concluded that urinary stones could be dissolved with hydromethanolic extract of *Pituranthos scoparius* roots and without the aid of surgical intervention. Consequently, the present study provides scientific proof for traditional claim of *Pituranthos scoparius* as antiurolithiatic. So, further *in vivo* studies are required to support the ethnomedicinal claim. Therefore, the present investigation will be supportive to the scientific documentation related *in vitro* studies. Correlation between *in vitro* and *in vivo* studies may be helpful to understand the molecular mechanism of litholysis process and to reveal phytochemicals of the extract responsible for dissolving or disintegrating renal calculi. Further studies need to isolation and purification of active phytoconstituents with potent antiurolithiatic activity.

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**Table 1.** Extraction yield and total phenolic content in different plants extracts

	<i>Cynodon dactylon</i> (L.)				<i>Pituranthos scoparius</i>				<i>Herniaria fontanesii</i> J.Gay	
	Aerial part		Roots		Aerial part		Roots		Aerial part	
	R (%)	TP (mg/g)	R (%)	TP (mg/g)	R (%)	TP (mg/g)	R (%)	TP (mg/g)	R (%)	TP (mg/g)
A	30,46	1,377±0,025	7,57	0,867±0,021	5,05	2,143±0,016	2,40	0,984±0,016	7,23	4,971±0,036
A.W	32,00	2,710±0,118	12,73	2,051±0,017	22,02	4,839±0,140	15,67	1,542±0,053	34,31	2,755±0,026
M	20,57	1,880±0,0276	13,91	1,333±0,017	12,72	3,58±0,132	10,49	6,230±0,091	37,27	2,489±0,013
M.W	16,57	5,160±0,197	12,90	0,822±0,015	10,44	2,133±0,069	16,21	4,745±0,249	38,83	4,926±0,030
W	20,13	2,759±0,026	13,42	1,369±0,051	19,43	4,607±0,075	18,01	2,754±0,022	25,65	0,968±0,042

(R (%): Extraction yield, TP: Total phenolic content (mg/g), A: Acetone (100%), A.W: Acetone/water (50%/50%), M: Methanol (100%), M.W: Methanol/water (50%/50%), W: water (100%).



Table 2. Phytochemical screening of all plants extracts

Plants	Part of plant	Phytochemicals																
		Solvents		tannins		Alkaloids		Anthocyanins	Starch	Flavonoids	Saponins	Coumarins	Sterols and triterpenes	Free anthracene derivatives	Combined anthracene derivatives			
		Gallic	Catechic	Mayer	Wagner	C.Heterosides	O. Heterosides								O. Heterosides with reduced genine	Reducing compounds		
<i>Herniaria fontanesii</i> J.Gay	Air part	A	-	+	-	+++	-	-	++	-	-	-	-	-	+	-	+	-
		A.W	-	+	-	+	-	-	+	-	-	-	-	-	++	-	+	-
		M	-	+	-	+++	-	-	+	-	-	-	-	-	+++	-	+	-
		M.W	-	++	-	+	-	-	+++	-	-	-	-	-	+++	-	+	-
		W	-	-	-	++	-	-	+	+	-	-	-	-	+++	-	+	-
<i>Pituranthos scoparius</i>	Aerial part	A	-	+	+	+	-	-	++	-	-	-	-	-	+++	-	+	-
		A.W	-	+	+	-	-	-	+	-	-	-	-	-	+	-	+	+
		M	-	+	+	-	-	-	-	-	-	-	-	-	+++	-	+	-
		M.W	-	++	-	-	-	-	+	-	-	-	-	-	+++	-	+	+
		W	-	+	+	+	-	-	+	+	-	-	-	-	+++	-	+	++
	Roots	A	-	+	-	+	-	-	-	-	-	-	-	-	+	-	+	-
		A.W	-	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+
		M	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-
		M.W	-	+	-	-	-	-	+	-	-	-	-	-	+	-	+	+
		W	-	+	-	++	-	-	+	+	-	-	-	-	+	-	+++	+
<i>Cynodon dactylon</i> (L.)	Aerial part	A	-	++	+	-	-	-	+	-	-	-	-	-	+++	-	+	-
		A.W	-	+	-	+	-	-	+	-	-	-	-	-	+	-	+	-
		M	-	+	+	+	-	-	+	-	-	-	-	-	++	-	+	-
		M.W	-	+	-	++	-	-	+	-	-	-	-	-	+	-	+	+
		W	-	+	+	-	-	-	+	+	-	-	-	-	+	-	+	+
	Roots	A	-	+	+	-	-	-	-	-	-	-	-	-	++	-	+++	-
		A.W	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+++	+
		M	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+++	-
		M.W	-	+	-	+++	-	-	-	-	-	-	-	-	+	-	++	-
		W	-	+	+	+	-	-	-	+	-	-	-	-	++	-	++	++

Average of 3 readings was taken: (+++) Very strongly positive, (++) Strongly positive, (+) Positive test, (-) Negative test.

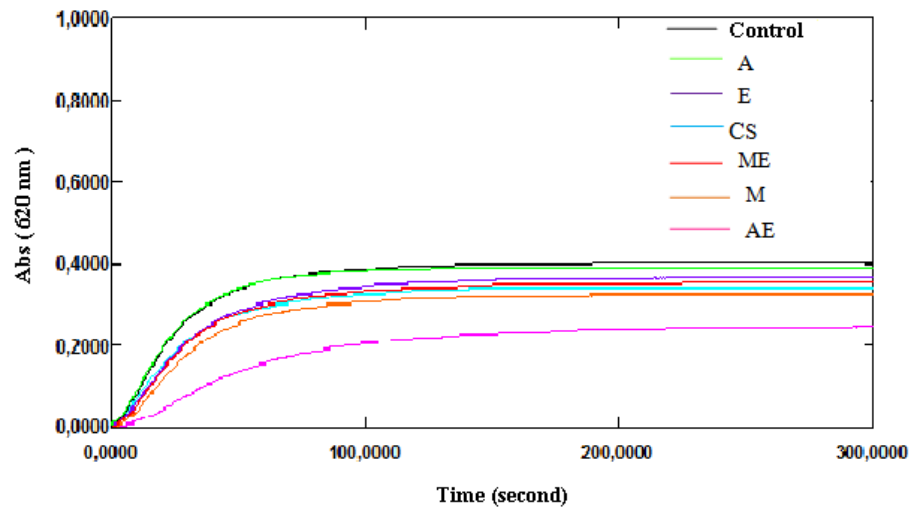
**Table 3.** Shows reduction of Calcium oxalate nucleus formation by plants extracts compared with control drugs.

	Percentage of inhibition (%)				
	<i>Cynodon dactylon</i> (L.)		<i>Pituranthos scoparius</i>		<i>Herniaria fontanesii</i> J .Gay
	Air part	Roots	Air part	Roots	Air part
Acetone	16,79 ±0,71	40,53 ±0,53	8,92 ±0,40	11,40 ±0,45	4,59 ±0,12
Acetone/ Water	35,88 ±0,62	31,79 ±0,62	40,22 ±0,67	30,43 ±0,55	47,05 ±0,75
Methanol	41,77 ±0,76	23,00 ±0,53	19,40 ±0, 41	36,63 ±0,52	24,69 ±0,85
Methanol/ Water	25,27 ±1,09	40,27 ±0,75	51,14 ±0,51	40,35 ±0,80	22,43 ±0,56
Water	12,12 ±0,61	50,59 ±0,61	22,04 ±0,48	18,69 ±0,75	11,34 ±0,99
		Tisane extract	Sodium citrate	Succinimide pharbiol	
		49,78 ±0,08	21,14 ±0,21	60, 39 ±0,49	

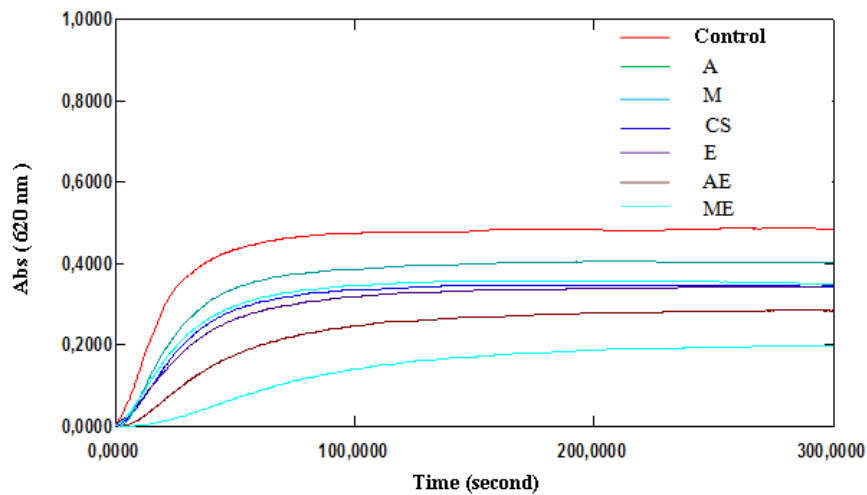
**Table 4.** Shows dissolution calculus by extracts of and standard drugs

	Percentage of dissolution (D%)									
	<i>Cynodon dactylon</i> (L.)				<i>Pituranthos scoparius</i>				<i>Herniaria fontanesii</i> J.Gay	
	Air part		Roots		Air part		Roots		Air part	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
Acetone	-	-	9,11	1,35	-	-	-	-		
Acetone/ Water	-	-	-	-	10,26	30,33			16,49	17,89
Methanol	8,96	3,07	-	-	-	-	-	-	-	-
Methanol/ Water	-	-	6,30	28,6	13,44	9,68	46,39	14,72	-	-
Water	-	-	14,68	5,45	-	-	-	-	-	-
			Tisane extract		Sodium citrate		Succinimide pharbiol			
			C1	C2	C1	C2	C1	C2		
			17,33	24,56	11,72	6,88	42,47	75,95		

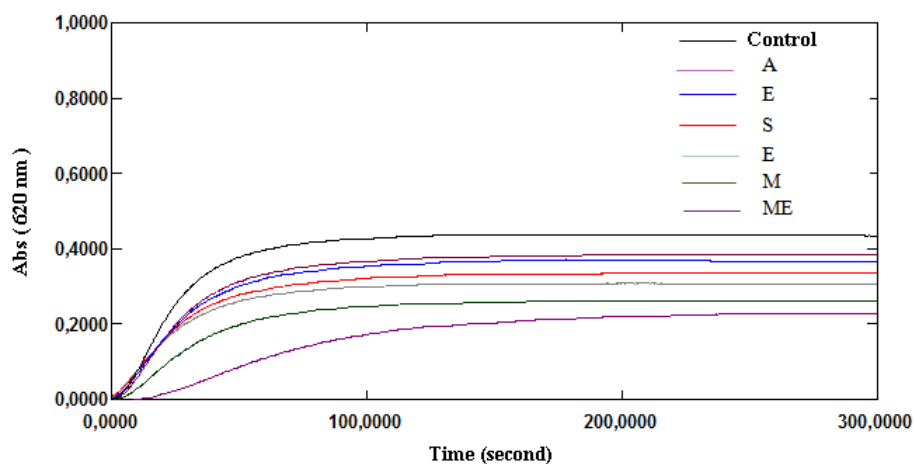
(C1: Uric acid; C2: Sodium urate)



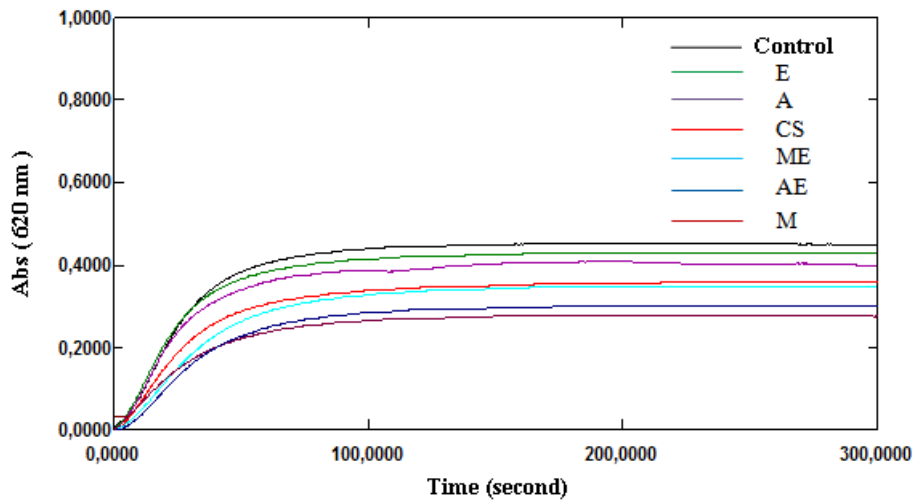
**Figure 1.** Change in turbidity without and with extracts from the aerial part of *Herniaria fontanesii* J. Gay



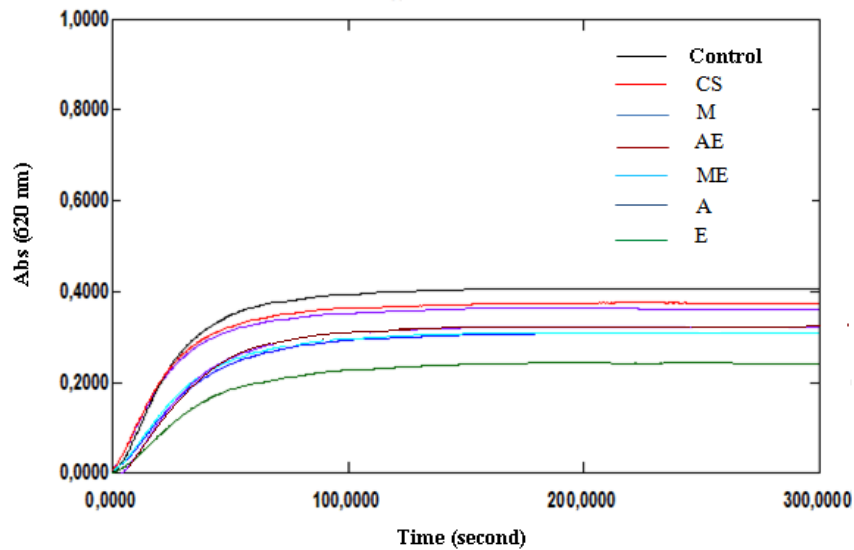
**Figure 2.** Change in turbidity without and with extracts from the aerial part of *Pituranthos scoparius*



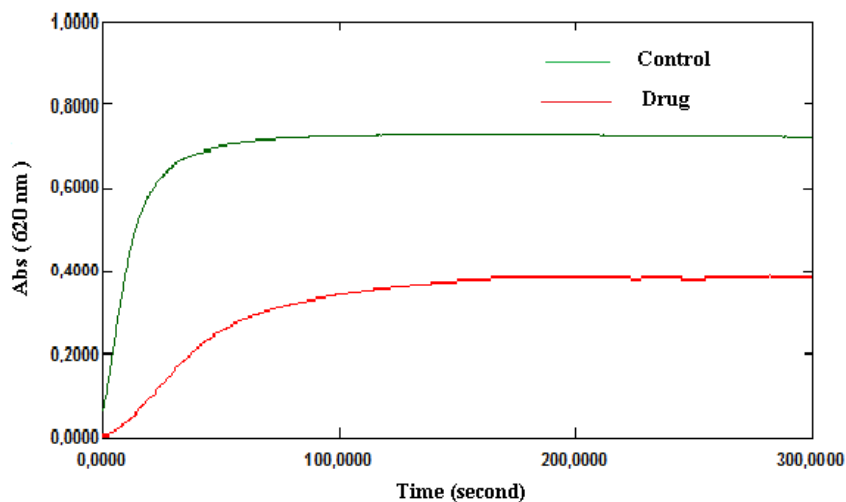
**Figure 3.** Change in turbidity without and with extracts from *Pituranthos scoparius* roots



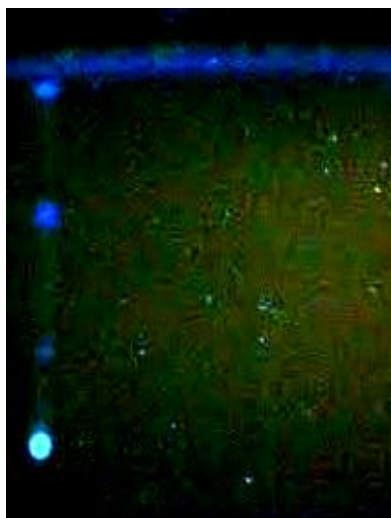
**Figure 4.** Change in turbidity without and with extracts from the aerial part of *Cynodon dactylon* (L.)



**Figure 5.** Change in turbidity without and with extracts from *Cynodon dactylon* (L.) roots



**Figure 6.** Change in turbidity without and with Succinimide pharbiol



**Figure 7.** One-dimensional TLC fingerprinting profile of hydromethanolic extract of *Pituranthos scoparius* roots under UV light (366nm).



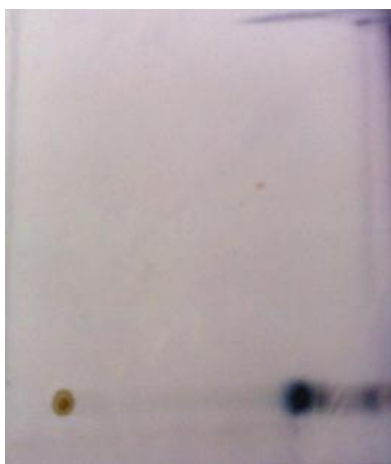
**Figure 8.** Two-dimensional TLC fingerprinting profile of hydromethanolic extract of *Pituranthos scoparius* roots under UV light (366nm).



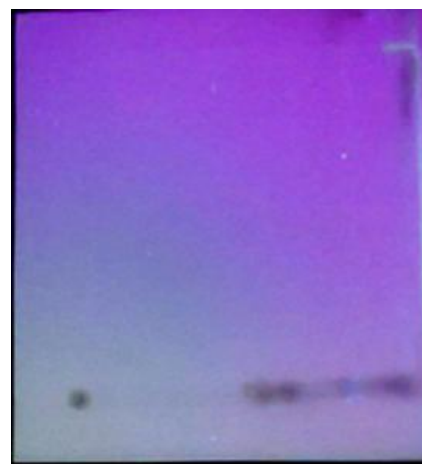
**Figure 9.** One-dimensional TLC fingerprinting profile of hydromethanolic extract of *Pituranthos scoparius* roots under UV light (254nm).



**Figure 10.** Two-dimensional TLC fingerprinting profile of hydromethanolic extract of *Pituranthos scoparius* roots under UV light (366nm) derivatized with  $AlCl_3$  (1 %)



**Figure 11.** One-dimensional TLC fingerprinting profile of hydromethanolic extract of *Pituranthos scoparius* roots derivatized with vanillin-sulfuric acid reagent



**Figure 12.** Two-dimensional TLC fingerprinting profile of hydromethanolic extract of *Pituranthos scoparius* roots under UV light (366nm) derivatized with Liebermann- Burchard reagent