The Rate of Dissolution and Crystallization of Kidney Stone in the Presence of *Hibiscus Sabdariffa* Extracts

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Abstract: Three *Hibiscus Sabdariffa* flowers extracts namely; chloroform, ethanol and aqueous were investigated for its ability to inhibit crystallization of calcium oxalate monohydrate crystals (COM) which is the major constituents of kidney stones. The results obtained showed that the different extracts of *Hibiscus Sabdariffa* have biological properties that act by inhibition rate of melting of calcium oxalate monohydrate stones. The effect of aqueous extract of *Hibiscus Sabdariffa* seeks to discourage the rate of melting of crystals of calcium oxalate from the top of the ethanol extract followed by chloroform extract. The same extracts of *Hibiscus Sabdariffa* also, act as inhibitors of calcium oxalate stones crystallization. The effect of aqueous extract of *Hibiscus Sabdariffa* on the rate of crystallization of calcium oxalate crystals was higher than the ethanol extract followed by chloroform extract. In conclusion, these results have been proved the folk medicine use of *Hibiscus Sabdariffa* for kidney stones and the extracts of it may be beneficial for the treatment of nephrolithiasis but a detailed preclinical and clinical study is required.

[Dalia I. Saleh, Samy F. Mahmoud, El-Sayed S. Abdel-Hameed. **The Rate of Dissolution and Crystallization of Kidney Stone in the Presence of Hibiscus Sabdariffa Extracts.** *Life Sci J* 2013;10(2):700-710]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 98

Key words: Nephrolithiasis, *Hibiscus*, Crystallization, Dissolution, Crystal growth.

1. Introduction

Nephrolithiasis, the formation of kidney stones, is a very painful disease that has afflicted a wide sector of human population since ancient times. There is no satisfactory drug available for use in clinical therapy then there is a need to explore more safe and cheap drugs from natural resources. The problem of calculi formation is observed and reported in all parts of the urinary tract, the kidney, the ureter and the urinary bladder, which may considerably vary in size (Havagiray *et al.,* 2010). Stone formation commonly occur due to inadequate urinary drainage, foreign bodies in urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities like vitamin A deficiencies, excess vitamin D, and metabolic diseases like hyperthyroidism, cystinuria, gout, intestinal dysfunction…. etc. (Butterweck & Khan, 2009).

Calcium-containing stones especially are the most commonly occurring ones (75-90%) followed by magnesium ammonium phosphate (10-15%) uric acid (3-10%) and cystine 0.5-1% (Yadav *et al.,* 2011). Calcium oxalate (CaOx) is the main inorganic phase found in kidney stones (-70%) (Bushinsky, 2001). Although both monohydrate (COM) and dihydrate (COD) polymorphs of CaOx can form, the former is much more common in stones (Lieske & Toback, 2000) and typically constitutes the core of kidney stones (Mandel & Mandel, 1989). In urine of stone-formers COM is the most abundant phase; it is, however, seldom excreted by healthy individuals, whereas COD crystals are routinely flushed out by urinary flow of both healthy people and stone-formers (Dyer & Nordin, 1967; Elliot & Rabinowitz, 1980). Multiple steps are involved in the formation of crystals, which are nucleation, crystal growth, crystal aggregation and crystal retention (Pareta *et al.,* 2011). Studies by Finlayson & Reid (1978) have shown that once nucleated, a crystal will pass into the urine before it grows sufficiently large to be retained in the nephron and subsequently form a stone. However, if not stabilized by adsorbed urinary compounds and excreted, COD crystals will undergo phase conversion to COM (Tomazic & Nancollas, 1979). In addition, membrane vesicles (Fasano & Khan, 2001), protein fragments (Grohe *et al.,* 2011), proteins, synthetic peptides (Wesson *et al.,* 1998; Grohe *et al.,* 2009; Chien *et al.,* 2009), poly-acids (Wesson & Worcester, 1996; Grohe *et al.,* 2006; Kirboga & Oner, 2010), bio- and block copolymers (Akyol & Oner, 2007; Akin *et al.,* 2008) and magnesium (Fasano & Khan, 2001) have been deemed important in producing COD crystals. It appears that all these compounds decrease the activation energy for COD formation and, thereby, catalyze the COD phase (e.g. via heterogeneous nucleation).

Management of stone disease depends on the size and location of the stones. There is no satisfactory drug available for use in clinical therapy then there is a need to explore more safe and cheap drugs from natural resources. From ancient periods, a number of herbal medicines have been found with potential effect in treating the problem of renal calculi (Khan *et al.,* 2010). Also, there are many reports on antiurolithiatic activity of various herbal extracts (Prasad *et al.,* 2007;Yadav *et al.,* 2011; Joy *et al.,* 2012).

It was reported that the two Hibiscus species; *H. sabdariffa* and *Hibiscus rosa sinensis* claimed as folk medicine for kidney stones (Jadhav *et al.,* 2009; Pachana, 2010; Nirmaladevi *et al.,* 2012: Kumar & Singh A., 2012).

In continuation of our work for exploring and studying plants with medicinal importance (Abdel-Hameed *et al.,* 2008; Abdel-Hameed, 2009; Dalia *et al.* 2010 ; Abdel-Hameed *et al.,* 2012) the three successive extracts; chloroform, ethanol and aqueous obtained from flowers of *H. sabdariffa* were investigated for its ability to inhibit crystallization of calcium oxalate monohydrate crystals (COM) which is the major constituents of kidney stones.

2. Materials and Methods

2.1. Chemicals

All solvents and reagents were analytical grade unless otherwise stated. Calcium carbonate, sodium oxalate, sodium chloride, ethanol, chloroform as well as other chemicals were obtained from Sigma Co.(St.Louis, Mo.,U.S.A.) . Deionized distilled water was used in the preparation of reagents and washing the glassware throughout.

2.2. Preparation of plant extracts and stock solution

The fresh flowers of *Hibiscus rosa-sinensis* were collected, cut and shade dried then finely powdered by electric mill and become ready for extraction. The plant powder (500 g) was used in the extraction process by Soxhlet extractor. In this process the extraction was started with chloroform for two hours followed by ethanol (2 hrs) and finally with water (2 hrs). At the end of each process, the filtrate was evaporated under vacuum using rotavapor affording dried extract ready for investigation.

Stock solutions of chloroform and ethyl alcohol extracts were prepared by dissolving in suitable volume of ethanol then completed by deionized distilled water. A suitable volume of saturated solution was taken to prepare different concentrations by dilution. Aqueous extract was also prepared by dilution of saturated solutions to get different concentrations.

2.3. Preparation of solutions

Pyrex glassware and analytical grade chemicals were used throughout. Water was purified by deionization followed by triple distillation and stored in a pyrex vessel under nitrogen. Solutions of carbonate free sodium hydroxide (0.1 M) and hydrochloric acid (0.1 M) were properly prepared. The sodium hydroxide solutions were standardized by acid-base titration using standard potassium hydrogen phthalate and standardized hydrochloric acid solutions by titration using phenolphthalein as an indicator.

2.4. Preparation of seeds

Calcium oxalate seeds were prepared by adding one liter of 0.1 M calcium chloride solutions to one liter of sodium oxalate solution (0.1 M) at 298 K at a rate of 250 ml per half an hour. The sodium oxalate solution was constantly stirred throughout the addition. The seed suspension was allowed to age with stirring for one day and was then filtered and the seed crystals were washed with deionized distilled water to remove surface contamination essentially chloride and oxalate ions. The seed crystals were aged for one month, then were re-filtered and washed further with deionized distilled water. The later process was repeated several times. The seeds were then filtered and dried. The seed material was then subject to x-ray powder diffraction studies, scanning electron microscope and the determination of specific surface area (SSA).

2.5. Measurements of surface area

It was reported that the specific surface area measured by the BET gas adsorption method could be different from those measured by Oilier methods (Wesson & Worcester, 1996). Thus, the specific surface area (SSA) was determined by the BET method applying equation (1):

$$SSA = 1/W\left(1 - P/P_o\right)\left(S_g / S_{gc}\right)V_c \frac{N^o A_{cs} P_o}{RT}$$

…… (1)

where:

W : the weight of solid,

Sg : the desorption single area,

S_{gc}: the single area of calibration,

V_c : the volume of calibration,

N° : Avogadro's number,

A_{cs}: the cross-sectional area of adsorbate molecule,

Po : the ambient pressure.

2.6. Calcium oxalate characterization

In the X-ray diffraction studies, the solid phase of calcium oxalate was characterized by x-ray powder diffraction using Cu-K radiation. The solid sample was well ground and mixed with internal standard, potassium bromide the ratio of about 4:1 by weight. The sample and standard were filled in a

rectangular cavity (1.5 cm × 1.0 cm × 0.05 cm) of a 3.8 cm × 3.8 cm × 0.2 cm aluminum solid holder and were slowly scanned at a speed of 10 / 4 20 = 10° to 90°. Scanning Electron microscopic micrograph of COM seeds is shown in Fig. (1).

In the scanning electron microscope study to obtain the shape of the seed crystals, Scanning Electron microscopic micrograph of COM seeds is shown in Fig. (2).

In the infra red study (IR) to insure that the prepared seed is COM, IR spectra were recorded by the IR spectrum of $CaC_2O_4 . H_2O$ Fig. (3).

In the thermal gravimetric analysis (TGA) study to obtain that the prepared calcium oxalate is monohydrate, The TGA was done on prepared seed is shown in (Fig. 4).

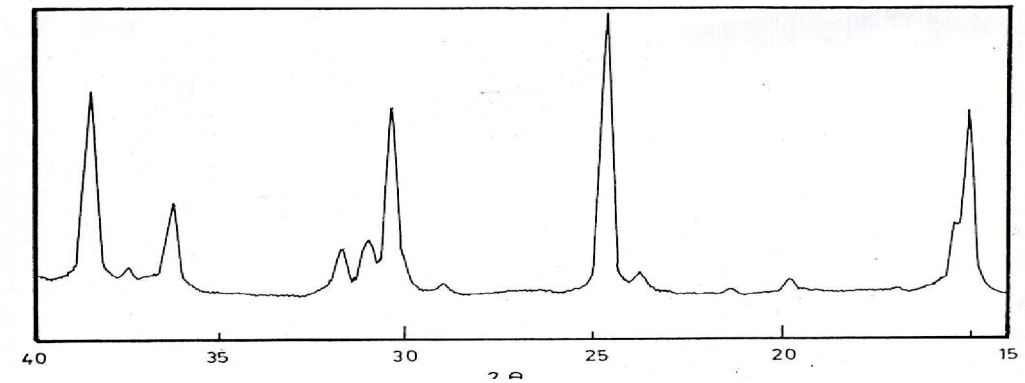

Fig. 1: X-ray powder diffraction studies for calcium oxalate crystals.

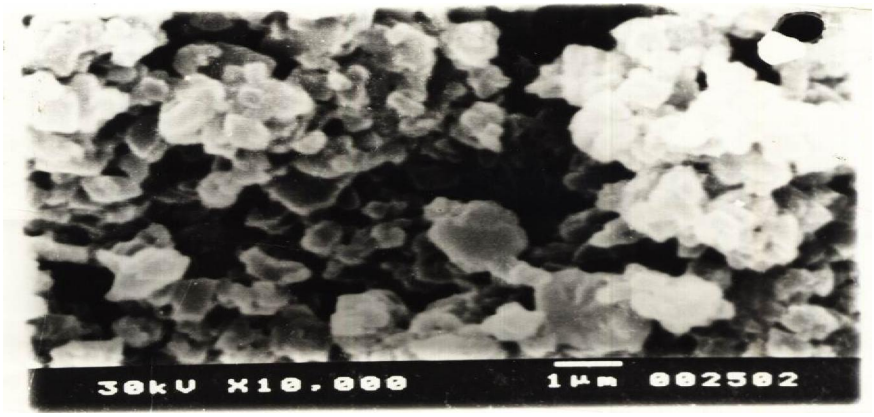

Fig. 2: SEM Micrographs of COM seeds.

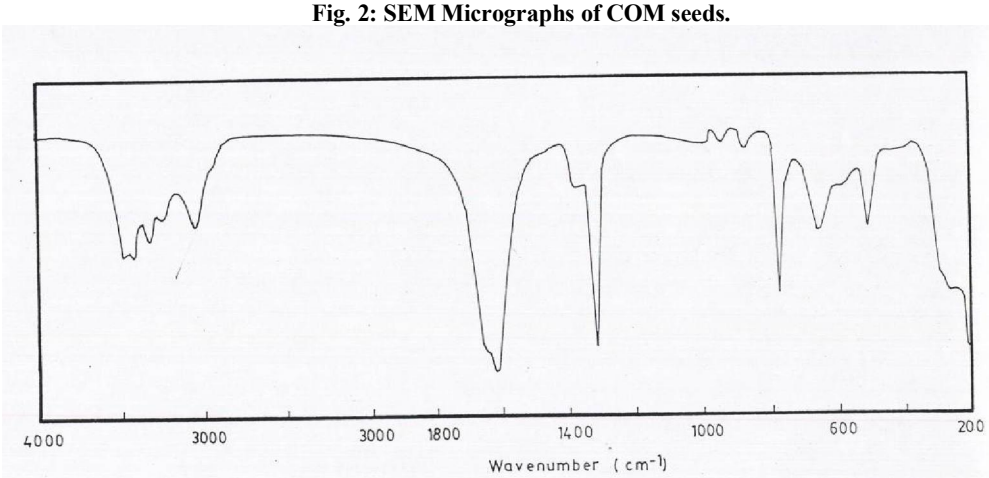

Fig. 3: IR Spectrum of calcium oxalate.

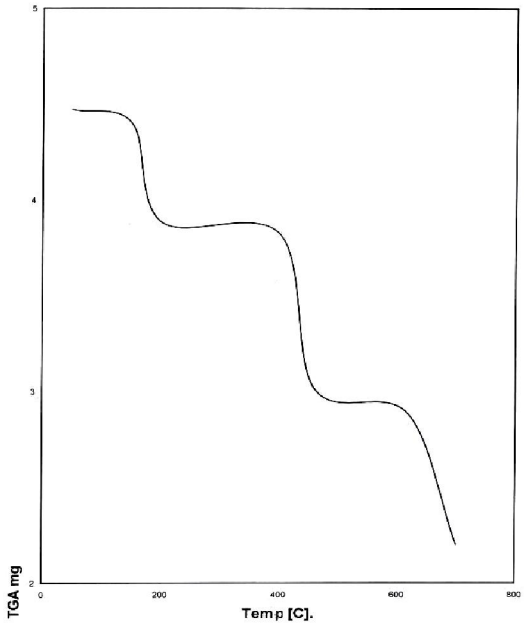

Fig. 4: Plots of temperature against TGA on calcium of oxalate crystals.

2.8. Potentiometric measurements

pH measurements were made using pH meter (Orion model 720A+). connected with a combined pH glass electrode. EMF measurements were made by calcium ion selective electrode, in conjugation with a calomel reference electrode. The combined pH glass electrode was checked before and after each dissolution or crystal growth experiment using the buffer solutions. The calcium ion selective electrode was checked using calcium chloride solutions with definite concentrations. If combined pH glass electrode measurements differed from the required, the electrode was reconditioned in warm HCl solution. In case of calcium ion selective electrode, if the measurements differed from the required, the electrode was put in solution of 10^{-2} M $CaCl_2$ for few days.

In dissolution experiments using pH-state, the studies were made at constant pH meter (Orion model 720A+); consisting of decimate impulsomate and stirrer was used to control the addition of titrant solution consisting of 0.15 M sodium chloride, into the reaction vessel. Since the impulsomate provides proportional control from the potential difference, the system was able to respond to a change of EMF of < 0.002 mV on the addition of reagents.

2.9. Dissolution experiments:

The crystal dissolution experiments were carried out in water thermo-stated double-walled Pyrex glass vessels. The cells were maintained at the required temperature (37 °C) by circulating thermo-stated water through the outer jackets. The cell contents were stirred with a magnetic stirrer and presaturated with nitrogen gas bubbled through the solutions during the experiments to exclude carbon dioxide.

In dissolution experiments, a measured volume of deionized, distilled water was transferred to the cell and a known volume of sodium chloride was added, then definite volume of calcium chloride solution was added followed by slow addition of known volume of sodium oxalate solution over a period of five minutes. The total volume was usually 300 ml and the pH was adjusted to the required value (6.0 ± 0.05) using standard sodium hydroxide solution or standard hydrochloric acid solution. Satisfactory stability of the under saturated solution was verified by constant pH reading for at least 30 minutes in experiments using pH-state, and by stability of EMF reading also at 30 minutes in potentiostate experiments. Following the addition of dry seed crystals, dissolution began immediately and combined pH glass electrode was used to control the addition of titrant solution consisting of 0.15 M sodium chloride in experiments using pH-state, while calcium ion selective electrode in conjunction with calomel reference electrode were used in experiments using potentiostate.

In addition, samples were periodically withdrawn and filtered at the reaction temperature through Millipore filters (0.22 M), prior to solution and solid-phase analysis.

2.10 Crystal growth measurements

Crystal growth experiments were carried out in water thermostated double-walled Pyrex glass vessel at (37 °C). A measured volume of deionized

distilled water was transferred to the cell followed by definite volumes of sodium chloride solution and calcium chloride solution, then a known volume of sodium oxalate solution was added slowly with constant stirring. The total volume was usually 300 ml and the pH was adjusted to pH = 6.5 ± 0.05 using standard solution of sodium hydroxide or hydrochloric acid. The stability of supersaturated solution was verified by constant EMF reading for at least 30 minutes. Then the dry seed was added and crystal growth began immediately. The calcium ion selective electrode in conjunction with Ag/Ag Cl electrode were used to control the addition of titrant solution consisting of (2.15×10^{-4} M) calcium chloride and (2.15×10^{-4} M) sodium oxalate solutions with definite volume of 1 M sodium chloride solution.

3. Results and discussion

Characterization of calcium oxalate formation is very important for the analytical and biological strategies. In this context, the quantitative studies of the ionic interactions in solutions of this electrolyte as well as the kinetics of its dissolution and crystal growth are critical challenge. Since, calcium oxalate is present in nearly all kidney and bladder stones (Singh *et al.,* 1988). In addition to growth kinetics, the inhibition of stone formation is another aim of active interest, since in normal urine the product of the ionic calcium and oxalate concentrations is more than one hundred times the value found in saturated solutions of the salt (Dalia, 2006). Calcium oxalate monohydrate (COM), a common constituent of kidney stones, usually occurs as irregular radial agglomerates which are normally formed by rapid precipitation (Grases *et al.,* 1988). In spite of the importance of studying the dissolution and crystallization of calcium oxalate, it is poorly developed, particularly from a kinetic point of view and still contains many unanswered questions. The simplest models of calcium oxalate stone formation describe it as taking place in for stages:

1- There must be a period of high super saturation during which crystal nucleation is "triggered off either homogeneously or; heterogeneously,
2- This is followed by a period of rapid crystal growth and / or aggregation when the primary particles accrete in size,
3- If this occurs sufficiently rapid, a particle may be generated which is just large enough to be trapped at some narrow point in the urinary system (the so-called free particle theory),
4- Alternatively it has been suggested that a primary particle may become attached to the walls of the renal tubules or collecting system through the participation of some gluing material (the so-called field particle theory). In both cases a nucleus is formed which constitutes a center around which a stone will form by the continued processes of crystal growth and adhesion.

In the present work, the rates of dissolution of COM ($K_{so} = 9.899 \times 10^{-8}$) have been investigated at 37 °C in absence and presence of trace amounts of natural products from Hibiscus extracts under saturated solution.

3.1. The effect of different extracts of *Hibiscus Sabdariff* rate of COM dissolution.

For this purpose, the effect of aqueous extract [Hi(a)], ethanolic extract [Hi(e)] and chloroformic extract of Hibiscus [Hi(c)] on the dissolution of COM have been measured. The experimental data obtained are summarized in tables (1 & 2). As can be seen, the data are given at the same relative under saturation ($\alpha = 0.09$). Each experiment was made in duplicate or triplicate for certainties using constant composition method in all experiments of the study. It can be seen from table (1) that a concentration of Hi(a) as low as 10^{-5} mol dm^{-3} experiments 1, markedly reduce the dissolution rates by at least 48.67 % times compared to that in absence of the additive at the same relative under saturation ($\alpha = 0.09$). In the case of Hi(e), its concentration as low as 10^{-5} mol dm^{-3} (experiment number 14) shows that the rate of dissolution reduced by 45.02%, at last at concentration 10^{-5} mol dm^{-3} of Hi(c) (experiment number 27) shows that the rate of dissolution decreased by 38.04 %. When the concentrations of the extracts was increased the rates of dissolution decreased due to blocking of active sites on the crystal surface by the additive molecule. On the assumption that the decrease in the rate of dissolution in the presence of different extracts of Hibiscus, reflect their adsorption at active dissolution sites on COM crystal surface, the degree of inhibition may be interpreted in terms of assemble Langmuir adsorption isotherm. This requires, linear relationship between the inverse of the relative function in rate R_o, $(R_o - R_I)$, and $[additive]^{-1}$; the results for the dissolution of calcium oxalate crystals in the presence of Hi(a), Hi(e) and Hi(c) at relative under saturation ($\alpha = 0.09$) are given in table (2). Applying Langmuir equation supports the assumption of surface controlled mechanisms. Chelating anions may be adsorbed at cationic sites and inhibit the dissolution when present at very low levels. When *Hibiscus Sabdariff* increased in concentration the amount of COM particles decreased. This is because Hibiscus contains mainly anthocyanin , cyaniding , delphinidin (Hong & Wrostlad 1990), succinic acid ,and oxalic acid (Petrova *et al.,* 2004).

According to the formulae of these compounds we can expect that the case of inhibition which may be due to adsorption of COO^-, OH^-, ~ H^+

groups which is discussed before; these groups act as a good medium for inhibiting the dissolution process of COM crystals (Pachana *et al.,* 2010). Compounds of positive charge increase the dissolution rate of COM so that the different acids in Hi (a) can decrease the dissolution rat.

On the other hand, ethanolic extract of *Hibiscus Sabdariff* Hi(e) contains glycosides, phenolic, amino acid, carbohydrates and flavanoids (Nirmaladevi *et al.,* 2012). The presence of coo^- group can act as a good medium for inhibiting the dissolution process of COM crystals (Dalia, 2006). It is clear in table (1) as it the coo^- group lower adsorption on the active sites on COM crystal and case the inhibition effect (Dalia, 2006).

The chloroformic extract of *Hibiscus Sabdariff* composition contains five new phytoconstituents (n-Nonacosan-13-one, n-Triacontane, n-Dotetracontane, n-Nonacosan-4-ol-18-one, n-Hentriacontan (Subramanian and Nair, 1972; Nakateni and Fukunaga, 1986; Shimizu and Tomado, 1993; Nakateni and Matsuoko, 1994; Anees *et al.,* 2004). Therefore, the presence of C=O and OH groups can reduce the dissolution effect (Dalia, 2006).

Table (1): Effect of different extracts of *Hibiscus Sabdariff* on the rate of dissolution of COM Tca : Tox = 1 : 1 at t = 37°C 10 mg seed, ionic strength = 0.15 mol dm^{-3} (NaCl) and σ = 0.09 using EMF.

Exp. No.	Additive / 10^{-7} mol dm^{-3}	Rate / 10^{-9} mol min^{-1} m^{-2}	% inhibition
1	Hi(a) 100	15.99	48.67
2	Hi(a) 95	16.185	48.07
3	Hi(a) 90	16.450	47.22
4	Hi(a) 85	16.965	45.57
5	Hi(a) 80	17.580	43.99
6	Hi(a) 75	18.132	41.82
7	Hi(a) 70	18.710	39.97
8	Hi(a) 60	19.970	35.93
9	Hi(a) 50	22.050	29.25
10	Hi(a) 40	23.615	24.23
11	Hi(a) 30	25.173	19.23
12	Hi(a) 20	27.081	13.11
13	Hi(a) 10	29.083	6.69
14	Hi(e) 100	17.136	45.02
15	Hi(e) 95	17.442	44.04
16	Hi(e) 90	17.886	42.61
17	Hi(e) 85	18.390	41.00
18	Hi(e) 80	18.971	39.13
19	Hi(e) 75	19.523	37.36
20	Hi(e) 70	20.187	35.23
21	Hi(e) 60	21.504	31.00
22	Hi(e) 50	23.070	25.98
23	Hi(e) 40	24.580	21.13
24	Hi(e) 30	26.020	16.51
25	Hi(e) 20	27.786	10.85
26	Hi(e) 10	29.492	5.37
27	Hi(c) 100	19.31	38.04
28	Hi(c) 95	19.620	37.05
29	Hi(c) 90	19.910	36.12
30	Hi(c) 85	20.270	34.96
31	Hi(c) 80	20.730	33.49
32	Hi(c) 75	21.123	32.23
33	Hi(c) 70	21.502	31.01
34	Hi(c) 60	22.780	26.91
35	Hi(c) 50	24.072	22.76
36	Hi(c) 40	25.230	19.05
37	Hi(c) 30	26.513	14.93
38	Hi(c) 20	28.291	9.23
39	Hi(c) 10	29.870	4.16

Table (2): Values of $R_o / (R_o - R_I)$ and $[inhibitor]^{-1}$ for dissolution of COM at 37°C, $\alpha = 0.09$ and $R_o = 31.167 \times 10^{-9}$ mol min^{-1} m^2.

Exp. No.	Additive / 10^{-7} mol dm^{-3}	Rate / 10^{-9} mol min^{-1} m^{-2}	10^5 [inhibition]$^{-1}$	Ro / (Ro – Ri)
1	Hi(a) 100	15.990	1.00	2.0536
2	Hi(a) 95	16.185	1.05	2.0800
3	Hi(a) 90	16.450	1.11	2.118
4	Hi(a) 85	16.965	1.18	2.195
5	Hi(a) 80	17.580	1.25	2.294
6	Hi(a) 75	18.132	1.33	2.391
7	Hi(a) 70	18.710	1.43	2.502
8	Hi(a) 60	19.970	1.67	2.784
9	Hi(a) 50	22.050	2.00	3.419
10	Hi(a) 40	23.615	2.50	4.127
11	Hi(a) 30	25.173	3.33	5.200
12	Hi(a) 20	27.081	5.00	7.628
13	Hi(a) 10	29.083	10.00	14.955
14	Hi(e) 100	17.136	1.00	2.221
15	Hi(e) 95	17.442	1.05	2.271
16	Hi(e) 90	17.886	1.11	2.347
17	Hi(e) 85	18.390	1.18	2.439
18	Hi(e) 80	18.971	1.25	2.556
19	Hi(e) 75	19.523	1.33	2.677
20	Hi(e) 70	20.187	1.43	2.839
21	Hi(e) 60	21.504	1.67	3.225
22	Hi(e) 50	23.070	2.00	3.849
23	Hi(e) 40	24.580	2.50	4.732
24	Hi(e) 30	26.020	3.33	6.055
25	Hi(e) 20	27.786	5.00	9.218
26	Hi(e) 10	29.492	10.00	18.607
27	Hi(c) 100	19.31	1.00	2.629
28	Hi(c) 95	19.620	1.05	2.699
29	Hi(c) 90	19.310	1.11	2.769
30	Hi(c) 85	20.271	1.18	2.860
31	Hi(c) 80	20.730	1.25	2.986
32	Hi(c) 75	21.123	1.33	3.103
33	Hi(c) 70	21.502	1.43	3.225
34	Hi(c) 60	22.780	1.67	3.716
35	Hi(c) 50	24.072	2.00	4.393
36	Hi(c) 40	25.230	2.50	5.250
37	Hi(c) 30	26.513	3.33	6.697
38	Hi(c) 20	28.291	5.00	10.837
39	Hi(c) 10	29.870	10.00	24.030

3.2. The effect of *Hibiscus Sabdariff* extracts on the crystallization of COM

Additives of both organic or inorganic nature play an important role in crystallization processes. It is important to know how the additives influence the crystallization process as well as the type and number of polar functional groups contained in additives molecule. Hydrophobic and hydrophilic regions, the molecular weight and concentration of additives and a close match between the spacing of acid groups and the spacing of cations of the crystal surface are considered among the factors that influence crystallization. It is proposed that the additives have two functions:

a- They could inhibit crystal growth by binding to the growth sites of the crystals,

b- They could act as a heterogeneous nucleator,

In general, changes in the rate of crystallization produced by the addition of foreign substances may result either from ocmplexation of the inhibitor, usually a chelating or sequestering agent, with the lattice cation and by adsorption of the molecules at active sites at the crystal surfaces. The latter of "threshold effect" may be induced through adsorption at much lower concentrations of the additive molecules. The influence of the inhibitors on crystal growth must be studied under highly reproducible conditions by the constant composition method described by Nancollas *et al.* (1979) and Grases *et al.* (1988).

In the case of Hi(a) we found that as the Hi (a) increase the crystal gross decrees as seen in table (3). Hence, the function groups of organic compound pigment and acid could transform the structure of COM to COD. This indicates that hibiscus may act as a good inhibitor for kidney stones since it induces the COD crystals that are easily excreted in urine (Pachana *et al.,* 2010). In particular , inhibition in urine will transform COM to COD (Jung *et al.,*2004;Thongboonkerd *et al.,* 2005). When the

Hibiscus Sabdariff concentration increase the amount of COM particles decrees this is because it contains anthocyanin, cyaniding, delphinidin (Hong & Wrostlad 1990); ascorbic acid (Wong et al., 2002; Fasoyiro et al., 2005) citric acid, Carvajal et al. (2009) succinic acid, and oxalic acid (Fasoyiro *et al.,*2005). Hence, these functional groups of organic compound pigment and acid could transform the structure of COM to COD (Pachana *et al.,* 2010).

In the case of Hi (e) there is also an acidic group which may be responsible on the inhibition effect of COM crystallization as in table (3). As it is seen the inhibition effect of the different extract can be arranged as; Hi(a) > Hi(e) > Hi(c). This is due to the acidity compounds in H(a) more than Hi(e) more than Hi(c).

From table (4) the KL values of different extracts of *Hibiscus Sabdariff* were calculated and it is found that KL values for Hi(a), Hi(e) and Hi(c) equal 14.26×10^4 dm^3 mol^{-1}, 10.53×10^4 dm^3 mol^{-1} and 5.41×10^4 dm^3 mol^{-1}. The values of KL obtain high adsorption affinity for the extracts.

Table (3): Effect of some *Hibiscus Sabdariff* extracts on the rate of crystal growth of calcium oxalate Tca : Tox = 1 : 1 at t = 37 °C 10 mg seed, ionic strength = 0.15 mol dm^{-3} (NaCl) and σ = 0.4 using EMF.

Exp. No.	Additive / 10^{-6} mol dm^{-3}	Rate / 10^{-9} mol min^{-1} m^{-2}	% inhibition
40	-	46.283	-
41	Hi(a) 100	34.56	25.34
42	Hi(a) 95	34.75	25.33
43	Hi(a) 90	35.01	24.36
44	Hi(a) 85	35.24	23.86
45	Hi(a) 80	35.51	23.28
46	Hi(a) 75	35.86	22.52
47	Hi(a) 70	36.19	21.81
48	Hi(a) 63	36.72	20.66
49	Hi(a) 60	37.02	20.01
50	Hi(a) 53	37.53	18.91
51	Hi(a) 50	37.82	18.29
52	Hi(a) 40	38.82	16.20
53	Hi(a) 30	39.98	13.62
54	Hi(a) 20	41.43	10.49
55	Hi(a) 10	43.44	6.14
56	Hi(e) 100	36.61	20.91
57	Hi(e) 95	36.97	20.12
58	Hi(e) 90	37.23	19.56
59	Hi(e) 85	37.58	18.80
60	Hi(e) 80	37.87	18.18
61	Hi(e) 75	38.19	17.49
62	Hi(e) 70	38.51	16.79
63	Hi(e) 63	39.03	15.67
64	Hi(e) 60	39.25	15.20
65	Hi(e) 53	39.83	13.94
66	Hi(e) 50	40.08	13.40
67	Hi(e) 40	40.91	11.61
68	Hi(e) 30	41.82	9.64
69	Hi(e) 20	43.12	6.83
70	Hi(e) 10	44.69	3.44
71	Hi(c) 100	38.08	17.73
72	Hi(c) 95	38.38	17.08
73	Hi(c) 90	38.62	16.56
74	Hi(c) 85	38.93	15.89
75	Hi(c) 80	39.28	15.13
76	Hi(c) 75	39.58	14.48
77	Hi(c) 70	39.93	13.73
78	Hi(c) 63	40.37	12.78
79	Hi(c) 60	40.63	12.21
80	Hi(c) 53	41.12	11.16
81	Hi(c) 50	41.39	10.57
82	Hi(c) 40	42.71	7.72
83	Hi(c) 30	43.02	7..05
84	Hi(c) 20	44.05	4.82
85	Hi(c) 10	45.24	2.25

Table (4): Values of Ro / (Ro / Ri) and [inhibitor]^{1} of crystal growth of calcium oxalate in presence of *Hibiscus Sabdariff* extracts at 37 °C and σ = 0.4 using EMF.

Exp. No.	Additive / 10^{-6} mol dm^{-3}	Rate / 10^{-10} mol min^{-1} m^{-2}	10^{5} [inhibition]$^{-1}$	Ro / (Ro – Ri)
41	Hi(a) 100	34.56	0.100	3.948
42	Hi(a) 95	34.75	0.105	4.013
43	Hi(a) 90	35.01	0.111	4.106
44	Hi(a) 85	35.24	0.118	4.191
45	Hi(a) 80	35.51	0.125	4.296
46	Hi(a) 75	35.86	0.133	4.440
47	Hi(a) 70	36.15	0.143	4.586
48	Hi(a) 63	36.72	0.159	4.840
49	Hi(a) 60	37.02	0.167	4.997
50	Hi(a) 53	37.53	0.189	5.288
51	Hi(a) 50	37.82	0.200	5.469
52	Hi(a) 40	38.82	0.250	6.202
53	Hi(a) 30	39.98	0.333	7.343
54	Hi(a) 20	41.43	0.500	9.537
55	Hi(a) 10	43.44	1.000	16.280
56	Hi(e) 100	36.31	0.100	4.886
57	Hi(e) 95	36.97	0.105	4.970
58	Hi(e) 90	37.23	0.111	5.112
59	Hi(e) 85	37.58	0.118	5.318
60	Hi(e) 80	37.87	0.125	5.501
61	Hi(e) 75	38.19	0.133	5.719
62	Hi(e) 70	38.51	0.143	5.954
63	Hi(e) 63	39.03	0.159	6.381
64	Hi(e) 60	39.25	0.167	6.581
65	Hi(e) 53	39.83	0.189	7.172
66	Hi(e) 50	40.08	0.200	7.461
67	Hi(e) 40	40.91	0.250	8.614
68	Hi(e) 30	41.82	0.333	10.324
69	Hi(e) 20	43.12	0.500	14.633
70	Hi(e) 10	44.69	1.000	29.054
71	Hi(c) 100	38.08	0.100	5.642
72	Hi(c) 95	38.38	0.105	5.856
73	Hi(c) 90	38.62	0.111	6.040
74	Hi(c) 85	38.93	0.118	6.294
75	Hi(c) 80	39.28	0.125	6.609
76	Hi(c) 75	39.58	0.133	6.905
77	Hi(c) 70	39.93	0.143	7.285
78	Hi(c) 63	40.37	0.159	7.827
79	Hi(c) 60	40.63	0.167	8.187
80	Hi(c) 53	41.12	0.189	8.984
81	Hi(c) 50	41.39	0.200	9.459
82	Hi(c) 40	42.71	0.250	12.954
83	Hi(c) 30	43.02	0.333	14.184
84	Hi(c) 20	44.05	0.500	20.727
85	Hi(c) 10	45.29	1.000	44.375

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3/13/2013