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Kinetic study of crystallization of calcium oxalate monohydrate in presence of *Zingiberofficinale* extracts

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ABSTRACT

Three *Zingiberofficinale* rout 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 *Zingiberofficinale* act as inhibitors of calcium oxalate stones crystallization. The effect of aqueous extract of *Zingiberofficinale* on the rate of crystallization of calcium oxalate crystals was higher than the ethanol extract followed by chloroform extract. In conclusion, these results have proved the folk medicine use of *Zingiberofficinale* 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

Keywords: Crystallization, *Zingiberofficinale*, Calcium oxalate stones, Kidney stones

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INTRODUCTION

Urinary system has the most widespread calculi in pathogenic formation (1-6).Whence minerals in the urine clump together and grow instead of being diluted so the urinary stone are developed (7). From analyzing of available data it appeared that calcium oxalate is the major part of nephrolithes in the citizens of different regions (5). By assuming various shapes and composed of many substances. The urinary calculi contain Soxlet, phosphate, uric acid and urate.

There are 75% calcium oxalate of all stones and 5% are composed of calcium phosphate (8). This problem does not dissolved until now and the world turned to find a solution, especially relating to human health and natural ways.

Ginger (*Zingiberofficinale*) is one of the world's best-known spices, and it has universally used throughout history for its health benefits .*Zingiberofficinale* Roscoe can be called the natural gold (9). The main constituents of ginger include volatile oil, phenolic derivatives (zingerone) and oleoresin (gingerols and shogaols) are main antioxidant compound in ginger (10).

Ginger is an underground rhizome of plant *ZingiberOfficinale* belonging to the family Zingibeaceae and now, it is considered a common constituent of diet worldwide (11). Over the world especially for its use in disorders of the gastrointestinal tract such as constipation, dyspepsia, nausea and vomiting (12). It was reported that ginger has medicinal properties against digestive disorders, rheumatism and diabetes (13). Ginger extract possesses antioxidative characteristic, It is reported that ginger pretreatment inhibited the induced hyperglycemia and hypoinsulinaemia (14-15). Other investigators have showed that the hypolipidemic effect of Ginger [16].In this study, have tried to dissolve the problem of urinary stone formation not only by saved natural compounds but also beneficial to human health.

EXPERIMENTAL STUDY

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., USA.). Deionized distilled water was use in the preparation of reagents and washing the glassware throughout.

Preparation of plant extracts and stock solution

The fresh roots of *Zingiberofficinale* were collected, cut and shade dried then finely powdered by electric mill to become ready for extraction. 500 gram of the plant powder were used in extraction process by Soxlet extractor. In this process has started extraction with chloroform followed by ethanol and finally with water, for two hours for each step. The filtrate has evaporated at the end of each process, using Rotavapor under vacuum to dry the extract, to be 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. In addition, aqueous extract was prepared by dilution of saturated solutions to get the different concentrations.

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 0.1 M carbonate free sodium hydroxide and 0.1 M hydrochloric acid 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.

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Preparation of seeds

Seeds of calcium oxalate were prepared by adding one liter of 0.1 M calcium chloride solutions to one liter of sodium oxalate solution (0.1 M) at 25°C at a rate of 500 ml per an hour. The sodium oxalate solution was constantly stirred throughout the addition. The seed suspension was allowed to mature 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 re-filtered and washed further with deionized distilled water. The later process was repeated several times. The seeds were then filtered and dried, and then subject to x-ray powder diffraction studies on COM, seeds is shown in figure (1). In the infrared study (IR) to insure that the prepared seed is COM, IR spectra were recorded by the IR spectrum of $CaC_2O_4 . H_2O$ figure (2).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 figure(3).

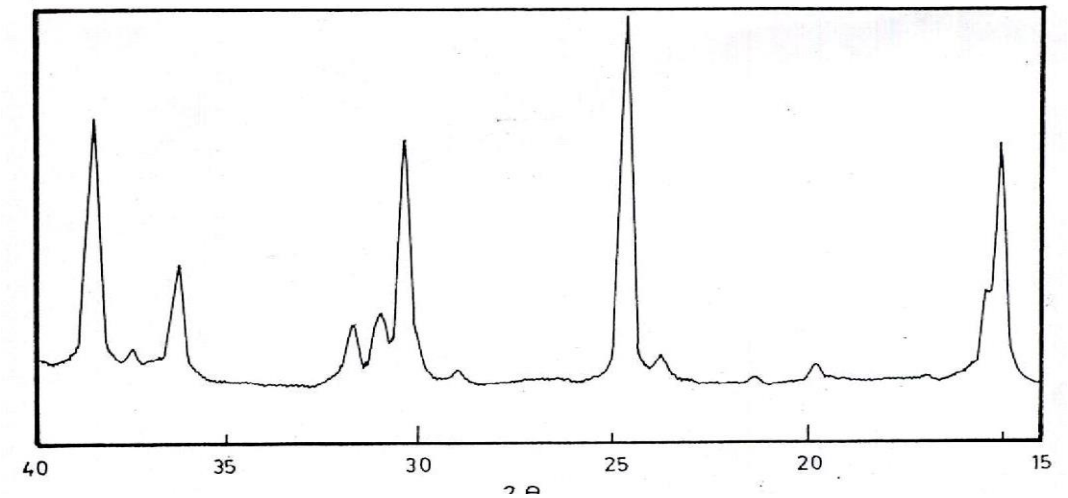

Figure 1: X-ray powder diffraction studies for calcium oxalate crystals

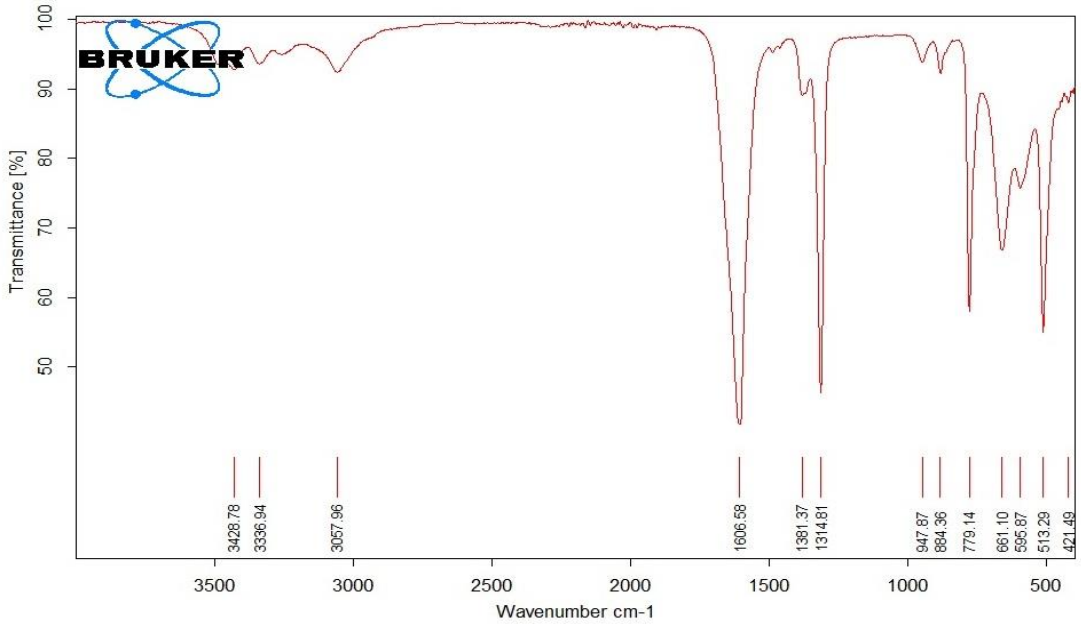

Figure 2: IR Spectrum of calcium oxalate.

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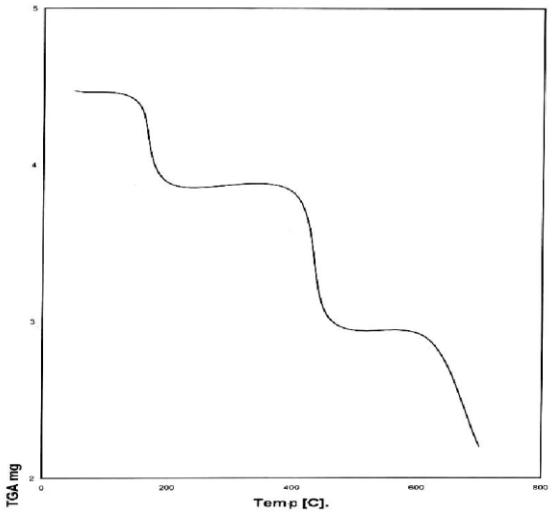

Figure 3: Plots of temperature against TGA on calcium of oxalate crystals.

Potentiometric measurements:

PH measurements were made using pH meter (Orion model 720A+). Connected with a combined pH glass electrode. The electromotive force (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 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} $MCaCl_2$ 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.

Crystal growth measurements

Crystal growth experiments were carried out in water thermo stated 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, and then a known volume of sodium oxalate solution was added slowly with constant stirring. The total volume was usually 300ml and the pH was adjusted to 6.5±0.05 using standard solution of sodium hydroxide or hydrochloric acid. The stability of super saturated 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/AgCl 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.

RESULTS AND DISCUSSION

Calcium oxalate is the major inorganic compound in urinary calculi (1). There are two process can increase crystal size as growth and aggregation. The crystal growth process is very slow. So, the body can get the urinary crystals out without growing into an appropriate size to be trapped in the duct of Bellini with an inner diameter of 7– 23 microns in a transit time across the kidney of 5–10 min (17). In this work, we evaluated the effect of different extracts of *Zingiberofficinale* on the crystal growth of calcium oxalate monohydrate at 37°C, trying to dissolve the urinary calculi by natural and saved materials.

ISSN: 0975-8585

The effect of *Zingiberofficinale* extracts on the crystallization of COM

Additives of both organic or in organic 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 consider 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 nucleate, In general, changes in the rate of crystallization produced by the addition of foreign substances may result either from complexion of the inhibitor, usually a chelating or sequestering agent, with the lattice cations and by adsorption of the molecules at active sites at the crystal surfaces. The latter of "threshold effect" may induced through adsorption at much lower concentrations of the additive molecules. The influence of the inhibitors on crystal growth must be studied under high lyre producible conditions by the constant composition method (18-20).

In the case of aqueous extract of *Zingiberofficinale* was found that, as the concentration increased, the crystal gross decrease as shown in figure (4). As this extract containing 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one. The volatile oil consists of mainlymono and sesquiterpenes; camphene, β-phellandrene,curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol,limonene, linalool, α-zingiberene (30-70%),β-sesquiphellandrene (15-20%), β-bisabolene(10-15%) and alpha-farmesene (9).These organic compounds have function groups as =O, OMe, OH and-OOH can transform the structure of COM to COD (Crystals of calcium oxalate dehydrate). This indicates that *Zingier officinal* can act as a good inhibitor for kidney stones since it induces the COD crystals that can be easily extracted in urine (21). In particular, inhibition in urine will transform COM to COD (22- 23). When the *Zingiberofficinale* concentration increases the amount of COM particles decrease this because it contains gengoral,,shogoal, paradol and methyl 6-iso gengoral (9,24,25). Hence, these functional groups of organic compound pigment and acid could transform the structure of COM to COD (21). In the case of ethanolic extract there are also (=OOCH3, OH^-) groups, (26, 27). Which may be responsible on the inhibition effect of COM crystallization as shown in figure (5). It is seen the inhibition effect of the different extract can be arranged as: $Z_{aq} > Z_{eth} > Z_{clor}$. This is due to the acidity compounds in Z_{aq} more than Z_{eth} more than Z_{clor}.

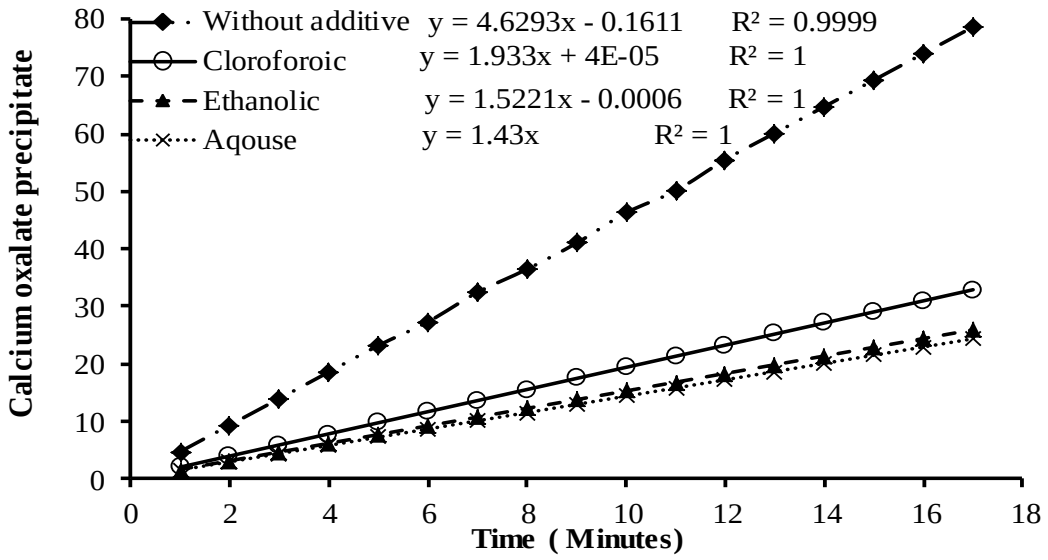

Figure 4: Plot of amount of calcium oxalate precipitated against time in presence of different *Zingiberofficinale* extracts.

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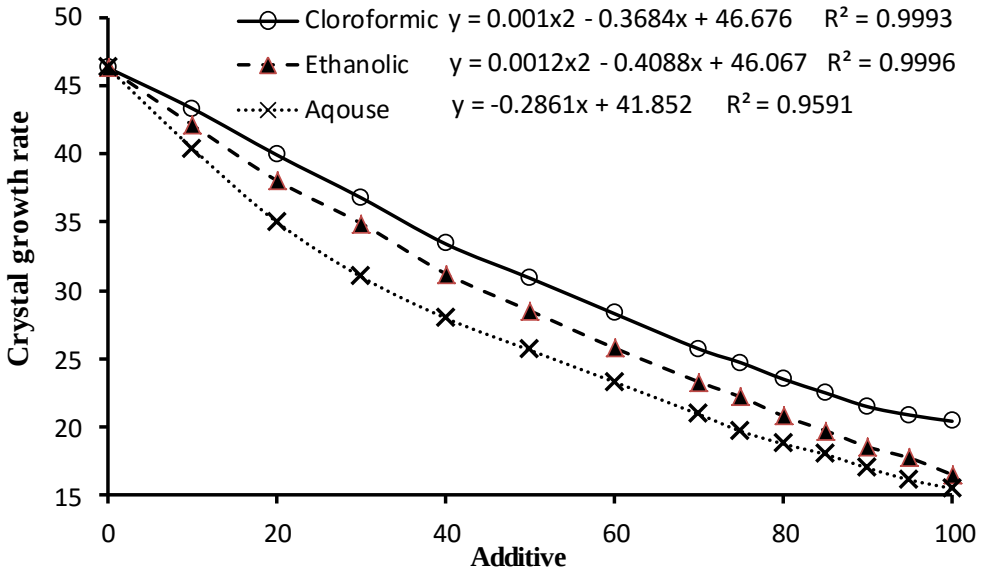

Figure 5: Plot of crystal growth rate of COM against (additive) of the Aqueous-, ethanolic-, chloroformic extract.

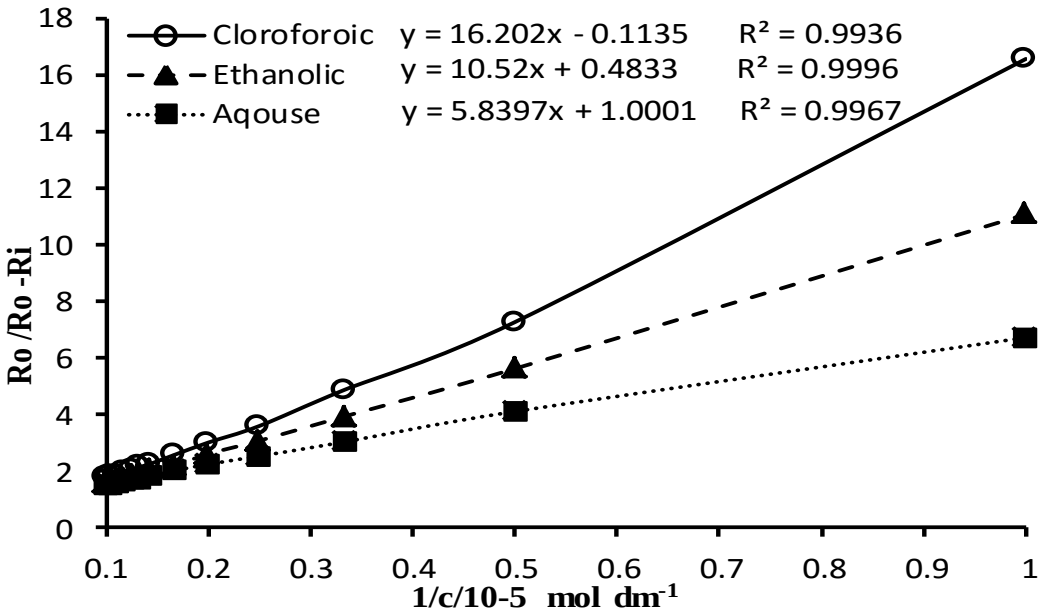

Figure 6: Values R/Ro-Ri against additive of crystal growth of COM in presence of *Zingiberofficinale* extracts ($Z_{aq.}$, $Z_{eth.}$, $Z_{clor.}$) at 37°C and σ = 0.4using EMF.

The value of affinity constants, $K_L$of different extracts of *Zingiberofficinale*were calculated by inverse slopes of the lines in figure (6), it is found that K_L values for Z_{aq}, Z_{eth} and Z_{clor} equal 13.56× 10^4 dm^3 mol^{-1}, 9.73× 10^4 dm^3 $mol^{-1}$and 3.83×10^4 dm^3 mol^{-1}. The values of K_L obtain high adsorption affinity for the extracts. So that the aqueous extract (Z_{aq}) was better than ethanolic (Z_{eth}) then cloroformic (Z_{clor}) due to their adsorption affinity.

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