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EFFECT OF DIFFERENT EXTRACTS OF AMBROSIA ON THE RATE OF DISSOLUTION AND CRYSTALLIZATION OF CALCIUM OXALATE CRYSTALS

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ABSTRACT: *Three extracts of ambrosia were prepared, aqueous extract Am (a), and ethanolic extract Am (e) and cloroformic extract Am (c) . The Potentiometric measurements, Dissolution experiments and Crystal growth measurements were studied on the these extracts. The results of this study showed that these extracts have ability to inhibit the rate of solubility of calcium oxalate stones, and the highest extract has the ability to inhibit the rate of formation of calcium oxalate was Am (a) followed by Am (e) followed by Am (c) .*

Keywords: *Ambrosia, Calcium Oxalate, X-ray differaction, SEM Micrographs, Crystalization.*

INTRODUCTION

Calcium oxalate is the main component of uroliths. There are three different hydrated forms of calcium oxalate, calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD) and calcium oxalate trihydrate (COT). The monoclinic COM is the thermodynamically most stable phase. Calcium oxalate crystals are the major component of most of the urinary calculi (Jian-Ming *et al.*, 2005).

The nucleation, growth and dissolution of mineral crystals are of particular importance in biological systems specially, calcium salts which are the major mineral components of them. The nucleation and growth of calcium oxalate hydrates, phosphates and carbonates polymorphs are frequently present in pathological deposits of mineral origins in the body (Saleh, 2002).

Concentrations of solutions when increased, a new thermodynamically stable phase is not formed until considerable degree of supersaturation has been achieved due to energy barrier which must be surmount before stable nuclei will form. The energy barrier formed due to the surface energy of these minute particles decrease in free energy on transferring solute from its more unstable state in supersaturated solution to the new phase is counter balanced by the increase in surface free energy of the nucleus. Once formed the nuclei are capable of growing until final equilibrium is reached in the growth stage (Mashaly, 1990).

With critical cluster formation, the incorporation of growth units at kink sites must overcome a free energy barrier. This is probably made up of rate determining steps for crystal growth (Burton, *et al.*, 1951 and Nielsen, *et al.*, 1987) such as,

1. Transport of lattice ions to the crystal surface by convection.
2. Transport of lattice ions to the crystal surface by diffusion though the solution.
3. Integration into the adsorption layer.
4. Adsorption at the solid liquid interface.
5. Diffusion within the hydrated adsorption layers.
6. Adsorption at a step representing the emergence of a lattice dissociation at the crystal surface.
7. Migration along the step.
8. Integration at a kink site on the step.
9. Partial or total dehydration of ions.

Crystal growth may be expressed in terms of several possible rate determining steps, including volume diffusion, adsorption, surface diffusion and integration. In addition, the integration of growth units into kink sites has been determined to approximate a parabolic rate law over a wide range of supersaturation (Nielsen, 1984) Polynucleation occurs when multiple clusters form and grow simultaneously at the crystal surface (Nielsen, 1984; Nernest, 1990 ; Nielsen and Toft 1984 and Hara and Reid 1973).

Although, technological progress of medicine prepared chemicals which have fast functional effects in many diseases, these medicines have bad (in some cases) dangerous effects on humanity. Modern researches proved that natural plants have the capacity of curing diseases more than chemical drugs and it does not have any bad side effects. This illustrates that it is important to use natural plants and its extracts in preparing medicine so we find that advanced countries are comitating how to import natural plants (seeds, oils, extracts) to decrease the dependence on the chemical drugs. In a previous work the dissolution of COM crystals was studied in presence of some alcoholic extracts of Ambrosia.

EXPERIMENTAL

Preparation of seed:

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 refiltered and washed further with deionized distilled water and (his 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).

Measurements of surface area:

In the present work, the specific surface area (SSA) was determined by the BET method applying equation (1):

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

where:

W : the weight of solid.
S_g : 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.
P_o : the ambient pressure.

It should be noted that the specific surface area measured by the BET gas adsorption method can be different from those measured by Oilier methods (Geissman and Matsueda 1968).

X-ray diffraction:

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° as shown in fig (1).

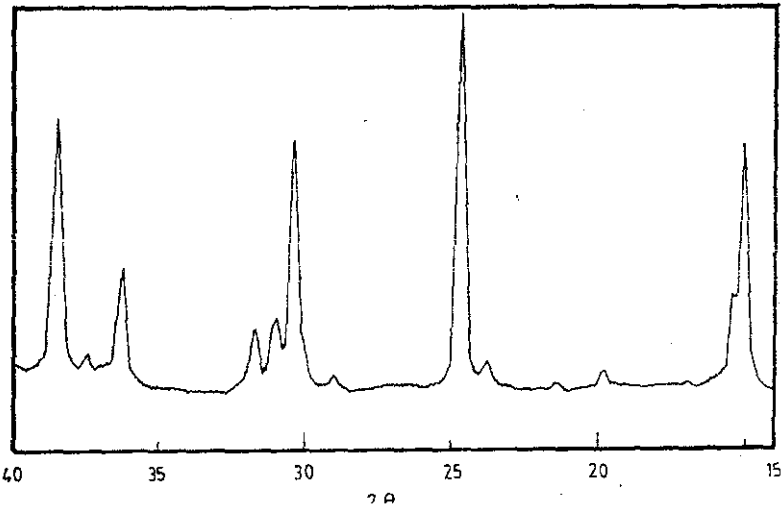


Fig. (1): X-ray powder diffraction studies for calcium oxalate crystals.
IR Spectra were recorded by the IR Spectrum of $CaC_2O_4 . H_2O$ fig. (2).

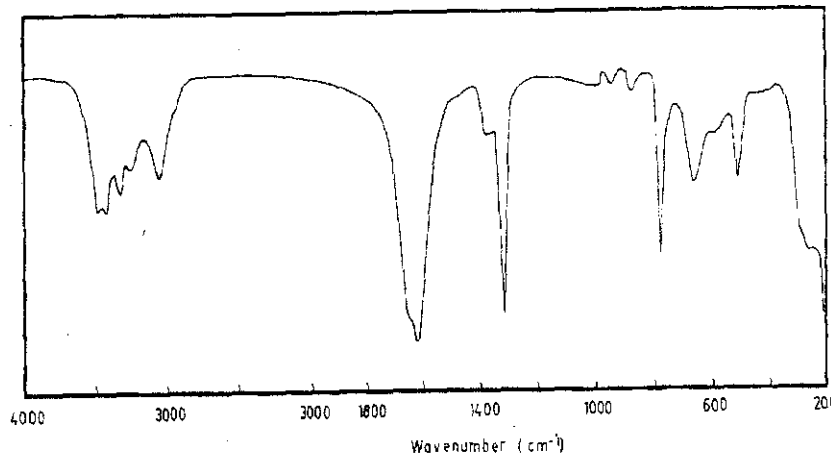


Fig. (2): IR spectrum of calcium oxalate.

Extraction and isolation of different extracts from Ambrosia:

Extraction is used to isolate soluble matter such as crude fat, additives, pesticides and critical minor constituents from complex material. The hexan extract was mold first, secondary chloroform extract, then ethanolic extract and at last aquas extract of ambrosia, they were made by using (soxtic system HT, 1043. Extraction Unit) made in Evleden. The extraction unit is supplied with eruice unite 1044 with hot oil to achieve solvent evaporation. The tube connecting the extraction and service units is also equipped with an air pump to evaporate the last traces of solvent from extraction cups. The manner of extraction can be summarized as follow:

The fresh plants were dried in shad then crushed, by using the (Micro-Feiniile-Culaui) MFC. Then the samples were inserted in a thimble and then into the extraction unit. The extract was evaporated using rota.vap. at 60 °C, dried and stored.

Preparation of extract solution:

Chlorofomic and alcoholic extracts were prepared by dissolving in suitable volume of ethanol then completed by deionized distilled water. A suitable volume of saturated solution were taken to prepare different concentrations by dilution. Aqueaus extract was also prepared by dilution of saturated solutions to get different concentrations.

Dissolution experiments:

The crystal dissolution experiments were carried out in water thermostated double-walled pyrex glass vessels. The cells were maintained

at the required temperature (37 °C) by circulating thermostated 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 $\pm$ 0.05) using standard sodium hydroxide solution or standard hydrochloric acid solution. Satisfactory stability of the undersaturated 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.

Crystal growth measurements:

Crystal growth experiments were carried out in water thermostated double-walled pyrex glass vessel at (37 °C). A measured volume of dc ionized distilled water was transferred to the cell followed by definite volumes of sodium chloride and calcium chloride solutions, 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 $\pm$ 0.05 by 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 Δg / Δg Cl electrode (Model 9050 Metrohm) were used to control the addition of tiirant 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

Effect of different extracts of Ambrosia on the rate of dissolution of COM:

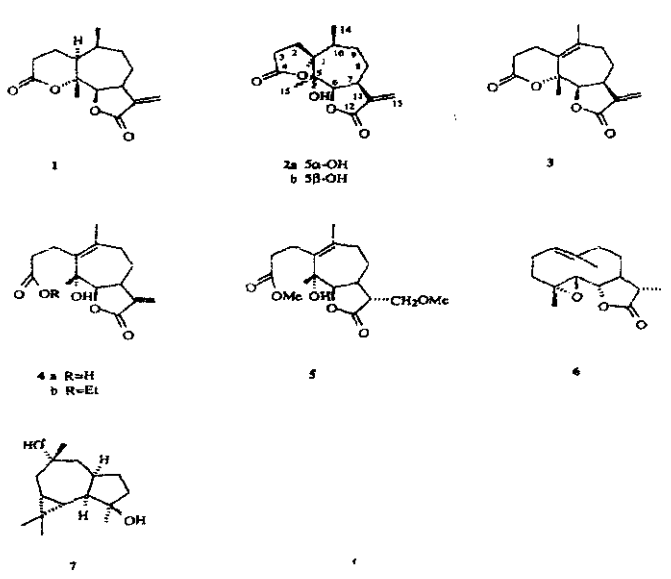
The different extracts of Ambrosia are isolated then we studied the effect of aqueous extract of Ambrosia [Am(a)] ethanolic extract of Ambrosia [Am(e)] and cloroformic extract of Ambrosia [Am(c)], on the rate of dissolution of COM crystals. The hixanolic extracts gave oil compound which was so difficult to dissolve in water.

The data for the effect of Am(a), Am(e) and Am(c) extracts are collected in figs. (3,4), the experimental data were obtained under conditions (σ = 0.09 , T = 37 °C and I = 0.15). Fig. (3) indicates the typical plot of extent of Ca^{+2} dissolved in the presence of Am(a), Am(e) and Am(c) as a function of time. The rates of dissolution of COM crystals in the presence of Am(a), Am(e) and Am(c) plotted against [additive] are shown in fig. (4). Such plotting indicate the influence of Am(a), Am(e) and Am(c) on the dissolution rates. The concentration of Am(a) as low as 10^{-5} mol dm^{-3}, markedly reduced the dissolution rates by at least 59.64 % compared with that in absence of additives under the same conditions. From fig.(3) it is detected that Am(e) at concentration 10^{-5} mol dm^{-3} ,it reduced the dissolution process of COM 55.35% and Am(c) also, the same concentration, it reduced the dissolution process of COM about 48.98 %.

Under the same condition (σ = 0.09, T = 37 °C, I = 0.15) when the concentrations of the additive was increased the rate of dissolution decreased due to blocking of the active sites on the crystal surfaces by the additive molecules of extracts.

Effect of aqueous extract of Am:

The aqueous extract of Ambrosia has good inhibition effect on the dissolution process on COM crystals as it contains different organic compounds according to the pervious studies (Stupp and Braun 1997 and Growford, *et al.*, 1968). From these compounds, the following can be mentioned according to G.L. Silva *et al* (Stupp and Braun 1997).



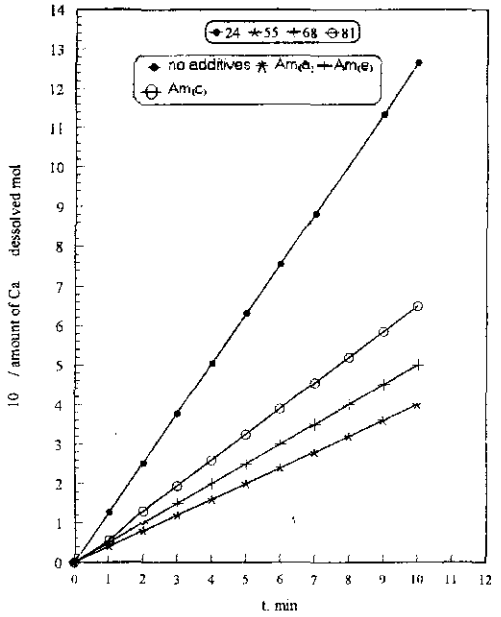


Fig.(3): Plots of amount of calcium dissoluted against time.

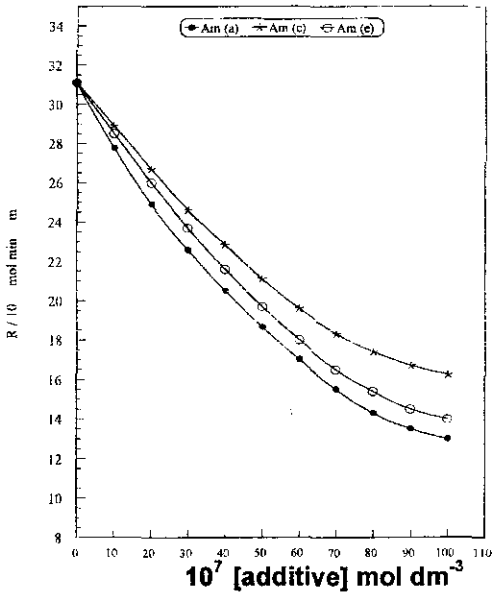


Fig.(4): Plot of rate of dissolution of COM against [additive] for different extracts of Am.

From the composition of the compounds found in Am(a) it is clear that there are different groups of = O, OH, S, S = O and there group may act as good inhibition in the additives of COM. It is known that the inhibitors which are anionic, are very good inhibitors for dissolution and crystalyzation of COM crystals specially those containing NH_2, OH, SO (Khider, 1995; Guozdev, *et al.*, 2004; Abdel Salam, *et al.*, 1984; Growford, *et al.*, 1968 and Saleh, 2002) which may adsorption on calcium ion sites. 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 the rate R_o / (R_o – R_l) and the reciprocal of inhibitor concentrations as in equation values of R_o / (R_o – R_l) and [additive]. For the dissolution of calcium oxalate crystals in the presence of Am extracts at relative undersaturation (σ = 0.09). The applicability of Langmuir model is illustrated in fig. (5); satisfactory linear relationship is obtained which confirm that the additives are adsorbed at the active sites on the crystal surface.

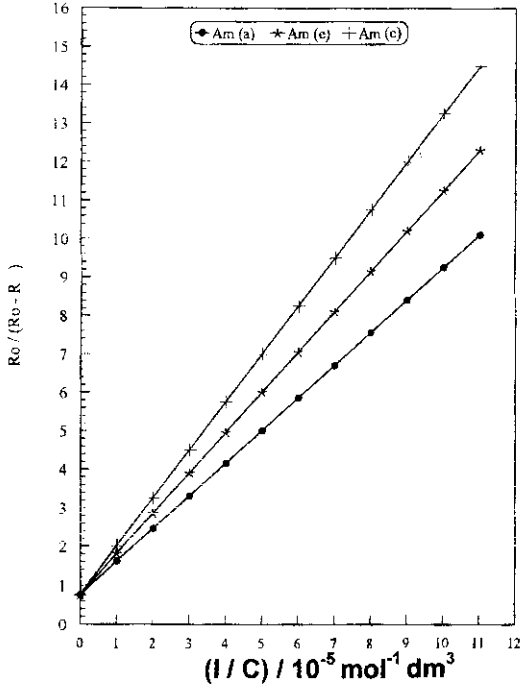
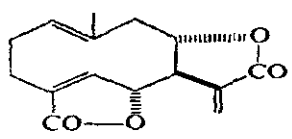


Fig.(5): Plots of Ro/Ro-R_l against (additives) of dissolution of COM in presence of Am(a),Am(e),Am(c) using emf.

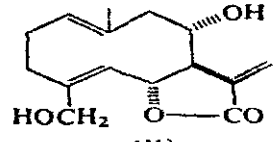
Effect of ethanolic extract of Am:

The effect of ethanolic extract of Ambrosia was studied and the results are collected in fig (3, 4) these show that the Am(e) act as an inhibitor for the

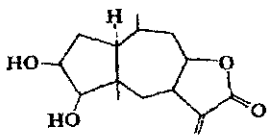
dissolution process of COM crystals as it contains many compounds as follow (Robertson, *et al.*, 1970; Ellis, *et al.*, 1993; Romo and Odrfguez-Hohn 1970; Poster, *et al.*, 1970; Felix, *et al.*, 1993; Geissman, *et al.*, 1973 and Vachneusk, *et al.*, 1976) :



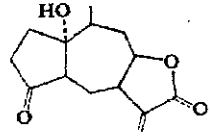
(I)



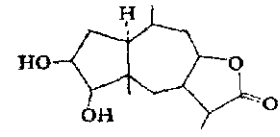
(II)



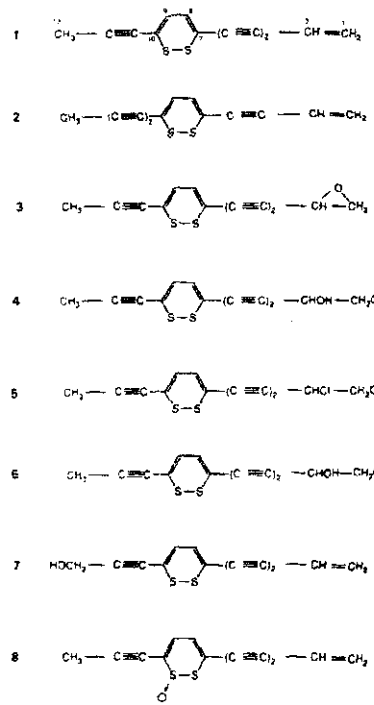
(I) Cumanin



(II) Peruvin



(III) Dihydrocumanin

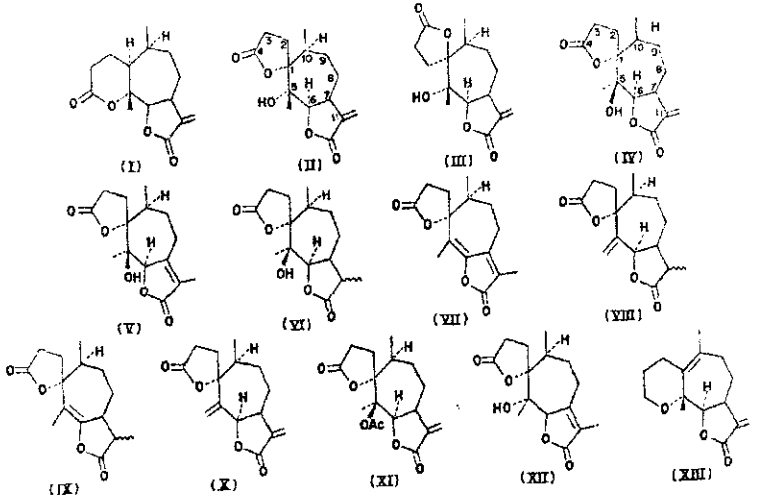


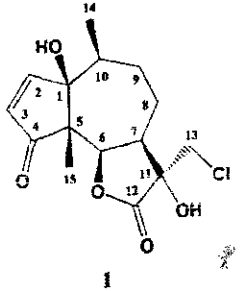
The last compounds refer to several groups which are found in Am(e) and these groups (OH, COO, Cl, C = O) make good inhibition for dissolution and

crystal growth. Influence of these groups may is the case or the inhibition effect of Am(e) on dissolution of COM as shown in Fig.(3).

Effect of cloroformic extract of Am:

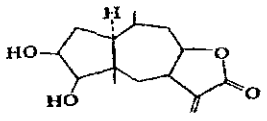
In cloroformic extract of Ambrosia, it has been shown by Werner Herz *et al.* (Balza and Neil 1990; Geissman, *et al.*, 1973; HiGo, *et al.*, 1971; Geissman, *et al.*, 1969; Porter and Mabry 1969; Mahmoud, *et al.*, 1999 and Delgado, *et al.*, 1988) that many compounds exist in this extract, of which the following were mentioned:



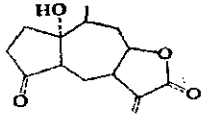


1

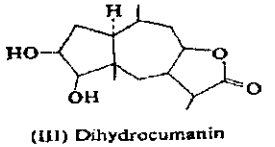
11□-hydroxy-13-chloro-11,13-dihydrohymenin.



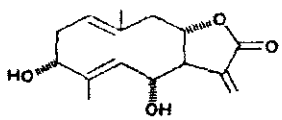
(I) Cumanin



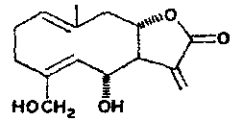
(II) Peruvin



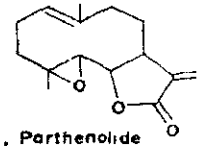
(III) Dihydrocumanin



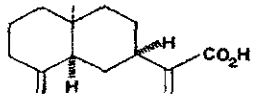
I. Chamissonin



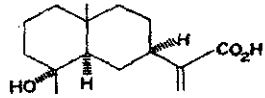
II. Artemisiifolin



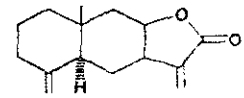
III. Parthenolide



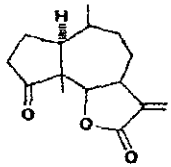
IV. Costic acid



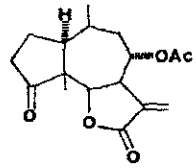
V. Iticic acid



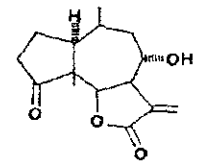
VI. Isoalantolactone



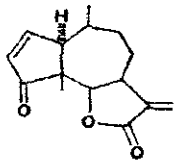
VII. Damsin



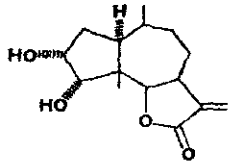
VIII. Confertiflorin



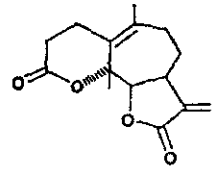
IX. Desacetylconfertiflorin



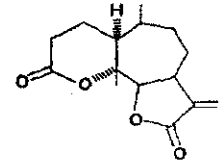
X. Ambrosin



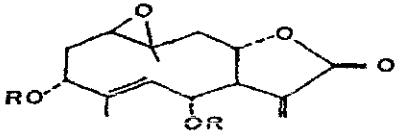
XI. Ambrosiol



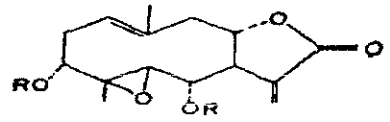
XII. Psilostachyin B



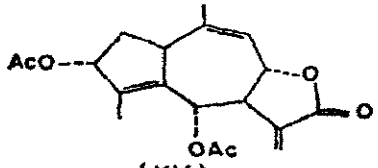
XIII. Psilostachyin C



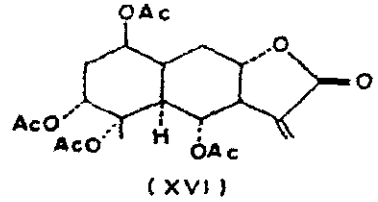
(XI) R = H
(XII) R = OAc



(XIII) R = H
(XIV) R = OAc



(XV)



(XVI)

Considering the last compouns, it can be observed that there are (COO, OH, = O) groups in these compounds which can lower adsorption on the active sites on COM crystal and case the inhibition effect.

From the values of inhibition ratios we can arrange the inhibition effect as follow Am(a) > Am(e) > Am(c).

Study of the effect of crystallization of COM in the presence of ambrosia extracts.

Proteins are known to play a key role in regulating biomineralization by controlling the shape size and often the phase of inorganic crystals (Nielsen, 1984; Addadi and Uleiner 1992; Mann, *et al.*, 1993; Zaremba, *et al.*, 1996 and Stupp and Braun 1997). Mechanisms by which organic molecules regulate biomineralization processes at both nucleation and growth stages include matrix assisted orientation of the crystal, face selective surface adsorption, and control of the crystal phase. Acidic proteins have been shown to get adsorbed and be overgrown by crystals of calcite, providing evidence that biopolymers do indeed co-crystallize with the mineral constituents of biogenic crystals. The mechanism by which macromolecules incorporate into the lattice of single biomineral crystals remains unknown, and little information is available regarding the structure of incorporated proteins or their orientation with respect to the unit cell of the inorganic crystal. Shedding light on the interactions between proteins with inorganic host lattices will aid in nanoscale materials design including the growth of composite crystals (Weiner and Addadi 1997) .

In the present work the rate of crystallization of COM is studied in the presence of different extracts ambrosia e.g. ethanolic, aqueous and chloroformic extracts .

In figs. (6,7) that the rate of crystallization of COM in the presence of aqueous, ethanolic and chloroformic extracts of Amprosia decrease the rate of crystallization in the order:

$$Am(a) > Am(e) > Am(c)$$

The values for different extracts of Ambrosia were calculated using Langmiur adsorption fig. (8), it is found that KL values for Am(a), Am(e) and Am(c) equal 16.67×10^4 dm^3 mol^{-1}, 14.28×10^4 dm^3 mol^{-1} and 6.25×10^4 dm^3 mol^{-1}respectively.

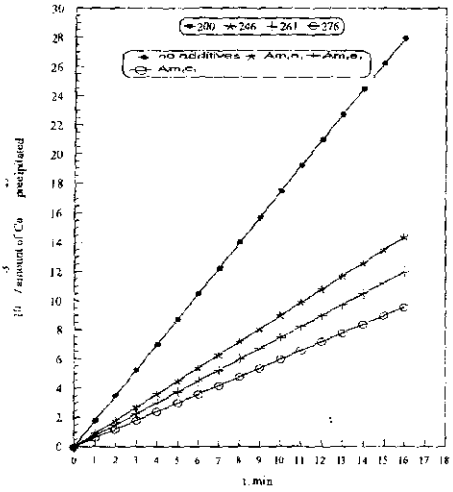


Fig. (6): Plots of amount of calcium oxalate precepitated against time in presence of some different Am extracts

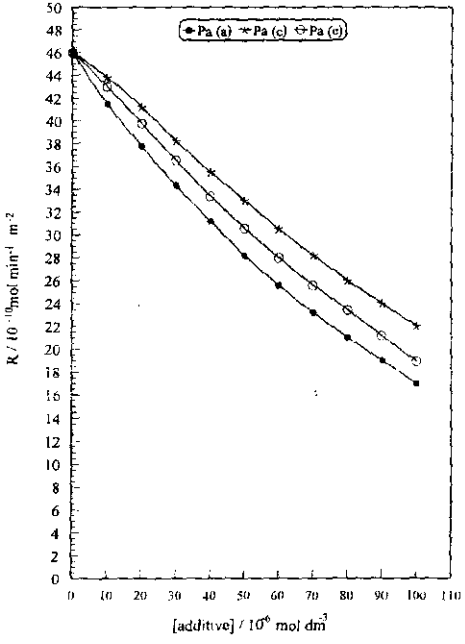


Fig. (7): Plot of rate of crystal grwoth of calcium oxalate against [additive] for Am(a), Am(e) and Am(c).

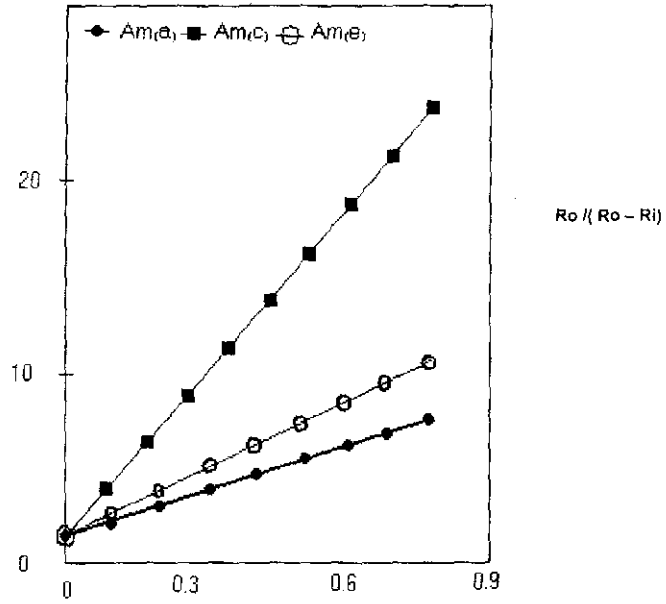


Fig.(8): Values of Ro/Ro - R_i against [additive] of crystal growth of COM in presence of Am(a),Am(e),Am(c) at 37 °C and σ =0.4 using emf.

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تأثير المستخلصات المختلفة لنبات الدمسيسة على معدل ذوبان وتكوين بللورات اكسالات الكالسيوم

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الملخص العربي

يهدف هذا البحث الى تحضير بعض المستخلصات الطبيعية من نبات الدمسيسة ودراسة مدى قدرتها على تثبيط معدل إذابة وتكوين حصوات اكسالات الكالسيوم وبصفة خاصة المستخلص المائى Am(a)، والمستخلص الإيثانولى Am(e) والمستخلص الكلوروفورمى Am(c) وكانت النتائج كالتالى :-

١- أن مستخلصات الدمسيسة لديها القدرة على تثبيط معدل الذوبان لحصوات اكسالات الكالسيوم .

٢- أن قدرة المستخلصات المختلفة على تثبيط معدل إذابة حصوات اكسالات الكالسيوم تتناسب طرديا مع القطبية وبالتالي يكون Am(a)>Am(e)>Am(c).

٣- أن أعلي مستخلص لديه القدرة علي تثبيط معدل تكوين اكسالات الكالسيوم كان Am(a)ثم يليه Am(e)ثم يليه Am(c).