Egypt. J. of Appl. Sci., 37 (5-6) 2022 *30-57*

IN VITRO KIDNEY STONE FORMATION, ANTIOXIDANT AND ANTICOAGULATION ACTIVITY OF BIOLOGICALLY ACTIVE EXTRACTS AND FRACTIONS OF KAFF MARYAM *(ANASTATICA HIEROCHUNTICA* L.*)*

Refaat A. Saber
Soil and Water Science Department, Faculty of Technology and Development, Zagazig University, Zagazig. Egypt.
E-mail: chem_refaat63@yahoo.com.

ABSTRACT:

Kaff Maryam (*Anastatica hierochuntica* L.) is believed to be very useful in Arab countries for treating various health disorders. Traditional medicine has been gaining it is recognized due to its importance in public health and the administration of infections. This work focuses on the identification and characterization of phenolic compound profiles in methanol and ethyl acetate extracts of *A. hierochuntica* L. High-performance liquid chromatography (HPLC) and gas chromatography (GC MS) were used to identify the different phenolics in both extracts for bioactive organic compounds in methanol extract as the main established of diagnostic tools for these compounds. Individual polyphenolics compounds were evaluated in two different solvent crude extracts using HPLC and the result recorded that major compound detected were E-Vallinic and Ellagic. Also, GC-MS analysis was performed to investigate the bioactive organic compounds of methanolic extract of *A. hierochuntica* L ., the results illustrated that, Benzyl nitrile was identified as a major chemical constituent according to the relative abundance followed by Benzene, (isothiocyanatomethyl) respectively. The purpose, of this study was to investigate the kidney stone formation, antioxidant, and anticoagulant activities of *A. hierochuntica* L extracts. The potential effect of *A. hierochuntica* L extracts on crystal growth of calcium oxalate monohydrate(kidney stone) and its inhibitive properties were investigated at 37^{o}C and pH =6.5 ±0.05. In addition, antioxidant and anticoagulation properties of *A. hierochuntica* L. activity were performed at different concentrations. The present research highlights the chemical composition and properties of *A. hierochuntica* L. that may be related to its beneficial effect. The present research highlights the chemical composition and properties of *A. hierochuntica* L. that may be related to its beneficial effect. The results in these article suggest that polyphenolics content

might be contribute to the high urolithiasis inhibition, antioxidant, and anticoagulation activity of this plant and establish it as a valuable natural antiurolithiasis, antioxidant, and anticoagulation source applicable in the drug industry.

Key Words: Kidney stone, Antioxidant activity; Anticoagulant activity; HPLC; GC-MS, *A. hierochuntica* L.

INTRODUCTION

In Egypt, herbal and natural plants were and still are prevalent among Egyptian native people for the treatment of many diseases. The importance of herbal and natural plants comes from the high biological properties and their effect in medicine than chemical drugs. Previous studies have indicated that the extracts of this plants have antioxidant, anticancer, antifungal and antimicrobial, activities**.** The biological importance of this plant is due to the presence of many flavonoids compounds in its extracts. Extracts of *A. hierochuntica* L contain many flavonoids, terpenes, glucosinolates, and other secondary metabolite compounds such as caffeoyl- and dicaffeoylquinic acids, apigenin,3,4-dihydroxybenzoic acid, glucocheirolin, glucoiberin, isovitexin, kaempferol-7-glucoside, luteolin, quercetin, isosilybins A and B, silybins A and B, and others **(Al Gamdi *et al.*, 2011).** Some of these compounds (such as apigenin, luteolin, and quercetin, were formerly identified in other types and have long been known for their medicinal properties **(Loguercio & Festi, 2011 and Agarwal *et al.*, 2013).**

This explains that important to use herbal, natural plants and their extracts in preparing medicine. Recently some scientists to call for returning depend on natural and medical plants (seeds, oils, extract) for treatment of several ailments and decrease the chemical drug dependence. A. *hierochuntica* L was found in the arid regions of Egypt, Saudi Arabia, North Africa and, Jordan, and can survive without water for long periods. The medicinal effect of herbal and medicinal plants came from bioactive chemical components which definite physiological actions on the living organism. The most important of these components are phenolic, tannins, flavonoid and alkaloids compounds **(Shariff, *et al.*, 2001)**. *A. hierochuntica* L. is a well-known in desert zone as medicinal plant. Novel melanogenisis inhibitor flavonoids with antioxidant potential show were isolated according to **(Nakashima, *et al.*, 2010).** *A. hierochuntica* L is known as Kaff Maryam in Egypt and Arab countries, where it is widely used frequently in traditional medicine recipes and consumed as a tea beverage. It is a powdered mixed with honey and

taken for the treatment of many conditions, in particular as a remedy for difficult childbirth and uterine hemorrhage **(Khalifa and Ahmad, 1980; El-Ghazali *et al.*, 2010).** In literature decoction of *A. hierochuntica L*.), used in folk medicine against diverse ailments such as asthma, gastrointestinal disorders, cough, malaria, wounds, depression, high blood pressure, epilepsy, heart disease, diabetes, and infertility **(Batanouny, 1999 and Sobhy *et al.*, 2011).** The whole plant contains flavonoids: luteolin-7- glucoside, isovitexin, kaempferol 7- glucoside, kaempferol 3-rhamnoglucoside, quercetin and lucitin. It also contains glucosinolates: glucoiberin and glucocheirolin. The fruits contain glucose, galactose, fructose, sucrose, raffinose and stachyose **(Kamboj, 2000 and Al-Gamdi, *et al.*, 2011).** Urolithiasis of calcium oxalate monohydrate (COM) is the more abundant in Egypt and the world. Calcium oxalate monohydrate COM aggregates and other mineral crystals (such as the dihydrate form of calcium oxalate, and various calcium phosphates), along with lipids and biopolymers, are found within kidney stones; but the aggregation of COM in particular is recognized as a vital factor in kidney stone development **(Robertson, 1981 and Grases,1992).** Renal stones formation are outcome of increased urinary supersaturation with posterior formation of crystalline particles. Calcium-containing stones are the most common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) and a mixture of both (45%) **(Chaudhary *et al.*, 2010).** Calcium oxalate stones are found in two different varieties, calcium oxalate monohydrate (COM), and calcium oxalate dihydrate (COD) **(Menon *et al.*,1988).**A number of studies have been carried out to understand the effect of various additives such as metallic ions and their complexes **(Grases *et al.*, 1989),** sodium dodecyl sulphate **(Skrtic *et al.*, 1986)** , a-keto glutaric acid **(Atanassova *et al.*,1996)** (a normal physiological constituent of urine), plant extracts **(Atamani and Khan , 2000),** maleic acid copolymers **(Bouropoulos *et al.*,1998)** and protein from human kidney **(Selvam and Kalaiselvi 2000)** on inhibition of calcium oxalate crystal growth.

A free radical may be defined as an atom or molecule that contains one or more unpaired electrons and is capable of independent existence **(Seifried *et al.*, 2007).**Reactive oxygen species (ROS) are the byproducts of many degenerative reactions in crop plants, which will affect the regular metabolism by damaging the cellular components. Reactive oxygen species generated by biological oxidation are active forms of

oxygen and include superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\cdot}$), singlet oxygen ($^1O_2$) and non-free radical species such as hydrogen peroxide (H_2O_2) **(Surh and Ferguson, 2003).** Increasing evidence highlights that reactive oxygen species (ROS) and oxygen-derived free radicals may contribute to a variety of pathological effects (e.g. DNA damages, carcinogenesis and cellular degeneration) and induce many diseases including aging, cancer, atherosclerosis, diabetes and rheumatoid arthritis **(Lien *et al.*, 2008).**

In recent decades, although, technological progress of medicine prepared chemically, there is an increasing effort in finding natural antioxidants compounds which are widely distributed in herbal and natural plant, which can end harmful oxidative stress reactions by means free radicals and would have vey power useful defense in protecting cells from intake free-radical **(Havsteen, 2002).** Industrial synthetic medicine have vast functional effects in many diseases this make some scientists to call for returning depend on natural antioxidant and medicinal plants for treatment the ailments**.** Because the medicinal characteristics of plants, researches should focus on medicinal plants that are rich sources of natural remedies. Since modern medicine has increasingly benefitted from exploring compounds present in traditional medicine and chemistry drug**(Abdallah and El-Ghazali, 2013).** Herbals and medicinal plants are vastly used to treat numerous human diseases **(Shylaja and Peter 2004).** Flavonoids, tannins, anthocyanins, and other phenolic constituents present in food of plant origin are potential antioxidants. Food rich in antioxidants plays an essential role in the prevention of some cancers and Alzheimer's diseases and cardiovascular. Blood clots within the blood vessels lead to blood clotting, as it is a major cause of sudden death due to strokes and heart failure. Thrombolytic, anticoagulant, antiplatelet agents reduce clots of the blood in vessels by dissolving or inhibiting clots **(Webster, 2001).** Heparin has pharmaceutical properties such as myocardial infraction and allergic condition, it is used mainly in thrombosis treatment but has side effect like hemorrhage, and it is expensive **(Kathiresan and Ravindran, 2006).** For this reason, it is necessary to explore alternative anticoagulants. Many researchers have found anticoagulants and recorded their activity in many of natural plants. Therefore, it is necessity and demand of time to explore alternative anticoagulants. The anticoagulant activities of different plant have been reported **(Pawlaczyk *et al.*, 2009)**. Therefore, the present study was carried out to investigate the impact of methanol and ethyl acetate

solvents on total phenolic content, total flavonoid content, HPLC profile of phenolic compounds, GC-MS analysis, kidney stone crystal growth, antioxidant and anticoagulant activities of *A.hierochuntica* plant.

MATERIALS AND METHODS

Preparation of plant extracts

A. hierochuntica L. plants were purchased from local market of Cairo, Egypt. The leaf buds parts were oven-dried at 45-50^{o}C, and then, crushed to powdered for uniformity using a mixer grinder and used for consecutive extraction , stored in an air tight sample jars at room temperature.10 g of the powdered of plant material was extracted by soaking in 100 ml of methanol and ethyl acetate for one day at room temperature in an orbital shaker. After one day, methanol and ethyl acetate extracts were separated from the residues by filtering through Whatman No. 1 filter paper. These processes were repeated twice on the residues of powdered sample with the same fresh solvents and extracts collected. The collected extracts were concentrated and released of solvent under reduced pressure at 40 °C, using a rotary evaporator. The dried crude concentrated extracts were weighed to then store in a refrigerator until used for analyses.

Determination of total phenolic content (TPC)

The total phenolic constituents in *A. hierochuntica* L were determined by the Folin-Ciocalteau spectrophotometric method described by **Matkowshi and Piotrowska, (2006).** Sample (1 g) was mixed with 10 mL 80% methanol in a dark bottle and shaking well for 2 hour. A volume of 0.250 mL was mixed with 0.250 mL Folin-Ciocalteau reagent and 0.50 mL of 10% sodium carbonate (Na_2CO_3), then the volume was completed to 5 mL with distilled water. After incubation in dark at room temperature for 30 min, the absorbance of the reaction mixture was measured at 725 nm against blank with a double beam Perkin Elmer UV/Visible spectrophotometer (UV- Visible EZ201, Perkin Elmer: USA). Gallic acid was used as the standard to produce the calibration curve. The results were expressed as milligram of gallic acid equivalents (GAE) per gram of dried sample.

Determination of total flavonoid content (TFC)

Total flavonoid (TFC) content was determined using colorimetric method as described previously by **Chang *et al.*, (2002).** Briefly,0.5 mL solution of each plant extracts in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room

temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. Total flavonoid contents were calculated as quercetin from a calibration curve. Total flavonoid contents were determined as quercetin equivalents (mg/g of dry weight). Three readings were taken for each sample and recorded the average of them.

Instrumentation

1. Analysis of polyphenol compounds by HPLC

Determinations of phenolics were estimated by reverse phase High performance liquid chromatography (HPLC) as described by the method of **(Pascale *et al.*, 1999).** Chromatographic separations were performed on a guard column Altima C18 analytical column (150 × 4.6 mm i.d.). The detection was recorded by diode array detection (DAD) and the volume of the injection was 10 µl. The mobile phase was used as a gradient with the three solvents, A (acetic acid, 2.5%): B (acetic acid, 8%): C ((acetonitrile). The best method was implemented in a gradient system with flow rate of 1 mL min^{-1} at 35°C as follows: at 0 min, 5%B; at 20min, 10%B; at 50min, 30%B; at 55min, 50% B; at 60min, 100%B; at 100min, 50%B and 50%C; at 110min,100%C until 120min. Phenolic compounds were estimated by external standard calibration at 280nm and expressed in mg g^{-1}dry matter of equivalent (+)-catechin for flavan-3-ols, equivalent coumarin for a polar aromatic compounds and equivalent quercetin-3-rutinoside for flavonols.

2. GC/MS analysis:

Gas chromatograph - mass spectrometer analysis of *A. hierochuntica* methyl extract was performed using an Agilent 7890A mass spectrometer coupled to an Agilent 7000 gas chromatograph. Basic organic and inorganic chemical profiles of plant and other ecological sample were focused on using GC-MS studies as one of the main established diagnostic instruments. Gas chromatography and mass spectral analysis of the *A. hierochuntica* methyl extract was implement by using Agilent GC–MS built with bonded-phase fused silica capillary column (30 mm · 0.25 mm ID; df = 0.25) and mass spectrum for identification of the corresponding metabolites with correlation by known spectra. Instrumentation of GC– MS operating key procedure for volatile and semi volatile organic compounds are as follows. highly helium reactive used as a carrier gas in column at the rate of flow 1.5 ml/min. The split less injection was maintained at 260 ^{o}C. The splitless injection mode was used with a split ratio of 40:1. The transfer line temperature was set at 260 ^{o}C. The mass analyzer (mz) was set at 60 eV,

electron impact source temperature at 200 ^{o}C, electron multiplier voltage at 1588 mV and solvents delay at 2 min. All scrupulous data were acquired by the full-scan mass spectra within the scan range of 50–400 amu. The oven temperature program was as follows: increased from 190 to 250 ^{o}C at a rate of 2 ^{o}C /min, and from 200 to 260^{o}C at a rate of 1 C/min. Finally, the obtained spectrum of methyl extract was compared with the standard known database in the NIST library and confirmed as exact compound **(Jan *et al.*, 2006).**

Crystal growth of calcium oxalate monohydrate (COM).

Preparation of seeds:

Calcium chloride and sodium oxalate solutions were prepared using the pure grade reagent with triply deionized water. Calcium oxalate monohydrate (COM) seed prepared by dropped $Na_2C_2O_4$ solutions to $CaCl_2$ in beaker 2Lwith continuously stirred and temperature was kept at 37^{0}C. The mixed solution was vacuum filtered using 10μm filter paper. COM seed crystals were washed and stored **(Abdel-al *et al.*, 2009).** Calcium oxalate monohydrate seed were aged for at least one month before use. Seed crystals were dried and then subject to thermo gravimetric analysis (TGA), determination of specific surface area (SSA), x-ray powder diffraction studies.

Crystal growth measurements

Crystal growth experiments were made in a Pyrex glass vessel of approximately 300 ml capacity. The solutions were maintained at the required temperature (37°C) by circulating thermostated water through the outer jackets. Working solutions were always magnetically stirred, and nitrogen gas was bubbled throughout experiment **(Hamza, and Nancollas, 1991)**.

Scavenging Activity on DPPH Radicals:

Quantitative measurement of radical scavenging properties of different *A. hierochuntica* L extracts was carried out based on determine the scavenging activity of the stable 1,1-diphenyl-2- Picrylhydrazyl (DPPH) free radical by widely used method **(Braca, *et al.*, 2001)** . Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined spectrophotometrically after 30 min, and the percentage inhibition activity was calculated from the following equation:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100.$$

Where; A_0 is the absorbance of the control and A_1 is the absorbance of the extract/ standard.

In the experiment, L-ascorbic acid was used as the positive control. The concentration of sample required to scavenge 50% of DPPH free radical (IC50) was determined from the curve of percent inhibitions plotted against the respective concentration.

Hydrogen Peroxide Scavenging Capacity

The ability of the *A. hierochuntica* L extracts to scavenge hydrogen peroxide was determined according to **Ruch *et al.*, (1989) .** A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM).Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *A. hierochuntica* L extracts and standard compounds were calculated:

% Scavenged [H_2O_2] = [(AC – AS)/AC] x 100

Where AC is the absorbance of the control and AS is the absorbance in the Presence of the sample of *A. hierochuntica* L extracts or standards.

Human plasma preparation

In the blood analysis unit at the University Hospital, Zagazig University, blood samples were collected from healthy individuals without a history of bleeding or thrombosis. The blood samples were placed in vials containing 2.5% sodium citrate solution on a centrifuge for 15 minutes at 5000 rpm at a temperature of 4°C to separate the plasma. The freshly separated plasma was used for coagulation time measurements.

Anticoagulant activity determined by prothrombin time (PT) test:

PT test was determined according to the method of **(Ekanayake, *et al.*, 2008)** with some modification. 200 µl of different concentrations of plant extracts (µg/ml) was mixed with 50 µl of citrated plasma. The mixture was incubated in a water bath for 5 min at 37 °C. Then 100 µl of PT reagent (Phosphoplastin RL, Thrmboplastin with calcium, R2 Diagnostics, South Bend, USA) was added and the time taken (sec.) for plasma clotting was recorded by stopwatch. 200 µl of bi-distilled water used as control instead of plant extracts.

Anticoagulant activity determined by activated partial thromboplastin time (APTT) test.

The method was used to measured APTT (**Ekanayake *et al.*, 2008),** with some modification. 50 µl of citrated plasma was mixed with

200 µl of different concentrations of plant extracts and incubated in a water bath at 37 °C for 1 min, then 50 µl of APTT reagent (Phospholin ES, Ellagic acid APTT reagent, R2 Diagnostics, and South Bend, USA) was added into the mixture. The mixture was incubated again for 5 min at 37 °C then 50 µl of $CaCl_2$ solution were added and the clotting time (min) was recorded by stopwatch. 200 µl of bi-distilled water instead of plant extracts were used as control.

RESULTS AND DISCUSSION

Total phenol and flavonoid contents of the extracts:

The total phenolic contents of *A. hierochuntica* L. of two extracts are represented in Table (1). The results indicated that the total phenolic content of methanol extracts and ethyl acetate had significant variations ranging from 41.6 to 28.53 (mg/g DW). The methanol extract had the highest total phenolic content (41.6mg/g DW), while the ethyl acetate had the lowest content 28.53 (mg/g DW).These results explained that the methanol extract possessed significant activity in releasing most secondary metabolites from leaves. This may be due to the fact that phenolic compounds are often extracted in higher amounts by using polar solvents such as aqueous methanol/ethanol **(Sultana *et al.*, 2007)** . Methanol is a relatively polar organic solvent compared to ethyl acetate, so most polyphenols that have been evaluated in this study are likely to be polycarbonate compounds. The main role of phenolic compounds in the disposal of free radicals has been emphasized in previous reports. Secondary metabolites in the plant are phenolic antioxidants. Its antioxidant activity is due to oxidative properties and structural structure which can play an important role in chelating transitional metals and scavenging free radicals **(Mohamed *et al.*, 2010) .** The results are consistent with previous studies, indicating that the extraction of phenolic compounds from plants increases with the increase of polarity of solvents **(Mohamed *et al.*, 2013).** The total flavonoid content of *A. hierochuntica* L. extracts is appear in table (1), Significant differences were observed depending on the solvent used for TF contents. The highest value of flavonoid (17.78 mg/g, DW) was observed in methanol extract followed by ethyl acetate extract (11.21 mg/g, DW) respectively. Methanol was more effective for total flavonoid extraction than ethyl acetate. Flavonoids are known to have anti-oxidant activity and have an effect on human health and nutrition. Flavonoids work through a mechanism scavenging or chelating processes **(Kessler *et al.*, 2003).** Flavonoids

were found to contain hydroxyl groups responsible for antioxidant properties in plants **(Das and Pereira; 1990).**

Table (1): Total phenolic and flavonoids contents of *A. hierochuntica* L. extracts

Sample	Total phenols (Gallic acid equivalent (mg/g)dray weight	Total flavonoids (Quercetin equivalent (mg/g)dry weight
Methanol extract	41.8	17.78
Ethyl acetate extract	28.53	11.21

HPLC analysis:

HPLC analyses of main polyphenolic compounds in *A. hierochuntica* L. extracts are shown in Table (2). Twenty-two phenolic compounds were identified in *A. hierochuntica* L plant extract. Flavonoids that bearing a large number of free hydroxyl groups on aromatic ring contain or sugar units are polar in character, such as methanol, ethanol, dimethyl sulphoxide, acetone and water. As for less polar flavonoids such as: isoflavones, flavones, flavanones, which carry a greater number of methoxyl groups, they dissolve in chloroform or ether. Because of the large diversity of phenolic compounds in the plant extracts, it is difficult to identify every compound, but it is not difficult to identify major and important phenolic compounds. HPLC analysis of major phenolic compounds in some medicinal plants has been developed **(Cai *et al.*, 2004 and Uddin *et al.*, 2014).**

The experimental results of high performance liquid chromatography analysis of polyphenolic compounds are shown in Table (2). Twenty-two phenolic compounds were identified in *A. hierochuntica* L plant extracts. The result in present study showed that the major phenolic acids identified in *A.hierochuntica* L different extracts Pyrogallol, E-Vanillic, Ellagic, Vanillic, Catechol, Chlorogenic, Caffeic, Salycilic, , Epicatechein, Ferulic, P-OH-benzoic, Catechein, 3,4,5-methoxy –cinnamic, Protocatchuic, Alpha-Coumaric, Coumarin, P-Coumaric,Gallic, Caffeic, Iso-Ferulic, 4-Amino –benzoic and Cinnamic. In previous studies, many authors proved the presence of different phenolic compounds and identified in *A. Hierochuntica* L extracts. In this concern, two novel skeletal flavanones, anastatins A and B were isolated from *A. hierochuntica* L, in addition to seven known flavonoids, eleven aromatic compounds, three phenylpropanoids, twelve lignanes and four flavonolignans **(Yoshikawa *et al.*, 2003).**

In addition, quercetin, isovitexin and 3- rutinoside of kaempferol and quercetin were also reported for A. Hierochuntica **(Marzouk *et al.*,**

2010). The polarity of extraction solvents in the present study also has an effect on the variation of individual phenolic compounds. This can be clarified by the identification of; P-OH-benzoic, Alpha-coumaric and salycilic acid in the methanol extract only. Results of the present study are in accordance with **Waszkowiak *et al.*, (2015),** which verified that the nature and polarity of extraction solvents have an impact on the solubility and extraction of the phenolic compounds from plant materials. Moreover, other phenolic compounds found in the extracts such as gallic acid and quercetin also possess beneficial effects on human health and eases oxidative stresses **(Pandey and Rizvi; 2009) .**

Table (2): HPLC profile of phenolic compounds provisional identified in the methanol and ethyl acetate extracts of *Anastatica hierochuntica* L

	Compound	Plant extracts			
		Methanol		Acetate	
1	Pyrogallol	mg/g 0.04624	ppm 46.24	mg/g 0.01012	ppm 10.12
2	Gallic acid	0.00121	1.21	0.00137	1.37
3	4-Amino -benzoic	0.00086	0.86	0.0011	1.10
4	Protocatchuic	0.00591	5.91	0.00294	2.94
5	Catechein	0.00688	6.88	0.00172	1.72
6	Catechol	0.03076	30.76	0.01154	11.54
7	Epicatechein	0.01219	12.19	0.00298	2.98
8	P-OH-benzoic	0.00805	8.05	----	ND
9	Chlorogenic	0.02694	26.94	0.00614	6.14
10	Vanillic	0.03152	31.52	0.01544	15.44
11	Caffeic	0.00112	1.12	0.00358	3.58
12	P-Coumaric	0.00319	3.19	0.00131	1.31
13	Ferulic	0.01009	10.09	0.00202	2.02
14	Iso-Ferulic	0.00109	1.09	0.00079	0.79
15	E-Vanillic	0.33902	339.02	0.12121	121.21
16	Alpha-Coumaric	0.00462	4.62	-----	ND
17	Benzoic	0.01478	14.78	0.02513	25.13
18	Ellagic	0.08703	87.03	0.06663	66.63
19	3,4,5- methoxy –cinnamic	0.00627	6.27	0.01183	11.83
20	Coumarin	0.00363	3.63	0.00048	0.48
21	Cinnamic	0.00075	0.75	0.00026	0.26
22	Salycilic	0.01109	11.09	-----	ND

GC/MS analysis:

The experimental result tabulated the results of the GC–MS analysis of the methanol extracts are given in Tables (3) and Fig 1. Gas chromatography-mass spectrometry results revealed presence that seventeen compounds were identified in methanolic extract of *A.*

hierochuntica L,. (Table 3 and Fig1). Benzyl nitrile was identified as a major chemical constituent according to the relative abundance followed by Benzene, (isothiocyanatomethyl)-,1,2-Dimethoxy-4-(2,3-dimethoxy-1-propenyl) benzene, 3',5'-Dimethoxyacetophenone, 5-Hydroxymethylfurfural, Benzoic acid, methyl ester, D-Allose, trans-13-Octadecenoic acid, methyl ester ,Hexadecanoic acid, methyl, γ-Tocopherol, 11,14-Octadecadienoic acid, methyl ester, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, cis-13-Eicosenoic acid, methyl ester and Benzaldehyde.

Table (3): phenolic compounds identified in methanol extract of *A.hierochuntica* L.

No	t_R (min.)	Proposed compound	Formula	M.W.
1	5.75	Benzaldehyde	C_7H_6O	106
2	8.85	Benzoic acid, methyl ester	$C_8H_8O_2$	136
3	10.56	Benzyl nitrile	C_8H_7N	117
4	13.91	5-Hydroxymethylfurfural	$C_6H_6O_3$	126
5	15.86	Phenol, 2-methoxy-4-(2-propenyl)- (Eugenol)	$C_{10}H_{12}O_2$	164
6	16.23	Benzene, (isothiocyanatomethyl)-	C_8H_7NS	149
7	18.39	Benzeneacetamide	C_8H_9NO	135
8	18.87	Benzoic acid, 2-(dimethylamino)ethyl ester	$C_{11}H_{15}NO_2$	193
9	21.11	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180
10	23.12	D-Allose	$C_6H_{12}O_6$	180
11	27.95	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
12	31.09	11,14-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
13	31.18	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
14	31.23	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292
15	31.99	1,2-Dimethoxy-4-(2,3-dimethoxy-1-propenyl)benzene	$C_{13}H_{18}O_4$	238
16	34.61	cis-13-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324
17	45.69	γ-Tocopherol	$C_{28}H_{48}O_2$	416

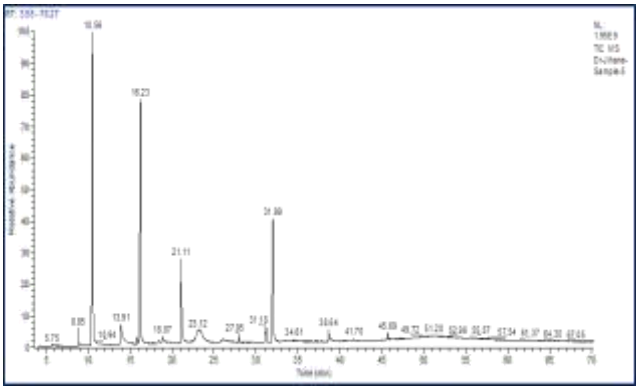

Fig (1): GC-MS chromatogram of methanol extract of *A. hierochuntica* L

Crystal growth:

X-ray diffraction, thermo-gravimetric analysis, scanning electron microscope and specific surface area (SSA) were investigated to confirm of chemical structure of calcium oxalate monohydrate seed crystals.

X-ray diffraction:

X-ray diffraction of variation prepared solids were determined by using a Philips diffractometer (XRG-3000 electronic instrument). 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 3.8 cm × 3.8 cm × 0.2 cm aluminum solid holders and were slowly scanned at speed of 10 / 4 20 = 10° to 90°. The value of 2θ for the major peaks of calcium oxalate monohydrate fig (2), similar to those reported to $CaC_2O_4 .H_2O$ with no evidence for to $CaC_2O_4 .2H_2O$ or $CaC_2O_4 .3H_2O$.

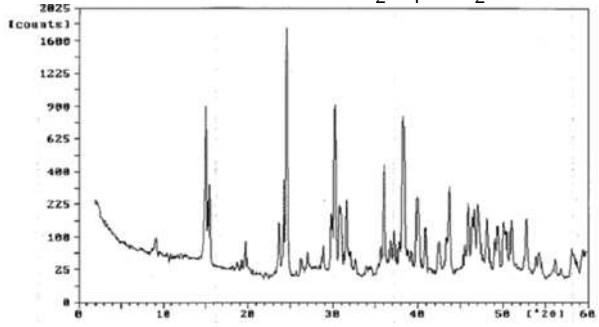

Fig (2): X-ray diffractoagram of calcium oxalate monohydrate crystals.

Specific surface area:

The specific surface area was calculated according to the **Brunauer–Emmett–Teller** (BET) method **(Brunauer, et al; 1938) applied** equation as follows:

$$SSA = 1/W.(1- P/P_O).((S_g /S_{gc}).vc. \quad N_g A_{cs} P_0 /RT \qquad (1)$$

Where:

W: The weight of solids. S_g: The desorption of single area. S_{gc}: Single area of calibration, Vc : Volume of calibration, N° : Avogadro's number, A_{cs}: Cross-sectional area of adsorbate Molecule, Po : Ambient pressure

The specific surface area of prepared calcium oxalate monohydrate being calculated by adsorption of krypton measured 3.37 m^2/g^{-1} .

Gravimetric analysis:

Thermal gravimetric analysis TGA, differential thermal analysis DTG and differential scanning calorimetry (DSC) study was done on prepared seed is shown in (Fig. 3).

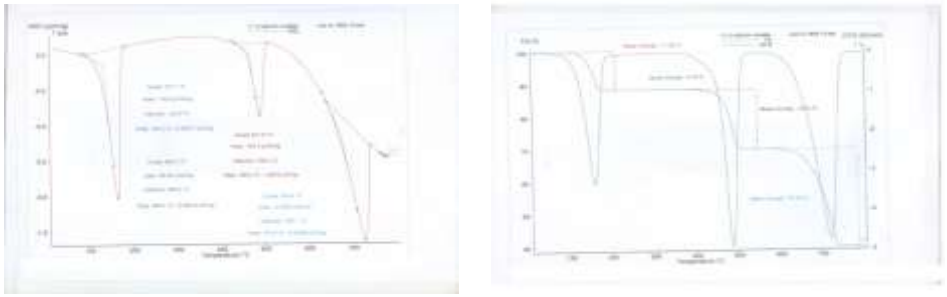

Fig (3): TGA and DTA of COM crystals.

Thermal gravimetric analysis TGA, differential thermal analysis DTG and Differential scanning calorimetry (DSC), study was done on prepared seed is shown in (Fig. 3) which indicate that removal one molecule of water at the first step t1 and conversion of $Ca_2C_2O_4$ to Ca CO_3 + CO in 2nd step at t_m and finally decomposition of $CaCO_3$ to CaO + CO_2.

Crystal growth of (COM) crystals experiments was investigated under experiment conditions T= 37 °C, I= 0.15 mol dm^{-3}, P^H =6.5 ± 0.05 at different σ values (where σ is the degree of super saturation) in absence and presence of inhibitors. Data in Fig (4), revealed that proportion relation between the supersaturation and rate of COM crystal growth in absence of inhibitors.

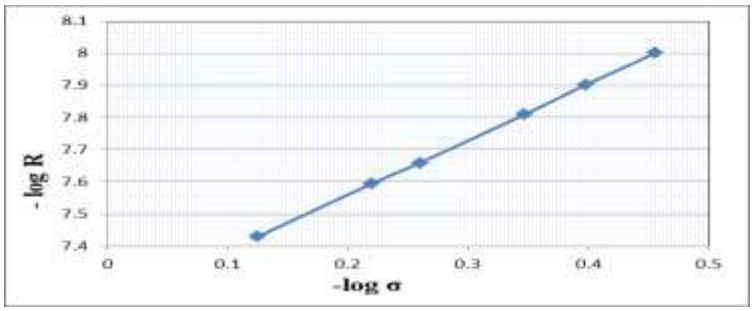

Fig (4); Plots of –log R against –log σ for crystal growth of calcium oxalate monohydrate crystals at 37 ˚C

Table (4) and Fig (5) gave an indication about the effect of methanolic and ethyl acetate extracts on the rate of crystal growth of COM. Crystal growth of (COM) crystals experiments was investigated under experiment conditions, t= 37^{o}C, I= 0.15 mol dm^{-3}, P^H =6.5 ± 0.05 at different σ values in absence and presence of inhibitors. The proportion relation between the supersaturation and rate of COM crystal growth in absence of inhibitors are shown in Fig (4). Crystal growth for

many springy soluble salts M_a A_b the rate of growth for seed surface area can be expressed by the equation (2)

$R = d[M_a A_b] dt = Ks \sigma^n$ ……(2)

Where s is proportional to the number of growth sites available on the seed crystals surface and n is the effective order of reaction. In general, the rates of crystal growth and dissolution of alkaline-earth metal salts are markedly inhibited by the addition of certain additives. Typical plot of -log σ against – log R (where σ supersaturation and R is rate of crystal growth of COM crystals, it was found that the effective order of crystal growth determined from the slop of these plot Fig (4) follow parabolic rate low with n≈2 which confirm surface controlled mechanism over rang of relative supersaturation (0.3> σ >0.8) . The possible inhibited effects of methanolic and ethyl acetate extracts for *A. hierochuntica* L., on the crystal growth of calcium oxalate were investigated in the presence of *A. hierochuntica* L., extracts. The experimental data summarized in Table (4) for both methanolic and ethyl acetate extracts, each experiment was made in triplicate for certainties. From the rate of profile is shown in Fig (5), where rate of crystal growth in the presence of methanolic and ethyl acetate, extract decrease with successive additions of additives. It can be seen that the concentration as low as 10^{-5} mol dm^{-3} for each additives reduced the crystal growth rates by as much as 81.76% and 64.56% compared to that in the pure solution at the same relative supersaturation in the presence of methanolic and ethyl acetate of *A. hierochuntica* L respectively.

The crystal growth rate decreased with an increase in the concentration of inhibitors, the decrease in crystal growth rate is due to blocking the active sites on the of COM crystal surface by inhibitor molecules. Where the anions adsorbed on cation sites and cation at anionic one on the surface.

Ca^{2+} (sol) ---------- Ca^{2+}(ads) (3)

OX^{2-} (sol) ---------- OX^{2-} (ads) (4)

Ca2+(ads) + OX^{2-} (ads) ---------- Ca C_2O_4 (ads) (5)

Ca C_2O_4 (ads) ---------- Ca C_2O_4 (lattice) (6)

when inhibitors adsorbed on the lattice sites, these preventing the transfer of calcium oxalate monohydrate units between adsorbed state as CaC_2O_4 (ads) --------- Ca C_2O_4 (lattice). The inhibitors in theses case are high sensitive to the morphology of the exposed crystal surface, which decreased the rate constant.

In literature among the chemical composition of *Anastatica Hierochuntica L* , Carbohydrate, protein **(Saranya *et al.*, 2019),** alkaloids, tannins, flavonoid and phenolic compounds **(Nakashim *et al.*, 2010)**. However Kaff-e-Maryum, lipids, amino acids. Coumarins, glycosides, betacyanin and terpenoids contained glucose, galactose, fructose, sucrose, raffinose and stachyose **(Al-Gamdi *et al.*, (2011) and Kamboj, (2000).** Furthermore, luteolin-7-glucoside, luteolin-7-glycoside, isovitexin, kaempferol 3-rhamnoglucoside, lucitin; glucosinolates including glucoiberin and glucocheirolin, and sterols were found in different parts of the plant (**Shah, et al;2014).** The effectiveness of integrated therapy of inhibitors can explained either by complexation of the inhibitor, usually a chelating or sequestering agent, with the lattice cation, or by adsorption of the additives at active sites on the crystal surfaces and sometimes may be both mechanisms can exist at the same time. Anionic inhibitor are very good inhibitors for dissolution and crystallization of COM crystals especially those containing -OH, -COO, -OCH_3, -NH_2 , -SO_3 and P-O-P **(Grases; 1988).**

The methanolic and ethyl acetate are extracts containing anionic group in their compound, which adsorbed effectively on cationic site and reduce the rate of growth of COM crystals. As the negativity of the additives increase on the surface of COM crystal, Ca^{2+} active sites nearly completely blocked and the crystal growth rate of COM crystal decrease. The methanolic and ethyl acetate extracts of *A. hierochuntica* L either organic or inorganic nature play an important role in crystallization processes. It is important to know how the additives influence the crystallization rate process as well as the type and number of polar functional groups contained in additives molecule of these extracts. Among the factors that influence crystallization are the molecular weight, hydrophilic and hydrophobic regions, and concentration of additives, and a close match between the spacing of acid groups and the spacing of cations of the crystal surface. The addition of *A. hierochuntica* L., extracts (additives), might be expected have the following functions:

(i) Interact chemically with the crystal surface to form complexes.(ii) Change in the characteristics of the adsorption layer of solid solution .(iii) They prevent crystal growth by binding to the growth active sites on the crystals surface.(iv) Being adsorbed on the crystal surface and physically block the active crystallization sites. (v) Alter the surface energy of the crystals . In general a specific inhibition of the rate of crystal growth is expected to take place at much lower concentration of the additives molecular than for simple complexation.

Table (4) ; Effect of methanol and ethyl acetate extracts on the rate of crystal growth of COM crystals at σ = 0.4

Type of extract	Additives / 10^{-6} mol dm^{-3}	Rate/10^{-9} mol $min^{-1}m^{-2}$	% Inhabitation	R_0 /R_0-R_i	10^5[inhibitor]$^{-1}$
Methanolic Extract		12.523			
	1.00	9.092	27.40	3.651	10
	2.00	7.031	43.86	2.28	5
	3.00	5.642	54.95	1.82	3.33
	4.00	4.745	62.11	1.61	2.5
	5.00	4.044	67.71	1.47	2
	6.00	3.403	72.83	1.37	1.67
	7.00	3.066	75.52	1.32	1.43
	8.00	2.746	78.07	1.28	1.1
	9.00	2.489	80.12	1.25	1.25
	10.00	2.284	81.76	1.22	1.00
Ethyl acetate extract		12.523			
	1.00	10.53	15.91	6.29	10
	2.00	9.117	27.20	3.677	5
	3.00	8.018	35.97	2.78	3.33
	4.00	7.078	34.48	2.296	2.5
	5.00	6.231	50.24	1.99	2
	6.00	5.662	53.99	1.852	1.67
	7.00	5.193	58.53	1.7	1.43
	8.00	4.907	60.81	1.64	1.25
	9.00	4.487	64.17	1.6	1.1
	10.0	4.189	64.56	1.57	1.00

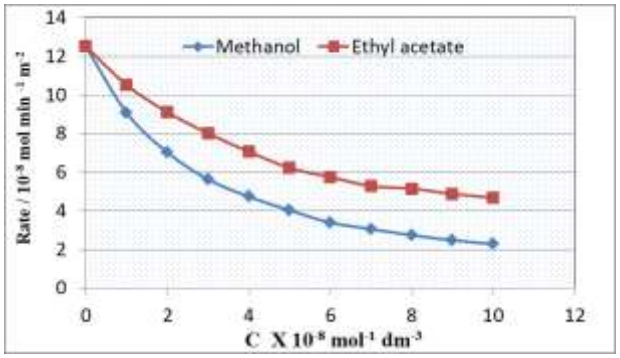

Fig (5): Plot of the rate of crystal growth of COM crystal against [methanol and ethyl acetate extracts] of *Anastatica hierochuntica* L at σ = 0.4, t = 37 ˚C and I = 0.15 mol dm-3.

On the assumption that the decreased crystal growth rate reflects the adsorption of both extractions at active growth sites, the influences of both inhibitors can be interpreted in the terms of a Langmuir isotherm **(Langmuir,1916)** as show in Table(4) and Fig (6) which indicate the

values of R_0-R_i /R_0 . According to these model at equilibrium, the rates of adsorption and desorption of solute on the surface are:

$$K_{ads}(1-\theta)\, C\, eq = K_{des}\ \theta \ldots\ldots\ldots(7)$$

Where θ is the fraction of active growth sites on the surface, occupied by inhibitor molecules and C_{eq} concentration of additives.

According to the surface coverage, θ the rate of crystal growth R_i, in the presence of additives is given by:

$$R_i = R_0\,(1-\theta) \ldots\ldots\ldots (8)$$

where R_0 the rate of crystal growth in absence inhibitor ,combining 7,8gives:

$$R_0 / R_0 - R_i = 1 + 1/K_{aff}\ .\ 1/C_{eq} \ldots\ldots\ldots(9)$$

Where K_{aff} is the affinity constant which equal K_{ads} / K_{de}, reflecting the adsorption affinity of solute molecules on crystal surface. The affinity constant K_{aff} is determined from the inverse of slopes of linear plot of R_0 / R_0 -R_i against $[inhibitor]^{-1}$ as show in Fig (6), where found to be equal 34 x 10^4 and 18 x 10^4 for methanolic and ethyl acetate extracts respectively. The values of K_{aff} reflect the ability of additives to inhibit the rate of crystal growth. The linear relationship and intercept of unity, strongly suggest that the mechanism of calcium oxalate formation may be the formation of monolayer blocking layer of additives ions at the surface of COM, so the additives act as inhibitors through the adsorption onto the active sites of COM crystals.

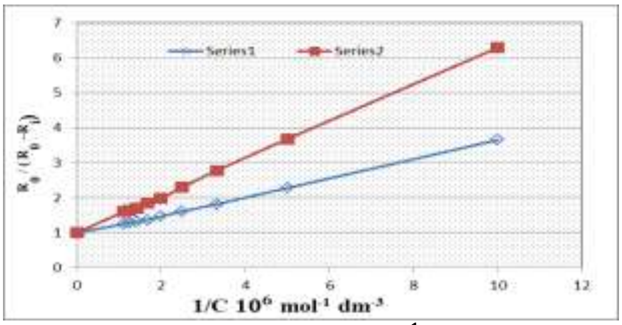

Fig (6). Plot of $R_0/(R_0 - R_I)$ against $[additives]^{-1}$ of crystal growth of COM in presence of methanol and ethyl acetate extracts.

Determination of DPPH free radical scavenging activity:

The DPPH radical scavenging activity of the methanol and ethyl acetate extracts are recorded in Table (5) and Fig(7). The fundamental role of antioxidants is to break the chain of radical reactions resulting from oxidation and antioxidants is divided in terms of their sources into

natural and manufactured. DPPH free radical scavenging activity is widely used in estimating free radical scavenging activities of antioxidants **(Gyamfi, 1999 and Lee *et al.*, 2002).** The antioxidant effect on DPPH radical scavenging due to their hydrogen donating ability. It is reduced to1,1- diphenyl-2-picrylhydrazine. The changes in colur (i.e. from deep-violet to light-yellow) can be measured spectrophotometric ally (**Azlim *et al.*, 2010)** .The DPPH radical scavenging activity result of the methanol and ethyl acetate extracts are recorded. The DPPH radical-scavenging activity of different *A. hierochuntica* L extracts, at different concentrations (50, 100, 150, 200 &250 µg /ml) is shown in Table (5). A concentration dependent scavenging activity was clearly demonstrated. At 250µl concentration, the inhibition of methanol, extract was determined as 79.17 and 69.63%, respectively in ethyl acetate extract as show in Fig (7). All studied samples, methanol and ethyl acetate extract had the weakest free radical-scavenging activity with an IC_{50} value of 88.76 and 130.72µg/ml, for methanol and ethyl acetate extracts respectively in compared with ascorbic acid as standard with IC_{50} = (44.11 µg/ml).

Flavonoids that carry a large number of free hydroxyl groups or contain sugar units are polar in character, and polar in solvent such as methanol, dimethyl sulfoxide, acetone and water. Flavonoids have several effective properties, including the following properties. Antioxidant, anti-inflammatory, antiviral, anti-hepatotoxicity, anti-bacterial, anti-high blood pressure and anti-cancer. Many traditional medicines and medicinal plants contain flavonoids as biologically active compounds. The antioxidant properties of flavonoids are present in fresh fruits and vegetables, which are believed to contribute to the prevention of cancer and heart disease **(Sobhy *et al.*, 2011)**.

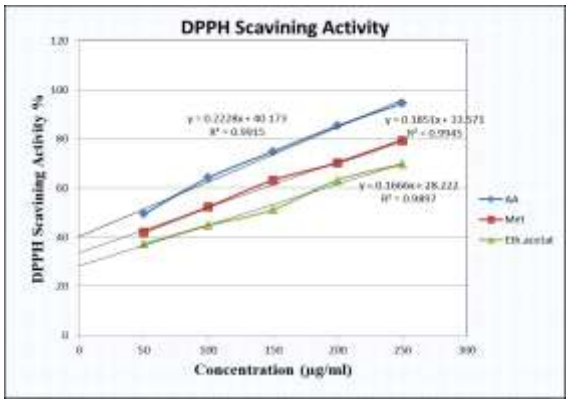

Fig (7): DPPH scavenging activity (%) for methanol, ethyl acetate extracts of *Anastatica Hierochuntica* L

Table (5): IC50 values of the antioxidant activities (methanol, ethyl acetate) of *Anastatica Hierochuntica* L extracts and ascorbic acid as standard against DPPH and hydrogen peroxide scavenging capacity.

Concentration µg/ml	DPPH scavenging activity			H_2O_2 scavenging activity		
	Ascorbic acid	Methanol extract	Ethyl acetate extract	Ascorbic acid	Methanol extract	Ethyl acetate extract
50	49.32	41.89	37.23	49.33	38.24	32.31
100	64.11	52.25	44.73	57.89	49.34	38.41
150	74.79	63.13	51.23	68.16	57.43	48.12
200	85.32	70.24	63.21	77.22	63.89	53.34
250	94.41	79.17	69.63	86.26	69.31	62.93
IC50	44.11	88.76	130.72	54.64	113.2	169.959

Hydrogen peroxide scavenging activity

The experimental results of the influence of methanolic and ethyl acetate extracts on hydrogen peroxide scavenging activity are shown in Table (5) and Fig (8)**.** These membranes show finite permeability to H_2O_2 in reliance on the membrane composition and on the term of membrane proteins ease H_2O_2 spread, known as aquaporin's **(Bienert *et al.*, 2006 & 2007).** Hydrogen peroxide can thus push through the plasma membrane and gain access to different subcellular compartments. However, it is unknown, whether different subcellular membranes show the same permeability to Hydrogen peroxide

Due to non-reactivity of hydrogen peroxide it is sometimes be toxic to cell because it may transformed to ($^{\bullet}OH$) hydroxyl radicals in the cells (**Gulcin *et al.*, 2010).** Scavenging activity of hydrogen peroxide in the presence of methanol and ethyl acetate extracts may be attributed to their phenolics, which can give rise electrons to H_2O_2, there for neutralizingH_2O_2 to H_2O **(Ebrahimzadeh, 2009).** The experimental results showed that the both extracts methanol and ethyl acetate had an effective hydrogen peroxide scavenging activity. As the antioxidant components present in the extracts are good electron donors, so lead to the conversion of H_2O_2 to H_2O. Figure (8) reports the hydrogen peroxide scavenging activity of various extracts of *A. hierochuntica* L. The results showed that methanol extract have scavenge the H_2O_2 radicals with an significant inhibition percentage of 38.24-69.31%, followed by ethyl acetate (32.31- 62.93 %) compared to the ascorbic acid as standard (49.33- 86.26) at the same concentration of extracts. Consequently, the eliminating of hydrogen peroxide was significantly for antioxidant defense in cell. According to the present findings, all the extracts might be used to provide a use full H_2O_2 scavenger for humans and foods.

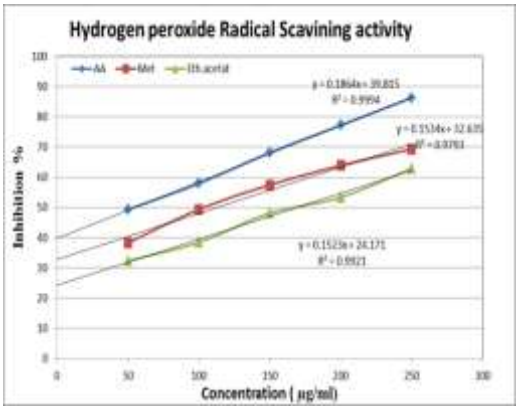

Fig (8) Hydrogen peroxide scavenging activity for methanol, ethyl acetate extracts of *A. Hierochuntica* L

Anticoagulant activity

In the present study, prothrombin time (PT) and activated partial thromboplastin time (APTT) were performed using normal human plasma to estimate the anticoagulant activity of methanol and ethyl acetate extracts of *A. hierochuntica* L. at different concentrations. The experimental results are presented in Table (6) reveale clear difference in the ratio of clotting time in PT and APTT assays depending on the type of extracts and concentration. Blood coagulation is the process by which blood clot is formed. Coagulation begins nearly promptly after an injury to the endothelium lining a blood vessel. Exposure of blood to the subendothelial space begin two processes: changes in platelets, and the exposure of subendothelial tissue factor to plasma factor VII, which ultimately leads to cross-linked fibrin formation. Platelets instantly form padlock at the position of injury; this is called primary hemostasis. Secondary hemostasis takes place simultaneously **(Furie and Furie, 2005).** Thus, the modern studying for antithrombotic, anticoagulant, and thrombolytic reagents from various plant sources have become a high priority in biomedical research**.** Phenolic and flavonoid compounds from the botanical origin are a prospective source of anticoagulant drugs **(Lamponi, 2021).**

In the present study, prothrombin time (PT) and activated partial thromboplastin time (APTT) were performed using normal human plasma to estimate the anticoagulant activity of methanol and ethyl acetate extracts of *A. hierochuntica* L at different concentrations. The experimental results presented in Table (6) revealed clear difference in the ratio of clotting time in PT and APTT assays depending on the type of extracts and concentration. In these work methanol and ethyl acetate extracts exhibited the highest anticoagulant activity as compared to the control. The methanol and ethyl acetate extracts significantly prolonged

the clotting time of plasma to 68 and 59 sec in PT test, correspondingly at 100 µg/ml concentrations as compared to 19 and 21 sec respectively in control. They also increased APTT time to 9.58 and 11.12 min, respectively at the same concentration 100 µg/ml concentrations as compared to 2.12 and 2.56 sec compared to control (Table 6). It was apparent that there were relative correlations between the different concentrations of *A. hierochuntica* L extracts required to inhibit clot formation and prolongation of prothrombin time. As concentration of methanol or ethyl acetate extracts, increase the blood coagulation process increased and the prothrombin time was inhibited. The obtained results may indicate that, the use *A. hierochuntica* L may increase the properties of prescribed anticoagulants. Secondary metabolites from the *A. hierochuntica* L extracts are potential source of anticoagulant drugs

Table (6) Prothrombin time (PT) and activated partial thromboplastin time (APTT) of methanol and ethyl acetate extracts of (*Anastatica hierochuntica* L.).

Concentration µg/ml	Methanol extract		Ethyl acetate extract	
	PT(sec)	APTT(min)	PT(sec)	APTT(min)
Control	19	2.12	21	2.56
20	24	2.34	22	4.96
40	28	4.12	25	5.54
60	39	5.46	33	6.67
80	52	7.46	47	8.48
100	68	9.58	59	11.12

CONCLUSION

The results in this research confirmed the opinion that several medical plants are emboldening resources prospective for inhibition of crystal growth of kidney stone, antioxidants, anti coagulation and could be successful as protective intermediary within the pathogenesis of several illnesses. It could be also used in support foodstuff against oxidative retro gradation. One of several medicinal plants that showed greatest potential effect against urolithiasis , oxidative stress coagulation activity is *A. hierochuntica* L., however must be followed these study by extensive phytochemical and pharmacological studies on these used plant .

Acknowledgments

The author appreciate Dr. Mohamed Alaa El-Din at the Hematology and Immunology Unit for helping to complete some analysis of the research in the laboratories of the General Fever Hospital in Zagazig City and many thanks to everyone that contributed to the success this work.

REFERENCES

Abdallah, E.M. and G.E. El-Ghazali (2013). Screening for antimicrobial activity of some plants from Saudi Folk medicine. Global J. Res. Med. Plants Indig. Med., 2(4):210-218.

Abdel-al, E.A. ; S. Daosukho and H. El-Shall (2009). Effct of supersaturation ratio and khella extract on nucleation and morphology kidney of stone. J. Crystal Growth, (311): 2673-2781

Agarwal, C. ; R. Wadhwa ; G. Deep ; D. Biedermann ; R. Gažák and V. Křen (2013). Anti-cancer efficacy of silybin derivatives - A structure-activity relationship. PLoS One; 8:e60074

Al-Gamdi, Noura ; M. William and C. Alan (2011). Tea prepared from *Anastatica hierochuntica* seeds contains a diversity of antioxidant flavonids, chlorogenic acids and phenolic compounds. Phytochemistry, 72:248-254.

Atamani, F. and S.R. Khan (2000) Effects of an extract from Herniaria hirsuta on calcium oxalate crystallization in vitro. Br. J. Urol. Int.; 85:621-625

Atanassova, S. ; K. Neykov and I. Gutzow (1996). Solubility, inhibited growth and dissolution kinetics of calcium oxalate crystals in solutions, containing α-ketoglutaric acid. J Crystal Growth: 160:148-153

Azlim, A.A. ; K.C. Ahmed Jalal; Z.I. Syed ; S.K. Mustapha ; M.R. Aisyah and R.K. Kamarul (2010). Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants leaves J., 17: 1077-1084

Batanouny, K.H.(1999). Wild Medicinal Plants in Egypt. The Palm Press. Cairo; (207)

Bienert, G.P. ; A.L. Møller ; K.A. Kristiansen ; A. Schulz ; I.M. Møller ; J.K. Schjoerring and T.P. Jahn (2006). Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes, J. Biol. Chem., 282:1183–1192

Bienert, G.P. ; J.K. Schjoerring and T.P. Jahn (2007) Membrane transport of hydrogen peroxide, Biochim. Biophys. Acta., 1758: 994–1003

Bouropoulos, K. ; N. Bouropoulos ; M. Melekos ; P.G. Koutsoukos ; G.C. Chitanu ; A.G. Anghelescu-Dogaru and A.A. Carpov (1998). The inhibition of calcium oxalate monohydrate crystal growth by maleic acid copolymers. The J. Urology, 159 (5): 1755-1761.22-

Braca, A. ; N.D. Tommasi ; L.D. Bari ; C. Pizza ; M. Politi and I. Morelli (2001). Antioxidant principles from Bauhinia terapotensis. J. Nat. Prod. (64): 892-895

Brunauer, S. ; P.H. Emmett and E. Teller (1938). Adsorption of Gases in Multimolecular Layers". J. American Chemical Society, 60 (2): 309–319.

Cai, Y. ; Q. Luo ; M. Sun and H. Corke (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74:2157-2184.

Chang, C. ; M. Yang ; H. Wen and J. Chern (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.

Chaudhary, A. ; S.K. Singla and C. Tandon (2010). In vitro evaluation of Terminalia arjuna on calcium phosphate and calcium oxalate crystallization. Ind. J. Pharm. Sci.,72(3):340–345.

Das, N. and T.A. Pereira (1990). Effects of flavonoids on thermal autooxidation of palm oil: structure activity relationship. J. Am Oil Chem Soc., (67): 255–258.

Ebrahimzadeh, M.A. ; S.F. Nabavi and S.M. Nabavi (2009). Antioxidant activities of methanol extract of *Sambucusebulus* L. flower. Pak J. Biol Sci., 12: 447-450

Ekanayake, P.M. ; C. Nikapitiya ; M. De Zoysa ; I. Whang ; S.J. Kim and J. Lee (2008). Novel antico.agulant compound from fermented red alga Pachymeniopsis elliptica. Eur Food Res Technol.; 227 (3): 897-903.

El-Ghazali, G.E. ; K.S. Al-Khalifa ; G.A. Saleem and E.M. Abdallah (2010). Traditional medicinal plants indigenous to Al-Rass province, Saudi Arabia. J. Med. Plant Res., (4): 2680-2683.

Furie, B. and B.C. Furie (2005). Thrombus formation *In Vivo*. J. Clin Invest., 115(12):3355-62.

Grases, F. ; L. Masarova ; O. Sohnel and A. Costa-Bauza (1992). Agglomeration of calcium oxalate monohydrate in synthetic urine Brit. J. Urol.; 70 (3): 240 .

Grases, F. ; C. Genestar and A. Millan (1989). The influence of some metallic ions and their complexes on the kinetics of crystal growth of calcium oxalate. J. Crystal Growth., 94: 507–512.

Grases, F. ; J. March ; G.F. Bibiloni and E. Amat (1988). The crystallization of calcium oxalate in the presence of amino acids. J. Crystal Growth., (87): 299-304.

Gulcin, I. ; Z. Huyut ; M. Elmastas and H.Y. Aboul-Enein (2010). Radical scavenging and antioxidant activity of tannic acid. Arab J. Chem.,3(1): 43-53.

Gyamfi, M.A. ; M. Yonamine and Y. Aniya (1999). Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. Gen. Pharmacol.;32:661-667.

Hamza, S.M. and G.H. Nancollas (1991). The dissolutions of calcium oxalate in the presence some of natural productes. J. Sci. Menoufia Univ., (V).265-273.

Havsteen, B.H. (2002). The biochemistry and medical significance of the flavonoids. pharmacol Ther., (96):67-202.

Jan, L. ; S. Nicolas ; K. Joachim and W. Lothar (2006) Gas chromatography mass spectrometry based metabolite profiling in plants, Nature Protocol.; 1(1):387-396

Kamboj, V.P. (2000). Antioxidant Content of Selected Medicinal Plants Used by Kaani Tribes of Kanyakumari District in Tamilnadu India. Curr. Sci.; 78(1):35.

Kathiresan, K. and V.S. Ravindran (2006). Muruganantham A. Mangrove extracts prevent the blood coagulate. Ind. J. Biotechnol., 5(2)52-254

Kessler, M. ; G. Ubeaud and L. Jung (2003). Anti- and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol (55):131–142.

Khalifa, T.I. and M.A. Ahmad (1980). Pharmacognostical study of certain species of Anastatica. PhD thesis, University of Cairo, Egypt.

Lamponi, S. (2021). Bioactive natural compounds with antiplatelet and anticoagulant activity and their potential role in the treatment of thrombotic disorders. Life (Basel). 15;11(10):1095.

Langmuir, I. (1916). The constitution and fundamental properties of solids and liquids. Part I. Solids. J. Am. Chem. Soc., (11), 2221- 2295

Lee, J.C. ; H.R. Kim ; J. Kim and Y.S. Jang (2002). Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten, J. Agric. and Food Chem.; 50:6490- 6496.

Lien, Ai Pham-Huy ; Hua He and Chuong Pham-Huy (2008). Free radicals, antioxidants in disease and health. Int. J. Biomed Sci., 4 (2): 89-96)

Loguercio, C. and D. Festi (2011). Silybin and the liver: from basic research to clinical practice. World J. Gastroenterol., 17: 288–301

Marzouk, M.M. ; M.A. Al-Nowaihi ; S.A. Kawashty and N.A. Saleh (2010). Chemosystematic studies on certain species of the family

Brassicaceae Cruciferae) in Egypt. Biochem. Syst. Ecol.; 38(4): 680-685.

Matkowshi, A. and M. Piotrowska (2006). Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. Fitoterapia.,(77):346–353.

Menon, M.D. ; B.G. Parulkar and G.W. Drach (1988). "Campbell's Urology", 7th ed., W. B. Saunders Company, New York.

Mohamed, A.A. ; A.A. Khalil and H.E.S. El-Beltagi (2010). Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (Hyphaene thebaica) Grasas Y Aceites.; 61 (1): 67–75.

Mohamed, Amal A. ; S.I. Ali and F.K. El-Baz (2013). Antioxidant and Antibacterial Activities of Crude Extracts and Essential Oils of Syzygiumcumyini Leaves. PLoS One. Apr 12;8(4):e60269.

Nakashima, S. ; H. Matsuda ; Y. Oda ; S. Nakamura and F. Xu (2010). Melanogenesis inhibitors from the desert plant *Anastatica hierochuntica* in B16 melanoma cells. Bioorg Med Chem.,(8) :2337-2345.

Pandey, K.B. and S.I. Rizvi (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2(5);:270-278.

Pascale, G. ; H. Mireille ; B. Patrick and J. Marie (1999).A. Antioxidant composition and activity of barley(Hordeum vulgare) and malt extracts and ofisolated phenolic compounds. J. Sci Food Agric.,79:1625-1634

Pawlaczyk, I. ; L. Czerchawski ; W. Pilecki ; E. Lamer-Zarawska and R. Gancarz (2009). Polyphenolic-polysaccharide compounds from selected medicinal plants of Asteraceae and Rosaceae families.Chemical characterization and blood anticoagulant activity. Carbohydr Polym., 77(3): 568-575.

Robertson, W.G. (1981). Factors influencing the crystallisation of calcium oxalate in urine - critique Scurr, D S, Bridge M. J. Crystal Growth.; 53(1): 182 .

Ruch, R.J. ; S.J. Cheng and J.E. Klaunig (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis.Jun;10(6):1003-1008.

Saranya, R. ; M.S. Ali and V. Dnuraha (2019). phytochemical, Fluorescence And Screeninggc-MC Analysisof Vaeious Crude

Extracts Of *Anastatica Hierochuntica* L. J. pharm. Chem. and Biol. Sci.,7(1):44-56.

Seifried, H.E. ; D.E. Anderson; E.I. Fisher and J.A. Milner (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. J. Nutr. Biochem.,(18): 567–579

Selvam, R. and P. Kalaiselvi (2000). A novel basic protein from human kidney which inhibits calcium oxalate crystal growth. Br. J. Urol. Int; (7): 86

Shah, A.H. ; M.P. Bhandari ; N.O. Al-Harbi and R.M. Al-Ashban (2014). Kaff-E-Maryam (*Anastatica Hierochuntica* L.): evaluation of gastro-protective activity and toxicity in different experimental models. Biol. Mreed, 6: 197.

Shariff, U.Z. (2001). Modern Herbal Therapy for Common Ailments. Nature Pharmacy Series.2001; (1): 9-84, Spectrum Books Ltd., Ibadan, Nigeria in Association with Safari Books (Export) Ltd. UK,

Shylaja, M.R. and K.V. Peter (2004). The functional role of herbal spices. In K. V. Peter (Ed.), Handbook of herbs and spices volume 2 (pp. 11–21). Cambridge, England.:Woodhead Publishing Limited. Google Books.

Škrtić, D. ; M. Marković and H. Füredi-Milhofer (1986). Precipitation of calcium oxalates from high ionic strength solutions IV. Testing of kinetic models. J. Cryst. Growth, 79: 791-6.

Sobhy, Eman A. ; M. Tailang ; S. Benyounes and K. Gauthaman (2011). Antimalarial and hepatoprotective effects of entire plants of Anastatic hierochuntica. Int. J. Res. Phytoch. Pharm.; (1): 24-27.

Sultana, B. ; F. Anwar and R. Przybylski (2007). Antioxidant activity of phenolic components present in barks of barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica, andEugenia jambolana Lam trees. Food Chem., (104): 1106–1114

Surh, Y.J. and L.R. Ferguson (2003). Dietary and medicinal antimutagens and anticarcinogens: molecular mechanisms and chemopreventive potential--highlights of a symposium. Mutat Res., 523-524:1-8.

Uddin, R. ; M.R. Saha ; N. Subhan; H. Hossain ; I.A. Jahan ; R. Akter and A. Alam (2014). HPLC-analysis of polyphenolic compounds in Gardenia jasminoides and determination of antioxidant activity by using free radical scavenging assays. Adv Pharm. Bull; 4(3):273-281.

Waszkowiak, K. ; A. Gliszczyńska-Świgło ; V. Barthet and J. Skręty (2015). Effect of extraction method on the phenolic and cyanogenic glucoside profile of flaxseed extracts and their antioxidant capacity. J. Am. Oil Chem. Soc.,92(11-12):1609-1619

Webster, C. (2001). Quick Look Series in Veterinary Clinical Pharmacology. Teton New Media, Wyoming, USA.PP 124-125.

Yoshikawa, M.F. ; T. Morikawa ; K. Ninomiya and H. Matsuda (2003). Anastatin A and B, New skeletal flavonoids with hepatoprotective activit ies from the desert plant Anastatica hierochuntica. Bioorg. Med. Chem. Lett., 13(6): 1045-1049.

النشاط البيولوجى لمستخلصات عشبة كف مريم ومكوناتها كمضادات لتكوين حصوات الكلى ومضادات الاكسدة وتخثر الدم معمليا.

رفعت أحمد ابراهيم صابر

قسم علوم الأراضى والمياة – كلية التكنولوجيا والتنميه– جامعة الزقازيق

كف مريم (*Anastatica hierochuntica* L.) من النباتات المفيدة جدًا في الدول العربية لعلاج الاضطرابات الصحية . لقد تم الاعتراف بالطب التقليدي نظرًا لأهميته في الصحة العامة وإدارة العدوى. يركز هذا العمل على تحديد خصائص المركبات الفينولية في مستخلصات الميثانول وخلات الإيثيل لكف مريم *A. hierochuntica* L. .تم استخدام كروماتوجرافيا السائل عالى الأداء (HPLC) لتحديد الفينولات المختلفة في كلا المستخلصين وكروماتوجرافيا الغاز (GC MS) من أجل المركبات العضوية النشطة بيولوجيًا في مستخلص الميثانول كأداة أساسية للتشخيص لهذه المركبات. تم تقييم مركبات البوليفينول الفردية في خام مستخلصين مختلفين من المذيبات باستخدام HPLC ، وسجلت النتائج أن المركب الأكثر تواجدا هو E–Vallinic و Ellagic.أيضا ، تم إجراء تحليل GC–MS لفحص المركبات العضوية النشطة بيولوجيا للمستخلص الميثانولي من *A. hierochuntica* L. ، وأوضحت النتائج أنه تم تحديد البنزيل نتريل كمكوِّن كيميائي رئيسي وفقًا للوفرة النسبية يلية البنزين ، (isothiocyanatomethyl) على التوالى. كان الغرض من هذه الدراسة هو فحص تكوين حصوات الكلى ، ومضادات الأكسدة ، ومضادات التخثر لمستخلصات *A. hierochuntica* L. تمت دراسة التأثير المحتمل لمستخلص A. hierochuntica L على نمو بلورات أوكسالات الكالسيوم أحادية الماء (حصوات الكلى) وخصائصها المثبطة عند 37 درجة مئوية ودرجة الحموضة = 6.5 ± 0.05. بالإضافة إلى ذلك ، تم إجراء خصائص مضادة للأكسدة ومضادة للتخثر لنشاط *A. hierochuntica* L. بتركيزات مختلفة. يسلط البحث الحالي الضوء على التركيب الكيميائي وخصائص *A. hierochuntica* L. التي قد تكون مرتبطة بتأثيرها المفيد. تشير النتائج في هذه المقالة إلى أن محتوى مادة البوليفينول قد يساهم في تثبيط تحص بولي ، ومضادات الأكسدة ، ونشاط منع تخثر الدم لهذا النبات ويثبت أنه مصدر طبيعي قيِّم لمضادات التحسس ومضادات الأكسدة ومضاد تخثر الدم المطبق في صناعة الأدوية.