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Inhibitory effects of *Ferula communis* L and *Verbena officinalis* L. extract on the crystal growth of calcium oxalate monohydrate

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ABSTRACT

The imbalance between promoters and inhibitors in the kidneys leads to Kidney stone formation or urolithiasis. The effects of aqueous and ethyl acetate extracts of *Ferula communis* L(Fc) and *Verbena officinalis* L (Vo), on the growth rate of COM crystals, were studied by using a constant composition technique in the absence and presence of inhibitors over the range of relative supersaturation (σ=0.23–0.70). The effect of I, pH, T, and size of particle seed crystal parameters was evaluated. Our results reveal that the aqueous extracts of Fc and Vo were found more effective than ethyl acetate extracts on crystal growth of $CaOx.H_2O$ experimentally, with the highest inhibition at 86.7% of aqueous (Fc) compare to 80.70 % for aqueous (Vo) in compare with 71.56% and 62.39% for ethyl acetate (Fc) and ethyl acetate (Vo) respectively at the same concentration and the same relative supersaturation degree. The dependence of the rates of COM crystallization in the presence of inhibitors was found to be second order suggesting a surface-controlled mechanism with n ≈ 2, these results were supported by the low value of activation energy Ea=5.004 kcal. The adsorption of the additive on the crystal surface can be interpreted in term of Langmuir adsorption isotherm, and K_L values determined in the presence of Aqua. (Fc), Aqua (Vo), Ethyl. acetate.((Fc)and Ethyl acetate (Vo) were 7.31 $x10^5$, 4.7x 10^5, 3.06x 10^5, 1.6 x 10^5 J/mol respectively indicating potent inhibitory influenced of these plant extracts.

***Keywords*:** Crystal growth, medicinal plants, constant composition method, calcium oxalate monohydrate, adsorption.

INTRODUCTION

Kidney stones in human consist of various organic and inorganic compounds. Calcium oxalate monohydrate is the main inorganic constituent of these stones. The mechanism of calcium oxalate renal calculi formation has attracted the medical scientists due to its widespread clinical occurrence and its difficult treatment (Worcester *et al*., 1993). Sparingly soluble salts of calcium oxalate have been investigated with respect to their pathological crystallization to form kidney stones (Worcester *et al*., 1993; Ryall *et al.*, 1986) and physiological crystallizations of calcium phosphates in bone and tooth mineralization (Romberg *et al*., 1986; Donnelly *et al*., 1989) or calcium carbonate (egg shells) and marine exoskeletal systems (Romberg *et al.*, 1986; Dominguez-Vera *et al*., 2000; Falini *et al*., 1996). These studies have been followed with several techniques including batch nucleation and seeded methods. Crystal growth of sparingly soluble salts in supersaturated solutions has been initiated either as a result of spontaneous nucleation and depletion of the reactants or by the addition of preformed seed crystals.

Calcium oxalate (CaC_2O_4) are generally present in various forms: $CaC_2O_4.H_2O$ calcium oxalate monohydrate (COM), $CaC_2O_4.2H_2O$ dihydrate (COD) and $CaC_2O_4.3H_2O$ the rarer trihydrate

(COT) (Thongboonkerd *et al*., 2006).The great dangerous form of calcium oxalate crystal is monohydrate form $CaC_2O_4.H_2O$ because it is great in size so, it is very sever in nephrolithiasis and urolithiasis pathogenesis because of their maximal affinity for renal tubular cells (Schroder *et al*., 1995; Aggarwal *et al*., 2010).

The components of kidney stones are changeable and depend on the living conditions of the patient; however, most kidney stones consist of calcium oxalate (Ca OX) (Raman, 2009). These stones are generated from the supersaturation of urine with Ca^{2+} and Ox_2^- ions, whereby the so-called precept of heterogeneous nucleation is present (Hautmann and Gschwendt, 2014; Gasser, 2019).

The presence of foreign substances may promote or inhibit the formation of various calcium oxalates crystals. In any precipitation system the condition of crystallization of CaC_2O_4 in common aqueous solutions is much different from those in a biological system, and the mechanism of the formation of calcium oxalate stones is not completely understood and a number of inquiries about inhibiting or promoting factors still remain unanswered (Ishwar Das *et al.*, 2004). Researchers studied the influence of foreign substance on the growth rate of COM crystals such as amino acid (Shen *et al.*, 2005), carboxylic acids and plant extracts (Mohamed *et al.*, 2007; de Cógáin *et al*., 2015) and metallic ions (Grases *et al*., 1989).

The natural plants have the ability of accruing diseases and have no bad side effects, therefore can be used in preparing medicine. The genus *Ferula* encompasses large number of species, most of them have been used as remedies in traditional medicine and their pharmacological influences are well authenticated either in human or veterinary medicine in various countries. *F. communis* L (Family Apiaceae) had been used as anti-cancer (Saleem *et al*., 2001), anti-diabetic (Iranshahi and Iranshahi, 2011), anti-bacterial, anti-ulcerative and anti-inflammatory effects (Li *et al*., 2015), treatment of stomach disorders, rheumatism, headache, arthritis and dizziness (Tamemoto *et al*., 2001).

It is a rich source of sesquiterpene and their derivatives sesquiterpene coumarins and prenylated sesquiterpene coumarins (Miski and Jakupovic, 1990; Miski and Mabry, 1985;, Valle *et al*., 1986). Also, it contains daucane esters (lapiferin and jaeskeanadiol benzoate, umbelliferone, umbelliprenin, and farnesiferol) (Abu Gabal *et al*., 2008). Daucane esters from leaves and seeds of is jaeschkeanadiol (Miski and Mabry, 1986 ; Lamnaouer *et al*., 1989). Also, *F. communis* has polysaccharides galactose, arabinose, glucuronic acid, and galacturonic acid (Youmbai *et al*., 2021), phenolic compounds as Chlorogenic acid, ferulic acid, quercetine, coumarin , tannic acid, and resorcinol and syringic acid (Rahali *et al*., 2018).

V*erbena officinalis* L. grows in all temperature regions of the globe and it acts as antibacterial (Hernández *et al*., 2000), antifungal and antioxidant (Bilia *et al*., 2008; Casanova *et al*., 2008), neuroprotective (Lai *et al.*,2006), besides it is analgesic (Calvo, 2006), anti-inflammatory (Calvo *et al.*, 1998; Deepak & Handa, 2000), antitumor against human choriocarcinoma JAR cells (Zhang Luo *et al.*, 2004), chronic lymphocytic leukemia cells (Martino *et al*., 2009), and solid tumor (Kou *et al*., 2013).

The main constituents isolated from *V. officinalis* L, include kaempferol, Flavonoids, apigenin 7-diglucuronide, luteolin 7-diglucuronide, luteolin 7-glucoside, luteolin 7-glucuronide apigenin, pedalitin 6-glucoside, and apigenin (Bilia *et al*., 2008; Calvo *et al.*, 1997). In addition there are pedalitin 6-galactoside, scutellarein 7-glucoside, scutellarein 7-glucuronide and scutellarein 7-diglucuronide (Harborne, 1993; Meng *et al.*, 2006), Hastatoside, Verbenalin, Pedalitin 6-O-(2-O-feruloyl)-

diglucuronide, Scutellarein 7-O-(2-O-feruloyl)-diglucuronide, Pedalitin 6-O-diglucuronide, Aucubin, Scutellarein 7-O-diglucuronide, 1,5-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, Scutellarein 7-O-glucuronide, Pedalitin 6-O-glucoside, Verbascoside and Isoverbascoside (Sheyla *et al*., 2011).

The present study aimed to evaluate the influence of ethanolic and aqueous extracts of (Fc) and (Vo), on the activity of crystal growth of COM crystals and measured the inhibitory activity of over range of supersaturation which reflect the percentage of crystal growth inhibition of COM.

MATERIALS AND METHODS

Calcium chloride, sodium oxalate, sodium carbonate, sodium chloride and sodium hydroxide and Ethyl acetate were analytical grade purchased chemicals from (fisher scientific company and Baker chemical company and El Nasr–Pharmaceutical Chemical Company).

Preparation of plant extract

F.communis L and *V. officinalis* L. were purchased from Harraz Herbs Company.The plant extract was prepared by boiling 100g of aerial part powder of *F. communis* L and *V. officinalis* L. in 400 ml distilled water for 1hour and left up night then filtering it with a Whatman filter paper twice then collected and concentrated using rotary evaporator under. Ethyl acetate extract was produced by macerated 100g from crushed and grinding the aerial part of plant for 72h with gentle shaking then filtrated and concentrated in a vacuum under reducing pressure rotary evaporator to obtain finally the crude extract of plant.

Preparation of seed:

Solutions of $CaCl_2$ and $Na_2C_2O_4$ were analyzed by passing aliquots through ion exchange resin (Dewix-50) in the hydrogen form. The eluted acids were titrating with standard NaOH solution of proper concentration using (Ph.Ph) phenolphthalein as indicator. All chemical solutions were prepared and kept in Pyrex vessels. $CaC_2O_4.H_2O$ seed was prepared in details by adding 1L of (0.01 M) $CaCl_2$ solution to 1L of $Na_2C_2O_4$ (0.01) at 25 ^oC at a rate of 500 ml/hour. $Na_2C_2O_4$ solution was continually stirred throughout the addition. The seed suspension was allowed with stirring for 24 hours then filtered then; the seed crystals were washed several times with distilled and deionized water to remove surface contamination due to chloride and oxalate ions. The seed crystals of sodium oxalate monohydrate were left for 30 days then refiltered and carefully washed with deionized distilled water and the wash process was repeated several times (Abdel-al *et al*., 2009**).** The seed was then filtered, dried and stored. The physical properties of seed crystal were characterized as calcium oxalate monohydrate (COM), by x-Ray powder diffraction (copper K x radiation, Phillips XRG 3000 Diffractometer). Particle sizes, were measured by single point BET nitrogen adsorption.

COM crystal growth by constant composition method:

Known volume of deionized distilled water was transferred to the cell and a measured volume of NaCl was added to the cell, then a definite volume of CaCl2 solution was added, followed by slow addition of known volume of Na_2C2O_4 solution over a period of five minutes. The total volume of the cell was usually 300 ml and the pH value was adjusted to the desired value (5±0.05), by using standard solution of NaOH and/ HCl solution. Satisfactory constancy of supersaturation solution was confirmed by constant emf reading for at least half hour. Specimens were periodically with draw then filtered via Millipore filters (0.22 M) for solution analysis.

Techniques:

Crystal growth experiments were carried out in double walled Pyrex glass vessel thermostated at 37°C. The vessel contents were stirred in presence of nitrogen gas dubbing to exclude atmospheric CO_2.

RESULT AND DISCUSSION

Kidney stone (also called renal calculi, nephrolithiasis or urolithiasis) are hard deposits made of minerals and salts that form inside your kidneys. Supersaturation solutions can differ in degree. It can be in the metastable extent where precipitation may take place only when stimulated by epitaxy or heterogeneous nucleation, or it can be in the unstable zone where quick spontaneous precipitation does occur. The limit between the two ranges, which can be called the spontaneous formation product, is not a fixed number but will depend upon the duration of incubation. Kidney stones are ordinarily composed of calcium oxalate monohydrate (COM) is the more thermodynamically stable form of calcium oxalate at room temperature and the formation of kidney stones is a result of increased urinary supersaturation with subsequent formation of crystalline particles. In the present study, a constant composition method has been used to investigate the crystallization of calcium oxalate in the absence and presence of methanolic and aqueous extract of *F. communis* and *V. officinalis* at the same relative supersaturation. The solid seeds of COM were confirmed by the physical properties.

X-ray Diffraction (XRD) and surface characteristics:

The solid phase of calcium oxalate monohydrate seed crystals was characterized by X-ray Patterns diffraction was determined using Cu-K radiation. The structure and morphology of calcium oxalate monohydrate seed crystals produced of various morphologies were recorded with Cu-Kα radiation source with double monochromator (λ=1.5405˚A) at 40 kV and 40 mA. The morphology properties of the specimens were recorded by physical adsorbing of (N2) at 77K using a Quantochrome Nova-Touch 4LX automated gas-sorption apparatus (USA).

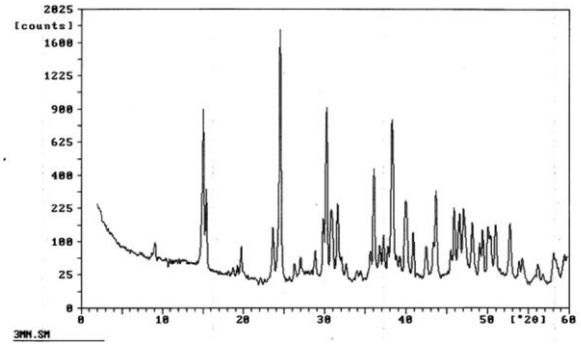

Fig. 1: X-ray diffraction crystallography pattern of calcium oxalate monohydrate crystals.

Determination of specific surface area of the prepared calcium oxalate monohydrate seeds:

COM crystals with different morphology were degassed at 80°C then, adsorption experiments were conducted at −195 °C by using an N2 adsorbent. The specific surface area of the seed crystals (SAA) was determined by applying Brunauer–Emmett–Teller (BET) equation. The pore-size distribution curves, average pore diameter, and pore volume were calculated using the Barrett–Joyner–Halenda method. (S_{BET}) of the crystals was calculated equal=3.37 m^2g^{-1}.

Thermo-gravimetric analysis (TGA):

The thermo-gravimetric analysis curves coupled with the mass spectrometry profile of calcium oxalate monohydrate seed crystals is shown in Figure (2). Three distinct stages of decomposition have been preceded. The 1st step tacks place between 45 and 250 °C and matches 12.33% of mass loss. This loss in mass is principally linked to the loss of H_2O (equation 1). The 2nd stage at 250 to 550 °C, corresponding to a very slight emission of CO_2 (equation 2). The 3rd stage occurs at 440 to 550 °C,

the maximum rate of emissions of CO and CO_2 which decreasing progressively until the disappearance of CO at 550°C (equation 3).

$$Ca_2O_4.H_2O \rightarrow CaC_2O_4 + H_2O \quad ....(1)$$

$$Ca_2O_4 \rightarrow CaCO_3 + CO_2 \quad .....(2)$$

$$CaCO_3 \rightarrow CaO + CO_2 \quad .....(3)$$

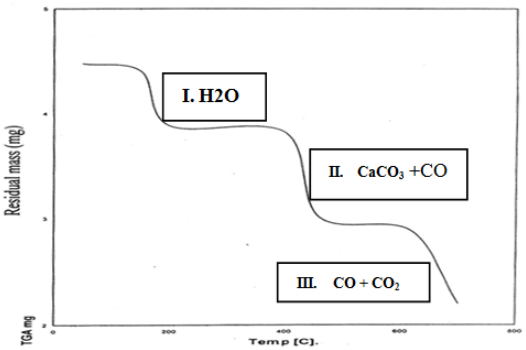

Fig. (2). Thermo-gravimetric analysis of calcium oxalate monohydrate seeds.

Scanning Electron Microscope (SCM) of calcium oxalate prepared seeds.

Typical SCM micrographs of the prepared COM seed crystals are shown in Figure (3). Ca $C_2O_4.H_2O$ (bi-axial positive) is strongly birefringent, and thus easily distinguishable from $CaC_2O_4.2H_2O$ (bi-axial negative) medium bi refringent, and $CaC_2O_4.3H_2O$ tri hydrate (uni- axial negative) weakly bi refringent.

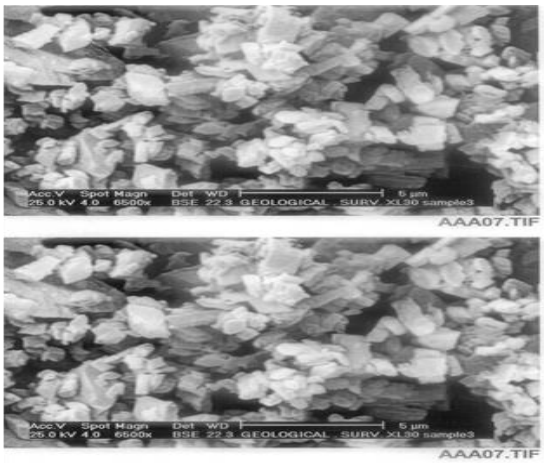

Fig. (3). SEM Micrographs of the prepared seeds of COM seeds.

Studying the mechanism of crystal growth of COM

The relative supersaturation "σ" for solutions containing the same concentrations of calcium and oxalate ions can be defined by the following relation:

$$\sigma = (\pi_0^{1/2} - \pi^{1/2})/\pi_0^{1/2} \quad .....(1)$$

Where π_0 is the solubility value at the same ionic strength (0.3 mol L^{-1} NaCl in the present study and π is the molar concentration product of calcium oxalate, $[Ca^{2+}]$ $[Ox^{2-}]$, in the solution.

In the present study, the rate of crystal growth of COM crystals at 37 ^oC, pH =5±0.05, ionic strength (I)=0.3 mol $dm^{-3}$and 10 mg seed crystals have been investigated in the absence and presence of ethanolic and aqueous extracts of *F. communis* and *V. officinalis*. The rates of crystal growth were studied at values of (σ) ranged from 0.23–0.7. The effect of change of the rates of crystal growth of COM against change in degree of supersaturation is shown in Table (1).

For numerous sparingly soluble salts, Ma and Ab, the rate of Crystal growth normalized for Surface area, can be expressed by equation (2)

$$R = d [ma\ A_b] /dt = k\ s^n \quad ..... (2)$$

Where K is the crystal growth rate constant, σ degree of supersaturation, S is proportional to the active growth sites available on the crystals surface and n is the order of reaction

Generally, the rates of crystal growth of alkaline-earth metal salts are noticeable inhibited in the presence of addition of certain inhibiters. The effective order of reaction can be obtained from the slope of typical plots of – log σ versus, – log R as shown in Figure (4).

Table (1): Growth of COM crystals. Tca^{+2}: Tox^{-2}=1:1 at t=37°C, pH=5±0.05, I=0.3(NaCl) mol dm^{-3}, and 10mg of seed crystals using E.M.F.

Exp. No	TCa^{+2} / 10^{-4} mol dm^{-3}	σ	-log σ	Weight Seed /mg	R X10^{-9} mol dm^{-1} min^{-2}	-log R
18	**2.452**	**23**	**0.638**	**10**	**2.51**	**8.61**
19	**2.591**	**30**	**0.523**	**10**	**3.09**	**8.52**
20	**2.790**	**40**	**0.398**	**10**	**6.54**	**8.18**
21	**2.991**	**50**	**0.301**	**10**	**10.47**	**7.98**
22	**3.190**	**60**	**0.220**	**10**	**15.12**	**7.82**
23	**3.390**	**70**	**0.155**	**10**	**19.07**	**7.71**
24	**2.790 a**	**40**	**0.398**	**10**	**6.540**	**8.18**
25	**2.790 b**	**40**	**0.398**	**10**	**6.541**	**8.184**
26	**2.790**	**40**	**0.398**	**20**	**6.548**	**8.1839**
27	**2.790**	**40**	**0.398**	**30**	**6.553**	**8.1836**
28	**2.790**	**40**	**0.398**	**50**	**6.564**	**8.1828**

a) Stirring speed 300 r.p.m **b) stirring speed 500 r.p.m**

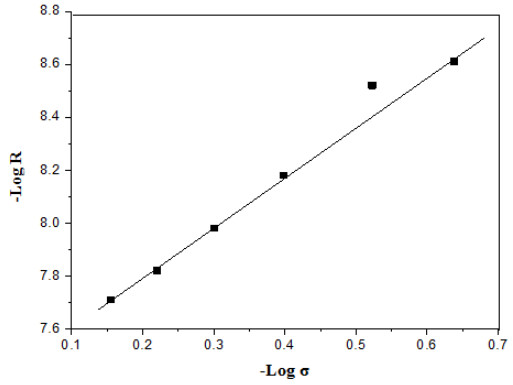

Fig (4): plot of –log R against –log σ for calcium oxalate monohydrate.

The effective order of crystal growth process of COM was n≈2, the value of n suggesting surface controlled mechanism over a range of relative supersaturation (0.23-0.7). The suggestion of surface controlled mechanism may also be corroborative by the observing the independence of the rates of the growth of COM crystals on the changes in stirring rate (fluid dynamics) as shown in Table (1) exp. 24, 25.

Effect of pH on the rate of crystal growth of COM:

In kidney either nephrolithiasis or urolithiasis, the pH of urine has been believed to modulate renal stone formation at different steps, inclusive precipitation, growth, aggregation. Moreover, pH is a significant factor that can increase the generation of solid phase and influence on the solubility of kidney stones (Worcester & Coe, 2008; McKay, 2010). Also, the pH medium is another important factor which affect on growth rate of COM crystals. The alteration of pH of physiological solution increases the solubility of calcium oxalate. In the present study the effect of pH variation at the same relative supersaturation (σ =0.4, I=0.3,T= 37°C and 10mg seed crystal) on the growth rate of COM crystals were studded. Figure (5) represent the relation between the change in pH on the growth of COM crystal which showed that, the rate of crystal growth increase with increase pH value until pH 8, which showed the highest value of the crystal growth rate corresponding to R=8.11x10^{-9} mol dm^{-1} min^{-2}, then gradually decrease in more basic medium. Juthatip *et al*. (2017) evaluated the formation of that the kidney stone in more acidic medium between (pH =4-5.5), where the acidic urine pH may promote calcium oxalate kidney stone formation, but the pH of basic urine may help to block calcium oxalate kidney stone formation.

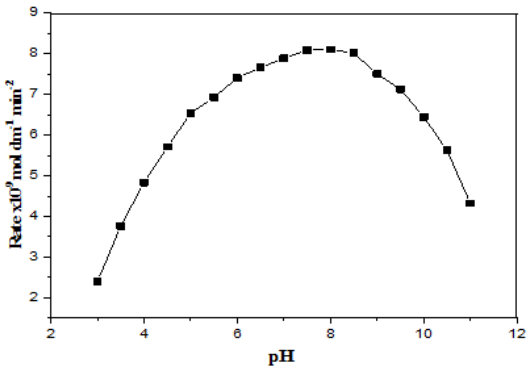

Fig (5): Effect of change of pH on the rates of growth of COM crystals at I = 0.3 mol dm^{-3}, σ= 0.4, t = 37^{o}C and 10 mg seed crystals.

Effect of change of ionic strength on crystal growth of COM

Ionic strength is another important factor affecting on crystal growth of calcium oxalate. The influence of ionic strength on COM crystal growth at 37^{o}C, pH=5.5, σ = 0.4, the weight of a seed of 10 mg, and at a range of ionic strength from 0.05–0.45 mol dm^{-3} congruous to human being urine (using sodium chloride solution) were studied. The experimental results showed that there was a decrease in the rate of crystal growth with increase the amount of ionic strength (Fig. 5). The influence of ionic strength on crystal growth can be attributed to a strong effect of repulsive electrostatic forces between like ions in elevation ionic strength solution, which prevents chemical interaction between Ca^{2+} and Ox^{2-} ions and hence decreases the nucleation probability (Moses *et al*., 2015). The ionic strength is essentially generated by Na and Cl which are present in higher concentrations than other component in urine (Guerra *et al*., 2005). When ionic strength increases, ion activities will decrease and consequently the quantity of substances which can be precipitated or crystallized from supersaturated solutions. This is elucidated by calcium precipitation in solutions with constant calcium and oxalate. However, increasing NaCl concentrations decreases the calcium oxalate precipitation. But, this influence cannot medicinally be used because a high sodium chloride intake stimulates urinary calcium and decrease citrate excretion (Ticinesi *et al.*, 2014). Increasing the ionic strength concentrated in urine does not protect from consequence precipitation because the influence of decreasing ion activity is predominately decrease by the increase of supersaturation due to the high ion concentrations. A high excessive production of urine remains therefore it is important for stone metaphylaxis.

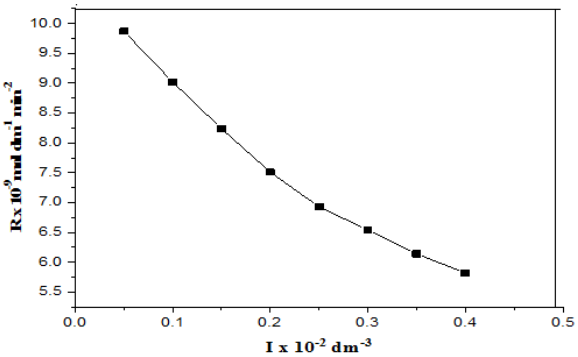

Fig (6). Effect of the change in ionic strength on crystal growth of COM at t=37^{o}C, pH =5, σ= 0.4 and 10 mg seed crystals.

Effect of change in temperature on COM crystal growth

Temperature has a distinct influence on the growth of salt crystals, either in height or area covered. The fast growth attained with the rise in temperature obviously shows the recurring patterns of the crystal. Though there is apparent difference between the samples of crystals growth at room temperature against those grown at rise temperature. The influence of variation in the temperature degree on the COM crystals were studded at pH=5, σ=0.4 and 10 mg seed crystals. The growth rates were calculated at different degree of temperature range 10, 15, 25, 30, 35 and 37^{o}C. Plotting log R against 1/T of various degrees of temperature gave

straight lines and the calculated (Ea) activation energy was 5.004 kcal. The small amount of activation energy (Ea) and independence of the of activation energy of the rates of crystal growth of COM on the stirring rate and the trivial change in rate, will ruling out bulk diffusion of electrolyte to the surface crystal as the rate controlling step and suggests an surface controlled mechanism for the rate growth of COM crystals.

Studying the mechanism of growth of COM crystals in the presence of additives:

The additives may have many influences on the process of crystal growth of calcium oxalate crystals, this effectiveness are: (i) formation complexes due to interaction of the biological compounds of the extracts with the crystal surface, (ii) the difference in the characteristics of the layer adsorbed on solid solution interface, (iii) the additives adsorbed on the surface and block the active sites on growing crystals, (iv) change the charge or the surface energy of the growing crystals. Generally a specified inhibition of the crystal growth rate is expected to occur at great lower concentration of the inhibitors molecule than complexation in simple form.

Constant composition method was used to investigate the potential inhibitory effectiveness of aqueous and ethyl acetate extracts of *F. communis* (Fc) and *V. officinalis* (Vo) on the growth rate of COM crystals at t=37^{o}C, I (NaCl)=0.3 mol dm-3 pH=5, σ=0.4 , 10 mg of seed crystals and stirring rates of 300 r.p.m. The experimental results were summarized in Table (2) at the same relative supersaturation.

Table (2): The effect of degree of supersaturation on the rate of growth of COM crystals in the presence aqueous and ethyl acetate extracts of Fc and VO at t=37 ˚C, pH =5±0.05, I = 0.3 mol dm^{-3} and σ =0.4.

			Aqua. (Fc)			Aqua. (Vo)			Eth.acetate. (Fc)			Eth.acetate. (Vo)		
$TCa^{+2}/10^{-4}$ mol dm^{-3}	Additives/10^{-7}Mol dm^{-3}	10^{6}[inhibitor]$^{-1}$	R x10^{-9}mol dm^{-1} min^{-2}	$R_o/(R_o - R_i)$	$(R_o - R_i)/ R_o$	R x10^{-9}mol dm^{-1} min^{-2}	$R_o/(R_o - R_i)$	$(R_o - R_i)/ R_o$	R x10^{-9}mol dm^{-1} min^{-2}	$R_o/(R_o - R_i)$	$(R_o - R_i)/ R_o$	R x10^{-9}mol dm^{-1} min^{-2}	$R_o/(R_o - R_i)$	$(R_o - R_i)/ R_o$
2.790	-		6.54	-	-	6.54	-	-	6.54	-	-	6.54	-	-
2.790	1	10	3.82	2.4	41.59	4.5	3.21	31.19	5.02	4.3	23.24	5.64	7.27	13.76
2.790	2	5	2.54	1.64	61.16	3.24	1.98	50.46	4.12	2.7	37.00	4.95	4.1	24.31
2.790	3	3.33	1.94	1.42	70.34	2.74	1.72	58.10	3.32	2.03	49.23	4.34	2.97	33.64
2.790	4	2.5	1.61	1.33	75.38	2.33	1.59	64.37	2.84	1.77	56.58	3.78	2.45	42.20
2.790	5	2	1.43	1.28	78.13	2.08	1.47	68.20	2.54	1.64	61.16	3.43	2.1	47.55
2.790	6	1.67	1.28	1.24	80.43	1.81	1.38	72.32	2.31	1.55	64.68	3.12	1.91	52.29
2.790	7	1.43	1.15	1.21	82.42	1.73	1.36	73.55	2.15	1.49	67.13	2.94	1.82	55.05
2.790	8	1.25	1.05	1.19	83.94	1.62	1.33	75.23	2.08	1.47	68.20	2.74	1.72	58.10
2.790	9	1.11	0.94	1.17	85.63	1.49	1.3	80.12	1.95	1.42	70.18	2.53	1.63	61.31
2.790	10	1	0.87	1.15	86.70	1.41	1.27	80.58	1.86	1.4	71.56	2.46	1.6	62.39

The rates of crystal growth of calcium oxalate monohydrate were plotted against various concentrations of different extracts (Fig.7). The plotting shows that as low as 10^{-6} mol dm^{-3} of each additive reduced the rate of crystal growth by 86.70 %, 71.56 % for aqueous and ethyl acetate extracts of Fc and 80.58% and 62.39% for aqueous and ethyl of Vo, respectively. The percentage of inhibition of the effect of

various extracts on the rate of crystal growth is due to blocking of the active sites on the crystal surfaces by the additive molecules of extracts. The results obtained suggesting the following order of inhibition; aqu. Fc > aqu. Vo> Eth. acetate. Fc > Eth. acetate. Vo.

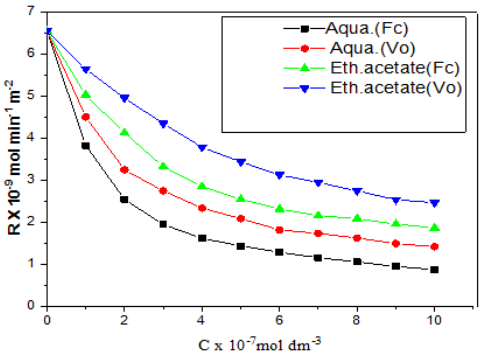

Fig: (7). Plot of the rate of crystal growth of calcium oxalate monohydrate crystal against Ethanolic and aqueous extracts of [Fc] and [Vo] at σ=0.4, =37 °C and I=0.3 mol dm^{-3}

The foreign substance might be particularly adsorbed at lattice sites and thus retarding the transfer of calcium oxalate monohydrate units between the adsorbed form on the crystal lattic [Ca C_2O_4 (ads) ⟶ CaC_2O_4 (lattice)].

Generally, the extracted molecules exert their effect via adsorption at active crystal growth sites on crystals. Chelating anions may be adsorbed at cationic sites and inhibit crystal growth when existing at very low levels. The main components of (Fc) involve sesquiterpenes (Iranshahi *et al.*, 2003 and Iranshahi *et al.*, 2009), Sesquiterpenes coumarin (Iranshahi *et al.*, 2010), sesquiterpene lactones (Kasaian *et al.*, 2014) and sulfur containing compounds (Iranshahi *et al.*,, 2003 b and Iranshahi., 2012) and polysaccharides galactose, arabinose, glucuronic acid, galacturonic acid and rhamnose (Youmbai ***et al.*, 2021).** The compound possessing -= O –,-OCH_3, N-N, -OH, -COO , and -NO groups that have an effective influence on the rate of crystal growth (grass *et al.*,1998).The presence of high number of compound possesses -OH , =O and –OCH_3 groups in their extracts act as a good inhibitors for calcium oxalate monohydrates crystals. In addition polysaccharides act as good inhibitors for crystal growth (Ishwar *et al.*, 2004).The existence of (–OH) hydroxyl group produce, a good inhibitor effect. Also Vo extracts include galactose, arabinose, glucuronic acid, and galacturonic acid which containing hydroxyle groups. On macromolecules, possibly through active sites formation at ionizable active group (OH–), anywhere as molecular weight of compound is high, the effectiveness of the inhibitors molecule increase is due to produce more computation with H_2O molecules on the crystal surface of COM. So as the concentrations of the extract molecules increase, the crystal growth rates of COM crystals decrease due to adsorbed the inhibitor molecules on the crustal surface and blocking of the active growth sites on the surface of crystals. The phytochemical analysis illustrated the presence of many compounds in the extracts of Fc and Vo that are famous for their biological importance and have shown in the treatment of many diseases such as kaempferol, Flavonoids, apigenin 7-diglucuronide, luteolin 7-diglucuronide, luteolin 7-glucoside, luteolin 7-glucuronide apigenin 7-glucoside, apigenin 7-galactoside, pedalitin 6-glucoside, and apigenin, which detected high pharmaceutical effects on the rate of crystal growth of COM. From the structure of the chemical composition of aqueous and ethyl acetate extracts of *F. communis* L and *V. officinalis* it was expected that they are good inhibitors for the growth rate of calcium oxalate monohydrate. The inhibition of calcium oxalate monohydrate may be explicate in terms of; a) sequestration of calcium ion in the solution, b) forming a complex with the

inhibitor molecules, c) specific adsorption on the crystal surface. Adsorption of inhibitors in the process of crystal growth mightily depends on the nature of substrate. The amount of limited growth factor increased nearly linearly with between the inverse of the relative reduction in rate, $R_0/(R_0-R_i)$, and the reciprocal of the inhibitor concentration, in the solution. The adsorption can be explained in terms of a langmuir-type isotherm (Amjad, 1987) according to the following relationship;

$R_0 /(R_0–R_i) = (K_LC)^{-1}$ Where, R_i and R_0 are the rates of crystal growth in the absence and in the presence of inhibitor respectively, KL is the adsorption affinity, and C is the concentration of additive.

The applicability of the Langmuir isotherm model is elucidated by the linearity of the plots in Figure (5). The adsorption, affinity constant, KL, given by the inverse slop of the lines in Table (2) and Figure (8) which conform the applicability of this simple adsorption isotherm at all supersaturation studied.

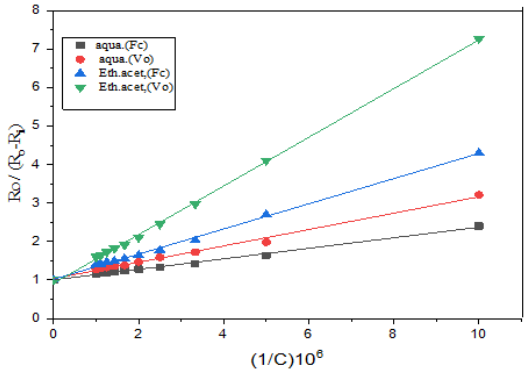

Fig. (8). Plot of $R_0/(R_0 –R_i)$ against $[(1/C)10^5]$.

The values of the adsorption affinity constant K_L were $7.31x10^5$, $4.7x10^5$, $3.06 x 10^5$ and $1.6x10^5$ mol^{-1} for aqueous and ethyl acetate, extracts of *F. communis* and *V. officinalis* respectively, at the same relative super saturation (σ=0.40). The values reflect the high adsorption affinity at low supersaturation in the presence of Fc and Vo inhibitors. It is evident from the results of the current study that, calculi of calcium oxalate in the body is a more complex phenomenon take place under dynamic condition in which urine continuously flows. The present in vitro study provides basic information to recognize the potent inhibitors. Both of these aqueous and ethyl acetate extracts contain numerous of complex macro-biomolecules that give altitude inhibition effect in the crystal growth of COM.

Conclusion:

Growth of COM crystals was studied using constant composition method. It was found that the crystal growth of COM follows surface controlled mechanism. The inhibition of growth of COM crystal by the effect of aqueous extract and ethyl acetate of the Egyptian edible plants *Ferula communis* and *Verbena officinalis* was studied and it was found that their aqueous extract produced high maximum inhibition of COM crystal growth than the their ethyl acetate extract in vitro conditions.

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التأثيرات المثبطة لمستخلص *Ferula communis* L. ، *Verbena officinalis* L. على النمو البلوري لأكسالات الكالسيوم مونوهيدرات

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المستخلص

يؤدي عدم التوازن بين المحفزات والمثبطات في الكلى إلى تكوين حصوات الكلى أو التحصى البولي. تمت دراسة تأثير المستخلصات المائية وخلات الإيثيل لكل من *Ferula communis* (Fc) ، *Verbena officinalis* (Vo) على معدل نمو بلورات أكسالات الكالسيوم أحادية الماء ، باستخدام تقنية التركيب الثابت في غياب ووجود مثبطات عند مدى فوق التشبع من (σ = 0.23 - 0.70). تم تقييم تأثير I (Ionic strength)، pH ، T(temperature) وتأثير التغير فى حجم بلورات أكسالات الكالسيوم. كشفت النتائج أن المستخلصات المائية لـ Fc و Vo كانت أكثر فاعلية من مستخلصات أسيتات الإيثيل على النمو البلوري بلورات أكسالات الكالسيوم أحادية الماء CaOx.H2O تجريبياً ، مع أعلى تثبيط عند 86.7٪ للمستخلص المائى (Fc) مقارنة ب 80.70% للمستخلص المائي فى حين كانت النتائج 71.56٪ و 62.39٪ لخلات الإيثيل (Fc) وخلات الإيثيل (Vo) على التوالي بنفس التركيز ونفس درجة فوق التشبع. كما وجد ان معدلات تبلور بلورات أكسالات الكالسيوم أحادية الماء في وجود المثبطات تتبع الرتبة الثانية (n≈2) مما يشير ألى انها تتبع ميكانيكية التحكم السطحى ، وقد تم الاستدلال على هذا من خلال القيمة المنخفضة لطاقة التنشيط (Ea = 5.004 kcal) . و يمكن تفسير امتزاز المادة المضافة على سطح البلورات من خلال نظام Langmuir للأمتزاز كما تم قد تم تقدير ثوابت الأمتزاز KL المحددة في وجد المستخلص المائى ومستخلص اسيتات الأثيل لكل من (Fc) , (Vo) والذى أشار إلى وجود التأثير المثبط القوي لهذه المستخلصات النباتية.